# Chapter 1

## Literature Review

### 1.1 Introduction

In the late 1800's numerous researchers described the existence of pathogens that were able to pass through bacteria-proof filters. This included observations by Pasteur on the rabies-causing pathogen in 1887, Ivanovsky's studies of tobacco mosaic disease in 1886 and the ability of the foot-and-mouth disease agent to pass through Berkfeld filters, as documented by Loeffler and Frosch in 1898. These researchers all concurred with the opinion of Pasteur, who believed that microbes were the causative agents of these virulent affections. It was Martinus Beijerinck who described the infectious pathogens as a *contagium vivum fluidum* in 1898. He was the first to propose that these were self-reproducing, sub-cellular entities that did not conform with features characteristic to bacteria in that these microscopically invisible organisms were filterable, inactivated by heat, and could not be cultured *in vitro*. Beijerinck's revolutionary ideas were strongly opposed, but subsequently obtained support from numerous independent studies on novel pathogens and eventually led to the establishment of a new branch of biological science called virology. Nucleic acid made its entrance into virus research in 1934 and the development of the electron microscope in the 1930's permitted the first visualization of these previously invisible pathogens. The study of viruses and ease with which they could be manipulated paved the way for the numerous discoveries in the field of molecular biology. The superficially simple, yet inherently variable and adaptive nature of these entities and their power to shape history ensures that virology will remain a perpetually relevant and dynamic discipline.

### 1.2 Picornavirus Taxonomy

Virus classification is the area of virology that best reflects the dynamic nature of this field. The International Committee on Taxonomy of Viruses (ICTV) was established in 1966 to address the need for a single, universal taxonomic scheme for viruses. Taxonomic status of known as well as newly discovered viruses is constantly being reviewed by this body (Wildy, 1971; Fenner, 1976; Matthews, 1979, Matthews, 1982; Francki *et al*. 1991; Murphy *et al.* 1995; van Regenmortel *et al.*

2000; Fauquet & Mayo 2001) due to the rapid accumulation of data and discovery of new viruses. In the 6$^{th}$ ICTV report (Murphy *et al*. 1995) 1 order, 50 families and 164 genera were recognized, by 1998 and interim review on virus taxonomy (Mayo & Pringle 1998) identified 2 orders, 54 families and 184 genera. Just 2 years later, in the 7$^{th}$ ICTV report 3 orders, 63 families and 240 genera are recognized (van Regenmortel *et al*. 2000).

### 1.2.1 Picornavirus genera

Foot-and-mouth disease virus (FMDV) belongs to the picornavirus family, a diverse group of non-enveloped, small RNA viruses which include economically important pathogens of humans and animals. The family name *picornaviridae*, is derived from '*pico*' referring to their small size and '*rna*' referring to their RNA genomes. On the basis of genome size and organization, virus replication strategy and sequence homologies, the family is divided into six genera comprising *cardiovirus, aphthovirus, rhinovirus, hepatovirus, enterovirus* and *parechovirus* (Table 1.1). These genera are further divided into antigenically distinct 'serotypes', a virological category equivalent to the 'species' taxonomic unit. Common properties within a serotype or species include sequence homologies, serological relationships, host range, pathogenicity and geographical distribution. In accordance with the ICTV accepted definition of a species, '*a virus species is defined as a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche*' (Van Regenmortel 1990). Members of a polythetic class should have one or more properties of the group, but there is no unique group characteristic which excludes or defines membership. Due to the complexity and variability of viruses and the importance of the species hierarchical level it is generally agreed that virus families and genera are best described monothetically (by a few defining characters), whilst species are best defined polythetically (Van Regenmortel 1990). The ICTV does not classify viruses below the level of species.

Most picornaviruses are specific to one or a handful of host species, with the exception of *encephalymyocarditis virus* (EMCV), which has been isolated from over 30 species, and FMDV which is believed to be capable of infecting around 200 mammal species. Viral transmission is usually horizontal and does not involve arthropod vectors, although EMCV has been isolated from mosquito and tick species. Picornavirus genera vary vastly in the number of member serotypes. Some genera are monotypic, eg. *Parechovirus*, whilst others

have over 100 distinct species (summarized in Table 1.1).

**TABLE 1.1** Summary of the assigned *picornaviridae* genera and their species composition

| Genus | Total number of species per genus | Virus name | No of species / serotypes per virus type |
|---|---|---|---|
| *Aphthovirus* | 8 | *foot-and-mouth disease virus* | 7 |
| | | *equine rhinitis A virus* (formerly *equine rhinovirus type* 1) | 1 |
| *Cardiovirus* | 2 | *encephalomyocarditis virus* | 1 |
| | | *Theiler's murine encephalomyelitis virus* | 1 |
| *Enterovirus* | 98 | *bovine enterovirus* | 2 |
| | | *coxsackievirus A* | 23 |
| | | *coxsackievirus B* | 6 |
| | | *echovirus* | 30 |
| | | *human enterovirus* | 4 |
| | | *human poliovirus* | 3 |
| | | *porcine enterovirus* | 11 |
| | | *simian enterovirus* | 18 |
| | | *Vilyuisk virus* | 1 |
| *Hepatovirus* | 2 | *hepatitis A virus* | 1 |
| | | *simian hepatitis A virus* | 1 |
| *Parechoviru s* | 1 | *human parechovirus type* 1 (formerly *human echovirus type 22*) | 1 |
| *Rhinovirus* | 104 | *bovine rhinovirus* | 3 |
| | | *human rhinovirus* | 101 |
| | | **TOTAL** | 213 |

**Sources**: Murphy *et al.* 1995; Fauquet & Mayo 2001

### 1.2.2 Picornavirus morphology

Picornavirus virions are icosahedral with no envelope and contain one molecule of infectious, positive sense, single stranded RNA (ssRNA), ranging from 7-8.5 kb in length. A small protein Vpg which is encoded by the 3B genome region is covalently linked to the 5' end of the genome and a poly (A) tract of variable length is located at the 3' terminus. The 25-35 nm capsid is composed of 60 protein subunits or protomers, each consisting of four proteins, VP1-VP4 (Fig. 1.1). VP1-VP3 are exposed on the surface, whilst VP4 is located internally at the pentameric apex of the icosahedron and contains a myristic acid molecule attached to the amino terminal glycine (Chow *et al.* 1987; Minor *et al.* 1995). VP1, VP2 and VP3 share an eight-stranded antiparallel ß-barrel configuration and differ from each other primarily in the loops connecting these beta-barrel segments (Palmenberg 1989). None of the viral proteins are glycosylated and with the exception of *poliovirus*, the virions generally lack lipids.

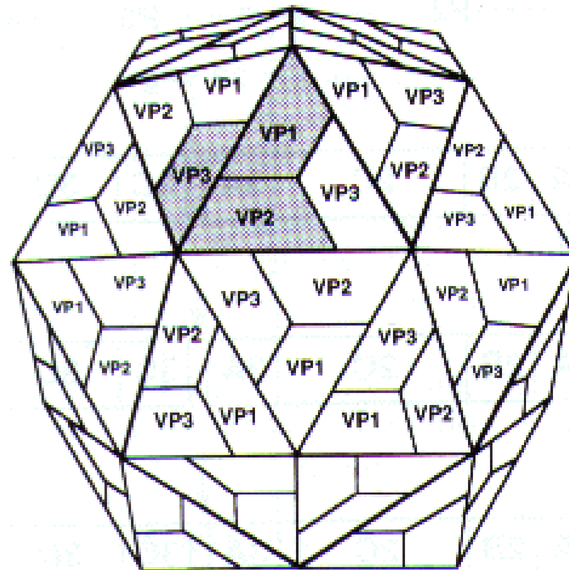


**Fig. 1.1** Diagram of the typical picornavirus icosahedral capsid showing the surface exposed VP1, VP3 and VP2 proteins. One of the 60 protomers, each of which contain an internalized VP4 protein is shaded. The pentameric apex about which the VP4 proteins are internally located, and the VP1 proteins are externally clustered, is indicated with a red arrow. Figure from Murphy *et al.* 1995.

### 1.2.3 Picornavirus genome organization and protein processing

Due to confusion arising from the naming of picornaviral proteins according to the molecular weight of the protein, the so-called L434 convention was adopted at the Europic `84 meeting held at Urbino, Italy to ensure universality of systematic nomenclature of viral proteins (Rueckert & Wimmer 1984). In accordance with the L434 diagram, L represents the leader protein, followed by the P1 region, which encodes four products A-D, which ultimately generate the VP1-4 coat proteins. Similarly, the P2 and P3 regions comprising three (A-C) and four (A-D) products respectively encode the non-structural proteins. FMDV differs from other picornaviruses in that it has 3 non-identical forms of Vpg encoded in the 3B genome region (Fig. 1.2). *Cardiovirus* and *aphthovirus* are the only picornavirus genera that have the L protein or leader proteinase. There are two in-frame translation initiation sites, resulting in two forms of the L protein, termed Lab and Lb in FMDV.

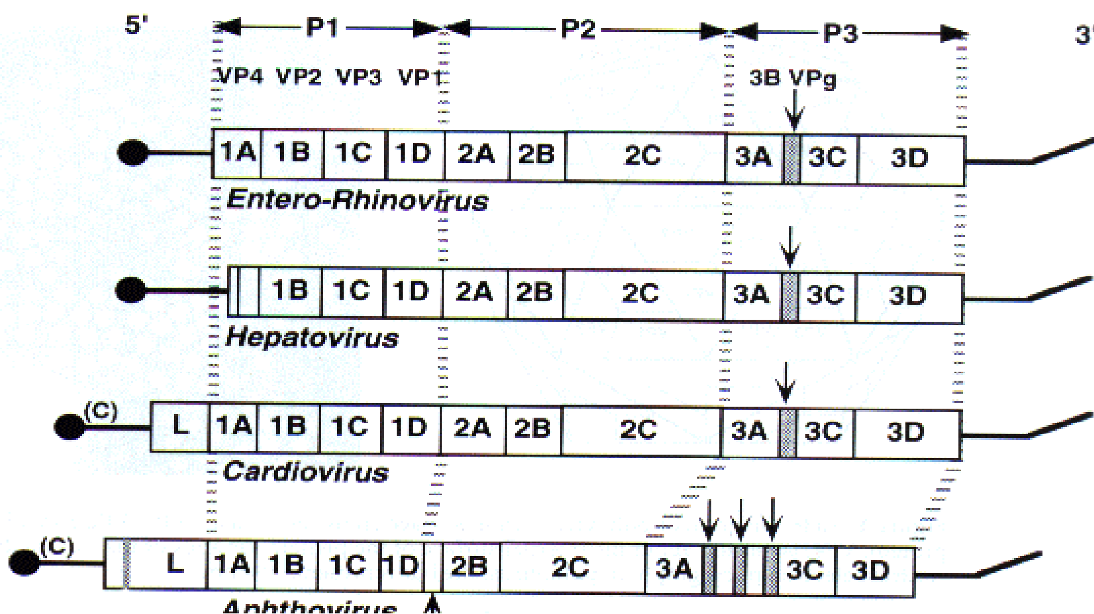


**Fig. 1.2** Genome organization of the *picornavirus* genera. Figure from Murphy *et al.* 1995.

## 1.3 *Foot-and-mouth disease virus*

FMDV is one of two members of the *aphthovirus* genus. The genus name is derived from the Greek word *aphtha* meaning 'vesicles in the mouth' and refers to the historical association of '*aphthovirus*' with foot-and-mouth disease (FMD) alone. Clinically FMD generally manifests as vesicles in the mouth and in the interdigital spaces and coronary bands of the hooves. Mortality in lambs, piglets and calves has been a major feature of some outbreaks. However, in older animals it rarely causes mortality. Virions are unstable below pH 6.5 and above pH 9, and have a buoyant density of 1.43-1.54 g/ $cm^3$ in CsCl. Seven immunologically distinct serotypes occur, namely types A, O and C, SAT types 1-3 and Asia-1. The classical, or European serotypes A, O and C were identified in the 1920's (Vallée & Carré 1922; Waldmann & Trautwein 1926). Recognition and typing of endemic African strains, the so-called SAT (South African Territories) types only commenced in 1948 (Brooksby 1958) and it was only in 1957 that a seventh serotype, Asia-1, was identified (Brooksby 1957). The different serotypes or FMD virus species differ genetically from each other by more than 50 % across the entire genome (Palmenberg 1989).

### 1.3.1 FMDV genome organization

FMDV has a single-stranded, positive sense RNA genome which is approximately 8400 bases long, and consists of a 5' non-coding region (NCR), a single large open reading frame and a short 3' NCR. It is polyadenylated on the 3' end and has a small virus encoded protein, Vpg covalently attached to the 5' terminus (Belsham 1993). The 5' NCR of FMDV is about 1300 nucleotides (nt) long, and far exceeds the length of other picornaviruses and typical cellular mRNAs which have 5'NCRs of 740nt and 50-100nt respectively. The first portion of the 5' NCR is termed the S fragment and is approximately 400 nt long. This is followed by the poly C tract, a homopolymeric tract of predominantly cytidyl residues which is 150-250nt long and which only occurs in *cardioviruses* and *aphthoviruses*. The last region of approximately 720 nt contains inverted repeats which are predicted to form pseudo-knots (Clarke *et al.* 1987). The internal ribosome entry site (IRES) which is immediately upstream of the first AUG initiation codon and is approximately 435 nt in length also occurs within this region (Belsham & Brangwyn 1990; Kuhn *et al.* 1990). The major portion of the FMDV genome consists of a single large open reading frame of 6996 nt encoding a polyprotein of 2332 amino acids (type O, Forss *et al.* 1984). Four distinct regions are distinguished for the polyprotein, namely the L, P1, P2 and P3. Another characteristic unique to FMDV is that there are three species of Vpg encoded by protein 3B, termed 3B1, 3B2 and 3B3. All encoded Vpg variants have been shown to be attached to the 5'

terminus of viral RNA (King *et al.* 1980).

### 1.3.2 Proteolytic processing of FMDV

The first protein processing event in FMDV is the L/P1 cleavage. Aphthovirus L proteinase cleaves co-translationally at its own C terminus and exists in at least two forms, $Lab^{pro}$ and $Lb^{pro}$, derived from initiation of translation at either of two in-frame AUG codons (Sangar *et al.* 1987). Both forms are able to cleave the L/P1 junction either in *cis* or in *trans* (Medina *et al*. 1993). Site-directed mutagenesis indicates that the L proteinases are thiol proteinases with the active site $Cys^{51}$ being conserved amongst FMDV and *equine rhinitis virus* (ERV) sequences (Piccone *et al.* 1995; Roberts & Belsham 1995). $L^{pro}$ also cleaves host-cell protein eIF-4G in *trans*, a feature it shares with $2A^{pro}$. This is a striking example of convergent evolution as the latter proteinase, although performing the same function, more closely resembles the small serine proteinase sub-class and has a different eIF-4G cleavage site to $L^{pro}$ (Kirchweger *et al.* 1994).

The second cleavage event occurs at the 2A/2B junction, and requires neither the L nor the 3C proteinases. It was shown that a 20 amino acid oligopeptide sequence corresponding to the FMDV 2A region and the N-terminal proline of 2B was able to mediate a co-translational cleavage (Ryan *et al.* 1991; Ryan & Drew 1994) and probably requires the three C-terminal residues (-NPG-) of 2A and the N-terminal residue (proline) of the 2B protein. These residues which are completely conserved are believed to be involved in 2A/2B cleavage do not conform to any known proteinase motifs. *Aphthovirus* polyprotein cleavage occurs between the C terminus of the 2A region and the capsid protein precursor (P1-2A), rather than between P1 and 2A as is the case with most other picornaviruses.

The 3C protease mediates P1 cleavage to produce 1AB (VP0), 1C (VP3) and 1D (VP1). After post-translational processing of the P1-2A, a myristate moeity is attached to the N-terminus of 1AB which appears to be critical for capsid stability (Chow *et al.* 1987). Cleavage of the capsid protein precursor, 1AB, encoding the VP0 protein (VP4 + VP2) only occurs on encapsidation of the virion RNA to form virus particles. This step is often referred to as ‘maturation cleavage’ is it is only at this stage that the four-chain subunits (VP1-VP4) are formed.

### 1.3.3 FMDV morphology

Amino acid alignment of FMDV with other picornaviruses, indicates that the loop regions connecting the beta-barrels in the VP1 and VP2 genes of foot-and-mouth disease virus are truncated (Palmenberg 1989), particularly in the regions contributing to the pentameric apex of the virion, where poliovirus and rhinovirus have major antigenic sites (Minor 1990). The atomic stucture of serotype $O_1$ BFS1860 (Acharya *et al.* 1989; Acharya *et al.* 1990) also indicates there is no pit or canyon in FMDV as is observed for other picornavirus genera capsids. There are two disordered regions within the VP1 gene that are exposed on the surface. The first known as the G-H loop extends from residues 133 to 158, and the second from residues 210-213 of serotype O viruses (Acharya *et al.* 1990; Logan *et al.* 1993). The C-terminal residues of VP1 within which this disordered region occurs runs clockwise over the virion surface from VP1, over VP3 to VP1 in the adjacent protomer where it lies close to the G-H loop. These regions correspond to those previously shown to be able to induce neutralising antibodies (Bittle *et al.* 1982; Strohmaier *et al.* 1982). Peptides corresponding to the VP1 gene G-H region of other serotypes inclusive of type A, O, C and SAT-2 strains were also shown to induce neutralizing antibodies to the homologous strain (Clarke *et al.* 1983). Disuphide bridges between the protomers of serotype O in which Cys-130 of VP2 is linked to Cys-134 of VP1 are known to occur in type O viruses, but not in serotype A viruses. Reduction of disulphide bonds favours a more disordered G-H loop structure (Parry *et al.* 1990).

Intact virus particles contain a single copy of genomic RNA, whilst virions lacking RNA are referred to as empty virus particles. The whole virus particle, empty virus particle and pentameric protein subunits have sedimentation coefficients of 146S, 75S and 12S, respectively. The 146S and 75S fraction elicit an immune response, whereas the presence of 12S protein resulting from disassembly of the virion by mild heat or acid treatment, does not induce neutralizing antibodies. 146S determination is therefore a critical part of vaccine production as it provides a measure of the number of intact virus particles which are able to evoke neutralizing antibody and so confer immunity.

### 1.3.4 FMDV receptor binding

Cell receptors are important determinants of viral host range and cell and tissue tropism. It is known that trypsin treatment abolishes FMDV infectivity (Brown *et al.* 1963) and reduces immunogenicity (Wild *et al.* 1969) and that the trypsin cleavage site is located within the major neutralization antigenic site in the VP1 gene spanning residues 140-160 (Bittle *et al.* 1982, Strohmaier *et al.* 1982). This portion of VP1 contains a highly conserved Arg-Gly-Asp (RGD) site which binds many extracellular ligands to integrin cell surface receptors (reviewed by Ruoslahti & Pierschbacher 1987). Early studies showed that RGD is important for the interaction of FMD virus with cellular receptors (Baxt & Becker 1990) and that SAT, A, O and C viral subtypes use different cellular receptors (Baxt & Barach 1982, Sekiguchi *et al.* 1982). The use of multiple receptors was recently confirmed when it was shown that FMDV is able to ultilize two distinct types of cells surface receptors, namely the heparan sulphate proteoglycans (HSPG; Jackson *et al*. 1996) and the RGD-dependent integrins, $\alpha_v\beta_3$ (Neff *et al.* 1998), $\alpha_v\beta_1$ (Jackson *et al.* 2000a) and $\alpha_v\beta_3$ (Jackson *et al.* 2000b). The $\alpha_v\beta_3$ and HSPG receptors are associated with virulence and loss of virulence following cell culture adaptation, respectively.

## 1.4 Genetic variation

The observed genetic variation in the FMD viral genome is the result of a two step process. Firstly, the replication of viral RNA is error-prone due to the absence of proofreading in the 3D-encoded RNA-dependent RNA polymerase. Secondly, competitive selection is continuously acting on the genome. Thus those mutants with a selective advantage in the prevailing environment, will be better represented than those with a selective disadvantage.

### 1.4.1 Mutation

The different genome regions of European serotype viruses accumulate mutations at between 0.9 x $10^{-2}$ and 7.4 x $10^{-2}$ substitutions per nucleotide per year (s/n/y) in persistently infected cattle (Gebauer *et al.* 1988). A study of the VP1 protein coding region of SAT-type viruses indicates that mutations become fixed in this portion of the genome at a rate of between 1.54 x $10^{-2}$ and 1.64 x $10^{-2}$ s/n/y in persistently infected African buffalo (Vosloo *et al.* 1996). FMD viral genes therefore evolve around $10^{6}$ times faster than nuclear eukaryotic genes due to increased misincorporation events, with roughly one substitution error occurring per genome replication cycle. This gives rise to a whole population of phylogenetically related FMD variants within a

single infected individual in accordance with the quasispecies concept (Domingo *et al.* 1992; Domingo *et al.* 1995 and references therein).

### 1.4.2 Selection

One of the major evolutionary mechanisms employed by RNA viruses is the prolific mutant production, detailed above. The immune system of an infected animal, which presumably provides a powerful selective force is another driving force in viral evolution. Evidence that mutations accumulate in the absence of immune selection (Diez *et al*. 1990) seem to accord with the neutral theory of molecular evolution and there has been much debate on whether FMDV evolution is driven by selection or whether the neutral theory applies.

The high mutation rate of FMDV makes this virus an ideal candidate for examining the molecular clock hypothesis, which is intrinsic in the neutral theory of molecular evolution. According to this theory, mutation rates for a given molecule would be equal for all organisms at all times. In contrast, positive Darwinian evolution would result in some sites within a molecule being more likely to change than others due to selective forces. In the latter case, a clocklike behaviour would not be observed. Two independent studies on field and laboratory strains indicate that selective forces are indeed working at sites within the immunogenic region of the VP1 protein (Fares *et al.* 2001; Haydon *et al.* 2001), although this was not supported by all datasets examined. These results indicate that antigenic variants benefit from a selective advantage in their interaction with antiviral antibodies produced by the host immune system.

### 1.4.3 Recombination

Recombination is another important process driving viral biology and evolution. In RNA viruses, recombination involves the exchange of genetic material between two nonsegmented RNA genomes resulting from polymerase ‘jumping’ during RNA synthesis. Polymerase jumping between different RNA templates is believed to be facilitated by enzyme pausing at sites of strong secondary structure (Wilson *et al.* 1988) and is consistent with the observation of RNA intermediates and the discontinuous nature of both DNA-dependent and RNA-dependent RNA synthesis in experimental systems (Lai 1992). Homologous recombination in RNA viruses (the exchange of two comparable genome regions) was first recognized in the family *picornaviridae*, in *poliovirus* (Hirst 1962) and FMDV (Pringle 1965) with reports in other RNA virus families,

such as coronavirus, following later (Lai *et al.* 1985).

Intratypic recombination occurs more frequently than intertypic recombination and it appears that recombination events in FMDV occur more readily in the 3' half of the genome, than in the capsid genes of FMDV (King *et al.* 1985; King *et al.* 1988). This is probably due to the higher degree of local secondary structure in the C-terminus half of the genome, promoting polymerase pausing (Wilson *et al.* 1988) and to the higher levels of sequence homology between non-structural genes as opposed to structural genes. It was also shown that recombination can involve single or multiple crossover events when two viruses of the same serotype co-infect cell cultures (King *et al.* 1982). Following demonstrations of intertypic recombination *in vitro* (McCahon *et al.* 1985; Giraudo *et al.* 1988), Krebs and Marquardt (1992) identified and characterised the first type O-C intertypic recombinant, from the field.

Recombination is not a frequent occurrence in most RNA viruses and it is unclear why picornaviruses and coronaviruses have such a high frequency of homologous recombination, but this process clearly provides an evolutionary advantage to these viruses. At its most extreme, recombination can give rise to a novel RNA virus following exchange of genetic material between two completely unrelated viruses in a naturally infected host. An example of this is *western equine encephalitis virus*, which appears to be derived from a crossover event between *Sindbis virus* and *eastern equine encephalitis virus* (Hahn *et al.* 1988). Recombination poses a real threat when attenuated vaccines are used, as reversion to virulence following natural infection of a vaccinated individual is likely given the high recombination frequency in FMDV.

### 1.5 Antigenic variation

One of the consequences of genetic variation through mutation, selection and recombination is that new antigenic variants are constantly being generated. In contrast to *poliovirus* where antigenic variation within a serotype seems to be of minimal epidemiological importance (Minor 1990), given that the existing vaccine strains function effectively despite being over 40 years old, antigenic variation within *aphthovirus* serotypes is extensive and therefore a critical factor in vaccine strain selection (Rweyemamu & Hingley 1984; Mateu *et al.* 1988). Not only is there no cross-protection between the seven FMDV serotypes (Brooksby 1982), but vaccination with one antigenic variant of a serotype does not necessarily protect an animal when challenged with a different virus of the same

serotype (Cartwright *et al.* 1982). Attempts to characterize the extent of the antigenic variation within a FMDV serotype led to the establishment of techniques whereby viral subtypes could be identified (Brooksby 1968). Initially over 60 different subtypes were identified by the World Reference Laboratory (WRL), but it quickly became apparent that there is a continuous spectrum of intratypic antigenic variants, making it difficult to identify specific subtypes (Pereira 1977). Subtype classification was therefore abandoned. Instead, the practical significance of the r-value was recognized (Rweyemamu *et al.* 1977) and is used as a measure of the suitability of a vaccine strain for a given outbreak situation, where r is:

$$r = \frac{\text{Activity of serum against the heterologous virus (field / outbreak strain)}}{\text{Activity of serum against the homologous virus (vaccine strain)}}$$

Thus a suitable vaccine strain is indicated by an r value greater than 0.4, whilst $r < 0.4$ indicates a poor antigenic relationship between the outbreak and vaccine strain. These cut-offs are based on the good correlation between the serum neutralizing antibody titre and protection from challenge in cattle (Rweyemamu *et al.* 1977).

### 1.5.1 Neutralizing sites of FMDV

Neutralizing monoclonal antibodies (MAb) have been used to map the antigenic sites involved in neutralization of FMDV. Most studies have focussed on the European serotypes (Robertson *et al.* 1984; Xie *et al.* 1987; Thomas *et al.* 1988; Barnett *et al.* 1989; Baxt *et al.* 1989; Kitson *et al.* 1990; Saiz *et al.* 1991; Crowther *et al.* 1993a; Lea *et al.* 1994; Aktas & Samuel 2000), with limited reports on neutralization sites of the Asia-1 serotype (Butchaiah & Morgan 1997; Marquardt *et al.* 2000) and the SAT-type viruses (Crowther *et al.* 1993b). Detailed antigenic profiles of the SAT-1 and SAT-3 serotypes have not been reported.

**TABLE 1.2** Summary of immunogenic sites identified in the surface-exposed structural protein genes of different foot-and-mouth disease virus serotypes

| Serotype | Antigenic sites; P1 gene | References |
|---|---|---|
| A | G-H loop; VP1<br>C-terminus region; VP1<br>H-I loop; VP1<br>B-C loop; VP1<br>B-C loop; VP2 | Robertson *et al.* 1984<br>Thomas *et al.* 1988<br>Baxt *et al.* 1989<br>Saiz *et al.* 1991 |
| O | G-H loop & C-terminus region; VP1 (Site 1)<br>B-C loop & E-F loop; VP2 (Site 2)<br>B-C loop; VP1 (Site 3)<br>VP3 (Site 4)<br>B-C loop; VP2<br>B-C loop (VP2) & G-H loop (VP1) | Xie *et al.* 1987<br>Barnett *et al.* 1989<br>Kitson *et al.* 1990<br>Crowther *et al.* 1993a<br>Aktas & Samuel 2000 |
| C | G-H loop; VP1 | Lea *et al.* 1994 |
| Asia-1 | G-H loop; VP1<br>B-C-loop; VP2<br>N-terminus; VP2<br>B-B knb; vp3 | Butchaiah & Morgan 1997<br>Marquardt *et al.* 2000 |
| SAT-2 | G-H loop; VP1<br>(amino acid positions 149, 156 & 158) | Crowther *et al.* 1993b |

Most epitopes mapped to loops connecting the ß-strands of the three surface exposed proteins. All studies confirmed that the major antigenic determinants of FMDV are situated in the VP1 gene, of which the G-H loop is the one of the most important neutralizing sites of the virus (Table 1.1). This region, spanning amino acid positions 140-160, protrudes from the virion surface and is highly exposed and mobile. The loop is critical for the process of receptor binding (via the highly conserved RGD motif) as well as being involved in neutralization of viral infectivity. Despite being a target for neutralizing antibodies, the RGD remains intact and experimental modification of amino acid sites in this region are often lethal (Mason *et al.* 1994; Mateu *et al.* 1996; Leipert *et al.* 1997). However, a serotype C escape mutant shown to contain

an RGG instead of the RGD, was viable in BKH-21 cells and antigenically distinct from the parent strain (Ruiz-Jarabo *et al.* 1999), leading to the conclusion that the mutant could enter the cells via an alternative receptor, other than through the RGD-binding integrin receptor, and that single amino acid replacements within the G-H loop, including the receptor recognition site can have profound effects on antigenicity. This finding supports the view that antigenic variation arises through two mechanisms. Firstly, a gradual increase in antigenic distance can be brought about by a steady accumulation of amino acid replacements within site A (G-H loop) of VP1 (antigenic drift), or alternatively, a rapid change can occur following an amino acid substitution at a critical site in the G-H loop (Martínez *et al.* 1991 and references therein).

## 1.6 Geographical distribution of FMD

FMDV has an essentially global distribution, with the exception of North America, Western Europe and Australia. The European serotypes occur in South America, the Caucasus, Africa, the Middle East and in Asia. Of these, serotype C occurs infrequently, with most outbreaks being due to serotype O (Kitching 1998 & 1999). Asia-1 is limited to the Asian continent and to the Middle East, whilst the SAT-types occur exclusively in sub-Saharan Africa, although rare incursions into other regions have been recorded. This includes the introduction of SAT-2 for the first time into Saudi Arabia and Kuwait in April and May, respectively, in 2000. Similarly, Asia-1 which has a restricted distribution was recorded for the first time in Greece in July 2000 (http://www.oie.int)

Until 2001, western Europe was free of the disease following intensive vaccination campaigns which ceased in 1991. Threats to disease security are however posed by regular outbreaks of the disease in southern and eastern Europe. These include the 1991, 1993, and 1996 outbreaks in Bulgaria, serotype O in Italy, Greece and Turkey in 1993, 1994 &1996 and 1995 & 1996, respectively. The threat posed to Europe by the close proximity and intensive trade in livestock in the Middle East where types O and A are endemic and where Asia-1 and SAT-2 have also been recorded is well-recognized. Of particular concern is the recent introduction of an exotic FMD serotype, Asia-1, into Greece. More recently, the Pan-Asian serotype O virus responsible for outbreaks throughout the Middle East, south-east Asia and South Africa (Knowles *et al.* 2000), was introduced into Great Britain. Prior to this, the UK had been free of the disease for over 30 years, with the last recorded epizootic occurring in 1967/8.

### 1.6.1 FMD serotype distribution Africa

FMD is endemic in sub-Saharan Africa, but absent from Madagascar (Kitching 1998). Six of the seven serotypes have been recorded on the continent (Brooksby 1972). Types A and O are widespread throughout sub-Saharan Africa, whilst type C occurs rarely (Fig. 1.3). The endemic SAT-types occur predominantly in southern and eastern Africa. SAT-1 and SAT-2 also circulate in West Africa and are the only serotypes to have made incursions into the Middle East. In North Africa serotype O is endemic in Egypt and Libya, but sporadic outbreaks occur in other countries within this region. Type O was introduced into Tunisia from the Middle East in 1989 from where it subsequently spread to Algeria and Morocco. Repeated incursions into Tunisia occurred in the 1990's presumably from Egypt and Libya (Samuel *et al.* 1999). The epidemiological situation is particularly complex in Kenya where numerous outbreaks due to serotypes SAT1, SAT2, O, A and C have been reported following the breakdown of the veterinary control programme. No other country has as wide a range of serotypes in circulation (Kitching 1998).

Due to poor reporting from the African continent, FMD is considered endemic in most African countries with only Morocco (based on serological surveillance), Swaziland, Lesotho, Zimbabwe, Namibia, Botswana and the Republic of South Africa being considered free of the disease by the OIE in 1999 (Kitching 1999). Reports of FMD in Africa from 1996-1999 point to an upward trend in the number of outbreaks (Table 1.3). These figures are unlikely to provide a true reflection of the situation in Africa, due to problematic reporting, but never-the-less represent the only official measure of the impact of this disease on the continent. The upward trend noted for Africa is reflected globally by an increase in outbreaks occurring in countries previously free of the disease. These include the type O outbreaks recorded in 1997 in Taiwan, in 2000 in Japan and in 2001 in the United Kingdom. Prior to this, these countries had been free of the disease for 68 years, 92 years and 32 years, respectively.

### 1.6.2 Foot-and-mouth disease in southern Africa

Clinical foot-and-mouth disease in cattle in southern Africa was first documented by Le Valliant in 1795 and recorded on numerous occasions in the 19$^{th}$ century by Kruger and Cummings, prior to the arrival of the rinderpest panzootic in southern Africa in 1896 (Bengis *et al.* 1987, Thomson 1994 and references therein). The decimation of cattle and wildlife by the rinderpest epidemic is believed to have led to the temporary disappearance of a large number of ungulate

diseases (Rossiter 1994), including FMD. After an absence of over 30 years, an outbreak was reported in 1931, in Zimbabwe for the first time in the 20th century. Shortly thereafter epizootics occurred in Botwana, South Africa and Zambia, after which FMD outbreaks became a regular occurrence in most southern African countries. The disease continues to threaten the agricultural trade and economic development in this African sub-region.

Six of the seven FMDV serotypes have been recorded in southern Africa. All three SAT-types occur in six of the ten southern African countries, namely South Africa, Zimbabwe, Namibia, Zambia, Botswana and Malawi. SAT-3 has not been recorded in Mozambique or Angola, but has been isolated from African buffalo in Uganda on two separate occasions (Hedger *et al.* 1973; Records of the Onderstepoort Veterinary Institute). The classical or European serotypes A and O (and C on rare occasions) have been identified in the Mozambique and Angola, in northern Namibia, Malawi and more recently in South Africa (Fig. 1.3). The latter serotypes are believed to be exotic to the southern African region, since unlike the SAT-types, antibodies to these classical serotypes do not occur in wildlife (Thomson 1994). Retrospective analysis of type O viruses causing outbreaks in Angola confirms that these outbreaks were due to introduction of the virus from either Europe or South America rather than from an endemic source (Sangare *et al.* 2001) as do studies on serotype A viruses from Malawi and Angola (Knowles *et al.* 1998). The situation in southern Africa contrasts with that in East Africa where type O and A viruses are representative of regional, endemic African genotypes, as indicated by molecular epidemiological studies (Knowles *et al.* 1998; Sangare *et al.* 2001). Furthermore, type O antibodies have been recorded in buffalo in Kenya (Anderson *et al.* 1979) and Uganda (Hedger *et al.* 1973). The latter reports should however be treated with caution as non-specific reactions have previously been recorded against type O in southern African buffalo populations (Condy *et al.* 1969; Records of the Onderstepoort Veterinary Institute).

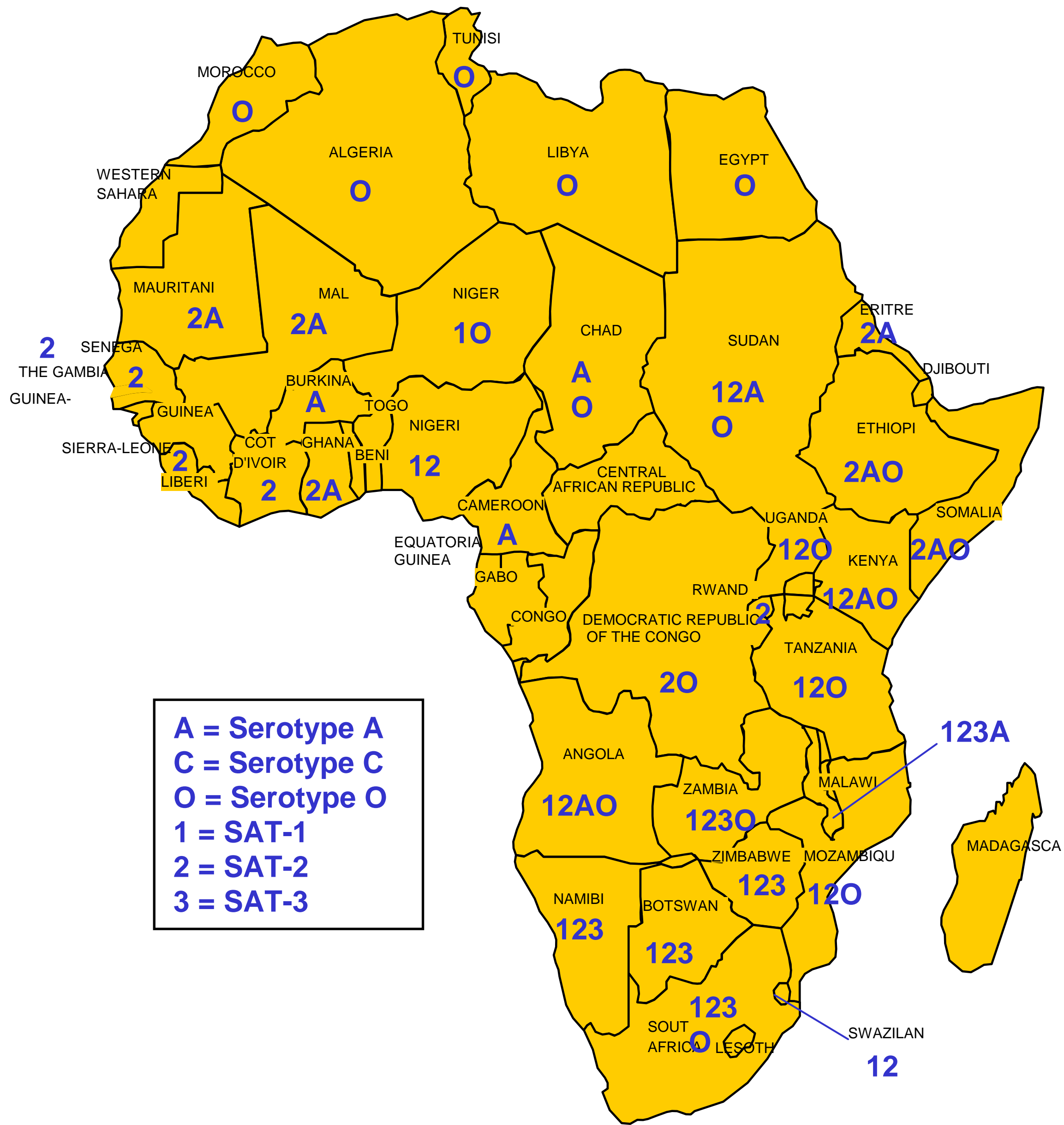


**Fig. 1.3** Distribution of FMDV serotypes in Africa

**TABLE 1.3** Number of FMD outbreaks reported yearly to the OIE, by African countries (1996-1999)

| Country / Territory | 1996 | 1997 | 1998 | 1999 | Total by country, 1996-1999 |
|---|---|---|---|---|---|
| Algeria | -- | 0 | 0 | 165 | 165 |
| Angola | -- | -- | 0 | -- | 0 |
| Benin | 20 | 23 | 3 | 59 | 105 |
| Botswana | -- | 0 | 0 | 0 | 0 |
| Burkina Faso | 56 | 20 | 35 | 90 | 201 |
| Burundi | -- | -- | -- | (+) | 0 |
| Cameroon | 2 | 5 | 3 | 10 | 20 |
| Cape Verde | -- | 0 | 0 | -- | 0 |
| Central African Republic | (+) | (+) | -- | -- | 0 |
| Chad | 42 | 4 | 30 | 18 | 94 |
| Comoros | -- | 0 | 0 | 0 | 0 |
| Congo (Democratic Rep of the) | -- | 1 | (+) | -- | 1 |
| Côte d'Ivoire | 7 | 7 | (+) | 1 | 15 |
| Egypt | -- | 1 | 0 | 0 | 1 |
| Eritrea | (+) | 4 | 27 | 3 | 34 |
| Ethiopia | 22 | 12 | 75 | 198 | 307 |
| Gabon | -- | 0 | 0 | 0 | 0 |
| Gambia | -- | 12 | (+) | (+) | 12 |
| Ghana | 58 | 33 | 60 | 22 | 173 |
| Guinea | -- | 0 | 0 | 9 | 9 |
| Kenya | 3 | 0 | 105 | 139 | 247 |
| Lesotho | -- | 0 | 0 | 0 | 0 |
| Libya | -- | 0 | 0 | 0 | 0 |
| Madagascar | -- | 0 | 0 | 0 | 0 |
| Malawi | -- | 0 | 1 | 0 | 1 |
| Mali | 8 | 3 | 9 | 18 | 38 |
| Mauritania | (+) | 8 | 1 | 4 | 13 |
| Mauritius | -- | 0 | 0 | 0 | 0 |
| Morocco | -- | 0 | 0 | 11 | 11 |
| Mozambique | 0 | 0 | 0 | 0 | 0 |
| Namibia | -- | 0 | 0 | 0 | 0 |
| Niger | 70 | 84 | 13 | 19 | 186 |
| Nigeria | -- | 3 | -- | 8 | 11 |
| Reunion (France) | -- | 0 | 0 | 0 | 0 |
| Rwanda | -- | (+) | (+) | -- | 0 |
| Sao Tome and Principe | -- | -- | -- | 0 | 0 |
| Senegal | 5 | 28 | 7 | 4 | 44 |
| Seychelles | -- | 0 | 0 | -- | 0 |
| South Africa | -- | 0 | 1 | 0 | 1 |
| Sudan | -- | 0 | 0 | 0 | 0 |
| Swaziland | -- | 0 | 0 | 0 | 0 |
| Tanzania | 105 | 55 | 19 | 287 | 466 |
| Togo | -- | 10 | -- | -- | 10 |
| Tunisia | -- | 0 | 0 | 2 | 2 |
| Uganda | 20 | 1 | 8 | 15 | 44 |
| Zambia | 1 | 1 | 0 | 4 | 6 |
| Zimbabwe | -- | 1 | 0 | 2 | 3 |
| **Total by year** | **419** | **316** | **397** | **1088** | **2220** |

1996: 14 countries reported FMD outbreaks; 1997: 21 countries reported FMD outbreaks; 1998: 16 countries reported FMD outbreaks; 1999: 22 countries reported FMD outbreaks

## 1.7 FMD in wildlife in southern Africa

One of the unique aspects of the disease in southern Africa is the role of wildlife in the epidemiology of FMD. Extensive serological surveys in which 3163 sera from 47 species were tested for antibodies to the three SAT-type viruses revealed that 18 artiodactyl species possessed antibodies to one or more serotype (Condy *et al.* 1969; Hedger 1976). In these and in subsequent studies the African buffalo (*Syncerus caffer*) consistently recorded the highest infection rates and antibody titres. Most buffalo populations in southern Africa are infected with the three SAT-type viruses, with the exception of those in the southern most distributional range, at the Hhluhluwe-Umfolozi game reserves (Esterhuysen *et al.* 1985) and Addo Elephant National Park. The high seropositivity rates in buffalo (approximately 80 %) together with the overlap in buffalo distribution and historical outbreaks of the disease (Thomson 1994) points to the role of this species in the epidemiology of the disease.

### 1.7.1 FMD in buffalo

Buffalo are believed to be the ultimate source of infection for livestock in southern Africa due to their ability to both maintain and transmit the disease. FMDV can persist in an isolated herd of buffalo for up to 24 years, whilst an individual animal can maintain the infection for up to 5 years (Condy *et al.* 1985). Furthermore, buffalo have unequivocally been shown to be the source of infection for cattle under both natural (Dawe *et al.* 1994a) and experimental conditions (Dawe *et al.* 1994b; Vosloo *et al.* 1996). However, transmission from persistently infected buffalo is believed to be an extremely rare event and numerous attempts to demonstrate transmission under experimental conditions have failed (Condy & Hedger 1974; Anderson *et al.* 1979; Hedger & Condy 1985; Bengis *et al.* 1986 ). Even acutely infected buffalo are not always capable of transmitting SAT viruses to cattle (Gainaru *et al.* 1986). The mechanism facilitating SAT-type virus transmission from persistently infected buffalo to cattle remains obscure, however transmission of virus from buffalo appears to occur readily when:

- There is close contact between the two species
- Buffalo are in an acute stage of infection and shedding large amounts of virus

FMDV is not transmitted vertically, however horizontal transmission occurs, with buffalo calves becoming infected for the first time at around 3-8 months after the waning of maternally-derived immunity (Condy & Hedger 1978). Due to synchronized breeding and calving of buffalo, large

numbers of calves will become infected at around the same time and shed large amounts of virus during the acute stage of infection (Gainaru *et al.* 1986). This results in environmental contamination and provides a potential source of infection for other susceptible cloven-hoofed species.

### 1.7.2 FMD in impala

In South Africa there is a regular cyclic occurrence of FMD epizootics (approximately every two years) in impala antelope, with sometimes severe signs of clinical infection. This contrasts with other southern African countries where clinical disease is not noted in these antelope and where they are not believed to play a significant role in the epidemiology of the disease (Anderson *et al.* 1993). Although studies have established that individual impala do not become carriers (Hedger *et al.* 1972; Anderson *et al.* 1975), it appears that the disease can persist in impala populations for between 6 and 13 months (Vosloo *et al.* 1992; Keet *et al.* 1996). The extended circulation of FMD virus in this antelope species has only been recorded in recent years in the southern half of the Kruger National Park, an area where impala population density is extremely high. Another unusual feature of the disease in impala in South Africa is that prior to 1982, most outbreaks were caused by SAT-1 virus, yet from 1982 until 1996, all outbreaks in impala were due to SAT-2 type viruses. This trend was recently halted by the recording of a SAT-1 outbreak in impala in 1998 (Records of the Onderstpoort Veterinary Institute).

Impala are particularly susceptible to the aerosol route of infection. In contrast to cattle and sheep which require 25 and 10 cell culture infective doses, respectively, for infection via the respiratory route, impala become infected on exposure to just one cell culture infection dose (Gainaru *et al.* Cited by Thomson 1994). Once infected, these antelopes secrete high levels of FMD virus 1-3 days prior to and 7-14 days after the development of lesions. They have been shown to precipitate infection in buffalo (Vosloo *et al.* 1996) and are also readily infected by buffalo shedding virus (Hedger *et al.* 1972), despite the two species not being in close proximity to each other.

The possibility that these antelope act as intermediaries in disease transmission was recognized in 1962 when clinical cases of FMD in impala were used as an indicator for predicting outbreaks in livestock (Meeser 1962). More recently when the risk posed by African buffalo to cattle in

close proximity to wildlife conservancies was assessed by computer simulated scenario-pathway analyses (Sutmoller *et al.* 2000), it was shown that infected antelope are capable of jumping the outer perimeter fence of a conservancy posed the greatest annual risk of FMD infection to cattle (1:5000). Impala and kudu (*Tragelaphus stresiceros*) antelope are the two species most likely to pose a threat due to their FMD infection rates and to their ability to clear perimeter game fences.

### 1.7.3 FMD in other antelope species

A number of antelope species have been experimentally infected with SAT-type viruses in order to determine carrier status, routes and amount of virus excreted as well as measuring antibody response, so that experimental and field data can be correlated. Of the species studied only kudu antelope (*Tragelaphus strepsiceros*) were conclusively shown to be persistently infected, with the carrier state of between 106 to 140 days being demonstrated (Hedger *et al.* 1972). Studies on eland (*Taurotragus oryx*), sable (*Hippotragus niger*) and wildebeest (*Connochaetes taurinus*) were either contradictory, or alternatively unable to show virus recovery past 28 days (Hedger *et al.* 1972; Anderson *et al.* 1975; Anderson 1980; Ferris *et al.* 1989).

### 1.7.4 FMD in *Suidae*

Experimental infection of warthog (*Phacochoerus aethiopicus*) and bush pig *(Potamochoerus porcus*) with SAT-2 type virus resulted in severe clinical signs of infection, antibody response to SAT-2 and transmission to in-contact animals. However no carrier status was demonstrated, nor did interspecies transmission of a SAT-1 from other wildlife species to these species occur. Antibody titres persisted past 300 days in warthog, but were low and of short duration (less than 100 days) in bush pig (Hedger *et al.* 1972). These results together with a lack of serological field data indicate that the bush pig is unlikely to play a role in virus maintenance or disease transmission in southern Africa.

### 1.7.5 FMD in elephant

The first case of FMD in an Indian elephant (*Elephus maximus*) was reported in 1935 (Ramiah 1935), but no virological data to support the circumstantial and clinical signs was presented until 1976, when natural infection of an Indian elephant with type O virus was detailed (Pyakural *et al.* 1976). The first confirmed report of FMD in African elephants (*Loxodonta africana*), also involved a European serotype ($A_7$ strain) and affected 15 elephants in a circus in Italy (Piragino

1970). Despite these isolated reports, negative results have consistently been obtained from serological surveys conducted on African elephants and no clinical cases of the disease have ever been reported on the African continent (Howell *et al.* 1973). Furthermore, elephants experimentally infected with SAT-2 type virus failed to react clinically or serologically (Hedger *et al.* 1972). These conflicting reports prompted the experimental infection of 9 African elephants in order to assess their role in the epidemiology of the disease in Africa. The results showed that the pathogenesis of the disease is similar to that observed for other susceptible animals. Elephants developed severe clinical signs of infection, were viraemic for up to 6 days and shed large amounts of virus, however, transmission to other elephants in close contact could not be demonstrated and antibody levels were extremely low (Howell *et al.* 1973). These results together with the lack of field evidence indicates that elephants are unlikely to play an important role as a natural host in the spread of FMD in the enzootic regions of Africa.

**1.8 FMD as a zoonosis**

Given the concern arising in the public mind following an outbreak of FMD, it is pertinent to mention that FMD is generally not believed to be a zoonosis in man. However a recent review of all available literature reveals that FMD has been recorded on rare occasions, with approximately 40 confirmed cases being reported in humans (Bauer 1997). Clinical signs vary from asymptomatic to severe, with blisters in the mouth and on the hands and feet. However healing is rapid. The virus types most frequently isolated from humans are type O, followed by type C and rarely type A. Antibodies to FMD are of short duration (< 16 weeks) and reach a peak at around 3 weeks after infection (Reviewed by Bauer 1997). Although humans are not readily infected with FMDV, it should be stressed that they can play a role as intermediaries in disease transmission, as demonstrated by the case of a non-infected individual that retained virus in his nasal passages for more than 24 hours before transferring the disease to susceptible cattle (Sellers *et al.* 1971).

## 1.9 The role of carriers in the epidemiology of FMD

The existence of a carrier state in cattle was first demonstrated by van Bekum and co-workers (1959). Carriers are defined as those animals from which virus can be isolated from the pharyngeal area more than 28 days after infection (Sutmoller *et al.* 1968). Although it is well established that FMD virus persists in buffalo (up to 5 years), cattle (up to 3 years), sheep (up to 9 months) and goats (for between 3-6 months), the mechanisms underlying persistence and the immunological pathways that eventually lead to viral clearance are not well understood. Host variation is however likely to play a role as different breeds of sheep and goats display differential abilities to act as carriers of the FMD virus (McVicar & Sütmoller 1968; Anderson *et al.* 1976). Of the livestock infected by FMD only pigs are believed to be incapable of establishing a persistent infection, although one recent isolated report has indicated that persistence in swine is possible (Merzencio *et al.* 1999). The mechanism of persistence is likely to depend on a number of factors including, viral replication (cytolytic versus lysogenic), target cell, host and strain variability.

### 1.9.1 Persistence *in vitro*

FMDV is usually cytolytic in cell cultures, but persistent infections have also been established in BHK-21 cells (De la Torre *et al.* 1985), resulting in multiple genetic and phenotypic variations in the persistent viral strain. Upon establishment of a persistent infection *in vitro*, it was shown that cells were resistant to superinfection with FMD viruses of the same or of different serotypes, but not to infection by unrelated viruses such as *encephalomyocarditis virus* (EMCV; De la Torre *et al.* 1985). It was later shown that it is not only the viruses that change during persistent infection, but the cells themselves undergo marked changes in morphology, resistence to infection and in growth characteristics (De la Torre *et al.* 1988; De la Torre *et al.* 1989). This coevolution between the persistently infected BHK-21 cells and the resident virus, was shown to produce viral variants of increased virulence for the parental cells and to have acquired the ability to bind heparin and to infect Chinese hamster ovary (CHO) cells (Escarmis *et al.* 1998). Comparison of the variant and parental viruses revealed amino acid differences involving sites at the five-fold axis of the viral capsid, namely, amino acid position 9 of VP3 (Asp -> Ala) and either amino acid position 108 (His -> Arg) or 110 (Gly -> Arg) of the VP1 gene (Escarmis *et al.* 1998). Drastic antigenic changes can occur *in vitro* in the absence of antibody selection during replication of FMDV in persistently infected cells (Bolwell *et al.* 1989; Borrego *et al.* 1993; Sevilla *et al.* 1996; Mbayed *et al.* 1997). The size of the initial population is a major factor in

determining the repertoire of antigenic variants evolving in viral quasispecies.

### 1.9.2 Persistence *in vivo*

Approximately 50 % of cattle infected with FMDV become persistently infected, irrespective of their vaccination status, or the serotype of the virus with which they were infected (McVicar & Sütmoller 1969). Persistent infection in cattle may promote the rapid selection of antigenic variants, in addition to being a virus reservoir as demonstrated by the rapid rate of fixation of mutations (0.9 x $10^{-2}$ to 7.4 x $10^{-2}$ s/n/y) in the VP1 protein of carrier cattle and the accompanying profound antigenic changes (Gebauer *et al.* 1988).

In order for a viral infection to be non-cytopathic, the target cells must be restrictive or non-permissive to the virus. Alternatively, viral variants with reduced cytopathogenicity which replicate at a lower rate must evolve. One or the combination of the afore-mentioned factors would ensure the long-term maintenance of the viral genome in host cells, but not necessarily avoidance of detection and elimination by the hosts' immune system. To achieve this, the virus would need to evade the immune system and / or establish persistence at an immunologically privileged site. The former requirement is readily achieved by the quasispecies nature of FMDV (Domingo *et al.* 1992), where genetic and antigenic variants are generated both in the presence and absence of immune selection (Gebauer *et al.* 1988; Diez *et al.* 1989; Domingo et al. 1993; Domingo *et al.* 1996; Vosloo *et al.* 1996). However, the pharyngeal area which is known to be the most common site of viral persistence is not considered an immunologically privileged site, and there is conflicting evidence regarding the ability of FMDV viral proteins to counteract the host immune defense (Moonen & Schrijver 2000; Haydon *et al.* 2001).

### 1.9.3 Possible modes of transmission of FMDV from carrier animals

One of the more perplexing problems regarding the role of persistently infected animals, and buffalo in particular, is that the mechanism whereby interspecies virus transmission occurs is unclear. In an attempt to clarify this, numerous studies have investigated possible means of transmission, without success. These include:

- ***Environmental contamination resulting from hunting***

The dissemination of virus through hunting and field dressing of an infected animal was tested

by Condy & Hedger (1974), and shown not to precipitate disease in susceptible animals in close proximity. This possibility is also unlikely as virus disassembly results when the pH in muscle tissue drops following the death of an animal (Bengis 1997).

- ***Stress induction***

Inducing stress in animals was proposed to be one means whereby the shedding of virus by carrier animals could be precipitated (Hedger & Condy 1985). This was tested in cattle through the administration of steroids and dexamethasone (Sutmoller & McVicar 1972; Ilott *et al.* 1997). The former chemical did not lead to increased viral activity in the pharynx, nor to transmission to non-infected in-contact animals (Sutmoller & McVicar 1972) and the latter, a known herpesvirus reactivator had the opposite effect and resulted in a drastic decrease in virus titres in oesophageo-pharyngeal (OP) fluid (Ilott *et al.* 1997).

- ***Mechanical transmission***

The blood feeding African buffalo fly, *Haematobia thirouxin potans* (Bezzi) was assessed for its ability to act as a mechanical vector of FMDV (Thomson *et al.* 1988). The fly failed to transmit FMDV to susceptible cattle after feeding on viraemic animals. The results indicate that this biting fly is an inefficient mechanical transmitter of SAT type viruses. Similarly, investigations into the potential of the ixodid tick, *Rhipicephalus zambeziensis,* the vector for Corridor Disease (CD) to transmit virus after feeding on viraemic animals was unsuccessful, despite virus survival of between 3 and 7 days being demonstrated in the tick (Van Vuuren *et al.* 1993).

## 1.10 Characterization of field strains of FMDV

Field strains of FMDV were initially characterized by subtyping, but as mentioned in section 1.5, the practical difficulties of discerning subtypes, given the continuous spectrum of antigenic variants, severely impedes the usefulness of this approach. From the 1980's onwards, the characterization of field strains was based on genetic methods and on $T_1$ oligonucleotide fingerprinting in particular (Domingo *et al.* 1980; Anderson *et al.* 1985; Anderson 1986; Carrillo *et al.* 1990). Briefly this approach involved two-dimensional electrophoretic separation of radioactively labelled RNA digested with $T_1$ ribonuclease. The resulting $T_1$ map represents about 5 - 7 % of the RNA genome (Domingo *et al.* 1980; Anderson 1986). Due to gel-to-gel variations, complexity of results and

difficulties in comparing large numbers of field strains with each other, alternative methods were investigated. Of these, the partial nucleotide sequence and analysis of the C-terminus end of the VP1 gene significantly improved the genetic resolution of virue relationships. In 1987 two reports on the application of this approach were published, one on poliovirus (Rico-Hesse *et al.* 1987) and one on FMDV (Beck & Strohmaier 1987), which revolutionized the field of picornaviral molecular epidemiology. The subsequent proliferation in similar studies has provided important epidemiological insights, including evidence of prolonged persistence of outbreak strains in the field (Samuel *et al*. 1999; Freiberg *et al.* 1999), trans-boundary virus transmission (Marquardt & Haas 1999; Samuel *et al.* 1999; Sangare *et al.* 2001) and the presence of virus types specific to different geographical areas (Saiz *et al.* 1993; Stram *et al.* 1995a; Knowles & Samuel 1997; Knowles *et al.* 1998). Outbreaks resulting from improperly inactivated vaccine and those in which there was no vaccine involvement have also been reported (Beck & Strohmaiher 1987; Krebs & Marquardt 1992; Suryanarayana *et al.* 1998). Most of the afore-mentioned studies have addressed the epidemiological situation of types O, A and C. In comparison to the European serotypes, few studies have addressed the continental genetic diversity of the SAT-types. Published studies have primarily focussed on the disease situation in South Africa (Vosloo *et al.* 1992; Vosloo *et al.* 1995; Keet *et al.* 1996) and Zimbabwe (Dawe *et al.* 1994a & 1994b), with only one study addressing genetic variation at a regional level (Knowles 1994). The latter reports were based on partial 1D nucleotide sequences corresponding to the C-terminal 150 to 200 nt of the VP1 protein generated by a direct RNA sequencing approach. The restriction on the length of sequence data generated for the VP1 protein of SAT-type viruses was primarily due to the lack of suitable primers with which to target this region. Difficulties in designing more appropriate primers also arose from the fact that the P1 regions of just two SAT-type viruses have been published (Brown *et al.* 1989; van Rensburg & Nel 1999).

## 1.11 Objectives of this study

From the review of the literature it is clear that numerous aspects of the molecular epidemiology of FMD in southern Africa remain to be clarified. In particular the mechanism whereby persistently infected buffalo transmit the disease to cattle and the role of impala as possible intermediaries in disease transmission remains obscure. It remains to be established whether buffalo viruses evolve independently in different geographical localities. The role of the African buffalo as the primary source of infection for wildlife and domestic artiodactyl species needs to be investigated further. In this regard, genetic characterization and comparison of field strains from diverse host species may provide insight into the epidemiology of the disease in specific regions. The greatest hurdle in conducting comprehensive molecular epidemiological studies is the lack of suitable PCR-based methods for amplification and characterization of the SAT-type viral genomes. The primary objective is therefore to develop and establish genetic characterization methods and apply these in an investigation of the epidemiology of the disease in southern Africa.

The aims of this study are therefore fourfold:

- To develop a PCR-based method suitable for the detection and genetic characterization of foot-and-mouth disease viruses prevalent in southern Africa

- To apply this new methodology in elucidating factors of regional epidemiological importance, namely:
  - The possibility that sexual transmission of foot-and-mouth disease with particular reference to the role of the African buffalo
  - The role of the impala antelope in disease transmission

- To assess the genetic variability of SAT-type FMD viruses in the buffalo maintenance host populations which occur in southern Africa, by applying the PCR-based methodology

- To use the buffalo virus genetic database as a reference for determining the origin of:
  - Historical outbreak strains in South and southern Africa
  - Recent outbreaks of the disease in livestock