CHAPTER 1

LITERATURE REVIEW

1. RIFT VALLEY FEVER

Rift Valley fever (RVF) is an acute or peracute zoönosis primarily affecting domestic ruminants (sheep, goats and cattle) in Africa and it is caused by a single serotype of the mosquito-borne Rift Valley fever virus (RVFV) (OIE Manual 1996). An undescribed virus disease of sheep, cattle and man from East Africa was reported in 1931 (Daubney, *et al.,* 1931). The disease was called enzootic hepatitis or Rift Valley fever and caused heavy mortalities among neonatal lambs on a farm in the Rift Valley in Kenya. About 95% mortality in very young lambs was reported and mosquitoes were suspected to be the vectors for this virus. Previously the virus was classified in a natural group with the viral agents of yellow fever and dengue of man however, it has been reclassified since. Since mosquitoes are the vectors of RVFV, climatic conditions, which favour mosquito breeding, are associated with outbreaks of RVF (Linthicum, *et al.,* 1999).

1.1 Epidemiology

Epidemics occur mainly in eastern and southern Sub-Saharan African countries and Madagascar. Countries in which major outbreaks and epidemics have occurred include Kenya, South Africa, Namibia, Mozambique, Zimbabwe, Zambia, Sudan, Egypt, Mauritania and Senegal (the first documented outbreak in West Africa, 1987). Smaller outbreaks, isolations and serological evidence have been recorded in Angola, Botswana, Burkina Faso, Cameroon, Central African Republic, Chad, Gaboon, Guinea, Madagascar, Malawi, Mali, Nigeria, Somalia, Tanzania, Uganda and Zaire (http:/www.cdc.gov/travel/).

The most recent outbreak in South Africa was in the Kruger National Park in 1999 where abortions and death occurred among buffalo, the virus could be isolated from other wild ruminants and disease occurred in humans (www.who.int). One of the most recent epizootics/epidemics in Africa occurred in East Africa in late 1997 and early 1998

(Linthicum, *et al.,* 1999) in Kenya and Somalia following heavy rains. In this outbreak, 300 people died in Kenya while an unknown amount of deaths occurred in Somalia. An extensive outbreak occurred in 1950-1951 in Kenya where 100 000 sheep died of the disease, accentuating the economic implications that a RVF outbreak might have. Major outbreaks occurred in South Africa in 1950-51 (Schulz, 1951) and an extensive epidemic in 1974-76 (www.who.int). In September 2000, an outbreak occurred in Saudi Arabia and Yemen. Within three months, 1100 human cases were reported of whom 11% died. This RVF epidemic represents the first documented transmission of the disease outside Africa. It was found that the virus isolated in the Yemen/Saudi Arabic epidemic is similar to the virus isolated in Africa in 1998 during the outbreak in Senegal (www.who.int).

Most of these outbreaks coincided with above average rainfall at irregular intervals, thus favouring the increase in mosquito populations (Davies, *et al*., 1985). An exception to the general rule of climate that plays a role has been an outbreak in Zimbabwe in 1969, which indicates that the association of wetter periods and RVF is unanimous and that other factors are involved. Because of wetter conditions prevailing over endemic and adjacent non-endemic areas, outbreaks can spread easily to non-endemic areas. Herd immunity that occurs among animals in endemic areas, the onset of colder conditions that suppress vector activity and an increase in predators of mosquitoes might lead to the disease to subside during an epidemic.

Epidemics such as the epidemic in Egypt in 1977 (598 deaths) as well as the outbreaks in Senegal in 1987 (224 deaths) and Saudi Arabia, Yemen in 2000 (outbreaks in countries not endemic to RVF) emphasize the importance of efficient control, diagnosis and surveillance of the disease. The Senegal epidemic was the first confirmed outbreak not alone of the disease in West Africa, but also of the disease in humans in West Africa. Egypt was "virgin" territory until an outbreak in Sudan in 1976 spread to Egypt causing the epidemic in 1977. These and other areas may be receptive for the disease if the vector is abundant and the concentration of domestic animals is high enough to facilitate in the amplification of the vector and virus during the transmission cycle (Shope, *et al*., 1982). The high titres obtained with plaque reduction neutralisation tests and Enzyme-linked Immunosorbent Assay (ELISA) to detect RVFV specific

immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies among camel sera collected from Niger, indicates that camel may be a sensitive and useful indicator of the presence of the virus in these regions (Mariner, *et al.,* 1995).

The fate of the virus during inter-epidemic periods and the reservoir of RVFV are still uncertain. It is thought that the virus exists in forests in a natural cycle (McIntosh, *et al.*, 1980) although no maintenance in transmission cycles in birds, monkeys, baboons or other wild vertebrates could be proven. Wild ruminants could play a role in the transmission cycle since positive diagnosis has been made of RVF in wild ruminants, i.e. during the outbreak in the Kruger Park in 1999. In South Africa, isolation of the virus from male and female aedine and mosquitoes confirmed that the virus is endemic to areas where livestock usually occur (McIntosh, *et al.*, 1980) and that maintenance of the virus takes place through transovarial transmission. Venereal transmission from transovarially-infected males to females could possibly also occur. The infected livestock and wild ruminants serve as a source of virus for feeding mosquitoes after initial infection. Ticks don't seem to play a role in the epidemiology of the disease (McIntosh, *et al.*, 1980) and contagion is not considered to be of importance. In contrast, non-vectorial transmission is important in humans, since blood, bodily fluids and organs (spleen, liver, and brain) of an infected animal is highly infectious (Shope, *et al.*, 1980). Laboratory and abattoir workers as well as veterinarians are thus at risk. Virus transfer from animal to animal has been reported during the epidemic in South Africa in 1974-76 upon vaccination with the same needle (Coetzer, *et* al., 1994).

1.2 Experimental infection

Virulence of the RVFV viral strain and susceptibility of the host animal determine the morbidity and mortality of RVF after natural infection. Discrepancies in disease patterns in experimental infection can be explained in terms of differences in the challenge rate, age, acquired immunity and reproductive status of the host (Shope, *et* al., 1982). The disease could not always be reproduced in non-pregnant animals after peripheral administration of the virus (Swanepoel, *et al.,* 1986), even though a high dosage of a virulent virus strain was used in such experimental infections.

1.3 Manifestations of RVF

Some variation occurs in disease patterns between groups of animals and epidemics. During an epidemic RVF can predominate in cattle or sheep at a stage or location that may change as the epidemic continues. An example of the variation is the difference in disease patterns between the disease described in 1931 (Daubney, *et al.*, 1931) and the epidemic that occurred in South Africa in 1951 (Schulz, 1951). During the epidemic in 1951, cattle were affected to a lesser extent than what was observed in 1931. Pathological differences were evident in the spleen, lungs and adrenal glands. During an epidemic, abortions and disease in ruminants along with disease in humans tend to be characteristic of an outbreak of RVF, although clinical signs of the disease tend to be non-specific (Shope *et al.*, 1982).

Major manifestations of RVF are known to be abortion in pregnant animals (related to the febrile illness of the dam) as well as disease of adult and neonatal animals (Coetzer, 1982). Colostral immunity against RVF can be conferred upon neonatal lambs by immune ewes. However, irreversible infection can occur if the lambs are attacked by sufficient quantities of mosquitoes. Humans become infected by handling of infected animal tissue or by mosquito bites. This results in symptoms of fatal hepatic disease, encephalitis or nonfatal, sometimes influenza-like infection. Aerosol and intranasal infection is very rare and exceptional, but have been demonstrated (Bishop, *et al.*, 1980).

RVFV is spread from the initial site of replication (lymph nodes) to the critical organs (spleen, liver and brain) where viremia is caused by the release of the virus. Damage is done due to the lytic effects of the virus (Coetzer, 1982). Incubation times of RVF in lambs and kids are from 12 hours and onset of the disease is characterized by a fever (often showing bi-phasic trends) exceeding 41°C with a 12-18 hour remission, subsiding prior to death (OIE Manual, 1996). Listlessness, disinclination to move or feed, abdominal pain and rapid and abdominal respiration are clinical symptoms during the peracute stage of RVF. Viremia may be demonstrable within 16 hours post infection and it persists until death, 36-42 hours later in lambs less than one week old. Maximum titres recorded of viremia are the highest in lambs and kids. In older animals, viremia is demonstrable 1 to 2 days post infection and it may persist for up to 7 days. Highest intensity of viremia occurs between the second and fifth day of the disease. RVFV was

shown to persist in the spleen of infected cattle for up to 31 days after a virulent challenge (Yedloutschnig, *et al.,* 1981).

The disease may be acute (mostly occurring under field conditions), peracute with sudden death or unapparent. Acute disease has an incubation time of 24-72 hours, a fever of up to 42°C lasting for 24-96 hours, anorexia, weakness, listlessness, an increased respiratory rate, at times foetid diarrhoea, blood-tinged nasal discharge and icteric symptoms with death occurring 2 to 8 days post infection. Pregnant animals can abort autolysed foetuses at any stage of gestation. Disease in calves resembles the described situation in lambs (Coetzer, 1982). Infection in adult cattle is sometimes unapparent or otherwise acute with a fever of 24-96 hours duration, symptoms of anorexia, a staring coat, lachrymation, salivation, nasal discharge, dysgalactia and foetid diarrhoea. Cows may also abort an autolysed foetus at any stage of gestation. Symptoms in humans are unapparent or influenza-like with ocular lesions, encephalitis or severe haemorrhagic hepatic disease occurring in some cases. Less than 1 % of the patients develop the haemorrhagic or encephalitic forms of the disease (Shope, *et al.,* 1982).

1.4 Immune response and resistance to RVF

RVFV is able to replicate in almost all cell types *in vivo,* with the liver and spleen being the major sites of virus replication. After infection, due to the destruction of the lymphoid tissue by the lytic effects of the virus, interferon and antibody responses are delayed and the hepatic effect of the virus cannot be controlled by the host responses (Coetzer, *et al.*, 1994). Antibodies were seen to become demonstrable within 2 to 3 days after infection (Swanepoel, *et al.,* 1986). During recovery, antibodies and macrophages facilitate clearance of the infection, the latter by production of interferon and induction of cell-mediated immune responses.

Resistant flocks or herds might be bred because of resistance being inherited in a simple Mendelian gene (Coetzer, *et al.,* 1994). Different species and even breeds of susceptible animals are known to differ in their relative susceptibility to RVF. Breeds of domestic ruminants exotic to Africa or areas where the virus is not endemic seem to be more susceptible to the disease (OIE Manual, 1996).

1.5 Significance of liver enzymes

An increase in serum levels of enzymes such as sorbitol dehydrogenase (SDH) and glutamate dehydrogenase (GLDH) occur early in the disease. GLDH levels increase significantly from the second day post-infection and 1 week after infection, levels decline to pre-infection levels, while SDH values increase from 4 days post-infetion (Swanepoel, *et al.,* 1986). GLDH is responsible for the conversion of α-ketoglutarate to L-glutamate (Brobeck, 1978). The variation in these enzyme levels is indicative of liver necrosis or necrotic hepatitis. Gamma-glutamyl transferase (GGT) levels remain the same during the disease (Swanepoel, *et al.,* 1986), indicating that the biliary tract is not affected during RVF. Aspartate aminotransferase (AST) catalyses the conversion of α-ketoglutarate and aspartate to oxalacetate and glutamate (Brobeck, 1978). Increasing levels of aspartate aminotransferase (AST) later on in the disease suggest hepatocyte degeneration. Alanine aminotransferase (ALT) levels show irregular and non-significant fluctuations confirming the fact that this enzyme test is of no use in herbivores (Swanepoel, *et al.,* 1986; Coetzer, 1982).

1.6 Pathology

The main pathological feature of RVF is hepatic lesions of diagnostic significance (Coetzer, 1982). The lesions (confined primary foci of necrosis) found in sheep and cattle are similar in infected animals and humans, with the most severe lesions occurring in aborted sheep foetuses and neonatal lambs. Lesions in adult sheep are less severe and not as widespread, although icterus occurs more evidently. Although the lesions seem to be similar among species, there are significant differences among different age groups, where younger animals have massive hepatic necrosis. Haemorrhages in the abomasum lead to the presence of free blood in the intestines. The spleen tends to be slightly enlarged with haemorrhages occurring in the capsule (Coetzer, 1982). The most striking microscopic feature is hepatic necrosis in all infected individuals, from there the description enzootic hepatitis (Schulz, 1951).

2. RIFT VALLEY FEVER VIRUS

2.1 Virus classification

Rift Valley fever virus is classified among the Bunyaviridae family of viruses in the *Phlebovirus* genus (Shope, *et al.*, 1980; Levy, *et al.,* 1994). There are five genera within the family Bunyaviridae: *Bunyavirus, Hantavirus, Phlebovirus, Nairovirus* and *Uukuvirus* (Levy, *et al.,* 1994). These viruses are arthropod-borne with a negative single-stranded RNA (ssRNA) genome. The Bunyaviridae family comprises more than 200 viruses (Bishop, *et al.* 1980), with the prototype virus of the family the Bunyamwera virus (Porterfield, *et al.*, 1975/76). The wild-type virus can be described as hepatotropic, viscerotropic or pantropic depending on the cellular tropism of the viral strain (Coetzer, *et al.* 1994).

2.2 Virus morphology

Characteristic of viruses belonging to this family is the uniform, spherical shape with a diameter of approximately 80-110 nm and a unit membrane envelope (Holmes, 1971). After glutaraldehyde fixation of tissue preparations containing the virus, the structural glycoproteins can be seen as 8-10 nm surface projections under the electron microscope (Donets, *et al.*, 1977; Obijeski, *et al.*, 1977). The particles contain an electron dense area of membrane surrounding the less electron dense space containing the nucleocapsid (Holmes, 1971).

2.3 Expression strategy

Although the Bunyaviridae family is considered as a family of negative-sense RNA viruses, some of these viruses - including the *Phlebovirus* RVFV - have a replication cycle that employs an ambisense expression strategy. An ambisense expression strategy involves transcripts and proteins that are found within the virus-infected cell thus suggesting positive- and negative-sense expression strategies (Levy, *et al.,* 1994). This implies that positive-sense RNA can directly act as mRNA, or these positive-sense RNA transcripts may act as templates for transcription of negative-sense transcripts that in turn can code for mRNA's or positive-sense viral genome species. For

example, the Punta Toro virus and the Sandfly Fever Sicilian virus, both *Phleboviruses,* was shown to employ negative-sense expression strategies (Ihara, *et al.*, 1984; Marriott, *et al.,* 1989).

2.4 Virus release

The virus is released from the infected cell by lysis or exocytosis (Obijeski, *et al.,* 1977). It was shown by electronmicroscopy that viral particles are associated within the cisternae and vesicles of the Golgi complex (Donets, *et al.,* 1977) and the viral proteins accumulate in the cytoplasm and at cellular membranes (Bishop, *et al.,* 1980). The virus develops in the cytoplasm of infected cells and maturation occurs by budding into the smooth-surfaced vesicles in the region of the Golgi system (Porterfield, *et al.,* 1975/76).

2.5 Virus characteristics

The host-cell derived lipid membrane of the RVFV particle renders it sensitive to lipid solvents such as ether, sodium deoxycholate and non-ionic detergents. The virus is more stable at pH 7-9 than at acidic pH values and it is rapidly inactivated at a temperature of 56°C, while at 4°C, the virus is stable in serum for several months and when stored in a freeze-dried state or at -60°C, even more so (Bishop, *et al.*, 1980; Levy, *et al.*, 1994). By using proteolytic enzymes to remove the exterior glycoproteins, the infectivity of the virus can be reduced significantly and the virus is inactivated rapidly by using ultraviolet light. Low concentrations of formalin and pH values below 6.8 also result in loss of infectivity. RVFV grows and produces cytopathic effects (CPE) and plaques on almost all primary cell cultures and continuous lines, with the exception of primary macrophages and lymphoblastoid cell lines (Swanepoel, *et al.*, 1986; Coetzer, *et al.* 1994).

2.6 Genome organization and expression products

The RVFV genome consists of three separately encapsidated single-stranded RNA segments, designated S, M and L according to the relative sizes of the segments (Robeson, *et al.*, 1979; Bishop, *et al.*, 1980; Levy, *et al.,* 1994). Genomic RNA segments have the potential to be organized in non-covalently closed circles (Bishop, *et al*, 1980;

Levy, *et al.,* 1994) because of the existence of complementary, genus specific conserved terminal sequences on the 5' and 3' termini (Suzich, *et al.,* 1990). The different sizes of the segments are respectively 0.3-9kb, 3.2-5kb and 6.5-14.4kb for the S, M and L segments (Levy, *et al.* 1994). The sedimentation coefficients of the genome segments are for the different segments L: 27-31S; M: 22-26S; S: 16-20S and the molecular weights are L: 2.6-2.8 x 10^6; M: 1.8-2.2 x 10^6; S: 0.7-0.8 x 10^6 (Bishop, *et al.*, 1980). The segmented genome of members of the Bunyaviridae family confers the capacity to the virus for antigenic shift. This occurs via segment reassortment of the genome as evidenced by naturally occurring reassortant viruses (Ushijima, *et al.*, 1981; Levy, *et al.*, 1994).

The S (small) segment's expression proceeds via the ambisense strategy (Suzich, *et al.,* 1990) thus both gene products from the S segment are synthesized from different templates by means of bi-directional coding. The nucleocapsid protein (N), a 20-30kDa protein, is synthesized from virus-complementary mRNA (Cash, *et al.,* 1979), while the much smaller non-structural Ns_s protein, is synthesized from a viral-sense mRNA. However, both the L (large) and the M (medium) segments have a negative-sense expression strategy (Suzich, *et al.,* 1990).

A viral RNA-dependent RNA polymerase has been found to be associated with the virus (Ranki, *et al.,* 1975). This enzyme is encoded by the L segment, while the M segment encodes for the two unique structural glycoproteins G1 and G2 of 55-70 kDa and 50-60 kDa respectively (Gentsch, *et al.*, 1979; Suzich, *et al.* 1990). In addition, it was also shown that two other non-structural proteins that have sizes of 14 kDa and 78 kDa each are also being expressed from the M segment.

2.7 Significance of the glycoproteins

The glycoproteins are formed by post-translational cleavage of a primary viral gene product. This implies that the M segment RNA is first translated into a polyprotein, which is subsequently cleaved to yield the different protein species (Obijeski, *et al.,* 1977). Many biological functions can be assigned to the M segment gene products (glycoproteins). These include attachment, fusion, hemagglutination, neutralisation and virulence, while the other proteins encoded by the genome may have modulating effects

on the viral virulence (Levy, *et al.*, 1994). With the use of monoclonal antibodies (mAbs), four antigenic determinants on the G2 glycoprotein have been identified of which three were shown to be involved in virus neutralisation (Gentsch, *et al.*, 1980; Keegan, *et al.*, 1986). After denaturation of the antigen (G2), three of the four mAbs still showed reactivity to the G2 glycoprotein, indicating that the corresponding epitopes are displayed in the employed expression strategy in a manner similar to the authentic glycoprotein. The sequences involved in antibody recognition were subsequently identified after cloning different regions of the G2 glycoprotein and using a bacterial system to express the proteins. In mapping the glycoproteins antigenically, it was shown that the identified epitopes had biological functions that correlated to antibody neutralisation and hemagglutination-inhibition (Besselaar, *et al.*, 1991). The epitopes that were recognised by the neutralising antibodies were thus accessible in a manner similar to the native proteins on the virus. The G1 glycoprotein plays an important role in pathogenesis and infectivity of the virus, although it is not protective. Antigenic mapping of G1 demonstrated four clusters of possibly interlinked epitopes.

The glycoproteins, G1 and G2 are synthesized in a seemingly similar way in Bunyavirus infected cells for several hours after infection, starting at about 4h post infection (p.i.). During this time, no evidence for proteolytic cleavage of the polyprotein encoded by the M segment could be shown. G2 and G1 are made for several hours and then the rate of synthesis declines. The G1 protein synthesis started about 4 hours p.i. which is rather late in the virus growth cycle, while synthesis of the G2 could not be properly followed because of co-migration with cellular proteins during electrophoresis (Pennington, *et al.*, 1977).

Nucleotide sequence differences of 0-4.5% and amino acid sequence differences of 0-2,4% for sections of the glycoprotein genes and proteins respectively, indicate little genetic variation between RVFV isolates, although distinctions could be made between isolates using oligonucleotide fingerprinting (Cash, *et al.*, 1981). The virus isolated during an outbreak in South Africa in 1951, did not differ from the isolate that caused the disease in 1931 in Kenia (Schulz, 1951) indicating the conserved nature of the recognised structural glycoproteins. Due to the conserved nature of these viral structural proteins, it can serve in a vaccine strategy against all the isolates.

3. DIAGNOSIS OF RIFT VALLEY FEVER

Diagnosis can be made from blood (preferably in an anti-coagulant), plasma, serum or organ samples by doing virus isolation or demonstrating virus specific antibodies (OIE Manual, 1996). Virus can be isolated either from blood during the febrile stage of the disease, from liver, spleen or brain of animals that died of the disease or from aborted foetuses. Suckling or weaned mice or hamsters are used extensively for virus isolation although not for identifying the virus. When infecting a cell line, CPE can be detected 1 to 5 days after inoculation or infection of the cells.

Viral antigen can be detected in infected tissue using several methods. Primary virus isolation can be done in small animals (hamsters or mice) or on cell cultures (OIE Manual, 1996). Immunofluorescence (IF) gives results in 24 hours or sooner. Complement fixation (CF), immunodiffusion (ID) and immunoperoxidase (IP) staining can also be used to detect viral antigen in tissue. However, results can't be obtained as soon as with IF. In serum of infected animals antigen can be detected by reversed passive heamagglutination or ELISA (OIE Manual, 1996; Coetzer, *et al.* 1994).

Virus specific antibodies can be demonstrated using CF, ID, ELISA, indirect IF, heamagglutination-inhibition (HAI), reversed passive HAI, radio-immunoassays (RIA), neutralisation of CPE, plaque reduction neutralisation (PRNT) and neutralisation tests in mice (OIE Manual, 1996; Swanepoel, *et al.,* 1986). Virus neutralisation is performed most commonly in detecting virus specific antibodies, since antibodies become demonstrable within three days. The 80% plaque reduction neutralisation titre ($PRNT_{80}$) test is recommended as the most specific and sensitive serological assay for RVF (Swanepoel, *et al.*, 1986; Mariner, *et al.,* 1995). This test is able to detect the earliest response (three days p.i.) against RVFV. Tests such as the virus neutralisation test, where live virus is used to detect the antibodies, are not recommended for use in countries where RVF is not endemic. By employing a capture ELISA the presence of virus specific antibodies could be demonstrated at 6-7 days p.i., distinguishing between recently infected animals and infections at an earlier stage on the basis of IgM presence (Niklasson, *et al.*, 1984). However, these ELISA systems also employ inactivated, whole virus as antigen.

Due to the effects of possible antigenic cross-reaction between different *Phlebovirus*es, neutralisation tests that may be the least affected by this would be the most suitable in diagnosis of the virus infection (OIE Manual 1996). Cross-reactions occur in HAI tests between all *Phlebovirus*es, while CF, ID and neutralisation tests proved monospecific for the different serotypes. Cross-reactivity in serological assays of other *Phlebovirus* specific sera for RVF was found to be negligible and it could be concluded that when comparing the pathogenicity and cross-reactivity of *Phlebovirus*es with RVFV, none are likely to cause disease or elicit antibodies that may be confused with RVF during diagnosis (Swanepoel, *et al.,* 1986).

4. CURRENTLY EXISTING CONTROL MEASURES AGAINST RIFT VALLEY FEVER.

Efforts to control the spread of RVF include biological and chemical control of the vector, moving the livestock to different areas that are well-drained, wind-swept and on a higher altitude, confining the animals to insect proof stables and immunisation. Of all these, immunisation is the only effective control measure, because the others are implemented too late or is impractical and expensive.

4.1 The live, attenuated Smithburn vaccine

4.1.1 Development of the Smithburn vaccine

The Smithburn vaccine strain (Smithburn, 1949) originated from the virulent Entebbe strain, which was isolated from mosquitoes captured in Uganda and subsequently inoculated in mice. The virus was passaged serially in mice in the process of identifying it. This passaged virus isolate lost tropism for the liver, but the neurotropic properties were retained (Smithburn, 1949). Smithburn then studied the possibility of using the modified virus as an immunizing agent. The neurotropic virus was found to be suitable as a vaccine even for use in newborn lambs. Inherited immunity could be demonstrated in newborn lambs for 5 months after birth following immunisation of the pregnant ewes. Although a decline in antigenic potency was observed with serial passaging of the virus, sufficient antigenic potency was retained to elicit high levels of

antibodies. The live vaccine is being produced today from the stock antigen derived from Smithburn's original neurotropic strain. Durable immunity in sheep can be obtained after a single inoculation with this vaccine, although problems are associated with the Smithburn vaccine.

4.1.2 Problems encountered with the Smithburn vaccine

The Smithburn vaccine may cause teratology and a percentage of the inoculated pregnant animals abort (Barnard, *et al.,* 1977). The cerebral hemispheres of the brain of a foetus are often absent and replaced by sacs filled with cerebrospinal fluid (hydranencephaly) and limited motion in multiple joints (arthrogryposis) occur in foetuses. These conditions are associated with *hydrops amnii* (edema of the amniotic sac) and prolonged gestation in ewes (Coetzer, *et al.,* 1977) when ewes are immunised with the live Smithburn vaccine strain at 42-74 days of gestation. Inoculation before this time (first trimester of pregnancy) could lead to early loss of the conceptus and teratology and inoculation at a later stage may lead to abortion, stillbirth or birth of viremic progeny (Coetzer, *et al.,* 1977). The maternal antibodies developed after vaccination at a late stage in gestation might give some degree of protection in the neonatal lamb in the form of colostral immunity. Although antibody levels obtained after vaccination of cattle with the Smithburn vaccine is lower in comparison to antibody levels obtained after vaccination with the killed vaccine, protection can still be obtained when vaccinating with the Smithburn vaccine (Barnard, 1979; Shope, *et al.,* 1982).

4.2 Inactivated virus vaccine

4.2.1 Development of the inactivated vaccine

Because low levels of antibodies are observed after vaccination with the Smithburn vaccine, the use of a formalin-inactivated (killed) vaccine is advised in vaccination of cattle (Shope, *et al.,* 1982). A wild-type highly immunogenic field-strain is used in the production of formalin-inactivated RVF vaccines (Barnard, *et al.*, 1977; Coetzer, *et al.,* 1977; Coetzer, *et al.,* 1994; OIE Manual, 1996). The virus can be inactivated using formalin, formaldehyde or beta-propiolactone (Barnard, 1979; OIE Manual 1996). The inactivated vaccine is used in pregnant sheep, cattle and in countries

where RVFV is not endemic. The inactivated vaccine differs from the virus used for the attenuated strain in that it is lethal when injected intraperitoneally in adult mice (OIE Manual, 1996).

4.2.2 Problems encountered after vaccination with the inactivated vaccine

Although the inactivated virus is safe to use in pregnant animals, large quantities of viral antigen need to be prepared, making it expensive to produce. Another disadvantage of the killed vaccine is that it induces short-lived immunity (Barnard, 1979). Inconsistent antibody titres are elicited after vaccination of sheep and annual vaccination with booster doses is necessary for sufficient protection against RVF (OIE Manual, 1996).

4.3 Immune response of animals vaccinated with the classical vaccines

Both vaccines are stored in lyophilised form with a titre of at least $10^{6,5}$ mouse LD_{50} per ml (OIE Manual 1996). The primary response in cattle when injected with both vaccines is similar (Barnard, 1979). After administration of a booster dose of the live vaccine, the antibody response is poor and only animals responding to the first dose will respond to the booster dose. Low immunogenicity in cattle vaccinated with the Smithburn vaccine is observed although immunisation occasionally was done successfully against RVF with this vaccine despite low neutralising antibody levels (Barnard, 1979). A booster dose of the inactivated vaccine stimulates antibody response in cattle significantly. Significant antibody titres could still be detected in cattle after 9 months (Barnard, *et al.,* 1977) using the inactivated vaccine. A variation in the antibody response in vaccinated sheep was observed after vaccination with the inactivated vaccine and despite a low serum-neutralising index, most of the inoculated sheep could resist infection of the virus. Sheep vaccinated with the Smithburn vaccine developed significant antibody titres and long-term protection (Coetzer, *et al.,* 1994; Morrill, *et al.,* 1997).

4.4 Vaccination of humans at risk

An experimental formalin-inactivated cell culture adapted RVFV vaccine (TSI-GSD-200) produced in the USA has been used for protection of humans at risk, i.e. laboratory workers and veterinarians, while the MP-12 chemically inactivated vaccine is an alternative not yet commercially available (OIE Manual, 1996; Pittman, *et al.,* 2000). Because of limited availability, the TSI-GSD-200 vaccine is not recommended for use in a public vaccination strategy (Shope, *et al.,* 1982; OIE Manual, 1996). Multiple doses are required for sufficient protection, however, the TSI-GSD-200 inactivated vaccine gives protection after multiple doses to vaccinees for approximately 6 years (Pittman, *et al.,* 2000). In situations such as epidemics, rapid protection is required. Single dose vaccination and high immunogenicity of the vaccine would then be desirable.

4.5 Other control measures

Due to the disease potential in humans and problems associated with the vaccines against RVF, the disease situation needs to be monitored continually in an efficient surveillance program. It is necessary to advance in the search for a safe vaccine as well as practical control measures to prevent outbreaks and spread of the virus in endemic areas as well as to areas free of the disease.

Israel implemented quarantine measures for imported livestock to prevent the disease from entering the country (Shope, *et al.,* 1982). Although quarantine measures are expensive, in an effort where most of the preventative measures are being taken in combination with each other, successful control might be obtained. However, the impracticality of such measures makes quarantine not an obvious choice in disease control. Livestock can also be moved to drier, wind-swept, higher altitudes where mosquito populations are smaller and high rainfall does not lead to the formation of temporary ground pools (dambos). Protecting animals from insects, specifically mosquitoes, would ensure less amplification of the virus in a transmission cycle.

5. DNA VACCINES

5.1 DNA vaccines - an overview

Definition: Genetic or DNA immunisation is the process of *in vivo* transfection, in which plasmid DNA that encodes protein based antigens is administered to an organism to raise a required immune response against the desired protein antigens (Kucerova, 1998). In this, eukaryotic expression vectors are used as plasmid backbones to produce the target proteins in the vaccinee. Genetic vaccines employ DNA (as plasmids) or RNA (as mRNA) as source of the genetic information that has to be transferred into the cells.

5.2 Development of DNA vaccination

In 1990 J.A. Wolff and colleagues set the direction of DNA vaccine research by reporting on a study done on mouse muscle, where RNA and DNA expression vectors containing genes for chloramphenicol acetyltransferase (CAT), luciferase and β-galactosidase where introduced into the skeletal muscle *in vivo*. Injection of the pure DNA or RNA directly into the skeletal muscle was shown to result in the expression of the cloned reporter genes at significant levels (Wolff, *et al.,* 1990). The study was aimed at directly introducing genetic information for gene therapy, instead of indirect introduction through changes in target cells. Functional CAT could be detected in extracts of the skeletal muscle. β-galactosidase activity could be detected after staining of sections of the muscles indicating the site of expression. A dose-response effect could also be observed when different amounts of luciferase reporter gene constructs where injected. The luciferase activity could still be detected after 60 days and it was reported that the DNA present in the cell is in a non-replicating circular extra-chromosomal form indicating that at that stage the exogenous DNA has not been integrated into the cellular genetic material.

Exact mechanism of entry of the polynucleotides into the cells remains uncertain, but very low amounts of the reporter gene products could be detected in other tissues as well. Muscle cells were successful in taking up the constructs because of the specific structural features and the low amount of connective tissue present. The DNA enters the

post-mitotic cell nuclei through the nucleopore in the same way as karyophilic macromolecules enter the cell (Dowty, *et al.,* 1995). The introduced gene has to be transferred across the cell membrane and entrance into the nucleus has to take place before the onset of transcription. For this purpose, modified recombinant viruses can be used as vectors to introduce the genetic information into the cells (Davis, *et al.,* 1993) although direct transfer was shown to work (Wolff, *et al.*, 1990). Entry of DNA into tissue did not seem to be the result of damage to the cells, because in cells at a distance from an injection site, β-galactosidase activity could also be detected when included for use as a reporter. (Danko, *et al.,* 1994).

5.3 Choice of an expression vector

The desired gene must be introduced into a mammalian expression vector containing the necessary control elements to enable stable expression of the protein in mammalian cells. These control elements that need to be included in a plasmid vector for vaccine production, are transcriptional control elements (promoter and enhancer, intron, polyadenylation signals), a replicon and a selectable marker (antibiotic resistance markers) for growth in bacterial cells (Danko, *et al.*, 1994). It was shown that after injection of a linear plasmid encoding the luciferase gene, luciferase activity could still be detected after 4 months (Danko, *et al.*, 1994), but by using other control elements in the same plasmid, the stable expression persisted for up to 180 days. The stability of infection was further influenced by the mitotic rate of the cells.

5.4 Delivery methods

DNA delivery methods include intramuscular injection (Wolff, *et al.,* 1990) and making use of jet injection systems, i.e. the gene-gun and particle bombardment (Fynan, *et al.,* 1993) that deliver the plasmid DNA intradermally. DNA can also be delivered by intravenous injection, inhalation and instillation - where the DNA will reach the mucosal surfaces. In delivering the DNA ballistically or via a jet injection system (gene-gun delivery), the DNA is precipitated onto a particle, i.e. gold-beads, and forced into cells with a high airpressure device (Leitner, *et al.,* 2000). On the other hand, cationic liposomes are used to enhance the delivery of DNA to each cell by complexing the DNA

with the liposomes to form microcapsules containing target plasmid DNA. Delivery of the DNA to more of the target cells will result in elevated levels of proteins being expressed.

The choice of a delivery method is influenced by the total amount of protein that can be produced, efficiency and stability of gene expression in the target cell and the repeatability as well as safety of the procedure (Danko, *et al.,* 1994). The stability of infection is influenced by the mitotic rate of the cells. Muscle cells can contain DNA even a year after infection without any sign of integration into chromosomal DNA (Wolff, *et al.*, 1990).

Improvement of the efficiency of cell-to-cell transfer of the delivered DNA or target gene may be obtained by using regenerating muscle as target tissue. Adeno- and retroviral vectors have been used to introduce DNA into the regenerating or mature cells along with plasmid DNA. The plasmid DNA gene transfer gave better results in both mature and regenerating cells in comparison to the other vector systems used (Davis, *et. al.,* 1993). In addition, cellular infiltration by the viral vectors was disadvantageous in comparison to plasmid DNA alone which had no such deleterious effect. Upon introduction of the plasmid DNA encoding the human growth hormone (hGH) DNA directly into the skin of mice with microprojectiles coated with plasmid DNA, it could be concluded that an immune response could be elicited by subsequent boosts and that this method of eliciting antibodies proves to be simple and time saving (Tang, *et al.,* 1992).

As mentioned, genetic vaccination can also include RNA as source of genetic information. The advantages of delivering RNA into the cell, is that RNA is transient in nature, it will not integrate into the chromosome and it will not cause insertional mutagenesis. However, the disadvantages of RNA are the instability, higher costs of production and storage and shorter term of expression leading to the need for booster injections for long-term protection (Waine, *et al.*, 1995).

The choice of tissue would depend on the route that needs to be followed. An intramuscular route seems to be the most efficient if the DNA is injected as an aqueous solution, but if the DNA is coated onto gold particles, the intradermal route seems to be the best (Davis, *et al.*, 1995). In targeting the post-mitotic muscle cells, the DNA is not being diluted out after several divisions and this could mean longer expression that can

result in a vaccination regime without booster doses being necessary (Dowty, *et al.*, 1995). Because some cells synthesize and secrete antigen more efficiently than others, the need for post-translational modifications to the protein will dictate the choice of target tissue. It is important to keep in mind that the method of injection has to be acceptable to the vaccinee and it has to be easy to administer.

Although seroconversion takes place after DNA immunisations, the amount of antibodies being produced varies with different models. However, it seems possible that antibody titres comparable to those elicited in traditional vaccines can be obtained. It has been found that even if antibody titres may be low, the seroconversion obtained in many instances can be sufficient for virus neutralisation and even protection against a lethal challenge (Dowty, *et al.*, 1995).

5.5 Immune response elicited by DNA vaccination

Structure of the plasmid backbone, amount of plasmid delivered into the cell, expression levels of the antigen, immunisation schedule, route of immunisation, target-tissue, number of immunisations, introns in front of the gene and toxicity of the antigen to the transfected cells are all factors that influence the immunogenicity of DNA vaccines. The strain of species to be immunised and the age of animals must also be considered as influencing factors on the immunogenicity of the DNA vaccine (Leitner, *et al.*, 2000). Efficacy of the antibody response is influenced by the expression vector, the method of DNA delivery and form of the expressed antigen.

Immunisation with DNA expressing HIV-1 genes could elicit humoral and cellular immune responses sufficient to protect naive chimpanzees from a challenge with HIV (Bagarazzi, *et al.*, 1998). The same constructs were found to immunologically potentiate infected humans (Ugen, *et al.*, 1998). Genetic immunisation has been done with constructs containing the measles virus hemagglutination and nucleoprotein genes. It was found that both humoral and cellular responses were elicited after immunisation with DNA constructs encoding the hemagglutination and nucleoprotein genes of the measles virus (Cardosso, *et al.*, 1996). However, the magnitude of the overall response could not be increased by additional injections. Humoral and cellular responses were obtained after vaccination of mice with DNA constructs encoding genes of the Bovine

Parainfluenza Virus type 3 glycoproteins when the genes were under the control of the human cytomegalovirus immediate-early promoter followed by intron A for stabilization of the RNA transcript (Van Drunen Little-van den Hurk, *et* al., 1999). The fact that DNA vaccines are able to elicit antibodies as well as raising a cell-mediated response means that invading pathogens can be blocked by the antibody and the pathogen infected cells can be destroyed by cytotoxic T-lymphocytes (CTL). This represents an ideal vaccine situation.

In DNA vaccination the antigen can be synthesized from the plasmid *in vivo* in the targeted cell, processed and subsequently presentated on the major histocompatibility complex (MHC) class I molecules on the surface of the target cell. $CD8^+$ T cells are stimulated by antigen presented on the MHC class I molecules on the surface of the target cell. On the other hand, a T-helper response (CD 4+ cell stimulation) has also been observed after plasmid immunisation. The exogenous antigen expressed and secreted from the targeted cell is thus presented on the MHC class II molecule on the surface of the APC (i.e. dendritic cells, Casares, *et al.,* 1997; Robinson, 1997) after endocytic uptake of the antigen by the APC. The following of MHC-I and MHC-II pathways and subsequent induction of CD8+ and CD4+ T-cell subsets, mimic the situation of a live virus vaccine or infection.

Although muscle cells are not professional antigen presenting cells, some antigens that seem to be immunogenic enough to elicit a strong immune response, are expressed and presented to the T cells (Leitner, *et al.,* 2000). Thus, the possible mechanism of induction of immunity by muscle cells is the production of antigen as a protein or peptides for antigen presenting cells (APC). On the other hand, the APCs such as dendritic cells may be transfected directly and these cells then migrate to the lymph nodes where B and T cell activation occurs. In the situation where the inoculation was through the skin, the dendritic cells are stimulated by the antigen and it migrates to the lymph nodes for eliciting an immune response.

Gene-gun immunisation of mice compared well to the situation of sub-lethal influenza challenging of mice as a vaccination strategy, in that plateau levels of antibody persisted for more than one year (Boyle, *et al.*, 1996). It was concluded that the long-term antibody persistence that was seen with the DNA plasmid vaccination was

associated with the presence of antibody secreting cells in the bone marrow and that the initiation of responses were facilitated by the inguinal lymph nodes. The protective antibody response was associated with much lower levels of antibodies when animals were vaccinated with the plasmid DNA construct than in the situation with a natural infection.

5.6 Significance of CpG motifs

In a natural situation, a microbe has surface molecules that act as immunostimulatory factors or adjuvants during infection and although DNA plasmids are administered without such adjuvants, the immune response that is induced is quite strong. The reason for this is the presence of immunostimulatory sequences, the so-called CpG motifs, in the DNA itself (Krieg, *et al.*, 1995; Hartmann, *et al.*, 1999; McCluskie, *et al.*, 1999; Leitner, *et al.*, 2000). A CpG motif consist of a cytosine molecule bound to a guanine molecule via a phosphodiesther linkage. These non-methylated, palindromic, dinucleotide sequences in DNA can activate monocytes, natural killer cells (NK), dendritic cells and B-cells in an antigen-independent manner. These sequences occur in the order of 20 times more in bacterial DNA than in DNA of higher eukaryotes and it consist of purine-purine-CpG-pyrimidine-pyrimidine (Pu-Pu-CpG-Py-Py) sequences (Krieg, A.M. *et al.*, 1995). The non-specific adjuvant activity of CpG sequences (Hassett, D.E., *et al.*, 1999) causes cytokine production - including that of interferon-gamma (IFNγ). Cytokines are important in virus infection control by stimulation of monocytes and macrophages (Robinson, H.L., *et al.*, 1997). Although IFNγ is needed for the induction of IgG_{2a} antibodies after intramuscular plasmid administration, the antigen specific CD8 + T cell responses could still be stimulated by DNA immunisation and CTL activity could be primed in the absence of IFNγ (Hassett, *et al.*, 1999). The virus-specific IgG could be induced and maintained, but the antibody response was skewed after DNA immunisation in the absence of IFNγ. This could be explained by the fact that IFNγ is involved in IgG class switching, inhibiting IgG_1 and enhancing IgG_{2a} responses.

5.7 Dosage requirements

The amount of DNA necessary for intradermal inoculation is much more than required for inoculation using the gene-gun because the transfection efficiency is much higher for the latter scenario, where the DNA is shot inside the cell compared to the former in which the DNA is introduced in the needle wound and not directly into the cell (Fynan, 1993). Dosage requirements for mice is 1 to 100 µg, for monkeys and calves 10 µg to 1 mg (when injecting intradermally or intramuscularly), while in the event of a gene-gun inoculation, only 10 ng to 10 µg of DNA is required. The amount of DNA needed is almost independent of the size of the animal that needs to be vaccinated (Robinson, *et al.*, 1997).

By injecting large amounts of plasmid and immunostimulatory sequences, the immune response is a biased Th1-type response. Smaller amounts of plasmid DNA or a low amount of DNA that is able to enter the target cell, such as when using the gene-gun inoculation method, will lead to a Th2-type response with the IgG_1 playing a dominant role (Barry, *et al.*, 1997; Leitner, *et al.*, 2000).

5.8 Optimisation of DNA vaccination strategies

As DNA vaccination strategies need optimisation, enhancement can be achieved by modifying the antigens (Rice, *et al.*, 1999). This can achieved either by deleting domains functioning in targeting the protein (Xiang, *et al.,* 1995; Cardoso, *et al.*, 1996; Lewis, *et al.,* 1996) or by adding sequences to target the antigen specifically (i.e. to either the class-I or class-II pathway). Specific epitopes can be used as minigenes or it can be included in a highly immunogenic sequence. In this way, B- or T- cell specific epitopes can be chosen according to the requirements for a specific vaccine.

Cytokines can be included in a vaccination strategy to direct or enhance a desired response (Lewis, *et al.*, 1997; Kim, *et al.*, 1998; Leitner, *et al.*, 2000). The cytokines can be included exogenously or the cytokine-encoding gene can be included in the plasmid construct. Because the DNA is usually injected into the muscle cells that are poor APC's and thus poor initiators of an immune response, the co-administration of immunostimulatory molecules could engineer the muscle cells sufficiently to act as APC's. In this way, the induction of a T-cell immune response can be facilitated by the

transfected muscle cells. It is important that the administered cytokines are specific for the type of response or direction of the response, because the different cytokines facilitate in different arms of the immune response (Lee, *et al.,* 1999). Physical linkage of the cytokine to the antigen can further enhance the immune response by ensuring the stimulatory effect in the local environment of the antigen where the responses occur. Chow, *et al.* (1997) found that IL-2 could stimulate the immune response to a Hepatitis B surface antigen a 100-fold when it is co-expressed in a plasmid vector.

A heterologous prime-boost-regimen has certain advantages over homologous boosting (Leitner, *et al.,* 2000). The efficacy of a virus-based vaccine may be hampered by an anamnestic response to the virus-based carrier, if given repeatedly. To include DNA immunisation in a vaccination strategy could prevent this, in addition to the fact that the immune response can be shifted in a Th1 direction. It may be important to administer the antigenically simplest vector first and the more complex vector or more potentially powerful antigen last. The HIV envelope protein genes were used in a recombinant DNA molecule as well as a recombinant vaccinia virus (Caver, T.E., *et al.*, 1999). Both these constructs and purified envelope proteins were used in a heterologous prime-boost regimen where the immune response could be enhanced a 100-fold in relation to a strategy where a DNA construct was used as booster.

Another way of optimising a DNA vaccination strategy could be to facilitate an increase in the replication of the DNA in the mammalian cell. A useful characteristic of members of the Alphavirus genus is the feature that the RNA encodes it's own RNA replicase, thus after infecting the cell, the replicase complex is being translated and this facilitates the replication of the viral RNA that encodes the genes of the structural proteins (Levy, *et al.*, 1994; Polo, *et al.,* 1998). This implicates that the structural protein genes can be replaced by that of the desired antigen, establishing a self-replicating DNA vaccine construct. Because replication takes place in the cytoplasm of the host cell, it is independent of the host cell's replication machinery. The advantages of a DNA vaccine with these features, are a broad host range of cells that can be "infected", much higher levels of expression and cytoplasmic replication (Leitner, *et al.*, 2000). This can lead to efficient MHC-I presentation due to the high amount of antigen present in the cell and the presence of ds RNA that may cause the system to trigger the release of cytokines such as

interferon molecules. When the transfected cells release antigen after transfection, it may be taken up by the local APC's leading to an enhancement of antigen being presented to the immune system. The self-replication also means that less of the vaccine can be administered to achieve protection if compared to conventional DNA vectors.

Targeting the DNA to specific tissue is possible, i.e. by adding asialoglycoprotein receptor ligands to the liposome-DNA complex for targeting hepatocytes or Kupffer cells (Templeton, *et al.*, 1997) or by expressing CD4A fusion proteins that target the expressed protein to antigen presenting cells (APC) (Deliyannis, *et al.*, 2000).

5.9 Limitations of currently used vaccines

Currently used vaccines - subunit vaccines, live attenuated vaccines and killed vaccines, have the following limitations:

Subunit vaccines:

- Inability of subunit vaccines to elicit CD8+ CTL response (induce long term immunity), thus necessitate the need for boosters.
- The production of the antigen is complicated and expensive.
- The antigen may be of low immunogenicity.
- Vaccine is not heat stable.
- High production cost.
- Purification is difficult.
- Incorrect folding of the antigen

Live attenuated vaccines:

- Live attenuated vaccines may revert to virulence.
- Vaccine is often not heat stable.

Inactivated or killed vaccines:

- Killed vaccines are unable to induce a CD8+ CTL response.
- There is a possibility of infection due to incomplete inactivation and loss of immunogenicity due to denaturation following inactivation in the production process of inactivated vaccines.

5.10 Advantages of DNA vaccines

An ideal vaccine should be safe, easy to administer, inducing the full range of immune responses, have a long-lasting effect, easy and inexpensive to manufacture, heat stable and the quality control should be simple yet accurate. According to Davis, *et al.*, (1995), DNA vaccines may have the following advantages:

- There is no risk of an attenuated organism mutating back to a virulent form.
- Potential long-lived gene expression, leading to sustained presentation of the antigen to the immune system.
- Sustained presentation would prevent the need for booster injections.
- Because of the *in vivo* synthesis of the antigen, the antigen is presented on the cell surface by MHC -I, leading to cell mediated immunity (CMI) due to the activation of CD8+ cytotoxic T-lymphocytes (CTL).
- No infectious agents are used in vaccination, eliminating the risk for infection in immunocompromised individuals.
- Production is easier, less expensive and it is simple to maintain quality control.
- DNA is heat stable, eliminating use of the cold chain.
- Multi disease vaccines can be produced by the inclusion of antigens from different infectious agents in the DNA construct or one can include different epitopes of the same or different antigens in the same construct.
- Immunostimulatory or immunomodulatory molecules can be administered to enhance the efficacy of the vaccine.
- The possibility that maternal antibodies may clear the antigen before the immune system has been stimulated doesn't exist in this scenario. Usually this leads to an unresponsiveness and higher susceptibility to tolerance.
- Advantages over problems associated with recombinant viruses such as pre-existing immunity, insertion mutagenesis, loss of attenuation and the occasional spread of inadvertant infection can be overcome by employing a DNA vaccination strategy.

5.11 Disadvantages of DNA vaccines

Disadvantages of DNA vaccines include the possibility of homologous recombination to occur between the genomic DNA of the vaccinee and the plasmid DNA injected (Davis, *et al.,* 1995; Prljic, *et al.,* 1999). This would result in foreign sequences being incorporated into the genome of the vaccinee. The possibility exist that disadvantageous consequences like auto-immunity or tolerance to an antigen may be induced by administration of the DNA vaccine or genes encoding an antigen in particular. Although these concerns have been raised (Beard, *et al.,* 1998), experimental evidence of these adverse effects has not been shown (Bagarazzi, *et al.,* 1998).

6. AIMS OF THIS STUDY

The most important aspect of any vaccine is the ability to induce a protective immune response. Protection against a wide variety of diseases and organisms has been obtained using DNA immunisation (Sedegah, et al., 1994; Barry, *et al.*, 1995; Xiang, et al., 1995; Van Drunen Littel-van den Hurk, et al., 1999) indicating that DNA vaccines have a potential as an alternative to classical vaccines.

Problems associated with classical vaccines currently used to protect animals against RVF can be summarised as:

- Abortions in sheep after vaccination with the live attenuated Smithburn vaccine.
- Low antibody titres in cattle after vaccination of the cattle with the live attenuated Smithburn vaccine.
- Teratology caused by the live attenuated Smithburn vaccine after vaccination of pregnant ewes.
- Only short term immunity induced by the inactivated vaccine in cattle.
- Large quantities of the antigen are required for the killed vaccine.

A variety of glycoprotein genes have been used in experiments where these genes were cloned into a mammalian expression vector and used as DNA vaccines (Ugen, et al., 1998; Van Drunen Littel-van den Hurk, et al., 1999). The results obtained from these

different experiments indicate that protective glycoprotein genes have been used successfully as target genes in a DNA vaccination strategy.

The advantages of DNA vaccines and the mentioned disadvantages or limitations of the currently used vaccines against RVFV, indicate that DNA vaccines might prove to be useful in developing a vaccine to protect susceptible animals against RVF. DNA vaccines have been tested successfully for protection against viral (Xiang, et al., 1995), bacterial (Barry, et al. 1995) and parasitic pathogens with (Sedegah, et al., 1994) and without intracellular phases (Waine, et al., 1995). It has been shown that manipulation and modulation of the immune response is possible by the addition of the genes of immunomodulatory molecules and optimisation of the plasmid backbone sequence (Leitner, *et al.*, 2000).

The aims of this study was to:

- Generate a plasmid DNA construct containing the protective glycoprotein genes of the Rift Valley fever virus and the gene encoding the Enhanced Green Fluorescent protein.
- The generated construct's performance as a DNA vaccine where mice (as a small animal model) and sheep (as a large animal model) were vaccinated against Rift Valley fever have to be evaluated in terms of neutralising antibody titres, evaluation of clinical signs, serum levels of liver enzyme and overall protection against RVF.
- In addition, subcloning of the glycoprotein genes into a bacterial vector system to evaluate expression of the glycoproteins. Expression products obtained could function as antigens in an ELISA based diagnostic assay to replace the whole virus that is being used as antigen. Development of an ELISA based assay that is more sensitive (less false negatives), less time-consuming, more affordable, less complicated and makes use of a recombinant protein as antigen would be the eventual outcome after a useful expression product could be obtained.
- A final objective of this study was to develop and evaluate a one-step RT-PCR. Such an assay would be advantageous in diagnosis of the disease

and during quality control in development of classical vaccines. Depending on the type of vaccine used in the future, the RT-PCR could assist in differentiating between vaccinated animals and naturally infected animals. The RT-PCR would assist in the timely diagnosis of RVF, be useful in a surveillance program and would reduce the risk in handling of whole virus.