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FOOT-AND-MOUTH DISEASE AND THE AFRICAN BUFFALO *(SYNCERUS CAFFER)*. II. VIRUS EXCRETION AND TRANSMISSION DURING ACUTE INFECTION

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ABSTRACT

GAINARU, M. D., THOMSON, G. R., BENGIS, R. G., ESTERHUYSEN, J. J., BRUCE, W. & PINI, A., 1986. Foot-and-mouth disease in the African buffalo *(Syncerus caffer)*. II. Virus excretion and transmission during acute infection. *Onderstepoort Journal of Veterinary Research*, 53, 75–85 (1986).

Three groups of young buffalo in captivity were infected by exposing them to similar buffalo in the acute stages of infection induced by needle inoculation with SAT 1 or 2 viruses. Clear foot lesions developed in most of the buffalo from which the relevant virus types were re-isolated. During the first week following infection virus was found in blood, nasal secretions, saliva, preputial secretions and faeces. Air samples collected in the immediate vicinity of acutely infected buffalo were also found to contain virus. However, the regularity of virus detection as well as the quantity of virus in buffalo specimens was generally lower than for cattle infected with viruses of the same type. Conversely, virus was detected in the nasal secretions or saliva of 3 buffalo up to 4 weeks after infection, a situation which has not been encountered in cattle.

Susceptible cattle and impala *(Aepyceros melampus)* were penned together with or in the immediate vicinity of infected buffalo and shared feeding and watering facilities with the buffalo. The pattern of transmission which emerged indicated that transfer of these viruses from buffalo to other species probably occurs only in the acute stages of infection and where there is direct physical contact between the species.

INTRODUCTION

The African buffalo *(Syncerus caffer)* is the only known free-living maintenance host for the SAT types of foot-and-mouth disease (FMD) virus (Hedger, 1976a) and because of the long association between game and FMD in southern Africa (Rossiter & Albertyn, 1947; Lamprecht, Buhr & Van der Merwe, 1956; Howell & Mansvelt, 1972) it is widely assumed that buffalo are the primary source of infection for domestic stock where effective control measures preclude cattle or other domestic stock maintaining the infection.

Furthermore, the finding that virus persists in the pharynx of buffalo infected with the SAT types of FMD for many years (Hedger, 1976a; Condy, Hedger, Hamblin & Barnett, 1985)—the animals have been termed carriers (Hedger, 1976a)—and the circumstantial association between FMD outbreaks in domestic stock and the presence of buffalo, has led to the belief that carriers are generally the primary source of infection for farm animals in Southern Africa. However, despite the recent report by Hedger & Condy (1985), carriers are, at least in general, inefficient transmitters of SAT type viruses (Bengis, Thomson, Hedger, De Vos & Pini, 1986; Anderson, 1986). The question therefore arises as to how efficiently buffalo transmit SAT viruses during acute infection. This is important because it is possible that young buffalo undergoing primary infection, rather than older carriers, provide the most likely source of infection both within buffalo herds and for domestic stock. The experiments described here were based on this premise.

The strategy employed was to infect young buffalo "naturally" by exposing them to members of the same species infected by needle inoculation. Thereafter, the inoculted animals were usually removed from the enclosure and replaced by susceptible cattle. Cattle that became infected were allowed to recover for 3 weeks and were then removed and replaced by fresh susceptible cattle. By repeating this process it was hoped that an indication of how long buffalo remained infectious after recovery from acute disease could be obtained.

MATERIALS AND METHODS

Animals

(i) *Buffalo*

Two groups of young buffalo were used in this work. The first consisted of 6 animals which were born in captivity to cows captured in the Kruger National Park (KNP) in the latter half of 1981 (Bengis *et al.*, 1985). They were all approximately 12 months old when these experiments commenced. As judged by their lack of demonstrable neutralizing antibody to SAT types 1, 2 and 3, they were susceptible to FMD (Bengis *et al.*, 1985). These buffalo were used in Experiments 1 and 2 (Table 3; Fig. 1).

The second group of 6 captive buffalo were kindly donated by the Natal Parks, Game and Fish Preservation Board and were captured in the Umfolozi Game Reserve during June, 1984. These animals were 12–18 months old when first exposed to FMD virus. The SAT types of FMD virus have been shown not to occur in the Umfolozi Game Reserve (Esterhuysen, Thomson, Flamand & Bengis, 1985) and other FMD types do not occur within the borders of the RSA. The buffalo had no demonstrable antibody to SAT 1, 2 and 3 viruses prior to experimental exposure to the disease. These buffalo were used in Experiment 3 (Table 3; Fig. 1).

For collection of specimens from buffalo they were immobilized using 3 mg etorphine hydrochloride[1] and 10–20 mg of xylazine HC1[2] inoculated intramuscularly using a projectile syringe fired from a compressed-air gun. To allow sufficient recovery time between immobilizations buffalo were sampled at intervals of not less than 2 days.

(ii) *Cattle*

The cattle used in these experiments were of various and mixed breeds and were all oxen aged 18 months or more. All had been reared in FMD-free areas, had not been immunized against the disease and showed no neutralizing antibody titres to the SAT virus types.

(iii) *Impala*

Fourteen 8–20 month old impala were captured in the Skukuza area of the KNP. All lacked demonstrable neutralizing antibody to SAT types 1, 2 and 3 prior to being introduced to pens adjacent to infected buffalo.

(iv) *Mice*

Two to 5 day old sucking mice (Onderstepoort conventional outbred strain) were used for virus titration.

(v) *Housing*

Buffalo, as well as cattle and impala which were kept in contact with them, were housed in isolation pens at

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[2] Division of Veterinary Services, P.O. Box 12, Skukuza 1350

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[1] M99, Rickett & Coleman, P.O. Box 32072, Mobeni 4060

[2] Bayer S.A. (Pty) Ltd, P.O. Box 143, Isando 1600

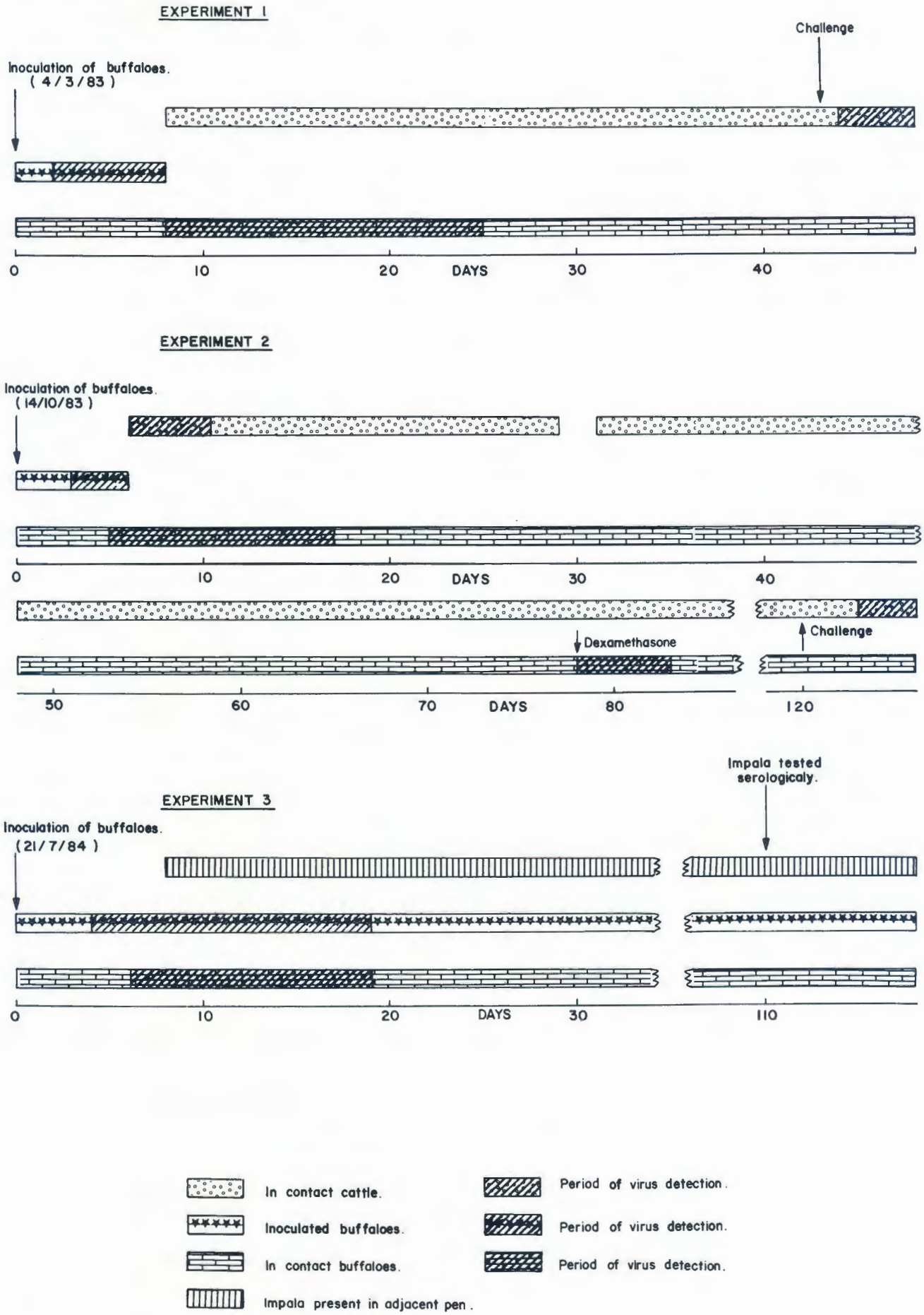

FIG. 1 The chronological sequence of events in Experiment 1, 2 and 3

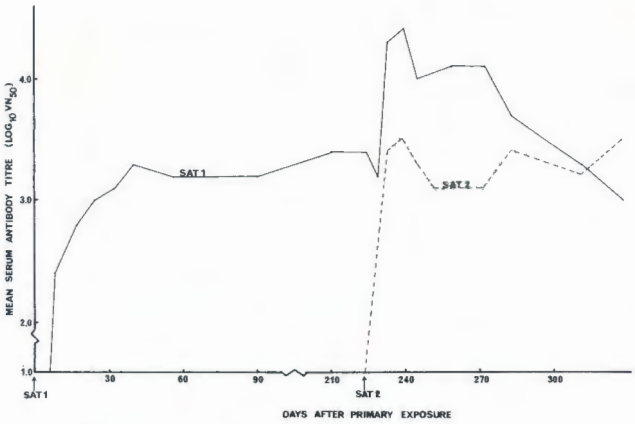

FIG. 2 The mean neutralizing antibody titres of 4 buffalo infected sequentially with SAT 1 and 2 viruses

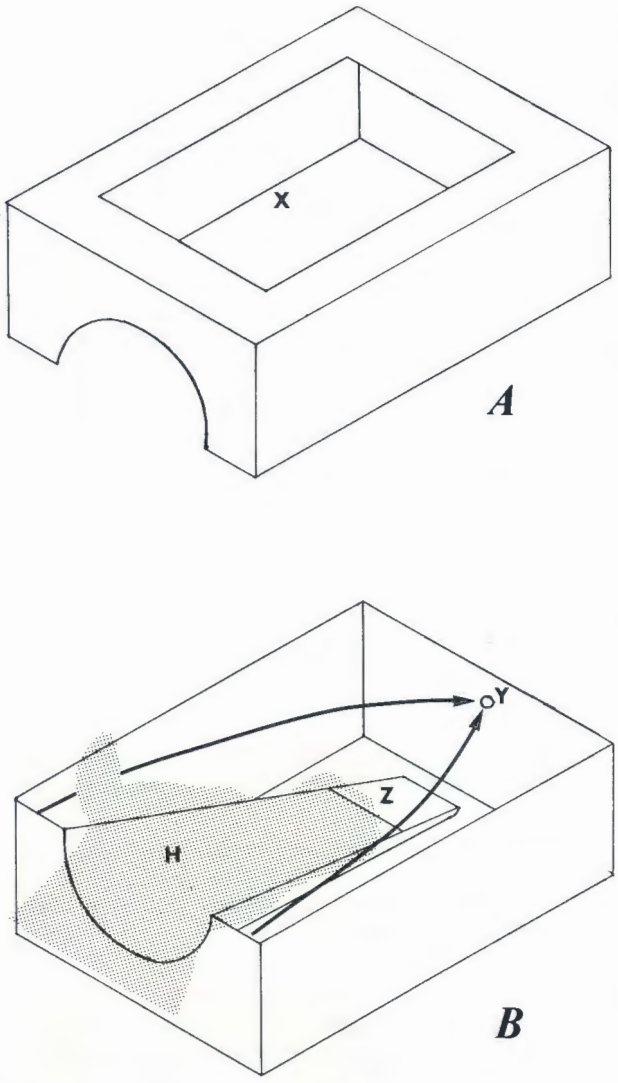

FIG. 3 Diagramatic representation of the head mask used for sampling expired air from buffalo and cattle. (Scale 1:20)

A & B—The 2 halves of the mask are shown separated for purposes of illustration. The arrows show the approximate direction of air movement.

X—Transparent viewing panel

Y—Port leading to the cyclone sampler

Z—Sloping head-rest

H—Position of the animal's head

Skukuza in the KNP. The lay-out of the pens used was described by Bengis *et al.* (1985). The KNP is a declared FMD enzootic area although only the SAT types have been identified there (Hedger, 1976b).

TABLE 1 Comparative titres of suspensions prepared from bovine tongue epithelium from cattle infected with SAT types of FMD virus in different systems

	Virus type	Titration system[3]		
		Cattle tongue	Sucking mice	IB-RS-2 cell cultures
SAR 17/80[1]	SAT 1	7,0	8,3	5,5
PHALAB/2–6/83/2[2]	SAT 2	8,0	8,7	6,5
SAR 2/81[1]	SAT 2	8,0	7,8	6,2
SAR 1/80[1]	SAT 3	6,0	7,9	5,8

[1] World Reference Laboratory Designation

[2] Designation of FMD Laboratory, Onderstepoort

[3] Log_{10} ID_{50} (cattle), LD_{50} (mice) or $TC1D_{50}$ (IB-RS-2 cells)/mℓ

In Experiments 1 and 2 (Table 3) the buffalo and cattle were alternated daily between pens B and C to facilitate cleaning (Bengis *et al.*, 1985). Due to the probability of injury to the latter, buffalo and impala (Experiment 3; Table 3) were not housed together but were held in adjacent pens, i.e. C and D respectively (Bengis *et al.*, 1985). The 2 species were however obligad to use the same water containers and hay racks.

The 6 cattle used to determine virus excretion following experimental infection (Experiments 4 & 5; Table 3) were housed in the high security stabling facilities at the FMD Laboratory, Onderstepoort. The salient features of this laboratory have been described elsewhere (Bruce & Pini, 1984). The stables were held at a negative pressure of approximately 50–60 Pa, a temperature of approximately 24 °C and underwent 10–15 air changes per hour.

Specimen collection for virus detection

Specimens from cattle were collected daily for 2 weeks after infection but buffalo were sampled at 2 day intervals or longer and sampling continued for at least 30 days after infection.

(i) Blood was collected from the jugular or tail veins into 5 mℓ vacuum tubes containing 0,05 mℓ 15 % EDTA (K_3) solution[1].

(ii) *Nasopharyngeal swabs* (NPS) consisted of a 400 mm piece of stainless steel welding wire to the end of which a swab made of cotton wool enclosed in cotton gauze was attached. The sterile swab was passed up the nasal passage as far as the soft palate before withdrawal. The end of the swab was then cut off into a bottle containing 5 mℓ of phosphate buffered saline (PBS), pH 7,4, incorporating 0,2 % bovine serum albumin.

(iii) *Conjunctival secretions* were collected by syringing 2 mℓ of PBS into the conjunctival sac and recovering as much of the fluid as possible.

(iv) *Preputial secretions* were sampled by flushing the preputial opening with 5 mℓ of PBS in the same way as for conjunctival secretions.

(v) *Urine* was collected directly into clean sterile bottles whenever possible.

(vi) *Faeces* (±1 g) was extracted from the rectum and a 10 % suspension in PBS prepared using a pestle and mortar. The suspension was clarified by low speed centrifugation.

(vii) *Air samples* were collected from either the immediate vicinity of infected cattle or buffalo or by means of a fabricated structure which enclosed the head of the

[1] Venoject, Terumo, Tokyo, Japan

TABLE 2 Recovery of SAT 1 virus from the cyclone sampler during experiments run to test the efficiency of the system

Method of testing	Virus introduced	Virus collected	Running time (min)	Proportion of virus recovered
				%
Virus introduced into the sampling fluid prior to commencement of sampling	5×10^{8}[1]	1×10^{8}	30	20
Virus released inside the head cover (normally enclosing the head of the animal)	4×10^{8}	7×10^{7}	45	18
Virus released inside the head cover (normally enclosing the head of the animal)	5×10^{8}	3×10^{7}	30	6

[1] Plaque forming units

TABLE 3 The methods of infection and responses of buffalo and cattle to SAT 1 and 2 viruses

Experiment No.	Species	No. animals	Virus (Table 1)	Mode of infection	Titre of inoculum ($\log_{10} ID_{50}$)	No. animals which developed FMD lesions	No. animals which developed pyrexia (≥39 °C)	4-fold increase in SVN titre to relevant virus (No. responding/ No. tested)
1A	Buffalo	2	SAR 17/80	Intramascular inoculation	7,5	2	NT	2/2
1B	Buffalo	4	SAR 17/80	Contact	NA	3	0	4/4
2A	Buffalo	2	PHALAB/ 2–6/83/2	Intravenous and submucosal inoculation	7,7 (i/v) 6,4 (s/m)	2	1	2/2
2B	Buffalo	4	PHALAB/ 2–6/83/2	Contact	NA	3	2	4/4
3A	Buffalo	2	PHALAB 2–6/83/2	Intramuscular inoculation	8,4	2	2	2/2
3B	Buffalo	4	PHALAB 2–6/83/2	Contact	NA	4	4	4/4
4	Cattle	4	SAR 17/80	Intradermolingual inoculation	6,3	4	4	4/4
5	Cattle	2	SAR 2/81	Intradermolingual inoculation	7,3	2	2	2/2

NT—Not tested
NA—Not applicable

immobilized animal (Fig. 3). The latter was constructed of a metal frame and plastic sheeting and was connected directly to the air sampler by means of a plastic tube. Air was sampled at the rate of approximately 700 ℓ/min using a cyclone sampler (Errington & Powell, 1969; Donaldson & Ferris, 1982). Sampling took place over 20 or 30 min periods and the collecting fluid consisted of PBS diluted 1:2 with distilled water (to compensate for evaporation, i.e. the concentration effect) containing 0,5 % bovine serum albumin and BRIJ 35 (lauryl ether of polyoxyethylene)[1].

All specimens which were not titrated immediately were stored at −70 °C or in liquid nitrogen in glass or polycarbonate bottles or vials. Where specimens were collected in the open air (i.e. in the KNP) care was taken to protect them from direct sunlight and to keep them cool until they were labelled and stored in liquid nitrogen. While cattle specimens were generally examined within 3 weeks of collection, buffalo specimens were mostly stored for several months prior to examination.

(viii) *Oesophageal/pharyngeal (O/P) specimens* were collected using the method described by Hedger (1968).

Viruses

Viruses used for infecting cattle and buffalo are shown in Table 1. In each case the virus was stored at −70 °C in 1 mℓ aliquots of a 10 % suspension of bovine tongue epithelium incorporating 5 % glycerol.

Virus titration

Ten-fold dilutions, prepared in PBS, of specimens which potentially contained FMD virus were titrated by intraperitoneal inoculation of 2–5 day old sucking mice. During a 6 day observation period any mouse which died or become ill was beheaded, skinned and gutted and the rest of the carcass ground up with a pestle and mortar and used to prepare a 10 % suspension in PBS containing antibiotics. The suspension was clarified by low speed centrifugation. This, as well as 10-fold dilutions thereof, was inoculated into IB-RS-2[2] tube cultures (4 tubes per dilution) which were examined daily for 3 days for the

[1] BDH Chemicals, Poole, England

[2] Pig kidney cell line obtained from Istituto Zooprofilattico Sperimentale, Brescia, Italy

TABLE 4 Comparison between virus isolation achieved from buffalo and cattle infected with SAT 2 viruses (Experiments 2, 3 & 5, Table 3)

	Species	Blood	Air samples	Nasal secretions	Saliva	Conjunctival fluid	Preputial secretions	Urine	Faeces
No. of animals with positive specimens/ No. tested	B	7/10	0/6*	5/10	6/6	0/6	4/6	ND	1/6
	C	2/2	1/2	2/2	2/2	2/2	2/2	1/2	0/2
Day of 1st virus detection (days)	B	1–5	NA	1–3	1–<6	NA	<2–10	ND	8–10
	C	1	2	1–2	1	1–4	1	2	NA
Duration of virus detection	B	1–4	NA	2–6 (26)	1–6	NA	1–≤7	ND	1
	C	3	1	6	6	1–2	3–5	1	NA
Titre range (log_{10} MLD_{50})	B	1,5–3,7	NA	0,5–5,2	1,3–4,3	NA	2,3–4,5	ND	1,4
	C	1,4–3,9	D	1,6–5,4	1,1–5,0	1,8–3,3	1,9–4,5	0,9	NA

B—Buffalo
C—Cattle
D—Virus detected but level too low to titrate
NA—Not applicable
ND—Not done
* Positive air samples were obtained from the whole group of buffalo in Experiment 2B but not from individual animals in Experiments 3A and 3B

TABLE 5 Comparison between virus isolation achieved from buffalo and cattle infected with SAT 1 virus (Experiments 1 & 4, Table 3)

	Species	Foot lesion epithelium	Blood	Air samples	Nasal secretions	Saliva	Conjunctival fluid	Preputial swabs	Faeces
No. animals with positive specimens/ No. tested	B	5/6	4/4	ND	6/6	ND	ND	ND	ND
	C	1/1 (1)	4/4	3/4	4/4	4/4	3/4	4/4	0/4
Day of 1st virus detection (days)	B	ND	<4	ND	2	ND	ND	ND	ND
	C	ND	1–2	2–3	1–3	1–2	2–5	1–3	NA
Duration of virus detection	B	ND	1–3	ND	2–6 (26)*	ND	ND	ND	ND
	C	ND	3–4	1	2–6	5–6	1–3	3–6	NA
Titre range (log_{10} MLB_{50})	B	6,2–8,0	3,3–4,8	ND	1,5–4,8	ND	ND	ND	ND
	C	8,3	1,3–5,2	D	1,6–5,6	1,3 5,8	2,0–4,0	1,8–5,7	NA

*(26)—Virus was isolated from nasal swabs of 2 buffalo approximately 26 days after infection after a 20 day period during which no isolation was achieved
(1)—Pooled specimen from 2 cattle
ND—Not done
NA—Not applicable
D—Virus detected but level too low to titrate

appearance of cytopathic effects (CPE). Cultures in which CPE developed were tested by microplate complement fixation (method recommended by the World Reference Laboratory for FMD, R. S. Hedger, personal communication, 1980) to check the type of the virus isolated.

Plaque titrations were performed on IB-RS-2 cells. The overlay consisted of 0,5 % agarose in Earles medium incorporating 5 % normal bovine serum. Incubation took place for 42 hours in an atmosphere of 5 % CO_2 in air.

Tube titrations were performed in IB-RS-2 and first passage bovine foetal kidney (BFK) cultures which were prepared using conventional techniques. The maintenance media used were a Hanks/Earles mixture supplemented with lactalbumin hydrolysate and 2 % bovine serum (IB-RS-2) or Eagles MEM containing 2 % bovine serum.

Serum neutralizing antibody (SVN) tests

SVN tests were performed in flat-bottomed microplate wells as described previously (Esterhuysen *et al.*, 1985).

Weather data

The data quoted here was obtained from records kindly made available by Mr C. J. H. van der Walt, P.O. Box X402, Skukuza 1350.

RESULTS

Virus titration systems

Table 1 shows that the mouse system used for virus titration was more sensitive in 3 of 4 viruses tested than intradermolingual titration in cattle tongues *in vivo*. This was so in all cases where mice and IB-RS-2 cell cultures were compared. Initially first passage tube cultures of

TABLE 6 Comparison between cattle and buffalo with respect to the quantity of SAT viruses in the 4 specimens which most frequently contained detectable infectivity

Specimen	No. animals with positive specimens / No. tested		Frequency distribution of titres in positive specimens									
			Cattle					Buffalo				
	Cattle	Buffalo	1,0–1,9[1]	2,0–2,9	3,0–3,9	4,0–4,9	5,0–5,9	1,0–1,9	2,0–2,9	3,0–vb43,9	4,0–4,9	5,0–5,9
Blood	6/6	11/14	5	2	5	6	1	6	0	5	2	0
NPS	6/6	9/14	5	7	5	6	6	3	7	2	6	1
Saliva	6/6	5/6	2	6	6	14	5	1	1	4	1	0
Preputial swabs	6/6	4/6	2	4	5	9	8	0	3	0	1	0

[1] Titre interval (log_{10} LD_{50}/mℓ or swab)

TABLE 7 Virus recovered from sequential nasopharyngeal swabs and oesophageal/pharyngeal specimens of 4 selected buffalo and 2 cattle

Species	Experiment No. (Table 3)	Animal No.	Specimen								
			NPS								O/P scrapings
Buffalo	1B	NA	1–2[1]	3–4	4–5	6–7	8–9	10–12	13–14	28–29	35–36
		3	2,5[2]	4,8	—	—	—	—	—	—	2,9
		4	2,0	2,5	2,3	—	—	—	—	3,8	—
		5	2,8	1,5	—	—	—	—	—	≥4,4	—
		6	—	3,7	—	—	—	—	—	—	—
Cattle	4	7455-5	5,0*	5,6	4,4	3,5	—	—	—	ND	ND
	5	52-7	5,4*	2,6	1,9	1,7	—	—	—	ND	ND

[1] 1–2 days after exposure to infection (buffalo) or virus inoculation (cattle)

[2] Log_{10} MLD_{50}/mℓ

* Times at which virus was also recovered from air samples

bovine foetal kidney (BFK) cells were used in parallel with mice but the sensitivity of different batches proved variable and BFK cultures were therefore discontinued (results not shown).

Performance of the air sampling system

The proportion of virus likely to be lost in the process of sampling air was investigated in 2 ways. Firstly, a known quantity of SAR 17/80, which had previously been adapted to growth in cell culture, was added to the collecting fluid prior to running the sampler. The quantity of virus in the sampling fluid at the start was then compared with that which remained after it had been run through the clyclone sampler (Table 2). In the second method, a known quantity of the same virus was nebulized inside the sampling hood [which in normal circumstances enclosed the head of the animal (Fig. 3)] using an Acorn Nebulizer[1] and compared with the quantity of virus finally collected. Table 2 indicates that the minimal quantities of virus recovered from cattle and buffalo (Tables 4, 5 & 7) were likely to have had a true value of approximately 1 log_{10} unit more than the actual quantities detected.

Extensive swabbing of the interior surfaces of the hood failed to deliver detectable quantities of virus which indicated that aerosol adsorption was unlikely to be the source of this loss.

[1] A. W. Staniforth, 15–16 Station Rd., Penarth, U.K.

Clinical responses of buffalo to infection with SAT 1 and 2 viruses

Apart from 1 animal, all the buffalo inoculated with or exposed to SAT types 1 and 2 developed clear FMD lesions on the feet. The lesions were confined to the interdigital cleft and, in some cases, were large, i.e. up to 5 cm in length. Vesicles which were seen prior to rupture were found to contain, in comparison with cattle showing similar lesions, relatively little fluid. Despite the extent of some lesions lameness was not apparent.

The animal which failed to show visible lesions behaved in this way on both occasions it was infected, i.e., in Experiments 1B and 2B (Table 3). That it did become infected was demonstrated by isolation of virus from NPS and it's antibody responses (Table 3).

Typical FMD lesions in the mouths of infected buffalo were never seen and excessive salivation was also not observed.

Temperature responses following infection were variable (Table 3).

Levels of infectivity in the body fluids, secretions and excretions of acutely infected buffalo and cattle

The results of attempts at isolating and titrating SAT viruses excreted by acutely infected buffalo and cattle are summarised in Tables 4 and 5.

From cattle, SAT 1 and 2 viruses were isolated consistently during the first week following infection from blood, NPS, saliva as well as conjuctival and preputial washings at titres $\geq 10^4$/mℓ or per sample (Tables 4 & 5).

Isolation from faeces was never achieved and although virus was recovered from the urine of 1 of the 2 animals tested, the titre of this specimen was lower than that of the preputial swab collected on the same day ($10^{0.9}$/mℓ vs $10^{4.5}$), i.e. the urine may have been contaminated by preputial secretions.

In buffalo, virus was recovered from all the above specimens with the exception of conjunctival washings and urine (which was not examined) during the week following infection. Virus was not, however, as consistently detected and titres were generally lower than in cattle (Tables 4, 5 & 6). There was no obvious difference in the behaviour of SAT 1 and 2 viruses in buffalo apart from viraemias which reached higher levels in SAT 1 infections than SAT 2; this was also the case in cattle (Tables 4 & 5).

In 2 buffalo (Experiment 1B; Table 3) virus was isolated from NPS taken 4 weeks after infection, i.e. after a period during which no isolation was made (Tables 5 & 7). Furthermore, the virus titre in the NPS of these 2 buffalo at 4 weeks after infection was higher than at any other time. Another buffalo, in Experiment 3B (Table 3), had $10^{1.3}$ MLD_{50}/mℓ of virus in its saliva 4 weeks after exposure to infected animals (i.e. those in Experiment 3A) while no virus was detected in 5 previous samplings. In the latter respect no comparison can be drawn with cattle since these were only sampled for 14 days.

Virus was detected in the air samples collected from 4 of 6 cattle when the head mask was used—on the second day following infection in each case (Tables 4, 5 & 7). On each occasion when virus was detected in air samples the tires of virus in NPS collected at approximately the same time were $\geq 10^5$ MLD_{50}/swab (Table 7). Similar samplings on buffalo were performed in Experiments 3A and B when no virus was recovered (Table 4).

In Experiments 2A and 2B air samples were collected in the open in the vicinity of 6 buffalo 4 and 5 days after 2 of them had been infected by needle inoculation (Fig. 1). By day 5 2 of 4 contact animals showed foot lesions from which SAT 2 virus was isolated. Virus was detected in the air samples on both occasions but when similar samplings (i.e. where the samples were taken from free air in the vacinity of infected animals) were carried out on cattle in the high security stables, virus could only be detected on 2 of 23 occasions (Bruce & Thomson, unpublished results, 1984). During the latter samplings care was taken to ensure that air was not dispersed by shutting off the supply air dampers and sealing the exhause outlets with plastic sheeting. Relative humidity was ≥60 %.

SVN responses of buffalo and cattle to infection with SAT 1 and 2 viruses

Infection in the buffalo and cattle listed in Table 3 was confirmed by demonstrating greater than 4-fold increases in SVN antibody to the relevant virus.

Fig. 2 depicts the mean neutralizing antibody responses of the 4 buffalo which were exposed to contact infection in experiments 1B and 2B (Table 3). This shows that following infection with SAT 1 virus, SVN titres reached a peak ($>10^{3.0}$ SVN_{50}) approximately 40 days after exposure and that this level was maintained for >7 months.

The SVN response to SAT 2 infection developed more rapidly and again persisted undiminished for more than 3 months. Infection with SAT 2 also resulted in an anamnestic response to SAT 1 with peak titres $>10^{4.0}$ SVN_{50}. These high levels, however, decreased by more than 1 $\log_{10}$ SVN_{50} over the following 3 months.

Both buffalo in experiment 3A (Table 3) developed detectable antibody responses within 6 days of being inoculated with SAT 2 virus while the 4 buffalo which acquired the infection by contact with the first 2 all had detectable responses within 10 days of the start of the experiment (results not shown).

Transmission of SAT 1 and 2 viruses from acutely infected buffalo to cattle and impala

In the first experiment (Experiment 1, Fig. 1) 2 of 6 young buffalo were infected by needle inoculation with SAT 1 virus (Table 3). Three of the 4 in-contact buffalo developed feet lesions typical of FMD 8–10 days later. At this stage the inoculated buffalo were removed from the pen and replaced by 2 susceptible cattle which remained in the pen until the end of the experiment. The cattle were examined every 2–3 days for lesions of FMD. Despite the fact that the in-contact buffalo were excreting detectable quantities of SAT 1 virus for at least the first 6 days after the introduction of the cattle (Fig. 1), the latter failed to develop FMD. This was demonstrated by their susceptibility to intradermolingual (idl) challenge inoculation ($10^{4.9}$ MLD_{50}) with virus recovered from lesion material from one of the 2 buffalo originally inoculated, as well as a lack of neutralizing antibody prior to challenge. Thus acutely infected buffalo failed to transmit FMD to susceptible cattle in the same pen.

Although forced to use common hay racks and drinking troughs, the buffalo and cattle avoided each other as far as possible so that it is unlikely that, in this experiment, the 2 species were ever in direct physical contact.

For Experiment 2 (Fig. 1) the same approach as for Experiment 1 was followed except that SAT 2 virus (Phalab/2–6/83/2) was used (Table 3). The buffalo were the same animals employed in Experiment 1, there being a 7 month internval between the 2 experiments (Fig. 2). In this experiment the cattle quickly acquired infection from the in-contact buffalo (Fig. 1). After allowing the cattle to recover from FMD they were removed 23 days after introduction into the buffalo pen and replaced by 3 further susceptible cattle (Fig. 1). For the next 47 days the newly introduced cattle associated freely with the buffalo but showed no signs of FMD and remained serologically free of antibody to SAT 2. On the 47th day the 2 buffalo orginally infected by needle inoculation were reintroduced into the pen. These plus one of the original in-contact buffalo were injected intramuscularly with 20 mg dexamethasone daily for 5 days. At the same time another 3 susceptible cattle were also introduced into the pen, i.e. there were 6 buffalo and 6 cattle together in the pen. The cattle again failed to develop FMD or neutralizing antibody to SAT 2. Fourty-five days later 2 of the cattle were challenged idl with $10^{4.3}$ ID_{50} of Phalab/2–6/83/2. These two animals developed FMD and transmitted the virus to the other 4 cattle within 5 days of inoculation.

All the cattle used in Experiment 2, unlike those in Experiment 1, mixed freely with the buffalo. The two species were observed to sleep together and interspecies grooming was also seen.

In Experiment 3 (Fig. 1) 2 members of a group of 6 buffalo were needle inoculated and 6 days later typical FMD lesions on the feet were observed in all 4 in-contact buffalo from which SAT 2 virus was isolated. Virus was present in specimens taken from these animals for up to 10 days after the needle inoculations were given (Fig. 1). Eight days after the start of the experiment a group of impala, which varied, due to nonrelated mortality, between 6 and 14 animals, was introduced into a pen immediately adjacent to the buffalo. Both species used common hay racks and drinking containers. Careful daily observation of the impala as well as periodic serological testing failed to identify any FMD infection in the impala (Fig. 1).

TABLE 8 Pertinent weather data during periods when virus was likely to have been excreted by infected buffalo during Experiments 1, 2 and 3 (Fig. 1; Table 3)[1]

Experiment No. (Fig. 1)	Date	Time	Total cloud (0–8)	Temperature (°C)		Relative humidity (%)	Wind		Rainfall[3] (mm)
				Thermograph	24 h minimum[2]		Direction (degrees)	Speed (m/s)	
1	12/3/83	0800	5	24,0	14,3	50	—	—	—
	12/3/83	1400	0	22,4	/[4]	28	—	—	/[4]
	12/3/83	2000	0	22,8	/	48	—	—	/
	13/3/83	0800	1	20,2	15,4	74	—	—	—
	13/3/83	1400	1	32,0	/	31	90	2,0	/
	13/3/83	2000	0	17,0	/	46	—	—	/
	14/3/83	0800	4	22,0	21,3	87	—	—	—
	14/3/83	1400	1	32,0	/	34	—	—	/
	14/3/83	2000	0	26,8	/	50	130	2,0	/
	15/3/83	0800	3	23,2	21,5	72	—	—	—
	15/3/83	1400	4	31,2	/	34	270	2,0	/
	15/3/83	2000	1	27,0	/	44	—	—	/
	16/3/83	0800	1	21,8	18,3	67	—	—	—
	16/3/83	1400	6	30,0	/	38	—	—	/
	16/3/83	2000	NR	NR	/	NR	NR	NR	/
2	20/10/83	0800	8	18,4	17,6	77	130	2,0	—
	20/10/83	1400	8	20,2	/	76	—	—	/
	20/10/83	2000	2	18,0	/	90	—	—	/
	21/10/83	0800	8	17,8	18,4 (sic)	92	—	—	6,3
	21/10/83	1400	8	23,0	/	58	180	2,0	/
	22/10/83	2000	8	19,2	/	70	180	5,0	/
	23/10/83	0800	6	20,2	16,6	48	180	2,0	0,6
	23/10/83	1400	7	25,0	/	40	130	2,0	/
	23/10/83	2000	1	26,0	/	58	90	2,0	/
	23/10/83	0800	1	22,9	14,2	50	90	5,0	—
	23/10/83	1400	1	30,0	/	34	360	5,0	/
	23/10/83	2000	0	28,0	/	36	50	2,0	/
	24/10/83	0800	1	25,0	17,0	54	130	2,0	—
	24/10/83	1400	5	37,0	/	26	360	2,0	/
	24/10/83	2000	8	20,0	/	82	180	5,0	/
3	29/07/84	0800	0	17,8	14,3	82	—	—	—
	29/07/84	1400	3	26,4	/	48	70	2,0	/
	29/07/84	2000	3		/	77	—	—	/
	30/07/84	0800	8	17/0	16,5	92	—	—	—
	30/07/84	1400	1	27/4	/	43	50	2,0	/
	30/07/84	2000	0		/	78	—	—	/
	31/07/84	0800	6	18,0	11,0	66	230	6,0	—
	31/07/84	1400	6	23,0	/	50	180	6,0	/
	31/07/84	2000	8		/	74	130	5,0	/
	01/08/84	0800	0	12,5	11,3	81	—	—	1,0
	01/08/84	1400	7	21,0	/	40	230	2,0	/
	01/08/84	2000	0	12,8	/	78	—	—	/
	02/08/84	0800	0	8,0	5,0	94	—	—	—
	02/08/84	1400	6	21,2	/	48	50	2,0	/
	02/08/84	2000	0	13,0	/	80	—	—	/

[1] Data kindly supplied by Mr C. J. H. van der Walt, Private Bag X402, Skukuza 1350

[2] I.e., the minimum temperature recorded since 2000 the previous day

[3] I.e., recorded in the previous 24 hours

[4] No data available

Prevailing weather conditions during transmission experiments

Details of the weather conditions existing while the buffalo were most likely to have been excreting virus and therefore potentially capable of transmitting the virus to other species in contact with them during Experiments 1, 2 and 3 (Fig. 1) are shown in Table 8.

DISCUSSION

The primary aim of this investigation was to establish to what extent and for how long FMD infected buffalo are likely to be infectious for other species, particularly cattle.

The 2 main routes by which the virus could be acquired by other species are inhalation and ingestion. So far as cattle are concerned, it has been concluded on the basis of analysis of outbreaks that the respiratory route is the more likely (Donaldson, 1983). Furthermore, higher infectivities are required to initiate infection by ingestion then by inhalation (Sellers, 1971). Conversely, Whyte (1976) has speculated that the most likely source of infection for African wild-life is drinking water contaminated by infected animals.

In the first experiment conducted to examine these 2 possibilities 4 buffalo in the acute stages of infection and with high concentrations of virus in both NPS and blood, failed to transmit SAT 1 virus to cattle in the same enclosure and sharing feeding and watering facilities with them (Experiment 1; Fig. 1). This surprising outcome was attributed mainly to the fact that the buffalo and cattle avoided each other as far as possible and it is unlikely that the 2 species were ever in direct physical contact. In addition, with the exception of the first 24 hours after the introduction of the cattle, daytime temperature were high and relative humidity low (Table 8). These conditions would have been disadvantageous for the survival of FMD virus and the vertical dispersion of aerosols would probably have been relatively great

(Gloster, Blackall, Sellers & Donaldson, 1981). Conversely, the morning and evening relative humidity readings and temperatures indicate that at night the conditions were likely to have favoured virus survival (Donaldson, 1983). The absence of wind or at most low wind speeds would have minimized lateral dispersion of any virus present in aerosols.

Probably because the cattle in the second experiment (Fig. 1), unlike those in the first, mixed freely with the acutely infected buffalo, SAT 2 virus was quickly transmitted to the cattle. In addition, for the first 3 days of the contact period, weather conditions were more favourable for virus survival than in Experiment 1 (Table 8). However, when the indicator cattle were replaced with fresh susceptible cattle approximately 4 weeks after the buffalo were first infected, no transmission occurred. This was despite the fact that they, like their immediate predecessors, associated closely with the buffalo, i.e. the 2 species slept together and cross species grooming was observed. Treatment of the buffalo with dexamethasone in an effort to mimic "stress" had no effect (Fig. 1).

In the third experiment, where impala were used as indicator animals, there was again no transmission (Experiment 3; Fig. 1). In this case the impala and buffalo were separated by a fence to prevent injury to the impala. However, the 2 species were obliged to use a common hay rack and watering facilities and were always within 30 m of each other. Since Experiment 3 took place in winter when night temperatures were comparatively low, relative humidity high and when little or no wind was present, the conditions for survival of virus in aerosols, at least at night, were ideal (Table 8).

Taken together these observations demonstrate that even acutely infected buffalo do not invariable transmit SAT viruses to other species in close proximity and that by 4 weeks following infection at least a high proportion of buffalo are no longer secreting infectious virus. Thus buffalo probably differ little from cattle in the latter respect (Hyslop, 1970; Graves, McVicker, Sutmoller & Trautman, 1971).

Although clinical FMD in free-living buffalo is of a low order (Hedger, 1976a), our finding of clear foot lesions in 11 of 12 experimentally infected buffalo is consistant with previous observations (Young, Hedger & Howell, 1972; Hedger, Condy & Golding, 1972; Anderson, Doughty, Anderson & Paling, 1979). However, the fact that a single animal on 2 occasions failed to show lesions while its cohorts did, indicates that inapparent infection does occur. FMD lesions in the mouths of infected buffalo have been reported (Young *et al.*, 1972; Hedger *et al.*, 1972) but they were less common and pronounced than the foot lesions. In these experiments no clear mouth or tongue lesions due to FMD were seen other than where infection was due to idl inoculation. In view of this, the practice of condemning buffalo carcasses derived from culling operations in the KNP in which mouth erosions or ulcers occur in the absence of feet lesions, should be re-evaluated. This is emphasised by the repeated failure of virus isolation attempts made on such lesion material (R. Bengis, unpublished observations, 1984).

The assay system used for virus estimation in buffalo and cattle specimens, i.e. titration in sucking mice, is not the most sensitive available; calf thyroid cultures are usually better in this respect (Snowdon, 1966; Donaldson, Herniman, Parker & Sellers, 1970; Hedger, 1976b). Logistical difficulties, however, prevented us from procuring calf thyroids for the production of these cultures. Nevertheless, as shown in Table 1, sucking mice generally detected infectivity more efficiently than idl inoculation of susceptible cattle, which is a sensitive method (Sellers, 1971). These results conflict with those reported by Hedger (1976b) who titrated O/P specimens from buffalo collected in the field in a number of systems and found that while idl titration in cattle and titration in calf thyroid cultures gave results which were comparable, sucking mice were much less sensitive ($\geq 10^{1,8}$/mℓ or sample). We can only conclude that the mice used here were more sensitive than those employed by Hedger (1976b). Such variation in mouse susceptibility has previously been encountered (Subak-Sharpe, 1961).

By comparing titres obtained from buffalo specimens with those from cattle it was hoped to provide more interpretable information than would be afforded by virus levels in buffalo specimens alone.

With the exception of conjunctival washings and urine, which was not tested, all specimens from cattle which yielded SAT virus did so in buffalo as well and, in most individuals of both species, virus was detected in 1 or more specimens up to 6 or 7 days after infection (Tables 4 & 5). However, virus detection in buffalo specimens was less consistent than from cattle and the titres tended to be lower (Table 6). This may have been at least partly due to the longer storage period of buffalo specimens.

In 3 instances in buffalo (twice from NPS and once from saliva) SAT virus was isolated up to 28 days after infection and after a period during which no isolation of virus was made from that animal (Tables 4, 5 & 7). Since cattle were only sampled for 2 weeks after infection it is impossible to be sure that similar results would not have been made from cattle although it has generally been found that virus excretion in cattle does not persist for more than 2 weeks (Cottral, 1969). It is possible that these late isolations orginated from virus which persisted in the pharynx of the buffalo, a common occurrence (Hedger, 1976a), but when O/P specimens were collected 1 week later the animals involved (No. 4 & 5; Table 7) did not have detectable virus in those specimens.

Because high virus titres in cattle faeces and urine have been recorded ($10^{5,5}$ and $10^{4,9}$ ID or IU_{50}; Sellers, 1971), it was surprising that poor virus recoveries were made both from cattle and buffalo excretions. However, Parker (1971) pointed out that, in cattle, virus is excreted per rectum in small quantities only and that the high concentrations of virus in cattle faeces collected from the floor is probably more a reflection of the total virus in the environment than rectal excretion. All the faecal specimens examined here were extracted directly from the rectum. Hyslop (1970) furthermore, found that viral content of cattle excretions to be low or variable. The one positive urine specimen we collected had a titre $10^{3,6}$ MLD_{50} lower than the preputial wash obtained at the same time so that contamination by preputial secretion may have been the origin of the virus in urine.

Anderson *et al.* (1979) measured virus in blood and nasal secretions of a buffalo after successive infections with SAT 2 and SAT 1. Viraemias at levels comparable with those reported here were found to last up to 6 days after infection while virus in nasal secretions persisted for up to 15 days although the maximal titre was only $10^{2,85}$ $TC1D_{50}$/mℓ, i.e. lower than the levels reported here (Tables 4 & 5). Young *et al.* (1972) found viraemias of $10^{3,7}$ $TC1D_{50}$/mℓ in 2 SAT 2 infected buffalo.

The SVN antibody responses of infected buffalo reached high levels following both virus inoculation and infection resulting from contact exposure. This was particularly so after buffalo initially infected with SAT 1 were infected 7 months later with SAT 2 (Fig. 2). Peculiarly, the SAT 1 response to this second challenge was much greater than that to SAT 2, probably reflecting the

phenomenon of "original antigenic sin". This is of importance in interpreting field data since it shows that the types against which sera show the highest titres are not necessarily those which most recently caused infection.

Antibody persistence in the buffalo was remarkable and probably reflects the propensity for SAT viruses to persist in the pharyngeal region of buffalo (Condy & Hedger, 1974). It was surprising therefore that after infection with SAT 1 virus, of 4 buffalo from which O/P specimens were collected 35–36 days later only 1 proved positive (Table 7).

By sampling the air in the immediate vicinity of infected animals, particularly with respect to cattle in the isolation stables where temperature, humidity and air currents could be controlled, it was hoped to arrive at a measure of effective aerosol excretion. In preliminary investigations with cattle in which air was sampled in stables housing groups of between 2 and 6 acutely infected animals (these animals were not part of this investigation), virus was only detected on 2 of 23 occasions (Bruce & Thomson, unpublished results, 1984). This poor recovery was unlikely to have been due to large-scale inefficiency of the sampling system since, at most, a little over 1 log of virus was lost in test samplings (Table 2). Conversely, on the only 2 occasions on which air samples were collected in the immediate vicinity of infected buffalo, virus was recovered in both instances. In view of the fact that the buffalo sampling took place in the open air this was an unexpected result. However, the weather at both sampling times was conducive to virus survival in aerosols i.e. the relative humidity was >60 %, temperature ≤21 °C and with little or no wind.

It is probable, although not proven, that FMD virus-containing aerosols are derived largely from the respiratory tract (Donaldson & Ferris, 1980; Sellers, 1983). We therefore treid to improve virus detection by sampling air directly from the head of both individual cattle and buffalo using the head mask shown in Fig. 3. This had some effect as 4/6 cattle sampled in this way had detectable virus in the air samples collected, albeit for 1 day only in each case (Experiments 4 & 5, Table 3; Tables 4 & 5). In buffalo, by contrast, no virus was detected in this way (Experiments 3A & 3B; Table 3). This was unexpected in view of the successful samplings conducted on buffalo in the open. A possible reason for this difference between cattle and buffalo is that while cattle air samples were usually inoculated into mice within hours of collection, buffalo specimens were stores in liquid nitrogen, often for several weeks, before processing. The one exception was when the buffalo were sampled in the open; in that instance mice were transported to the KNP for inoculation. It is also possible that the virus recovered during open air sampling of buffalo was derived from a source other than expired air. In view of the low virus content of buffalo excretions, what this source might be is not clear. Whatever the case, the inability of buffalo to transmit SAT viruses unless there was direct contact between them and the cattle or impala, indicates that air-borne virus derived from buffalo is unlikely to be infectious for other species unless they are in the immediate vicinity of the infected buffalo.

As Hyslop (1965) had previously found, in all the positive air samples tested only a proportion of mice inoculated with undiluted sample succumbed to FMD. This indicates that the threshold of virus detection in this sampling system is at about the level of respiratory excretion by cattle. This finding also accords with results obtained by Donaldson and colleagues using European types of FMD virus (A. Donaldson, personal communication, 1984). It was therefore impossible to derive accurate titres for the quantity of virus excreted as aerosols by cattle. However, by taking into account the proportion of mice which died, the volume of sample inoculated (0,05 mℓ per mouse), the fluid volume of the sample collected (±50 mℓ) and the time over which collection occurred (usually 30 min), it was calculated that positive specimens would represent $10^{2.2}$–$10^{2.8}$ MLD_{50}/30 min sample. If 1 $\log_{10}$ of virus probably lost during sampling (Table 2) is added and the 30 min sample result extrapolated to a 24 hour excretion period, it is likely that positive samples represented between $10^{4.7}$ and $10^{5.3}$ MLD_{50} of virus excreted per day. This figure is close to the peak 24 h excretion rate for cattle of $10^{5.4}$ given by Sellers (1971) for the European types of FMD virus. The latter figure, however, does not, so far as we are aware, incorporate a correction factor for the proportion of virus lost during sampling.

In the field it is unlikely that more than a minority of buffalo would excrete infectious quantities of virus simultaneously because infection with the SAT types in most large buffalo populations is endemic (Hedger, 1976a; Esterhuysen *et al.*, 1985). For this reason buffalo would not be expected to provide an intense source of air-borne infection which would pose a threat to other species, especially cattle, which sample a large air volume (Gloster *et al.*, 1981). It may be, however, that infection of young animals in breeding herds occurs epidemically particularly in view of the seasonal breeding pattern of buffalo. In most air-borne FMD outbreaks in Europe pigs, which individually excrete approximately 1 500 times more virus than cattle (Donaldson, 1983), have usually been a primary source of infection (A. I. Donaldson, personel communication, 1985).

We conclude that under normal field conditions only buffalo in the acute stages of infection with SAT type viruses (which would probably mean only young animals) are likely to provide a direct potential source of infection for other species and then only where close contact occurs. However, indirect transmission of FMD from buffalo to other species remains a possibility for example, by ox peckers (Whyte, 1976) and arthropods (Cottral, 1969).

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REFERENCES

ANDERSON, C. E., DOUGHTY, W. J., ANDERSON, J. & PALING, R., 1979. The pathogenesis of foot-and-mouth disease in the African buffalo *(Syncerus caffer)* and the role of this species in the epidemiology of the disease in Kenya. *Journal of Comparative Pathology*, 89, 541–549.

ANDERSON, E. C., 1986. Potential for the transmission of foot-and-mouth disease from African buffalo *(Syncerus caffer)* to cattle. *Research in Veterinary Science*, 40, 278–280.

BENGIS, R. G., THOMSON, G. R., HEDGER, R. S., DE VOS, V. & PINI, A., 1986. Foot-and-mouth disease and the African buffalo *(Syncerus caffer)*. I. Carriers as a source of infection. *Onderstepoort Journal of Veterinary Research*, 53, 69–73.

BRUCE, W. & PINI, A., 1984. The new foot-and-mouth disease laboratory at Onderstepoort. *Proceedings of the 13th World Congress on Diseases of Cattle*, Durban, 1984, 52–55.

CONDY, J. B. & HEDGER, R. S., 1974. The survival of foot-and-mouth disease virus in African buffalo with non-transference of infection to domestic cattle. *Research in Veterinary Science*, 16, 182–185.

CONDY, J. B. & HEDGER, R. S., 1978. Experiences in the establishment of a herd of foot-and-mouth disease free African buffalo *(Syncerus caffer)*. *South African Journal of Wildlife Research*, 8, 87–89.

CONDY, J. B., HEDGER, R. G., HAMBLIN, C. & BARNETT, I. T. R., 1985. The duration of the foot-and-mouth disease carrier state in African buffalo (i) in the individual animal and (ii) in a free-living herd. *Comparative Immunology Microbioloy and Infectious diseases*, in press.

COTTRAL, G. E., 1969. Persistence of foot-and-mouth disease virus, in animals, their products and the environment. *Bulletin de L'Office International des Epizooties*, 71, 549–568.

DONALDSON, A. I., 1983. Quantitative data on airborne foot-and-mouth disease virus: Its production, carriage and deposition. *Philosophical Transactions of the Royal Society, London,* B302, 529–534.

DONALDSON, A. I., HERNIMAN, K. A. J., PARKER, J. & SELLERS, R. F., 1970. Further investigations on the airborne excretion of foot-and-mouth disease virus. *Journal of Hygiene,* 68, 557–564.

DONALDSON, A. I. & FERRIS, W. P. 1980. Sites for release of airborne foot-and-mouth disease virus from infected pigs. *Research in Veterinary Science,* 29, 315–319

ERRINGTON, F. P. & POWELL, O. E., 1969. A cyclone separator for aerosol sampling in the field. *Journal of Hygiene,* 67, 387–399.

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.

GLOSTER, J., BLACKALL, R. M., SELLERS, R. F. & DONALDSON, A. I., 1981. Forecasting the airborne spread of foot-and-mouth disease. *Veterinary Record,* 108, 370–374.

GRAVES, J. H., MCVICKER, J. W., SUTMOLLER, P. & TRAUTMAN, R., 1971. Contact transmission of foot-and-mouth disease from infected to susceptible cattle. *Journal of Infectious Diseases,* 123, 386–391.

HEDGER, R. S., 1968. The isolation and characterisation of foot-and-mouth disease virus from clinically normal herds of cattle in Botswana. *Journal of Hygiene,* 66, 27–36.

HEDGER, R. S., 1976a. Foot-and-mouth disease in wildlife with particular reference to the African buffalo *(Syncerus caffer). In:* ANDREW PAGE, L. (ed.). Wildlife Disease, 235–244. New York: Plenum Plublishing Corporation.

HEDGER, R. S., 1976b. The maintenance of foot-and-mouth disease in Africa. Ph.D thesis, University of London.

HEDGER, R. S., CONDY, J. B. & GOLDING, S. M., 1972. Infection of some species of wild life with foot-and-mouth disease virus. *Journal of Comparative Pathology,* 82, 455–461.

HEDGER, R. S. & CONDY, J. B., 1985. Transmission of foot-and-mouth disease from African buffalo virus carriers to bovines. *Veterinary Record,* 117, 205.

HOWELL, P. G. & MANSVELT, P. R., 1972. The epizootiology of foot-and-mouth disease in the Republic of South Africa. *Bulletin de L'Office International des Epizooties,* 77, 629–634.

HYSLOP, N. St. G., 1965. Secretion of foot-and-mouth disease virus and antibody in the saliva of infected and immunized cattle. *Journal of Comparative Pathology,* 75, 111–117.

HYSLOP, N. St. G., 1970. The epizootiology and epidemiology of foot-and-mouth disease. *Advances in Veterinary Science,* 14, 261–307.

LAMBRECHTS, M. C., BUHR, W. H. B. & VAN DER MERWE, J. P., 1956. Observations on the transmission of foot-and-mouth disease to game and controlled transmission of the disease from game to cattle and vice versa by means of contact. *Journal of the South African Veterinary Medical Association,* 27, 133–137.

PARKER, J., 1971. Presence and inactivation of foot-and-mouth disease virus in animal faeces. *Veterinary Record,* 88, 659–662.

ROSSITER, L. W. & ALBERTYN, A. A. L., 1947. Foot-and-mouth disease in game. *Journal of the South African Veterinary Medical Association,* 18, 16–19.

SELLERS, R. F., 1971. Quantitative aspects of the spread of foot-and-mouth disease. *Veterinary Bulletin,* 41, 431–439.

SELLERS, R. F., 1983. Discussion. *Philosophical Transaction of the Royal Society, London,* B302, 96.

SUBAK-SHARPE, H., 1961. The quantitative study of foot-and-mouth disease virus in unweaned mice. II. Studies with additional mouse strains and comparison of some methods of titration. *Archiv für die Gesamte Virusforschung,* 11, 39–63.

SNOWDON, W. A., 1966. Growth of foot-and-mouth disease virus in monolayer cultures of calf thyroid cells. *Nature,* 210, 1079–1080.

WHYTE, I. J., 1976. Aspects of impala (*Aepyceros melampus* Lichtenstein) behaviour and ecology which may affect the epizootiology of foot-and-mouth disease in the Kruger National Park. Certificate in Field Ecology, Dissertation. University of Rhodesia.

YOUNG, E., HEDGER, R. S. & HOWELL, P. G., 1972. Clinical foot-and-mouth disease in the African buffalo *(Syncerus caffer). Onderstepoort Journal of Veterinary Research,* 39, 181–184.