London School of Hygiene and Tropical Medicine

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THE MAINTENANCE OF FOOT AND MOUTH DISEASE IN AFRICA

Thesis presented for the Degree of Doctor of Philosophy

by

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Note

Some of the work in this thesis has already been published under the following references:-

Hedger, R. S., (1968). The isolation and characterisation of foot-and-mouth disease virus from clinically normal herds of cattle in Botswana. J. Hyg. Camb. 66, 27-36.

Hedger, R. S., Condy, J. B. & Falconer, J., (1969). The isolation of foot-and-mouth disease from African buffalo (Syncerus caffer). Vet. Rec. 84, 516-517.

Hedger, R. S., (1970). Observations on the carrier state and related antibody titres during an outbreak of foot-and-mouth disease. J. Hyg., Camb. 68, 53-60.

Hedger, R. S., (1972). Foot-and-mouth disease and the African buffalo (Syncerus caffer). J. Comp. Path. 82, 19-28.

Hedger, R. S., Condy, J. B. and Golding, Susan M. (1972). Infection of some species of African wildlife with foot-and-mouth disease virus. J. Comp. Path. 82, 458-461.

Hedger, R. S., Forman A. J. and Woodford, M. H. (1973). Foot-and-mouth disease virus in East African buffalo. Bull. Epiz. Dis. Afr. 21, 99-101.

Throughout the thesis reference is made to the seven distinct immunological types of the virus of foot-and-mouth disease which are as follows:-

In Europe, Asia, South America, northern and central Africa:	Types O, A and C
In Africa, south of the Sahara:	Types SAT (South African territories) 1, 2 and 3.
In Asia	Type Asia 1 (Type SAT1 has also occurred in the Middle East)

Within each type there are different but antigenically related strains classified as subtypes or subtype variants.

Although the term "carrier virus" is used throughout this thesis to signify virus isolated from an animal after the acute phase of infection, this does not imply any differences between strains of virus recovered from carrier animals and strains from cases of typical acute foot-and-mouth disease.

PART I

ABSTRACT and GENERAL MATERIALS & METHODS

CHAPTER 1 - ABSTRACT

The object of the work presented was to elucidate the maintenance of foot-and mouth disease (FMD) in Africa, to assess its risks and to define guidelines for its control.

Part II shows that in cattle the carrier state is a natural sequel of infection but that the virus is maintained in domestic animals only for a limited period in the absence of reinfection. Transmission of infection from carriers is infrequent.

Part III describes investigations in free-living buffalo and shows that they are maintenance hosts of foot-and-mouth disease Virus (FMDV) which appear to have achieved an equilibrium with the virus so that it causes them little harm. A single herd may maintain several virus types simultaneously for prolonged periods with a low probability of spillover into other species.

Part IV describes antibody surveys and experimental infections in African wildlife. FMD antibody was demonstrated in 18 cloven hoofed species from only a few of which virus has been isolated. Kudu, impala, warthog and bush pig were shown to be susceptible to experimental and contact infection, and elephant to experimental but not contact infection. The carrier state was demonstrated only in kudu.

In the general discussion the following points are made:-

1) Although cattle may carry virus for up to 2½ years, the risk of their transmitting the infection is low.

2) Where FMD is endemic, successful routine prophylactic vaccination eliminates both disease and virus from domestic stock and also apparently from associated susceptible wild species other than buffalo.

3) Buffalo maintain FMDV independently of cattle but, in the absence of visible lesions, normally shed

insufficient virus to infect other species.

4) Infected cattle however shed sufficient virus to infect susceptible wild animals (e.g. impala, kudu) which in turn may infect other cattle.

5) This cycle of infection can be broken by vaccination of domestic stock, protecting both the livestock and wild life industries.

CHAPTER 2 - MATERIALS AND METHODS

Materials and methods are presented here as, in most respects, they are common to the work presented in all parts of this thesis.

1.1 Introduction

The main tool used in the study of the carrier virus state in foot-and-mouth disease (FMD) is the probang, which, subsequent to and during these studies has been used, not only as an epizootiological tool to determine extent of infection and to elucidate disease history, but also for retrospective and prospective diagnosis and in the assessment of safety of stock prior to interterritorial movement. The demand by importing countries for the probang testing of cattle, sheep and goats has steadily increased and currently 3,500 - 4,000 samples for animals intended for export are being tested annually at Pirbright.

2.2 Collection and handling of samples

Oesophageal/pharyngeal (O/P) samples were collected in cups similar to those described by Sutmöller and Gaggero (1965). These cups (Figure 1) are now commonly described as probangs. Smaller cups 1¼" deep with a diameter of $^3/_4$" were used for calves. The cup was passed through the mouth into the pharynx and by dorsal and lateral movements attempts were made to scrape quickly the surface epithelium of the pharynx and soft palate. The cup was then passed down into the upper portion of the oesophagus and withdrawn, preferably after the animal had swallowed, depositing the pharyngeal scrapings together with mucous and saliva into the cup. Care was taken to ensure that each specimen contained some visible cellular material.

Samples collected in the morning from cattle which have passed the night in dry and dusty kraals often contain quantities of fine soil and faecal matter. Many may contain variable amounts of rumenal contents. When it was not practical to water

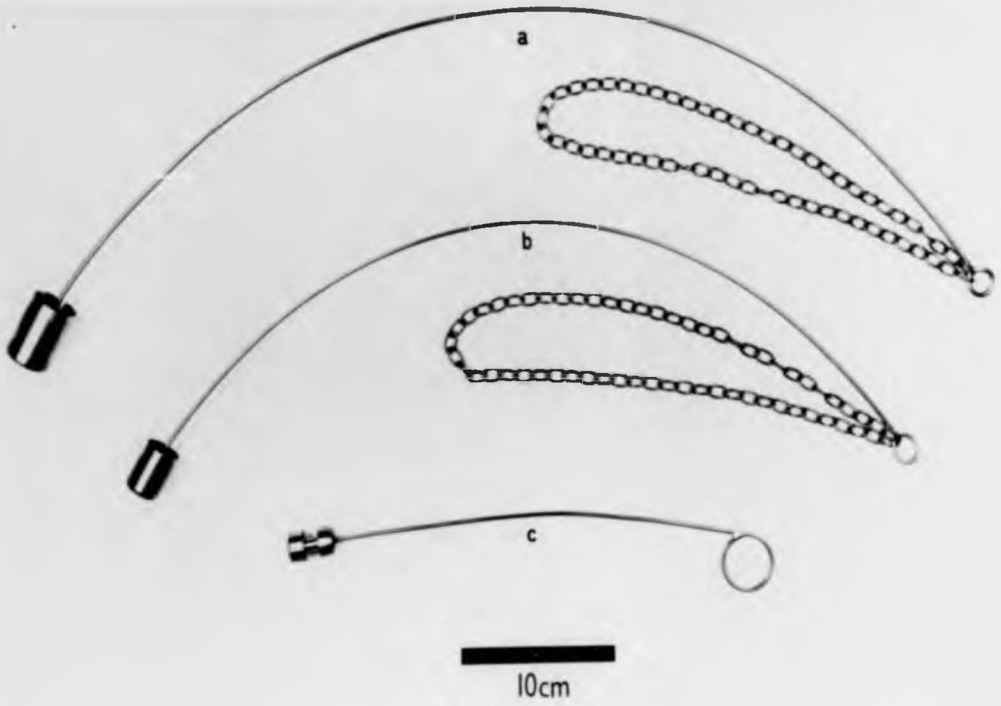

Fig 1. Sputum cup or probang

a) for adult cattle b) for calves c) for sheep and goats

animals prior to sampling and samples collected were grossly contaminated with acid ruminal contents animals were drenched with water or normal saline and then resampled. O/P samples from immobilised buffalo similarly were frequently grossly contamined with overflow from the rumen due to induced muscular relaxation, especially in animals which had been recumbent for some time.

In Europe and in other well watered areas adequate quantities (5 - 10 ml.) of oesophageal/pharyngeal fluid are not difficult to obtain. In dry countries, however, where available grazing may be a long distance from water, animals are frequently so dehydrated that little or no fluid may be collected in the cup. Satisfactory samples were obtained from such animals by rinsing their mouths with a few millilitres of water or collecting medium immediately before collection.

The cups were disinfected and cleansed with sodium hypochlorite and rinsed several times in a series of containers of water between animals. All equipment was autoclaved or boiled at the conclusion of sampling.

Immediately after collection each sample was mixed with an equal quantity of 0.08 M phosphate (PB) buffer containing 0.01% bovine serum albumen and 0·001% phenol red indicator and antibiotics, subdivided into aliquots in bijou bottles and placed on dry ice. Antibiotics were included to give a final concentration per ml. in the medium of:-

Penicillin	1,000 i.u.
Neomycin sulphate	100 units
Polymyxin B sulphate	50 "
Mycostatin	100 "

It was noted that O/P samples uncontaminated with rumen contents were alkaline and when added to equal quantities of the more usual 0·04 M PB at pH 7·6 resulted in high pH values (mean of 14 samples pH 8·16±0.16). The use of 0·08 M PB resulted in a lower pH (mean of 9 samples pH 7·55± 0·05).

In buffalo and other wild species which had been shot, difficulty was often experienced in obtaining usable samples with a probang after death owing to relaxation of the tissues and the presence of large amounts of blood in the pharynx in most of the animals. The pharynx was therefore exposed by cutting down through the intermandibular space, and scrapings of the pharyngeal walls and soft palate were taken with a curette or scalpel.

2.3 Transport and storage of samples

As other workers (Burrows, 1966; van Bekkum, et al., 1966) have noted that samples quickly lose their infectivity when stored at 4° C. or -20 C., comparative parallel tests of O/P samples containing minimal virus stored at 4° C. and in dry ice at -70° C. were carried out at Pirbright before the commencement of these studies. There was a steady loss of virus titre in samples stored at +4° C., but on dry ice, following an initial loss probably due to freezing and thawing, the virus titre remained constant over the 30 days of the experiment.

Samples after collection were therefore immediately frozen and stored on dry ice or in liquid nitrogen and transported as soon as possible to the laboratory in boxes lined with 2 inch polystyrene with adequate dry ice for the journey. To maintain adequate stocks of dry ice over many weeks in remote areas with ambient temperatures reaching 43° C., 4 in. thick polystyrene lined cavity walled metal boxes large enough to hold four 56 lb blocks of dry ice were used. When the dead space was filled with vermiculite, stocks of dry ice lasted up to 2 weeks without replenishment.

2.4 Isolation of virus

Isolation and titration of virus was carried out on primary monolayer cultures of calf thyroid cells in 6 in. x 5/8 in. tubes on roller drums as described by Snowdon (1966) who showed calf thyroid cells to be more sensitive to unmodified FMDV than BHK21 cultures, unweaned mice, secondary pig kidney

cultures or cattle by intradermolingual inoculation. O/P fluid in 0·2 ml amounts was inoculated into each of not less than 10 tubes per sample. Cytopathic effects (CPE) were normally apparent by 27 hr. after inoculation of the tubes but a final check reading was made at 48 hr. In some cases isolation from duplicate buffalo O/P samples was made in 2 year old Devon steers by intradermolingual titration (Henderson, 1949) and in unweaned 5 to 6 day old mice by intraperitoneal inoculation (Skinner, 1953). The behaviour of representative buffalo carrier strains was also studied in BHK 21 cells (Stoker & Macpherson, 1964) and in IB-RS-2 cells (de Castro, 1964).

2.5 Separation of strains in virus mixtures

Aliquots of original or isolation passage material were incubated separately with equal volumes of 1 in 5 dilutions of the three SAT type-specific sera for one hour at 18° C., then titrated in ten-fold dilutions on calf thyroid cell monolayers in roller tubes. Tissue culture fluid from tubes showing CPE at the highest dilution contained the separated virus, the identify of which was confirmed by complement fixation (CF) tests on the tissue culture supernatant after further passage. The assumption was that, even in the absence of complete neutralization, the preponderance of the unneutralized strain at limit dilution would mask and outgrow any residual partially neutralized virus of the other strain.

Where virus mixtures were demonstrated, the results were confirmed by repeating the tests on duplicate O/P samples. In two cases, virus mixtures were confirmed by the production of mixed infections in cattle inoculated with O/P samples.

2.6 Virus specificity

The specificity of all virus strains isolated was confirmed by complement fixation tests using the microtitre method described by Casey (1965), modified so that virus serum mixtures were incubated in the presence of complement for 30 minutes at 37° C. prior to the addition of sensitised sheep erythrocytes, and the degree of lysis was estimated from the pattern of unlysed cells deposited in each well.

Representative virus strains were adapted to guinea pigs by serial passage and specific antisera were prepared by the method described by Davie (1964). Cross CF tests were carried out in tubes (Brooksby, 1952; Davie, 1964) and antigenic differences were sought between these strains, vaccine strains and previously established subtype reference strains. The subtype screening of the Uganda virus isolates (Chap. 4.4) was carried out using the microtitre CF method described by Darbyshire, et al (1972).

2.7 Sera

Animals were bled from the jugular vein using disposable syringes. 18 gauge 1½" needles were adequate even for the thickest skinned buffalo. Sera were separated on the day of collection using a small variable speed portable centrifuge* developed for field use to run off either a 6 V. or 12 V. car battery. The sera were decanted into bijou bottles (approx. 3 ml. serum/bottle) to which one drop of an antibiotic mixture had previously been added. The antibiotics were reconstituted so that the single drop contained sufficient to bring the concentration per ml. of serum to:-

Penicillin	100 i.u
Neomycin sulphate	100 units
Polymyxin B sulphate	50 "
Mycostatin	100 "

For many of the cattle and all the captured wild animals in the field surveys, an anticoagulant (EDTA) was added to the blood and an aliquot was placed on dry ice, as with the O/P samples for viraemia studies. The remainder of the blood with EDTA was centrifuged at the end of each day and the plasma decanted. Thus, after sampling, it was not necessary to wait for clotting, and the haemolysis so often associated with rough handling of clotted blood in hot conditions was avoided. Prior to using EDTA, to check possible differences, a series of sera and plasma, taken simultaneously from the same animals, were tested in parallel in virus neutralisation tests with almost identical results.

* Luckham Ltd., Labro Works, Burgess Hill, Sussex.

Sera were generally but not always transported frozen on dry ice and in the laboratory stored at -20° C. prior to test. All sera were inactivated for 30 mins. at 56° C. before testing. Some of the earlier game sera from Rhodesia, heavily contaminated with bacteria and fungi, were inactivated at 62° C. for 1 hour following centrifugation.

2.8 Serum neutralisation tests

In the early stage of this study sera were assayed by the cell metabolic inhibition test (CMI) or colour test (Martin and Chapman, 1961) using primary monolayers of pig kidney cells. The virus strains used in the test were those most recently isolated from cattle in the area and used in current vaccines, but not necessarily identical to the strains isolated from buffalo or previously infecting the other animals. Before use in tests, viruses were adapted to pig kidney cells by serial passage. Later this test was modified by the use of BHK 21 cells in Microtiter* plates, and more recently neutralisation tests have been carried out using monolayers of either BHK 21 cells or IB-RS-2 cells on disposable flat bottomed tissue culture grade Microtiter plates.

Sera for test were diluted 1 in 4 in a cell growth medium consisting of Eagles medium with the addition of 10% tryptose phosphate broth, 1% glucose, 4% normal ox serum, 0.001% phenol red and 100 i.u./ml each of penicillin, polymyxin, neomycin and mycostatin, and 0.05 ml amounts were used to prepare two fold dilution series on microtitre plates using 0.05 ml diluting loops. Pretitrated virus, diluted in cell growth medium to contain an estimated 100 TCD_{50} virus doses per 0.05 ml, was then dropped into each well using 0.05 ml dropper pipette. Following incubation at 37° C for 1 hour, 0.025 ml of cell suspension, containing 2.5×10^6 cells/ml, was added to each well and the plates sealed with pressure sensitive adhesive cellulose tape. Controls in each test included an homologous antiserum of known titre, a cell control, a medium control and a virus titration from which the actual amount of virus used in the test was calculated.

* Flow Laboratories, Irvine, Scotland

Plates were incubated at 37° C and monolayers were normally confluent within 24 hours. CPE was usually sufficiently advanced at 24 - 48 hours for preliminary reading of the test to be made using an inverted microscope and an indexing substage specially designed to take a microtitre plate (I.S. Caie, to be published). Routinely the test was completed on the morning of the third day (at approximately 68 hours) when the plates were unsealed and without prior removal of the culture fluid or washing, immersed in 10% formol saline for 30 minutes, achieving simultaneous disinfection and cell fixation. Plates were then removed from the formol saline, shaken to remove excess fluid and without rinsing immersed in a bath containing 0.05% methylene blue in 10% formalin for a further 30 minutes, following which they were rinsed in a bath of tap water before being read macroscopically.

Wells with 50% or greater evidence of CPE were considered to be infected and neutralisation titres were expressed as the reciprocal of the final dilution of serum present in the serum virus mixture at the 50% end point estimated according to the method of Kärber (1931).

Tests were considered to be valid when the actual amount of virus used per well in the test was between $10^{1.5}$ and $10^{2.5}$ TCD_{50} and when the titre of the reference serum was within two fold of its expected titre estimated from the mean of previous titrations. When the controls were outside these limits the results were discarded and the tests were repeated.

Prior to changing the CMI test with pig kidney cells on macroplates to a microtest with BHK 21 cells on microplates, comparative trials were carried out with a series of sera against different virus types using both techniques with similar results (Dawe, P.S., personal communication). Similar preliminary trials were carried out before adopting

stained monolayers on flat-bottomed plates. This latter technique has been found to be more definitive and objective than the CMI tests and results can be read as early as the second day.

Serum neutralisation tests in mice were a modification of the method used for measuring virus infectivity described by Skinner (1953).

0.2 ml quantities of a two-fold dilution series of each serum were added to equal quantities of pretitrated virus suspension and the mixture was incubated at 37° C for 1 hour before being inoculated intraperitoneally into randomised 5/6 day old "P" strain mice using 5 mice per dilution. Each mouse received 0.03 ml. and the dilution of virus suspension in the serum/virus mixture was such that each 0.03 ml contained 400 mouse ID/50. Serum controls at the lowest dilution of each serum and a virus titration were included in each test.

2.9 Immobilisation and capture

Except where otherwise stated all buffalo and some other species were immobilised for sampling and later released. Immobilisation was by intramuscular injection using metal projectile syringes fired from Cap Chur* guns. Dose rates of 5 to 6 mg. etorphine hydrochloride (M99, Reckitts) or 60 mg. fentanyl citrate (Sublimaze, Janssen) combined with either 30 mg. acepromazine maleate (Acetyl promazine, Boots) or 300 mg. azaperone (Suicalm, Janssen/Crown Chemicals) were used in all ages of buffalo. Estimated weights of darted animals varied from 200 to 800 kg. Reversal of narcosis was affected with cyprenorphine hydrochloride (M285, Reckitts) injected into a branch of the auricular vein.

In the earlier surveys in Botswana and Uganda, buffalo were darted at a range of 10 to 15 yards from a moving vehicle. Using several pre-loaded guns it was often possible to dart a number of animals on first contact. Immobilisation generally occurred in 10 to 15 minutes, longer in some larger animals.

* Palmer Chemical & Equipment Ltd., Inc, Douglasville, George, USA.

During this period the buffalo often covered a considerable distance, frequently retreating into thick bush or forest, necessitating the use of trackers to follow them. As a precaution, captured animals were roped before sampling. Calves were sometimes captured by hand. In the relatively open areas of the Q.E. National Park in Uganda where buffalo were accustomed to the presence of tourists in motor vehicles, it was possible to approach to within 30-40 yards and to dart from a stationary vehicle.

Although aggression was encountered during capture, little was shown by recovered animals which had received a tranquillising drug in addition to the immobilising agent. Relatively long acting tranquillisers however are probably contra-indicated in areas where predators abound and as sampling teams became more experienced, they dispensed with their use.

In later surveys in Botswana, helicopters were used for locating and darting and the methods have been described in detail by Dräger (1974) and Dräger et al (in press). The helicopter is used for the finding, the herding after darting, and the precise locating of all the darted animals after immobilisation. The sampling teams remain in vehicles on the ground and are directed by the helicopter. Two way radio communication is helpful but not essential if a suitable system of signals is arranged. Sampling teams raised the immobilised buffalo at their own discretion. Normal procedure was for the helicopter pilot to cut out a small group of 20 - 30 buffalo from the main herd, and to herd them into the open. Ideally, six buffalo of the selected age groups were darted at one time. When immobilisation was complete, the remaining unaffected animals were driven off by the helicopter and two sampling teams moved in. Allowing a maximum of fifteen minutes for the securing, sampling and release of each animal, all the buffalo were sampled within 45 minutes of going down. In this way casualties due to recumbency or overdosing were minimal.

Numbered metal ear tags were placed in both ears of each animal for future identification and yellow or red paint was sprayed on horns, flank and rump for ease of recognition. All animals were clinically examined before release, particularly for present or past lesions of FMD. Estimates of the age of buffalo were based on dentition, horn configuration and growth as described by Pienaar (1969).

Antelope however are difficult to dart, not only from the ground but also from the air due to their small size and ability to take evasive action. Use was therefore made of the helicopter on occasion to drive groups of antelope into large holding pens constructed of plastic sheeting suspended from wires slung between trees. Marksmen on foot were able to approach close enough to the antelope in these pens for darting.

2.10 Experimental infection

The infection, examination and sampling of the captive wild animals during experimental infection necessitated in most cases anaesthetisation or immobilisation using sodium thiopentone (Intraval, May and Baker) or M99/etorphine hydrochloride, the latter administered in projectile syringes fired into the gluteal region. Reversal of narcosis was affected by M285/cyprenorphine hydrochloride by intravenous injection. For examination and sampling it was necessary to cast each individual elephant. Thus practical considerations, including the well being of the subjects, influenced the frequency with which samples could be taken.

P A R T II

THE CARRIER STATE IN DOMESTIC ANIMALS UNDER FIELD CONDITIONS

CHAPTER 3 - INTRODUCTION

The manner in which the virus of foot-and-mouth disease is perpetuated between outbreaks of clinical disease has long exercised the minds of livestock owners, and for many years there has been speculation that recovered animals might transmit disease to other susceptible animals. Many observant field veterinarians, particularly in countries where the disease has been enzootic and where control has not included the slaughter of affected animals, have been convinced that some outbreaks of disease could only be explained by the existence of virus carrier animals.

In some diseases, e.g. rinderpest and swine fever almost all infected animals develop clinical signs. In others, e.g. polio in man and pseudorabies in pigs, infection is frequently inapparent, the animal acquiring infection for a brief period and becoming immune without it having been noticed. Some viruses rarely, if ever, produce recognisable disease in animals they infect. After infection the causative organism may be eliminated from the body or persist in the carrier or latent state. The duration of persistence varies and may be lifelong as in herpes in man or relatively short as in polio. Cattle may remain carriers of Brucella abortus for years. Not all carriers however shed the infecting agent and some shed intermittently. Some inapparent infections which persist as latent infections may recrudesce when aggravated; the classic example being herpes in man, when non specific stimuli such as fever, menstruation, gastro intestinal or even psychic upsets can cause recrudescence (Kaplan, 1969). Shope (1964) described the latent infection of pigs with swine influenza virus and how a sudden adverse change in the weather activates the virus causing coughing and sneezing to start simultaneously in pigs over a wide area.

Rapp and Jerkofsky (1973) have defined a persistent infection as one in which infectious virus is continually released, even in the presence of circulating antibody and in the absence of signs of disease, and a latent infection as one in which at least the genome of the virus is present but infectious virus cannot be recovered except during episodes of overt disease. Although FMDV may be recovered from cattle for very short periods prior to the appearance of clinical signs (Burrows, 1968(b):. Hedger & Dawson, 1970), there

is no evidence however to suggest the occurrence of latent infection which at a later date may be triggered to cause disease. There is however epizootiological evidence that animals recovered from foot and mouth disease may become virus carriers and be responsible for spreading infection.

It has been suggested that the last recorded outbreaks of FMD in Australia in 1871-72 may have been due to the importation of carrier animals from Britain (Pullar 1964-5). It is difficult to imagine how, in those slow days of sail, active infection could have persisted in shipboard animals during the many weeks out of contact with land.

During the outbreak of FMD in Denmark in 1893-95, the disease reappeared on four large farms on which outbreaks had occurred 6 - 12 months previously. In each case, only calves born since and cattle brought in since the previous outbreak were affected. Attempts were made to stamp out the disease by slaughter of all the new animals, but disease again broke out on one of these farms twelve months after the second outbreak. Again, only the new animals were affected (Anon., 1901). The source of these outbreaks could not be determined, nor, during this period, was there any trace of FMD elsewhere in the country (Bang, 1912). The possibility of recovered carrier animals being responsible for the recrudescence of disease on these farms cannot be overlooked. Bang, postulating that infection may persist for a long time in a recovered animal, also cites the case of a Dutch bull which, after undergoing a period of quarantine, was introduced into the far north of Sweden, where FMD broke out a few months later.

In Great Britain, two outbreaks of disease which occurred following the 1922-24 epidemic, when the slaughter policy was temporarily abandoned, may have been due to carrier animals. One outbreak in Lancashire in 1924 was attributed to 2 recovered animals having been purchased from a farm infected with FMD 8 months previously (Anon., 1925). It was suggested also that an outbreak in Yorkshire in 1925 was due to the purchase of a bull and a heifer from a farm infected 15 months previously (Anon., 1926).

In a survey of FMD outbreaks in Switzerland from 1920-27, Burgi (1928) estimated that about 3% of recovered cattle remained carriers for 5 to 6 months and probably for as long as a year.

Outbreaks of disease in cattle along trek routes in some African territories have on occasion been attributed to the movement of apparently clinically normal animals. In more recent years in Kenya the existence of the carrier state in cattle presented a feasible explanation for the recurrence of FMD on some of the larger ranches among previously uninfected animals and calves up to a year after the previous outbreak. Recurrences were sufficiently frequent to persuade some veterinarians not to allow the movement of susceptible stock on to previously infected farms without prior vaccination (unpublished data).

In South Africa, on the other hand, control of the disease has been achieved by the cordonning off and aphthisation (deliberate infection by inoculation) of all susceptible stock in the infected area. Following a period of quarantine after the clinical end point of disease, restrictions have been lifted and normal movements of animals resumed. In spite of the apthisation of many thousands of animals through the years no subsequent outbreaks of disease have been attributed to recovered carrier animals (M.C. Lambrechts, personal communication).

The existence of the carrier state in cattle recovered from FMD however remained conjecture until 1959 when Van Bekkum, Frenkel, Frederiks and Frenkel demonstrated the intermittent recovery of Type A FMD virus (FMDV) in the saliva of 10 of 13 cattle for up to 5 months after recovery from disease. In 1965 in South America, Sutmoller and Gaggero, using Van Bekkum's sampling techniques, detected FMDV in the oesophageal/pharyngeal (O/P) fluid of 14 of 25 animals, 6 months after natural infection; 10 animals were carrying Type C virus and four, Type A.

Burrows (1966), working with strains of three types of FMDV recovered virus from 27 of 30 steers up to 26 weeks following

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Burrows (1966a), working with strains of three types of FMDV recovered virus from 27 of 30 steers up to 26 weeks following

experimental infection. Although the numbers of animals in his experiments were small, his results suggested that the virus strain and type may be important in determining the duration of carrier states and the titre of carrier virus recovered. Van Bekkum et al (1966) also demonstrated the persistence in cattle of virus strains of different types for variable periods after experimental infection: type A virus for 7 weeks, type O for 8 weeks and type C for 14 weeks after intradermolingual infection.

FMDV has been recovered from sheep for up to 5 months after experimental infection (Burrows, 1968a).

Burrows (1966a), working with experimentally infected cattle showed that the main sites of carrier virus replication were the mucosae of the pharynx and the dorsal surface of the soft palate. Van Bekkum et al (1966) also concluded that the cells lining the pharynx were the main sites of virus production in recovered cattle.

Little was known, however, of the prevalence of the carrier state under natural conditions in the field in areas where FMD is enzootic and where, owing to the emphasis on livestock and meat exports, knowledge of the ecology of FMDV assumes considerable importance.

Part II of this thesis describes investigations into the carrier state in domestic animals under field conditions in three such African territories under four main headings:-

Chapter 4 - The prevalence of virus carriers following outbreaks of disease and the characterisation of the virus strains isolated
Chapter 5 - The duration of the carrier state
Chapter 6 - The relationship of serum neutralising antibodies to the carrier state
Chapter 7 - The transmission of infection by carrier animals.

CHAPTER 4 - THE PREVALENCE OF VIRUS CARRIERS FOLLOWING OUTBREAKS OF DISEASE AND THE CHARACTERISTICS OF VIRUS STRAINS ISOLATED

4.1 Type SAT3 virus - Botswana

4.1.1 Prevalence

The first survey was of 360 clinically normal cattle and 60 goats in three localities on the Botletle River in Northern Botswana where, for many years, FMD has occurred periodically (see map p.86). The word Botletle is said to be synonymous with FMD and the river, normally the only permanent open water source in the area, is a focus of animal concentration both domestic and wild from many miles around.

In the first locality (Rakops) there had been an outbreak of FMD earlier in the year, with the last clinical cases 7 months before the survey. In the second (Lake Dow) there had been severe infection 12 months previously; and in the third, in eastern Ngamiland, a considerable distance from recently infected areas, there was no history of FMD or vaccination in the cattle under test. The outbreaks in the first two localities were due to type SAT3 virus, subtype 4, strain Becl/65. During attempts to control the disease, cattle and goats had been vaccinated with a bivalent attenuated vaccine incorporating a type SAT3 strain (SA57/59) and a type SAT1 strain (RV11). The SAT3 vaccine strain was a different subtype from the field strain and did not completely control or prevent infection.

The cattle (Bos indicus), mainly long horned with pre-thoracic humps, were native-owned indigenous Tswana stock which were ranched under very harsh conditions with a low standard of husbandry. They were selected from a number of different herds at each cattle post, but under the prevailing conditions of husbandry the cattle at each post may, epizootiologically, be considered as members of one herd. The goats were of the local breed and were adult. There were no sheep in the area.

The localities from which the cattle and goats were drawn, the numbers of cattle sampled, the percentages of carrier animals, and details of the previous FMD history are summarised in Table 1. The total number of cattle at each cattle post during the survey varied from 500 to 1700, thus the sample represented between 3·5 and 12% of each population. Although attempts were made to choose balanced samples including males and females in equal proportions, emphasis was placed on older animals thought more likely to have been among the infected animals during recent outbreaks.

TABLE1. Summary of the results of a carrier virus survey in cattle in three localities in Botswana

Locality	Cattle Post	No. of Cattle Sampled	Positive Carriers	%	Period since last clinical infection
Rakops	Sokwani I	60	12	20	7 months
	Sokwani II	60	13	23	7 months
	Tsienyani	60	11	18	7 months
Lake Dow	Goi	60	2	3	12 months
	Machana	60	12	20	12 months
Ngamiland	Manwelo's	60	0	0	No history of infection

At each cattle post ten adult goats were also sampled, where possible, including animals reported by their owners to have been affected in the recent outbreak. No virus was recovered from any of the goats.

The percentage of carrier cattle at the three Rakops cattle posts was similar at approximately 20%. 12 months after clinical disease, at Goi, the carrier prevalence was lower (3%). The higher prevalence (20%) at Machana at a similar period after infection may have been due in part to intermingling and movement of cattle following the withdrawal of quarantine restrictions. Virus was isolated from one calf said to have been born after the last outbreak of FMD in the area. The serum neutralising (SN) antibody titres of this calf and its dam were high (1/178) but no virus was isolated from the single O/P sample taken from the dam. All the virus strains isolated were type SAT3.

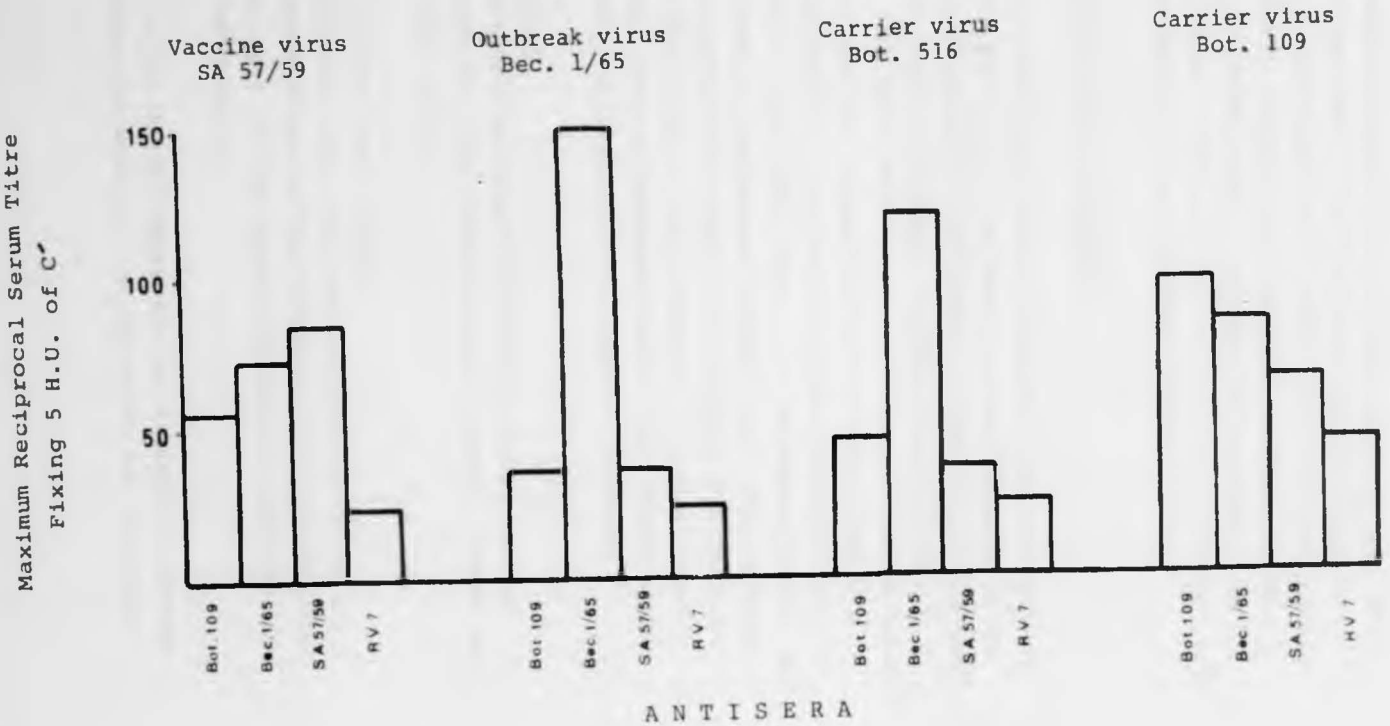
There was no apparent relationship between the incidence of carriers and sex or age in this survey.

4.1.2 Characterisation of virus strains isolated

All the carrier virus strains isolated were screened by CF tests against a range of type SAT3 subtype specific antisera. The resultant profiles (histograms) indicated not only an antigenic difference of some strains from the outbreak strain of virus, but also at least one antigenic difference between strains of carrier virus isolated from different animals in the same herd. None of the strains isolated however was similar to the vaccine strain (SA57/59). Figure 2 compares the profiles of two carrier virus strains with the outbreak strain and the vaccine strain of SAT3 virus administered to the cattle earlier in the year. Bot. 109 was isolated 7 months after, and Bot. 516 twelve months after, clinical end point.

Specific antisera were prepared in guinea pigs to both these carrier strains, and cross CF tests (Davie, 1964), carried out in tubes with these strains, the outbreak

Figure 2. Complement Fixation tests comparing two carrier virus strains with the outbreak and vaccine strains (Hedger, 1968)



strain and the vaccine strain. Table 2 illustrates their relationship expressed as R values (Ubertini et al, 1964): The carrier strain (Bot. 156) and the outbreak strain (Bec. 1/65) are identical (R = 100%). The carrier strain (Bot. 109) however, though of a similar subtype to the outbreak strain, shows some antigenic difference from it both by its R value (71%) and by its profile. Neither show a close relationship to the vaccine strain.

4.2 Type SAT1 Virus - Botswana

This survey was made immediately following an outbreak of FMD due to type SAT1 virus in 1968, in the Satau area of the Chobe River district of Botswana, close to its northern border with the Caprivi Strip. In this remote area, isolated from the rest of Botswana by a game reserve to the south and east, and by tsetse fly to the west, FMD has occurred infrequently. The last recorded outbreak was in 1950, also due to type SAT1 virus. In September 1967, the area was included in the annual prophylactic vaccination campaign which covers the whole of northern and western Botswana. At the time of the outbreak the herds were partially immune having received their first vaccination with a bivalent inactivated SAT1 and SAT3 vaccine.

4.2.1 Prevalence

The cattle were native-owned indigenous Tswana stock; grazing was communal with herds mixing freely. Herds were examined in three areas:-

i) The infected area (SATAU)
ii) A contiguous area, the grazing grounds of which overlapped those of the infected area (Katchekau)
iii) A clean area in the same district 70 miles to the East (Kazangula)

The sampling in the three areas was an attempt to detect possible previous disease in the district and to study

TABLE 2. The relationship of two carrier strains, the field outbreak strain and the vaccine strain of virus expressed as R values

BOT. 109	100%			
BOT. 516	70%	100%		
BEC. 1/65	71%	100%	100%	
SA. 57/59	40%	30%	22%	100%
	BOT. 109 (Carrier)	BOT. 516 (Carrier)	BEC. 1/65 (Outbreak)	SA. 57/59 (Vaccine)

NOTE:

Identical strains of virus have an R value of 100% equivalent to a cross-fixation product (CFP) of 1.0 (Davie, 1964) while antigenically different strains have lesser values depending on the degree of difference. The precise degrees of difference which classify the relationship of virus strains is difficult to define. Davie (1964) arbitrarily classified strains with cross fixation products of 0.5 (R = 70%) or more as in the same subtype group, and Brooksby (1968) suggested the following classification:-

	R
Type difference	10%
Subtypes widely different	10% - 32%
Subtypes different	32% - 70%
Differences within subtypes	70% - 100%

Forman, (1974) however, working with purified viruses on microplates suggests that a more realistic level for subtype variation would be R = 25%. He also pointed out that strains could not be placed in groups such that all strains within a group are more closely related to each other than to strains in other groups.

possible transmission of carrier virus to contiguous cattle following withdrawal of quarantine restrictions after the outbreak.

The immune or partially immune herds in the infected area (SATAU) comprised about 1200 head and fell roughly into three groups:-

1. Herds with an approximate 70% morbidity
2. Herds with an approximate 25% morbidity
3. Herds in contact with diseased herds but in which no clinical disease was recorded.

Only clinically normal cattle which had showed no signs of disease were sampled. Carrier virus was recovered from animals in all three groups. Table 3 shows the incidence of carrier virus recovery in each group in the infected area and also in the contiguous and clean areas. All the virus strains isolated were shown in CF tests to be type SAT1.

TABLE 3. The recovery of carrier virus from clinically normal cattle in infected, contiguous and clean areas

	No. of Animals Sampled	No. Virus Positive	% Carriers in clinically normal animals
INFECTED AREA (SATAU)			
Herds with 70% morbidity	27	23	85
Herds with 25% morbidity	18	6	33
Herds with 0% morbidity	20	6	30
CONTIGUOUS AREA (KATCHEKAU)	30	0	0
CLEAN AREA (KAZANGULA)	60	0	0

In the presence of active infection, some of the apparently immune animals may have been in the prodromal stage of infection (Burrows, 1968) or undergoing subclinical infection. Whole blood was therefore examined in parallel with the O/P samples for the presence of viraemia, none was detected nor were fresh cases of disease seen on subsequent weekly inspections.

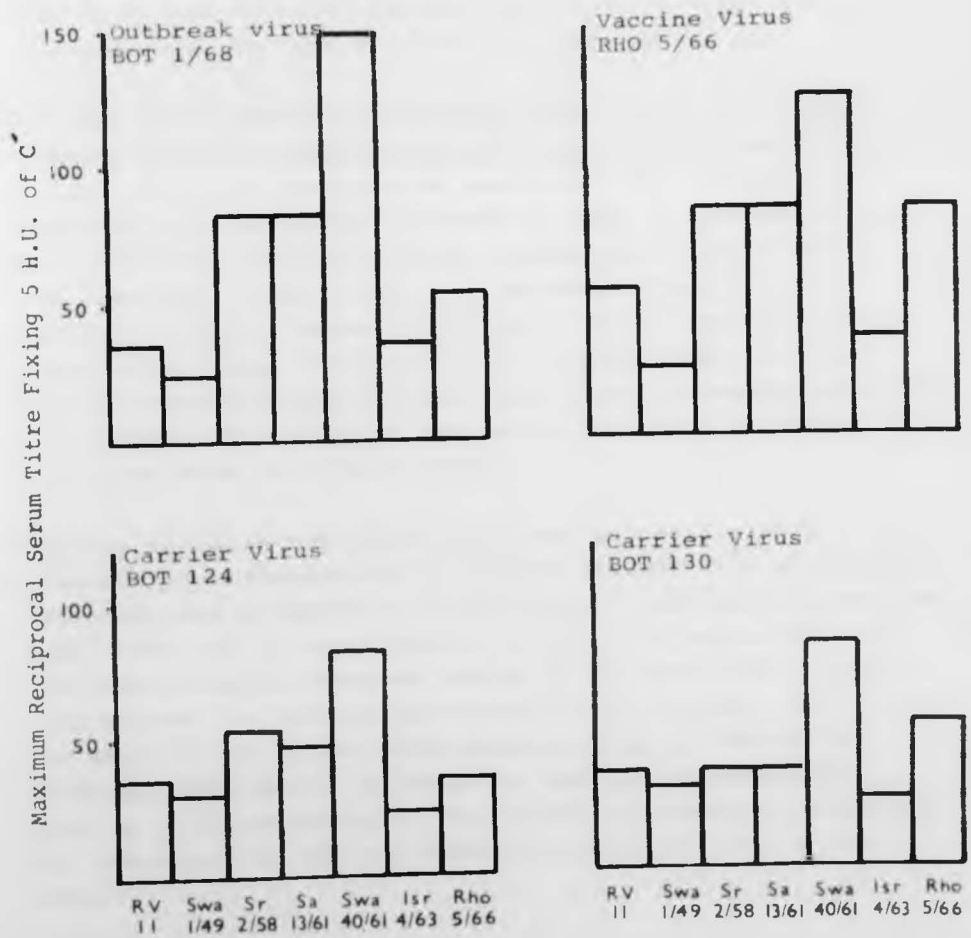
4.2.2 Antigenic Studies

Antigenic differences had been observed between strains of type SAT3 carrier virus from animals in the same herds (Chap. 4.1). Whether variation in the virus had occurred at the time of the outbreak or following the outbreak during the carrier state could not be determined. In this survey, therefore, in addition to the O/P samples from clinically normal animals, lingual epithelium from vesicular lesions was collected from a number of affected animals and virus isolations made; all viruses isolated were typed SAT1.

All the virus strains isolated, both from O/P samples and from lingual epithelium, were screened in CF tests against a range of subtype specific antisera, including the type SAT1 strain used in the vaccine (RHO 5/66). No significant antigenic differences were observed among the 40 strains isolated during and immediately after the outbreak. Figure 3 illustrates the comparison of two of the carrier strains (Bot 124 and Bot 130) with a strain isolated from a clinical case (Bot 1/68) and the vaccine virus (Rho 5/66).

Virus from one of the epithelium samples was adapted to grow in guinea pigs for the production of a specific antiserum. In cross CF tests this strain was similar to the vaccine strain RHO5/66 with a CFP (Davie, 1964) of 0.83 equivalent to an R value (Ubertini et al. 1964) of 91%. RHO 5/66 is a standard World Reference Laboratory (WRL) reference strain originating from an outbreak in cattle in Rhodesia in 1966.

Figure 3. Results of Complement Fixation Tests comparing two carrier virus strains with the outbreak and vaccine strains of virus



4.3 Type A Virus - Malawi

In 1968, a routine prophylactic vaccination scheme was introduced in northern Malawi in an attempt to create a buffer zone against the southward movement of FMD virus types exotic to southern Africa. Prior to its introduction a survey was carried out north of Karonga and along the banks of the Songwe river to determine the immune and disease status of the cattle population. Outbreaks of disease, usually associated with disease in neighbouring Tanzania, had occurred periodically in this area, the last outbreak, due to Type A virus two years previously.

O/P and serum samples were taken from cattle at a number of crushes prior to vaccination and again six months later during the first vaccination campaign. All animals were examined clinically for evidence of past or present disease with negative results. Table 4 shows the results of the O/P samples: Type A virus was recovered from 1 of 190 sampled two years after, and from 1 of 168 two and a half years after known infection. The latter animal had no neutralising antibody to Type SAT2 virus. However, antibody in a number of animals in the group indicated previous infection with this virus type.

One way CF tests indicated that the two carrier virus isolates were antigenically similar (Figure 4). A specific antiserum was prepared in guinea pigs to one of the isolates (Mal 1/68) and it was compared in cross CF tests with two available vaccine strains, Kemron A and Ken 42/66. Table 5 illustrates the results expressed as "R" values. Mal 1/68 was shown to be in the same subtype group as Ken 42/66, isolated from cattle in Kenya in 1966 and subsequently used as a vaccine strain. Ken 42/66 was therefore selected for inclusion in the multivalent vaccine for use in the area.

TABLE 4. The recovery of carrier virus from a clinically normal population of cattle in Northern Malawi

Cattle Crush	No. Sampled	No. Virus Positive	Virus Type	Months after Last outbreak	Amount of Virus (Tubes/10)	Reciprocal SN Antibody Titre
1968						
Kaporo	60	1	A	24	8/10	178
Mwafilaso	65	0		23		
Mwasulama	65	0		24		
1969						
Mwafilaso	60	0		30		
Mwenipera	60	1	A	30	4/10	≤ 6
Marakamogho	48	0		30		

Fig 4. Complement Fixation Tests Comparing the Two Malawi Type A Carrier Virus Strains

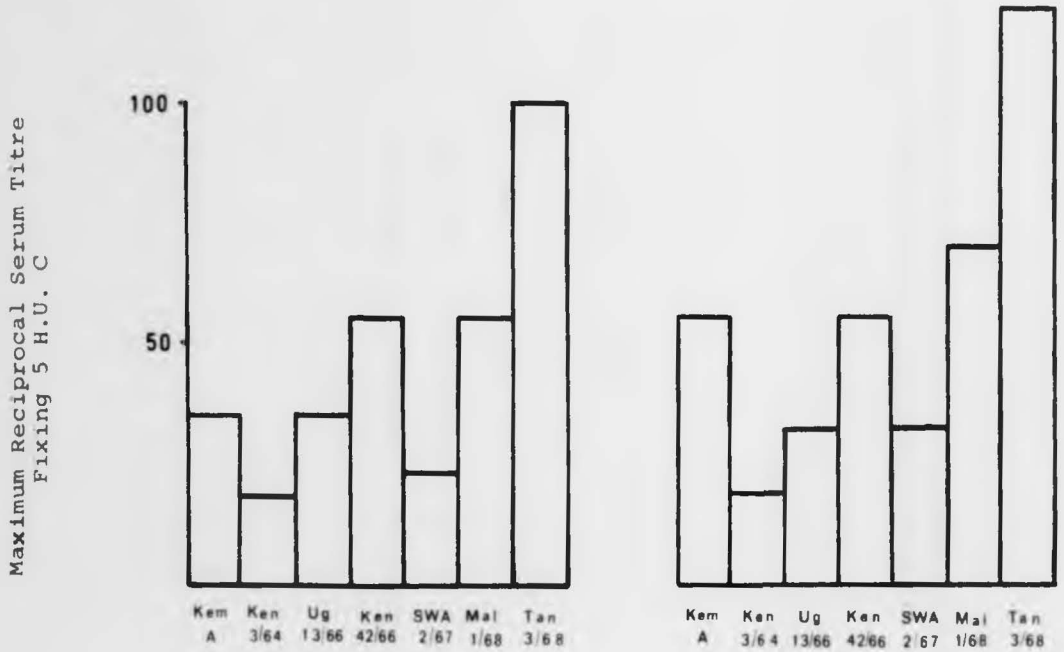


TABLE 5. The relationship of the carrier virus Mal 1/68 with two available vaccine strains expressed as R values

Mal 1/68	100%		
Ken 42/66	67.5%	100%	
Kem A	24%	46%	100%
	Mal 1/68 (Carrier)	Ken 42/66 (Vaccine)	Kem A (Vaccine)

4.4 Type SAT2 Virus - Uganda

Three months after an outbreak of FMD in 1970 due to type SAT2 virus, probang samples from two groups of cattle on the Ankole/Masaka Ranching Scheme in western Uganda were examined for carrier virus. The first group were cross bred Boran, Friesian, Brown Swiss and Red Polls on the Ruhengere Experimental Station, and almost all of them were known to have been infected. The second group were Ankole cattle on a neighbouring ranch reported not to have been infected.

The results (Table 6) confirm the reported history of disease, and illustrate how the techniques of carrier virus recovery can be used to define the extent of infection following a recent outbreak.

Quarantine restrictions had been lifted and it was of particular interest to determine the amount of virus being harboured by the carrier animals. Duplicate O/P samples from all the positive animals were therefore titrated in primary bovine thyroid monolayers in roller tubes. Titres varying from $10^{1.7}$ to $10^{4.0}$ (geometric mean $10^{2.2}$) per ml. were recorded.

4.5 Type O Virus - Uganda

Following reports of unconfirmed FMD at Buruli in Central Uganda 3 - 4 months previously in Ankole-cross cattle, about to be used for a type SAT2 vaccine trial, probang samples were taken from adult animals said to have been affected. Type O virus was isolated from three of ten animals sampled, confirming the history of disease and identifying the virus type responsible.

TABLE 6. The isolation of carrier virus from recovered cattle in Western Uganda

Herd	History	Number Sampled	Positive Carriers	Virus Type	% Positive
Ruhengere	Infected 3m previously	30	10	SAT2	33
Ranch No. 1	Reported Not infected	30	0	-	-

CHAPTER 5 - THE DURATION OF THE CARRIER STATE

5.1 Type SAT3 Virus

Following the initial survey for carrier virus along the Botletle River in northern Botswana (Chap. 1.1), representative groups of cattle were sampled at regular intervals in the Rakops locality. Although the author was not able personally to carry out all the repeat samplings, continuity of sampling staff was maintained where possible. Under prevailing local conditions it was not always practical to resample all the same animals, but groups were taken from the same herds on each occasion and included numbers of animals previously identified as virus carriers.

Table 7 shows that virus may persist in carrier animals for up to 2 years following infection with this strain of virus. Since the completion of this survey similar representative groups of cattle have been monitored periodically for carrier virus with negative results during a programme of disease surveillance in connection with campaigns of routine prophylactic vaccination.

Table 8 shows the incidence of virus recovery in individual animals and illustrates how, in some animals, virus recovery may be intermittent. This was demonstrated on one occasion when a particular animal (No. 208) was sampled in error twice within minutes by different operators; virus was recovered from one sample but not the other. Nevertheless the results suggest that when no virus is recovered from an animal on either of two successive samplings, then it is unlikely to be a carrier.

The end point of virus recovery from animal No. 669 was not determined as she was not presented for later samplings.

TABLE 7. The recovery of Type SAT3 carrier virus in representative groups of cattle on the Botletle River at varying periods after infection

	Months Post Infection								
	7	9	12	14	16	19	23	27	40
Number positive	38	1	2	3	4	2	1	0	0
Number tested	180	61	44	70	57	55	60	50	50
% virus positive	21	1.6	4.5	4.5	7	3.5	1.7	0	0

TABLE 8. The Incidence of Type SAT3 virus recovery in individual animals at varying times after infection

Animal No.	Months Post Infection						
	7	9	12	14	16	19	23
109	+		-	-	-	-	
126	+	-	+	-	+	-	-
132	+		-	-	-	-	
142	+	-	-	-	-		-
144	+	-	-		-	-	
208	+		-	-	+		
209	+			-	+	-	-
259	+		-	-	-	-	
669	+	+	+	+	+	+	+
720				+		+	-
741				+			

5.2 Type SAT1 Virus

Following the outbreak of Type SAT1 infection in cattle on the Chobe River in Northern Botswana (Chap. 4.2) available animals in the infected area and contiguous area were resampled at six monthly intervals. Table 9 shows that carrier virus persisted in the infected herds for 12 to 18 months after infection. All strains isolated were typed SAT1. It was not possible to distinguish by their carrier status, those animals which had been clinically affected from those which had not. Of the 13 animals virus positive six months after infection, 5 had not been clinically infected, and one of two animals positive 12 months after post-infection (p.i.) had developed no disease. Repeated sampling in the vaccinated contiguous herds (Katchekau) failed to reveal spread of carrier virus following resumption of normal contacts after the withdrawal of quarantine restrictions.

A number of carrier virus strains isolated at periods after the outbreak were screened in CF tests against a range of subtype specific sera but no antigenic difference from the original outbreak strain was observed.

TABLE 9. The persistence of virus in carrier animals following Type SAT1 infection on the Chobe

	Infected Area (Satau)			Contiguous Area (Katchekau)		
	No. Examined	Virus Positive	%	No. Examined	Virus Positive	%
During Outbreak	93*	63	68	30	0	0
6 months p.i.	34	13	28	30	0	0
12 months p.i.	37	2	5	30	0	0
18 months p.i.	35	0	0	30	0	0

* Includes both clinically affected and unaffected animals at time of outbreak

5.3 Type SAT2 Virus

Following the survey of cattle on the Ankole/Masaka ranching scheme in Western Uganda for carrier virus (Chap. 4.4) a repeat sampling was made of cattle said to have been infected nine months previously on the Ruhengere Experimental Station. Table 10 shows that 11% of the animals sampled were carrying virus nine months after infection. Unfortunately only three of the animals previously shown to be carrying virus at three months p.i. were available for sampling. Virus was recovered from these three and from only one of the 33 previously untested animals. The results may therefore under-estimate the extent of persistence of this virus type in previously infected animals.

TABLE 10. The persistence of Type SAT2 virus in carrier cattle in Uganda

	No. Sampled	No Virus Positive	% Positive
3 months after infection	30	10	33
9 months after infection	36	4	11

5.4 Type O Virus

Attempts to isolate FMDV in the field from goats which had been infected seven months previously were unsuccessful (Chap. 1.1). It was known however that sheep may carry virus for up to 5 months after experimental infection (Burrows, 1968) and McVicar & Sutmöller (1968) reported the recovery of carrier virus from goats, infected by intranasal inoculation and by contact, for up to 28 days after infection. As the normal duration of the carrier state in goats following infection was not known, adult British Saanen milch goats were experimentally infected at Pirbright both by intradermocoronary inoculation and by contact, and O/P samples were examined at regular intervals on tissue cultures of primary bovine thyroid cells. The virus used was Type O (Strain BFS 1860) isolated from cattle during the 1967/68 outbreak of disease in the West Midlands of England.

Table 11 summarises the results. Apart from marked agalactia, the clinical response of all the goats, whether infected by contact or inoculation, was of a very low degree and would probably have passed unnoticed in non-lactating goats in the field. All seven goats exposed to contact infection became infected and exhibited viraemia, but no lesions were observed in three of them. Lesions in the others were minor and transient. In six, virus persisted for only a few days (6 - 12) after infection; in three, virus persisted longer (22, 41 and 67 days). Virus recovery was intermittent. Neither mode of infection nor the severity of signs appeared to influence the carrier status. The animal which carried virus longest was infected by contact and, apart from agalactia, showed no clinical evidence of infection. Both the other longer term carriers however showed lesions; one was infected by inoculation and the other by contact.

TABLE 11

THE RECOVERY OF VIRUS FROM OESOPHAGEAL / PHARYNGEAL SAMPLES IN GOATS

Animal No.	DAYS 1	2	3	4	5	6	7	8	9	10	11	12	14	16	19	22	26	29	33	36	40	43	47	54	55	56	60	63	68	75	82
JJ 73	-	NT	L +	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JJ 74	-	NT	L +	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
JJ 72	-	NT	-	-	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	+	-	+	-	-
JK 6	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JK 7	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JK 8	-	-	+	L +	NT	NT	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JK 9	-		-	+	+	L +	+	+		-		-	-		+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-
JK 10	-		-	-	-	L -	-	+	+	-			-		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JK 27	-		-	+	L +	+	+	+	+		+	-	-	-	-	-	-	-	-	-	-	-	DIED								

= Viraemia
L = Appearance of Lesions
NT = Not Tested
\+ = Virus Recovered
\- = No Virus Recovered

JJ 73 & JJ 74 - Intradermo-coronorary Band Infection
JJ 72 - JK 27 - Contact Infection

CHAPTER 6 - THE RELATIONSHIP OF SERUM NEUTRALISING (SN) ANTIBODIES TO THE CARRIER STATE

6.1 Type SAT3 Virus

The association of high SN antibody titres with the carrier state has been reported (Sutmöller & Gaggero, 1965; Burrows, 1966 and Van Bekkum et al., 1966). During the survey of cattle in Northern Botswana for carrier virus (Chap. 4.1) their sera were tested in the CMI test against the strain of SAT3 virus (Bec 1/65) isolated during the recent outbreak: 46 of the 50 carriers had high SN antibody titres but four, all adults, had no detectable SN antibody. Their sera were therefore also assayed for antibodies against their respective homologous carrier viruses in mouse neutralisation tests but results were negative.

Table 12 compares the individual antibody titres of a group of carrier animals from one of the cattle posts 7 months after infection with those from one 12 months after infection. In addition an attempt was made to assess the amount of pharyngeal virus and each sample was inoculated into ten tissue culture tubes. Although the number of tubes positive gives some indication of the amount of virus in the sample, the range covered is only ten-fold and differences within this range cannot be regarded as significant, nor used to establish a correlation between antibody and carrier status. It can be deduced from the summed values, however, that more virus seemed to be present in the 7 month group than in the 12 month group.

Table 13 compares the geometric mean SN antibody titres of groups of virus positive animals with groups of virus negative animals at each of four cattle posts. Analysis of these titres in a Student's "t" test showed that there was no significant difference ($t = 1{\cdot}72$, $0{\cdot}1 > P < 0{\cdot}05$) between the mean titres obtained from the two classes of animals.

TABLE 12. Virus isolations and serum antibody titres against the outbreak virus (Bec. 1/65) in individual animals at two representative cattle posts

Sokwani II (7 months after infection)			Machana (12 months after infection)		
Animal Number	Tubes/10 Positive	Recipr. SN Titre	Animal Number	Tubes/10 Positive	Recipr. SN Titre
208	10	128	514	3	-
220	10	128	516	3	≤6
195	8	45	517	3	64
186	7	90	536	3	128
217	7	256	555	2	64
212	4	64	522	1	≤6
180	4	128	526	1	178
218	3	16	534	1	≤6
174	2	178	537	1	90
209	2	128	540	1	22
224	2	128	558	1	90
197	1	≤6	566	1	178
205	1	22			

TABLE 13. The geometric mean SN antibody titres of groups of virus positive and virus negative animals at different cattle posts

Cattle Post	Virus Positive	Range	Virus Negative	Range
Sokwani I	275 (12)*	90-1024	88 (48)	≤6-256
Sokwani II	64 (13)	≤6-256	112 (47)	≤6-708
Tsienyani	128 (11)	≤6-256	95 (49)	≤6-512
Machana	42 (12)	≤6-178	54 (48)	≤6-355
TOTAL	93 (48)	=6-1024	81 (192)	≤6-708

* Number in group

6.2 Type SAT1 Virus

Immediately following the outbreak of FMD on the Chobe River in Northern Botswana (Chap. 4.2), cattle which had been clinically affected, and vaccinated cattle which had not, were tested for SN antibodies to Type SAT1 virus (strain Rho 5/66), a strain closely related to the outbreak strain. Clinically unaffected animals were grouped as carriers or virus negative animals. The possibility that some of the latter may have been undetected carriers, was appreciated.

Table 14 shows that when animals in a herd partially immune following vaccination are exposed to natural infection in the field, many with immunity sufficient to protect them from clinical disease become carriers of virus.

TABLE 14. Geometric mean reciprocal antibody titres in groups of clinically affected and non affected animals in infected and non affected herds

	Clinically infected animals	Carriers (no overt disease)	Virus negative animals
INFECTED AREA (SATAU)			
1) FMD in herds	812 (28)*	363 (29)	603 (16)
2) No FMD in herds		794 (6)	166 (13)
3) Total	812 (28)	389 (35)	338 (29)
CONTIGUOUS AREA (Katchekau)	-	-	112 (29)
CLEAN AREA (Kazangula)	-	-	112 (22)

* Number of animals in group

In herds with clinical FMD the antibody titres in affected animals were generally higher than in those which resisted disease, but carriers appear to have lower antibody levels than virus negative animals. In contrast, in unaffected herds, titres in carrier animals were markedly higher than in non carriers. The overall antibody levels of both carriers and virus negative animals were however similar. The generally higher titres of unaffected animals in the infected area compared with those in the contiguous and "clean" areas may have been due to their earlier revaccination undertaken as a disease control measure.

Tables 15 and 16 show the reciprocal SN antibody titres and results of virus examination of groups of immune cattle which became virus carriers without developing disease, and groups of clinically affected animals both at the termination of the outbreak and again six months later. At six months p.i. SN antibody titres of both groups had dropped considerably. Although convalescent titres in the clinically affected group had been approximately two-fold higher than in the unaffected group, six months later their SN antibody levels were similar.

These results confirm the finding with SAT3 virus (Chap. 6.1) that following disease there is no apparent correlation between the carrier status of animals and their antibody titres.

TABLE 15. Virus isolations and reciprocal serum antibody titres in cattle which resisted clinical infection during outbreak

Animal Number	Immediately after infection: Recipr. SN titre	Immediately after infection: Tubes/10 Positive	6 months after infection: Recipr. SN titre	6 months after infection: Tubes/10 Positive
97	178	10	64	0
109	178	10	45	0
116	178	10	45	0
118	178	10	90	9
119	355	10	90	0
121	178	3	90	0
128	178	10	45	0
147	512	10	45	6
149	256	10	32	0
151	1400	3	64	0
153	128	10	90	4
155	1024	1	128	2
Geometric mean	282		64	

TABLE 16. Virus isolations and reciprocal serum antibody titres in cattle clinically affected during the outbreak

Animal Number	Immediately after infection: Recipr. SN titre	Immediately after infection: Tubes/10 Positive	6 months after infection: Recipr. SN titre	6 months after infection: Tubes/10 Positive
96	1,400	10	45	0
80	2,800	N/T	45	0
81	355	10	45	0
87	1,400	"	45	5
90	90	"	45	3
91	256	"	45	1
92	256	"	64	2
95	1,024	"	45	0
107	708	"	32	0
130	512	"	64	7
132	1,024	"	22	0
134	708	"	64	10
Geometric mean	607		49	

6.3 Type SAT2 Virus

Three months after an outbreak of FMD due to Type SAT2 virus in western Uganda (Chap. 4.4), recovered animals in Ruhengere Experimental Station, and animals reported to have been unaffected on a neighbouring ranch, were sampled for carrier virus. Their sera were tested against Type SAT2 virus (strain Ken 3/57), isolated from an outbreak of disease in cattle in Kenya. This strain was different from but related to the outbreak strain with an R value of 41% (A J Forman, personal communication).

The mean antibody titres (Table 17) of the carrier animals and the virus negative animals were similar and there is no apparent correlation between the amount of virus being harboured by an individual and its SN antibody titre. No neutralising antibody to Type SAT2 virus was demonstrated in the 30 animals sampled on the neighbouring ranch reported to have been disease free, nor was any virus recovered. This result resembles the situation in Europe where many thousands of animals have been tested prior to inter-territorial movement for carrier virus and antibodies with negative results.

Following infection, where high antibody levels are found in a sample of a population, carrier virus will also be recoverable from that population. Conversely, if significant antibody titres cannot be demonstrated in some animals in a population then it is highly unlikely carrier virus will be recovered.

TABLE 17. Reciprocal neutralising antibody titres in carrier and non carrier animals following Type SAT2 infection in Uganda

Carriers			Non-Carriers		
Animal Number	Log 10 virus per ml. O/P sample	Recipr. Titre	Animal Number		Recipr. Titre
1217	4.0	178	1216	NVI *	90
473	2.5	178	1863	"	22
1213	2.5	355	1849	"	90
1210	1.8	256	1840	"	128
254	1.8	32	482	"	22
1845	1.8	32	1211	"	64
737	1.8	178	1205	"	355
1209	1.7	32	1839	"	32
398	1.0	22	1218	"	64
1208	Trace	64	320	"	128
Geometric mean		79			69

* NVI = No virus isolated

CHAPTER 7 - TRANSMISSION OF CARRIER VIRUS

7.1 Field observations

On some large ranches in northern Kenya, outbreaks of FMD have occurred through the years with regularity. Sometimes, where geographical conditions and other factors allowed, it had been the practice to split very large ranches into two separate administrative units for quarantine purposes on the confirmation of FMD in one isolated section. The quarantine of the infected section was vigorously controlled and supervised by local veterinary officers until a minimum of six weeks had elapsed after the clinical end point of the disease had been established by inspection. Quarantine restrictions were then lifted following inspection of all stock by a veterinarian. The ranch would again be administered as a single unit and internal movements of stock for purposes of breeding, dipping, inoculation etc. would be resumed. On some of these ranches, which carried several thousand head of cattle, FMD due to the same virus type broke out again within a few months to a year after the previous clinical end point. Not infrequently this occurred in the absence of any overt disease elsewhere in the district and when epizootiological investigation failed to reveal any outside source of infection.

On investigation of these re-occurrences of disease it was rewarding to closely question the indigenous herdsmen whose knowledge of the individual animals in the herds for which they were responsible was often considerable. When morbidity in a herd was low, and many animals were not clinically affected, the investigator was frequently informed that individual unaffected animals had "had it last time" and that the majority of clinically affected animals had for various reasons "not had it last time". Such reasons included a particular animal not having been in the infected section of the ranch during the last outbreak, or having been a calf during or not born until after the infection.

These re-occurrences or recrudescences of disease clearly indicated the possibility of a carrier state in cattle and suggested that carrier animals were responsible for the initiation of clinical disease in susceptible animals.

In Kenya, in the post-war years, the virus types responsible for almost all outbreaks of FMD were established. In the large scale farming areas of the highlands, virus samples from every infected farm and ranch were typed. Until 1960, only Types A and 0 had been recorded. In that year however an outbreak of disease due to Type SAT2 virus occurred on a European ranch in north Kenya for the first time. The outbreak was quickly controlled and disease spread to only two contiguous ranches. Eight weeks after clinical end point on the first ranch, quarantine restrictions were withdrawn and forty Boran cattle were sold for breeding. All these animals had been seen to be clinically infected during the outbreak and prior to movement they were clinically examined on two separate occasions. They were found to be recovered and free of any evidence of disease.

The cattle were moved by rail to a farm in the Highlands approximately 250 miles to the west and very far removed from any possible source of infection due to Type SAT2 virus. On arrival they were grazed in a field bordering one in which other cattle were grazing. Contact was possible over and through the wire fence. Two months after the arrival of the Boran animals, FMD broke out in these contact cattle. The virus responsible was typed as SAT2. There was no other infection in this region due to Type SAT2 virus nor had this virus type been previously recorded there.

This incident provided strong epizootiological evidence of the existence of carrier animals and of the transmission of disease from them to other animals.

7.2 Carrier Transmission Experiment

This experiment, mentioned briefly by Falconer (1972), was designed to study the possibility of spread of FMDV from carrier cattle to susceptible cattle under natural conditions in the field in Botswana. The design included the herding together of 20 susceptible indigenous cattle with a similar number of carrier animals over an extended period with the inclusion of stress in the form of simulated trekking in drought conditions.

An isolation enclosure of 10 acres was built within a mile square outer security fence close to the field laboratory at Motopi (see map Figure 5, page 86) in a cattle-free area of northern Botswana. It was made stock proof with a seven foot high chain-link fence embedded in concrete and surmounted by externally overhanging barbed wire strands. Strict disease security precautions were taken and disinfection and showering facilities were provided for staff entering and leaving the enclosure. The enclosed area was flat white Kalahari sand with a cover of thornbush and dry grass varying from dense thorn thickets to small open glades. A water trough, salt lick and and cattle crush were provided in the enclosure together with a deep fenced-off pit to enable rapid disposal of infected animals in the event of successful transmission with resultant clinical disease. A small thornbrush kraal was built, into which the animals were herded at night.

16 Afrikander cattle with no history of disease or vaccination were brought 600 miles to Motopi by lorry from a FMD-free area in southern Botswana. Prior to movement, they were clinically examined and on 2 occasions O/P samples were screened for FMDV and sera tested for neutralising antibodies against all three SAT type viruses with negative results. At Motopi before the start of the experiment, they were again clinically examined and screened for SN antibodies and virus.

Carrier animals were selected by screening O/P samples from cattle previously found to be carriers and ear tagged following a Type SAT1 outbreak of disease at Satau on the Chobe River six months earlier. A larger number of positive virus carriers were identified, but, probably because of the remoteness of the area and primitive influences, the owners of only four carrier animals agreed to sell. Even exchange at the rate of one indigenous carrier steer for two improved in-calf Afrikander heifers was refused. The four carrier animals were transported by lorry through extremely difficult country over three days involving considerable stress. Five days after their arrival in the isolation enclosure, virus was no longer recovered from one of them (No. 92, Table 18).

Carrier and susceptible animals were grazed and browsed together as one herd during the day and were close-herded in a small thorn kraal at night to simulate local conditions. Cattle watered once daily from a hand filled trough and had free access to a single salt lick compounded with coarse sand and cement. Veld hay was fed to supplement the limited grazing in the enclosure. All animals were clinically examined twice daily, morning and evening. Temperatures were taken, mouths were opened and tongues examined but muzzles and tongues were not handled.

During the last two weeks of the experiment, the stress of a long trek to the abattoir or railhead was simulated by driving the cattle daily around the inside perimeter of the enclosure during the heat of the day for four and a half hours (about 15 miles). The supplementary veld hayration was reduced and the cattle were watered every other day. Salt was continually available.

To avoid unnecessary handling and trauma, O/P and serum samples were taken only at the start and finish of the experiment. During the period of stress, when intercurrent disease was suspected, blood smears were taken for diagnostic purposes.

Tables 18 and 19 show the results of the virus isolation attempts and the SN antibody titres of both groups of animals at the start and finish of the experiment.

The specificity of all viruses isolated was checked by CF test. The carrier group were slaughtered at the end of the experiment and, in addition to O/P samples taken before death, samples of soft palate, pharynx and interdigital skin were taken at post mortem. All samples were virus negative. No virus was recovered from the susceptible group of cattle.

Sera were assayed for antibodies against Type SAT3 virus in addition to SAT1 virus as the carrier group had been vaccinated with a bivalent SAT1 and SAT3 vaccine six and twelve months previously. At the end of experiment no SN antibodies, which could have indicated virus transmission, were detected in the susceptible animals.

No clinical signs of FMD were seen in either group of cattle during the course of the experiment. The scrub grazing and rough walking imposed during the simulated trekking resulted in considerable trauma to the tongues, mouths and feet. By the end of the experiment five animals had become lame due to trauma. During the period of stress, intercurrent disease occurred in both the carrier and susceptible groups. Trypanosoma congolense infection became clinically apparent in two of the carriers (Nos. 92 and 153) necessitating their withdrawal from the daily trekking. One of the susceptible cows showed muscular incoordination on the 9th day of stress but recovered after vitamin A, D and E injections; another aborted a three month foetus on the same day.

TABLE 18. Carrier transmission experiment; virus isolation

	Animal Number	Tubes +ve/10 at selection (28/6/68)	Virus Type	Tubes +ve/10 Start of exp. (12/7/68)	Virus Type	Tubes +ve/20 End of exp. (26/8/68)
	92	2/10	SAT1	0/10		0/20
CARRIER	130	7/10	SAT1	5/10	SAT1	0/20
GROUP	153	10/10	SAT1	2/10	SAT1	0/20
	155	4/10	SAT1	4/10	SAT1	0/20
SUSCEPTIBLE	817	0/30 *	-	N.T.	-	0/20
GROUP	819-834	0/30	-	N.T.	-	0/20

* Sampled on three separate occasions between 2/6/68 and 18/6/68. Each sample was inoculdated separately into 10 roller tubes of primary bovine thyroid cells.

TABLE 19. Carrier transmission experiment: Reciprocal SN antibody titres

	Animal Number	At selection (28/6/68) SAT1	At selection (28/6/68) SAT3	Start of experiment (12/7/68) SAT1	Start of experiment (12/7/68) SAT3	End of experiment (28/8/68) SAT1	End of experiment (28/8/68) SAT3
	92	64	≤6	90	≤6	90	8
CARRIER	130	64	≤6	90	≤6	90	≤6
GROUP	153	NT	≤6	90	≤6	90	≤6
	155	128	≤6	128	≤6	128	22
		(2/6/68)					
SUSCEPTIBLE	817	≤6	≤6	≤6	≤6	11	≤6
GROUP	819-834	≤6	≤6	≤6	≤6	≤6	≤6

CHAPTER 8 - DISCUSSION OF PART I

8.1

The demonstration of virus carriers among cattle following natural outbreaks of FMD due to strains of 5 of the 7 immunological virus types shows that the carrier state is a natural sequel to infection. Many virus infections causing acute disease, e.g. rinderpest, measles and influenza, result in a solid immunity without long-term excretion of virus. FMDV is therefore unusual in this respect. Not all cattle become demonstrable carriers but a very high proportion may be shown to be carriers immediately after infection.

The duration of the carrier state in individual cattle following infection varies and may depend on a number of factors including the strain of virus, the animal's breed and type, nutritional status, conditions of husbandry and inter-current disease. Van Bekkum et al (1959) reported the recovery of type A virus more than 5 months after infection. Sutmöller and Gaggero (1965) recovered types A and C viruses in the field six months after disease and Burrows (1966) recovered type SAT3 virus from cattle 6 months after experimental infection. Composite results drawn from animals in Africa over a number of years (Table 20) show that although the majority of cattle have ceased to excrete virus a year after infection, a number continue to carry virus for up to 2½ years. Straver et al (1970) in a later study in Holland have had similar results reporting the persistence of a type C virus strain in one cow for 24 months. After infection, there is not only a gradual tailing off in the number of animals harbouring virus, but also in the amount of virus being excreted. The overall picture suggested that virus strain has little influence on the duration of the carrier status in the field.

TABLE 20. Percentage cattle from which virus could be isolated at different periods post infection

1m	3m	6m	7m	9m	12m	19m	23m	24m	30m
85(1)*	33(2)	28(1)	20(3)	11(2)	20(3)	3.5(3)	1.7(3)	1(A)	1(A)
	33(0)		23(3)		3(3)				
			18(3)		4.5(1)				
					5.4(2)				

* Virus Type, SAT1, 2, 3 Type A or O

Virus recovery from individual animals may be intermittent and Van Bekkum (1959) has emphasised that a negative result from a single O/P sample does not necessarily indicate that virus is not being excreted, and suggested that the amount of virus in the "saliva" of an individual may vary at different times. Intermittency of virus recovery from an individual and the variation in isolation rates from groups of animals may be due however to a number of factors including the individual operator, the handling of the samples after collection and differences in the susceptibility of the systems used for virus isolation. Recently samples have been split into aliquots at the time of sampling before freezing on dry ice. On occasion, following disappointing isolation rates, attempts have been made to isolate virus from the duplicates and triplicates on fresh cell cultures of different origin with much more consistent results.

In sampling, emphasis has generally been placed on older animals more likely to have been involved in past outbreaks of disease. More recent work in buffalo (Part II) shows that, in that species, the frequency of virus isolation and the amount of virus being excreted is greatest in animals 1 to 3 years old. Although emphasis on younger cattle might have

yielded better results in terms of virus isolations, no correlation has been found between the sex or age of sampled animals and their carrier status.

The persistence of an attenuated vaccine virus strain in the pharynx of vaccinated cattle and its transference from them to contact animals has been reported in South America by Auge de Mello et al, (1966). Our results in Botswana however failed to show persistence of attenuated strains of type SAT1 and SAT3 viruses following vaccination. Prior to the survey of cattle for carriers on the Botletle River in Botswana, the majority of animals had been vaccinated twice seven to eight months previously, with a vaccine incorporating attentuated strains of type SAT1 and SAT3 viruses. Although virus isolations were made from 50 of 360 cattle sampled, all isolates were typed SAT3 and shown to be either identical to or related to the outbreak strain of virus. The difference between these results and those of de Mello may be due to different characteristics of the attenuated virus strains used. Further investigations were not possible as attenuated vaccines lost popularity in Africa and only inactivated vaccines have since been used.

Available evidence of the occurrence of carrier goats is scanty. McVicar and Sutmöller (1969) recovered strains of types O, A and C viruses from goats 28 days after infection. We failed to recover virus from goats in the field 7 months after type SAT3 infection. However our limited experimental study showed that after infection with a type O virus only 3 of the 9 goats carried virus for more than a few days; the longest period of virus recovery after infection was 67 days. Goats are therefore probably similar to sheep from which Burrows (1968a) recovered virus for 1 to 5 months after infection. Carrier goats may thus not be of great importance in the epizootiology of FMD. It is emphasised however that during active infection, goats, like sheep, often show little or no clinical evidence of disease and yet may be excreting virus and capable of widespread dissemination of the disease.

The association of high SN titres with the carrier state has been previously reported (Sutmöller and Gaggero, 1965; Burrows, 1966 and Van Bekkum et al, 1966). Our results are in general agreement and indicate that, following infection, a population with high antibody levels will contain carriers.

However no SN antibodies could be detected in 4 of the 50 carriers identified during the survey on the Botletle River for SAT3 virus (Chap. 4.1) or in the one type A carrier in Malawi in 1969 (Chap. 4.3). Auge de Mello et al, (1966) were also unable to detect antibody in virus positive cattle in contact with animals given an attenuated vaccine, suggesting the level of infection in these animals was insufficient to stimulate an antibody response. It is tempting to assume that our failure to detect antibody in the five carriers, all adult, is also evidence of the transference of small amounts of carrier virus from animal to animal without clinical or serological reaction. No further cases of disease occurred in these populations and it is therefore unlikely the animals were in the early stages of disease. It is well known, of course, that some animals fail to show a detectable SN antibody response to primary sensitisation with virus and others only show a minor response which quickly wanes. The absence of antibodies in some virus positive animals does not rule out therefore the possibility of their having been infected with virulent virus at the time of the outbreak.

These findings suggest caution in the acceptance of negative serological results in individual animals as criteria of safety for interterritorial movement.

Carrier and non carrier animals cannot be distinguished by their SN antibody titres nor is there correlation between the amount of virus being excreted by an animal and its SN antibody titre. Although comparisons must depend on our ability to detect carrier virus, results in cattle do not suggest that the presence of carrier virus or the level of infection influences the persistence of circulating neutralising antibody.

Van Bekkum et al, (1959) mentioned that some vaccinated animals which had shown no signs of clinical disease during outbreaks were found to harbour virus. Our results confirm this. During the type SAT1 outbreak on the Chobe River in Botswana (Chap. 4.2) animals with sufficient immunity to protect against disease also became carriers and subsequently carried virus for as long as those which had been clinically affected.

Sutmöller et al, (1968) subsequently exposed susceptible and vaccinated cattle to FMDV in varying amounts by different routes and found that a high percentage of cattle became carriers after pharyngeal and intra-nasal exposure. When virus established itself in the pharynx, multiplication took place in spite of preinfection serum antibody and without lesions in many cases. The proportion of animals which became carriers depended not only on their immune status but also on the degree of virus exposure. Our observations with type SAT1 virus in a natural outbreak of FMD (Chap. 4.2) confirm this. When exposure to virus was high (herds with an approximate 70% morbidity), 85% of animals sampled which failed to develop disease became carriers. When exposure was lower (approximately 25% morbidity in the herd) only 33% became carriers. When exposure was limited to daytime contact with infected animals in other herds with which they grazed, the proportion was also low (30%).

In the first group the mean SN titre of the 4 animals which resisted disease and from which carrier virus was not isolated was 1/1318 compared with a mean of 1/247 for the 23 animals which did become carriers. The numbers are small but suggest the possibility that a sufficiently high titre of circulating antibody may protect an animal not only from clinical disease but also from becoming a carrier.

The mean SN antibody titre of the virus negative animals in the herds with active infection was considerably higher than the mean in the clinically unaffected herds suggesting that an active infection in the herd may boost the neutralising antibody of the animals which resist disease. Further observations, however, including the assay of sera before as

well as after herd infection, would be needed to corroborate this.

Vaccination both before and after an outbreak appeared to have little effect on the duration of carrier status (Chap. 6.2). Although, with this strain of type SAT1 virus, antibody titres were higher in clinically affected animals than in unaffected animals during and immediately after the outbreak, titres waned quickly and six months afterwards there was no observable difference between the two groups. In spite of vaccination completely protecting some animals and affording partial protection to others it did not obscure or mask the disease, and diagnosis presented no great difficulty.

Within each of the seven immunologically distinct types of FMDV, strains have been isolated which differ from one another antigenically and it has long been suspected that antigenic changes may result from the spread of virus through a partially immune population. Hyslop and Fagg (1965) reported the experimental serial passage of a type SAT1 virus through partially immunised cattle resulting in an immunologically distinct variant after the 34th serial passage. Hyslop (1965) further showed that strains of type SAT1 virus, serially passaged in tissue culture in the presence of increasing concentrations of specific antisera, showed an increased ability to multiply in those sera. It might also be expected that, in the presence of antibody to an infecting strain, antigenic mutants are more likely to be selected. A study of type SAT3 carrier virus strains (Chap. 4.1.2) showed at least one antigenic difference between strains of virus isolated from different animals in the same herd indicating the possibility of such change occurring in the carrier state. No antigenic changes were observed however in the virus strains isolated from individual animals at varying periods after infection. It was not possible to know whether the origin of all the carrier strains was the particular virulent strain isolated during the outbreak, antigenic variation having occurred subsequently in the carrier state, or whether there was antigenic heterogeneity among the virus strains present during the outbreak.

No significant antigenic differences were observed however in the 40 strains isolated during and immediately after the type SAT1 outbreak on the Chobe (Chap. 4.2). This suggests that antigenic homogeneity is probably normal during the virulent stage of an outbreak. With this virus strain no antigenic differences from the outbreak strain were observed in carrier strains isolated 6 and 12 months later. Significant antigenic change in the carrier state under natural conditions may be rare, but there is suggestive field evidence of its occurrence. For example, in a particular cattle population on the banks of the Kafue River in the Namwala district of Zambia, outbreaks of disease due to type SAT2 infection occurred in 1960, 1964 and 1965. On each occasion a different virus subtype was identified (unpublished data). No other outbreaks of FMD were reported elsewhere in Zambia during these years. The cattle population was isolated from the rest of the country by Tsetse fly to the north and south, swamps to the east and a National Park to the west. The local people do not keep sheep and goats. Such movement of animals as occurs is generally outwards for slaughter. It seemed likely therefore that persisting carrier virus may have changed antigenically sufficiently to overcome the immunity of its host or cattle in contact with that host thus initiating disease on the two latter occasions. Conditions in the field in Africa however are often complex and the possibility of these two outbreaks having been due to spread of virus from wild buffalo cannot be entirely excluded.

The initiation of disease by carrier animals may normally involve the transference of virus to other susceptible animals. However certain conditions such as stress may lead to enhancement of multiplication, and even virulence, of the virus causing overt disease in the carrier itself with consequent shedding of large amounts of virus and enhanced risk of spread of disease. This would be most likely when the carriers' circulating antibody had waned to a low level. Clinical disease did not recur in the small sample of four cattle from which carrier virus was isolated in the absence

of demonstrable SN antibody (Chap. 6.1). Thus it may not be circulating antibody alone, normally present in carriers, which protects against generalisation and initiation of disease. Acquired cell-mediated immunity may have protected these animals from clinical manifestation. Although reduced pathogenicity to cattle has been reported in some strains of carrier virus (Kaaden et al, 1970), infectivity for cattle is retained. R Burrows (personal communication) produced clinical FMD with generalisation in susceptible cattle by intradermolingual inoculation of O/P fluid taken from a carrier steer 16 weeks after infection with SAT3 virus (strain Bec 3/64). O/P fluid frequently contains only small amounts of virus, and large quantities, up to 4 ml. were used to produce disease. Under natural conditions the amounts of carrier virus likely to be transferred from animal to animal are generally too small to initiate disease. It is possible that certain trigger factors provide a more favourable environment for the growth of carrier virus and consequent appearance of clinical disease in its host. These may include, in addition to possible antigenic change, immuno-depressive factors such as stress due to long trekking, starvation and intercurrent disease.

Field veterinarians in the past, hypothesising that recovered animals act as carriers of FMDV, have suggested that some outbreaks of disease could only be explained by the transmission of virus from apparently healthy cattle to susceptible animals with which they are in contact. Field observations (Chap. 7.1) indicate that virus persisting in recovered cattle on large ranches may be responsible for the reappearance of disease at a later date in previously uninfected animals, and there is strong evidence (Chap. 7.1) that carrier virus may spread to fully susceptible animals and be responsible for the dissemination of disease over long distances. Carriers seem likely to be one of the mechanisms perpetuating FMDV in a population. However, the duration of the carrier state is limited; if infection does not spread to other animals during this period, it will die out in that population unless more carriers are introduced. Observations suggest however that initiation or dissemination of disease

by carrier animals is rare and influenced by factors not well understood.

The isolation of virus on numerous occasions from a calf born after an outbreak of clinical disease (Chap. 4.1.1) appears to provide evidence of the transmission of persisting virus from one animal to another within the herd in the absence of visible disease. It cannot be stated that the source of this virus was the calf's dam although her antibody titre suggested recent infection with virus.

The transmission of carrier virus from animal to animal has not yet been demonstrated experimentally. Van Bekkum et al (1959) reported negative results when susceptible or vaccinated cattle were introduced into herds known to be carrying virus. Later Burrows (1966a) reported no clinical or serological evidence of transmission when four susceptible cattle were housed in close contact with 6 carriers of Type SAT3 virus from the 9th to 14th week of convalescence.

The experiment described here was carried out with local cattle under natural conditions in Africa but its value was reduced by the small number of carriers finally involved. It is worth recording, however, because the opportunities for further similar experiments may not easily recur, and the failure of virus to transfer from the carrier group to the susceptibles suggests that, certainly with this strain of virus, initiation of infection by carriers some months after infection does not readily occur.

Various factors which might have predisposed to transmission of virus under natural conditions were introduced into the experiment. Close animal to animal contact was ensured in the small thorn kraal into which the animals were herded at night. The animals all watered at the same trough at the same time. Lack of grass in the enclosure ensured browsing with constant epithelial trauma which is a feature of the tongues and mouths of animals on free range in unimproved low veld grazing. Such trauma would allow easy access of

virus to susceptible cells. Cattle in Africa coming to lick salt will often salivate profusely, almost explosively, and salivation by virus excreting animals may favour transmission. A single rough surfaced salt lick was used by all animals ensuring saliva to mouth contact from animal to animal.

Stress and consequent intercurrent disease introduced into the experiment may be immunosuppressive factors which favour transmission or even recrudescence of clinical disease in virus carriers. However not only was there no transmission of virus but following the period of stress, virus could no longer be recovered from the carriers. It is unfortunate that, to avoid unnecessary handling and trauma, artificial factors which might influence the spread of virus, O/P samples were not taken at regular intervals throughout and the exact point at which the carriers ceased to excrete measurable quantities of virus was not determined. Virus was still being recovered from some animals in their herd of origin 6 months later.

8.2 Applications of carrier virus techniques

Some applications of the techniques for the detection of virus carriers used and developed during these studies have been briefly mentioned. One has been in retrospective diagnosis. In parts of the world where the economic value of individual animals is low, the effects of, and therefore importance of FMD is minimal in the eyes of the stockholder. Consequently, particularly in pastoral areas where distances are great and communications difficult, reporting of disease is frequently haphazard and delayed. A field veterinarian, arriving to make an initial diagnosis often finds infection has passed through and no suitable lesion material remains from which virus could be isolated. Our results however show that, following infection, FMDV may be isolated from a high proportion of recovered cattle for considerable periods. Thus the examination of O/P samples from a small number of representative animals will allow isolation and characterisation of the virus type involved.

These techniques also have a potential application in prospective or anticipatory diagnosis, as FMDV may be isolated from the pharynx of cattle in contact with disease, up to 5 days prior to the appearance of lesions. During the 1967/68 epizootic of FMD in the West Midlands of England, plans were in fact prepared to use the probang for prospective diagnosis in in-contact animals which may have been incubating disease.

As epizootiological tools, the techniques have proved useful in determining the disease history in a population and delineating the extent of infection. In areas where vaccination is being considered, the monitoring of the cattle population for carrier virus has provided a means to assess the disease threat from within that population in terms of virus type and subtype.

The value of the probang test in the assessment of the safety of stock prior to international movement was quickly noted and a number of countries now routinely require negative tests prior to the importation of cattle and sheep.

P A R T III

THE CARRIER STATE IN GAME WITH PARTICULAR REFERENCE TO THE AFRICAN BUFFALO (SYNCERUS CAFFER)

CHAPTER 9 - INTRODUCTION

In many African territories, particularly where FMD is endemic there has long been considerable interest in the relationship between wild animals, domestic stock and the disease. Many species of animals have been reported as having been infected (Macaulay, 1963) and FMDV has been isolated from some species in the natural state (Hooper Sharpe, 1937; Meeser, 1962; World Reference Laboratory for FMD, unpublished data). The implication of free-living wild animals in the initiation and dissemination of the disease, however, has generally been speculative.

Although the association of the African buffalo with rinderpest (Henning, 1965) and with East Coast Fever (Brocklesby & Barnett, 1966) is well established, less is known of its association with FMD. In an extensive random survey of 39 species of African wildlife from a number of countries over a five year period, Condy, Herniman and Hedger (1969) reported significant antibody titres to one or more FMD virus types in 16 species, all cloven-hoofed. Of these 16 species, the highest and most consistent antibody titres were recorded in the African buffalo. Condy (1970), in the course of natural outbreaks of FMD in cattle in Rhodesia in 1965 and 1966, attempted on two occasions to infect captive buffalo artificially with strains of Type SAT2 virus isolated from infected cattle. Although four of the six buffalo used were apparently susceptible to Type SAT2 virus with no demonstrable SN antibody at the time of infection, Condy failed to produce convincing evidence of clinical disease in animals inoculated by both intramuscular and intradermolingual routes. However, high specific SN antibody titres developed in the inoculated animals.

It seemed likely that the buffalo played a significant role in the perpetuation of FMDV in the wild, but apart from the inclusion of this species in a check list of non-domestic animals reported as having been infected with FMD (Macaulay, 1964), no record could be found of the confirmation of disease

in or isolation of virus from this species under natural conditions. Indeed, Lees May (1960) describing an outbreak of Type SAT1 infection in a herd of cattle kept in Wankie Game Park in Rhodesia, mentioned that among the wild animals, infection was seen only in kudu (Tragelaphus strepsiceros) and test killings of buffalo in contact revealed no signs of infection.

Apart from direct clinical observation there are two methods by which the occurrence of infection in wild life can be studied. Firstly a search may be made for antibody in the sera of animals, and secondly attempts can be made to isolate virus from both clinically affected and normal animals. The development of sophisticated techniques for the immobilisation of wild animals has now made possible, in suitable areas, the sampling of comparatively large numbers of a free living population.

Part II of this thesis describes the use of these techniques in studies of the African buffalo in which it is shown to be a maintenance host of FMDV. The results are described under six main headings:-

Chapter 10 - The isolation of virus
Chapter 11 - Detailed studies of virus incidence and persistence in a wild buffalo population
Chapter 12 - The antigenic characterisation of buffalo virus isolates and their relationship to cattle strains
Chapter 13 - The properties of buffalo virus strains in other systems
Chapter 14 - The persistence and transmission of virus in captive buffalo
Chapter 15 - The occurrence of clinical FMD in buffalo.

CHAPTER 10 - THE ISOLATION OF VIRUS

The origin of the 1968 Type SAT1 virus FMD outbreak in cattle in the Satau area of the Chobe River District of northern Botswana (Figure 5, Page 86) was not determined. No FMD was present elsewhere in Botswana nor had neighbouring territories reported disease in the adjoining areas. The last recorded outbreak in the district had been in 1950, also due to an SAT1 virus strain. Surveys for carrier virus, in 1968, among unaffected herds outside the immediately affected area, failed to reveal undetected infection in cattle. Contact between domestic stock and wild life is generally indirect and there was reported contact of some of the infected herds with buffalo in November 1967, six weeks before the confirmation of disease in the cattle. An attempt was therefore made in November 1968 to isolate FMDV from buffalo shot in control operations in the area using methods developed for the detection of carrier virus in cattle. Eight buffalo were sampled and Table 21 shows the result of virus isolation attempts from pharyngeal scrapings and of testing of their sera for SN antibody. Several buffalo had significant antibody titres to more than one of the three virus types (SAT1, 2 and 3) previously recorded in southern Africa. Two animals numbers 6 and 9, had titres against types O and A viruses, types not previously recorded in southern Africa. Their significance is not known but as these 2 animals also had higher titres to all three SAT virus types, they may have been cross-reactions.

The sampled buffalo were apparently clinically healthy before death, and examination of mouths and feet revealed no evidence of present or past lesions of FMD. No sign of disease was seen in their herds of origin nor in herds of other susceptible species, e.g. impala, with which they had been in contact. FMDV was isolated from two buffalo; one strain was type SAT1, the type responsible for the localised outbreak in cattle eleven months earlier. Astonishingly, the other was type SAT2, a virus type not previously identified in Botswana since regular typing of outbreaks was started in

TABLE 21. Virus isolation from and serum antibody assay of buffalo shot in Botswana in 1968

Animal Number	Virus Isolated	Tubes/10 Positive	Reciprocal serum antibody titres SAT1	SAT2	SAT3	O	A
1			128	64	45	≤6	≤6
2			45	8	16	"	"
3			11	22	11	"	"
4	SAT1	2	178	32	11	"	≤6
5			64	22	22	"	"
6			355	128	178	22	45
7	SAT2	6	45	90	8	≤6	≤6
9			256	256	256	11	90

1948. Representative groups of cattle sera collected during the previous five years from different parts of Botswana were tested against type SAT2 virus with negative results, thus no evidence was revealed of undetected infection with this type in domestic stock in the country during this period.

In the southeast of Rhodesia, during the same year, colleagues, using similar techniques, sampled groups of buffalo shot on two separate occasions on Hippo Valley Estates. Table 22 shows the results of the samples sent to this institute from the first group of ten buffalo. There was no clinical FMD in domestic stock during 1968. The last outbreak of FMD in the area had been due to type SAT2 virus in August 1967, and, at the time of sampling the buffalo, SAT2 virus was still being recovered from O/P samples from previously infected cattle. The isolation of type SAT1 virus was therefore surprising, particularly as type SAT1 virus had not been recorded in cattle in the area since 1962.

Table 23 shows the result of a second sampling later in the year. Both types SAT1 and SAT3 virus were isolated from this small group. Type SAT3 infection had not been recorded in the country for 15 years.

Clearly FMDV can survive for considerable periods in populations of buffalo with a low probability of transference to domestic stock.

Although high SN antibody titres to type SAT2 were demonstrated in some of the buffalo in Rhodesia (Tables 22 and 23), no type SAT2 virus was isolated. The numbers of animals sampled were small but the results did not suggest implication of the buffalo in the initiation of type SAT2 infection in cattle the previous year. However, a year later, in 1969, two out of five buffalo sampled in the same area were harbouring type SAT2 virus, while one of the remaining three was harbouring type SAT1 virus.

TABLE 22. Virus isolation from and SN antibody titres from first group of buffalo shot in Rhodesia

Animal Number	Virus Isolated	Tubes /10 Positive	Reciprocal serum antibody titre SAT1	SAT2	SAT3	O	A
25			≤6	-	11	≤6	≤6
26			"	22	45	"	"
27			"	128	64	"	"
28			"	512	16	"	"
29			"	90	64	"	"
30			"	64	32	"	"
31			128	64	90	"	"
32	SAT1	2	≤6	256	64	"	"
33			"	90	16	"	"
Bull calf			"	708	32	"	"

TABLE 23. Virus isolations and SN antibody titres from second group of Rhodesian buffalo

Animal Number	Virus Isolated	Tubes /10 Positive	Reciprocal serum antibody titre SAT1	SAT2	SAT3	O	A
1			45	32	355	8	≤6
2			64	-	355	≤6	"
3	SAT3	3/10	128	32	45	16	8
4			45	64	90	8	≤6
5			45	8	64	≤6	"
6			32	22	≤6	"	45
7	SAT1	1/10	45	355	22	"	≤6

Later in 1969, all of four buffalo sampled in the Wankie Game Reserve in north western Rhodesia were carrying type SAT1 virus. The last outbreak of FMD in cattle in this area, however, had been due to a type SAT2 virus in 1966. Again in 1969, virus was isolated from two of three buffalo sampled at Mujiri in the Zambesi Valley. One was type SAT1 virus and the other type SAT3. Due to tsetse fly, however, there is little chance of contact between buffalo and cattle in this area of Rhodesia.

Similarly in South Africa in 1970, types SAT2 and SAT3 virus strains were isolated from pharyngeal scrapings from buffalo shot during culling operations in the Kruger National Park; and type SAT3 virus was isolated from 1 of 8 clinically normal buffalo captured for experimental purposes. While clinical disease due to type SAT2 virus had been confirmed in impala in the Park, and in cattle on bordering farms in 1968 and 1969, infection due to type SAT3 virus had not been recorded in the Republic since 1959, eleven years previously.

Although high antibody titres to one or other virus type were recorded in the Rhodesian buffalo from which virus was isolated at Hippo Valley (Tables 22 and 23), the highest serum antibody titre did not necessarily correspond with the virus type isolated from the pharynx. Exceptional care was taken both in the field and laboratory to ensure correct identification of O/P and serum samples and no explanation for this could be given. It is emphasised that no signs of present or recent FMD were seen in any of the buffalo sampled, in their herds of origin or in herds of animals with which they were in contact. During the sampling of animals in Botswana and Rhodesia in 1968, no outbreaks of disease were reported in either country.

CHAPTER 11 - DETAILED STUDIES OF VIRUS PERSISTENCE IN WILD BUFFALO POPULATIONS

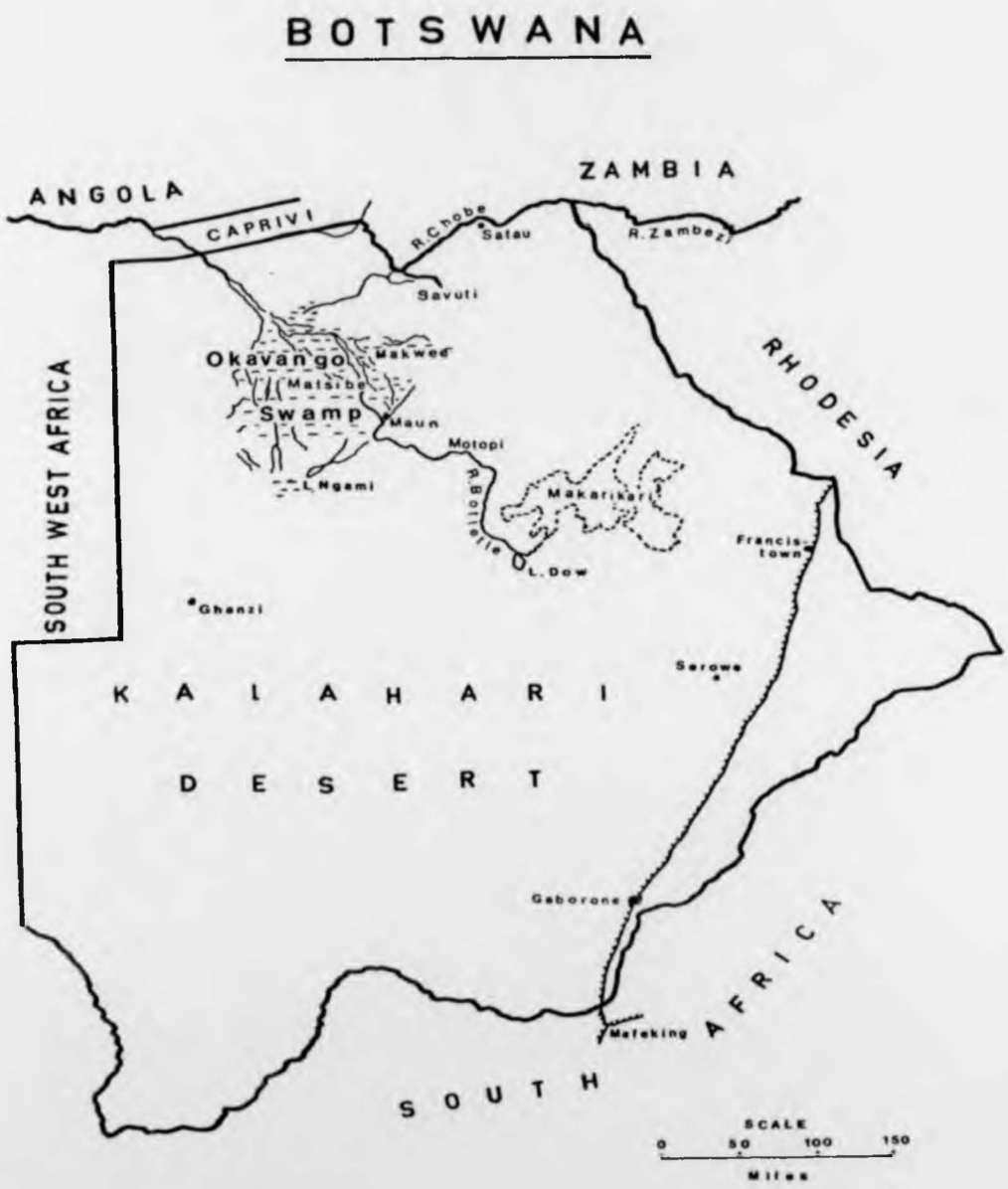
11.1 Botswana

In the initial study, buffalo were sampled in an area within the western boundary of the Chobe National Park where the Savuti Channel flows into a swamp on the borders of the Mababe Depression in northern Botswana, (approx. 18°30'S, 23°50'E) (Figure 5). Sampling took place 18 months after the last outbreak of FMD in domestic stock in Botswana, which occurred at Satau about 60 miles to the north.

The dry season was selected when water-drinking species of game, including buffalo, were concentrated around permanent water. The buffalo population in the area was estimated at approximately 3,000 head, individual herds varying from 10 to 300 animals. It is thought that at least 9 separate herds were sampled but selection was limited by difficulties of terrain and making contact. Following disturbance, herds often fragmented, detached animals sometimes remaining as smaller groups and sometimes joining other herds. When a large population of free-living buffalo is centred on permanent water there is probably considerable intermingling of animals from different groups, and the sample taken was therefore probably representative of the population as a whole. Buffalo may travel considerable distances and herds may disperse over wide areas with the onset of the rains. Their exact movements and degree of contact with domestic stock is however unknown.

Other species abounded in the area and were in close and frequent contact with the buffalo. Predominant were impala (*Aepyceros melampus*); tsessebe (*Damaliscus lunatum*); wildebeeste (*Connochaetes taurinus*); warthog (*Phacochoerus aethiopicus*); giraffe (*Giraffa camelopardalis*); hippopotamus (*Hippopotamus amphibius*); and elephant (*Loxodonta africana*).

Figure 5


BOTSWANA
ANGOLA
ZAMBIA
CAPRIVI
R. Chobe
Satau
R. Zambezi
Savuti
Okavango
Makwee
Matsebe
Swamp
Maun
Motopi
L. Ngami
R. Boteti
Makarikari
L. Dow
Francis-town
SOUTH WEST AFRICA
RHODESIA
Ghanzi
Serowe
K A L A H A R I
D E S E R T
Gaborone
Mafeking
SOUTH AFRICA
SCALE
0 50 100 150
Miles

62 buffalo of all ages, representing an approximate 2 per cent of the population, were sampled over a three-week period. Of this total, 52 animals were immobilised, captured and released after sampling. For comparison with previous work (Chap. 10), ten buffalo were shot and pharyngeal scrapings were taken in place of O/P samples.

FMDV was isolated from 35 of the 62 animals; 34 virus isolations were made from the 52 animals captured and sampled while alive, and 1 from the 10 animals shot and sampled after death. Strains of all three SAT virus types were isolated. More than one virus type was isolated from seven of the animals (Table 24).

No evidence of disease or lameness was observed in the herds of buffalo seen or sampled. Clinical examination of the animals sampled, especially of the mouth and feet, failed to reveal signs of current or past infection with FMD. In many samples virus was, however, present in surprisingly high titre (up to $10^{3.9}$ $tcid_{50}$ per ml. of sample) suggesting recent infection with more than one virus type.

Indeed viraemia with type SAT1 virus in the absence of clinical signs was demonstrated in one yearling buffalo (No. 10), in addition to isolation of virus from its pharynx. Supporting evidence that this was a recent infection was provided by the absence of type SAT1 antibodies in this animal (Table 25).

Strains of the three SAT type viruses were isolated in roughly equal proportions. While it was difficult to be certain of individual herd identities there appeared to be no correlation between virus types and herds of origin sampled.

Table 24 shows the age and sex of each animal sampled with the virus type and titre of each positive sample. There was no correlation between sex and carrier state. Virus was isolated from 14 of the 24 males sampled and from 21 of the

TABLE 24

AGE AND SEX OF BUFFALO IN RELATION TO VIRUS ISOLATED (after Hedger, 1972)

Age less than 1 year		Age 1-2 years				Age 2-3 years				Age 3-4 years				Age 4-6 years				Age 7 yrs or more			
Animal No.	Sex	An. No.	Sex	Virus type (SAT)	Virus titre	An. No.	Sex	Virus type (SAT)	Virus titre	An. No.	Sex	Virus type (SAT)	Virus titre	An. No.	Sex	Virus type (SAT)	Virus titre	An. No.	Sex	Virus type (SAT)	Virus titre
5	♀	10*	♂	1	>1.5 **	17	♂	2	3.38	8	♂	3	<1.0	1	♀	3	<1.0	3	♂	1	<1.0
7	♀	15	♀	2	2.69	29	♀			11	♀	1,2	3.89	2	♀	2	1.38	6	♂	3	<1.0
14	♂	22	♀	1	<1.0	32	♂	3,2	1.17	12	♀	1	<1.0	4	♀			9	♀	3	<1.0
27	♀	24	♂	3,1,2	1.85	38	♀	1	3.88	13	♀	1	<1.0	19	♀	2	1.0	16	♀		
49	♂	37	♀			39	♀	3,2	2.5	18	♀	3	1.0	21	♀			20	♀	2	<1.0
54	♂	46	♀	3	2.38	40	♀	2,3	2.0	30	♀			26	♀			23	♂	1	<1.0
61	♂	45	♀	3	<1.0	41	♂	3,2	3.5	31	♂	1	<1.0	28	♀			25	♀		
		47	♀			44	♂	3	<1.0	36	♂	1	1.0	48	♂	1	<1.0	33	♀	2,3	<1.0
						52	♀	2	<1.0					50	♀	2	<1.0	34	♀		
														53	♀	1	<1.0	35	♀		
														62	♂			42	♀	1	<1.0
																		43	♂		
																		51	♂		
																		55	♂		
																		56	♀		
																		57	♀		
																		58	♂		
																		59	♂		
																		60	♂	3	<1.0

*Viraemia demonstrated. ** Titre expressed as $\log_{10}$/ml.

38 females. No virus was recovered from young calves, but virus recovery was nearly twice as frequent in animals 1 and 3 years old (84%) as in older animals, (47%); the highest virus titres occurred mainly in the younger animals (Figure 6). All but one of the mixed infections also occurred in younger animals; all three SAT virus types were isolated in each age group and there was no apparent correlation between virus type and age.

Table 25 shows the SN antibody titres of the individual buffalo in the various age groups in relation to their carrier status. The sera were tested against type SAT1 virus strain Rho 5/66, (isolated from cattle with FMD in Rhodesia in 1966), antigenically similar to the strain involved in the most recent SAT1 outbreak in Botswana in 1968; type SAT2 virus strain SWA1/69, (from a recent outbreak in neighbouring South West Africa in 1969) and type SAT3 strain Bec 1/65 (from the last recorded outbreak due to SAT3 virus in Botswana in 1965). The titres demonstrated in the buffalo might have been higher had homologous strains of virus been used in the tests.

The majority of buffalo possessed significant antibody titres to more than one virus type and many had high titres to all three types. High titres to each of the three virus types occurred in each age group and there was no marked difference in the apparent immunity status from group to group, nor was it possible to draw distinctions between different herds by the level or type of their antibody titres.

While the highest neutralising antibody titre correlated with the virus type isolated in some animals (e.g. Nos. 8 and 36) there was no correlation in others (e.g. Nos. 12 and 48). This apparent anomaly had been noticed previously in animals sampled in Rhodesia (Chap. 6).

In previous game serum surveys, antibody titres of 1 in 32 or greater had been regarded as significant (Condy et al, 1968). On this basis all the calves with the exception of No. 7, possessed significant antibody titres to all three virus types.

Fig 6. Age Incidence, Amount of Virus Excretion and Percentage Virus Recovery in 62 Buffalo

Figure 6 90

TABLE 25

BUFFALO RECIPROCAL SERUM ANTIBODY TITRES (after Hedger, 1972)

Animal No.	Age less than 1 year Virus types (SAT) 1	2	3	Animal No.	Age 1-2 years Virus types(SAT) 1	2	3	Animal No.	Age 2-3 years Virus types (SAT) 1	2	3	Animal No.	Age 3-4 years Virus types (SAT) 1	2	3	Animal No.	Age 4-6 years Virus types (SAT) 1	2	3	Animal No.	Age 7 yrs or more Virus types (SAT) 1	2	3
5	64	90	128	10*(1)**	6	11	45	17(2)	128	178	32	8(3)	45	22	178	1(3)	45	22	128	3(1)	355	178	355
7	90	22	64	15(2)	128	355	355	29	22	128	11	11(1,2)	128	355	355	2(2)	32	45	32	6(3)	45	45	355
14	256	355	128	22(1)	45	178	22	32(2,3)	178	355	256	12(1)	32	128	178	4	90	64	64	9(3)	355	90	128
27	90	128	708	24	11	45	6	38(1)	90	6	178	13(1)	90	22	178	19(2)	6	64	16	16	256	355	256
49		N/S		37(1,2,3)	178	90	64	39(2,3)	64	128	90	18(3)	90	355	512	21	178	128	90	20(2)	128	355	64
54	90	90	128	45(3)	64	355	64	40(2,3)	32	45	90	30	32	355	64	26	45	32	178	23(1)	90	45	178
61	64	128	178	46(3)	128	178	6	41(2,3)	6	64	90	31(1)	128	45	178	28	45	256	178	25	64	90	512
				47	90	64	45	44(3)	90	45	90	36(1)	128	90	32	48(1)	45	256	32	33(2,3)	45	128	708
								52(2)	90	256	256					50(2)	708	178	128	34	64	90	64
																53(1)	90	90	128	35	128	22	128
																62	256	90	90	42(1)	178	90	90
																				43	32	178	90
																				51	32	45	90
																				55	256	90	90
																				56	128	355	178
																				57	45	90	32
																				58	22	90	32
																				59	128	178	64
																				60(3)	6	90	45

* Viraemia demonstrated. ** Virus type isolated. N/S = no sample.

Calf No. 7 which was only a few days old, and had a titre of only 1 in 22 to SAT2 virus, nevertheless had significant titres to both types SAT1 and 3. Calf No. 14 was estimated to be approximately two weeks old, Nos. 5 and 7 three to four weeks, and Nos. 54 and 61 four to five months old. There was no sign of infection in any of these calves nor was virus isolated. This antibody was therefore considered to be colostrally transferred maternal antibody.

In the 1 and 2 year old groups, this maternally derived immunity had waned and several young animals were susceptible to one or more virus types. For example, No. 24 was susceptible to types 1 and 3 viruses and No. 10 to types 1 and 2. No. 10 in fact was viraemic and SAT1 virus was isolated from its pharynx confirming its susceptibility. Animal No. 46 had lost antibody to type SAT3 infection and the isolation of virus from its pharynx in the absence of detectable viraemia suggested prodromal infection. Almost all the adult animals (four years or older) had antibody to all three virus types. There was no obvious correlation between the serum antibody titre of an individual and its carrier status, nor was it possible to infer any chronological pattern of disease or infection by serological examination of thevarious age groups.

11.2 Uganda

Following the initial detailed study in Botswana, a similar smaller survey was carried out in 1970 in two ecologically different areas in Uganda where the prevalent FMD virus types in cattle had been O, A and SAT2. A total of 33 buffalo were sampled, 9 in the Mburo Game Reserve south east of Mbarara where cattle had recently been affected with FMD (Type SAT2); and 24 in several areas of the Queen Elizabeth (Q.E.) National Park in southwest Uganda. The buffalo in the Mburo Reserve were shot by Game Department officials in control operations but the animals in the Q.E. Park were immobilised for sampling and later released. The number of buffalo in the Mburo Reserve was not accurately known but the sample probably

represented between 1 and 3 per cent of the population. The 24 animals sampled in the Q.E. Park represented 0.12% of the known population of 20,000 buffalo.

Virus was isolated from 4 of the 9 buffalo from the Mburo Reserve and from 5 of the 24 from the Q.E. Park. Table 26 shows the results of virus isolation attempts and antibody assay of the sera of the 9 Mburo animals and includes foetuses of varying term from three of the cows which were shot. The Game Department selected adult animals for culling but three of the four virus isolations were made in animals under 5 years old. Three animals were carrying type SAT1 virus and only one, type SAT2 virus, the cause of recent outbreaks in cattle in the adjoining ranches. High SN antibody titres to all three SAT type viruses were recorded. Type O antibody was also found in some of the buffalo which also had high titres to the SAT type viruses. In the absence of isolation of type O virus, the significance of these antibodies remains in doubt.

Antibodies to all three SAT virus types were demonstrated in two of the gravid cows (Nos. 5 and 6) and type SAT1 virus was isolated from one of them (No. 6). Virus was not recovered from the foetuses nor were any antibodies demonstrated in their sera. One foetus was judged to be almost full term and the other in the seventh or eighthmonth of gestation. No signs of FMDV were observed in the buffalo sampled or in their herds of origin.

In the Q.E. Park, buffalo were sampled in five areas north of the Kazinga Channel (which separates Lake George and Lake Edward), in one area just south of the channel, and also in the extreme south of the Park near the Congo border. In the Q.E. Park, buffalo populations are relatively static (M Woodford, personal communication) and there is little intermingling of herds. South of the Kazinga Channel, and particularly in the Ishasha area, buffalo are reported as having had little or no contact with cattle in Uganda for many years. They do, however, cross the Ishasha River into

TABLE 26. Virus isolation and serum antibody titres of buffalo shot in the Mburo Game Reserve in Uganda

Animal Number	Age Years	Virus Isolated	Reciprocal serum antibody titre SAT1	SAT2	SAT3	O	A
7	2-3		128	45	≤6	8	≤6
8	2-3	SAT1	≤6	90	≤6	≤6	≤6
1	3-4	SAT2	≤6	90	≤6	≤6	≤6
6	4-5	SAT1	178	128	90	64	≤6
	Foetus		≤6	≤6	≤6	≤6	≤6
5	6		178	64	64	16	11
	Foetus		≤6	≤6	≤6	≤6	≤6
9	6		8	≤6	≤6	≤6	≤6
	Foetus		≤6	≤6	≤6	≤6	≤6
4	8	SAT1	128	32	178	11	≤6
2	8		128	90	90	22	≤6
3	12		64	64	45	32	11

the Parc National Albert in the Congo but is believed unlikely that they would have had contact with cattle there.

Table 27 shows the results from buffalo in the Q.E. Park. All three SAT type viruses were isolated but type SAT3 virus was isolated only in the Ishasha area, south of the Kazinga Channel. No type SAT3 virus was isolated from buffalo captured north of the Channel, nor was there serological evidence of infection with that virus type. No clinical evidence of past or present FMD was seen in sampled buffalo, their herds of origin or other wild life seen during the sampling. Reports were received of lame buffalo in the Ankole Channel area two years previously, but confirmation of FMD was lacking.

Over a period of several years, prevalent virus types associated with outbreaks of FMD in domestic stock in Uganda have been types O, A and SAT2. Types SAT1, 2 and 3 were isolated from the buffalo but no conclusive evidence of their infection with the exotic European virus types was demonstrated.

The significance of the type O SN antibodies in some of the buffalo both at Mburo and in the Q.E. Park is doubtful. In all cases except one (No. 31) higher titres to all three SAT virus types were recorded, and in No. 31 which also had a titre of $^{1}/_{64}$ to type SAT1 virus the titre to type O was only $^{1}/_{22}$. In previous random surveys (Condy et al, 1969) titres below $^{1}/_{32}$ have been regarded as possibly non-specific in the absence of other evidence of infection.

The most recent recorded outbreak of disease due to type SAT3 virus was in Botswana 4½ years previously and this virus type had not been previously identified further north than Rhodesia.

11.3 Continuing study

11.3.1 Buffalo

The results so far presented show that whenever or wherever sufficient numbers of clinically normal free-living African

TABLE 27

Virus Isolations and Serum Antibody titres of Buffalo captured in the Queen Elizabeth National Park, Uganda

Area	Number	Age Years	Virus Isolated	Reciprocal Serum Antibody Titre SAT 1	SAT 2	SAT 3	O	A
North of Kazinga Channel								
Nyamagasani	10	6		90	40	<6	8	<6
	11	8-9		90	512	<6	11	<6
Kayonja	29	1		<6	<6	<6	<6	<6
	12	2½	SAT 2	32	256	8	11	<6
	13	2½		<6	<6	<6	<6	<6
	16	8		8	45	<6	<6	<6
	15	(calf of 16) 6 weeks		32	45	<6	<6	<6
	14	10		64	90	<6	<6	<6
Kasenye	18	1½		16	<6	<6	<6	<6
	17	11-12		45	90	8	<6	<6
Mweya Peninsular	25	4		8	45	8	<6	<6
	33*	5½		8	11	<6	<6	<6
	32*	6½		22	8	8	<6	<6
	31	7		64	<6	<6	22	<6
South of Kazinga Channel								
Ankole Channel	21	1½-2	SAT 1	45	64	128	16	<6
	19	2		<6	<6	8	<6	<6
	24	3	SAT 2	90	64	45	<6	<6
	23	9		64	64	256	22	<6
	24**	10 days		16	<6	<6	<6	<6
	20	9		90	<6	<6	<6	<6
Ishasha	28	2½	SAT 3	32	64	45	<6	<6
	27	4	SAT 3	22	45	64	11	<6
	26	10		90	64	45	<6	<6

* Captive Buffalo held separate from the main population
** Believed to be calf of No. 23

buffalo have been sampled, FMDV has been isolated and significant SN antibody titres to FMDV demonstrated. The buffalo is thus probably a maintenance host of FMDV in Africa. Criticism might be levelled at this conclusion because each survey was of a particular population at a particular time. The survey in Botswana was therefore continued and widened to include two further separate buffalo populations in the Okavango in Ngamiland. Using more sophisticated techniques, including helicopters for locating and darting the animals, a total of two hundred buffalo were sampled in 1972, and again in 1973 in three different areas, including the Savuti where the first large scale sampling took place in 1969 (Figure 5, Page 86). The new areas were Makwee and Matsibe in the Okavango delta. Both studies were carried out at the same time of year, in the dry season, when water drinking species were concentrated around permanent water.

Table 28 shows the percentage virus isolation rates from all areas from year to year. These figures are remarkably similar and show no indication of a change in the virus status of the buffalo in spite of the absence of observed or reported disease in either domestic or wild animals during this period.

TABLE 28. The overall percentage of FMDV isolation in Botswana buffalo in different years

	Total Sampled	Virus Isolated	Percentage
1969	69	35	56.5
1972	201	117	58.5
1973	202	123	61.5

Table 29 shows that all three SAT virus types were isolated in each area and that the percentage virus recoveries from area to area and from year to year in each area were similar. Where multiple virus type isolations were made from the same animal, only the predominant virus type has been included in these figures.

TABLE 29. FMDV isolations from 3 separate buffalo populations in Botswana 1969 - 1973

Area	Number Sampled	SAT1	SAT2	SAT3	% Virus Positive
Savuti 1969	62	13	9	13	57
1972	115	38	11	13	54
1973	76	19	24	11	71
Matsibe 1972	43	14	3	6	54
1973	57	15	9	2	47
Makwe 1972	43	15	10	7	74
1973	69	18	16	9	61

There was no great variation in the amounts of virus being excreted from area to area or from year to year. In 1969, 60% of the virus titres recorded were less than $10^{1.0}$/ml and titres among the remainder vared up to $10^{3.9}$/ml; in 1972, 38% were less than $10^{1.0}$/ml, remaining titres varying up to $10^{3.2}$/ml; and in 1973, 46% were lower than $10^{1.0}$/ml with titres of the remainder varying up to $10^{4.2}$/ml. The lower titres are similar to those of carrier cattle at varying stages after infection, and the higher titres to those of similar samples from cattle in the prodromal stage of disease or soon after infection. As in the earlier survey, virus

recovery was considerably more frequent in the younger age groups. Figure 7 shows the percentage of animals from which virus was isolated in the different age groups, during the two successive samplings of 201 and 202 buffalo respectively. The curves are very similar to that obtained in 1969 (Figure 6) the highest virus titres occurring mainly in animals between 1 and 4 years old.

In contradistinction to the results obtained in 1969 virus was isolated from 50% of the calves under 1 year old in 1972 and 1973. However, no virus was isolated from animals less than 5 months old.

The occurrence of multiple infections, which had been demonstrated in the 1969 survey, was confirmed in 1973 when more than one virus type was isolated from five of the buffalo. Two animals were carrying types SAT1 and 2 viruses, two of them types SAT1 and 3 viruses and one was carrying both SAT2 and 3 virus types.

Although the small numbers make interpretation difficult, there appears to have been an increase in the overall number of type SAT2 virus isolations made in 1973 particularly in the Savuti area (Table 30); the increase was twofold overall and threefold in the Savuti herds.

All animals were ear tagged at the time of sampling and numbered collars were fitted to a limited number. Some which retained their collars were resampled when opportunity presented. In addition, in 1973, three animals were recaptured after 8 days and further samples taken. Table 31 shows that in sampled buffalo, as with cattle, the recovery of virus from O/P samples from individual animals could be intermittent. It was unfortunate that the three animals sampled on 2.10.1973 were carrying only minimal amounts of virus. The considerable stress imposed on these buffalo by their being darted twice from the air, immobilised and captured, might have been thought to favour the excretion of virus. On the second sampling, however, virus in minimal quantity, was recovered from only one of them.

Fig 7. Age Incidence and Percentage Virus Recovery in Buffalo in Two Successive Years

TABLE 30. Percentage virus isolations of the total number of buffalo sampled (Botswana)

1) All areas combined:-

	SAT1	SAT2	SAT3
1972	31%	12%	13%
1973	26%	24%	11%

2) Savuti:-

1969	21%	15%	21%
1972	33%	10%	11%
1973	25%	32%	14%

TABLE 31. Results of tests on buffalo sampled on successive occasions

Animal Number	Virus Isolated	Antibody Titre SAT1	SAT2	SAT3	Virus Isolated	Antibody Titre SAT1	SAT2	SAT3
	1969				1972			
29		22	128	11	SAT1	1024	64	708
	1972				1973			
43	SAT1	256	90	45	SAT1	64	22	64
48	SAT1	64	355	11	SAT1	90	90	22
127	SAT1	708	32	90	SAT1	22	45	178
25		178	90	64	SAT2	45	32	178
45		128	64	178	SAT2	45	32	90
151		128	178	90	SAT2	45	32	90
	2.10.1973				10.10.1973			
119	SAT2	45	45	90		128	64	90
142	SAT2	45	32	90		90	64	90
152	SAT2	128	45	256	SAT2	178	64	178

Type SAT1 virus was again recovered in 1973 from three animals shown in 1972 to be SAT1 virus carriers. Antibody titres to this virus type were however waning.

No virus was isolated from animal No. 29 in 1969 and SN antibody levels then suggested this animal had probably been infected only with type SAT2 virus. Three years later however, SAT1 virus was isolated from this animal and a very high antibody titre confirmed a probably fairly recent infection. A high SAT3 SN antibody titre suggested that this animal had probably been infected also with type SAT3 virus in the intervening period.

Under the conditions in which animals were darted and immobilised, it was not easy to capture both the dam and the calf, nor was it possible on some occasions to be certain of the relationship between a calf and the cow which it followed during the stress of capture. In a few instances however, cows were identified with their calves and were captured together. A comparison of the results from each cow and calf is given in Table 32. Estimated ages of the calves varied from 5 - 9 months.

Any transferred colostral immunity had waned in the calves of cows Nos. 60 and 73 but as cow No. 73 was carrying SAT1 virus her calf's antibody level was probably still high enough to protect it. The SAT1 antibody titre of the calf of No. 137 which is higher than that of its dam suggests previous infection with this virus type, but it is susceptible to ype SAT2 virus.

No virus was isolated from cows Nos. 113, 179 and 167, yet virus in high titre was isolated from their calves. The high virus titres combined with the moderate antibody titres suggest recent infection in these calves, probably not necessarily from their own dams, but from other animals in the herd.

TABLE 32. Buffalo cows and their calves: a comparison of results of virus isolations and antibody titres

Animal Number	COWS Antibody Titre SAT1	SAT2	SAT3	Virus Isolation	CALVES Antibody Titre SAT1	SAT2	SAT3	Virus Isolation	Estimated age (Months)
60	45	45	32		8	8	≤6		6 - 9
73	≥355	128	90	SAT1	22	22	≤6		5 - 6
137	32	90	256		178	8	32		6
113	256	512	512		64	45	64	SAT1 (3.2)*	9
179	90	178	178		32	22	22	SAT1 (3.0)	±6
167	11	64	128		64	32	90	SAT2 (2.5)	5 - 6
127	708	32	90	SAT1	90	355	90	SAT1 (+)	=6

* Titre $\log_{10}$/ml

\+ Not titrated

11.3.2 Observations in other species

During the periods of sampling, no signs of sickness or lameness were seen in the many thousand animals of other wild species, known to be susceptible to FMD, which were in contact with the buffalo. The degree of contact varied but at times was close when different species were sharing a small waterhole.

During the sampling of buffalo in Botswana in 1969, two impala, 3 tsessebe and 9 warthog were sampled and examined. No virus was detected and no signs of present or past lesions were observed in the mouths or on the feet. No antibodies to SAT1, SAT2 or SAT3 virus types were demonstrated in their sera. In 1972, a further 51 wild animals, including impala, tsessebe, warthog, wildebeeste, lechwe (Kobus leche), reedbuck (Redunca arundinum) and two kudu, which were in contact with the buffalo were sampled with similar negative results.

During the 1973 buffalo sampling, a further 70 animals including larger numbers of kudu and sable antelope (Hippotragus niger), species not frequently sampled previously, were similarly examined.

No virus was isolated nor were clinical signs of disease seen. SN antibody titres to FMDV were demonstrated however in the sera of three kudu and one sable antelope.

No FMD has occurred in domestic stock in Botswana since January 1968. In neighbouring territories, outbreaks due to SAT2 virus occurred in Ovamboland in South West Africa, several hundred miles to the west of the sampling areas, in 1969 and 1970; in the Ngoma area of the Caprivi Strip in 1971; and at Kazangula, 250 miles to the east in Rhodesia, in 1970. It is unlikely that the sampled buffalo populations had contact with cattle in any of these areas.

CHAPTER 12 - ANTIGENIC STUDIES ON BUFFALO VIRUS ISOLATES

Strains of virus perpetuated in buffalo may differ from those in cattle or may undergo antigenic change while in buffalo. A knowledge of the strains of virus persisting in buffalo populations and their relationship to vaccine strains is also important when considering the degree of risk to vaccinated cattle which may come in contact with buffalo. Therefore limited studies have been made of selected strains of virus isolated from buffalo in Botswana and Uganda.

12.1 Botswana

A specific antiserum was produced in guinea pigs to a strain of each virus type isolated from buffalo in 1969, and these strains were compared in cross CF tests with the strains most recently occurring in cattle in the area and incorporated in current vaccines.

The selected buffalo type SAT1 strain was shown to be widely different from the vaccine strain Rho 5/66 with a CFP of 0.034, equivalent to an "R" value of 18%. Rho 5/66, isolated from cattle in south eastern Rhodesia in 1966, had been shown previously to be similar to the strain of virus causing a localised outbreak of disease in cattle in northern Botswana in 1968 (Chap. 4.2.2).

The selected buffalo SAT2 strain was similar to the subtype group of strains occurring in cattle in South Africa, Mosambique and Swaziland in 1969, and in Rhodesia 1970. Its CFP and 'R' value with the reference strain SA3/69 were 0.568 and 75% respectively. It differed however from the vaccine strain isolated from cattle in neighbouring South West Africa in 1969 (CFP 0.228, 'R' = 48%). Although SAT2 virus infection had been recorded in South West Africa, Angola, Zambia, Rhodesia and in the eastern Transvaal in South Africa, it had not been recorded previously in domestic stock in Botswana.

The selected SAT3 strain was shown to be different from, though related to, the SAT3 strain (Bec 1/65) isolated from cattle in Botswana in 1965 and since used as the vaccine strain. The CFP of the buffalo strain to Bec 1/65 was 0.254, equivalent to an 'R' value of 50%. No outbreaks of disease due to SAT3 virus had been recorded in domestic stock since 1966 in Southern African or elsewhere.

12.2 Uganda

Results of tests carried out by the World Reference Laboratory at Pirbright suggest that the strains of type SAT1 virus isolated from the Mburo Game Reserve and the Q.E. Park are probably similar and that these strains are probably more closely related to a strain of virus isolated from buffalo in Botswana in 1969 than to strains isolated from cattle in East Africa (A J Forman, personal communication).

Results of similar preliminary tests suggest the type SAT2 virus strains isolated from buffalo in both Mburo and in the Q.E. Park are probably related to strains previously isolated from cattle in Kenya and Uganda (Figure 8). Type SAT3 virus had not previously been isolated in East Africa and a specific antiserum was produced in guinea pigs against one of the buffalo strains of virus from the Q.E. Park. This strain was then tested against the subtype reference strains of type SAT3 virus in cross CF tests and shown to be a new subtype (Figure 9).

Figure 8. Comparison of Uganda Buffalo SAT2 virus strains with East African Vaccine Strains isolated from cattle

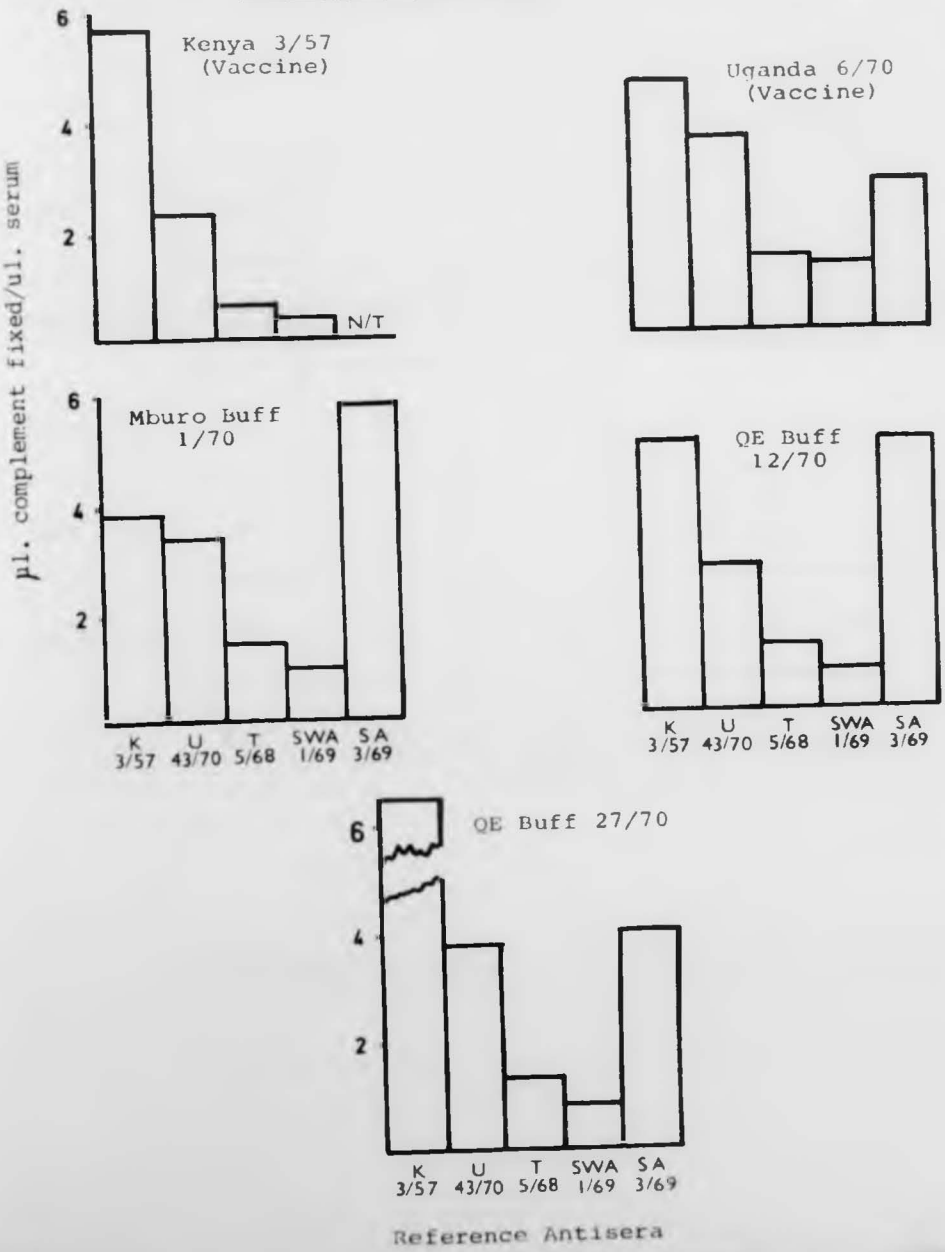


Figure 9. The relationship of the Uganda buffalo type SAT3 virus strain (Ug. 27/70) with the reference strains from Southern Africa

	RV7	Bec 20/26	SA 57/59	Bec 1/65	Ug 27/70
RV7	100*				
Bec 20/61	31	100			
SA 57/59	46	19	100		
Bec 1/65	62	31	58	100	
Ug 27/70	23	18	21	29	100

* Percentage "R" value

CHAPTER 13 - THE BEHAVIOUR OF BUFFALO VIRUS STRAINS IN OTHER SYSTEMS

The possibility that strains of virus isolated from wild buffalo in the absence of signs of disease might be modified or attenuated for other hosts or cell cultures, particularly cattle, was considered. The pathogenicity of buffalo strains was however indistinguishable from that of other unmodified field strains of FMDV in tissue cultures of calf thyroid, IBRS-2 and BHK cells and in unweaned baby mice. Selected strains of types SAT1 and SAT3 virus adapted readily to guinea pigs, producing generalization in 48 hours after 3 passages. A third strain, type SAT2, was highly myotropic in guinea pigs, causing 100 per cent mortality from myocarditis on the first passage. This necessitated the preparation and use of a homologous vaccine for the production of specific antiserum for cross CF tests.

As buffalo viruses might have become modified for domestic animals, a strain of each type was inoculated intradermolingually into two 2-year-old Devon steers. The inoculum was the original O/P sample. One susceptible uninoculated animal was left in contact with each pair of inoculated animals. Vesicles typical of FMD were evident at the sites of inoculation in all animals 18 hours after inoculation and generalization involving severe lesions in 3 or 4 feet occurred in all animals within 72 hours. With each virus strain, contact infection took place, with production of classical FMD. Incubation periods varied from 4 to 7 days. At the termination of the experiment, 21 days after infection. 2 animals remained carriers of SAT1 virus, and 2 carriers of SAT3 virus. Virus was no longer recovered from the animals infected with SAT2 virus.

The differences in the susceptibility of the different systems to the unmodified buffalo virus strains examined (Table 33) were of the same order as those reported by Snowdon (1966) for FMDV strains isolated from cattle.

Table 33. Titration of Buffalo O/P samples in different systems

Titration System	Animal Number & Virus Type		
	B11/69 (SAT1)	B17/69 (SAT2)	B41/69 (SAT3)
Cattle (Intradermolingual)	3.3*	2.9	3.1
Primary BTY Cells	3.5	3.2	3.5
BHK Cells	1.8	=1.0	1.3
Unweaned Mice (Intraperitoneal)	=1.5	NVR**	NVR

* Titres $\log_{10}$/ml of sample

** No virus recovered

CHAPTER 14 - VIRUS PERSISTENCE IN CAPTIVE BUFFALO AND ITS NON-TRANSFERENCE TO DOMESTIC CATTLE

14.1 The duration of the carrier state and persistence of antibody titres

The repeated sampling of populations of wild buffalo in Botswana over a number of years has shown that FMDV can survive in free living herds for very considerable periods. Little was known of the duration of the carrier status in individual buffalo.

In South Africa, 6 months after natural infection of a group of eight young captive buffalo with a type SAT2 virus strain, all the animals were found to be carriers (Young et al, 1972). Amounts of virus present in the O/P samples varied from $10^{2.0}$ to $10^{3.5}$ $tcid_{50}$/ml. (Table 36 Page 121). During the continuing study of free living buffalo herds in Botswana, three animals found to be carriers in 1972 were still carrying the same virus type when recaptured a year later (Table 31 Page 102).

Opportunity for further study of the duration of the carrier state and persistence of SN antibody titres occurred in Rhodesia. Ten buffalo were captured as young animals, hand reared and kept in captivitiy in a remote area, 20 miles from the nearest domestic animals, and where all wild ruminants had been eradicated, during anti-tsetse fly operations. These experiments were carried out jointly with Dr J B Condy of the Veterinary Research Laboratory, Salisbury, Rhodesia.

The buffalo were kept under observation for 2½ years and sera and O/P samples were taken at intervals. During this period three calves were born. Table 34 shows that individual buffalo may carry type SAT1 and SAT2 virus for as long as 2½ years (Nos. 50, 53 and 85).

The ability of buffalo to carry more than one type of virus at the same time and over an extended period was also demonstrated, confirming earlier observations (Chap. 11.1).

Table 34. (From Condy and Hedger, 1974)

Virus Isolations and Reciprocal Antibody Titres of the Carrier Buffalo throughout the Course of the Experiment

Animal No.		Date 28.3.70	26.4.70	29.5.70	3.7.70	7.9.70	16.11.70	1.5.71	8.6.71 (50) 27.7.71 (52)	14.7.71	29.10.71	3.3.72	29.4.72	3.8.72
50	Virus isolated	2*	1†	2	1	1	Neg	Neg			Neg			1
	SAT 1 antibody	90	45	32	64	90	128	256	1400	256	90	90	128	128
	SAT 2 antibody	45	45	16	16	32	22	8	⩽6	⩽6	⩽6	⩽6	⩽6	⩽6
528	Calf of No. 50										Neg			Neg
								born	1400	1400	64	8	8	8
								6.6.71	22	16	⩽6	⩽6	⩽6	⩽6
52		1	1	1	1	1	1	Neg			Neg			Neg
		90	128	178	178	355	256	355	1400	512	355	128		16
		⩽6	⩽6	⩽6	⩽6	⩽6	⩽6	45	32	11	45	11		⩽6
527	Calf of No. 52										Neg			1
								born	1400	1400	64	8	⩽6	45
								25.7.71	45	11	⩽6	⩽6	⩽6	8
53		1	1	1	Neg	1	1	Neg			Neg			1
		45	45	90	178	256	512	355			1400	256		355
		22	45	45	32	45	45	64			128	64		64
529	Calf of No. 53													Neg
											born		90	22
											31.12.71		22	⩽6
84			Intro-	Neg	Neg		Neg	2			2			2
			duced		⩽6	⩽6	⩽6	⩽6			⩽6	90	11	128
			24.9.70		⩽6		⩽6	64			512	90	90	22
85		1	1+2	1	1	1	Neg	Neg			1			2
		178	256	178	128	128		355			1024	11	256	355
		32	45	45	32	45	64	32			178	90	64	64
86		1	1	Neg	Neg	1	Neg	Neg			1+2			Neg
		256	178	64	256	256		128			90	90	90	11
		64	32	32	32	32		16			16	22	22	45
		↑ 1 steer introduced						↑ 6 cattle introduced Feb. 1971						

* Virus type SAT 2
† Virus type SAT 1

High SN antibody titres also persisted over periods in excess of 2½ years without apparent reinfection.

14.2 Transmission of virus

14.2.1 Buffalo

At no time during the course of this experiment in Rhodesia were clinical signs of FMD seen in the buffalo. However, buffalo to buffalo transmission did occur. Calf No. 527, (Table 34) previously virus negative, became infected with type SAT1 virus at approximately 1 year old, when its titre of conferred maternal antibody had waned. Although no clinical disease was observed, the isolation of virus at a titre of $10^{2.3}$ $tcid_{50}$/ml from the pharynx, combined with a rising antibody titre, provided evidence of recent infection. Similarly, buffalo No. 84, negative for virus and antibody on three successive samplings after introduction into the herd, showed evidence of type SAT2 infection by a rising SN antibody titre and the continued isolation of that virus type from its pharynx. The development of antibody to type SAT1 virus in this animal in significant amounts suggested infection with this virus type also, but virus was not isolated.

Within 48 hours of birth, the SN antibody titres of the calves were similar to those of their dams in the two cases recorded. Detectable SN antibody persisted in the calves for from 3 to 7 months.

Two cows shown to be carriers of type SAT1 virus during pregnancy were negative for FMDV at parturition, but their SN antibody titres at this time were the highest recorded. Subsequent to parturition their antibody titres dropped and virus was again recovered from two of them.

14.2.2 Cattle

Six unvaccinated cattle from a FMD free area, shown to be susceptible to FMD by repeated failures to demonstrate SN

antibodies or to isolate virus from O/P samples, were kept for 18 months in close contact with 10 buffalo, five of which had been shown to be carriers. One steer was kept with the buffalo for 30 months (Table 34). During this period, the carrier buffalo continued to harbour virus, and buffalo to buffalo transmission occurred (Chap. 14.2).

During the day, the buffalo and cattle were herded together, being permitted to graze at will and watering from a pool in a stream. At night, due to the bullying nature of one buffalo, the entire group could not be kept in the same pen, so buffalo and cattle were separated by a horizontal pole partition, with 12 inch spaces between poles allowing muzzle to muzzle contact.

It has been suggested that some FMD outbreaks have originated during game culling operations in which the viscera of (infected) wild animals were left to contaminate the cattle grazing (Condy & Hedger, 1974). Therefore, on two separate occasions during this study, 2 buffalo cows shown to be Type SAT1 virus carriers were slaughtered at the entrance to the holding pens, where they were cut up and eviscerated as if by hunters.

At no time during the course of the experiment were clinical signs of FMD seen in the susceptible cattle, nor were FMD SN antibodies demonstrated, or FMDV recovered from O/P samples.

14.3 Virus maintenance and size of buffalo population

The maintenance of a virus depends on a number of factors including the size of the available host population. For an infection to persist in a restricted area there must be a continuous replenishment of the population of susceptible hosts at a rate compatible with the frequency of successful transmission required for maintenance (Smith, C.E.G., 1964). This, in turn, must depend on reproduction unless new hosts migrate in, and be further dependent on the duration of both infectivity and immunity following infection. Black

Table 35 Virus Isolations and SN Antibody Titres from an isolated Buffalo Herd on Island 117 in Lake Kariba

Number	1973 Virus Type Isolated	1973 Tubes/10 Positive	1973 SN Antibody Titres SAT1	1973 SAT2	1973 SAT3	1974 Number	1974 Virus Type Isolated	1974 Log_{10} Virus Titre/ml.	1974 SN Antibody Titres SAT1	1974 SAT2	1974 SAT3
9			64	64	16	15**	SAT2	1.7		N/S	
11	SAT1	10	64	64	16	17			11	<6	<6
15*	SAT2	10	178	178	22	20**			64	11	32
18	SAT2	4	45	64	22	27	SAT2	3.0	<6	128	<6
20*	SAT2	9	178	128	90	28	SAT1	<1.0	64	32	16
24	SAT1	7	355	32	32	34				N/S	
26				N/S		36			<6	8	<6
29	SAT1	1		N/S		37			256	45	178
31	SAT1	2	128	256	64	42**	SAT2	2.5	11	90	<6
35	SAT2	1	128	64	22	44**	SAT2	1.5	<6	128	8
38	SAT2	5	178	64	45	47	SAT1	2.3		N/S	
40	SAT2	7	90	64	22	48**	SAT1	1.0	128	8	90
42*	SAT2	10	22	90	11	49	SAT2	1.5	32	45	11
44*	SAT2	8	45	16	11	50	SAT2	<1.0	45	22	16
46	SAT2	8		N/S		53	SAT1	1.3		N/S	
48*	SAT1	9	256	45	64	54	SAT1	3.7	178	45	45
58*	SAT1	3	256	128	90	56	SAT1	1.0	90	16	8
70			90	64	32	57	SAT1	<1.0	128	45	178
						58**			90	32	45
						59	SAT2	1.5		N/S	

* Animal also samples in 1974
** Animal also sampled in 1973
N/S No sample

(1966), studying island communities and quoting Hope Simpson (1954), has postulated that a critical community size of from 350,000 to 500,000 may be necessary for the maintenance of measles virus and as small a population as 1,000 for the maintenance of varicella. Although the samplings of free living buffalo so far described have been from populations of many thousand animals, the study of the small group of captured buffalo (Chap. 5.2) suggests that FMD virus may be maintained in a much smaller population.

It was important therefore to study a small, free living population of buffalo isolated from contact with other animals and possible reinfection. In the Bumi Hills area of Rhodesia in 1962, the rising waters of Lake Kariba induced a herd of approximately 100 buffalo to seek the safety of high ground which later became an island, four to five miles long by approximately half a mile wide, separated by deep water from the mainland. On this island, designated Island 117, the buffalo and antelope (including impala, waterbuck and kudu) have since remained completely isolated. A competent Game Authority asserts the only species to swim periodically to and from the island is elephant.

In experiments carried out with Dr J B Condy numbers of these buffalo were immobilised and sampled in August 1973, and again in August 1974. Following isolation, population pressures on the island had necessitated culling operations by the Game authorities, and at the time of the first sampling in 1973, there were 57 buffalo in the herd. 18 of these were sampled in 1973 and 20 in 1974. The result (Table 35) demonstrate maintenance of FMDV in an isolated population of 50 to 100 animals.

The very high rate of virus isolation may have been partly due to the method of sampling. The buffalo were driven by helicopter through a funnel into a large enclosure of plastic sheeting, suspended from wires slung between trees and posts, and left there overnight. The next day they were darted from outside with a minimum of excitement and disturbance.

Only type SAT1 and SAT2 viruses have to date been isolated from this herd. The type SAT3 SN antibody titres in some animals may be due to heterotypic responses following infection with one or both of the other virus types. Six carriers sampled in 1973 were resampled in 1974 and four were still carrying virus.

At no time was clinical disease seen in the buffalo or other susceptible species on the island, yet the high virus isolation rates and high titres of virus recovered (up to $10^{3.7}$/ml) indicate infection possible within the last 6 or 8 months. Animal No. 44, a calf in 1973, may well have been then in the prodromal stage of infection for its SN antibody titre to SAT2 virus was eightfold higher when sampled in 1975.

In 1974 forty of the impala and three waterbuck (Kobus ellypsiprymnus) resident in the island were also sampled. All animals were clinically normal, no virus was isolated nor were SN antibodies demonstrated.

CHAPTER 15 - CLINICAL FOOT AND MOUTH DISEASE IN BUFFALO

In the preceding chapters attention has been drawn to the complete absence of clinical FMD or evidence of healed lesions in free living buffalo sampled in Botswana, Rhodesia and Uganda.

In the Kruger National Park (KNP) in South Africa, during the course of four separate epizootics covering a five year period prior to 1970, lesions considered to be characteristic of FMD were only very rarely observed in buffalo in spite of abundant clinical evidence of the disease in impala (Young et al, 1972 collaborative work). Between March 1970 and February 1971, while the disease was still prevalent in the Park, a more detailed examination was possible during culling operations. Only 22 out of 1,285 buffalo showed lesions suggestive of FMD. The lesions, however, were described as small, and suitable epithelium samples could not be collected. Various specimens of epithelium from suspected lesions, lymph nodes, tonsillar tissue and scrapings from the pharynx from 14 of these buffalo were examined for virus. All were negative except for one pharyngeal scraping from which Type SAT2 virus was isolated. The failure of buffalo to show obvious clinical FMDV was demonstrated by Condy (thesis) in Rhodesia in 1965. He inoculated 2 buffalo intramuscularly and 2 intradermolingually with a suspension of infected cattle tongue epithelium during an outbreak of type SAT2 infection. No lesions were seen in three of the animals but they did develop high SN antibody titres. In the fourth animal, which was shot seven days after infection, a small interdigital lesions was observed in the hind foot. In 1966, Condy again infected 2 more captive buffalo intradermolingually with a field strain of type SAT2 virus. Apart from minimal reactions at the inoculation sites of one animal, no clinical signs or lesions were seen but again both animals developed high SN antibody titres. The buffalo used in these experiments were apparently susceptible and had no pre-existing neutralising antibodies to type 2 virus.

However during the course of a type SAT2 epizootic of FMD amongst free living game animals in the KNP in 1971, a group of sub-adult buffalo held in captivity became naturally infected. In the early stages of the epizootic, in anticipation of spread of disease to this group, sera and O/P samples had been taken. One of them (No. 1) was identified as a type SAT3 carrier with high SN antibody titres to all three SAT virus types, but absence of antibody indicated that the remainder were susceptible (Table 36).

All previous work indicates that the absence of antibody in 8 of 9 buffalo of this age is unusual. Seven of the 8 had been captured when only a few days old and one when approximately 6 months old. All had been in captivity from 1½ to 4 years prior to the experiment. It is assumed that these animals were not carrying virus at the time of capture and that any colostral immunity had long since disappeared. Buffalo No. 1 was captured as a two year old and had been in captivity for a year.

Signs of disease in this captive group were seen seven weeks later during an experiment involving the artificial infection of a group of elephant in a nearby pen with type SAT2 virus. Initially three buffalo were affected; spread was rapid and within seven days all but one (No. 1) of the buffalo appeared to have been infected (Young et al, 1972). The three animals first infected were tranquillised and examined closely: lesions were confined to the dental pad, palate, lips and dorsum of the tongue. Vesicles were very small (approximately 1 cm in diameter) although in one animal a lesion in the hard palate measured 2.5 cm. On the feet, similar small vesicles were found on the coronary band and in the interdigital cleft. Type SAT2 virus was isolated from vesicular epithelium of these animals and one had a titre of $10^{6.5}/tcid_{50}$ gm. Viraemia was demonstrated in two of them, one with a titre of $10^{3.7}/tcid_{50}$ ml. Recovery was complete in 3 weeks and no clinical evidence of previous infection could then be detected (Young et al, 1972).

TABLE 36. Recovery of carrier virus and the immune response of buffalo and in-contact cattle following infection (After Young, Hedger & Howell, 1972)

Animal Number	Age Years	Recovery of carrier virus: Before Infection	Recovery of carrier virus: 6 months after infection	Neutralizing antibody titres: Pre-infection SAT1	Pre-infection SAT2	Pre-infection SAT3	6 months Post-infection SAT1	6 months Post-infection SAT2	6 months Post-infection SAT3
Buffalo									
1	3	SAT3	SAT2 - 2.0*	265	90	355	178	90	178
2	1½	Neg.	SAT2 - 2.3*	≤6	≤6	≤6	≤6	256	≤6
3	1½	"	SAT2 - trace	"	"	"	"	178	"
4	2	"	SAT2 - 2.0	"	"	"	"	355	"
5	4	"	SAT2 - 2.5	"	"	"	"	256	"
6	3½	"	SAT2 - 2.7	"	"	"	"	1024	"
7	2½	"	SAT2 - 2.5	"	"	"	"	128	"
Cow									
1		"	Neg.	8	"	"	"	32	"
2		"	"	≤6	"	"	"	64	"
3		"	"	"	"	"	"	≤6	"

* $\log_{10}$/ml of sample

Natural transmission of the disease to cattle took place when three steers in a pen adjoining the buffalo became infected with the same strain of virus 11 - 17 days later. Although the three steers showed classical FMD lesions in the mouth and all four feet, no carrier virus was isolated from them 6 months after infection. Neutralizing antibody titres in their sera taken at the same time were also of a very low order compared with the buffalo.

CHAPTER 16 - DISCUSSION

Although it has been shown (Part II) that in cattle the carrier state is a natural sequel to infection with FMDV, its duration in an immune cattle population is limited. Routine prophylactic vaccination of cattle and goats in Botswana has resulted in the disappearance of disease from domestic stock. This has been confirmed by continued surveys of cattle in which, after 2¼ years, no carriers were found.

Where free-living susceptible animals exist, however, side by side with domestic animals, they may play an important role in maintaining the virus. The part played by the African buffalo, the only remaining wild cattle-like ruminant species to roam portions of its ancestral range freely in large herds, is of particular interest.

The use of immobilisation techniques has made practicable and acceptable the sampling of certain wild animal populations in Africa, and our results show that wherever suitable samples of wild buffalo have been examined for FMDV virus, it has been found. Virus has generally been isolated from a high proportion of animals and in high titre from many, in the complete absence of clinical signs. Buffalo thus provide a reservoir of infection and, although overspill into other species occurs only rarely, they represent a hazard to unprotected domestic stock.

All three SAT virus types have frequently been isolated from the same populations and even from the same herds of buffalo. When numbers of animals sampled have been small (Chap. 10) and only one or two virus types have been isolated, high SN antibody titres to the remaining types in some animals have usually indicated recent infection with them also. The isolation on a number of occasions of more than one virus type from individuals was remarkable as this rarely appears to occur in carrier cattle exposed to infection with another virus type. The existence of multiple virus types both in populations and individuals explains previous puzzling

results when high SN antibody titres to more than one virus type were found and when the highest titres have not necessarily corresponded with the virus type isolated. The maintenance within buffalo populations of virus in an area also explains some of the unexpected results obtained in earlier wildlife serum surveys.

In spite of the absence of clinical signs in the various buffalo populations sampled in Botswana, Rhodesia, Uganda and more recently in Zambia, the high incidence of virus isolation, the high SN antibody titres, and the large amounts of virus harboured, especially in young animals, all indicate recent infection. Recent infection at the time of sampling was in fact confirmed by the demonstration of viraemia in a yearling animal at Savuti (Chap. 11.1).

Although buffalo strains of virus appear to have achieved equilibrium with their natural hosts causing them little harm, they are unmodified in other systems and fully pathogenic for cattle. Selected strains of each of the three SAT virus types inoculated intradermolingually into susceptible cattle produced severe disease with generalisation which quickly spread by contact to other cattle (Chap. 13).

The results of the surveys in Botswana in 1969 and in succeeding years (Chap. 11.1 and 11.3), suggest a maintenance mechanism of FMDV in buffalo. Adult cows generally have high SN antibody titres to the prevalent virus types, but although high titres were present in young calves, no virus was isolated from them. Calves therefore probably receive sufficient colostral immunity to protect them against early infection. However as maternal immunity wanes, calves become susceptible at different times to one or more virus types during the first years of life.

The higher virus titres in animals 1 to 3 years old indicate that infection with the prevalent virus types occurs then. Although clinical signs are usually absent, infection in susceptible animals results in viraemia followed by an immune

response with a high titre of type specific antibody. Thereafter they remain carriers excreting virus of one or more types for very long periods. The experiment in Rhodesia during which captive buffalo were monitored for 2½ years supported this hypothesis (Chap. 14, Table 34). The high SN antibody titres in the three cows before parturition, and the similar titres in the calves within 48 hours of birth, suggest possible colostral concentration of antibody occurs as in cattle after infection with FMDV (Stone and DeLay, 1960). Demonstrable antibody disappeared after a few months, one of the calves subsequently becoming susceptible to and then infected at approximately 1 year old with SAT1 virus, a virus type being carried by several of the adult animals. Similarly another older buffalo without demonstrable SN antibody introduced into the group became infected with type SAT2 virus. In neither case were clinical signs observed.

The majority of mature buffalo sampled in southern Africa had antibody to all three SAT virus types. Individual virus titres in some old animals were sometimes lower, and virus carriers were less frequent in the older groups but it is not known whether they become susceptible to reinfection.

Available evidence indicates that infection is horizontal rather than vertical. Four foetuses have been examined, three in Uganda (Chap. 11.2) and one in Botswana. Although high SN antibody titres were demonstrated in three of the dams and two of them were in fact carrying virus, the foetuses had neither virus nor SN antibody. In the Rhodesia experiment (Chap. 14, Table 34) calf No. 527 became infected approximately a year after its dam had apparently ceased to harbour virus. In Botswana a limited number of calves have been captured and sampled with their dams but comparison of the respective SN antibody titres and virus isolations (Chap. 11.3, Table 32) does not suggest transmission from cow to calf.

A continued cycle of infection in younger animals is favoured by the calving and behavioural habits of the buffalo. Although there are seasonal calving peaks, calves are dropped throughout the year providing a continual supply of

susceptible animals and the amount of virus excreted, perhaps spasmodically, by subclinically infected and carrier animals is apparently sufficient to initiate infection. Buffalo herds tend to be large and there is close animal-to-animal contact. In the dry season whole populations concentrate near permanent water, but move away when the rains provide surface water. During this movement buffalo travel considerable distances and fragment into smaller herds. When the dry weather returns, animals susceptible to one or more types of virus are brought into contact with virus excretors. It has been pointed out (Chap. 14.3) that virus maintenance depends on the size of the host population. With FMDV in domestic animals, rapid transmission from animal to animal rapidly exhausts the supply of susceptibles and prolonged maintenance could only be expected in a very large population. However the samplings in 1973 and 1974 of the small herd of buffalo, isolated on Island 117 in Rhodesia since 1962, show that FMDV can be maintained in a herd as small as 50 - 60 animals. The study on ten captured buffalo (Chap. 14.1) in which a calf born during the experiment became infected and subsequently a virus carrier suggest that virus might be maintained in an even smaller group; Asymptomatic infection of an adult buffalo (No. 84) also occurred. Virus maintenance in a small group must depend on infection persisting in individual animals for long enough and in sufficient quantity to infect new susceptible animals as they come along, for example from calving peak to calving peak. FMDV does persist in individual buffalo for very considerable periods: free living buffalo in Botswana were carrying type SAT1 virus in 1972 and were still carrying it when resampled a year later. In South Africa, captive buffalo were still harbouring virus in considerable titre (up to $10^{3.5}$tcid$_{50}$/ml) six months after infection, and in Rhodesia (Chap. 14) virus has been shown to persist in individual captive animals for as long as 2½ years.

Thus the wild African buffalo is a maintenance host of SAT type strains of FMDV. The virus is well adapted to its host and, as with many other virus infections in their maintenance hosts, e.g. enterovirus in man and many arboviruses in their maintenance hosts, infection is normally inapparent. After

infection the animals become immune virus carriers for long periods. The reported incidence of clinical disease in African buffalo has always been low (Young et al, 1972) and suspected cases have usually been unsubstantiated by virus isolation. Nevertheless clinical disease does occur (Chap. 15): 8 of 9 young buffalo held captive in the Kruger National Park in South Africa became clinically affected during nearby experimental infection of elephants and consequent liberation of very large amounts of virus. Their pre-infection SN antibody titres however indicated that these buffalo were fully susceptible to all three SAT viruses, a situation highly unlikely to occur in free living populations with the constant interplay of the three virus types.

In contrast to some other areas, outbreaks of FMD have occurred with relative frequency in susceptible species in the Kruger National Park, and it may well be that the strains causing clinical signs, albeit of low degree, in buffalo have originated from other species, probably cattle, and were not buffalo-adapted. The absence of clinical signs may thus depend on the inter-relation between strains of virus well adapted to buffalo and the antibodies which many of these animals possess.

The transference of virus from buffalo to domestic animals is a rare occurrence under normal conditions. Type SAT2 virus, isolated frequently from buffalo in Botswana (Chaps. 10 and 11), has never been recorded in domestic stock in that country and the level of immunity achieved by type SAT1 vaccination in cattle is unlikely to have afforded protection against the widely different buffalo SAT1 strain. In Rhodesia, types SAT1 and 3 viruses were isolated from buffalo; type SAT1 infection had not occurred in cattle in the area for six years and SAT3 virus had not been recorded in the country for 15 years. Similarly, in South Africa in 1970, type SAT3 virus was isolated in buffalo 11 years after the disease had last occurred in domestic stock. Type SAT3 virus was isolated from buffalo in Uganda (Chap. 11.2) and had never previously been recorded in East Africa.

Yet overspill of virus into domestic cattle does sometimes occur e.g. the reoccurrence of type SAT1 infection in cattle on the Kafue River in Zambia in 1973, 14 years after the last outbreak; and the reappearance of type SAT3 in cattle in south east Rhodesia in 1974 after an absence of 20 years. This virus type had however been isolated from buffalo in the area in the intervening years (Chap. 10).

The mechanism of spread from the buffalo maintenance host to domestic stock still needs study in greater depth because of its importance in assessing the probable success of vaccination campaigns. Its infrequency may be partly due to the transience of normal cattle/buffalo contact, and, in northern Botswana, to the immunity achieved in domestic animals by prophylactic vaccination. Although transmission almost certainly occurred from clinically affected buffalo to susceptible cattle in a neighbouring pen in South Africa (Chap. 15), no transmission occurred when a group of clinically normal carrier buffalo were held in the closest possible contact with susceptible cattle for 18 months in Rhodesia (Chap. 14.2.2). During this period the buffalo continued to harbour virus and buffalo to buffalo transmission of virus did occur.

Cattle may become infected if exposed to sufficient virus, but the amount of virus excreted by subclinically infected buffalo is apparently usually insufficient for transmission. If the virus behaves in buffalo as in cattle then maximum virus excretion would be expected during the period of viraemia which precedes the formation of secondary vesicles. In a large buffalo population viraemia must be quite frequent in young animals and yet no signs of disease have been seen in the many thousands of susceptible wild species observed in contact with them during periods of sampling.

Under African conditions, transmission from viraemic buffalo to another species is therefore unlikely in the absence of overt lesions. How then does transmission take place? The hunting and killing of a viraemic animal, the distribution of its meat among cattle owners and consequent availability of large amounts of virus to cattle makes an attractive

hypothesis for the mechanism of transfer. It would explain the infrequency of overspill of virus into the cattle population. Of nearly 800 buffalo sampled at random by us, only one has been found to be viraemic.* Moreover viraemia is commonest in young animals, which the hunter would normally avoid. The chances of a hunter therefore killing and returning with a viraemic animal are very low.

* Subsequent to the preparation of this work, viraemia has been demonstrated in a second calf, one of 167 buffalo sampled in Botswana at the end of 1974.

PART IV

FOOT AND MOUTH DISEASE IN OTHER SPECIES OF AFRICAN WILD LIFE

CHAPTER 17 - INTRODUCTION

There has been considerable circumstantial evidence in many African territories of the implication of non-domestic species, particularly wild ruminants, in the initiation and spread of FMD in domestic stock. Although a large number of wild species have been reported clinically infected (Macaulay 1963, 1964; Condy, 1970), very few cases have been substantiated by virus isolation or the demonstration of significant antibody titres.

In Rhodesia, in 1932, virus isolated from fresh foot lesions of a kudu shot during an outbreak of disease in cattle was inoculated into cattle which developed typical FMD (Hooper Sharpe, 1937). This virus was subsequently classified in 1948 as type SAT1. In South Africa in 1956, a kudu was inoculated with material from infected cattle and developed lesions on all four feet in 42 hours. Vesicular fluid from lesions yielded type SAT1 virus (Lambrechts et al, 1956). Meeser (1962) reported the isolation of type SAT3 virus from diseased impala shot during an outbreak of disease in cattle on the borders of the KNP in South Africa. In 1965/6 in Rhodesia, Condy (1970) attempted to infect captive wildebeeste by intramusucular and intradermolingual inoculation of vesicular fluid from infected cattle without producing any obvious clinical signs. Low antibody responses were however produced in three of the seven wildebeeste. Two warthogs inoculated in the bulbs of the heel with the same material developed disease with generalisation on all four feet.

Very little however is known of the relative susceptibility and incidence of FMD in most African species of wild life, or of their role if any in transmitting infection to domestic animals. A commonly held opinion, was voiced by Hobday (1964) in Northern Rhodesia, who observed that the disease appears to persist at waterholes where, in the dry season, fairly close contact occurs between wild and domestic animals. He believed that infection in "game" animals was normally of a very low grade producing few or no lesions and spreading very slowly. In times of drought, however, sufficiently

close contact occurred at watering points for transmission from infected wild animals to cattle, thus initiating an epizootic. This opinion was endorsed by Macaulay (1963) who quoted examples in South Africa and along the stockroutes in Ngamiland in Botswana. When the species susceptibility to FMDV and its pathogenesis in each species has been more closely studied, indiscriminate reference to "game" animals or wild life as a source of new foci of infection may be replaced by identification of perhaps one or two species which particularly favour its survival and dissemination.

Studies of FMD in buffalo have been described in some detail in Part II of this thesis. Part III presents the results of studies in other species of African wild life described under the following headings:-

Chapter 18 - The susceptibility of different game species
Chapter 19 - The infection of some game species

Much of this work was carried out jointly with Dr J C Condy in Rhodesia and the section on infection of elephants was carried out jointly with Drs P G Howell and E Young in South Africa.

CHAPTER 18 - THE SUSCEPTIBILITY OF VARIOUS WILD SPECIES

18.1 Serological Survey

Apart from direct clinical observation, there are two methods for the study of the incidence of infection in wild life: firstly serology, and secondly attempts to isolate virus from both clinically affected and normal animals. The 1962 rescue operations of wild life trapped by the rising waters of Lake Kariba in Rhodesia presented a unique opportunity for the collection and study of sera from African wild life. The survey, started then, was continued with the aid of various game cropping schemes, casual hunting and game control measures against the tsetse fly and was extended to cover widespread areas of Rhodesia (Lees May and Condy, 1965). The survey has since been broadened to include available wild life sera from other African territories principally Botswana, Zambia, Uganda, South Africa, South West Africa, Kenya and the Central African Republic.

Assay of SN antibodies was carried out by the cell metabolic inhibition test (colour test) using monolayers of primary pig kidney cells (Martin and Chapman, 1961) and modifications of this test using BHK cells on microtitre plates. More recently neutralisation tests have been carried out on tissue culture grade microplates using IB-RS2 cells, surviving cell monolayers being fixed in formalin and stained with methylene blue for reading (Golding et al, in press).

Where possible, each serum was tested against a suitable strain of each of the types of virus known to have occurred in the region. The choice of strains depended on availability, ability to grow well in tissue culture and antigenic similarity to current or recently isolated strains from the area. Initially, established laboratory strains were used, the behaviour of which in tests against cattle sera was well known; these included RV11 (SAT1) and RV7 (SAT3) isolated in Southern Rhodesia in the nineteen thirties and Rho 1 (SAT2) isolated from a Northern Rhodesia field sample

in 1948. More recently field strains, antigenically more similar to current outbreak strains, have been adapted for use in the test.

Type SAT1 strains used have included SWA 4/62, isolated from an outbreak of disease in South West Africa in 1962 and similar to field strains isolated from Rhodesia and Bechuanaland at the time, and Rho 5/66, isolated from Rhodesia in 1966 and similar to the strain involved in the outbreak in Northern Botswana in 1968 (Chap. 4.2). Type SAT2 strains have included SA 106/59, a strain isolated from a field outbreak in South Africa in 1959, SR 1/65, isolated from cattle in Southern Rhodesia in 1965, and also SA 3/69 or SWAZ 1/69, both isolated from an outbreak of disease involving South Africa, Swaziland and Mozambique in 1969. Recently a Rhodesian strain, Rho 2/72, antigenically different from previously isolated strains, has been used in some tests.

The choice of type SAT3 strain has been limited by the relative infrequency of outbreaks due to this virus type in cattle in Southern Africa. The stock strain SA 106/59, derived from an outbreak in cattle in South Africa, was replaced in 1965 by a new strain from an outbreak in Bechuanaland in 1965. This strain has since been used extensively, no further outbreaks of SAT3 type infection being recorded until 1974 when an antigenically similar strain caused a limited cattle outbreak in southeastern Rhodesia.

Table 37 lists the number of sera of each species tested and includes the numbers of animals which showed significant SN antibody titres to SAT1, 2 or 3 virus types. A total of 3,166 sera from 47 species were tested, and 18 species, all cloven hoofed, were shown to have significant SN antibody titres to one or more SAT type viruses. As only small numbers of sera could be obtained from some species, the order in which the species are placed is not necessarily indicative of their relative susceptibility to FMDV or to the prevalence of infection in that species. Nevertheless, the

TABLE 37

SERUM NEUTRALISING ANTIBODIES TO FMDV IN AFRICAN WILD ANIMALS

Species	No. tested	No. with titres >1/32
Buffalo - Syncerus caffer	1080	948
Kudu - Tragelaphus strepsiceros	172	38
Warthog - Phacochoerus aethiopicus	314	23
Impala - Aepyceros melampus	323	21
Topi - Damaliscus korrigum	48	15
Sable Antelope - Hippotragus niger	47	12
Wildebeeste - Connochaetes taurinus	69	7
Duiker - Sylvicapra grimmia	25	5
Bushbuck - Tragelaphus scriptus	39	4
Tsessebe - Damaliscus lunatus	59	3
Grysbuck - Raphicerus sharpei	10	2
Reedbuck - Redunca arundinum	22	2
Roan Antelope - Hippotragus equinus	5	2
Eland - Taurotragus oryx	22	1
Waterbuck - Kobus ellipsiprymnus	19	1
Oryx - Oryx gazella	2	1
Giraffe - Giraffa camelopardalis	8	1
Thompson's Gazelle - Gazella thomsoni	2	1
Lechwe - Kobus leche	115	0
Steenbok - Raphicerus campestris	19	0
Bushpig - Potamoecherus procus	17	0
Springbok - Antidorcas marsupialis	11	0
Kongoni - Alcelaphus cokei	3	0
Kob - Kobus kob	2	0
Kyala - Tragelaphus angasi	2	0
Suni - Nesotragus livingstonianus	1	0
Klipspringer - Oreotragus oreotragus	1	0
Grant's Gazelle - Gazella granti	1	0
Oribi - Ourebia ourebi	1	0
Elephant - Loxodonta africana	353	0
Hippopotamus - Hippopotamus amphibius	58	0
Rhinoceros - Diceros bicornis (Black) Diceros simus (White)	4	0
Zebra - Equus burchelli	37	0
Camel - Camelus dromedarius	9	0
Springhare - Pedetes capensis	15	0
Cape Hare - Lepus capensis	10	0
Baboon - Papio ursinus	11	0
Genetcat - Genetta genetta	6	0
Civet cat - Viverra civetta	1	0
Leopard - Panthera pardus	1	0
Porcupine - Hystrix africaeaustralis	2	0
Wild dog - Lycaon pictus	1	0
Small rodents (various)	204	0
White tailed mongoose - Ichneumia albicauda	2	0
Vulture - Pseudogyps sp.	1	0
TOTAL	3166	

highest and most consistent antibody titres were recorded in buffalo; 88% had significant SN antibody levels.

Clinical disease has often been reported in kudu and impala and significant titres were found in numbers of these. Clinical disease has also been reported in warthog, sable antelope, wildebeeste, duiker (Sylvicapra grimmia), bush buck (Tragelaphus scriptus), grysbok (Raphicerus sharpei), roan antelope (Hippotragus equinus), eland (Taurotragus oryx), waterbuck and oryx (Oryx gazella), (Macaulay, 1964) and in all of which SN antibody titres have been demonstrated. Reports of clinical disease in these species have however rarely been confirmed by virus isolation, and caution is needed in their interpretatioh. On the otherhand, SN antibody was demonstrated in topi (Damaliscus korrigum), tsessebe, reedbuck, giraffe and Thomson's gazelle (Gazella thomsoni) species in which FMD has as yet not been reported.

Many of the sera were obtained at random and there is little information for epizootiological interpretation of the results. There is however considerable information available on the 1,020 sera collected in Rhodesia (Lees May & Condy 1965, Brooksby, 1968., Condy et al, 1969). In the Zambesi valley around Lake Kariba, the game population is mainly static with little contact with domestic stock due to the presence of the tsetse fly. SN antibodies were detected in a number of buffalo and one impala in this area although there was no record of FMD occurring within 100 miles during the past 25 years. Type SAT3 SN antibodies were detected in sera from buffalo near Wankie in north west Rhodesia, yet FMD had not been recorded in this area since regular typing of all disease outbreaks was started in 1950. The majority of outbreaks in Rhodesia occur on the large ranges in the south-east. Here large numbers of wild animals and domestic stock are in closer contact, grazing the same pastures and using the same watering points. During droughts, wild animals concentrate and migrate from ranch to ranch. It is in this area that the greatest number of positive game sera was obtained. Antibodies to virus types SAT1 and 2, the prevailing types in cattle,

were recorded in various species. Buffalo also had SN antibodies to type SAT3 virus, a type not recorded in cattle for more than 15 years.

The majority of the lechwe sera were obtained during and after an extensive outbreak of FMDV in cattle on the Kafue Flats in Zambia. On this flood plain, vast herds of these antelope intermingle with large numbers of cattle. The lack of clinical evidence of disease and of antibody in the lechwe, in spite of extensive infection in the cattle, suggest this species was not involved in the initiation and spread of this outbreak and is probably not normally susceptible to FMDV under natural conditions.

The observation of clinical disease in a very susceptible species, impala, in South Africa however has provided advance warning of disease outbreaks among domestic stock (Meeser, 1962).

Sera have been obtained from various species in Botswana during the sampling of buffalo carriers with which they were in contact. Antibody was demonstrated in some kudu and sable antelope but not in impala, warthog, bushbuck, tssessebe and wildebeeste (species in which SN antibodies were recorded elsewhere) or in lechwe.

Table 38 shows the range of SN antibody titres recorded in those species considered to be positive. Brooksby (1968) has emphasised that with wild animal sera, definition as positive or negative based on an arbitrary level transferred from work in cattle may very well be misleading. Serum samples from many species other than buffalo have been lower than the titres that would have been expected from cattle. The significance of specific antibody titres in animals depends on a number of factors which include the time since infection, the rate of decline of existing convalescent titres, the species and age of the animals, and whether the virus used in test is heterologous or homologous. In the majority of cases in this survey, apart from species, these factors were unknown and the upper range of titres demonstrated in each

TABLE 38. Range of FMDV type specific reciprocal antibody titres recorded in positive animals

Species	Range of titres		
	SAT1	SAT2	SAT3
Buffalo	≤3 - 1400	≤3 - 1024	≤3 - 1400
Kudu	≤3 - 355	≤3 - 64	≤3 - 178
Warthog	≤3 - 512	≤3 - 355	≤3 - 64
Impala	≤3 - 512	≤3 - 256	≤3 - 178
Topi	≤3 - 90	≤3 - 512	≤3 - 45
Sable antelope	≤6 - 355	≤6 - 22	≤6 - 32
Wildebeeste	≤6 - 45	≤6 - 64	≤6 - 8
Duiker	≤3 - 64	≤3 - 45	≤3 - 8
Bushbuck	≤6 - 32	≤6 - 45	≤6 - 22
Tsessebe	≤6 - 45	≤6	≤6 - 32
Grysbuck	≤6 - 90	≤6 - 90	≤6 - 11
Reedbuck	≤3 - 64	≤3 - 45	≤3 - 6
Roan antelope	≤6 - 45	≤6 - 22	≤6
Eland	≤3 - 512	≤3 - 11	≤3 - 22
Waterbuck	≤3 - 16	≤3 - 32	≤3 - 22
Oryx	≤3 - 512	≤3	≤3
Giraffe	≤6	≤6 - 45	≤6
Thompson's Gazelle	≤6	≤6 - 32	≤6

species might have been considerably higher had it been possible to use homologous strains of virus in the tests.

Some comment is necessary, therefore, on the interpretation of the results. A positive correlation between the SN antibody titres in cattle in cell metabolic inhibition tests using the homologous strain of virus, and protection following the intradermolingual challenge with the homologous cattle strain of virus has been demonstrated by Martin and Chapman (1961) and Mowat and Martin (unpublished data quoted by Burrows et al, 1963). Cattle with titres of 1:45 or greater, withstood challenge in most instances. Subsequent experience with the test has shown that in random sampling of sera from cattle occasional non-specific reactions up to 1:32 may be recorded. In general, these are recognisable when the sera are tested against a number of virus types, as similar titres are recorded against each virus used in the test. A low titre (for example, 1:16), may be considered specific against a certain virus when there are no demonstrable titres against other viruses used in the test, and when there is a recent history of FMD or FMD vaccination in the animals concerned. Evidence of recent experience with FMDV may be clinical or, in the absence of any clinical history, may be indicated by high specific titres in other animals of the same group.

In this survey, where the majority of samples have been collected at random, only titres of 1:32 or greater have been regarded as significant.

18.2 Virus isolations

18.2.1 Clinically affected animals

Through the years numerous specimens from various species of African wild animals suspected of being affected with FMD have been submitted for virus isolation and confirmation of disease. The isolation of virus from a kudu (Lambrechts et al, 1956) and impala (Meeser, 1962) has already been mentioned. In recent years specimens from wild animals sent to the World

Reference Laboratory for FMD at Pirbright have mainly originated from the Kruger National Park where FMD is said to be endemic among the large concentrations of game animals, and many outbreaks of disease can be traced to this area (Howell and Mansvelt, 1972).

Table 39 shows the specimens received and virus isolations made from suspected cases of FMD in wild animals in the Park from 1958 to 1974. Unless otherwise stated the material has been epithelium or epithelial scrapings from suspected lesions in the mouth or on the feet. On occasion when lesion material has not been abundant, specimens from both mouth and feet have been submitted in the one bottle.

The virus isolations illustrate the changing pattern of infection in the KNP as first one virus type and then another sweeps through susceptible animals supporting the conclusion that FMD is enzootic.

Most specimens have been from impala indicating the clinical disease is probably most obvious in this species. Clinical FMD probably also occurs more frequently in impala than in other species, for virus was isolated from 36 of the 56 suspected cases. Although numbers of specimens from other species have been smaller, no virus was isolated from 17 suspected cases in wildebeeste and from only one of the 23 suspected buffalo: the positive sample was from a six-month calf.

Virus titres of from $10^{7.0}$ to $10^{8.5}$ $tcid_{50}$/gm of epithelial tissues have been recorded in impala. The titre of the buffalo samples was $10^{5.7}$/gm.

18.2.2. Clinically normal animals

Following infection with FMDV, cattle, sheep, goats and African buffalo, become virus carriers for variable periods of time (Parts II and III). Domestic pigs on the other hand do not carry virus for more than a few days after infection

TABLE 39. Virus isolations from suspected cases of FMD in wildlife in the Kruger National Park 1958 - 1974

Month/Year	Species	Number	Virus Isolations	Type
12/58	Impala	5	3	SAT3
2-3/59	Impala	3	2	SAT3
	Wildebeeste	1	-	
	Sable Antelope	1	-	
4-8/59	Impala	5	5	SAT3
10-12/59	Impala	5	5	SAT2
3-11/60	Impala	5	3	SAT2
8/64	Hippopotamus	3	-	
10-12/67	Impala	10	3	SAT2
3-9/68	Wildebeeste	16	-	
	Elephant	2	-	
	Buffalo	8	-	
	Kudu	1	-	
	Impala	2	1	SAT2
4-6/70	Impala	3	-	
	Buffalo	6	1	SAT2*
8/71	Impala	7	7	SAT1
	Buffalo	2	-	
2/72	Buffalo	1	-	
10-11/73	Impala	8	4	SAT1
	Buffalo	1	1	SAT1
8-9/74	Impala	3	3	SAT2
	Buffalo	6	-	
	Buffalo	3	2	SAT2+
11-12/74	Impala	9	8	SAT1

* Virus isolated from pharyngeal scrapings, other specimens negative

\+ Virus isolated from retropharyngeal lymph nodes

Table 40 The O/P Sampling of Wildlife Species other than Buffalo 1969 - 1974

	Impala	Wilde-Beeste	Warthog	Lechwe	Tsessebe	Sable Antelope	Kudu	Spring-bok	Bush Buck	Harte-beeste	Water Buck	Hippo	
1968 Rhodesia	112					1							
1969 Rhodesia	10(1)*												
Botswana	2		9		3							2	
S Africa	34						1	9	10	10			
1970 Botswana	20	20			10								
1971 Botswana	5	6	6				2						
1972 Botswana	15	10	9	5	9		16(3)						
1973 Botswana	21		1	10	9	13							
Zambia				31							3		
1974 Rhodesia	40												
Botswana	3		1	10	3	10(6)	3(1)						
TOTAL	262	36	26	56	34	24	22	9	10	10	3	2	494

* Numbers in brackets indicate number of animals with SN antibodies.

NOTE: The results of 89 of the impala in 1968 and of all the animals samples in Botswana in 1970 have been mentioned previously (Condy, 1970, Falconer, 1972)

(R Burrows, personal communication). Serological evidence indicates that at least 17 species of African wild animals apart from buffalo are susceptible to FMDV and disease has been confirmed in two of them, impala and kudu, by virus isolation. Attempts have therefore been made on numbers of occasions in Rhodesia, Botswana, South Africa and Zambia to isolate virus from O/P samples from animals of various species captured or shot during sampling or control operations. Table 40 summarises these results. A total of 493 O/P samples from 11 species were examined with negative results. Included in the table are 89 impala previously published by Condy (thesis, 1970) and 10 wildebeeste, 10 hartebeeste and 9 springbok mentioned by Falconer (1972).

As the demonstration of minimal virus in O/P samples demands considerable attention to detail in the handling of samples following collection and in the methods used for virus isolation, it is emphasised that on almost every occasion when specimens from antelope were examined with negative results, virus was isolated from similar specimens taken from buffalo at the same time. Demonstrable SN antibody levels were rarely present except in buffalo. One of 10 impala sampled in Rhodesia in 1969 had a 1 in 178 titre against type SAT1 virus, and similar titres against type SAT3 virus were present in 2 of 16 kudu sampled in Botswana in 1973 (Table 40). Another kudu, sampled in the same area in 1974, had a titre of 1 in 128 to SAT1 virus. At the same time significant type SAT1 titres (range 1/45 to 1/355) were recorded in 6 of 10 sable antelope. This was surprising as they had no signs of past disease and no FMD had been recorded in either domestic and wild animals in Botswana since 1968.

The absence of disease history in the sampled animals makes it difficult to assess the negative virus findings in terms of whether or not a particular species becomes a carrier after infection, and the low incidence of SN antibody in known susceptible species such as impala and kudu does not suggest recent infection. The failure to isolate virus in other free living species over a number of years, while virus was constantly being isolated from buffalo (Part 3), does suggest, however, that species other than buffalo are probably not normally important in the maintenance of infection in the absence of disease in cattle.

CHAPTER 19 - THE INFECTION OF SOME SPECIES OF WILD ANIMALS

19.1 Rhodesia

To learn more of the course of FMD infection, the carrier status and the significance of antibody titres in wild animals, groups of kudu, impala, wildebeeste, warthog, bush pig (Potamochoerus porcus), buffalo and a single elephant were captured when young and together with four bovine controls were held in isolation in a FMD free area in Rhodesia pending an opportunity to carry out an infection experiment. Pens housing each species were adjoining and of open construction. Animals of the same species were allowed to mix freely and no attempt was made to isolate the different species. To avoid the establishment of a fresh focus of infection this experiment could be carried out only on infected and quarantined premises during a natural outbreak of disease. When such an outbreak occurred on a ranch on the banks of the Zambesi river at Kazangula, the experimental animals were transported 700 miles in cattle trucks to isolation pens of open construction, adjoining one another, in the infected area.

Infection was by intradermolingual inoculation of 1 ml. of a 1 in 10 suspension of freshly ground tongue epithelium from a naturally infected bovine cow at approximately 10 different sites in all species, except warthogs and bush pig which were inoculated subdermally in the bulbs of the heels of all four feet (Burrows, 1966b). Under prevailing field conditions, preinfection determination of the titre of the challenge virus was not possible but, following a 5 day delay in transit to the laboratory in England, during which the unpreserved virus suspension reached unknown ambient temperatures, a titre of $10^{4.7}$ calf thyroid cell tcid/ml was recorded. From experience elsewhere (Howell et al, 1973, Sellers, 1975) the virus titre at the time of inoculation was expected to be in excess of 10^8 cattle infective doses/ml. The infecting virus was typed SAT2 (strain Rho. 4/70). Four additional epithelium samples from cattle in the infected herd were typed SAT2 and an assay of 40 sera from convalescent

cattle on the ranch revealed the presence of type SAT2 antibodies only. Unfortunately 2 of the buffalo in the experiment were long term carriers of SAT1 virus, and it was subsequently shown that one other was viraemic with SAT1 and probably excreting large amounts of this virus at the time of experimental infection with SAT2.

The virus strains used in serum neutralisation tests were Rho 5/66 (SAT1), isolated from cattle in Rhodesia in 1966 and similar to the most recent outbreak in Botswana in 1968, and SA 3/69 (SAT2), isolated in South Africa 1969 and subsequently shown to be similar to the outbreak strain (Rho 4/70).

RESULTS

19.1.1 Cattle

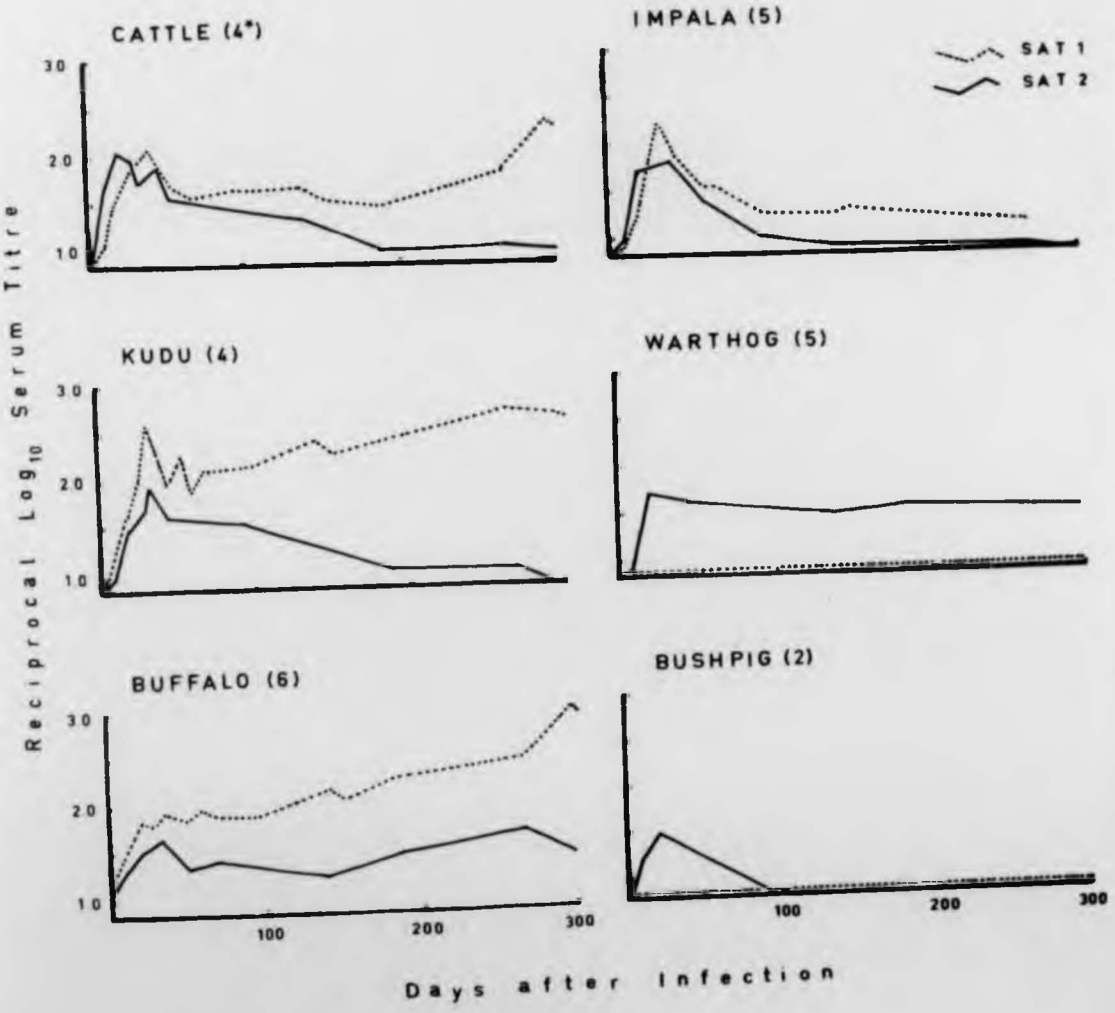
Following infection, two adult cows and 2 eighteen-month old heifers used as controls showed classical lesions of FMD with generalisation to the feet within 48 hours followed by a normal but low grade antibody response to SAT2 (see Figure 10). Although clinical signs of a second virus infection were not observed, the SAT2 antibody responses were followed by similar rises in SAT1 titres, and 2 of the animals became long term carriers of the latter virus.

19.1.2 Buffalo

The 6 buffalo aged 1 to 4 years had been screened for FMDV and FMD antibodies on several occasions prior to the experiment. Two had been carriers of SAT1 for 200 days. At the time of arrival on the infected ranch, and prior to artificial infection of all groups with SAT2, one previously uninfected buffalo was shown to be viraemic with SAT1. Another became viraemic with SAT1 the following day.

No clinical signs were apparent in the buffalo nor were vesicularlesions observed at the sites of inoculation with SAT2. Detailed examination, however, revealed very small

Figure 10. Geometric mean SN antibody responses to FMDV in different species (Hedger et al, 1972)



*Number in group

interdigital lesions on one or more feet of 4 of the 6 animals and single very small ruptured lesions, from which SAT1 was isolated, on the dental pad of 2 of the animals. Antibody responses to both virus types occurred in all animals.

Virus was recovered from O/P samples of the 6 buffalo 46 days after infection. Five were carrying SAT1, and one SAT2. A month later one of the SAT1 carriers was carrying a mixed infection of both virus types. The carrier state persisted in all animals to 206 days after infection, and at the conclusion of the experiment, 292 days after infection, 2 animals were still excreting SAT1. The maximum virus titre recorded was $10^{4.7}$ $tcid_{50}$/ml. of O/P sample (Table 41) and virus titres of up to $10^{3.5}$ $tcid_{50}$/ml were recorded 7 months after infection.

19.1.3. Kudu

The ages of the kudu varied from 1 to 3 years. After infection, viraemia was demonstrated in 3 of the 4 animals (two SAT2 and one SAT1) and vesiculation of the tongue, muzzle and all 4 feet, including the accessory digits, was observed in all the animals. Lesion material from 1 animal viraemic with SAT2 was typed as SAT1 thus demonstrating a mixed infection. Antibody responses to SAT1, similar to those detected in cattle and buffalo, occurred in all animals and persisted at a high level throughout the experiment.

SAT2 responses were lower and shorter (see Figure 10). All the kudu became carriers of SAT1 and the carrier state persisted from 106 to 140 days.

19.1.4. Impala

Type SAT2 viraemia was demonstrated in 4 of the 5 inoculated impala. Lesions on tongue and 4 feet occurred in all animals, including one left uninoculated as a contact control. From this animal, however, which died 10 days after infection, SAT1 in high titre ($10^{6.53}$/g.) was isolated from lingual

TABLE 41. Range of virus titres recorded in different species

	Viraemic blood	Vesicular epithelium	O/P sample
Buffalo	2.7 - 3.2 *	2.17+	1.67 - 4.7
Kudu	2.2 - 3.7	3.5 - 5.33	
Impala	3.7 - 4.36	6.53	
Warthog	3.0 - 4.7	5.0 - 7.0	
Bush Pig	4.2	4.0 - 6.83	

* Log_{10} primary bovine thyroid tcid $_{50}$/ml.

\+ Log_{10} primary bovine thyroid tcid $_{50}$/gm.

lesion material. Antibody responses to both virus types were lower than in cattle, buffalo and kudu and they did not persist at a significant level beyond 300 days. Carrier virus was not recovered from this species.

19.1.5 Wildebeeste

None of the 4 wildebeeste aged from 1 to 4 years used in the experiment succumbed to artificial or natural infection nor were antibody responses recorded. No virus was detected in bloods taken on the second day after intradermolingual inoculation of virus.

19.1.6 Warthog

Artificial infection of all 5 warthog by intradermal inoculation of the bulbs of the heel with SAT2 was successful. Viraemias ($10^{3.0}$ to $10^{4.7}$ $tcid_{50}$/ml.) were demonstrated and virus of high titre (up to $10^{7.0}$/g.) was isolated from vesicular epithelium from feet lesions. Lesions also occurred on the foreleg kneeling pads and accessory digits. A single lesion was seen on the gum of one animal. Contact infection with SAT2 took place very rapidly in two uninoculated warthog. Antibody responses to SAT2 were similar to those of impala, but persisted longer. Warthog did not become carriers. Natural cross-infection with SAT1 did not occur although the warthog were in close contact with infected animals of other species throughout the experiment.

191.1.7 Bush Pig

Both bush pigs reacted to SAT2 inoculation with classical signs of FMD. Viraemia was demonstrated in one of the animals and severe vesicular lesions with subsequent thimbling were observed on all 4 feet and accessory digits of both animals. Snout lesions were observed in one animal. Virus in high titre was isolated from lesion material (Table 41). Antibody responses following infection were low and short. No virus was isolated from O/P samples taken after infection.

19.1.8 Elephant

The single elephant, aged 2 years, failed to react either clinically or serologically to artificial or natural infection with either virus. No carrier virus was recovered from subsequent O/P samples.

19.1.9 Comparative species susceptibility

Severe clinical disease occured in cattle, kudu, warthog and bush pig. Disease was less severe in impala and clinical signs were inapparent in buffalo, although both these species were highly susceptible to virus. No evidence of infection was observed in the wildebeeste or the single elephant.

Cattle, kudu, buffalo and impala, but not pigs, proved susceptible to natural cross infection with type SAT1 virus of buffalo origin. The degree of exposure of the porcines to this virus type may have been less than of the other species, due to the construction of the pens, as natural transmission of type SAT2 infection of bovine origin from inoculated warthogs to uninoculated controls took place readily.

Carrier virus was recovered from kudu for up to 5 months after infection and from buffalo for much longer. Virus titres in kudu O/P samples were considerably lower than those of buffalo. No carrier state was demonstrated in impala, warthog, bush pig or in the single elephant.

The degree and duration of the SN antibody response varied between species (Figure 10 and Table 42). Titres after infection in buffalo, kudu and impala to type SAT1 virus were similar to those in cattle. While high titres persisted for a comparatively short period in impala, their persistence in kudu and buffalo (both species which became carriers) was of longer duration than in cattle. Titres after infection in warthog and bush pig (species which suffered severe clinical infection) were lower and of shorter duration.

TABLE 42. Highest individual post infection reciprocal SN antibody titres recorded

	SAT1		SAT2	
Cattle	1400	(195*)	256	(139)
Kudu	1400	(550)	178	(112)
Buffalo	1400	(398)	128	(81)
Impala	1024	(166)	178	(68)
Warthog	≤6		178	(56)
Bush Pig	≤6		45	(33)

* Group mean of highest individual titres

19.2 South Africa

19.2.1 FMD in elephant

In areas where game conservation is practised, the population density of certain species has increased enormously. This applies to the African elephant in the Kruger National Park. When the export of elephants to areas free of FMD was considered, it became necessary to establish its possible role in the epizootiology of the disease in Africa.

Circumstantial evidence implicating elephant in the spread of FMD in Africa has been vague, and comment has been directed more at the facility with which these animals break through fences, and the possibility of their bulk mechanically carrying virus, rather than at their possible susceptibility to infection. Ramiah (1935) described a case of FMD in an Indian elephant cow (Elephas maximus), which it was stated had been in close association with active infection amongst cattle; this case was not corroborated by the isolation of virus or demonstration of antibody. Piragino (1970) reported an outbreak of FMD amongst a group of 15 African elephants in Italy; the disease was confirmed by the isolation and identification of type A FMDV. Again it was suspected that the source of virus was infected cattle in the vicinity. In 1975 an outbreak was confirmed in a number of Indian elephants in Nepal by the isolation of type O FMDV (World Reference Laboratory, unpublished information). Virus titres of $10^{4.7}$, $10^{6.2}$ and $10^{8.2}$ $tcid_{50}$ per gm. of tissue were recorded in individual epithelial samples. Further clinical and epizootiological information was not forthcoming.

However, in Africa, 353 sera from free living elephant collected at random in a number of countries have been tested against strains of appropriate virus types with negative results, notwithstanding these sera were frequently taken during outbreaks of FMD in domestic stock and in other game, in which SN antibodies were demonstrated. Further, there have been no confirmed clinical observations of the disease in Africa (Howell et al., 1973).

19.2.2 Experimental Infection

a) Experimental animals

During culling operations in various regions of the KNP young, weaned, orphaned elephants were captured and transported to an isolation camp, where they were held for 9 to 12 months before the experiment. Their ages were estimated at 1½ to 3 years. The elephants were divided into four groups of three, each group accommodated in a separate pen.

b) Experimental procedure

On the basis of a preliminary titration of the infectivity of the stock virus suspension in suckling mice, three dilutions were prepared. 0.5 ml. of the virus suspension selected was equally distributed through six needle tracks over two sites, three on the anterior, and three on the posterior aspect of the dorsum of the tongue. In each group, one elephant remained uninoculated while the remaining two received one of the three dilutions of virus. When retitrated in the tongues of local cattle, the stock virus gave an infectivity titre of $10^{8.5}$ cattle ID_{50}/ml and, in the laboratory, $10^{9.4}$ $tcid_{50}$/ml in calf thyroid cells. In the volume of 0.5 ml. inoculated into the tongues of the elephants, the amount of virus administered was estimated at 2×10^6, 2×10^4 and 2×10^2 cattle ID_{50} respectively.

c) Virus

The virus used was identified as type SAT2 and was collected from a vesicle on the tongue of a buffalo showing clinical FMD. It was one of a number of buffalo and impala involved in an epizootic in the northern regions of the Park during autumn 1970. A portion of this sample was inoculated intradermolingually into the tongue of a bovine heifer and epithelium from a vesicle which developed 18 hours later was used to prepare the stock virus suspension. This virus was considered to be homologous with the virus strain (SA 3/69) used in the SN tests, which had been isolated 6 months previously from cattle outbreak close to the Park boundary.

RESULTS

d) Clinical

Following intradermolingual inoculation, the incubation period was short and primary vesicular lesions were present at the sites of inoculation in the elephants receiving the highest dose (2×10^6 cattle ID_{50}) within 24 hours. Primary lesions were evident in all the inoculated elephants, except two, by 48 hours. The lesions were multiloculate and progressive up to several cm. in diameter. Although the epidermis of the elephants' tongue is thin and delicate, vesicles were distended and firm. They appeared to be subdivided by numerous fine septae and, on puncture, the vesicular fluid was not readily evacuated.

In the elephants which reacted to infection, there was an apparent relationship between the rate of progress of the lesions and the virus dose administered. However, of the two elephants which failed to react, one had received an estimated 2×10^2 cattle ID_{50} and the other the maximum dose of 2×10^6 ID_{50}. In the latter, areas of necrosis along the needle tracks were observed.

Secondary vesicles appeared in the affected animals on the buccal surfaces, dorsum of tongue and commisures of the lips, 6 to 7 days after infection, and lameness was apparent between the 5th and 8th days. The feet were swollen and extremely painful; vesicular lesions appeared round the toe nails which progressed to complete separation of the sole in some cases. Regeneration of the mouth epithelium was slow, with scarring still obvious after 3 - 4 weeks. The soles regenerated but the feet remained deformed and signs of infection were still obvious when the elephants were slaughtered 10 months later. The lesions and clinical observations made of individual elephants in this experiment has been described in detail elsewhere (Howell et al., 1973). No signs of disease were detected in the four control animals in contact.

TABLE 43 THE INFECTIVITY OF TISSUE AND BLOOD SAMPLES FROM EXPERIMENTALLY INFECTED ELEPHANTS

Virus Dose (Cattle ID_{50})	Elephant No.		DAYS AFTER INFECTION 1	2	3	4	6	7	9	10	13	17
2×10^2	1	Blood	-	-		-		-	-	-		
2×10^2	4	Tongue Epith					6.0(m)					
		Pedal Epith										1.8
		Blood		-	4.0		5.0		-			
Control	3	Blood	-	-		-		-	-	-		
2×10^4	2	Tongue Epith						4.7(m)				
		Pedal Epith							7.0(m)		Euthanasia	
		Blood	-	-		-		1.0(m)	-	-		
2×10^4	5	Tongue Epith		8.2								
		Pedal Epith									3.4	Died
		Blood		trace	1.0(m)		1.0(m)		-			
Control	6	Blood		-	-		-		-			
2×10^6	7	Tongue Epith	7.2	8.4								
		Pedal Epith										4.0
		Tongue Vesic Fluid		8.2								
		Blood	-	trace		-		-		-		
2×10^6	8	Blood	-	-		-		-		-		
Control	9	Blood	-	-		-		-		-		
2×10^6	10	Tongue Epith	6.4									
		Blood	-	3.9		-		-				
2×10^6	11	Tongue Epith	9.0									
		Pedal Epith									3.4	
		Pedal Ves. Fluid							1.0			
		Blood	1.2		3.0		2.2		-			
Control	12	Blood	-		-		-		-			

NOTES: - signifies sample taken but no virus isolated

titrations carried out in primary bovine thyroid cells and expressed as $\log_{10}$ amounts per g. of tissue or ml. of fluid.

(m) titrations carried out in 5 d.o. unweaned mice.

e) The infectivity of tissues

Table 43 shows the results of the virus assay of lesion material and blood from both the infected and in contact control animals. Lesion material from affected elephants contained high concentrations of virus. Titres of from $10^{6.0}$ to $10^{9.03}$/gm were recorded in tongue epithelium while vesicular fluid from one elephant gave a titre of $10^{8.3}$/ml. Epithelial samples from the feet taken at an early stage of development were less infective ($10^{1.8}$ to $10^{4.0}$/gm) although one sample titrated in baby mice yielded $10^{6.0}$/gm. On occasions, up to 50 ml. of fluid was aspirated from vesicles on the feet. Amounts of virus in this fluid varied from $10^{1.0}$ to $10^{4.0}$/ml.

Viraemia was detected in all the clinically affected animals. Virus was present in the blood of elephant No. 11 twenty four hours after infection, and in the others 24 to 48 hours later. Viraemia persisted for up to 6 days in elephants which had received both high and low concentrations of virus. The titre of virus in the blood reached peaks of $10^{4.0}$ to $10^{5.0}$ $tcid_{50}$/ml. and was independent of the original concentration of virus inoculated. On no occasion during the 21 day period after infection, in which sampling was undertaken, was virus detected in the blood of the uninoculated controls or the two elephants (Nos. 1 and 8) which failed to respond clinically to infection.

O/P samples taken from the elephants 38, 62 and 91 days after infection were all negative for carrier virus.

f) The immune response

The immune response of the infected elephants is given in Table 44. Examination of the pre-infection serum samples revealed no trace of antibodies to either of the three selected SAT type antigens. Maximum antibody titres were detected on the 21st day after infection with the exception of elephants No. 1 and 8, thus providing further confirmation of the failure to infect these two elephants experimentally.

TABLE 44. Detection of serum neutralising antibodies in elephants after experimental infection with foot-and-mouth disease virus

Dose of Virus	Elephant Identification	Neutralising antibody titre to antigen SA3/69 (SAT 2) Days after infection 0	7	8	18	21	38	63	91
$2x10^2$	No. 1	≤6	-	≤6	-	≤6	≤6	≤6	≤6
	4	≤6	≤6	-	-	45	22	11	16
Control	3	≤6	-	≤6	-	≤6	≤6	≤6	≤6
$2x10^4$	2	≤6	-	≤6	Euthanasia				
	5	≤6	≤6	-	22	Died			
Control	6	≤6	≤6	-	-	≤6	≤6	≤6	≤6
$2x10^6$	7	≤6	-	11	-	45	32	16	16
	8	≤6	-	≤6	-	≤6	8	≤6	≤6
Control	9	≤6	-	≤6	-	≤6	≤6	≤6	≤6
$2x10^6$	10	≤6	≤6	-	-	45	32	22	16
	11	≤6	≤6	-	-	64	32	22	22
Control	12	≤6	≤6	-	-	≤6	≤6	≤6	≤6

The absence of immune response in the control animals confirmed that these animals had not become infected through contact. Compared with other species, the humoral immune response was low and the decline in the SN antibody concentration rapid.

CHAPTER 20 - DISCUSSION

The successful implementation of control measures against FMD in Africa where domestic stock co-exist with various wild species, some of which are susceptible to FMDV, requires a knowledge, not only of the behaviour of each species, but also of which are susceptible, of how infective they can be and thus of the degree of threat they pose when infected and their possible roles as maintenance hosts of the virus.

Very little work has been done on these problems and most field observations in the past have not been corroborated by virus isolation or demonstration of antibody. The serological survey presented here shows that in addition to buffalo, 17 species of African wildlife, all cloven hoofed, are susceptible to FMDV.

The significance of antibody titres in animals is dependent on a number of factors which include the time since infection, the rate of decline of convalescent titres, the species and age of the animals, and the relationship of the test virus to the infecting virus. Many of these factors were unknown and the antibody titres in each species in the survey might have been higher had wholly homologous virus been used in the tests. Comparatively small numbers of some species were sampled, often in the absence of any history of FMD. The absence of antibodies in certain species does not therefore necessarily indicate their insusceptibility, e.g. no antibody was demonstrated in the bush pig sampled in the survey, but this species later proved very susceptible to both artificial and contact infection (Chap. 19.1.17).

As non-specific reactions up to 1 in 32 are sometimes recorded in random cattle sera, only titres at least as high were regarded as significant. Some species, of course, may react to infection with lower convalescent titres than others, and some titres regarded as inconclusive in this survey may have been significant, or even indicative of recent infection. For instance, Condy et al (1969) infected by inoculation 4 wildebeeste, 2 buffalo and 2 cattle with type SAT2 virus of

bovine origin: convalescent SN antibody titres of the buffalo and cattle were of the order of 1 in 1,000 but those of the two wildebeeste which reacted were only 1 in 64. Convalescent titres of experimentally infected elephants (Chap. 19.2.2) were also low and two months after infection would have been regarded as probably insignificant.

FMD has been confirmed in 2 of the free-living species ser - positive in this survey, kudu and impala, and there are reports of its occurrence in others, e.g. warthog, sable antelope and wildebeeste. In some, e.g. topi and tsessebe, clinical disease has not yet been reported. Although caution is needed in the interpretation of negative results, especially if the numbers are small and there is no history of disease, the negative results in a fairly large number of lechwe in close contact with cattle during and after a widespread FMD outbreak in Zambia suggest this species may not normally be susceptible to natural infection.

In the Rhodesian series in the Zambesi valley, where there is little cattle/game contact and where there was no record of FMD for 25 years, except for one impala, antibody was recorded only in buffalo (also shown to be carrying virus). In the southeast however, where the majority of outbreaks occur, there is closer cattle/game contact and it was here that most of the antibody in wild animals was recorded. The inference is that, although some wild species are susceptible and may help disseminate infection, the origin of their infection is probably cattle, themselves shedders of large amounts of virus.

In Botswana, where the cattle are vaccinated and FMD has been eradicated in domestic stock, the position is similar to the Zambesi valley. Numbers of known susceptible species, such as impala and warthog, in close contact with virus shedding buffalo, have been monitored for antibodies and virus over several years with negative results. The recent demonstration of antibodies in sable antelope and kudu however is surprising, for since buffalo sampling started in 1968, there has been a close surveillance of game animals by the wildlife and veterinary authorities and by the sampling teams, and no signs

of disease have been observed either in cattle or in wild animals.

The failure to isolate virus from O/P samples from nearly 500 random samples from other wild species over a number of years, while virus was continually being isolated from buffalo, suggests that other species are probably not normally important in the maintenance of FMDV in the absence of disease in cattle. However, apart from impala, numbers of each species sampled have been small and more work is indicated before definite conclusions can be made.

During the years 1958 to 1974, in apparent contrast to other areas in Africa, FMD occurred with regularity in the Kruger National Park, and it is possible that here the situation may be different. The removal of human predators and restriction of movement has, over many years, resulted in populations of some species becoming so great that culling is necessary. In some areas there are concentrations of certain species such as impala. To the north, west and south, an effective game fence prevents normal contact between the animals in the Park and cattle in South Africa, but the eastern fence which is the Mocambique border is incomplete (Howell and Mansvelt, 1972) and frequent cattle/game contact is possible there. Virus isolations, mainly from impala, illustrated how virus of one type, and then another, swept through the Park. Virus of all three SAT types, often in high titre, was isolated from 36 of 56 suspected clinical cases in impala, indicating their susceptibility to FMD. No virus was isolated from the 17 suspected wildebeeste and from only one of the 23 suspected cases in buffalo. Buffalo nevertheless maintained virus independently, the virus types isolated from them not necessarily being related to the types causing disease in the impala and cattle. For example, types SAT2 and SAT3 were isolated from buffalo in 1970, and the following year type SAT1 caused disease in impala. type SAT3 virus had not been recorded in South African since 1959, eleven years previously.

A more precise way in which to study the susceptibility of, the course of infection in, the significance of antibody

titres and the carrier state of wild animals is, of course, by experimental infection of captive groups.

In the Rhodesian experiments, while it is unfortunate that superimposed infection with SAT1 virus from a carrier buffalo occurred undetected at the beginning of experimental infection with SAT2, the results illustrate the complexity of the problems in areas where FMD may be endemic in animals of varying susceptibility, and highlight the interplay of virus strains which may occur during an outbreak of disease. The difficulties in carrying out this work in primitive conditions in the context of an outbreak of FMD in cattle were enhanced by distance from the laboratory and techniques necessary for handling partially tame and wild animals. The titre of the infecting virus suspension was unknown at the start of the experiment and probably considerably higher than that recorded later. The virus titres of lesion material from the different species may also have been higher for the same reasons. The results are of value, however, as there is little record of similar work and a similar opportunity may not readily recur.

Classical FMD occurred in kudu, impala, warthog, bush pig and in the control cattle, but although very small lesions were observed in some buffalo when examined closely, clinical signs were not readily apparent. The lack of clinical or serological response to both natural and artificial infection in wildebeeste supported previous observations (Condy, 1970) and indicates that this species may be highly resistant to infection.

The carrier state was demonstrated in cattle, buffalo and kudu, the species in which antibody titres were highest and of longest duration. This may suggest a connection between persisting virus and continuation of high antibody titres. In kudu the carrier state persisted for from 3½ to 4½ months, and, in this respect, they may be similar to sheep. In other species, (e.g. impala, warthog and bush pig) which did not carry virus, antibody titres were lower and of shorter duration.

In contrast to the other species, both warthogs and bushpigs experienced a pure type SAT2 infection. This may indicate that they were less suceptible to aerosol infection, for natural infection with SAT2 virus took place rapidly in two uninoculated controls in direct physical contact with the inoculated pigs.

Previous evidence suggests that FMD does not occur naturally among elephants in Africa. We have confirmed however, the elephants' susceptibility to experimental infection and that the pathogenesis of the disease is similar to that in other susceptible species after inoculation. Vesicular lesions developed at the inoculation sites followed by viraemia lasting up to 6 days, and the appearance of vesicular lesions on the feet 5 - 8 days later.

Quantitative assays showed that virus reached high concentrations in both vesicles and blood and that large quantities of virus must have been shed. The lack of transmission to the four susceptible elephants kept in close confinement with the infected animals suggested that natural transmission would be highly unlikely in the wild where contact would be much more transient. The particularly severe involvement of the feet suggested too that if the disease had occurred frequently it would have been recognised. Notwithstanding that scar tissue from the experimental infection persisted for more than 10 months, similar lesions have not been observed in over 3,500 elephants culled and examined in the KNP over the previous 4 years. Thus the elephant does not appear to play an important role as a host in the transmission of FMD in Africa.

PART V

GENERAL DISCUSSION

CHAPTER 21 - GENERAL DISCUSSION

The design and successful implementation of control measures against FMD in Africa depend on a proper understanding of how the virus is maintained, both in domestic and wild animals, particularly between recognisable outbreaks of disease. Also important is an appreciation of the risks to domestic animals and how these risks may be monitored.

21.1 The Maintenance of FMD

In domestic animals, especially in large populations, the most obvious mechanism of virus perpetuation is direct transmission from diseased to susceptible hosts, and for cattle the most likely scurce of infection is other cattle, or sheep, goats or pigs with which they are in close and continuous contact. Affected animals shed virus in vesicular epithelium, vesicular fluid, saliva, milk, faeces, urine, semen and vaginal secretions and probably in other body fluids, during and immediately after periods of maximal viraemia. Indirect transmission may occur through the dissemination of virus in meat, milk, hides or other animal products; or through fomites (contaminated inanimate objects) such as vehicles, utensils, bedding, fodder etc. Virus may also be transmitted mechanically by living vectors, especially man who combines freedom of mobility with rapid transportation. In recent years (e.g. Sellers and Parker, 1969) attention has been focussed on the airborne excretion of virus and its probable dissemination in aerosols over long distances in conditions of high relative humidity.

Virus may persist undetected in a form of disease described by Falconer (1972) as occult, particularly in semi immune populations where the disease is endemic: only a few animals are affected at any one time and clinical signs are minimal. Diagnosis requires the detailed examination of the tongue and mouth of every animal in the herd. Typically examination of a herd of perhaps 1000 animals reveals perhaps 2 or 3 with discreet mouth lesions but no other clinical signs.

Relatively small amounts of virus are shed and the disease may either spread slowly by subclinical serial infection through the herd or die out. Such sporadic minimal disease may pass unnoticed until affected animals are brought into contact with fully susceptible animals, perhaps from another area, while being moved along a stockroute, or perhaps when disease appears in young calves whose maternal immunity has waned. The shedding of large amounts of virus by freshly infected fully susceptible animals may then be sufficient to break the dwindling herd immunity acquired from previous infection and disease spreads. Falconer (1972) suggested that stress factors such as trekking or the rigorous daily inspection of animals in quarantines may cause eruption of the minimal form of disease into the typical virulent form.

Infection in a herd may also persist in carrier animals. The demonstration of virus carriers following natural outbreaks of FMD, due to strains of 5 of the 7 immunological types, shows that in cattle the carrier state is a natural sequel to infection; but virus is maintained in domestic animal populations by carriers for only a limited period in the absence of re-introduction of infection. Although the majority of carrier cattle have stopped excreting virus by the end of the first year after infection, individual animals may harbour virus for periods of up to 2½ years. Sheep carry virus for shorter periods, from 1 - 5 months (Burrows, 1968a), up to 9 months (McVicar and Sutmoller, 1969). Our observations suggest goats carry virus for still shorter periods. Pigs have not been shown to carry virus for more than a few days after infection.

In Africa the role of numerous wild species, particularly those which share their grazing with domestic stock, in the maintenance of virus must also be considered. We have shown (Part III) that the African buffalo is a maintenance host of FMDV in which the virus appears to have achieved an equilibrium causing it little harm. The three SAT virus types have a wide geographical distribution in buffalo and are capable of persisting for very long periods, probably

indefinitely, in relatively small populations, without clinical signs of disease and independent of infection in other species. Buffalo strains of virus remain nevertheless fully pathogenic for cattle. In this respect, FMD is similar to some other virus diseases, for example malignant catarrhal fever (MCF) of cattle. However, in contrast to MCF, where a high proportion of the wild maintenance hosts (blue wildebeeste) is viraemic and viraemia may be prolonged (Plowright, 1963), FMD viraemia in buffalo is short and has been demonstrated infrequently. Available evidence thus suggests that transmission of FMDV among buffaloes is more usually from carrier animals than from animals in viraemia. Buffalo usually become infected during the first three years of life after maternal immunity has waned: thereafter animals remain carriers for very long periods. All three SAT virus types may be maintained simultaneously in the same herd, and, on occasion, multiple virus types may be isolated from the same animal. The only reports of clinical FMD in buffalo have been from South Africa where highly competent observers, engaged in wildlife research, keep a constant surveillance of wild animals in the KNP. Nevertheless Young et al (1972) reported lesions suggestive of FMD in only 1.7% of 1,285 culled buffalo carcases examined when FMD was present in other wild animals in the Park. Many of the suggestive lesions may have been unrelated to FMD for virus was recovered from only one of 23 suspected cases from which lesion material was tested between 1953 and 1974. Thus the overall picture in South Africa is probably not very different from that elsewhere in Africa where occasional suspicious lesions have been seen but virus not isolated.

A survey of over 3,000 sera from 47 species from several territories over a number of years showed that 17 other wild species, all cloven hoofed, were also susceptible to FMDV. The absence of antibody in other species, especially where only small numbers were tested, often in the absence of any history of disease in the area, does not necessarily exclude their susceptibility. FMD has been reported in many species, but confirmed by virus isolation in only a few, notably

impala and kudu. The experimental infections reported here of captive animals of several species in the field demonstrated classical FMD in kudu, impala, warthog and bushpig, with natural spread to impala and warthog. The disease was particularly severe in kudu and wild pigs but lesions also occurred on the tongue and all four feet of the impala. In the buffalo only close examination after immobilisation revealed very small interdigital vesicles in some animals. Kudu, impala, and buffalo (but not wild pigs) were susceptible to superimposed natural infection with a Type SAT1 virus of buffalo origin, but clinical signs were absent in the buffalo. Wildebeeste were not susceptible to either virus.

Elephants proved susceptible by intradermolingual inoculation but, in spite of very close contact and the very large amounts of virus shed by inoculated animals, natural transmission to uninoculated controls did not take place.

Kudu carried virus for up to 5 months after infection and therefore probably resemble sheep and goats as carriers. The carrier state was not demonstrated in impala, wildebeeste, warthogs, bush pig or elephant. Anderson et al (1975) were unable to demonstrate carriers among impala or wildebeeste either in field surveys or after experimental infection and concluded that neither were likely to develop clinical disease and therefore contributed little to the spread of disease. Their inability to produce overt disease in impala in East Africa emphasises that strain differences (both in virus and possible host) have an important bearing on comparative species susceptibility.

SN antibody responses were highest and most prolonged in buffalo, kudu and in the control cattle, all species which are naturally infected and develop carrier states. Responses were lower and shorter in impala, and similar to those of wildebeeste in earlier experiments in wildebeeste (Condy, et al, 1969). None of these species became carriers and they may be subsidiary or incidental hosts. The antibody response

in experimentally infected elephants was also low in spite of severe disease. This species is probably not normally naturally infected in Africa. There was no correlation between zoological classification and species susceptibility.

21.2 The risks to domestic animals

Assessment of the risks of infection to domestic animals in any country depends on whether or not infection exists in that country. If it does, the various factors involved include its geographical distribution, the distribution, population densities and behavioural habits of susceptible species, domestic and wild, and whether infection can be readily detected by disease. Important also is whether, and in what species, there are carriers and the risk they represent. The probability of infected animals infecting others depends not only on their relative infectivities and susceptibilities, but also on the degree of contact and climatological considerations. Disease control policies are also important. If infection is absent from a country particular attention must be paid to the nature and integrity of boundaries and the standards of disease reporting and certification in both neighbouring and exporting countries.

The geographic distribution of infection both within and without the territory is of prime importance both in terms of virus type and, where vaccination is being considered, of virus subtype.

A knowledge of the susceptibilities of different species and of their distributions and population densities is important in Africa where domestic and wild animals frequently share the same habitat. The susceptibilities of domestic species are well know, but those of wild animals have remained largely a matter of conjecture. The evidence presented here helps to define the susceptiblities to FMDV of several important wild species.

It is important to know whether infection can be readily

detected in a population by disease. Clinical signs are often transient in sheep and the disease may pass unobserved in goats. Cattle are normally marker species in which disease is evident, but in the minimal sporadic form a smouldering infection may be undetected. The work presented shows that although disease may readily be detected in some wild species, e.g. kudu, impala and warthog, it usually passes unnoticed in buffalo, the most important maintenance host.

It is also important to know which species can become carriers and with what frequency, the duration of their carrier states and the risk carriers present to domestic stock. Cattle, sheep, goats, buffalo and kudu appear to be the main important species in this respect. The epizootiological importance of carriers is however still debated. In South Africa, in the past, aphthisation (infection by inoculation) has been used for control for many years, stock being accepted for slaughter six months afterwards. Many animals moved must have been carriers, but no fresh outbreaks of disease were attributed to them. The fact that animals were destined for slaughter may have reduced the risk of their contact with other normal healthy animals (Brooksby, 1971). On the other hand, a number of earlier writers in Europe (Chap. 3) have suggested that outbreaks were due to carrier animals. The initiation or spread of disease by carrier animals is rare but our observations in Kenya (Chap. 7.1) show that it does occur and is associated with movement, allowing virus carriers to come into contact with completely susceptible animals. We failed to achieve experimental transmission by carriers but the numbers involved were small and the amount of virus being excreted minimal. A feature of FMDV is its very large number of immunologically distinct types and subtypes and, while virus is persisting in the carrier state in a large number of animals, in the presence of antibody for a considerable time, the initiation of infection due to the emergence of an antigenic variant is possible. Antigenic change has not yet been demonstrated in carrier viruses in the field, but the three SAT2 outbreaks in 1960, 1964 and 1965 in cattle in the Namwala district of Zambia, each due to a different subtype, provide strong suggestive evidence of its occurrence (Chap. 8.1).

Although goats and sheep, in which clinical signs are often minimal, play important roles in the spread of FMD, they carry virus for relatively short periods and are probably not so important as cattle as long term maintenance hosts. Kudu are probably in a similar category.

Virus may persist for very long periods in individual buffalo and probably indefinitely in even small herds (Chap. 14.3); but under normal circumstances they represent only a slight risk to other species. Frequently the virus type isolated from buffalo has borne no relationship to the types involved in disease in associated domestic stock in the area. For instance, in Botswana, type SAT2, never recovered in cattle, was frequently isolated from free living buffalo. Similarly type SAT3, isolated from buffalo in Zambia and Uganda, had not been recorded previously in either country.

The probability of infected animals infecting others depends not only on their infectivity and the susceptibility of the animals at risk, but also on the population densities of the species involved, their behavioural habits and the degree of contact which may occur between them. Husbandry considerations, particularly the provision of fencing and water, influence the probability of infection especially from one species to another, domestic or wild. Hobday's observation (1964) that the disease appears to survive at waterholes where there is fairly close contact between wild and domestic animals, suggesting the persistence of FMDV in wild species, has been echoed by many careful observers. However, in dry weather, not only wild animals, but also cattle, often from widely dispersed areas, concentrate at sources of permanent water. There would be closer contact between intermingling cattle, some susceptible, and, where the disease is enzootic, other carriers, than there would be with wild animals. Under these conditions transmission from carrier to susceptible cattle can occur (Chap. 7.1) but transmission from carrier buffalo to cattle (Chap. 14.2.2) or to other susceptible species (Chap. 11.3.2) does not occur readily. The failure of this very infectious virus to transmit to other species may be due to the transience of normal contact between animals of different species combined with the relatively small amounts

of virus excreted by buffalo in the absence of overt lesions. The mechanism of spread to other species, which occurs rarely, may not be simple and demands further study. The possibility of vectors such as biting flies or ticks should not be overlooked. It may be however that the chance killing and butchering of a viraemic buffalo with the consequent availability to cattle of large amounts of virus is a possible mechanism of spread. It would explain the infrequency of overspill, for viraemia is of short duration and rarely demonstrated.

Buffalo are not the only wild species susceptible to FMD. In Botswana, however, where domestic stock are vaccinated, our failure to find disease, virus, and in most cases antibody, in other species in contact with carrier buffalo over a number of years suggests that wild species other than buffalo are normally not important as maintenance hosts in the absence of disease in cattle. Nevertheless overt clinical disease in wild animals, with the shedding of large amounts of virus may result in cross infection to other wild species and to domestic animals. There has been considerable circumstantial evidence of the involvement of wild life in the initiation and spread of FMD in Africa: sometimes with antibody demonstrated and, on occasion, virus isolated. Much of the sampling has been carried out in the context (and excitement) of outbreaks in cattle and it has seldom been possible to define the precise history of the outbreak and whether the disease had spread from wild animals to cattle or vice versa. Reports of disease in wildlife prior to its appearance in domestic stock have been few. Hooper Sharpe (1937) reported what were believed to be 3 month-old lesions in a kudu shot at the beginning of an outbreak of FMD in cattle, and in recent years, in the Kruger National Park, outbreaks have occurred in impala prior to the disease being diagnosed in cattle on bordering farms. The origin of infection of the impala however was not determined and may have been infected cattle in neighbouring territories.

The effectiveness of disease control measures also affects the probability of infection by influencing the degree of contact and the susceptibility of animals at risk.

The assessment of the threat from outside the territory depends on the standard of disease reporting and control in neighbouring and exporting territories and on the efficiency of import controls. In Africa, the integrity of frontiers, especially when they do not form significant geographical barriers such as the Kalahari Desert in west and southwest Botswana, is particularly important as, in the absence of expensive game-proof fences it is seldom possible to restrict the movement of wild animals. Large rivers have a special significance, for while they may be efficient barriers to the movement of susceptible wild animals, the people on either side are often of the same ethnic origin and movement across is frequent. The possibility of birds conveying infection over long distances should not be overlooked but evidence of their implication has usually been coincidental. The possibility of windborne aerosol infection across frontiers is more real and is influenced by climatological factors. In the dry, hot cattle-producing areas of Africa long distance transmission is unlikely since virus would be rapidly inactivated by a combination of high temperature, low relative humidity and high levels of ultra violet light (Sellers, 1972).

21.3 Monitoring the risks

Assessment of the risk of infection from neighbouring territories and from spread of clinical disease in one's own territory can be aided by monitoring the virus type (and where appropriate subtype) responsible for all outbreaks and extensions of outbreaks. Subtype identification is important when vaccine breakdown is suspected or when virulent disease appears in a partially immune population. It may be also used to aid the tracing of the origin of an outbreak.

Determination of the prevalence of the carrier state is an important aspect of monitoring the threat. In most animals the carrier state is associated with high SN antibody titres, thus the examination of a random sample of O/P samples with

negative results and demonstration of the absence of high SN titres can be reasonable evidence of freedom of a herd or population from infection. While it is not possible, without sampling every animal, to be certain that none is infected, a negative result on a single random sample of 200 animals would give a 95% level of confidence that not more than 2% of animals in the herd or population might be carrying virus (Geigy Scientific Tables, 6th edition: binomial distribution). Except at herd level, samples may not be truly random and are influenced by such factors as accessibility, owner cooperation and the strategic position in relation to disease threat. The confidence levels will be increased if the herds most at risk are selected for sampling and if the same herds and same animals are sampled on successive occasions with negative results (T. F. de C. Marshall, personal communication). In the absence of clinical disease in the population, less than 2% of animals carrying virus would probably signify that infection had occurred at least 18 months earlier (Part II, Table 20). Remaining carriers would be excreting minimal amounts of virus and therefore unlikely sources of further infection.

The monitoring and surveillance of disease entities in wild animal populations are necessary pre-requisites to the prevention, control and eradication of major diseases of domestic stock (Hayes, 1974). Where cattle are not protected by vaccination, monitoring of wild animals for infection may provide forewarning of possible outbreaks. More important, when domestic stock are vaccinated, the monitoring of wild maintenance hosts for virus can define the disease threat from within the country and aid the selection of appropriate vaccines.

The development of sophisticated techniques, such as the projectile syringe and the use of helicopters, has made possible the large scale sampling of certain wild animal populations, particularly buffalo, a maintenance host of FMDV and the most likely wild reservoir of infection in Africa. Virus has been isolated from a very high proportion (more than

50%) of animals sampled, thus the sampling of much smaller numbers by more humble techniques (Chap. 10) is likely to yield the information required.

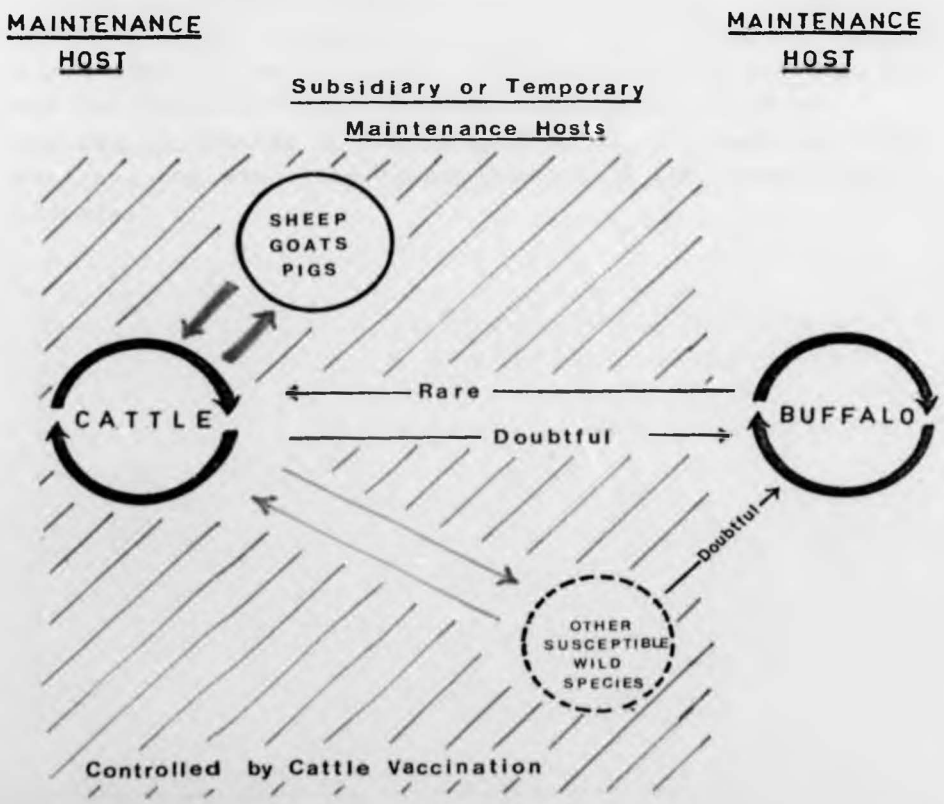
The testing of sera for antibody can also be a useful form of monitoring in some circumstances, particularly in hunting or control areas where it may be possible to maintain a continuing survey of all animals shot over considerable periods. The interpretation of negative results however requires caution and it is difficult to relate positive titres from a single sampling to the probable time of infection.

21.4 The control of FMD in Africa

Control measures against FMD are aimed at either eradicating the sources of infection, influencing the degree of contact between the sources of infection and susceptible hosts, and the immunisation of animals at risk by vaccination or aphthisation, or any combination of these. In countries with land borders, eradication by slaughter of infected animals is seldom viable, particularly in developing areas of Africa where susceptible species of wild life abound. Control policies directed at reducing the degree of contact between infection and animals at risk are mainly administrative and include import controls, quarantine, cordons, movement controls and the creation of stockfree zones. Such policies are often disruptive, they interfere with trade and may be politically or socially unacceptable. When withdrawn they do not take into account possible dangers from carrier animals or infected wild animals. Aphthisation, still occasionally used, involves the declaration and cordonning off of an infected area followed by the deliberate infection of all susceptible stock in it, so that they simultaneously acquire immunity and the disease quickly dies out. There are many objections to this practice, not least of which, in Africa, is the creation of a highly concentrated source of infection for susceptible wild species which may then perpetuate the virus. Vaccination may be used either an an emergency measure in the face of an outbreak or strategically on a regular routine basis. The former has met with mixed success in Africa and,

even if successful, protects animals for a comparatively short period following the outbreak. The latter, aimed at maintaining a level of immunity in the population as a whole has met with success in several African countries, notably so in Botswana where routine vaccination, started in 1966, has resulted in the eradication of FMD from domestic stock. Furthermore, since its institution, no evidence of infection has been found in impala, warthog, lechwe, tsessebe, wildebeeste, hartebeeste, springbuck and bushbuck, many of which were sampled when sharing the same pasture and water-holes as virus excreting buffalo.

Thus, within the limits of present knowledge, the probable role and relative importance of various species in the maintenance of FMDV in Africa could be represented thus:-



Successful routine vaccination of all domestic stock breaks this cycle and results in the eradication of virus as well as disease. Opinion is divided on the necessity of vaccinating sheep and goats as well as cattle, particularly after the initial period when disease is no longer observed, as it is unlikely virus would be maintained in them for a long period. If infected, however, and infection often passes unnoticed, they would present a serious hazard to cattle. Until the mechanism of spread of FMDV from buffalo to cattle is elucidated, vaccination of both cattle and small stock is advisable if the aim is eradication of infection rather than control. The evidence from Botswana suggests also that vaccination of domestic stock also results in the disappearance of disease from susceptible wild hosts other than buffalo which maintain FMDV independently of infection in other species. In the absence of clinical lesions they are however unlikely to infect other wild species. Providing an adequate level of immunity is maintained in domestic animals there seems to be no reason, from the point of view of FMD, why the livestock and wild life industries should not peacefully coexist to the benefit of all in countries whose heritage includes free living species of the larger wild animals.

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