CHAPTER 1

The past, present and future of foot-and-mouth disease vaccinology: A Literature Review

1.1 General introduction

Foot-and mouth disease (FMD) is a highly contagious acute viral infection, affecting all cloven-hoofed animals. Its host range in domestic animals include cattle, sheep, goats and pigs, while a number of wild life species such as antelope, both water and African buffalo, camels, llama and giraffe are also susceptible. Although mortality rates are generally low (less than 5%), FMD has a major economic impact on the livestock industry. The direct losses are mainly due to reduced production of meat, milk and other animal products during an outbreak. Spread of the disease is usually rapid with a short incubation period of ± 2 to 8 days (Thomson, 1994).

Transmission of the disease is either direct or indirect. The most general route is direct contact between infected animals that excrete the virus, and susceptible animals. FMD virus is present in all secretions and aerosols derived from the respiratory tract during the infectious period. Indirectly, the disease can be transmitted by viral contaminated objects or materials, such as tissues and animal products (e.g. milk and meat), while humans and vehicles have also been implicated in the mechanical transmission thereof (Sellers, 1971). Although airborne transmission occurs very rarely and is very much dependent on climate, several outbreaks occurring by this means have been reported in northern Europe (Donaldson, 1979; Fogedby *et al.*, 1960; Gloster *et al.*, 1982).

The FMD virus can persistently infect ruminants for periods ranging from a few weeks to years. In such cases, virus can be isolated from the oesophagus and throat fluids by probang extractions (van Bekkum *et al.*, 1959). In cattle, this carrier status has been shown to be responsible for outbreaks of the disease up to two years post infection

(Thomson, 1996). The African buffalo (*Syncerus caffer*), known to maintain the virus for five years in an individual animal and at least 24 years in an isolated herd (Condy *et al.*, 1985), can transmit the disease to cattle (Hedger & Condy, 1985; Dawe *et al.*, 1994; Vosloo *et al.*, 1996). The mechanism of transmission is however not clearly understood, but recent reports indicate that sexual transmission of the disease between buffalo and cattle is a possibility (Bastos *et al.*, 1999).

Early clinical signs include fever, dullness and a fall in milk yield. These signs are however rapid in onset and are followed by the development of lesions in the mouth and on the feet (Figure 1.1). The lesions result in lameness and discomfort in the mouth such as grinding of the teeth. Young domestic animals infected with FMD virus, may die suddenly due to myocarditis (Thomson, 1994).

Initially, control of FMD involves containment of the disease, although the ultimate aim should be the total eradication thereof. A systematic approach in endemic areas usually starts with mass vaccination, followed by the control of sporadic outbreaks. The status of "freedom of disease with vaccination" and consequently the status of "freedom of disease without vaccination" is then awarded to free areas by the Office des Epizooties (OIE). The International Animal Health Code of the OIE defines each of these stages which also entails specific international trading implications for live animals and animal products. Although a very important aspect in the control of FMD, vaccination is only one of several zoo-sanitary measures essential for successful control. Other measures include the control of animal movement, prohibition of importation of susceptible animals and animal products from high risk areas, appropriate diagnostic testing of susceptible animals prior to importation and the application of quarantine measures as well as the slaughtering of infected and susceptible, in-contact animals, depending on the overall epizootiological situation. The authority responsible for effective control should also have access to specific fields of expertise. These include epidemiology, animal health legislation, risk analysis, logistics, training and education (Garland, 1999).

B

A

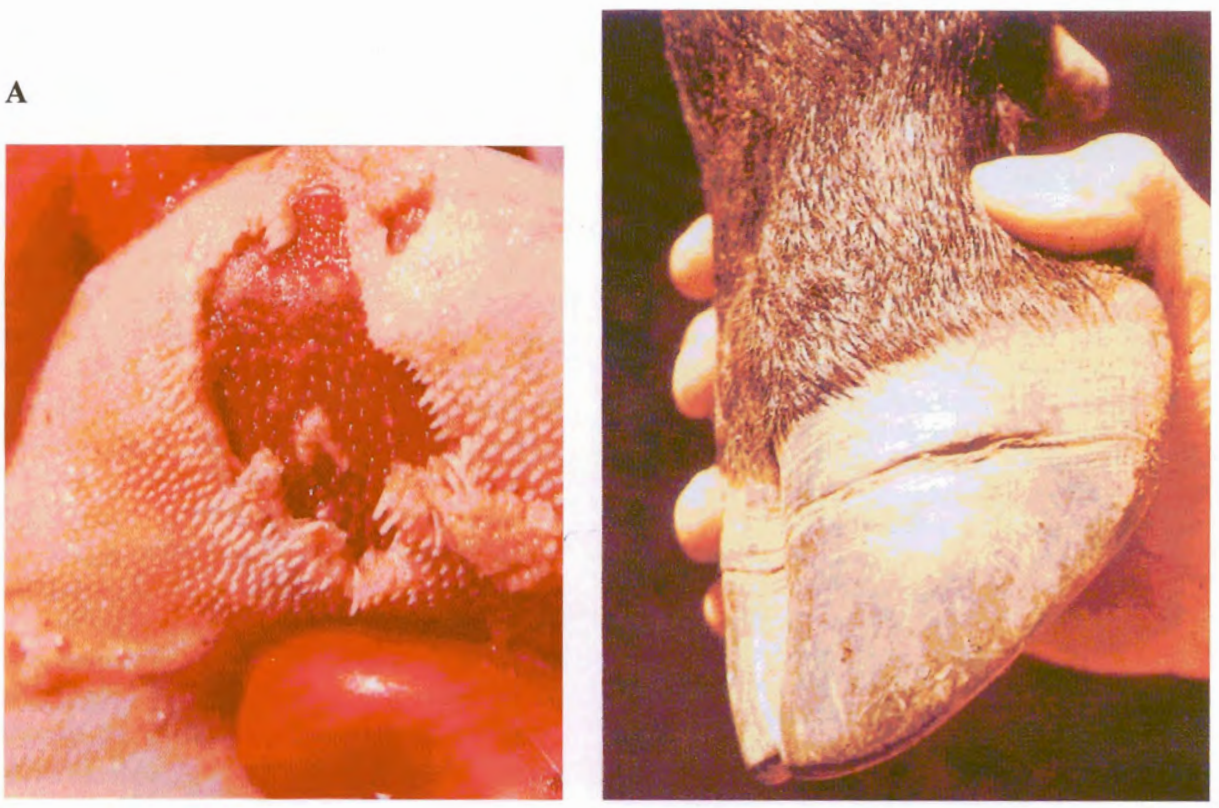

Figure 1.1: Examples of foot-and-mouth disease lesions. A: Ruptured vesicle on the tongue of an ox. B: A faulty hoof of an ox that has recovered from foot-and-mouth disease (Taken from Thomson, 1994).

An integral part of control is the rapid and accurate diagnosis of FMD. Due to the highly infectious nature of the virus, handling thereof as well as diagnosis is restricted to high-security laboratories. Diagnosis involves clinical evaluation, identification of virus and viral antigens during early stages of infection and serology in cases where the infection is lasting longer than two weeks (Thomson, 1994).

Positive viral identification entails viral isolation from clinical specimens such as lesion material and probang extractions on primary pig kidney cells (PK) as well as the detection of viral RNA through the polymerase chain reaction (PCR). As it is a relatively new introduction, the use of PCR is however restricted to only a limited number of laboratories. Enzyme linked immunosorbent assays (ELISAs) and the virus neutralization tests (VNT) are used for antigen and antibody identification and assay (Thomson, 1994; Garland, 1999).

1.2 A historical perspective of foot-and-mouth disease outbreaks

The first descriptions of foot-and-mouth disease were given by Fracastorius in 1546. He described a "contagious affection" which occurred in cattle in 1514 in what is now known as Italy that was characterized by "a certain rawness and small pustules in the mouth" (Bulloch, 1927). Frequent occurrence of the disease was reported during the 17^{th} and 18^{th} centuries in France, Germany and Italy, but was recorded in the United Kingdom for the first time only in 1839. FMD appeared for the first time in South America in Argentina in 1871 (Brown, 1986), probably introduced from Europe. During the first half of the 20^{th} century, pandemic outbreaks of the disease raged through Europe. Through extensive vaccination programs, FMD was gradually controlled and later eradicated from western Europe (Brown, 1992). In southern Africa the first reports on FMD date back to 1780 when le Vaillent described a disorder in cattle which "attacked the feet of oxen. In some cases the hoofs dropped off". The first official report was obtained in 1892, although older inhabitants of the region described a disease which resembles FMD long before the official recordings were made. Due to the rinderpest outbreak of 1896, FMD "disappeared" until 1931 when an outbreak re-occurred in Zimbabwe (Thomson, 1994).

Although prophylactic vaccination has been stopped in 1991 in the European Union, its disease-free status is often threatened. Examples of outbreaks include Italy (1993), Greece (1994, 1996 & 2000[1]) and east European countries such as Bulgaria (1991, 1993 & 1996) and Turkish Thrace (1995 & 1996). In 1997 the only outbreaks reported were in Armenia and Georgia (Kitching, 1998; Kitching 1999; Leforban, 1999). Several reports involving type O outbreaks in Georgia and Kazakhstan were however, received from May to July 2000[1].

FMD in the Middle East, Central and east Asia is wide-spread. Outbreaks occur frequently in dairy herds in Saudi Arabia, probably introduced from Asia and Africa. Just recently, an outbreak of SAT 2 has been confirmed in these dairy herds[1]. This is the first incidence of SAT 2 outside the continent of Africa as far as could be determined. The epizootic subsequently spread to Kuwait as well. In countries such as India, parts of China, Thailand and Cambodia FMD is endemic. Control is complicated in certain countries through unstable political situations, lack of supporting legislation and religious considerations (Kitching, 1998; Kitching, 1999).

A very serious outbreak of type O occurred in Taiwan in 1997. The pig-adapted strain swept through the entire island within four months (Dunn *et al.*, 1997). Taiwan had been free of foot-and-mouth disease since 1929. It was subsequently controlled through extensive vaccination and slaughtering. The total economic loss in Taiwan is estimated at approximately US$ 380 million (Yang *et al.*, 1999). Following the disastrous 1997 outbreak in Taiwan, the countries of the Far East reviewed their national management policies regarding surveillance, diagnostic vaccine stocks, legislation etc. to be better prepared for future emergencies (Yagasaki, 1999). Despite these actions, South Korea has had numerous outbreaks of the disease during 1999 and 2000, while Japan reported two incidents of FMD recently – the first in 92 years[1].

Due to the significant improvement in the control program in South America, Uruguay was declared free of disease in 1995, while Argentina, Paraguay and the southern states of Brazil are free with vaccination. Recent reports of FMD outbreaks have, however,

[1] See Disease Information Webpage of the OIE (http://www.oie.int/Info/a_dsum.htm)

been received from these free-zone areas in Paraguay, Argentina and Brazil[2]. Outbreaks were also reported in Bolivia, Colombia and Ecuador. Despite these recent outbreaks, the situation has vastly improved in South America (Kitching, 1998; Kitching 1999).

An outbreak of type O swept through northern Africa during 1989 and 1990, starting in Tunisia and spreading to Algeria and Morocco (Samuel *et al.*, 1999). Currenly, Morocco is free of the disease. As serotype O is endemic to Egypt and Libya, outbreaks in the region and neighboring countries occur frequently. FMD is endemic in sub-Saharan Africa, with the exception of Madagascar. Countries such as Ivory Coast, Mali, Rwanda, Senegal, Tanzania, Uganda, Malawi and Kenya[2] reported several outbreaks in recent years. This is however, not a true reflection of the epidemiology of the disease. Poor surveillance and diagnostic facilities are possibly responsible for the poor reporting of the disease in the region (Kitching, 1998; Kitching, 1999).

In southern African countries, the disease is mainly restricted to game parks where it persists in African buffalo populations. Occasionally, it spreads from wildlife to domestic cattle populations. Such an outbreak of SAT 2 occurred in 1997 in Zimbabwe where impala or kudu jumping fences, were implicated as intermediaries in transmission between buffalo and cattle (Thomson, 1999). The role of antelope in the transmission from buffalo to cattle was again reported with the independent outbreaks of SAT 1 and SAT 3 in Zimbabwe in 1999[2]. Buffalo were also involved in the 2000 outbreak of SAT 1 in cattle on an experimental farm in the controlled area of South Africa (A. D. S Bastos, personal communication). This was the first outbreak of disease in domestic animals since 1983 in the country. A much more threatening outbreak of FMD was reported during September 2000 in the disease-free zone of South Africa[2]. This type O virus, previously exotic to South Africa, forms part of the Pan Asian topotype, which has caused numerous outbreaks in the nineties throughout the Middle East, Asia and the Far East[2] (O. Sangare, personal communication). This outbreak emphasized the need for stricter movement control of animals and animal products world-wide.

[2] See Disease Information Webpage of the OIE (http://www.oie.int/Info/a_dsum.htm)

1.3 Foot-and-mouth disease virus properties

1.3.1 Classification

The causative agent of FMD is the foot-and-mouth disease virus (FMDV) which belongs to the family *Picornaviridae* (Ruekert, 1996). This family of positive strand RNA viruses contains nine genera, namely *Entero-*, *Rhino-*, *Cardio-*, *Aphtho-*, *Hepato-*, *Parecho-*, *Erbo-*, *Kobu-* and *Teschoviruses* (Stanway *et al.*, 2000). Together with equine rhinitisvirus A (Li *et al.*, 1996), FMDV make up the genus *Aphthovirus*. Identified by Loeffler and Frosch in 1897 as the first filterable agent, FMDV played an important role in virus research. Seven distinct serological types were identified using cross-protection experiments. Types A, O and C were recognized in the 1920's by French and German scientists, while the South African Territory types 1, 2 and 3 as well as Asia-1 were identified at the Animal Virus Research Institute at Pirbright, England, in the late 1940's and early 1950's respectively (Brooksby, 1982).

1.3.2 Physical properties

A well-known characteristic of FMDV is its lability below pH6. The acid instability varies however with strains, isolates and passage history (Domingo *et al.*, 1990). This characteristic of *aphthoviruses* is shared by the *rhinoviruses*, while the *entero-* and *cardioviruses* can survive pH as low as 3 (Ruekert, 1996). FMD virions have a diameter of 27-28nm, are spherical in shape and exhibit icosahedral symmetry. Seventy percent of the virion consist of protein, 30% RNA and a small amount of lipid (Putnak & Phillips, 1981). The sedimentation constant of virions in sucrose gradients (146S), another well-known characteristic of FMDV, is widely used in vaccine manufacture for the determination of intact virions (Barteling & Vreeswijk, 1991).

1.3.3 Genome organization and viral structure

The FMD viral genome is a positive sense, single stranded RNA genome approximately 8 500 nucleotides in length. A schematic representation indicating the characteristics of the genome as well as the encoded proteins, is shown in Figure 1.2.

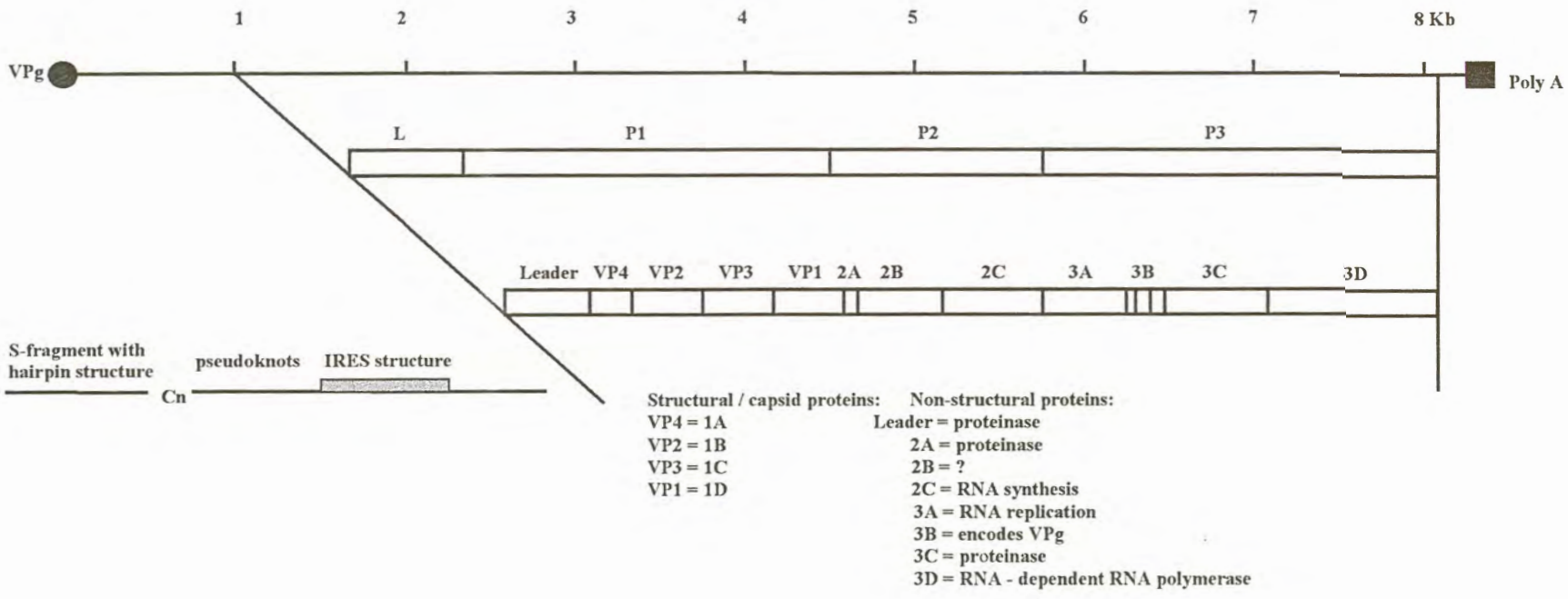

Figure 1.2: Schematic representation of the foot-and-mouth disease virus genome. The VPg-linked RNA strand, the four polyprotein precursors (L, P1, P2, P3), the mature viral proteins as well as the location of the 5' UTR secondary structures are indicated.

Covalently linked to the 5' untranslated region (5' UTR) of the genome, is a small virus-encoded protein, VPg (viral genome-linked protein). The gene (3B) encoding VPg is located in the P3 region of the open reading frame (ORF). Although the exact function of the protein is not clear, it has been implicated in RNA synthesis and possibly virion formation (Falk *et al.*, 1992). A poly(C) tract of unknown function is located approximately 150 to 370 bases from the 5' end (Harris *et al.*, 1976; Rowlands *et al.*, 1978). Although initial reports suggested that this region play a role in virulence, it was subsequently shown that even a virus with a poly (C) tract of 2 residues, was virulent in mice (Rieder *et al.*, 1993). The region, 100-420 bases in length (Black *et al.*, 1979; Escarmis *et al.*, 1992), separates the small (S) fragment of the genome from the large fragment (L). No open reading frames exist in the S fragment. Secondary structure predictions suggest the sequence to fold into a hairpin structure (Newton *et al.*, 1985).

The L fragment contains a series of pseudoknots (Clarke *et al.*, 1987a), the internal ribosome entry site (IRES) and an ORF. To the 3' end of the pseudoknots, the highly structured IRES is found. This region contains extreme secondary structures and directs cap-independent internal initiation of protein synthesis (Jackson *et al.*, 1990). The ORF encodes a polyprotein that is cleaved during translation by viral encoded proteinases. Several protein precursors (P1, P2 and P3) are produced that are then further processed into structural and non-structural proteins. The proteinases responsible for these cleavages, the Leader, 2A and 3C, are discussed in more detail in Chapter 3. The four viral proteins, VP1, VP2, VP3 and VP4, are structural proteins and form the capsid of the virus.

The exact function of the 2B region (Figure 1.2) is unknown, while it has been implicated in host range determination in *rhinoviruses* (Lomax & Yin , 1989). Due to the presence of three NTP-binding domains (GXXXXGK, DXXG, NKXD) in the 2C protein, it is evident that this region interacts with nucleic acids and is therefore also implicated in RNA synthesis (Dever *et al.*, 1987; Hodgman, 1988). According to Gorbalenya and co-workers (1989a & 1989c), the protein exhibits motifs characteristic of the helicase superfamily. The 3A-gene product has been shown to be membrane

associated, a prerequisite for RNA replication and is involved in the initiation of vesicle formation (Weber *et al.*, 1996). Recently, a 3A protein containing a deletion, was implicated in viral virulence (Beard & Mason, 2000). As mentioned previously, the 3B gene encodes three forms of the VPg protein. The 3D gene encodes a RNA-dependent RNA polymerase and is highly conserved in the picornavirus family (Stanway *et al.*, 1984). The functional domain includes the GDD (Gly-Asp-Asp) motif which shows similarity to the YXDD (Tyr-Xxx-Asp-Asp) motif found in other RNA viral polymerases (Kamer & Argos, 1984). Downstream of the 3D gene, is the 3' UTR and the poly (A) tract, both variable in length.

The P1 polyprotein precursor undergoes several proteolytic processing events to produce VP0 (VP4 + VP2), VP3 and VP1 (Domingo *et al.*, 1990; Belsham, 1993). The N-terminus of the precursor is myristoylated (Chow *et al.*, 1987) which has been shown to be essential for capsid assembly/stability. Following proteolytic processing, the VP0 precursor has a myristate moiety attached to the N-terminus. VP0 is cleaved possibly through auto-catalysis and the four viral proteins (VP1-4) form the virus particle. The icosahedral capsid (Figure 1.3) consists of 60 copies of each of the four proteins. VP1-3 are exposed, while VP4 is located internally and can also be seen as an N-terminal extension of VP2 (Acharya *et al.*, 1989). The N-terminal region of VP1 and the C-terminus of VP4 are packed together at the three-fold symmetry axis and interact with the viral RNA which induces protein-protein interaction, thereby contributing capsid stability (Curry *et al.*, 1997).

Five copies of VP1 are situated around the five-fold symmetry axis, while VP2 and VP3 are situated at the two-and three-fold axes of symmetry, respectively. Together they form the protomeric subunit. The spatial arrangement of VP1-3 corresponds with other picornaviruses, although the surface of the particle is smoother, lacking the peaks and depressions of other picornaviruses (Acharya *et al.*, 1989; Fry *et al.*, 1990). Due to the smaller size of VP1 with respect to other picornaviruses and the fact that it is being directed towards the five-fold axis, a hole is formed that leads from the surface into the interior through the β annulus. The latter is formed by the N-terminal

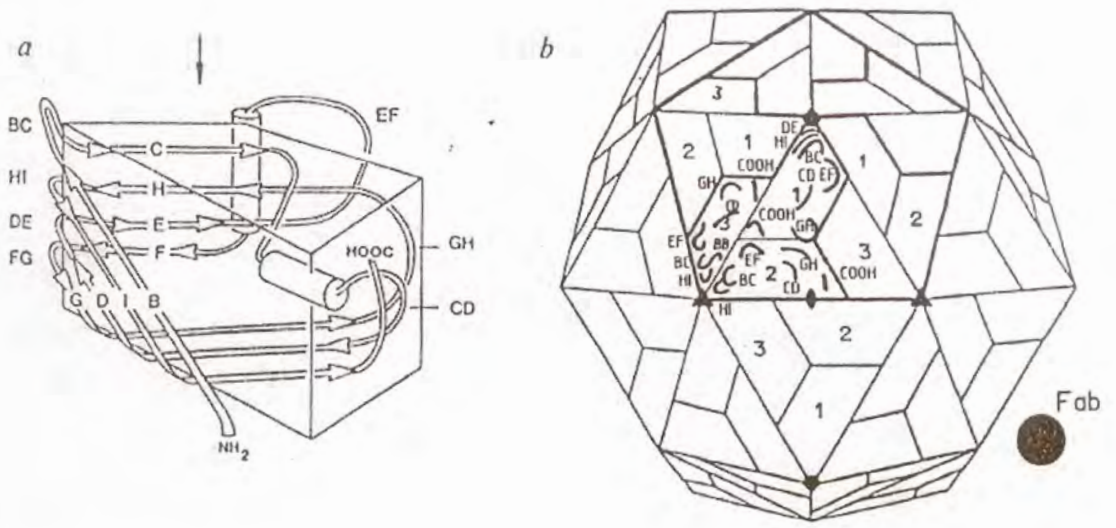

Figure 1.3: Structural representation of the picornavirus capsid proteins. A: The core of each protein (VP1, VP2, VP3) is an 8-stranded β-sandwich. The β-strands are indicated as B, I, D, G, C, H, E and F and are joined through connecting loops. Black arrow indicates the capsid surface. B: The capsid is formed by 60 biological protomers. Each protomer is composed of one copy of VP1, VP2 and VP3. The icosahedral symmetry axes are labaled 5*x* pentagon, 3*x* triangle and 2*x* oval. Most surface loops of VP1, VP2 and VP3 are indicated. The area of an antibody footprint is also indicated (Fab) (Taken from Mateu, 1995).

extensions of 5 VP3 molecules. The residues of VP3, forming this region, are highly conserved.

Characteristic of FMDV is its acid lability. At pH 6.8, the viral particle is disrupted and the pentameric subunits are produced with the release of VP4 and the RNA. From the structure, the high density of histidine residues on the pentamer interface is evident. As the pK of the histidine side chain correlates with the pH of pentamer dissociation, Acharya and co-workers (1990) speculated about the involvement of the histidine residues in the dissociation of the capsid. It has subsequently been shown that His-142 in VP3 is linked to the acid-induced disassembly of the capsid (Ellard *et al.*, 1999).

1.3.4 Overview of infectious cycle

Viral multiplication occurs in the cytoplasm of the host cell. Initiation of the infectious cycle (Figure 1.4) involves attachment of the virion to a specific cell receptor. The cell attachment site of the virus has been shown to be located on a highly mobile G-H loop on the surface of the VP1 protein containing a conserved Arg-Gly-Asp (RGD) sequence (Fox *et al.*, 1989; Logan *et al.*, 1993; Lea *et al.*, 1994; Mason *et al.*, 1994). The RGD consensus is also present in other proteins known to bind to the cell surface receptor family, the integrins (Hynes, 1992; Ruoslahti & Pierschbacher, 1987). Several integrin receptors have been implicated in the cell adhesion of FMDV. These include not only the vitronectin receptor (integrin $\alpha_v\beta_3$) (Berinstein *et al.*, 1995; Jackson *et al.*, 1997; Neff *et al.*, 1998), but also the integrins expressed in epithelial cells, $\alpha_5\beta_1$ (Villaverde *et al.*, 1996; Jackson *et al.*, 2000a) and $\alpha_v\beta_6$ (Jackson *et al.*, 2000b). Field isolates of FMDV have been shown to utilize the integrin receptors for internalization, whereas several cell cultured adapted viruses have been shown to utilize heparin sulfate proteoglycans (Jackson *et al.*, 1996; Sa-Carvalho *et al.*, 1997).

The receptor position the virion favorable for entering the cell. The mechanism of RNA entry into the host cell for FMDV is not exactly clear, although the involvement of VP4 in the release of the viral genome, has been shown by Knipe and co-workers (1997). Contrary to the case with cellular mRNA, picornaviral RNA is not capped, but contains a small protein, VPg. This protein is covalently linked to the 5' end of the

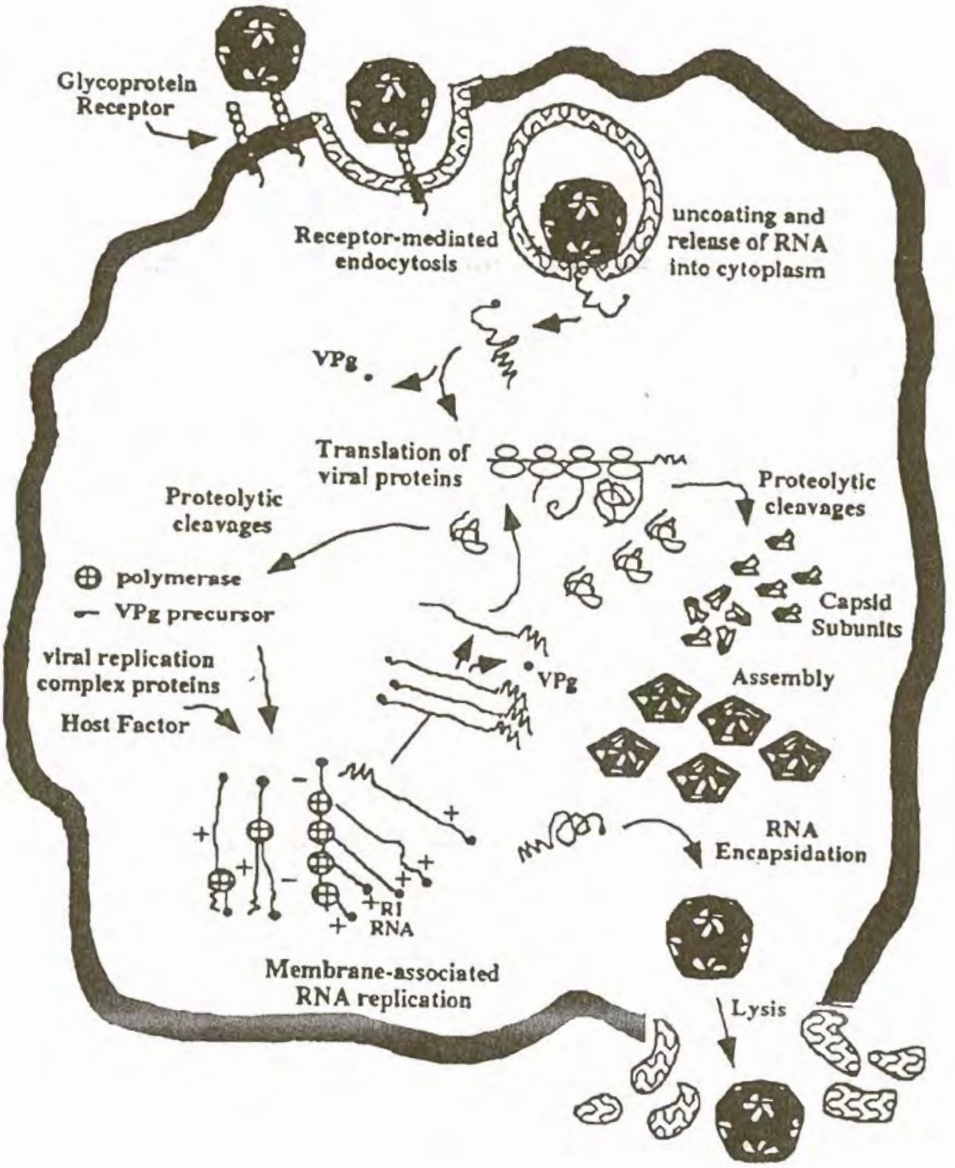

Figure 1.4: Overview of the poliovirus infectious cycle. (Taken from Ansardi *et al.*, 1996)

viral RNA and is removed in the cytoplasm (Lee *et al.*, 1977). Synthesis of the polyproteins is therefore initiated cap independent at the internal ribosome entry site (IRES). Characteristic of the picornaviral IRES structure, is the presence of an oligopyrimidine tract at the 3' border of the structure, followed by an AUG triplet. Translation is usually initiated at this AUG for *cardio-* and *aphthoviruses* (Pelletier *et al.*, 1988).

Several translation initiation factors, involved in cap-dependent initiation, have also been implicated in the translation initiation at the picornaviral IRES structure. For FMDV, these include the eukaryotic initiation factor (eIF) 4B (Meyer *et al.*, 1995) as well as the cellular polypyrimidine tract-binding protein (PTB) (Niepmann, 1996). The latter forms part of the 48S and 80S ribosomal initiation complexes formed with the IRES structure and plays an important role in the stimulation of internal translation initiation (Niepmann *et al.*, 1997). The RNA strand directs synthesis of the polyprotein which is cleaved into individual proteins as synthesis progress (Rueckert, 1996).

The Leader proteinase is the first protein to be synthesized and cleaves itself from the rest of the polyprotein whilst the latter is still being synthesized. The Leader then cleaves eIF-4G which results in the inhibition of cap-dependent mRNA translation of the host cell and the selective advantage of the uncapped viral RNA translation (Devaney *et al.*, 1988; Belsham & Brangwyn, 1990; Ohlmann *et al.*, 1995). It was also shown that the cleavage of eIF-4G by the Leader proteinase supports internal initiation by the IRES elements (Ohlmann *et al.*, 1996; Ohlmann *et al.*, 1997; Ziegler *et al.*, 1995). With the progression of polyprotein synthesis, the polyprotein is cleaved into viral proteins, mainly by the 3C proteinase. The latter is also responsible for the cleavage of the nuclear protein histone H3, which could be related to the host cell transcription shutoff (Falk *et al.*, 1990; Tesar & Marquardt, 1990). The cleavages of eIF-4G and histone H3 are therefore implicated in an almost complete break down of host cell functions during viral infection.

Synthesis of the positive sense viral RNA occurs on the smooth endoplasmic reticulum and is initially extremely rapid to generate many positive-sense copies. With the

expansion of the positive sense copies, a greater fraction is translated, processed and packaged into virions (Ruekert, 1996). Virion assembly involves the formation of protomers, five of which then assemble into pentamers (12S), followed by the packaging of plus-stranded VPg-RNA to form provirions. Twelve pentamers associate together to form either empty capsids (lacking RNA) with a sedimentation coefficient of 75S or provirions (146S) (Grubman *et al.*, 1985). The final step in mature virion formation is the cleavage of VP0 to form the mature four-chain subunits. Viral particles are released from the host cell by infection-mediated disintegration of the host cell (Ruekert, 1996).

1.3.5 Antigenic properties

Initial experiments suggested the presence of a major immunodominant site for FMD located within the G-H loop of VP1 (Bachrach *et al.*, 1975; 1979). Subsequent research demonstrated the involvement of multiple sites independent of the G-H loop for FMD (Domingo *et al.*, 1990 and references therein. Table 1.1 summarizes the immunodominant sites determined for types A, O and C.

Table 1.1: Description of immunologically important secondary structure elements (taken from Mateu, 1995)

AXIS	SECONDARY STRUCTURE ELEMENTS	TYPE O	TYPE C	TYPE A_{10}	TYPE A_{12}	TYPE A_{22}	TYPE A_5
5X	VP1 B-C	3					
	VP1 H-I			4	1		
2X	VP1 G-H	1a,5	A	1	2	1	
	VP2 E-F	2					
3X	VP2 B-C	2	D	3			1
	VP3 B-B	4	D	5			
	VP3 B-C			3			
	VP3 H-I			3			
	VP1 C_T	1b	C,D	2	3,4		2
	VP3 E-F			3			
	VP3 G-H				4		

Kitson and co-workers (1990) identified four independent immunodominant sites for serotype O (O_1Kaufbeuren). The positions of these sites have been located on the related strain O_1BFS (Acharya *et al.*, 1989). Site 1 involves the G-H loop and C-terminus regions of VP1, while sites 3, 2 and 4 are located on loops of VP1, VP2 and VP3, respectively. Another functionally independent site was described later, although its physical position might overlap with site 1 (Crowther *et al.*, 1993a). Three independent sites for serotype C (C-S8c1) have been determined and located on the structure of the same strain (Mateu *et al.*, 1989; Mateu *et al.*, 1990; Lea *et al.*, 1994). Site A is located on the G-H loop of VP1 and site C on the C-terminus region. Site D involves regions from VP1, VP2 and VP3. Multiple sites have been identified for different subtypes of serotype A. Sites identified for A_{10} (Thomas *et al.*, 1988) include the G-H loop (site 1), C-terminus region (site 2), another site in VP1 and several regions in VP3. Baxt and co-workers (1989) identified four immunodominant sites for A_{12}, also involving the G-H loop and C-terminus regions as well as a further epitope on VP1 and a fourth on VP1 and VP3.

The antigenic structure of the site present on the G-H loop of VP1 display a high level of complexity. This includes the involvement of overlapping and non-overlapping epitopes as well as the fact that some of these epitopes are conformational-dependant or discontinuous epitopes (Domingo *et al.*, 1990). The latter by definition implies the involvement of amino acid residues on different protein segments and is therefore dependent on the conformation of the protein. Continuous epitopes involve residues located in a single protein segment although strictly speaking, most of these epitopes will be discontinuous as not every residue is necessarily involved in antibody binding. Continuous epitopes are however distinguished from discontinuous ones by their ability to be mimicked by peptides (Mateu, 1995). Following the above description of continuous and discontinuous epitopes, serotypes A and C display continuous epitopes, while serotype O contains discontinuous epitopes as well (Mateu, 1995).

1.4 Vaccination against Foot-and-mouth Disease

The effectiveness and consequently success of vaccination against human and animal infectious diseases are well known. In fact, vaccination or immunization has been responsible for increasing the life-span of humans more than any other 20th century medicine (Plotkin, 1993). The contribution of active vaccination has been crucially important in the prevention and control of major infections such as diphteria, mumps, rubella, tetanus, poliomyelitis, measles, yellow fever as well as the eradication of smallpox (Moxon, 1990). Due to it's very important impact on the economics of livestock and poultry industry, vaccination also remains to be the most cost-effective method to manage the onslaught of infectious diseases in the veterinary field (Babiuk, 1999).

Yearly vaccination programs against foot-and-mouth disease were introduced in 1952 in the Netherlands and soon followed by other European countries. The number of outbreaks decreased drastically and in 1992 Western Europe was declared free of the disease by the OIE. Eradication of FMD in South America is also progressing well with several countries in the region being free of disease (see section 1.2). The success in South America is mainly due to extensive vaccination programs, co-ordinated by the Pan-American Centre for FMD in Rio de Janeiro (Barteling & Vreeswijk, 1991; Brown, 1992).

Several important factors ensuring vaccine efficacy should be considered during a vaccination campaign. These include satisfactory potency and safety as well as the implementation of an acceptable quality assurance system (Doel, 1999). According to the European Pharmacopoeia, potency testing involves the vaccination of three groups of five cattle with three dilutions of the vaccine. These animals are then challenged 2-4 weeks post vaccination with a virulent strain from the same type as the vaccine. A 50% protective dose (PD50) of 3 or more is required (Van Oirshot, 1999). Due to the nature of FMD vaccines (killed or inactivated virus), it is imperative that a high standard of safety is maintained. Previously, several outbreaks of FMD in Europe were reportedly linked to vaccination (King *et al.*, 1981; Beck & Strohmaier, 1987). The preservation

of the cold chain, a long shelf-life and vaccine strain selection are other important factors to consider. The vaccine should also be used as prescribed by the manufacturer (Doel, 1999).

A protective immune response to FMDV entails the induction of neutralizing antibodies and macrophage activity, thus involving both B- and T helper-lymphocyte dependent responses (McCullough *et al.*, 1992 and references therein). These facts should be taken into consideration during vaccine design. Furthermore, to obtain and sustain rapid and long-lasting herd immunity, the frequency of vaccine administering is essential. Usually, annual and biannual vaccination is required. Other factors influencing the immune response, include the quality of antigen, type of adjuvant used during formulation and physical condition (age and health) of animals (Doel, 1999; Van Oirschot, 1999).

1.4.1 Conventional, inactivated vaccines

The availability of high concentrations of viral antigen, properly inactivated viral preparations and the addition of non-toxic adjuvants to stimulate the desired immune response are essential for inactivated viral vaccine production (Barteling & Vreeswijk, 1991). In the 1920's Vallée and his co-workers showed that FMDV could be killed or inactivated by formaldehyde without losing its antigenicity. However, it was only during the early 1950's when Frenkel developed the large-scale *in vitro* production of natural host tissue to cultivate the virus, that mass vaccination against FMD was initiated (Barteling & Vreeswijk, 1991; Brown, 1999). Aided by the discovery of antibiotics, Frenkel cultivated virus on bovine tongue epithelium, followed by viral harvest, adsorption onto aluminium hydroxide and inactivation with formaldehyde (Gillespie & Frenkel, 1955). The use of aluminium hydroxide as an adjuvant was discovered during the 1930's first by Schmidt and later by Waldmann. Although this was a simple production system and there was also no need for viral adaptation to the culture system, a low level of bacterial and fungal contamination was ever present. It was also impossible to increase production during outbreak situations (Barteling & Vreeswijk, 1991).

1.4.1.1 FMD viral production in cell lines

Following the development of tissue culture technology, the growth of FMDV on primary cells from pigs and cattle was investigated. It was soon shown that baby hamster kidney cells (BHK 21, clone 13) was better host cells for production of the virus (Mowat & Chapman, 1962). Production of FMDV on BHK cells in monolayer (roller bottle system) can easily be used for large-scale production, but this is a labour-intensive and prone to contamination.

Alternatively, different types of "multi-surface systems" have also been investigated over the years. These include the Girogen system, which is made up of long glass tubes fixed around a shaft inside a cylindrical fermenter (Girard *et al.*, 1979). Cells grow over the inner and outer surfaces of the tubes. The use of glass beads packed in a stationary fermenter has also been shown to be effective in the production of FMDV on BHK monolayer, although the method has presumably not been applied on an industrial scale (Spier & Whiteside, 1976). Micro-beads in suspension is well known in the production of inactivated vaccines for human use, such as polio vaccine (Van Wezel, 1967). FMDV production using this system was attempted experimentally, but probably due to financial implications was not used for vaccine production (Barteling & Vreeswijk, 1991).

With the adaptation of BHK 21 cells to grow in suspension culture (Capstick *et al.*, 1962), large-scale production of cultures was possible. Although FMDV production on BHK monolayer is considered to be better than production in suspension culture, most vaccine factories today use the latter technology. This is probably due to the fact that it is less labour intensive, less prone to contamination and much easier for large-scale production of vaccine.

1.4.1.2 Inactivation of FMDV

Viral inactivation is the most crucial step in the production of vaccine. As mentioned previously, several outbreaks in Europe have been linked to improperly inactivated virus particles present in the vaccine. The safety of a vaccine batch is usually determined with inactivation kinetics. The minimal accepted safety level should be

below $-5\log_{10}$ plaque forming units for a 100 liter batch (Barteling & Vreeswijk, 1991). Following the historical method of formaldehyde inactivation, $Al(OH)_3$ gel is adsorbed to the viral particle. This gel is however toxic for cells, making proper *in vitro* inactivation kinetics determination impossible. This problem was solved by ultracentrifugation of the virus – $Al(OH)_3$ complex. During this procedure, the virus and the gel are separated, enabling one to collect and titrate the virus and subsequently determining inactivation kinetics (Barteling & Woortmeijer, 1984).

Inactivation using aziridines has been shown to be a better option than formaldehyde as inactivation is rapid with first order kinetics enabling extrapolation to predict the safety of the batch (Brown *et al.*, 1963; Bahnemann, 1973; Barteling & Vreeswijk, 1991). However, these compounds are highly toxic. A substitute chemical, bromoethylamine hydrobromide (BEA), is available and is transformed into the active substance ethylenimine at pH > 8 (Bahnemann, 1975). It is still necessary to check for residual live viral particles in the vaccine batch.

1.4.1.3 Vaccine formulation

To ensure a proper immune response, the inactivated antigen must be adsorbed to an immune stimulant or adjuvant. Only three adjuvants have been applied to FMD vaccine production: the aqueous adjuvants $Al(OH)_3$ and saponin, and oil emulsion. With the adsorption of $Al(OH)_3$ to the antigen, followed by formaldehyde inactivation, initial monovalent vaccines had a dose volume of 60 ml. Following the concentration of the antigen – $Al(OH)_3$ complex and the addition of purified saponin, a final dose volume of 5 ml was obtained for a trivalent vaccine (Barteling & Vreeswijk, 1991).

Problems were however encountered with the vaccination of pigs. With the addition of diethylaminoethyldextran (DEAED) to the antigen – $Al(OH)_3$ complex, efficient protection was obtained. Due to the high price of DEAED, this is however not a viable financial option for vaccine manufacturers (Leeuw *et al.*, 1979). Improved protection of pigs was also obtained using oil emulsion vaccines. According to this method the antigen is emulsified in a mineral oil in the presence of an emulsifying agent (Freund & Thompson, 1945). Injected intramuscularly, cattle developed adverse reactions. By

reduction of the dose volume and with subcutaneous administration, the problem could be solved. An advantage of oil emulsion vaccines is the longer lasting immune response in comparison to the aqueous vaccines (McKercher & Graves, 1977). When the water in oil emulsion is emulsified once more, a double emulsion (water in oil in water) is obtained (Herbert, 1965). The advantage of the double emulsion is its lower viscosity during injection.

An exciting new development in the field of vaccine technology is the controlled-release microsphere formulations, which mimic repeated vaccinations. The antigen is contained in a polymer and is released as a pulse 1-6 months post injection. The pulse is dependent on the rate of polymer degradation, which is determined by the composition and molecular weight of the polymer (Cleland, 1999). An example of the application of microspheres containing antigen in viral vaccines is the work done by Hilbert and co-workers (1999) with influenza A. Eight weeks post injection of mice with a microsphere formulation, the same level of neutralizing antibodies could be detected as for two administrations four weeks apart with conventional vaccine. This new development holds promise for single administration, providing prolonged protection.

*1.4.2 **Alternative vaccines***

The technology used to produce killed or attenuated conventional vaccines has not changed much over the past 200 years (Babiuk, 1999). With the advances in immunology and especially molecular biology, new techniques are being developed to address emerging infectious diseases, improve current vaccines and to address diseases that previously evaded conventional vaccines, including malaria and HIV (Ada, 1997). New approaches to vaccinology include: (i) heterologous gene expression in recombinant vectors; (ii) attenuated vectors containing deleted genes; (iii) combination vaccines containing multivalent antigens to protect against more than one disease simultaneously; (iv) DNA vaccination; (v) regulation of the immune response and (vi) new microcarrier systems for immunomodulation delivery (Kurstak, 1999). Another very important application of new technologies would be the design and refining of

vaccines to produce the "ideal" vaccine. In table 1.2 a list of parameters for a so-called "ideal" vaccine is given.

Table 1.2 Parameters of an "ideal" vaccine (taken from Babiuk, 1999)

1. Efficacy greater than 90%
2. Effective after a single dose
3. Long-lived immunity (preferably life-long)
4. Effective when given orally (no need for injections)
5. Induces a wide range of appropriate responses (mucosal, humoral, cellular)
6. Low cost
7. Compatible with local management practices
8. Compatible with co-administration of other vaccines
9. Stable (genetically/thermally)
10. High safety

Evidence for FMD outbreaks originating from improperly inactivated viral particles present in conventional vaccine preparations, prompted researchers to explore alternative means of vaccination against FMD. Several different options were and are still being investigated and include heterologous gene expression, using different expression systems, DNA vaccination as well as the construction of synthetic peptides.

1.4.2.1 Heterologous gene expression

The development of recombinant DNA technology provided the means for the expression of FMD proteins. One of the first reports described the cloning and expression of VP1 (Figure 1.2) in *Escherichia coli*, but only partial protection against the homologous strain was achieved (Kleid *et al.*, 1981). The VP1 region was targeted due to the presence of the major immunogenic site. Despite the enthusiasm generated by this and other reports (Boothroyd *et al.*, 1981; Küpper *et al.*, 1981), immunological levels induced were 100-1000 times lower than equivalent amounts of the intact virion (Domingo *et al.*, 1990). This suggested the presence of important antigenic regions on other parts of the P1 region. Other systems used to express the VP1 protein include hepatitis B core proteins (Clarke *et al.*, 1987b) and bacterial fusion proteins (Broekhuijsen *et al.*, 1987; Winther *et al.*, 1986).

Several systems for the expression of P1 have been tested. *Escherichia coli* expression of P1-2A, part of 2B and 3C, resulted in the efficient synthesis and processing of the structural protein precursor as well as the formation of empty capsids (Lewis *et al.*, 1991; Grubman *et al.*, 1993). In spite of this, the capsid assembly process was found to be inefficient. This is probably because no myristoylation occurs in *E.coli*, and the toxic effect of the 3C proteinase. Expressing the cassette P1-2A+3C in insect cells using a baculovirus system, resulted in the efficient myristoylation of VP0 and the processing of capsids proteins (Roosien *et al.*, 1990; Belsham *et al.*, 1991). The same results were obtained with expression of this cassette in mammalian cells, showing that myristoylation of the capsid protein precursors occurs independently of other viral proteins (Belsham *et al.*, 1991).

The same construct (P1-2A+3C) was also expressed constitutively in a vaccinia virus system, but no empty capsids were obtained (Abrams *et al.*, 1995). Upon expression in an inducible system, the efficient processing of the capsid proteins was obtained as well as the formation of empty capsids. The myristoylation region on VP4 was mutated and the mutated cassette was again expressed in the constitutive system. Although the correctly processed capsid proteins were expressed, the formation of empty capsids could still not be obtained. This result is probably due to 3C toxicity. Recently, it was shown that protection with homologous challenge in mice could be obtained using a recombinant vaccinia virus expressing only the P1 region of FMDV strain C3Arg85 (Berinstein *et al.*, 2000), thereby avoiding the 3C proteinase toxicity.

Recently the expression of the VP1 protein in transgenic plants (*Arabodopsis* and Alfafa) has been demonstrated (Carrillo *et al.*, 1998; Wigdorovitz *et al.*, 1999a). Although protective immune responses were obtained in mice, the production of recombinant antigen was very low. A tobacco mosaic virus-based vector was subsequently used for the expression of the VP1 region in plants. Mice injected with foliar extracts from infected leaves, developed an immunogenic response to the VP1 protein and were protected against virulent challenge (Wigdorovitz *et al.*, 1999b).

1.4.2.2 DNA vaccination

DNA vaccination involves the direct introduction by needle innoculation or particle bombardment of host tissue with plasmid DNA. The result is expression of antigenic proteins by transfected cells (Whalen, 1996). DNA vaccination resembles a viral infection since the biosynthetic machinery of the host is used for protein synthesis. Due to the ease of production and stability of DNA, any desirable virus may be used to produce a potential DNA vaccine, side-stepping the difficult strain selection process for the production of conventional inactivated vaccines. Additional advantages include the ability to elicit cell-mediated immune responses and the elimination of risks associated with the use of live agents. DNA vaccination may potentially render protection against diseases that previously evaded conventional vaccination. The amount of antigen produced can be low, resulting in a lower humoral immune reponse in comparison to traditional antigen-containing vaccines (Robinson *et al.*, 1993; Xiang *et al.*, 1994).

Several safety concerns regarding DNA vaccination exist. Although no evidence for the integration of foreign DNA into the host genome has been obtained yet, it does remain a possibility. In addition, production of anti-DNA antibodies may also lead to auto-immune disease. Again, no evidence for the production of such antibodies has been obtained thusfar (Manickan *et al.*, 1997). Several DNA vaccine trials in the livestock and poultry industry have been carried out (Beard & Mason, 1998), with the DNA vaccine developed against avian influenza being the most successful. The hemaglutinin protein is expressed under the control of the cytomegalovirus (CMV) promotor. Upon heterologous challenge, higher levels of protection were obtained with the DNA vaccine than with the conventional inactivated vaccine (Kodihalli *et al.*, 1997). Despite these encouraging results, problems with delivery systems are generally encountered. The real challenge for DNA vaccination in the veterinary field involves therefore the improvement of delivery and potency of low doses of DNA in order to achieve similar levels of efficacy as obtained for conventional vaccines.

Although empty FMD capsids could be obtained with the expression of regions of the FMDV genome in different systems, the antigen yield remained low due to the toxic

effect of the 3C proteinase on the different host cells. To address this problem, DNA vaccines were designed for the production of empty capsids in animals.

One approach entails the construction of the cassette P12X3C using the pcDNA3 plasmid, which contains the strong type II promoter from the CMV immediately early gene as well as the bovine growth hormone (BGH) polyadenylation site. The P1/2A region as well as the 3C proteinase-coding region was inserted into this vector to produce the P12X3C cassette (Chinsangaram *et al.*, 1998a). It was shown that the expected viral proteins (VP0, VP3, VP1 and 3C) could be processed. Several variations of this cassette were also constructed. The plasmid (pP12X3C-mut) contains a point mutation at the active site of the 3C proteinase. Plasmid piP12X3C contains an intron, but piIP12X3C contains an intron and the FMDV IRES structure, upstream of the P1 region (Chinsangaram *et al.*, 1998a). The intron was included for enhanced expression in mammalian cells as well as immunogenicity (Beard *et al.*, 1999). The addition of the FMDV IRES structure was included to investigate whether cap-independent translation would have an effect on protein expression levels. These constructs were evaluated in mice and administered through gene gun inoculations. Plasmid piP12X3C was shown to be more immunogenic than pP12X3C. The addition of the IRES structure did not seem to have an effect on the immunogenicity. Both pP12X3C and pP12X3C-mut were able to elicit an antibody response, but only pP12X3C was able to produce neutralizing antibodies. The importance for the production of empty capsids to maintain conformational epitopes, was therefore confirmed again (Chinsangaram *et al.*, 1998a).

Another approach in the development of FMD DNA vaccines involves the engineering of the genome to produce attenuated FMD virions. To be able to manipulate the FMDV genome, an infectious cDNA clone, $pRMC_{35}$, was constructed (Rieder *et al.*, 1993). Attenuation was subsequently achieved through the deletion of the coding region of the Leader proteinase (Piccone *et al.*, 1995a). This proteinase is responsible for the inhibition of translation of the host-cell mRNA during infection. It was shown that the Leader-less virus is less virulent than the wild type virus (Brown *et al.*, 1996). Upon evaluation of the construct as a vaccine in cattle, two of the three animals did not

develop lesions, but did show signs of infection post challenge (Mason *et al.*, 1997). Partial protection was also observed upon evaluation in swine (Chinsangaram *et al.*, 1998b).

Attenuation was also achieved through the deletion of the receptor-binding site RGD (McKenna *et al.*, 1995). The virus is consequently unable to spread between normal cells, but can be propagated in cells containing a novel, genetically engineered receptor (Rieder *et al.*, 1996). Cattle vaccinated with the RGD-deleted mutants did not show signs of infection and were protected upon challenge (McKenna *et al.*, 1995). The RGD-deleted mutants were subsequently incorporated into the pcDNA3 vector containing the CMV promotor as well as the BGH site (Ward *et al.*, 1997). This construct, pWRMHX, produced non-infectious FMDV particles in cells and animals. Swine inoculated with this replicating DNA vaccine did not show any signs of disease and produced neutralizing antibodies to FMDV. Through the insertion of an intron onto the 3D region of the genome (pWRMHiX), infectivity was enhanced (Beard *et al.*, 1999). Evaluation of pWRMHX, pWRMHiX, piP12X3C and piIP12X3C in swine through gene gun inoculations showed that neutralizing antibodies could be obtained for all the DNA vaccine candidates. However, levels were much lower than preliminary studies in mice. Only pWRMHX and pWRMHiX were able to render significant protection upon challenge (Beard *et al.*, 1999). Although promising results were obtained, neutralizing antibody titres are much lower than that elicited with conventional inactivated vaccines. Improvements in plasmid design as well as alternative delivery systems could increase infectivity and therefore improve immunogenicity in animals (Beard *et al.*, 1999).

Recently, a recombinant replication-defective human adenovirus serotype 5 containing the P1-2A region of FMDV as well as the 3C region (Ad5-P12X3CWT) was constructed and investigated as a potential DNA vaccine candidate (Mayr *et al.*, 1999). Viral proteins VP0, VP3 and VP1 could be detected upon infection of human 293 cells. Swine inoculated intramuscularly with this construct developed neutralizing antibodies and were partially protected upon challenge. A variation of this construct, which contains a mutation in the proteinase, was not able to elicit neutralizing antibodies in

mice (Mayr *et al.*, 1999). In a similar study, only the P1 region was incorporated into the adenovirus-based vector (Sanz-Parra *et al.*, 1999). Partial protection in cattle was obtained only after a second round of combined subcutaneous and intranasal vaccination. Improvement of potency as well as the efficacy of the Ad5-P12X3CWT construct, are currently being examined (Mayr *et al.*, 1999).

1.4.2.3 Synthetic peptide vaccines

The production of synthetic peptides as a means to vaccination has the advantage of being chemically stable and defined. Due to the simple structure of these peptides, manipulation thereof can be readily accomplished. Incorporating of both B and T cell epitopes in the design of synthetic peptides, an appropriate immune response might be obtained (Brown, 1993; Sobrino *et al.*, 1999).

Following the elucidation of the viral capsid structure of FMDV, peptide vaccine candidates were constructed against the major immunodominant site (residues 140-160) located in the G-H loop of the VP1 protein. Initial evaluation of these peptides in guinea pigs, mice and cattle seemed promising (Bittle *et al.*, 1982). Although a peptide comprising of residues 140-160 of VP1 as well as residues 200-213 at the C-terminus end of the protein was able to protects upon challenge, immunogenicity was substantially lower than that elicited by conventional vaccines (DiMarchi *et al.*, 1986).

A large-scale evaluation of peptide vaccines directed against an Argentinean serotype C isolate was carried out recently (Taboga *et al.*, 1997). Four peptides containing B and T cell epitopes present in VP1 were tested. These peptides were defined as A (site A), AT (site A as well as a T-cell epitope), AC (site A as well as the C-terminus region) and ACT which included all three linearised epitopes. Upon challenge with the homologous virus, the highest level of protection was obtained with the more complex ACT peptide, although protection levels were still lower than 40%. The lack of solid protection reflects the difficulty in reproducing the immunogenicity of the entire viral capsid through the much simpler synthetic peptides. This is probably due to the inadequacy of T-cell epitopes to provide help to B-cell epitopes, the inefficient

presentation of the peptides to the immune system as well as the highly variable nature of the RNA virus (Sobrino *et al.*, 1999).

A better understanding of the B-and T-cell interaction as well as the inclusion of T helper epitopes will lead to improved strategies for synthetic peptide vaccines (Sobrino *et al.*, 1999). A T-cell epitope on VP4 has been shown to be recognised by T lymphocytes from vaccinated cattle (Van Lierop *et al.*, 1995) and swine (Sobrino *et al.*, 1999). This epitope, when positioned in tandem with the immunodominant site (B cell epitope) in VP1, rendered help to the B cell epitope and could therefore be a likely candidate to provide T cell help (Blanco *et al.*, 2000).

Improvement of peptide stability and presentation are also important factors to take into consideration. Promising results have been obtained recently through the use of retro-inverso peptides. These peptides are more resistant to proteinase degradation, thus enhancing immunogenicity. An all-D retro peptide corresponding to the immunodominant region of type A_{12} was linked to activated keyhole limpet haemocyanin and oil-adjuvanted before the inoculation of swine. These animals were challenged eleven days post-vaccination with the homologous virus. Only one animal from nine was completely unprotected, two developed small lesions, while the remaining six animals exhibited no clinical signs of disease (Nargi *et al.*, 1999).

1.5 Vaccination in sub-Saharan Africa – Aim and Scope of this study

As discussed earlier in this review (see sections 1.2 and 1.4), several parts of the world have been declared free of FMD by the International Animal Health Code of the OIE. These regions include the countries in the European Union, Uruguay and Paraguay in South America as well as several disease-free zones. Such an internationally recognized FMD-free zone status was given to South Africa in 1996 by the OIE. This status is of immense value to the country as animal and other agricultural products to the amount of approximately US$ 3.2 billion are exported annually (Agricultural News, 1998). The free-zone in South Africa include major parts of the country, with the exception of the northeastern region of the country (inclusive of the Kruger National

Park), while similar zones have also been awarded to Namibia and Botswana. Eradication of FMD in sub-Saharan Africa is however, highly unlikely due to the presence of large numbers of the free-living maintenance host, the African buffalo (*Syncerus caffer*). These animals provide a potential source of infection for domestic livestock (Dawe *et al.*, 1994) and other wildlife, such as impala (*Aepyceros melampus*) (Bastos *et al.*, 2000).

At present, the disease is controlled in sub-Saharan Africa essentially through vaccination, restriction of animal movement and frequent inspections of animals in the controlled areas (Hunter, 1998). However, the inactivated vaccines currently in use have several disadvantages. These include the inability to adequately cover the extent of antigenic variation within the SAT types in different regions. Antigenic and genetic characterization of field isolates have revealed that these viruses evolve independently and rapidly in different geographical areas, across international borders (Esterhuysen, 1994; Vosloo *et al.*, 1995; Bastos *et al.*, 2001). Vaccines therefore need to be custom-made to specific geographic areas to be effective. However, the adaptation of field isolates as high-antigen producing and stable vaccine strains is presently a cumbersome, time consuming and expensive process.

As an alternative method to address the problem of antigenic variation in different geographic areas, a combined approach between conventional inactivated vaccine technology and recombinant molecular biology, is proposed. The strategy entails the development of recombinant FMD viruses with the construction of a full-length cDNA clone of a suitable vaccine strain. The production of such recombinant viruses has been achieved previously for the A_{12} serotype (Sa-Carvalho *et al.*, 1997; Almeida *et al.*, 1998; Beard & Mason, 2000). The antigenic characteristics of such recombinant viruses can then be manipulated by exchanging the genetic determinants for antigenicity, i.e. specific regions of the genes encoding structural proteins. The recombinant viruses will then be applied in the manufacturing of conventional, inactivated vaccines.

It is therefore envisaged to develop custom-made vaccines to foot-and-mouth disease, employing recombinant FMD viruses, in quick response to an outbreak situation in a specific problematic area. The aim of this study was therefore to:

(I) investigate factors that could possibly influence the construction of such recombinant or chimeric viruses between the European and SAT types;

(II) the construction of a chimeric virus and the subsequent evaluation thereof, utilizing parameters important in commercial vaccine manufacturing.