1. INTRODUCTION

Foot and mouth disease (FMD) is a viral infection capable of rapid horizontal spread between infected and susceptible cloven-hoofed animals. In the vast majority of cases transmission occurs following physical or close contact between acutely infected and susceptible individuals: high levels of FMD virus occur in all secretions as well as aerosols derived predominantly from the respiratory tract of animals for 1 to 3 days prior to, and for 7-14 days after, the development of lesions (97, 30, 67, 98, 38, 113). Urine and faeces contain variable but generally lower quantities of virus (67, 114). Less frequently the virus is spread mechanically between infected and susceptible animals by contaminated animal products (e.g. milk and meat), fomites, vehicles or people (67). Rarely, FMD virus is transported over long distances by air-borne aerosols across land (for up to about 10 km) or water surfaces (sometimes hundreds of kms) if suitable climatic and other circumstances prevail (52, 38).

Apart from these varied means of transmission, it has been suspected for about 100 years that cattle recovered from FMD are also sometimes able to initiate outbreaks of the disease. Despite the long period which has elapsed since this possibility was first advanced and the many studies that have been conducted, less is understood about this mode of transmission than any of the others. Circumstantial evidence indicates that it occurs rarely but whether such transmission requires a special set of circumstances or is merely an infrequent stochastic phenomenon remains to be determined.

During the initial stage of infection in cattle, the virus has been shown to occur predominantly in the mucosa of the pharynx, soft palate and anterior oesophagus as well as associated mucus despite the fact that lesions develop mostly in the skin at the horn-hoof junction of the feet and buccal mucosa, viz: following spread of the virus from the initial site of replication in the pharynx (119, 22, 24, 87). Inspired virus-containing aerosols are the usual source of infection for farm animals although circumstantial evidence indicates that initial infection in outbreaks involving pigs often occurs by the oral route. This is despite the finding that higher doses of virus (about 1000-fold more than for respiratory infection) are required to initiate infection by the oral route in pigs (111). The size of the aerosol particles (mean about 6µm) largely determines where in the respiratory tract they are deposited which may be anywhere from the nasal passages to the alveoli (51). This being so, it is difficult to explain why, in cattle at least, most virus is found predominantly in the region of the pharynx. Part of the explanation may be as advanced by Burrows, et al., (24) who contend that the pharynx “is exposed to virus directly by inhalation and ingestion and indirectly to virus cleared by the muco-ciliary mechanisms from both the nasal passages and the lungs and from ingested virus regurgitated during ruminating”. A recent study has found viral nucleic acid in alveolar septa of cattle within 6-18 hours of exposure to virus-containing aerosols (20) indicating early lung involvement. Not only is the pharyngeal mucosa involved early in the pathogenesis of FMD in cattle but in this species, as well as a number of other ruminants, the virus may persist at that site for months and sometimes years after recovery when the virus can no longer be detected in any other organ or tissue. In pigs by contrast, initial viral replication following respiratory exposure appears to take place in the lungs (111) and FMD viruses do not persist either in the lungs or the pharynx (see below).

Animals in which FMD virus persists for more than 4 weeks in the pharyngeal region are commonly referred to as carriers (10, 93, 126) although demonstration of viral persistence is, in itself, insufficient to fulfil the requirements of a carrier in the epidemiological sense1. To satisfy the requirements of a carrier the virus must not only persist in an animal but that animals must be able to transmit the infection. Labelling all animals persistently infected with FMD virus as carriers is unfortunate because it has resulted in the assumption that any persistently infected animal is potentially able to transmit the disease (108). While that may be so, it may equally not be the case. For this reason an attempt is made in this paper to draw a distinction between mere viral persistence and true carrier status.

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1 Martin et al. (1987) define a carrier as an infected animal that spreads pathogenic or potentially pathogenic organisms, yet remains clinically normal.
The identification of carriers of FMD and understanding their role in the initiation of outbreaks is becoming increasingly important. The reasons for this are, firstly, that significant progress in ridding various regions of the world from FMD has been achieved in the recent past (e.g. Western Europe and some parts of South America) and it is economically important for these regions to maintain or improve their status in this respect. This is particularly so now that it is possible for countries to obtain recognition of their disease-free status by the OIE. Together with the liberalization of international trade in animals and agricultural products, which is promoted by the World Trade Organization, this is likely to increase the possibility of carriers introducing FMD to regions which have achieved disease-free status because not only are carriers more difficult to identify than diseased animals, but the animal populations of disease-free countries are fully susceptible in the absence of immunization. In the second place, the carrier state is at least one of the means by which African buffalo (*Syncerus caffer*) maintain SAT-type viruses (59, 60, 116). Maintenance of SAT-types by African buffalo means that countries in Africa which are fortunate to possess wildlife in abundance have to balance the ecological and other benefits of conserving these animals with the restrictions on trade in animal products which their existence engenders (114). A vital consideration is therefore whether the regulations governing international trade in livestock and their products are appropriate in the light of our current understanding of the role of carriers, both wild and domestic, in the transmission of FMD.

### 2. BIOLOGICAL SIGNIFICANCE OF CARRIERS

Infections with high reproductive rates, *viz.* those capable of rapid spread such as FMD virus, have the intrinsic disadvantage that they quickly run out of susceptible animals to infect (animals recovered from the infection are usually immune) unless the host population is very large. To overcome the possibility of what amounts to auto-extinction such infections employ a number of strategies. Most commonly they either vary the antigens which induce immunity and so circumvent the immune response of the host population (9) or establish carriers which maintain the infection in individual animals until a sufficient number of susceptibles is recruited into the population (usually animals born after the epizootic whose maternal immunity has waned) to sustain another epidemic (127). FMD virus is unusual in employing both these strategies and there is good reason to believe that antigenic variation in FMD viruses is, at least to some extent, dependent on events in the pharyngeal region of persistently infected animals.

### 3. HISTORICAL PERSPECTIVE

Ironically, the best evidence for carriers being important in the initiation of outbreaks of FMD are historical accounts of this disease occurring in circumstances where it was reasonably certain that no new animal introductions had occurred and where no alternative explanation other than the involvement of carriers could be advanced. These cases have been cited repeatedly in other reviews and therefore no attempt is made here to repeat them other than to provide a list of references in which these accounts can be found: (71, 13, 21, 86, 91, 88, 107, 17, 102, 67, 64, 117).

### 4. CURRENT UNDERSTANDING OF VIRAL PERSISTENCE IN DIFFERENT SPECIES

#### 4.1. Cattle

Most published investigations into persistence of FMD address the situation in cattle in which it has indisputably been shown that in a variable proportion (frequently more than half) of animals, FMD virus of the relevant type can be recovered from oesophageo-pharyngeal (OP) specimens collected using a small beaker attached to a wire handle (so-called probang or probang-cup) originally developed by Grae & Tallgren (118, 107, 64) one month to several years after infection (118, 106, 22, 19, 57, 30, 58, 105, 4, 80, 60, 56). These investigations indicate that all 7 FMD virus types are capable of inducing persistent infection lasting up to 42 months (56). However, individual strains of virus probably differ in efficiency in this respect although the observation that the dose of virus to which animals are exposed influences the number of persistently infected animals which result (117) and the probability that individual animals vary in their ability to sustain persistent infections (109) are confounding factors.

Irregularity in the presence of FMD viruses from OP secretions collected by probang sampling of persistently infected animals (*Table 1*) makes this conventional method for detecting persistent infection inherently unreliable. The use of the polymerase chain reaction (PCR) for identifying FMD virus in OP specimens collected using probangs has been found by some to be more sensitive than virus isolation using cell cultures (87, 41) but OP specimens may contain substances which interfere with PCR and therefore a schedule involving a combination of cell culture inoculation and PCR for detecting persistent infection has been proposed (66). Nevertheless, lack of precision in collecting the correct specimen makes probang testing essentially a 'hit or miss technique', particularly when performed by inexperienced (sicians. The possibility of using serological methods for this purpose and especially to differentiate between cattle with antibody induced by vaccination as
opposed to infection is currently being investigated in a number of laboratories. Apparently promising data on an enzyme-linked immunotransfer blot assay have been published (16) but further evaluation is required.

In cattle the sites of viral persistence, based on measurement of infectivity of tissues recovered at necropsy from animals infected experimentally with virulent virus, are predominantly the mucosa of the pharynx, the dorsal soft palate and anterior oesophagus (22, 23). However, in cattle infected with virus strains attenuated for that species, virus was most frequently recovered from and occurred at highest titre in the tonsillar region of the pharynx (23). Subsequent investigation using the polymerase chain reaction (PCR) has indicated that viral replication occurs mostly in the pharynx of cattle on the basis that anti-sense RNA was regularly demonstrable there but not in the anterior oesophagus. The implication is that virus present in the anterior oesophagus does not originate there. Most virus in OP specimens is possibly associated with mucus (119).

It remains to be determined which cells support viral replication in the pharynx and what replication strategy the virus employs. This is the major conundrum of research in this field: why has no-one yet been able to do this despite considerable effort? That there has been no lack of serious attempts by competent researchers to identify the cells in which persistent virus replicates is belied by the paucity of publications in this regard (126). Techniques such as immuno-histochemistry, immunofluorescence and in situ hybridization have so far failed for reasons which are not clear. Histology has likewise been of little benefit because no obvious microscopic lesions have been observed in the pharynx of animals with persistent infection (93). Apart from finding negative sense RNA in the pharynx which is indicative of active replication taking place (87), the replication strategy of persistent infections in FMD is unknown although there has been speculation in this regard (126, 94).

Table 1
Parameters of persistent foot and mouth disease virus infection in cattle

<table>
<thead>
<tr>
<th>Proportion of animals with virus in OP secretions (%)</th>
<th>Duration of viral persistence (months)</th>
<th>Virus levels (log10/ml)</th>
<th>Virus present intermittently in OP secretions</th>
<th>Persistence detected in vaccinated (V) or unvaccinated (UN) animals</th>
<th>Reference</th>
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<tr>
<td>77</td>
<td>(E)</td>
<td>4 - 8</td>
<td>“small”</td>
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<td>V and UV 118</td>
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<td>35</td>
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<tr>
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<td>56</td>
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<td></td>
</tr>
<tr>
<td>100</td>
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<td>0.3 - 2.9 pfu</td>
<td>Yes</td>
<td>V and UV 22</td>
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<td>20</td>
<td></td>
<td>8</td>
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</tbody>
</table>

E: experimental investigation  F: field investigation  NS: not stated
pfu: plaque-forming unit  TCID<sub>50</sub>: tissue culture infectious doses 50%

The original report by Van Bekkum and colleagues (118) on identification of persistent virus in the OP secretions of cattle recovered from FMD is a milestone of research into FMD because not only was it the first
report of viral persistence in the OP region of cattle but they also described the major features of the phenomenon which have subsequently been confirmed by other investigators, viz:

i) in a high proportion of cattle viral persistence in the OP may occur following infection and the proportion of persistently infected animals decreases as the time since infection increases, most animals clearing the infection within 4 to 5 months (Table 1); in the experiment they described 10/13 (77%) animals became persistently infected and this lasted for 5.5 - 8 months. Van Bekkum et al., (118) used the term “saliva” to describe the OP specimens which they examined, which subsequently led to some confusion because FMD virus is not recoverable from true saliva for more than about a week after infection (113),

ii) usually only low levels of virus are recoverable from OP secretions and the virus may also only be detectable intermittently: furthermore, virus levels tend to decrease with time (Table 1),

iii) immunized animals subsequently exposed to infection may become persistently infected even if they do not develop the disease, viz. following inapparent infection (Table 1).

So far there is no indication that any particular sex or age-group of cattle is more prone to develop persistent infection with FMD viruses (57, 54). Whether the apparent greater susceptibility to FMD of Bos taurus breeds as compared to those belonging to Bos indicus (84) is reflected in differences in the abilities of these two species to support persistent infection remains to be determined. A wide range of cattle breeds have been identified with persistent FMD infection and there is no obvious breed predilection (94) although there are anecdotal reports from Zimbabwe that Brahman cattle are more efficient at maintaining persistent infection than other cattle breeds.

Superinfection of the bovine pharynx with different types of FMD virus has been demonstrated and two or more viruses may co-exist in such circumstances for extended periods of time (109, 54).

Earlier reports of viral persistence on the feet of cattle, in their uro-genital tracts (the virus detected in concentrated urine) and in blood (83, 18, 124, 125) were not confirmed by Van Bekkum and colleagues (118) and nor have they been since (22, 30). There is, however, a statement in a discussion document to the effect that FMD virus was detected intermittently in the semen of a bull for up to 42 days (31, 117) and there is a claim that 7/22 bulls free of FMD for at least 6 months had FMD virus in their semen (89). There are no other reports either to support or contradict these findings.

4.2. Sheep

Persistent infection of sheep with FMD viruses has been less extensively studied than in cattle but persistence lasting up to 12 months has been found (78, 100). In normal circumstances individuals probably maintain the infection for no more than 1-5 months (23). A recent study conducted in Anatolia (Turkey) found that 16.8% of 469 sheep probanged following an FMD outbreak had persistent infection in contrast to 18.4% of cattle in the same locality, although how long after the outbreak the specimens were collected was not stated (54). In four sheep mixed infection with types A and O was identified. Conversely, in a field survey of indigenous sheep in Kenya there was no evidence that persistent infection followed natural infection with a variety of FMD virus types (5). Differences in susceptibility to FMD between breeds of sheep as well as variation in pathogenicity of virus strains for sheep may explain the discrepancy in findings between Anatolia and Kenya (48, 78, 5).

Burrows (23) found that in sheep persistent virus was recovered most frequently and in highest titre from the tonsillar area and not, as in cattle, from the mucosa of the pharynx and dorsal soft palate.

4.3. Goats

In an experimental study 93, 56, 44, 37, 25, 12 and 6 percent of Indian goats were found to have persistent virus (type not stated) in their OP secretions for 2, 3, 4, 5, 6, 7 and 8 weeks after infection respectively while 7/116 goats in an infected flock had persistent infection at a time estimated to be 2-3 months after infection (103). By contrast, in a well conducted experimental study in Kenya involving type O and SAT 2 viruses, there was no development of persistent infection among 24 experimentally infected goats despite the fact that a high proportion of animals had high virus titres (up to 104.0 /ml) in OP specimens collected from them soon after infection (5). As part of the same study, only 1 of 346 goats probanged in 5 endemic localities in Kenya was shown to be persistently infected in contrast to 10/676 persistent infections detected in cattle in the same localities.
This limited data indicates that goats, like sheep, develop persistent infection less frequently and for shorter periods of time than do cattle.

4.4. Pigs

The balance of evidence is that pigs do not continue to harbour FMD viruses for longer than 8-10 days and certainly no longer than 28 days after infection (99, 117, 85) in spite of the fact that they excrete about 3000-fold more virus during the acute stage of infection than do cattle (38). Free-living African suids, *viz.* warthogs (*Phacochoerus aethiopicus*) and bushpigs (*Potamochoerus porcus*) also do not harbour FMD virus past the stage of acute infection (59).

It remains to be ascertained why most ruminants and pigs differ in their ability to sustain persistent infection. Ideas in this regard have been advanced by Salt (94).

4.5. Water buffaloes

It is ironic that the behaviour of FMD viruses in water buffaloes (*Bubalus arnee*), which are domesticated in several areas of the world, has been less extensively documented than is the case for wild African buffalo (*vide infra*) but it is dangerous to extrapolate the features of FMD virus interaction of African buffalo to water buffalo because they belong to different genera despite the similarity in their appearance. Furthermore, the SAT virus types usually associated with African buffaloes are unique to Africa. Water buffaloes, in contrast to the African variety, regularly develop lesions characteristic of FMD although their susceptibility to FMD and the severity of the lesions appears to be variable, ranging from severe to inapparent (92, 82). In experimentally infected water buffaloes viral persistence lasting 2 months has been detected in OP secretions (82) and there is a report from Brazil which provides indirect evidence for the virus persisting for up to 24 months in these animals (53).

4.6. Llamas

The available evidence, although limited, indicates that llamas (*Lama glama*) do not harbour FMD virus in the pharyngeal region beyond the acute stage of infection (32, 72).

4.7. Deer

Of the 10 species of deer recorded as having been infected with FMD virus (61), a number have been assessed for their ability to maintain persistent infection. Among five species which occur in Britain, fallow (*Dama dama*) and sika (*Cervus nippon*) deer regularly developed persistent infection while red deer (*Cervus elaphus*) did so occasionally. Roe (*Capreolus capreolus*) and muntjac (*Muntiacus muntjak*) deer, on the other hand, did not (45, 49). By 63 days after infection half of the fallow deer still had detectable virus in their OP secretions (45) and in both fallow and sika deer virus titres in OP secretions averaged 104.6 TCID\(_{50}\) per sample at 28 days after infection (49).

White-tailed deer (*Odocoileus virginianus*) in the United States of America were shown to maintain FMD virus for up to 11 weeks (77).

4.8. African buffaloes

Most free-living populations of African buffalo (*Syncerus caffer*), in southern Africa at least, have high infection rates with SAT-type FMD viruses, the exception being at the southern-most limit of their distribution (43). In the Kruger National Park in South Africa, for example, more than 80% of buffalo of all ages are serologically positive to the three SAT virus types when tested by the blocking ELISA (11) although the proportion of positives identified by neutralization tests is lower (113). Rates of persistent infection determined by identifying FMD viruses in OP specimens of buffalo are also high, varying from 40 - 60% of animals sampled (59, 60, 6, 8). However, in some groups of buffalo much lower rates of persistent infection have sometimes been found but it is not clear whether these lower rates reflect the true situation or merely inefficient sampling and processing techniques (112). The titre of virus in OP secretions may be as high as 105.2/ml during the acute stage of infection but by a week after infection generally drops to lower levels; nevertheless, titres greater than 104/ml have been reported (6, 33). Individual animals may maintain the infection for periods of at least 5 years (27) but it appears that a significant number of animals fail to maintain persistent infection for prolonged periods because the proportion of persistently infected animals falls after reaching a peak in the 1-3
4.9. Antelopes

Although serological evidence of infection in at least 15 species of antelope has been recorded (28, 61, 55, 7) demonstration of viral persistence has only been reported in kudu (*Tragelaphus strepsiceros*) in which the virus was detected for between 106 - 140 days in OP secretions after artificial infection (59). In the same investigation two wildebeest (*Connochaetes taurinus*) had SAT 1 virus in their OP secretions for 45 days after infection but in a subsequent study (3) no persistence in this species was demonstrable. In eland (*Taurotragus oryx*) virus was recovered from OP secretions for up to 32 days in one study (2) but not in another (4). Transitory persistence - up to 56 days - was found in sable antelope (*Hippotragus niger*) (44). Experimental studies have failed to provide evidence of viral persistence in impala (*Aepyceros melampus*) (59, 3) which, among antelope in southern Africa, are more frequently affected by FMD than any other (115, 7).

5. IMMUNE RESPONSES IN RELATION TO PERSISTENT INFECTION

Subsequent to the finding by Van Bekkum *et al.*, (118) that immunized cattle which resisted challenge infection nevertheless became persistently infected, Sutmöller (109) found that vaccinated, passively immunized and immunologically naive cattle became so with equal facility. The apparent inability of immune responses to prevent or influence the course of persistent infections has been supported by other investigations (22, 57, 108, 105). Conversely, more contemporary studies have indicated that vaccinated animals may be less prone to persistent infection than non-immunized cattle (90, 35) and it was previously speculated that very high antibody level may provide resistance in this regard (58). Field data from East Africa where the incidence of persistent infection was found to be higher in non-vaccinated areas than areas where routine immunization was practiced supports the contention that vaccination may have an effect (4) although this finding could have been the result of immunization depressing viral activity.

Once persistent infection is established there appears to be continued systemic immunological stimulation because, in most animals, the humoral antibody response to the relevant virus type is more protracted than expected and this has been found to apply not only to cattle but African buffalo and kudu as well (58, 63, 79). To complicate the issue, a small proportion of persistently infected cattle have no detectable humoral antibody (57, 109) which means that the presence of persistent infection in individual animals cannot be excluded on the basis of a lack of circulating antibody.

Levels of local neutralizing antibody in the pharynx are higher in persistently infected cattle in comparison to animals recovered from the infection (75) and specific IgA levels are also higher whether the animals are vaccinated or not (94). This indicates that, as is usually the case for the humoral response, persistent virus in the pharynx provides a continuous immune stimulus. It is not known whether persistently infected cattle that lack circulating antibody similarly fail to produce a local immune response. The treatment of OP specimens with trichlorofluoroethane has been found in some laboratory studies to increase the titre of virus recovered, presumably by dissociating virus/antibody complexes (109, 80), but this is not a universal finding.

Any possible role for cell mediated immune responses in FMD infection remains largely a matter of speculation (126, 94).

6. GENOMIC AND ANTIGENIC VARIATION ASSOCIATED WITH PERSISTENT INFECTION

Like most other RNA viruses, FMD virus probably exists as quasispecies, *viz.* complex mixtures of related genomes that act as a whole (42, 65, 36, 73). This is consequent upon the lack of proof-reading mechanisms during RNA replication which enables nucleotide substitutions to occur at a rate more than a million-fold greater than is the case during DNA replication (65). Constraints of functionality preclude limitless variation and the rate of fixation of mutations in persistently infected cattle is $0.8 \times 10^{-2}$ to $7.4 \times 10^{-2}$ substitutions per nucleotide per year, which is higher than the rate that occurs during acute disease episodes when horizontal transmission is the rule (36, 73). Intra- and intertypic recombination between two viruses which may co-exist in the pharynx of persistently infected animals provides another mechanism whereby more dramatic mutational events may occur (76, 70). Despite the potential for rapid antigenic evolution during persistent infection among FMD viruses, the extent to which this occurs in the field is not clear because conflicting results have been published (Table 2) and also there are no unequivocal reports that antigenic or other phenotypic variations observed have been biologically significant.

<table>
<thead>
<tr>
<th>Genomic or phenotypic variation identified in foot and mouth disease</th>
<th>viruses involved in persistent infection of animals</th>
</tr>
</thead>
</table>

- 92 -
Data on phenotypic variation (e.g. plaque size, infectivity for a particular species, antigenic variation and virulence) in viruses recovered from persistently infected cattle is summarized in Table 2. Intuitively it might be assumed that one or other of the broad findings is likely to be generally correct, viz. either viral variation tends to occur in persistently infected cattle or it does not. However, Holland et al. (65) have observed that even among RNA viruses which tend to evolve rapidly, long-term stasis of viral genomes is sometimes observed and may be equated with the evolutionary concept of “punctuated equilibrium” that is becoming increasingly accepted in evolutionary biology (104). They further contend that rapid evolution of RNA viruses is promoted by conditions which lead to loss of population equilibrium caused by environmental change. This could result from sequential infection of new hosts or, more likely in the context of persistent infection, cell types. Immune selection exerted by antibody present in the pharyngea of persistently infected animals may be an example of environmental change although it has been clearly demonstrated that antigenic variation may occur in the absence of immune pressure (37). Therefore the apparently conflicting reports on the occurrence of antigenic or other phenotypic characteristics (Table 2) are not necessarily contradictory.

Whatever the mechanism(s), it is clear from sequencing of a portion of the ID (VP1) gene of SAT-type viruses recovered from persistently infected African buffaloes in the Kruger National Park in South Africa, which covers a relatively small area with 17 - 30 000 free-living buffaloes, that the extent of genomic heterogeneity is considerable (Figure 1). This intratypic heterogeneity appears greater than has been found for genomic variation in viruses recovered from persistently infected domestic animals in regions of similar or greater size and supporting larger numbers of animals (121, 69). This could be due to the lack of human intervention in FMD transmission in wildlife reserves, i.e. enabling a high proportion of animals to become infected for longer periods of time.

**Figure 1**

**Neighbour-joining tree (Jukes & Kantor correction) of SAT 2 viruses recovered from oesophageo-pharyngeal specimens from buffalo in the Kruger National Park (South Africa) between 1986 and 1995**

(Dendrogram provided by A.D. Bastos, Onderstepoort Institute for Exotic Diseases, South Africa)
The tree was derived from nucleotide sequences of a portion of the 1D gene of each isolate (121, 122, 123). Values at the nodes indicate the percentage support determined by 500 bootstrap replicates.

Recent experimental work with African buffalo persistently infected with both SAT 1 and SAT 2 viruses has provided the interesting observation that despite the fact that two co-existant FMD viruses that persisted in individual buffalo developed nucleotide substitutions at approximately the same linear rate (1.54% and 1.64% nucleotide substitutions per year), the SAT 2 isolates underwent rapid and dramatic antigenic change whereas the SAT 1 viruses showed no perceptible change in this respect (123). Without any other obvious explanation it is assumed that this is a chance phenomenon, i.e. the substitutions in the SAT 1 isolates occurred in regions of the 1D gene which made no significant antigenic difference while in the SAT 2 isolates changes occurred at immunologically important sites. An alternative explanation is that the SAT 2 virus was produced from cell cultures and plaque purified whereas the SAT 1 virus was derived from natural infection so that the adaptive requirement on the SAT 2 virus was greater than for SAT 1. Dawe et al., (33) in a similar experiment, found that virtually no genomic variation in a SAT 2 virus occurred in buffalo over a period of 5 months.

It is likely that other phenotypic changes in FMD viruses involved in persistent infections may equally depend on whether mutations occur at positions of the genome controlling those characteristics or not. Since available evidence suggests that mutational events occur at similar frequencies throughout the length of the FMD virus genome (120), chance is likely to be the principal determinant of such events.

7. TRANSMISSION OF FOOT AND MOUTH DISEASE BY CARRIER ANIMALS

To date there are no unequivocal reports of the transmission of FMD from persistently infected domestic livestock to susceptible animals other than the circumstantial data available from the references cited in the section on historical perspective and the events associated with SAT 2 outbreaks in cattle in Zimbabwe between 1983 and 1991 (121, 56) discussed below. This is despite FMD virus having been shown to be “externalized” by persistently infected cattle when they cough (109). There are, conversely, many reports on the failure of attempts to effect FMD transmission from persistently infected to susceptible animals, especially cattle (81 [cited by 102], 118, 96, 19, 105, 108, 109, 110, 68, 14) and it is a fair assumption that more attempts at carrier transmission have been made and not reported because of the negative findings.

Despite the lack of direct evidence for FMD transmission by persistently infected domestic animals, there are instances where the infection appears to have been transmitted by carrier cattle without the development of clinical disease in the recipient (107, 108, 57, 58). In these cases the probability is that small amounts of infectivity were transferred to the contact animals but whether that is the reason for the development of subclinical infection is a matter of speculation. Doses of virus as low as 10 - 25 TCID50 have been shown to be capable of causing the disease, as opposed to the
infection, in cattle and sheep (50, 39) and it is possible that doses lower than that may sometimes result in inapparent infection. What makes some of these instances even more remarkable is that the recipient animals became viraemic without subsequently developing a detectable humoral antibody response (50, 108, 12, 5, 40).

There is good evidence that carrier cattle were intimately involved in the causation of a series of seven SAT 2 outbreaks which occurred in Zimbabwe between 1983 and 1991 one of which, centred on Gweru in 1989, spread widely on the central plateau of the country (Figure 2). By a combination of careful field observation, good livestock movement records and genome sequencing of viruses obtained from cattle involved in the outbreaks (56, 121, 69), it has been shown with a considerable degree of certainty that carrier cattle were the origin of at least two of these outbreaks: on Delken Farm in the Mutorashanga District and the other at Whaddon Chase Farm in the Midlands Province on 23/7/89 and 24/10/91 respectively.

Figure 2
Map of Zimbabwe showing locations relevant to FMD outbreaks in which carriers are believed to have been involved (from 1987 to 1991)

Note: Although the farms Delken and Whaddon Chase are geographically close to each other, they are separated by a dyke (high ridge) which makes direct access between the farms difficult.

In the Delken Farm outbreak, the SAT 2 virus isolated from the 1989 outbreak at Delken Farm was shown by nucleotide sequencing to differ by less than 4% from the virus involved in the 1987 outbreak at Blackwaters Ranch in the Insiza District, hundreds of kilometres away. Movement records showed that cattle from Fountains Farm (surrounded on three sides by Blackwaters Ranch and initially thought to have escaped infection in 1987), were moved to Msimbi Farm in the Shamva District on 13/2/87, i.e. 39 days before the infection at Blackwaters Ranch was detected on 24/3/87. On 16/8/88 the cattle were moved to Delken Farm, viz. after a period in excess of 18 months on Msimbi Farm. Clinical disease was detected on Delken Farm on 27/3/89, i.e. approximately 7 months after the arrival of the cattle originating from Fountains Farm.

The Whaddon Chase outbreak was traced back to cattle which originated from Aberfoyle Farm approximately 21 months earlier. Aberfoyle Farm, like Fountains Farm in the Delken outbreak, apparently escaped the disease in early
May 1989, but lies adjacent to two properties (Zhangwe and Wonder Rock) which did become infected. The cattle involved had been vaccinated on three occasions (15/5/89, 2/6/89 and 15/8/89) before they were moved to Witham Farm on 16/2/90. After a stay of about 9 months they were again moved to Pama Farm where they stayed about 3 months and were then sent to a feedlot at Whaddon Chase in September 1991. FMD was diagnosed in young cattle from Lions Den that had mixed with the feedlot cattle on Whaddon Chase for one day on 24/10/91. The viruses from Zhangwe/Wonder Rock and Whaddon Chase were shown to be very similar by sequencing studies (56). Furthermore, when 176 cattle on Aberfoyle were probranged on 11/11/91 after the suspicion had arisen that carriers from that farm had caused the outbreak at Whaddon Chase, one persistently infected animal was identified, viz., approximately 20 months after the cattle that eventually arrived at Whaddon Chase had left Aberfoyle (56).

Thus in both these instances the carrier cattle were infected more than two years before the disease was transmitted and had on numerous occasions been in contact with other susceptible cattle to which the infection was not transmitted. Furthermore, in the case of Whaddon Chase Farm it appears that the carriers were able to transmit the infection during a contact period lasting less than 24 hours.

The opinion has been expressed that “mucosal disease” has been involved in the initiation of carrier transmission in Zimbabwe although the basis for this conclusion is not clear (56).

In contrast to the situation in domestic livestock, true carrier status has been unequivocally demonstrated for African buffalo (62, 33, 34, 123) although even in this species most attempts at effecting transmission between persistently infected and susceptible buffalo or from persistently infected buffalo to cattle have been unsuccessful (25, 26, 6, 61, 15, 46). Given this inefficiency of transmission by carrier buffalo it is unlikely that these animals are the usual source of infection for susceptibles in breeding herds and it has been postulated that “childhood epizootics” are largely responsible for the high infection rates found in most free-living populations of buffalo (see above) (116). However, in such circumstances carriers are probably important for the survival of SAT type viruses in interepizootic periods within buffalo herds (116).

Two interesting aspects were revealed by the two experiments in which carrier transmission from buffalo to cattle was demonstrated (33, 123). Firstly, in both instances transmission only occurred some months (5 and 10 respectively) after the start of the experiments. Furthermore, in the Zimbabwean experiment in which the buffalo and cattle were in contact throughout, no transmission occurred between the buffalo and cattle at the time when the buffalo were acutely infected, viz. in the first 7-10 days (33), a phenomenon previously encountered in South African experiments (115, 46). Secondly, in both experiments male buffalo and domestic cows were represented among the animals involved: in other similar experiments during which FMD viruses were not transmitted either the sexes of the animals is not clear or steers were used (25, 6, 15). This gives some support to speculation on possible sexual transmission by carrier buffalo.

Putative factors which may precipitate (“trigger”) transmission between carrier buffalo and cattle have been a subject of debate for many years in southern Africa, e.g. stress (62, 46) and “mucosal disease” (see above) but there is little foundation for such speculation at present.

8. CONCLUSION

Historical data derived principally from early European experience with FMD as well as more recent observations from southern Africa indicate that in rare instances carrier cattle are able to initiate outbreaks of FMD more than two years after becoming infected. African buffalo, which are more efficient carriers than cattle, are likewise rarely able to transmit SAT type viruses to susceptible cattle with which they come into direct contact. Mechanisms whereby transmission is effected by either cattle or buffalo remain uncertain. The possibility that sexual activity is involved should be investigated.

Persistent infection for varying periods has been identified in a number of other ruminant species, both domestic and free-living, but evidence that they have ever precipitated outbreaks of FMD is lacking. Among domesticated species water buffalo should be examined more carefully with respect to their ability to sustain persistent infection and perhaps transmit the virus.

Lack of information on the cells which support viral replication in the pharyngea of persistently infected ruminants and the replication strategy of persistent FMD viruses precludes an effective understanding of the carrier state in FMD. A more reliable diagnostic test for identifying persistent infection in the pharyngeal region of cattle is also an urgent requirement.

In some persistently infected buffalo, and this may be the case for cattle as well, significant antigenic change of the infecting virus sometimes occurs which could potentially render formerly effective vaccines less so in the event of a new outbreak being initiated by carriers.
REFERENCES


