CHAPTER 4

Construction of a chimeric foot-and-mouth disease virus between serotypes A and SAT 2: Comparison with wild type SAT 2 in terms of antigenicity, growth properties and thermal stability[1]

4.1 Introduction

Effective vaccination in sub-Saharan Africa requires the use of custom-made vaccines for specific geographic localities, due to the genetic and antigenic variability of the SAT types of FMDV. Comparison of r-values (expression of antigenic relationships between viruses) determined for ZIM/7/83/2 (a west Zimbabwean strain) and KNP/19/89/2 (originating from the Kruger National Park, South Africa) in relation to isolates from the Kruger National Park, revealed much lower r-values for ZIM/7/83/2 than the KNP strain (Esterhuysen, 1994). The serological data demonstrated therefore the closer relatedness of the KNP strains. A similar study investigating the antigenic relationships of isolates originating from northern and southern Zimbabwe, indicated these isolates to belong to antigenically different groups (Bauman & Esterhuysen, 2000). These results were confirmed genetically which clearly showed the closer gene relationships of isolates originating in the same geographical region (Vosloo *et al.*, 1995; Bastos *et al.*, 2001). Taken together, these results are indicative of the independent evolution of FMDV in different geographical regions and argue thus for the use of custom-made vaccines.

The use of such vaccines implies unfortunately the screening of various field strains, usually buffalo isolates. Adaptation of these viruses to baby hamster kidney (BHK) cells have proven to be a tedious process. In addition, these viruses do not produce high amounts of stable antigen. The screening process is also a cumbersome, labor intensive and therefore an expensive process. It is thus proposed to develop chimeric

[1] Parts of the work presented here have been performed at the Plum Island Animal Disease Center (USA) under the supervision of Dr. P.W. Mason and sponsored by the International Atomic Energy Agency.

or recombinant viruses, which would facilitate the manipulation of the antigenicity of a particular virus to be used in the production of custom-made, but conventional, inactivated vaccines.

The construction of full-length cDNA copies for the foot-and-mouth disease virus was at first hampered due to the presence of the poly (C) tract in the FMDV genome. However, in the early 1990's such a full-length cDNA copy was constructed for type O containing 32C residues in the poly (C) tract (Zibert *et al.*, 1990). Since it was previously shown that picornaviral RNA is infectious (Alexander *et al.*, 1958; Colter *et al.*, 1957), synthetic RNA's were transcribed from the cDNA copy and used to infect cells. Subsequently, the construction of such cDNA copies of the foot-and-mouth disease virus proved to be a powerful and successful tool for manipulating the characteristics of the virus.

Using a full-length cDNA clone containing the genetic backbone of type A_{12} and a poly (C) of 35 (Rieder *et al.*, 1993), several chimeric viruses were constructed. These include the exchange of the immunodominant site, the G-H loop, of A_{12} with that of O_1BFS and C_3Resende. Inactivated vaccines prepared from these chimeric viruses induced antibodies in guinea pigs which neutralized both serotype A and either types O or C (Rieder *et al.*, 1994). The external capsid regions (partial P1 region) for two different type O isolates, O_1Campos (Sa-Carvalho *et al.*, 1997; Almeida *et al.*, 1998) and OTai (Beard & Mason, 2000) were also successfully inserted into the A_{12} infectious cDNA clone, yielding infectious FMDV. The construction of such chimeric viruses employing the SAT type viruses has, however, not yet been attempted.

In this study the construction of a chimeric virus between serotypes A and SAT 2 is described by exchanging the external capsid protein coding region of the A_{12} cDNA clone with that of ZIM/7/83/2. The viable chimeric virus was cultivated in BHK cells and subsequently compared to the wild type ZIM/7/83/2 virus in terms of immunogenicity, growth properties, antigen production and thermal stability.

4.2 Materials and Methods

4.2.1 Viral and bacterial strains

The SAT 2 strain described in Chapter 2 (ZIM/7/83/2 - passage history: B1BHK5B1) was used in this study. *Escherichia coli* MAX Efficiency DH5α™ competent cells (genotype: F⁻ϕ80d*lac*ZΔM15Δ(*lac*ZYA-*arg*F)U169 *deo*R *rec*A1 *end*A1 *hsd*R17(r_K^-,m_K^+) *pho*A *sup*E44 λ⁻ *thi*-1 *gyr*A96 *rel*A1) was obtained from Life Technologies and used in the transformation experiments.

4.2.2 RNA extraction and cDNA synthesis

RNA was extracted from the tissue culture sample (250 µl) using the phenol-based Trizol reagent (750 µl) (Life Technologies) according to the manufacturer's specifications, followed by a chloroform extraction and isopropanol precipitation. The extracted RNA (5 µl of 20 µl)) was subsequently used as template for cDNA synthesis. MMLV RT (Life Technologies) and an antisense oligonucleotide located on the 2A/2B-junction site (Figure 4.1), cDNA-2A (5'-CGCCCCGGGGTTGGACTCAACGTCTCC-3') (binding nucleotides 2055 to 2088 on MAWT12), was used for the reverse transcription reaction carried out for 1h at 42°C.

4.2.3 PCR amplification

The external capsid coding region (VP2, VP3,VP1) of ZIM/7/83/2 was amplified using the AdvanTaq™ DNA Polymerase available from Clontech. The reaction was carried out in the presence of 0.2 mM deoxynucleotides as well as 0.4 µM of oligonucleotides: cDNA-2A and cDNA-VP2 (5'-CGGAATATTGACAACACGACACGGTACAA CCAC-3') (binding nucleotides 4002 to 4025 on MAWT12) (Figure 4.1). Both cDNA-2A and cDNA-VP2 were designed using data generated for ZIM/7/83/2 P1 region (Chapter 2). Reaction conditions comprised of an initial denaturing step of 60 sec at 94°C followed by 15 cycles of 30 sec at 94°C and 8 min at 68°C. A final extension step of 50 min at 68°C was followed by a cool-down step of 90 min at 15°C. The resulting amplicon was analysed by agarose gel electrophoresis and recovered from the agarose gel using a freeze-thaw method. This method involved the freezing and thawing (three times) of the gel slice in the presence of 100µl STE buffer (100mM

Tris-HCl, pH 7.5; 100mM NaCl; 1mM EDTA) and 0.01% SDS followed by buthanol extractions (twice) and an ethanol precipitation.

4.2.4 Cloning of SAT 2 external capsid coding region into A_{12} infectious cDNA clone

The two oligonucleotides, cDNA-VP2 (*SspI*) and cDNA-2A (*XmaI*), were designed with restriction enzyme sites to facilitate the cloning of the amplified external capsid coding region into the A_{12} full-length cDNA clone, MAWT12 (Rieder *et al.*, 1993). Following the restriction enzyme digestion of the purified amplicon and MAWT12 (treated with alkaline phosphatase, Roche), ligation reactions were carried out using the Rapid DNA Ligation kit from Roche according to the specifications of the manufacturer. The ligation mixture (3µl) was transformed into 20 µl of *E. coli* MAX Efficiency DH5α™ competent cells. Large-scale preparations of putative positive full-length clones (FLC) were isolated from 20ml *E. coli* cultures using the Wizard Plasmid Prep kit (Promega). Construction of the FLC was verified by sequencing of the restriction site using an ABI PRISM 377 DNA Sequencer from Perkin Elmer and a sense oligonucleotide in the VP4 region (5'-TCAACACACACAACCAACACTCA-3') (P307) as well as an antisense oligonucleotide in the 2B region (5'-GCATCTGGTTGATTGTGTCTACC-3') (P308) (P.W. Mason, personal communication).

4.2.5 RNA synthesis

To facilitate RNA transcription, 5 µg of the purified plasmid DNA was linearized with *Not I* to facilitate RNA synthesis . This step was followed by a proteinase K digestion, phenol and chloroform extractions as well as a final ethanol precipitation. RNA transcription was carried out with the MEGAscript™ T7 kit from Ambion at 39°C for 2 h. The quality of the RNA was analyzed on agarose gel electrophoresis (1% agarose gel) and the concentration of the newly synthesized RNA was determined by comparison to a known standard. All reactions were carried out using only RNA-grade reagents.

4.2.6 Transfection of BHK cells with infectious viral RNA

Baby hamster kidney (BHK) cells were transfected with the transcribed RNA by means of either electroporation or lipofectin reagent (Life Technologies). Approximately 15 μg infectious RNA together with 7.5 x 10^6 cells were used in the electroporation experiment. The electroporated cells were subsequently transferred to basal medium eagle (BME) (Life Technologies) containing 10% bovine calf serum (Hyclone). Five milliliters of the cell-medium mixture was transferred to a 35 mm well and incubated for 5h at 37°C in CO_2 incubator after which the cells were rinsed with BME containing 1% calf serum and incubated overnight with BME + 1% calf serum. Cells were frozen and thawed the next day and passed further on BHK cells. Aliquots were stored following each step. For transfection by means of lipofectin, RNA was diluted with opti-MEM (Life Technologies) followed by a 15-20 min incubation at room temperature with the lipofectin reagent. This lipofectin-RNA complex was then overlayed on pre-prepared cells (6 x 10^5 cells / 35 mm well) and incubated for 5 h at 37°C in a CO_2 incubator. The RNA containing medium was then replaced with 0.6% gum tragacanth in MEM (modified eagles medium) containing 1% calf serum and incubated in the 37°C incubator for 48-72h. The wells were stained with crystal violet.

4.2.7 Viral titrations on IB-RS-2 cells

The newly synthesized chimeric virus between types A and SAT 2 was passaged four times on BHK cells and stored at -70°C to be used in the subsequent characterizing experiments. The determination of viral titrations for the A12/SAT2 chimera (BHK4) and the wild type ZIM/7/83/2 (B1BHK5B1BHK1) was carried out in flat-bottomed microtitre plates (Nunc). Roswell Park Memorial Institute (RPMI) medium (Sigma) was used to prepare 0.5$\log_{10}$ dilutions of the viral stocks. RPMI medium (50μl) together with 50μl of the virus dilutions were plated out per well and incubated for 1h at 37°C. The test was performed in 8 fold. Following the incubation period, 100μl of a cell suspension (0.3 x 10^6 / ml IB-RS-2, a pig kidney cell line in RPMI medium containing 5% fetal calf serum (Delta Bioproducts)) was placed out per well. The microtiter plates were incubated at 37°C for 72h in a CO_2 incubator. Results were read directly with an inverted microscope. The calculated viral titers were expressed as $TCID_{50}$/50μl (tissue culture infectious doses) (Esterhuysen, 1994).

4.2.8 Plaque titrations on IB-RS-2 and BHK cells

Petri-dishes (50 mm diameter) were prepared with IB-RS-2 and BHK cells with a concentration of 5 x 10^6 cells / dish and incubated at 37°C to be confluent in 48h. The two viruses were diluted in RPMI medium using $\log_{10}$ dilutions. After the media was removed from the dishes containing the confluent cells, 200 µl of the viral dilutions were added and incubated for 30 min at 37°C. A RPMI medium-agarose mixture (5 ml), containing 5% normal bovine serum and 0.1% agarose (Merck) was added to each dish, left to set and incubated for 48h at 37°C and CO_2. All titrations were done in duplicate. Plaques were stained using a methylene blue solution (1% w/v methylene blue dissolved in absolute EtOH, added to an equal volume of formaldehyde and 8 x PBS).

4.2.9 Virus neutralization test

The two viruses were tested against cattle sera that were raised against the wild type ZIM/7/83/2, KNP/19/89/2 and a mixture of the two sera. The sera were generously provided by J. J. Esterhuysen. These sera were diluted in serial twofold dilutions (50µl) in 8 columns across a microtitre plate. The viral dilutions used ($0.5\log_{10}$ apart) were calculated to straddle the 10^2TCID_{50} dose. 50µl of the virus dilutions were plated out on the microtitre plate in three fold and incubated for 1h at 37°C. Subsequently, 100µl / well of cell suspension (0.3 x 10^6 IB-RS-2 cells in RPMI medium containing 5% fetal calf serum) was added and incubated for 72h at 37°C and CO_2. Results were read using an inverted microscope. A virus titration for each test was included to be able to determine the actual virus titer and dose for the specific experiment. Serum titers were expressed as the $\log_{10}$ reciprocal of the dilution, which protected 50% of cultures from that dose of virus. The final endpoint titer of the serum was determined as the log reciprocal of the dilution which protected 50% of cultures from 10^2TCID_{50} of virus (Esterhuysen, 1994).

4.2.10 Determination of 146S content

To determine the concentration (µg/ml) of the 146S viral particle, 0.5 ml of a sample was layered carefully on top of a 15 – 45% sucrose gradient. These gradients were

subsequently centrifuged in an ultracentrifuge at 285 000 x *g* and 6°C for 52 minutes. The protein content was then detected on an UV-detector and chromatographically registered on a recorder. The 146S concentration was calculated from the surface area of the relevant peak multiplied by an emission constant, $E_{254}^{1\%} = 73$ (Lei, 1978).

4.2.11 Determination of growth kinetics

In order to determine the relative growth rates and antigen production of wild type ZIM/7/83/2 and A12/SAT2 on BHK cells, an inoculum of 1 virus particle per 100 cells were used. Virus titers determined with plaque titrations (section 4.2.8) on BHK cells were used. Roller bottles containing 10^8 BHK cells / ml were incubated for 1h with the virus at 37°C after which 80 ml of RPMI (containing no fetal calf serum) was added and incubated at 37°C. Samples of the supernatant were taken at different time intervals. The viral titers and 146S content of these samples were subsequently determined.

4.2.12 Thermal stability testing

The same infection rate used in section 4.2.11 was applied to determine the thermal stability of the two viruses investigated. Viruses were grown till their highest point of antigen production as determined during the growth kinetics. Each virus was harvested and stirred for 1h in the presence of 1% chloroform at 4°C. This mixture was centrifuged for 10 min at 2 000 rpm and the supernatant was divided into 9 aliquots for each virus. Four of the aliquots were left at 4°C for 21 days and the rest at 37°C for the same duration. One sample was directly tested for its 146S content, whilst the remaining samples were determined in 7 day intervals.

4.3 Results and Discussion

4.3.1 Construction of a chimeric virus between serotypes A and SAT 2

The 2.2 kb external capsid protein coding region (VP2-VP3-VP1), of the SAT 2 isolate, ZIM/7/83, (B1BHK5B1) was amplified from cDNA and cloned into the *SspI* and *XmaI* sites of the A_{12} full-length cDNA clone, MAWT (constructed by Rieder *et al.* (1993)) (Figure 4.1). As ZIM/7/83/2 does not contain the *SspI* and *XmaI* restriction enzyme

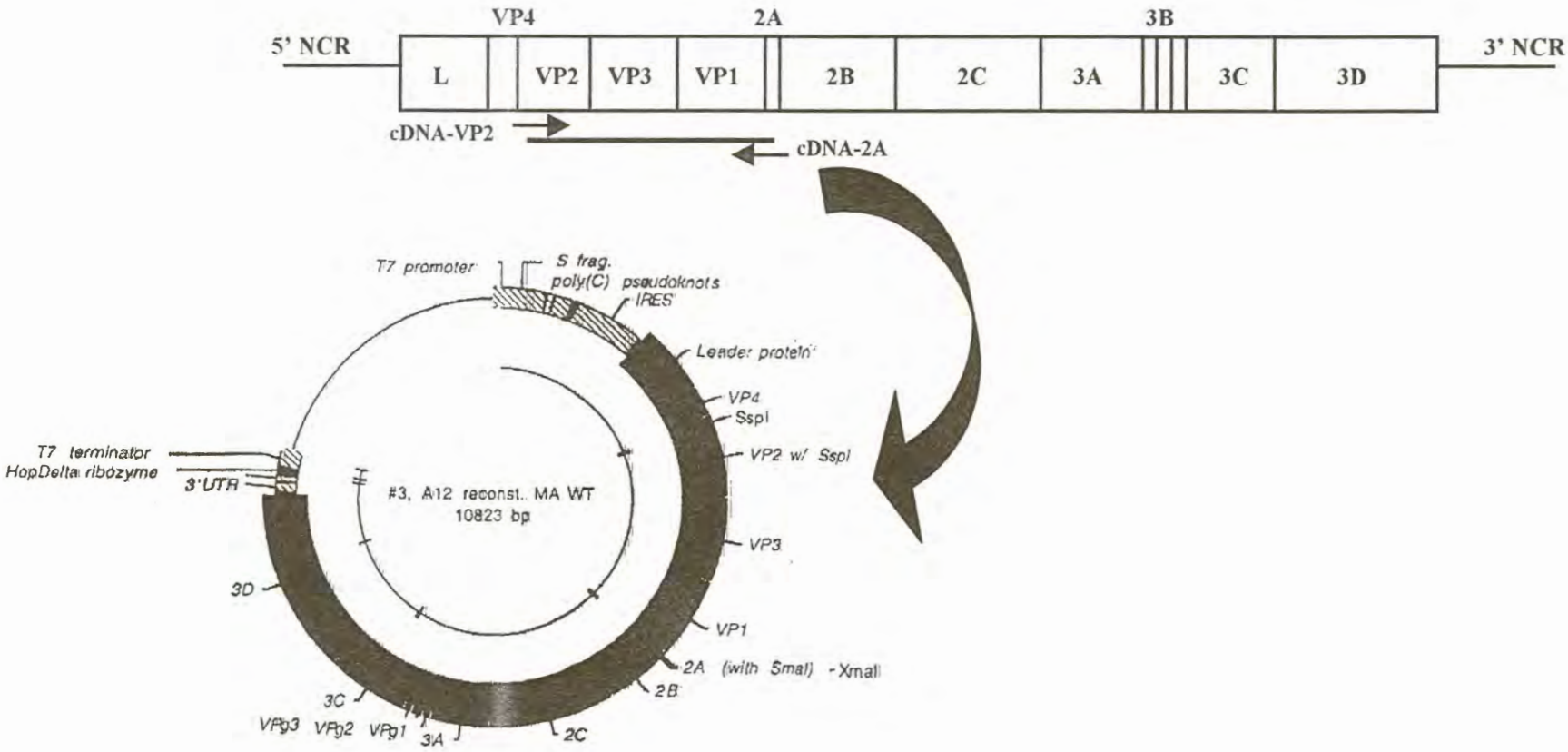


Figure 4.1: Schematic representation of the construction of the chimeric virus between serotype A and SAT 2. The external capsid protein coding region amplified by cDNA-VP2 and cDNA-2A, was cloned into the SspI and XmaI sites of MAWT, which contains the FMDV genome, T7 propmoter and terminator regions as well as the HepDelta ribozyme.

sites, these sites had to be engineered during the design of oligonucleotides cDNA-VP2 and cDNA-2A. In order to achieve this, a single amino acid change (Val (GTC)-Leu (TTG), both polar) was introduced in the VP2 region of ZIM/7/83/2 (Figure 4.2). The corresponding external capsid-coding region of the A_{12} clone was removed by restriction enzyme digestion and that of ZIM/7/83/2 was successfully inserted into the cDNA clone, creating a chimeric construct between types A and SAT 2. The successful construction of the chimeric clone, pA12/SAT2, was verified with nucleotide sequencing (Figure 4.2). The sequence of the newly constructed pA12/SAT2 corresponds to that of a computerized version of the construct, verifying the type A genetic backbone as well as the SAT 2 external capsid-coding region. The receptor-binding region (RGD) of the foot-and-mouth disease virus was also found to be unaltered.

In vitro-synthesized RNA's generated from several pA12/SAT2 clones were used to transfect BHK cells (Figure 4.3). Several clones were screened before infectious viral particles could be obtained (results not shown). A flow-diagram (Figure 4.4) indicates the subsequent steps followed to obtain an infectious A12/SAT2 chimeric virus. The viability of the RNA derived from #14A12/SAT2 was confirmed through plaque titrations on BHK monolayer. Viral particles generated from this clone (#14) were therefore harvested. The partial nucleotide sequence of the external capsid-coding region of the chimeric virus was again determined, verifying the isolation of the correct virus (results not shown). The ability of the chimeric #14A12/SAT2 virus to form plaques on BHK cells following transfection (Figure 4.4), suggests that the SAT 2 structural proteins were compatible with the type A 3C proteinase.

It is worthy to note that viable chimeric virus could not be obtained following the initial construction of a chimeric virus between types A and SAT 2. This construct was derived from the pGEMZIM/7/83/2 plasmid generated previously (Chapter 2). Direct amplification from cDNA using a high fidelity polymerase did however yield the viable chimeric virus, #14A12/SAT2. These results together with the sequence differences indicated in the VP1 region (Figure 4.2), emphasize the need to determine the nucleotide sequence of the structural-protein-coding region of clone #14. Since no

Figure 4.2: The nucleotide sequence comparison of a computerized construction of the A12/SAT2 chimera (A12ZIM/7/83/2), the wild type ZIM/7/83/2 P1 region (WTZIM/7/83/2) as determined previously (Chapter 2) and the nucleotide sequence as determined for the newly constructed pA12/SAT2 (clone #14) with oligonucleotides P307 (A) and P308 (B). The two oligonucleotides used for cloning, cDNA-VP2 (A) and cDNA-2A (B) as well as the junction sites are indicated in bold with the restriction enzyme sites used during cloning, indicated in bold and in italics. Differences obtained in VP1 between pA12/SAT2 and the wild type SAT 2, are indicated in italics. The receptor-binding region (ArgGlyAsp) in VP1 is also indicated in bold and underlined.

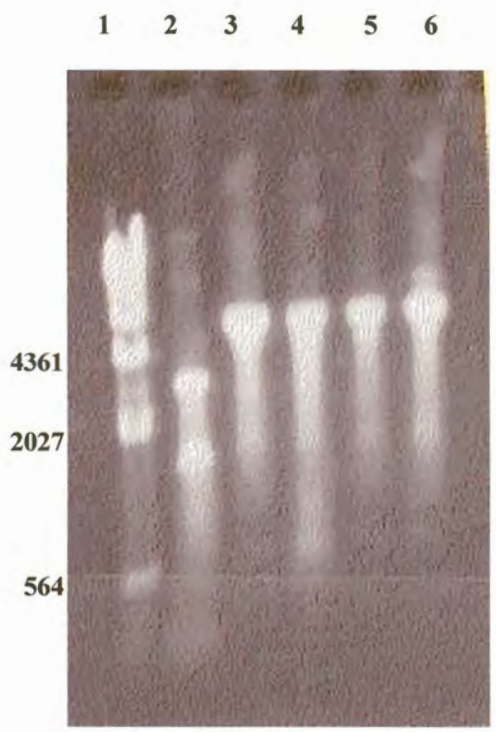


Figure 4.3: Agarose (1%) RNA gel indicating the *in vitro*-synthesized RNA's generated from the different pA12/SAT2 clones. Lane 1 containes the molecular weight marker (λ-DNA digested with *Hind III*), lane 2 RNA standard of known concentration and lanes 3-6, clones #7, #14, #15 and #21 derived from pA12/SAT2.

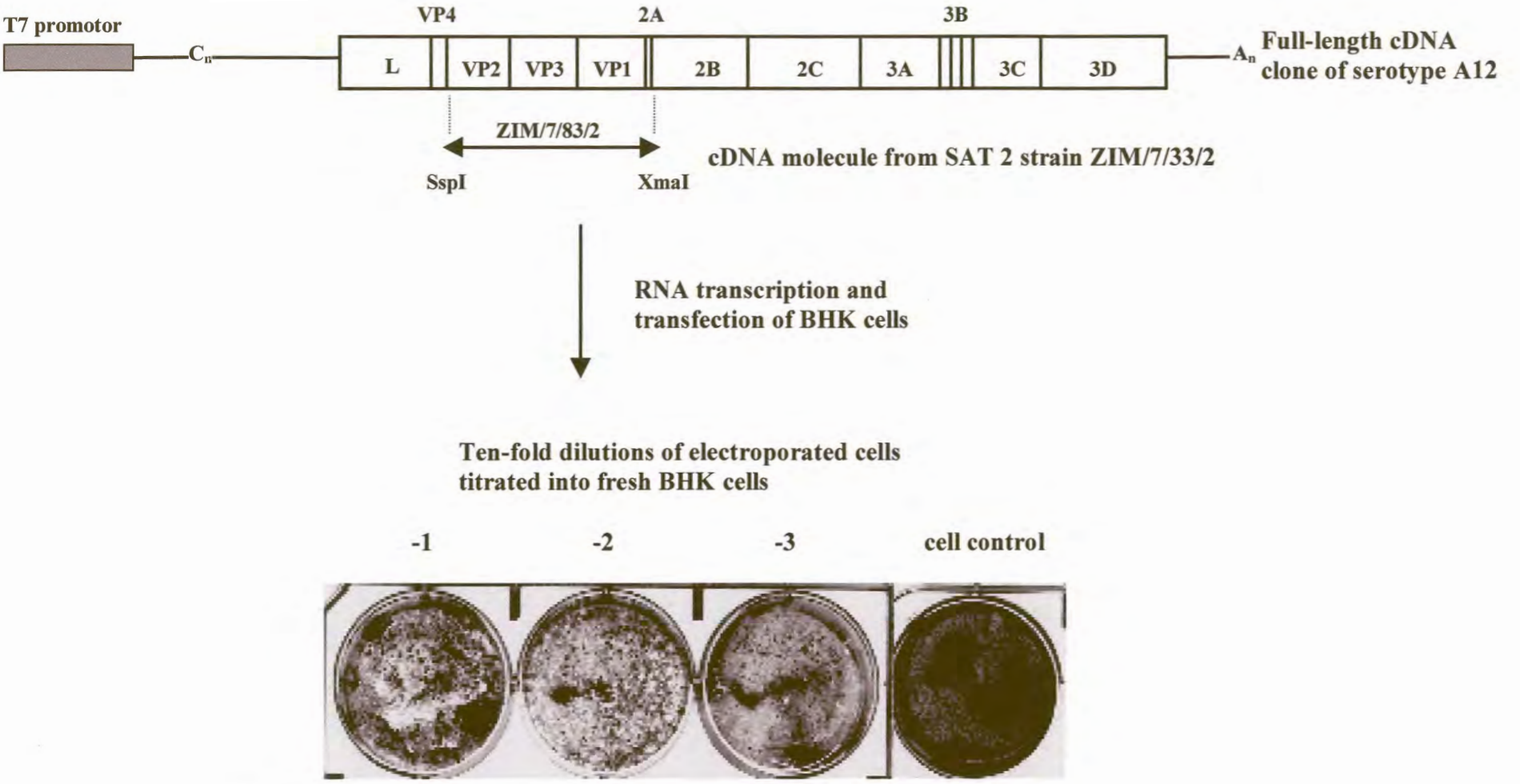


Figure 4.4: A flow-diagram indicating the construction of the chimeric virus between types A and SAT 2. The viability of clone #14 is shon with ten fold dilutions, clearly indicating the foci formed.

proof-reading enzyme was used, PCR artifacts could have been generated during the initial amplification of the region since no proof-reading enzyme was used.

4.3.2 Comparison with wild type SAT 2

The chimeric or recombinant virus, #14A12/SAT2, was compared to the wild type SAT 2 virus, ZIM/7/83/2, in terms of immunogenicity, growth properties, antigen production and thermal stability.

4.3.2.1 Immunogenicity

The unavailability of monoclonal antibodies raised against SAT 2 and ZIM/7/83/2 in particular, necessitate the use of the virus neutralization test (VNT) to investigate the immunogenicity of the chimeric virus. The wild type ZIM/7/83/2 and the recombinant virus were tested against sera raised against ZIM/7/83/2, another SAT 2 virus originating from the Kruger National Park, KNP/19/89/2, representing a different lineage, as well as a mixture of the two sera. The results indicate similar antigenic profiles for the two viruses, with the serum titer obtained against the KNP/19/89/2 strain being the lowest (WT ZIM/7/83/2 = $10^{2.2}$; #14A12/SAT2 = $10^{2.3}$) (Figure 4.5). Although it is not possible to conclude that the epitopes on the viral capsids of the two viruses are identical, the results obtained from the serological assays indicate the immunogenicity of the wild type and recombinant viruses to be similar.

4.3.2.2 Growth properties and antigen production

The growth properties of the recombinant virus were investigated by studying both plaque formation and growth kinetics and comparing it to that of the wild type virus. Plaque titrations were carried out on BHK and IB-RS-2 (pig kidney) cells (Figure 4.6). For both, ZIM/7/83/2 and #14A12/SAT2, plaques obtained on BHK cells were much smaller than on IB-RS-2 cells. Although the plaques obtained for the chimeric virus were extremely small compared to that of wild type ZIM/7/83/2, the plaque morphology of the viruses seemed to be similar. The similarity in morphology is expected since the receptor-binding region (RGD) of #14A12/SAT2 was shown to be intact (Figure 4.2). It was previously shown that with the exchange of only the G-H loop containing the RGD region of O_1BFS and C_3Resende, plaque morphologies

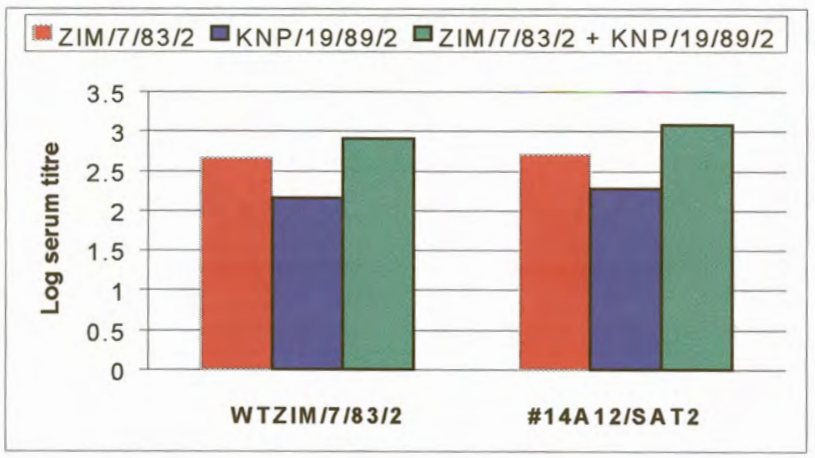


Figure 4.5: Immunogenic profile of wild type ZIM/7/83/2 and #14A12/SAT2 chimeric virus as determined with the virus neutralization test using sera raised against ZIM/7/83/2, KNP/19/89/2 and a combination thereof.

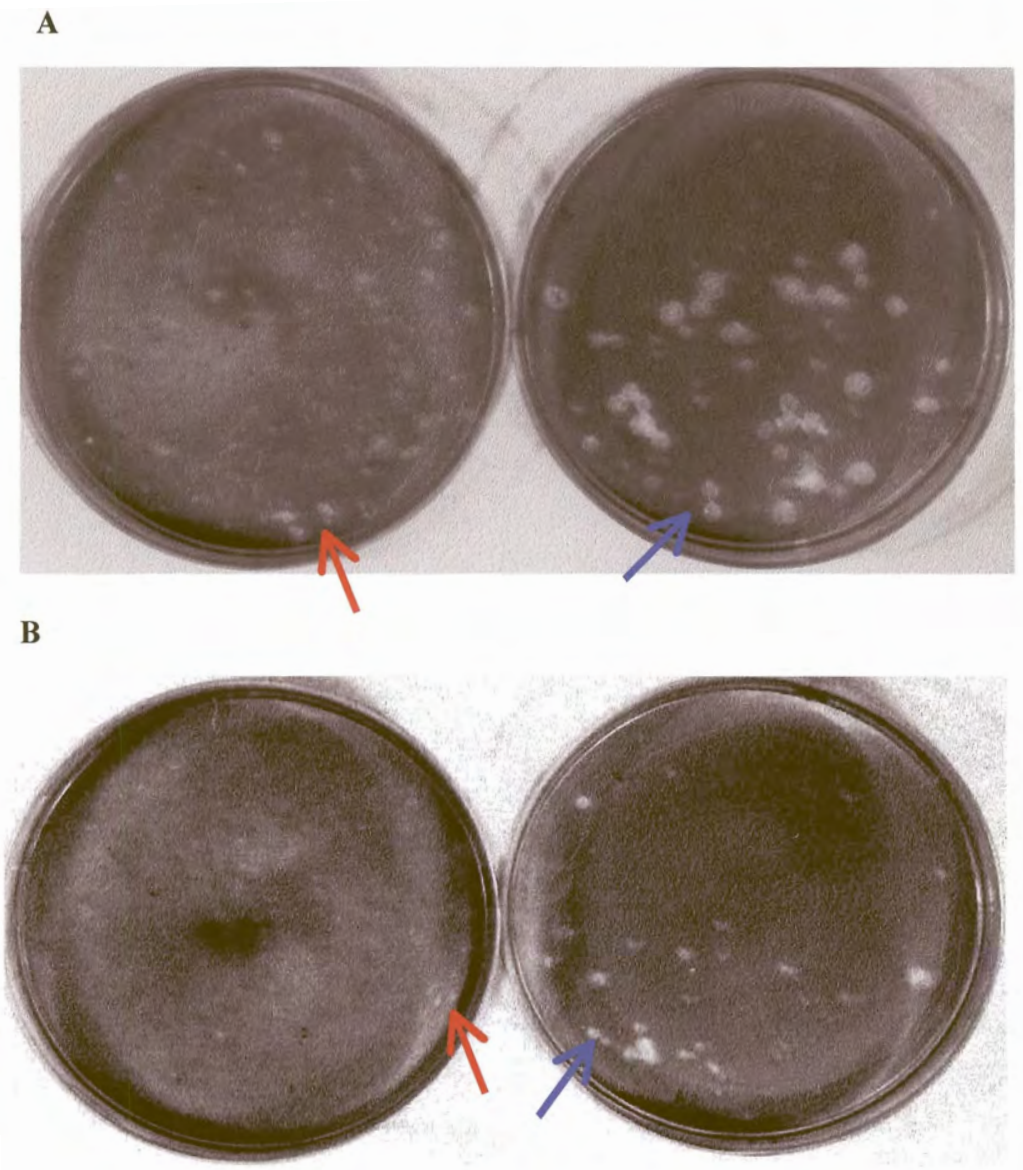


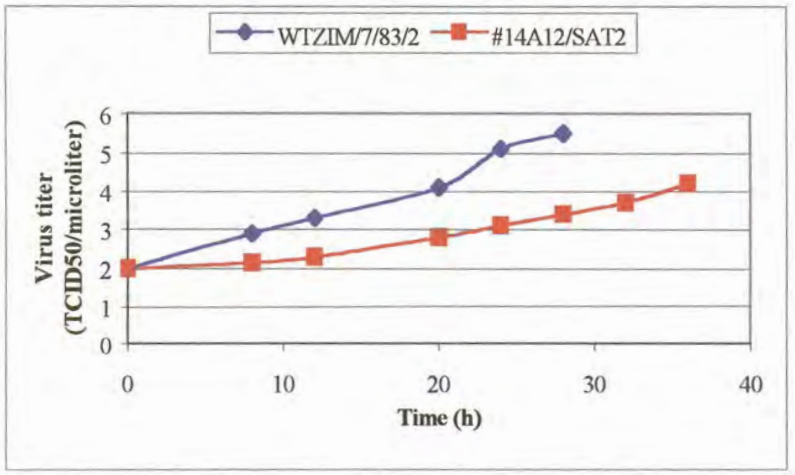
Figure 4.6: Plaque morphology as determined for WT ZIM/7/83/2 (A) and #14A12/SAT2 (B). The red arrows indicate foci on BHK cells and the blue arrows on IB-RS-2 cells.

similar to that of the wild type O_1BFS and C_3Resende were obtained (Rieder *et al.*, 1994).

A reduction in growth rate could explain the extremely small plaques obtained for the recombinant virus and was subsequently confirmed with growth studies (Figure 4.7). Using titers as determined with the plaque assays on BHK cells as well as a high multiplicity of infection (m.o.i) rate, the comparative growth rate of the wild type ZIM/7/83/2 and chimeric virus, #14A12/SAT2 was investigated. It was previously observed that ZIM/7/83/2 reaches high titers after 24 – 28h (results not shown) and therefore the experiment for this virus was discontinued after 32h. It was however found that #14A12/SAT2 reaches high titers after only 36h. The rate of antigen production was also seen to be reduced (Figure 4.7). The highest concentration of 146S content for ZIM/7/83/2 (1.64 µg/ml) was obtained after 28h, whilst the highest 146S yield for #14A12/SAT2 (1.29 µg/ml) was obtained after only 36h.

A reduced growth rate for a recombinant virus (A_{12}) had been reported previously (Rieder *et al.*, 1993). In this instance the recombinant in question had a poly (C) tract of only 2 residues. Plaques of less than 1 mm were obtained for this virus, whereas plaques of 5 to 8 mm were obtained for a recombinant containing a poly (C) tract of 35 residues. The latter virus ($A_{12}C_{35}$) also displayed a similar growth rate in BHK cells than the wild type A_{12} virus, achieving high titers within 24h (Rieder *et al.*, 1993). In the current study the A_{12} genetic backbone contains a poly (C) tract of 35 residues. Following the transfection of BHK cells with the synthetic RNA, the chimeric #14A12/SAT2 virus were passed an additional 4 times on BHK cells to allow the poly (C) tract to elongate. Previously, chimeras containing the identical A_{12} genetic background (poly (C) tract of 35 residues) were passed only twice on BHK and achieved high titers within 24h (Sa-Carvalho *et al.*, 1997). Nevertheless, since the actual length of the poly (C) tract of the #14A12/SAT2 chimera has not been determined, the role it might play in the slower growth properties observed for this virus can not be ruled out.

A



B

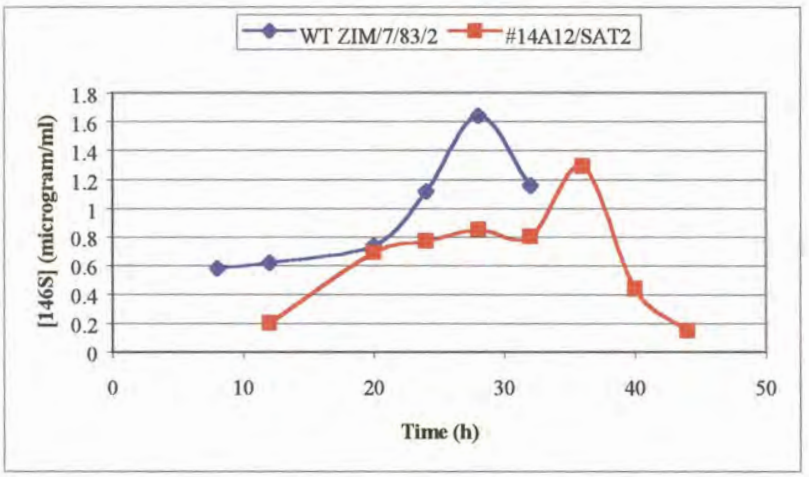


Figure 4.7: Comparative growth characteristics of wild type ZIM/7/83/2 and #14A12/SAT2 chimeric virus. (A) Virus growth as determined for ZIM/7/83/2 (28h) and #14A12/SAT2 (36h). (B) Antigen production (146S particle) as determined for ZIM/7/83/2 (28h) and #14A12/SAT2 (36h).

Similar growth properties for the wild type A_{12} and the recombinant virus derived from a full-length cDNA clone of the A_{12} genome containing 35 C residues were observed, reaching high titers within 24h (Rieder *et al.*, 1993). Although not performed under the same laboratory conditions, the growth rate as determined for the wild type ZIM/7/83/2 correlate well with that of A_{12}. Ideally, the recombinant #14A12/SAT2 virus should therefore display similar growth properties. The extended growth rate of 8h observed for the chimera could thus be indicative of inefficient replication of the SAT 2 capsid region by the A_{12} nonstructural proteins. Although only the VP0/VP3 cleavage site differs between A_{12} and ZIM/7/83/2, the regions adjacent to the P1 cleavage sites differ quite extensively between the two viruses (see Chapter 3, Figure 3.7). These junction sites might be important in the recognition of the processing site by the 3C proteinase. Another explanation for the slower growth rate observed for the chimeric virus could therefore be sub-optimal processing of the SAT 2 P1 region by the A_{12} 3C proteinase.

4.3.2.3 Thermal stability

The same infection rate (high m.o.i) employed during the growth studies were used to investigate the thermal stability of the chimeric virus. The chimeric virus was harvested after 36h, and the wild type after 28h. Incubation in the presence of 1% chloroform was carried out to lyse the cells and therefore to release cell-associated virus particles. Following centrifugation the supernatants of the two viruses were incubated at 4°C and 37°C for 21 days. From Figure 4.8 it is evident that the wild type ZIM/7/83/2 is stable at 37°C, exhibiting little variability in the 146S yield. The virus also seemed to be stable at 4°C. However, following a very low initial 146S yield for #14A12/SAT2, a 4 to 8 fold increase in 146S yield was obtained at 4°C and 37°C after 7 days. This result can be explained in terms of the aggregation of the viral particles. These viral particles aggregate with one another and therefore escape the UV-photometric measurement (Lei, 1978). The formation of these complexes may be caused by either electrostatic forces or hydrophobic interaction between viral particles, or both (S. J. Barteling, personal communication). Once the aggregates start to dissociate, the yield in 146S might increase drastically. In this case, higher yields for the chimeric virus were obtained after 7 days, but a decrease in 146S concentration followed over the next two weeks.

These results are indicative of the #14A12/SAT2 chimeric being less stable than the wild type ZIM/7/83/2. The stability of the A_{12} virus derived from the A_{12} cDNA clone has however not been determined under these conditions. It is therefore unclear at this stage whether the #14A12/SAT2 chimeric virus display similar thermal stability than the A_{12} virus or not. In addition, it is important to note that the thermal stability testing was carried out on the cell harvest and not on BEA/formaldehyde inactivated virus. The chemical inactivation process might also influence capsid stability. The results presented here may therefore not be a true reflection of the overall capsid stability of the viruses investigated.

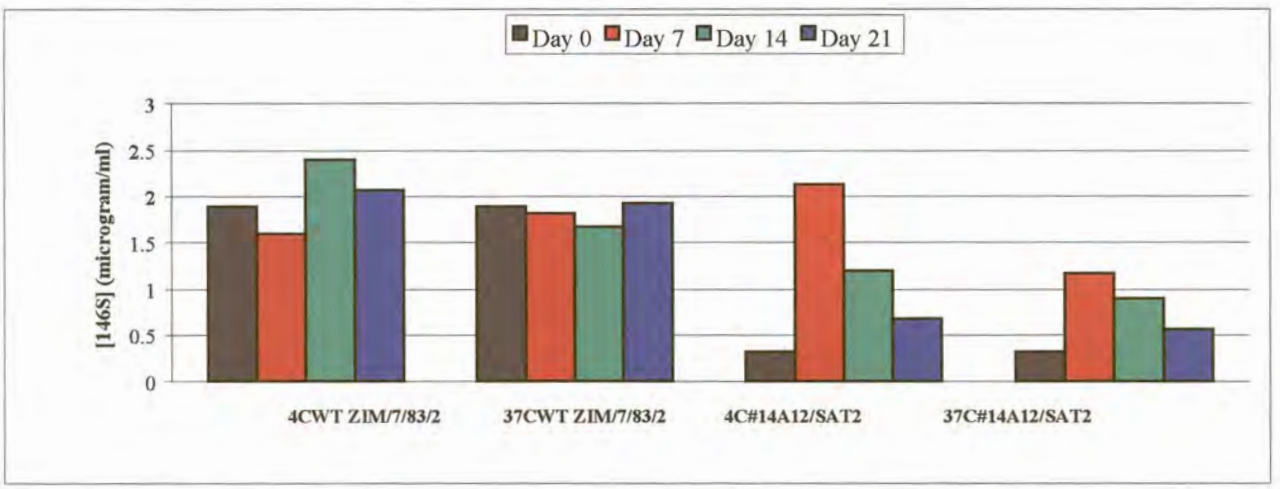


Figure 4.8: Thermal stability as determined for the WT ZIM/7/83/2 and #14A12/SAT2 chimeric virus at 4°C as well as 37°C, was carried out over a 21 day period. The amount of intact virions [146S] is presented in µg/ml.

4.4 Conclusions

In this chapter the construction of a chimeric cDNA clone between serotypes A and SAT 2 was described. The resulting recombinant virus was derived from infectious RNA transcribed from clone #14. This clone was obtained following the insertion of the external capsid-coding region of ZIM/7/83/2 cDNA into the A_{12} full-length cDNA clone, replacing the A_{12} structural-protein-coding region with that of ZIM/7/83/2. The resulting recombinant virus, #14A12/SAT2, was subsequently compared to the wild type SAT 2, ZIM/7/83/2. The results obtained indicated that the two viruses display almost identical immunogenicity, although the intactness of the epitopes could not be compared due to the unavailability of monoclonal antibodies against SAT 2. Comparisons in growth rate between the chimera and the wild type SAT 2 virus, ZIM/7/83/2, indicated the chimera to be a slower antigen producer, although comparative antigen yields could be obtained. Despite these yields, the chimeric virus constructed in this study between types A and SAT 2 is possibly not an ideal candidate for inactivated vaccine manufacturing due to the lack of stability displayed by this virus. The reason for the observed thermal instability is however unclear.

Although a viable chimeric virus could be constructed between the two serotypes, it is possible that the A_{12} replicating cDNA clone is not optimal for the construction and subsequent production of chimeric viruses employing the SAT type viruses originating from southern Africa. Alternatives should therefore be investigated.