

CHAPTER 4

CONCLUDING REMARKS

DNA vaccines offer numerous advantages over conventional vaccines for RVFV, although the conventional vaccines used to protect animals from the disease can lead to life-long protection (Smithburn, live, attenuated virus), or short-term protection for vaccinated cattle (inactivated, wild-type virus). For DNA vaccines, the advantages include sustained life-long presentation of the antigen in all vaccinees, activation of CD8+ CTL's, no reversion back to virulence, eliminating the use of the infectious agent in the vaccination, ease of production, lower cost, simpler quality control and elimination of the need for a cold chain. Because Rift Valley fever is a zoonotic disease, handling of the virus is not desirable. DNA vaccination offers a strategy that excludes handling of the infectious agent, thus it would be preferred above the currently used live, attenuated Smithburn (although the Smithburn strain in specific poses no real threat to human health) or inactivated vaccines.

It has to be remembered that when the efficiency of a DNA vaccine is evaluated in terms of neutralising antibodies after vaccination, the neutralising anti-RVFV antibodies elicited are monovalent polyclonal antibodies. When using monovalent polyclonal antibodies to detect whole virus, the test results (eg ELISA signal) obtained could be expected to be lower in comparison to antibodies detected when directed against the whole virus (polyvalent polyclonal antibodies). The low neutralising antibody titre could still be representative of a vaccine that confers significant levels of protection.

In this first study of a DNA vaccine for RVF, the construct has been developed and evaluated via a vaccination trial against virulent RVFV challenge. By employing an indirect immunofluorescence assay, it was observed that the RVFV glycoproteins were expressed from the DNA vaccine construct in mammalian cells, although at lower levels than what was expected. Subsequent vaccination of mice led to a delay of death after challenging of the mice with a virulent strain of RVFV. An indication that the target gene of the DNA vaccine was expressed and induced seroconversion in sheep, was obtained by testing the serum of the vaccinated sheep in a virus-neutralising antibody assay. The neutralising antibody levels were low compared to what is expected in a

situation where the live or inactivated vaccines were used. The group that received the vaccine via the intradermal route had the highest virus neutralising antibody titres. However, no appreciable levels of protection were observed. Under circumstances of controlled infection, it has been observed that sheep participating in vaccination trials did not show symptoms of the disease although they were challenged with virulent virus strains and even unvaccinated control sheep developed only mild infection (Swanepoel, *et al.*, 1986). In this study such an observation was also made. Even though the sheep were challenged with a virulent isolate and clinical signs did manifest in the animals, it was not an indication of a severe infection. It is not clear whether the weak performance of the DNA vaccine in the target animal is due to low level of antigen.

The DNA vaccination strategy needs further optimisation in terms of gene modifications (addition of leader signal sequence, truncation of any membrane insertion signals) to optimise expression and secretion of the target antigen to increase the levels of neutralising antibodies and to obtain protection levels against the disease in the target animals. Optimisation procedures also need to include route of administration, immunomodulation and immunostimulation and heterologous prime-boost strategies.

The currently used diagnostic ELISA format involves two assays that enable one to discriminate between early and late infection on the basis of IgM and IgG detection. In these assays, inactivated whole virus is used as the antigen. Binding of the virus to the microtitre plates is poor and therefore a double sandwich system capturing the RVFV specific IgM antibodies is used. A recombinant protein antigen could shorten the time it takes to complete such an assay by eliminating additional steps and eliminate handling and culturing of the virus, making the assay safer and less expensive.

In this study an attempt was made to express the glycoprotein antigen (G2G1) from the subcloned plasmid constructs in a bacterial expression system. Expression in this specific system proved to be unsuccessful in terms of yield when the full-length fragment encoding the glycoproteins from the RVFV M segment was used, but preliminary results using the pRSET phage-facilitated bacterial expression system using a truncated form of the gene is promising (data not relevant for this thesis). Sequences upstream of the translational start site were removed during the cloning of the truncated G2 gene fragment that only represents 43% of G2.

Future objectives regarding expression of the antigen include the use of a Baculovirus expression system or a yeast system i.e. *Pichia pastoris*. A major advantage of these systems is that glycosylation of gene products takes place in contrast to the case in bacteria. Although protein yield would be lower in comparison to what could be obtained in a bacterial system, post-translational modifications could be performed in these systems. Antigens resembling the naturally generated proteins could thus be produced.

Currently used diagnostic assays detect either the antigen or the antigen specific antibodies. Again, because RVF is a zoonosis, it is not desirable to produce antigen (virus) for use in these assays and virus culturing is time consuming and expensive. Detection of early infection is possible with the ELISA assay detecting the IgM antibodies, but in some situations, it is not sensitive enough, since false negative results are obtained. A molecular based diagnostic tool such as a RT-PCR to detect the virus RNA genome can eliminate virus culturing and handling. Such an assay can be optimised to increase sensitivity. The RT-PCR assay that was developed in this study was shown to detect virus RNA in the serum samples from animals that participated in the DNA vaccine trial. Virus RNA could also be detected in blood spiked with virus. The level of sensitivity of the RT-PCR assay equals the detection of 0,25 TCID₅₀ in the reaction after extraction of RNA, indicating the ability to detect non-viable virus particles as well. This corresponds to a sensitivity level of 1 TCID₅₀/ml in the sample material to be evaluated. The RT-PCR has to be validated with respect to specificity (inclusion of unrelated but similar viruses as well as related viruses). A fully optimised, validated and evaluated RT-PCR assay can result in establishing a less time consuming and more user-friendly molecular diagnostic assay. Such a RT-PCR could furthermore be used as a tool in the production of RVFV vaccines since it could quantify the viral amounts and viral load. A PCR-based assay to discriminate between vaccinated and non-vaccinated individuals would be useful in monitoring outbreaks and would facilitate in the epidemiological study of the disease.