final report

Project code: P.PSH.0558

Prepared by: Wilna Vosloo, Nagendra Singanallur, Jacquelyn Horsington, Jacqui Morris
CSIRO-Australian Animal Health Laboratory

Date published: March 2014

ISBN: 9780958582506

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Foot-and-mouth disease risk management project

Report for Phase 1 (December 2010 – November 2013)

This is an MLA Donor Company funded project.

Through the MLA Donor Company, the Australian Government provides financial support to equal any private funding for innovative research projects.

Meat & Livestock Australia and the MLA Donor Company acknowledge the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Foot-and-mouth disease (FMD) continues to be a serious threat to the agricultural economy of Australia; therefore it is essential to ensure a high level of preparedness and an effective response in the face of a disease incursion. At present viruses belonging to serotypes O and A are circulating in South East Asia, due to its proximity the biggest perceived threat to Australian livestock industries. An important aspect of control will be vaccination and access to quantitative data to support a decision whether or not to vaccinate. Results in cattle, sheep and pigs indicated protection soon after vaccination in most cases with resultant decrease in virus excretion into the environment, but efficacy depended on the species, challenge virus and route of challenge. During outbreaks, rapid diagnosis will be essential and swabs (nasal, saliva and faecal), oro-pharyngeal scrapings (probangs) and non-invasive diagnostic surveillance tools were evaluated. Most swabs tested positive for viral RNA both before and after clinical disease in all species, while rope sampling was comparable with individual saliva swabs from pigs with acute infection. The knowledge gained will in future directly impact on the choice of FMD virus vaccine strains for inclusion in the Australian vaccine bank and provides the country with the opportunity to appropriately address issues regarding emerging strains, be better prepared to control an outbreak and use vaccines in a cost effective manner to minimise the inevitable disruption to trade in export and domestic livestock markets resulting from an outbreak.

Executive summary

Foot-and-mouth disease (FMD) continues to be a serious threat to the meat and livestock industries in Australia. It is of high importance to perform research and build capacity both here and in neighbouring countries to improve diagnostic capability and increase our understanding of the epidemiology and pathogenesis of FMD virus (FMDV) strains in the region. It is also essential that vaccine efficacy studies in relevant animal species are performed to verify that the currently available vaccines protect against newly emerging strains of this continually evolving virus. These actions, as implemented in the first phase of this project, are paramount to ensure a high level of preparedness and an effective response in the face of an incursion.

This report provides a brief overview of the work performed for Phase 1 of the FMD Risk Management project. The project focused largely on testing vaccine efficacy using vaccine strains held in the Australian vaccine bank against viruses currently circulating in South East Asia (SEA). A number of experiments were performed involving cattle, sheep and pigs to ascertain not only whether the vaccines will protect animals against clinical disease, but to also determine the course of disease through viraemia (blood) and the amount of virus excreted in secretions such as saliva, nasal fluids, and faeces. In addition, the immune response to the vaccines was measured. Due to the restrictions on importing live FMDV into Australia, several overseas institutes with animal facilities where experiments and laboratory assays involving FMDV can be performed were engaged as collaborators for this work.

A major focus for the vaccine trials has been the investigation of suitable challenge models. Even though FMDV is highly infectious, in the context of efficiently conducting expensive experiments with ethics and resource limitations, it is necessary to select a challenge path (frequently injection of virus) that is more likely to generate disease than the natural exposure paths (direct contact and aerosol). This is accepted as a limitation of FMDV research and adds to the complexity of interpreting results.

At present, both serotypes O and A are circulating in SEA. It was therefore necessary to determine whether at least one of the serotype O and A antigens included in the vaccine bank will provide early protection in pigs against viruses circulating in the region. The O1 Manisa and A Malaysia 97 vaccines were tested against serotype O and A SEA viruses, respectively, by vaccinating pigs and challenging them at early time points post vaccination. In addition, the experiments were designed to determine whether vaccination lowers virus excretion thereby preventing infection of pigs in close, but not direct contact with vaccinated and infected animals.

Upon virulent challenge using injection into the heel of the bulb, protection was in excess of 75% 4 and 7 days post vaccination (dpv). Transmission did not seem to occur between pigs in close, indirect contact, despite the detection of viral RNA in some swabs from the contact pigs in the serotype O experiment. For both serotypes, the quantitative RT-PCR results of the swabs taken of secretions showed that virus excretion was less and for shorter periods of time in the vaccine groups when compared to the unvaccinated groups.

During 2010 there was a widespread outbreak of FMD in South Korea caused by a serotype O virus. Reports indicated that the current vaccines may not be efficacious and Merial developed an experimental vaccine using the outbreak virus. AAHL, the Pirbright Institute and Merial collaborated to compare the newly developed

O/SKR/2010 and the currently used O1 Manisa vaccines in cattle and pigs when challenged with the O/SKR/2010 virus.

In cattle the O1 Manisa vaccine had a lower potency (PD_{50} = 3.47) compared to the O/SKR/2010 vaccine (7.94), as could be expected from a heterologous vs. homologous challenge. Both vaccines protected all 5 cattle when administered at a full dose. However, regardless of vaccine, animals sero-converted to the non-structural proteins (NSP) indicating that vaccination did not prevent virus replication using the intra-dermalingual route of infection, although it did prevent clinical disease in a number of animals. When comparing overall viral RNA levels in swabs, vaccination reduced the viral load and can therefore assist with disease control by preventing large-scale contamination of infected premises. It can be concluded that the high potency O1 Manisa vaccine should provide protection to cattle in Australia if a virus related to O/SKR/2010 should be introduced.

The newly developed O/SKR/2010 and O1 Manisa vaccines were also compared in pigs challenged with O/SKR/2010 by injection into the bulb of the heel. When pigs were challenged 5 dpv, all animals displayed generalised disease by 2 days post challenge (dpc), suggesting neither vaccine offered protection. As this could be due to a high virus challenge dose, the dose was decreased and the pigs were challenged 21 dpv to allow a comparison between the two vaccines. Neither vaccine provided full protection. It is not clear why the vaccines that provided 100% protection to cattle at full dose failed to protect pigs but is in line with previous findings that immune responses in pigs are decreased compared to cattle. It seems therefore that the currently available vaccines will not be fully protective in pigs if an outbreak of a virus related to O/SKR/2010 should cause an outbreak in Australia.

Based on the outcome in cattle and pigs, it was decided to also test the efficacy of the O1 Manisa vaccine in sheep. However, firstly a number of infection routes were compared to determine the route that results in the most reproducible clinical signs to guide future work in sheep. Both the intra nasal instillation (INP) and coronary band (CB) routes of infection caused reproducible disease in sheep with rapid progression to observed clinical signs when infected with O/SKR/2010. Contact transmission and aerosol exposure also infected all sheep, but the progression to disease was more variable, consistent with natural exposure.

There are different interpretations of the vaccine dose to be used in sheep. Most manufacturers recommend half a cattle dose, i.e. 1ml, but this is not based on scientific merit. For that reason, we compared different doses of the O1 Manisa vaccine in sheep and challenged with O/SKR/2010 at 7 and 14 dpv. Since the vaccine is of high potency, it was decided to first test whether lower doses of O1 Manisa vaccine could protect sheep against CB infection with O/SKR/2010 7 dpv. This was followed by an experiment where sheep were given double doses of vaccine and challenged at later time points. In all cases, sheep showed clinical signs. It was not clear whether the challenge model and dose of challenge virus using the CB inoculation route was too high to mimic natural conditions. Therefore, another vaccine study was performed where infected sheep were used as donors to infect sheep via direct contact at 4 dpv which is more consistent with natural challenge. All of the vaccinated sheep were fully protected upon challenge. The RT-qPCR results from probang samples suggested that approximately 50% of the infected sheep became carriers 35 dpc, indicating that probangs could be a suitable diagnostic sample once lesions have healed.

An experiment was performed to investigate the early pathogenesis of FMDV infection in pigs. First it was necessary to establish a model of direct-contact infection with an O isolate from Vietnam. The results from the model study indicated an inconsistency in the development of clinical disease in pigs following heel pad

inoculation at the infectious dose used. However, a period of 2 hrs of direct contact between infected and susceptible pigs was sufficient for FMDV transmission in pigs. The study is ongoing.

As part of the vaccine efficacy testing in pigs, the use of cotton ropes as a diagnostic tool for FMDV was investigated. Compared to saliva swabbing and visual examinations, rope sampling is far less laborious for people and is stress-free for animals. The ease in which oral fluid samples from ropes were collected and extracted makes rope sampling an extremely useful method of sample collection that may complement FMD monitoring efforts in pig populations. With further validation of the specificity and sensitivity of detection, this may be a cost effective, non-invasive sampling tool to detect FMD in a pen considering that susceptible, unvaccinated pigs will rapidly infect each other due to direct contact.

The project has demonstrated the success of using swabs (in cattle, sheep and pigs) and probangs (in cattle and sheep) as diagnostic tools. Often clinical material such as vesicular fluid or epithelial flaps are not available, either because the animals are in the incubation phase and are yet to develop lesions or the lesions have started healing. In addition, clinical signs in sheep and goats are frequently mild or inapparent, and lesions on the coronary band can be obscured by wool/hair. These studies demonstrated that virus can be found in excretions both before and after clinical signs are observed and swabs or probangs can be recommended for diagnostic use in the face of an outbreak.

The samples collected during these efficacy trials are used to validate diagnostic assays, and progress has been made to obtain regulatory approval to import samples that pose no risk to the livestock industries in Australia allowing AAHL to perform the assays locally and ensure staff are familiar with running the assays. Developing technologies to perform deep sequencing using RNA extracted from material collected during the trials is making progress overseas.

It is clear that there is significant variation in the severity of disease caused by different isolates, emphasising the need to investigate more isolates from the region. In addition, it seemed that differences in the duration of excretion may occur, depending on the virus. There were no significant differences between nasal and saliva swabs obtained from pigs and both samples are suitable to detect disease. In sheep, in oral swabs appear to be a slightly more reliable indicator of FMDV status than in nasal swabs. However, significant variation was observed between individual animals. Viral RNA was found in the faeces of pigs, but not in sheep. Although faecal swabs are not a sample of choice for diagnostics due to lower sensitivity observed in pigs compared to nasal and saliva swabs, these results indicate that environmental viral contamination from faeces may be more prevalent when pigs are infected compared to sheep. However, more data need to be collected to verify this conclusion.

One of the vulnerabilities identified and addressed as part of the project is lack of capability and capacity in SEA to diagnose FMD and perform genetic and antigenic characterisation on isolates from the region. Efforts are ongoing to engage the OIE FMD Regional Reference Laboratory, based in Pakchong, Thailand, to collaborate on training and capacity building. Training was provided to Vietnamese staff in bio-security and in the execution of animal experiments with exposure to Australian animal ethics regulations. AAHL staff also provided on-the-job training to scientists in the laboratories whilst working there. A training workshop for Vietnamese staff was held at AAHL and scientists were sponsored to attend scientific meetings abroad. Locally, the project has appointed two research scientists and one research assistant. Several AAHL staff have had the opportunity to participate in the animal and laboratory experiments, thereby ensuring more staff are familiar with clinical

FMD and laboratory assays. In addition, one staff member from the Department of Primary Industries in Western Australia participated in an animal trial.

The knowledge gained in this project will in future directly impact on FMD response policy including the selection of FMDV vaccine strains to be kept in the Australian vaccine bank. It provides the country with the opportunity to appropriately address issues regarding newly emerged strains, be better prepared to control an outbreak and use vaccines in a cost effective manner. In addition, it offers Australian governments and industries access to firsthand knowledge on FMDV strains that are potential risks enabling them to respond to FMD policy questions and have access to quantitative data for disease modelling.

The project has enabled Australia to make a significant contribution to FMD research, not only locally, but internationally as supported by the annual external scientific reviews. Australia now has a significant position in the Global FMD Research Alliance by virtue of its international collaborations and scientific contribution. The collaboration with various prestigious research groups puts the country in a very strong position regarding access to expertise not locally available.

As a whole, the experiments performed to date indicate that the vaccine strains in the Australian vaccine bank are likely to protect cattle, sheep and pigs against clinical disease. However, since not all vaccines provided full protection, it is clear that vaccination needs to be used in combination with other control options such as movement control and strict on- and between-farm bio-security. The viruses are constantly evolving in the field and novel viruses may be introduced from other regions of the world. It is therefore necessary to continually monitor isolates to ensure diagnostic assays will detect any emerging strains and predict vaccine efficacy by *in vitro* methods like vaccine matching studies, substantiated by *in vivo* challenge.

This project has addressed the seven objectives and made significant progress towards the ensuring that Australian livestock industries, and the Australian community, will be better prepared to minimise the inevitable disruption to trade and domestic livestock markets that would be caused by an outbreak of FMD. The research team is now well set up to continue a high level success in Phase Two that will continue from 2014 to early 2017.

Table of Contents

1 Background

Foot-and-mouth disease (FMD) is one of the most infectious viral diseases affecting cloven-hoofed ruminants and pigs. Although it does not cause mortality in adult animals, the production losses and economic impacts due to trade embargoes can be severe. Australia has been free of FMD since 1872 and this status, together with the absence of various other infectious diseases, provides Australia with lucrative export markets.

The disease occurs as seven distinct serotypes (O, A, C, Asia-1, SAT1, 2 and 3) with specific geographic distribution. There is little to no cross-protection between the serotypes and added to that, large amounts of genetic and antigenic variation within each serotype can lead to vaccine failure.

Foot-and-mouth disease is of major concern to the Australian livestock industries. The disease has the potential to cause losses in excess of $50 billion per year, and compounding this, many years of lost revenue due to restrictions placed on the export of Australian produce. The government and the industries are investing in several pre- and post-border mitigation measures to prevent disease introduction; one being a FMD virus (FMDV) antigen bank for use in the event of an outbreak. However, there has been little investigation into vaccine efficacy with a heterologous FMDV challenge due to cost and a prohibition on the use of live FMDV in Australia.

FMD is endemic in many parts of the world and occurs in most countries in South East Asia (SEA), through its proximity the biggest perceived risk to Australia's agricultural economy. For this reason industry and the federal government are funding a project, called the Foot and Mouth Disease Risk Management Project (FMDRMP), focusing on aspects of FMD such as protection of various cloven-hoofed species using the vaccine strains in the bank, pathogenesis of SEA viruses in equivalent Australian domestic species, validation of diagnostic assays, molecular epidemiology of FMD in SEA and capacity building in the region as part of our pre-border mitigation.

The antigen bank will be activated should an outbreak of FMD occur in Australia. However, due to costs, the current bank contains a limited number of strains and it is not known how well these strains will protect against viruses currently circulating in SEA. Data on the efficacy of the vaccines would potentially influence the decision to vaccinate rather than cull animals. The vaccine efficacy studies and related laboratory assays were all performed offshore since no live FMD virus is allowed in Australia.

2 Project objectives

The main objectives of the project are to:

- Gain comprehensive knowledge about FMD virus strains that pose a geographic high risk to Australia and their comparable likely behaviour in Australian livestock species
- Devise control strategies tailored to Australian circumstances and store appropriate bulk serum-derived reagents for future Australian use
- Improve laboratory diagnostic capability for FMDV to rapidly isolate or detect FMDV and confirm a primary diagnosis by providing AAHL staff the opportunity to work with live virus off shore and so gain experience with FMD culture and recognition of cytopathogenic effects (CPE)

- Validate diagnostic tests (including DIVA) for use in local animal species and breeds, and to conduct genetic 'fingerprinting' (sequencing) on the virus(es) isolated in support of molecular epidemiology and vaccine selection
- Enhance the epidemiological and virological understanding and thus help model virus spread
- Include FMD vaccination response policies in AUSVETPLAN and associated national standard operating procedures that are soundly technically based
- Have experimental data on the efficacy (in terms of protection against challenge with circulating high-risk virus isolates) of the vaccine strains and the vaccine potency in Australia's FMD vaccine bank, to maximise the benefits arising from investment in the vaccine bank and to inform FMD response planning.

The outcome of the project is that Australian livestock industries, and the Australian community, will be better prepared to minimise the inevitable disruption to trade and domestic livestock markets that would be caused by an outbreak of FMD.

3 Methodology

3.1 Efficacy of a high potency O1 Manisa monovalent vaccine against heterologous challenge with a FMDV O Mya98 lineage virus in pigs 4 and 7 days post vaccination

To determine whether the O1 Manisa vaccine is efficacious against the type O viruses currently circulating in SEA, pigs were vaccinated and challenged at early time points post-vaccination. In addition, the experiment was designed to determine whether vaccination lowers virus excretion thereby preventing infection of pigs in close, but not direct contact with vaccinated and infected animals.

In all experiments described in this report, we used the vaccine at the high payload (>6PD_{50}) and as a double-oil emulsion formulation, as would be used in Australia in the event of an outbreak. All the vaccines were formulated from the antigens held in the Australian Vaccine Bank by Merial.

Animal ethics approvals were obtained from the AAHL Animal Ethics Committee (AEC) (AEC1465 and 1497) and all work was performed according to the Australian code of practice for the care and use of animals for scientific purposes. The animal experiments were performed in collaboration with NAVETCO and RAHO6 based in Ho Chi Minh City (HCMC), Vietnam.

3.1.1 Preparation of pig challenge virus

Eight healthy pigs (Landrace cross-bred) were used to prepare pig adapted O/VIT/2010 (Mya 98 strain), a virus that caused outbreaks in Vietnam during 2010. Tissue culture adapted virus was inoculated either into the footpad of the left forelimb at multiple sites (2 ml; 0.1 ml/site in each digit) or intravenously (1 ml) into the ear vein and intramuscularly (1 ml) on the mid neck region. Two pigs were used for each route. The animals were observed for the development of generalised disease and development of lesions on the other feet, mouth, snout and tongue. Epithelium from the lesions was harvested and a 10% suspension prepared. Two additional pigs were inoculated with 1 ml of the 10% suspension into the footpad of the left forelimb at multiple sites. Epithelium was again harvested and two more pigs inoculated as described above. A 10% suspension was prepared from the collected vesicular

lesions and titrated. This constituted the pig challenge virus and was stored in aliquots at -80^{o}C until used.

3.1.2 Pig immunisation and challenge

Three groups of 10 pigs were divided into sub-groups consisting of 5 pigs each (Figure 3.1.1). Groups O-V7 (n=5) and O-V4 (n=5) were vaccinated with 2ml double-oil adjuvant O1 Manisa monovalent vaccine (>6PD$_{50}$) intramuscularly in the neck and challenged on 7 and 4 days post-vaccination (dpv), respectively. Group O-UV (n=5) was not vaccinated but consisted of unvaccinated challenged controls. Groups O-UVC7, O-UVC4 and O-UVC (n=5 each) were used as unvaccinated indirect contacts and housed in the same room as O-V7, O-V4 and O-UV, respectively, but with a physical partition consisting of a waist-high steel wall between the groups (Figure 3.1.1). The animals were not in direct physical contact and had separate feed and water troughs but shared the same air handling facility.

The animals in groups O-V7, O-V4 and O-UV were challenged with $10^{5.0}$ TCID$_{50}$ of the pig adapted challenge virus by inoculation in two sites in the left-hind footpad (0.2 ml/site). The animals were observed for 14 days for the development of generalised disease and appearance of secondary lesions of FMD. Clinical material including nasal secretions, saliva and faeces were sampled daily using cotton swabs for virus isolation and viral genome detection. Clotted blood for serum was collected on -7, -4, 0, 5, 7, 10 and 14 days post-challenge (dpc).

The animals in groups O-UVC7, O-UVC4 and O-UVC were also observed for clinical disease and samples taken daily as described above. Clotted blood for serum was collected on days 0, 5, 7, 10 and 14 dpc.

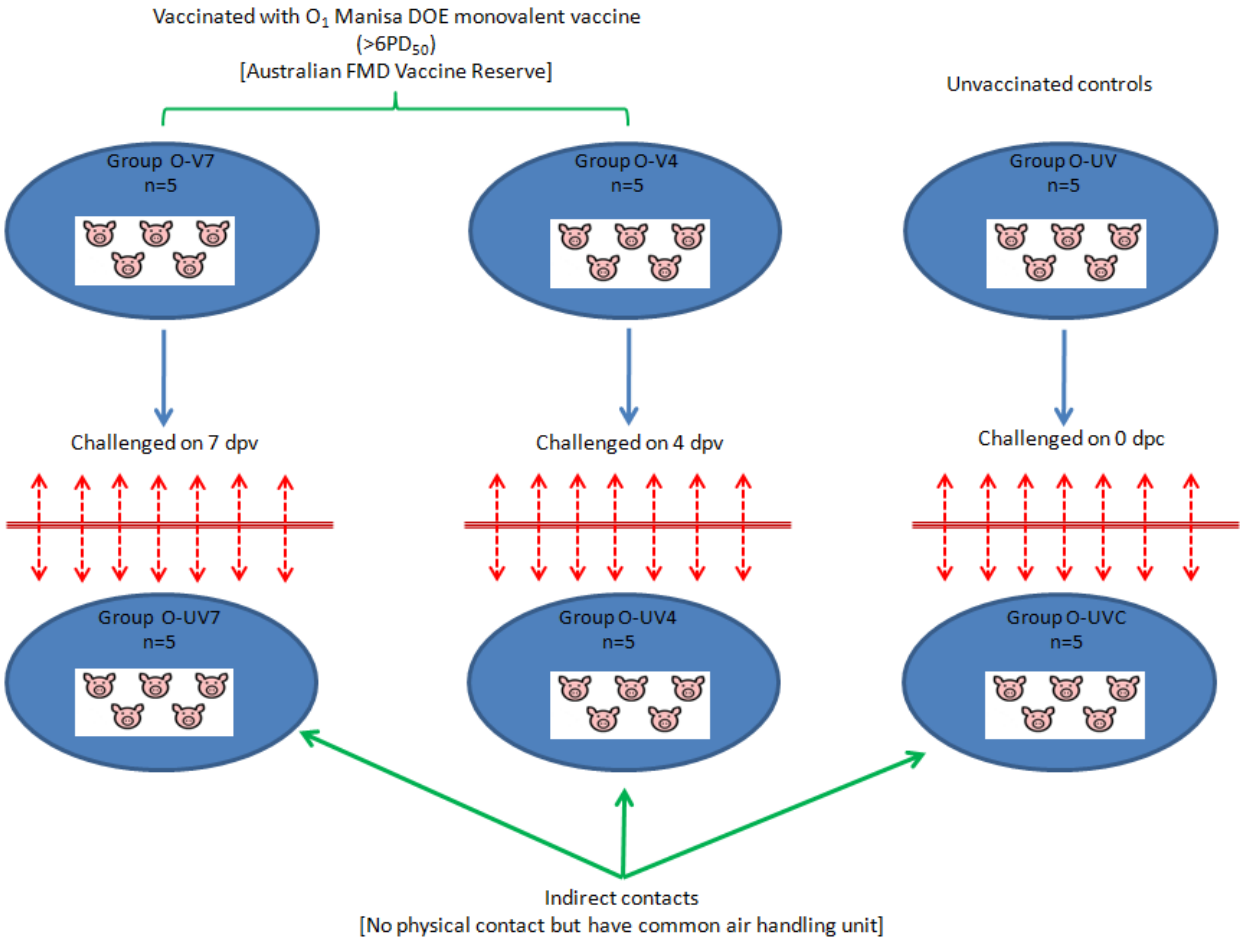

Figure 3.1.1 Experimental layout and housing of challenge and indirect contact groups for studying the efficacy of high potency O1 Manisa vaccine against O/VIT/2010 (Mya-98 strain) pig challenge virus

3.2 Efficacy of high potency A Malaysia 97 monovalent vaccine against heterologous challenge with a A/VIT/2005 SEA-97 FMDV lineage virus in pigs 4 and 7 days post vaccination

At present, both serotypes O and A are circulating in SEA and it was therefore necessary to also determine whether the serotype A vaccine will provide early protection in pigs against regional viruses.

Animal ethics approvals were obtained (AEC1514 and 1571). The animal experiments were performed in collaboration with NAVETCO and RAHO6 based in HCMC, Vietnam.

3.2.1 Preparation of pig challenge virus

Five healthy pigs (Landrace cross bred) were used to prepare pig adapted A/VIT/2005 (SEA-97 lineage), a virus that caused outbreaks in Vietnam in 2005 as described in 3.1.1. Clinical material was stored at -80^{o}C as a 10% solution.

3.2.2 Titration of A VIT 2005 SEA-97 pig adapted virus

Four apparently healthy pigs were used to titrate the A/VIT/2005 pig adapted virus. A 10% suspension of the ground up vesicular epithelia was diluted from 10^{-1} to 10^{-8} and 0.1–0.2 ml of the diluted inoculum was administered intradermally to the four limbs of each pig. Development of lesions at the sites of inoculation was scored at 24, 36, 48, 60 and 72 hours post inoculation.

3.2.3 Pig immunisation and challenge

The experimental design and layout was as described in 3.1.2 except that the vaccinated and challenged groups consisted of 8 pigs each (A-UV, A-V4 and A-V7) and 5 pigs were used for the in contact groups (A-UVC, A-UVC4, A-UVC7). Groups A-V7 and A-V4 were vaccinated with A Malaysia 97 monovalent vaccine (>6PD_{50}) and challenged on 7 and 4 dpv, respectively. Group A-UV was the unvaccinated challenged controls. Groups A-UVC7, A-UVC4 and A-UVC were used as unvaccinated indirect contacts and housed in the same room as A-V7, A-V4 and A-UV, respectively, as described in 3.1.2.

The animals in groups A-V7, A-V4 and A-UV were challenged with $10^{5.0}$ $TCID_{50}$ of the pig adapted A/VIT/2005 and sampled as described in 3.1.2. Clotted blood for serum was collected on -7, -4, 0, 5, 7, 10 and 14 dpc. The animals in groups A-UVC7, A-UVC4 and A-UVC were also observed for clinical disease and samples taken daily as described in 3.2. Clotted blood for serum was collected on days 0, 5, 7, 10 and 14 dpc.

3.3 Comparison of the potency of O1 Manisa and O/SKR/2010 high payload vaccines in cattle using O/SKR/2010 (Mya-98 lineage) as challenge virus

During 2010 there was a widespread outbreak of FMD in South Korea caused by a serotype O virus. Reports from the field indicated that the current vaccines may not be efficacious and Merial developed an experimental vaccine using the outbreak virus. AAHL, the Pirbright Institute and Merial collaborated to compare the newly

developed O/SKR/2010 and the currently used O1 Manisa vaccines in cattle and pigs.

Animal ethics approvals were obtained from the AEC (AEC 1570) and the Institutional Animal Ethics Committee of the Pirbright Institute, United Kingdom (PPL 70/7253). The potency tests were performed at the facilities at Pirbright following the European Pharmacopeia.

3.3.1 Experimental design

Fifteen Holstein-Friesian cross-bred cattle were randomly allocated to each of the 2 vaccines (monovalent double-oil adjuvant O1 Manisa and O/SKR/2010 vaccine both at >6PD_{50}) and further divided into groups of 5 animals each (Figure 3.3.1). Potency tests were performed by vaccinating the groups of 5 cattle each with either neat, 1/4 and 1/16 dose. All cattle were challenged with O/SKR/2010 cattle challenge virus 21 dpv. Three cattle were used as unvaccinated controls. The animals were observed daily for appearance of clinical signs of FMD up to 8 dpc and again at termination at 14 dpc.

Clotted blood for serum was collected on 0, 7, 13, 14, 18 dpv and 0-8, 10 and 14 dpc. Saliva samples were collected on 0–8, 11 and 14 dpc. Probang samples were collected on 0, 2, 4, 6, 8, 11 and 14 dpc.

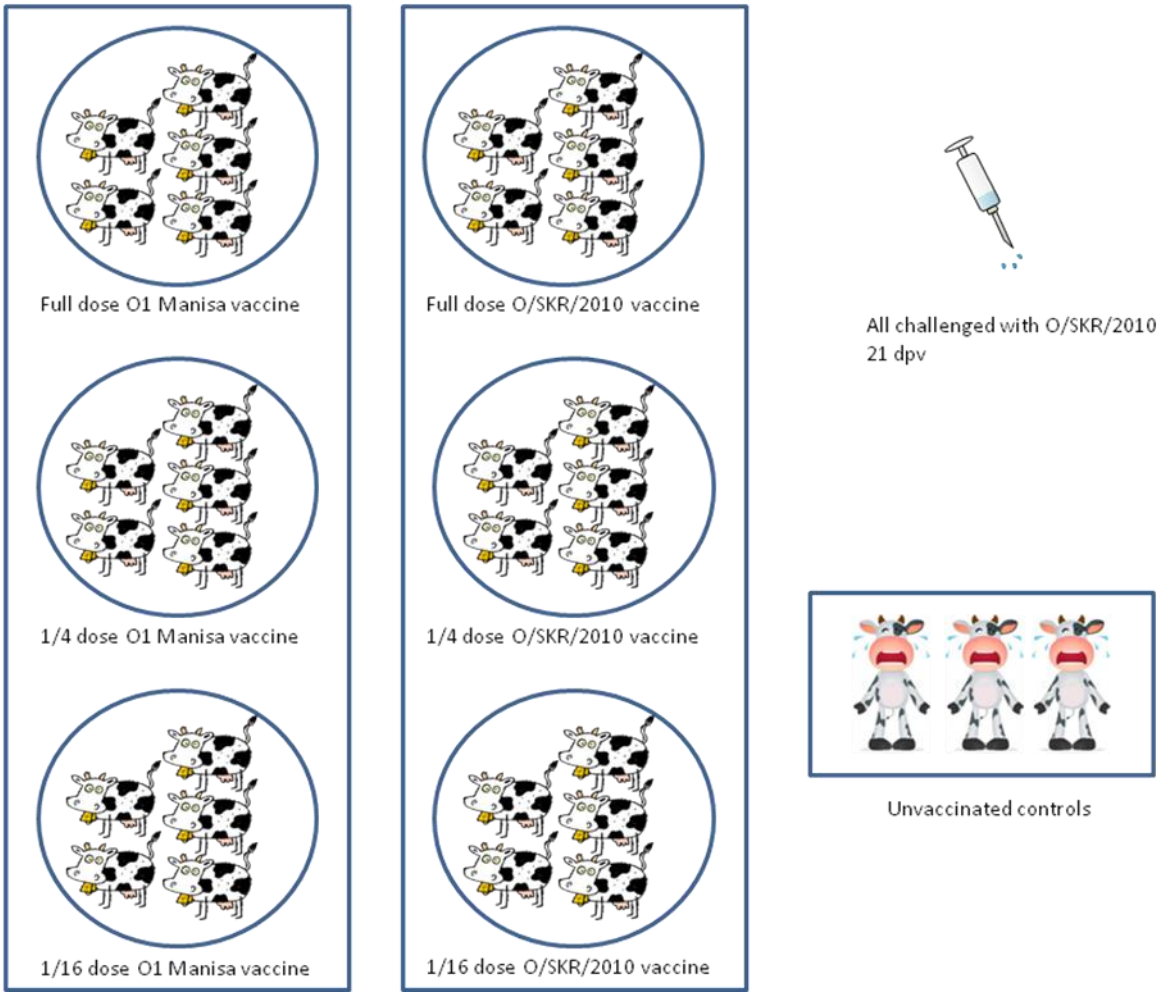

Figure 3.3.1 Experimental design of the cattle potency study where one group was vaccinated with a full, ¼ or 1/16 dose of O1 Manisa vaccine and the other group with similar dilutions of O/SKR/2010 vaccine. All animals were challenged with O/SKR/2010

3.4 Comparison of the vaccine efficacy of O1 Manisa and O/SKR/2010 in pigs using O/SKR/2010 as challenge virus

The newly developed O/SKR/2010 and O1 Manisa vaccines were also compared in pigs. Only 5 pigs were used for each vaccine and both vaccines were administered as a full dose. In the first trial the pigs were challenged 5 dpv, followed by a trial where they were challenged 21 dpv.

The animal experiments were approved (UK - AC000397, AC000429, AC000427; AAHL - AEC 1595) and performed at the Pirbright Institute.

3.4.1 Adaptation of O/SKR/2010 virus in pigs

The virus was adapted to cross-bred Landrace pigs as described in 3.1.1.

3.4.2 Titration of O Mya 98 (O/SKR/2010) cattle adapted virus

After performing the 5 day vaccination challenge, a titration of O/SKR/2010 was performed in four healthy pigs. The virus was diluted to 10^3 $TCID_{50}$/ml and 10^2 $TCID_{50}$/ml and inoculated into the foot-pad of both hind limbs at multiple sites (0.1 ml in total) of 2 pigs per dilution.

3.4.3 Vaccine efficacy trials

Two groups of 5 pigs each were vaccinated with a full dose of O1 Manisa and O/SKR/2010 and challenged either 5 or 21 dpv. Two pigs were used as unvaccinated controls for each study. All groups were kept in separate rooms and monitored and sampled daily.

All pigs, including controls, were bled on the day of vaccination. At 5 dpv the pigs were challenged with at least 10^4 $TCID_{50}$/ml of O/SKR/2010 virus by inoculation in three sites in both of the hind feet pads, 0.1 ml in total. The animals that were challenged at 21 dpv received the same volume by a similar route but at 10^3 $TCID_{50}$/ml. The groups were observed daily for the development of generalised disease and appearance of secondary lesions of FMD. Clinical material including nasal secretions, saliva and blood were collected daily for virus isolation.

3.5 Vaccine efficacy trials with high potency O1 Manisa monovalent vaccine against FMDV O/SKR/2010 (Mya-98 strain) in sheep 4 days post vaccination

Based on the outcome in cattle and pigs, it was decided to also test the efficacy of O1 Manisa vaccine in sheep at the dose recommended by the manufacturer. However, since a direct needle challenge does not mimic a natural route of infection, infected sheep were used as donors to infect sheep vaccinated only 4 days prior to contact.

All the protocols for experimentation with live sheep were approved by the AEC (AEC 1637) as well as the ethics committee at the National Centre for Foreign Animal Diseases, Winnipeg, Canada, where the experiment was performed.

3.5.1 Vaccine efficacy trials

Rideau Arcott-Ile de France cross-bred sheep of 6–12 months age, weighing approximately 40 kgs, were used.

The vaccine efficacy experiment consisted of two groups, where one group was vaccinated 4 days and the control group was not vaccinated prior to exposure to infected donor sheep (Figure 3.5.1).

Vaccination: Group 1a (n=8) was vaccinated with 1 ml O1 Manisa double-oil emulsion monovalent vaccine (> $6PD_{50}$) intramuscular on the anterior neck region of 4 days prior to challenge.

Control group: Group 2a (n=4) was the unvaccinated control group.

Challenge of donor sheep (Group 1b (n=8) and 2b (n=4)): The sheep were challenged by coronary band injection using 500 µl ($0.5 \times 10^{6.8}$ $TCID_{50}$) of lamb kidney (LK) cell passed O/SKR/2010 (Mya-98) virus. After 24 hours the sheep in Groups 1a and 2a were moved into the rooms with the sheep from Groups 1b and 2b, respectively, for direct contact challenge. Two in-contact sheep were moved with

two donor sheep in 6 designated rooms so that each room had 4 animals (Figure 3.5.1).

All of the sheep were monitored for the development of FMDV-specific clinical signs such as pyrexia, lameness, salivation, development of vesicles etc. Clinical samples were collected from the donor and in-contact animals from 0-10, 14, 21, 28 and 35 dpc, including vesicular fluid and tissue material if present, oro-pharyngeal and nasal swabs and saliva and faecal swabs (in duplicates), clotted and K3-EDTA/heparinised blood for virus isolation and reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR).

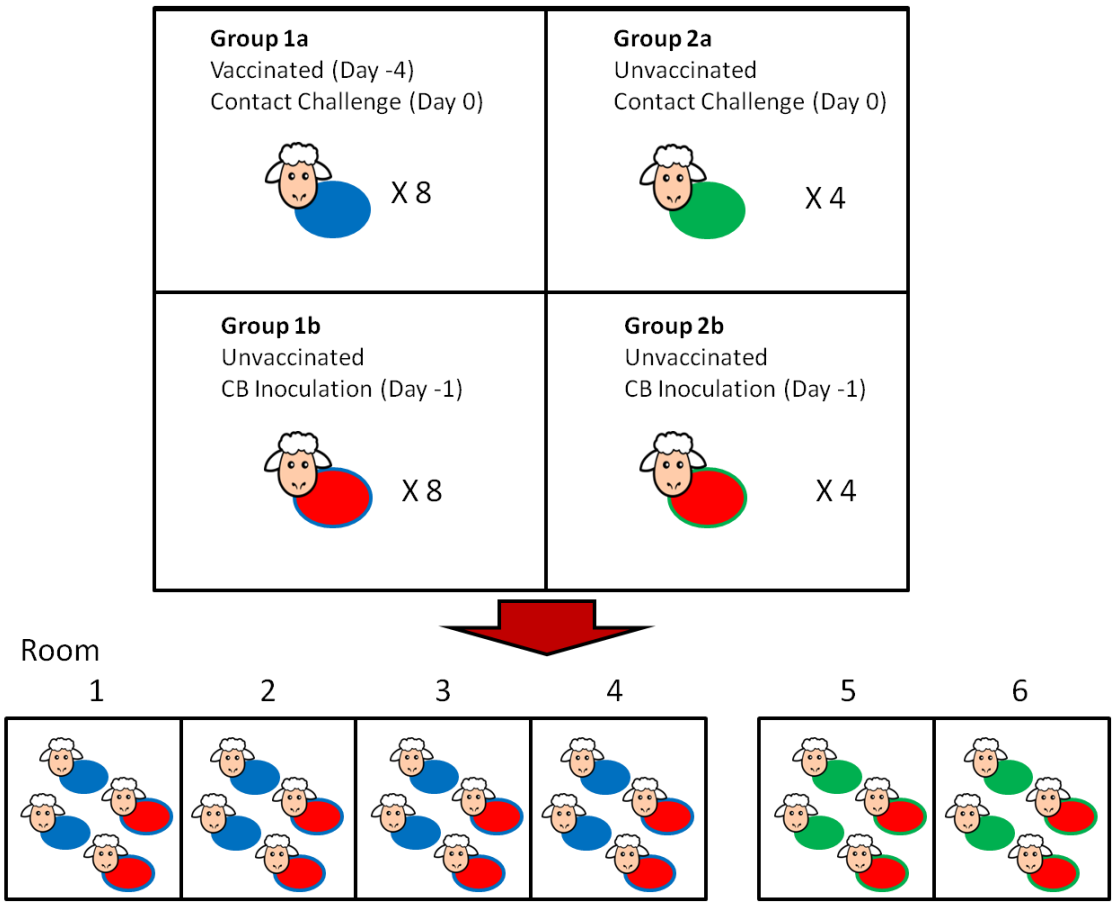

Figure 3.5.1 Experimental layout of the sheep vaccinated with O1 Manisa 4 days prior to challenge with O/SKR/2010

3.6 Vaccine efficacy trials with O1 Manisa monovalent vaccine against challenge with O/SKR/2010 (Mya-98 strain) in sheep

Few *in vivo* experiments have been described for sheep and a number of infection routes have been used without a comparison to determine the route that results in the most reproducible clinical signs and infection success. It was therefore decided to compare a number of routes to guide future work in sheep. In addition, there are different interpretations of the vaccine dose to be used in sheep. Most manufacturers recommend half a cattle dose for sheep, i.e. 1ml, but this is not based on scientific merit. For that reason, we compared different doses in sheep using the O1 Manisa vaccine and challenge with O/SKR/2010 at different time points post-vaccination.

All the protocols were approved by the AAHL ethics committee (AEC 1636) as well as the Plum Island Animal Disease Centre (PIADC), United States of America ethics committee.

This work covered a number of different experiments that were performed sequentially. The challenge virus O/SKR/2010 was adapted and titrated in cattle at PIADC.

3.6.1 Identify the most reproducible route of inoculation

For Experiment 1, phase 1, different routes of challenge were compared to gain information on the most reproducible route of infection during sheep challenge studies. The routes were intra-naso-pharyngeal instillation (INP), aerosol exposure and inoculation (AEI) and CB inoculation. Eight cross-bred Dorsett sheep were used in each group. Two sheep were euthanized 24 hours and 2 more after 48 hours for pathogenesis studies from each route of inoculation. The remaining 4 sheep were kept for a total of 9–10 days for observation, lesion scoring and sampling.

For the INP inoculation, 2 ml of inoculum was deposited into the nasopharynx using a flexible, 14 gauge, silicone catheter inserted through one nostril (Figure 3.6.1).

For the AEI route of challenge, sheep were inoculated with the challenge virus by instilling 2ml of the virus suspension by aerosol using a nebuliser (Figure 3.6.1).

For Experiment 1, phase 2, direct transmission from infected to susceptible sheep was tested as a route of challenge. Four sheep were infected by injecting 200 µl of challenge virus into the CB, just above the hoof, and these animals were observed and sampled as comparison with the other routes of infection. Forty eight hours after they were infected, they were placed with susceptible (contact) sheep. Two sheep in the contact group were euthanized after 48 hours and two more after 72 hours and samples were collected after necropsy for pathogenesis studies. At each point where 2 contact sheep were euthanized, one of the CB infected sheep was also removed to ensure the ratio between infected and naive sheep remained 1:2. All the remaining sheep were observed for a period of 10 days as described for Experiment 1 phase 1 (Figure 3.6.2).

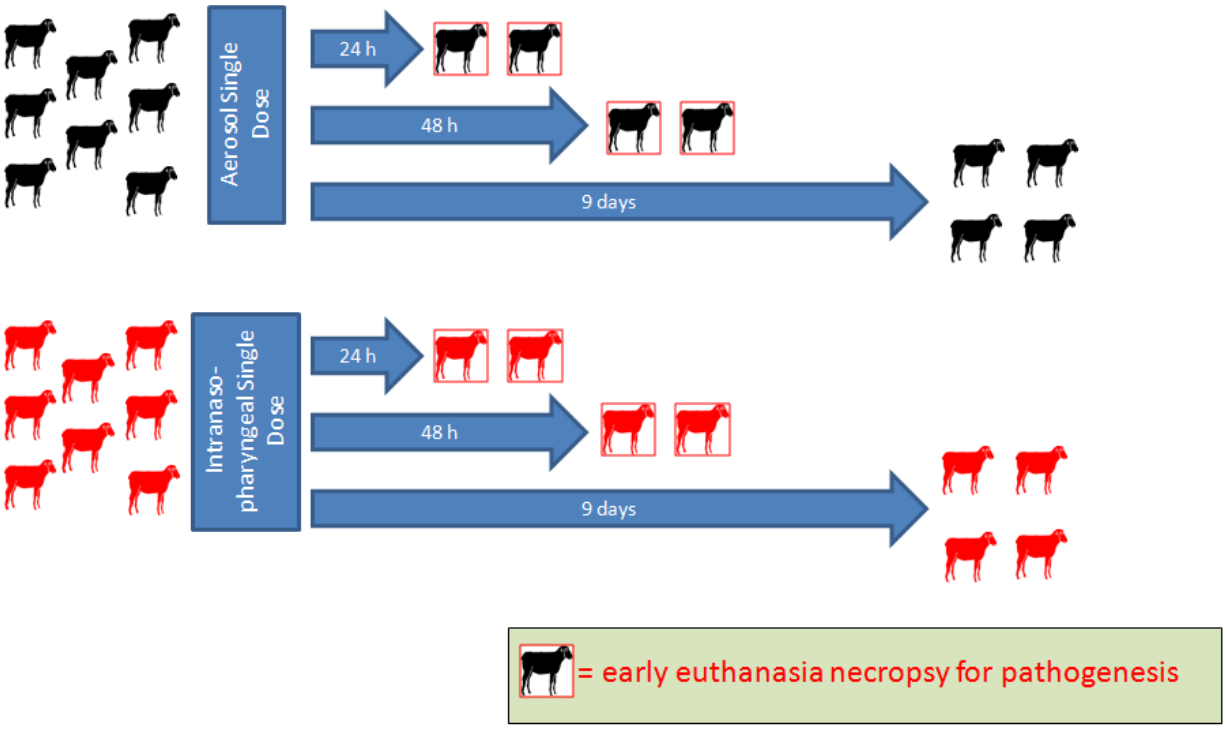

Figure 3.6.1 Experimental design to determine the optimal route for infecting sheep using O/SKR/2010 and either INP or AEI

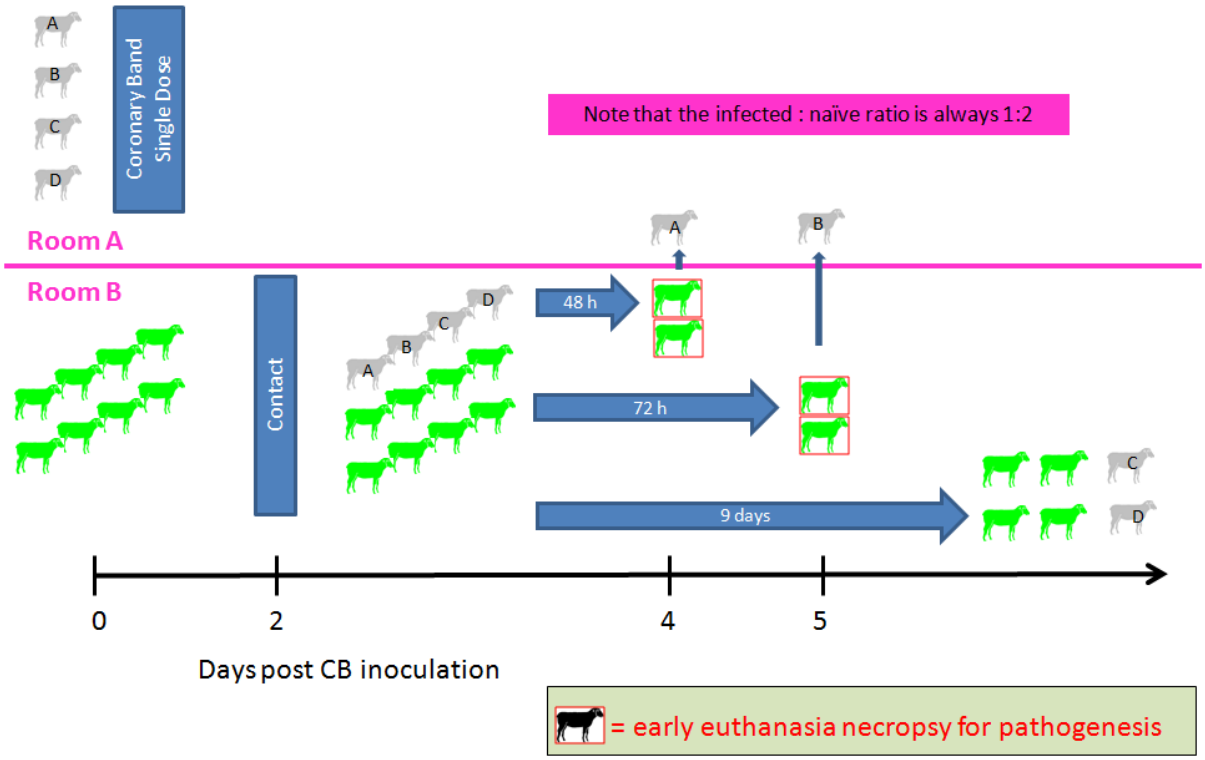

Figure 3.6.2 Experimental design to determine the optimal route of infection in sheep using O/SKR/2010 and direct contact

3.6.2 Vaccine efficacy trials

Experiment 2 phase 1 was to determine a vaccine dose response. Three groups of 7 sheep each were administered with three different dilutions of O1 Manisa double-oil emulsion monovalent vaccine (> 6 PD_{50}) (Group 1; full dose (1ml), Group 2; ½ dose and Group 3; ¼ dose) intramuscularly. Four naive controls were included (Group 4a). All the animals were challenged 7 dpv by CB injection with 200 µl of with the O/SKR/2010 challenge virus (10^6TCID_{50}/ml). The animals were kept up to 10 dpc and samples taken (Figure 3.6.3).

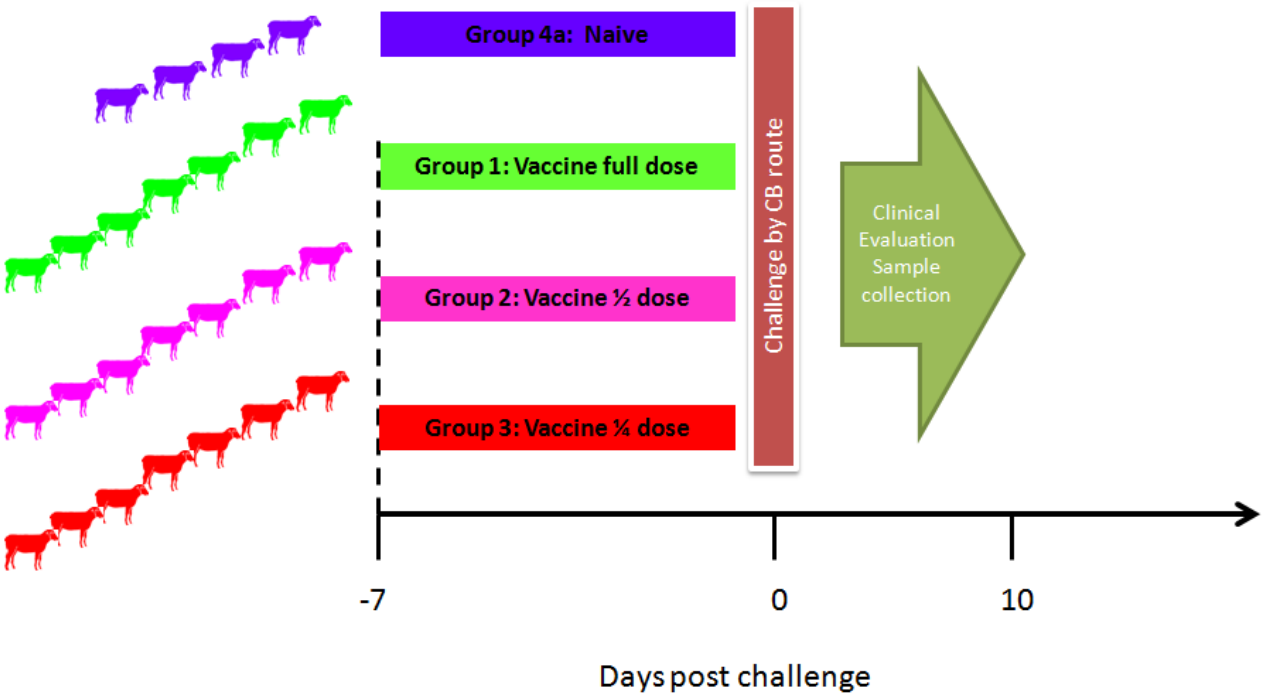

Figure 3.6.3 Experimental design to determine the vaccine dose needed to protect sheep against infection 7 dpv

In Experiment 2 phase 2, one group of 7 sheep was administered 1 ml of vaccine and challenged at 14 dpv (Group 5) while 2 additional groups of 7 sheep each received 2 ml of vaccine and were challenged at 14 and 7 dpv (Group 6 and 7 respectively). Four naive controls were included (Group 4b). All the animals were challenged by CB injection with the O/SKR/2010 challenge virus. The animals were clinically examined for 10 days and followed until day 35 to determine if carrier sheep were present (Figure 3.6.4).

All the sheep were monitored for the development of FMDV-specific clinical signs. Samples were collected from the challenged and in-contact animals at regular intervals for 9 days, including vesicular fluid and tissue material if present, oro-pharyngeal and nasal swabs and saliva and faecal swabs, clotted and K3-EDTA/heparinised blood. Probang samples were taken on 14, 17, 21, 24, 28, 31 and 35 dpc. Samples were taken for virus isolation, RT-qPCR and serology.

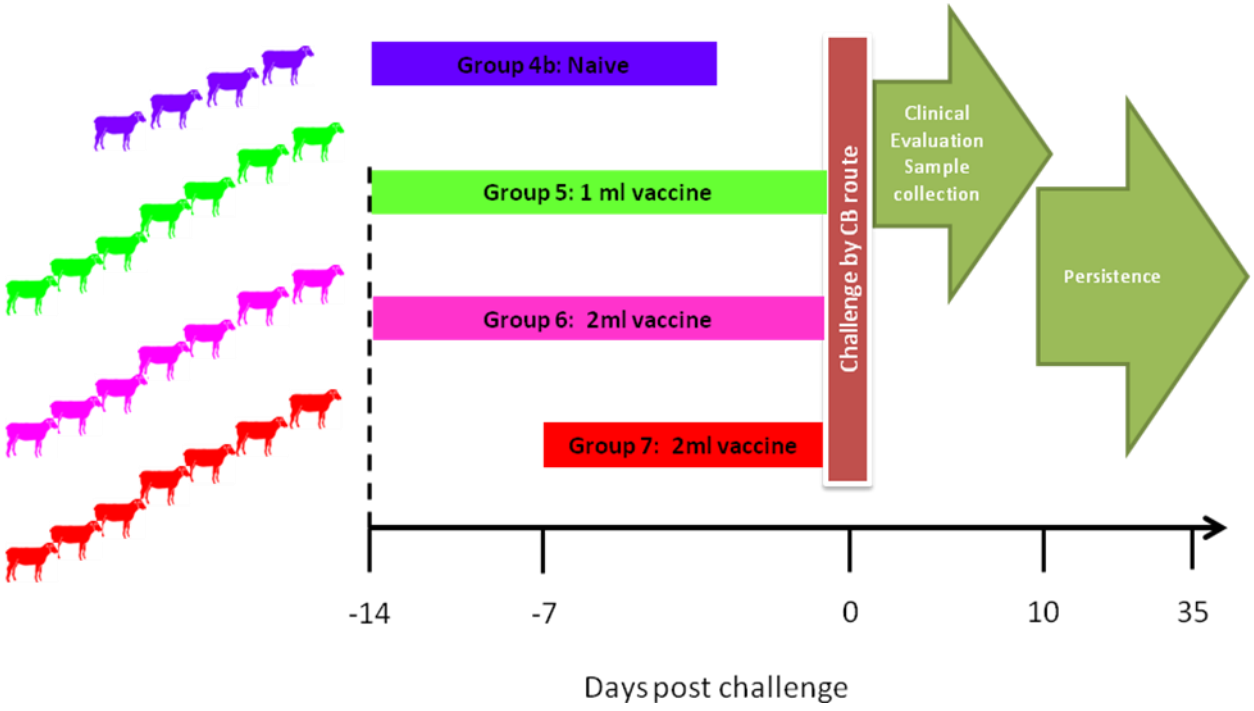

Figure 3.6.4 Experimental design to determine the optimal vaccine dose to protect sheep against challenge 7 and 14 dpv and to investigate the impact of vaccination on the establishment of a carrier state

3.7 Early pathogenesis in pigs infected with O/VIT/2010

To establish a model of direct-contact infection with FMDV O/VIT/2010 (Mya-98 strain) in pigs and to examine the early pathogenesis of this strain in pigs infected following exposure to pigs with clinical disease.

The early pathogenesis of FMDV infection in pigs infected with FMDV O/VIT/2010 (Mya-98 strain) was performed under EAC 1647 at the NAVETCO facility, Vietnam.

3.7.1 Establishment of a direct-contact infection model in pigs

A model of direct-contact infection (a natural route of infection) was established by comparing the development of clinical disease in naive pigs after different periods of contact with diseased pigs that had been infected by heel-bulb inoculation.

Cross-bred Landrace pigs of approximately 3 months of age were used. Groups of pigs (2 each) were allowed to have direct contact with 2 donor pigs that were infected 48 hours prior to contact and showed lesions. One group was exposed for 2 hours, then removed and kept for a further 48–72 hours to determine if they had become infected during the 2 hour contact period. This was repeated with more groups, but contact was 4, 6 and 48 hours. The contact animals were monitored for development of FMDV-specific clinical signs such as pyrexia, lameness and the development of vesicles. When the pigs displayed such signs they were sedated for clinical sampling and euthanized for necropsy. If no clinical signs were present the animals were euthanized at 72 hrs post-exposure.

3.7.2 Pathogenesis study

Based on the model, a 2 hour contact period was sufficient to ensure both contact pigs became infected. However, infection of the donor group was adjusted where 2 donors were infected via the heel-bulb 72 hours and another 48 hours prior to contact to allow clinical disease to develop. Groups of 2 pigs each were allowed 2 hours direct contact with the 3 donor pigs, removed and either immediately slaughtered (2 hours post contact) or at 4, 6, 8, 10, 12, 24 and 48 hours post exposure. Prior to euthanasia, blood as well as nasal, oral and faecal swabs were taken. At necropsy, tissues were collected and placed in formalin and lysis buffer. These included the dorsal tip of snout epithelium, rostral tip of the lower lip, coronary band epithelium of the left hind leg, coronary band epithelium of the right hind leg, left popliteal lymph node (LN), left superficial inguinal LN, tongue, left mandibular LN, left mandibular salivary gland, ventral superficial cervical LN, tongue, epiglottis, paraepiglottal tonsil, soft palate, lingual tonsil, hard palate, pharyngeal tonsil, retropharyngeal LN, dorsal superficial cervical LN, mid trachea, hilar LN, lung – cardiac lobe, lung – apical lobe, lung - diaphragmatic lobe, thymus, bronchi – bifurcation, heart, spleen, liver – mid right lobe, stomach, kidney, duodenum, mesenteric LN, jejunum, ileum, ileocaecal LN, spiral colon, caecum, rectum and any visible lesions.

3.8 Collection of oral fluids using cotton ropes as a sampling method to detect FMDV in pigs

3.8.1 Experimental design

Cotton ropes were used to examine its use as a diagnostic tool for FMDV in pigs. These ropes were used to collect oral fluids during the pig infection experiments described in 3.1 and 3.2. Separate ropes were used for each group and were collected daily. Saliva samples were collected daily from the buccal cavity of each pig using sterile cotton swabs. RT-qPCR was performed and an internal 18S RNA control was used. Quantitative PCR was done on the saliva samples.

3.8.2 Statistical analysis

The results of the rope samples for each group were compared with the combined daily results of the saliva swabs for the same group. A group of animals were deemed positive for virus RNA in the saliva swabs if at least one of the animals in the group was positive. The group estimates for the rope and saliva samples were used to calculate prevalence of disease, test sensitivity and specificity, predictive values (probabilities for true positive, true negative, false positive, and false negative) and positive and negative likelihood ratios and their 95% confidence intervals using a 2x2 contingency table. The agreement between the two sampling procedures was compared using Kappa statistics (κ).

4 Results and discussion

4.1 Efficacy of a high potency O1 Manisa monovalent vaccine against heterologous challenge with a FMDV O Mya98 lineage virus in pigs 4 and 7 days post vaccination

4.1.1 Vaccine efficacy trial

The virus was successfully adapted to pigs after 3 passes and used to infect the 5 vaccinated pigs in each of groups 7 and 4 dpv (O-V7 and O-V4) and the unvaccinated group O-UV.

All animals in group O-UV showed generalised disease within 48–72 hours post-challenge. One pig died 2 dpc and necropsy showed infarction in the epicardium, referred to as 'Tiger Heart', a syndrome previously described for FMDV infection. The other four animals showed lesions on all four feet, snout, lower lip and tongue between 2 and 4 dpc. On 9 dpc the pigs breached the steel wall and a small hole was created where pigs from groups O-UV and O-UVC could have direct contact. None of the contact animals in group O-UVC showed disease until 13 dpc when one pig showed generalised disease, with lesions on the feet and tongue, and was subsequently removed. At 14 dpc, necropsy examination revealed heart lesions on another pig, though no other lesions were observed. The other three animals remained clinically normal up to 14 dpc when the experiment was terminated.

Group O-V7, which was challenged at 7 dpv, included one pig that showed generalised disease 2 dpc with secondary lesions on all 3 feet other than the site of inoculation. No lesions were noticed in the mouth, tongue and snout. One animal showed lines of infarction on the heart musculature at necropsy at 14 dpc. None of the contact pigs (O-UV7) showed any lesions.

One pig in group O-V4, which was challenged at 4 dpv, demonstrated generalised disease with secondary lesions developing on feet other than the site of inoculation and a lesion on the lower lip 3–4 dpc. Another pigs had a lesion on the tongue 4 dpc that had burst by 5 dpc and then healed. No lesions were observed on the feet or snout. All pigs remained clinically negative in group O-UVC4.

4.1.2 Quantitation of FMDV RNA by RT-qPCR from nasal swab samples

The nasal swabs indicated that viral RNA was present in the infected animals of groups O-UV and O-V7 as early as 1 dpc. One pig in O-UVC4 was also positive on that day. All the animals of group O-V4 were positive 2 dpc while the in-contact pigs for this group (O-UVC4) were positive at 4 dpc. No viral RNA was detected in the nasal swabs from groups O-UVC7 and in O-UVC only at 14 dpc. Viral RNA was detected in the nasal swabs from the other groups up to day 14 when the experiment was terminated. The mean copy numbers/swab of FMDV genome in nasal secretion is shown in Figure 4.1.1.

4.1.3 Quantitation of FMDV RNA by RT-qPCR from saliva swab samples

Groups O-UV and O-V7 had viral RNA in saliva at 1 dpc, followed by group O-V4 at 2 dpc. At 4 dpc RNA could be detected in all groups except O-UVC7 that never showed any RNA throughout the experiment. From 5 dpc the recovery of RNA was not consistent, especially in O-UVC4 where positive animals were found only on 4, 10, 11, 13 and 14 dpc. Given that these pigs never showed clinical disease, it

probably indicates that virus excretion was at very low levels. Figure 4.1.2 shows the mean copy numbers/swab of FMDV genome in saliva.

.

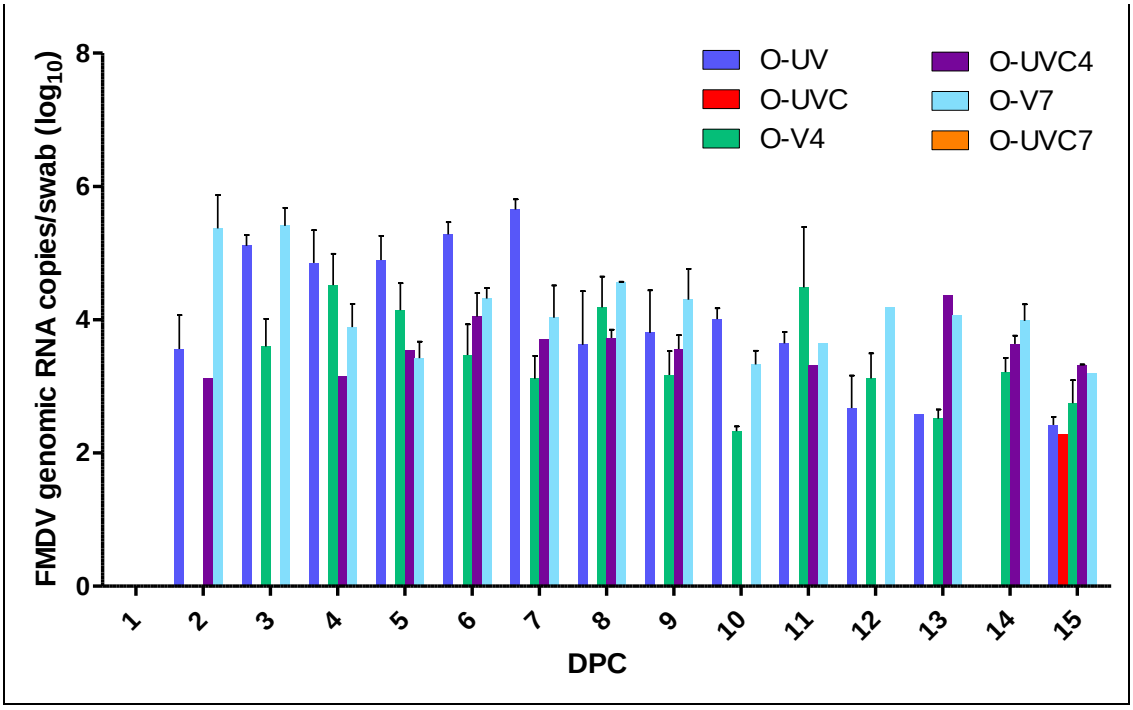

Figure 4.1.1 Mean FMDV RNA copies per group per day in nasal swabs

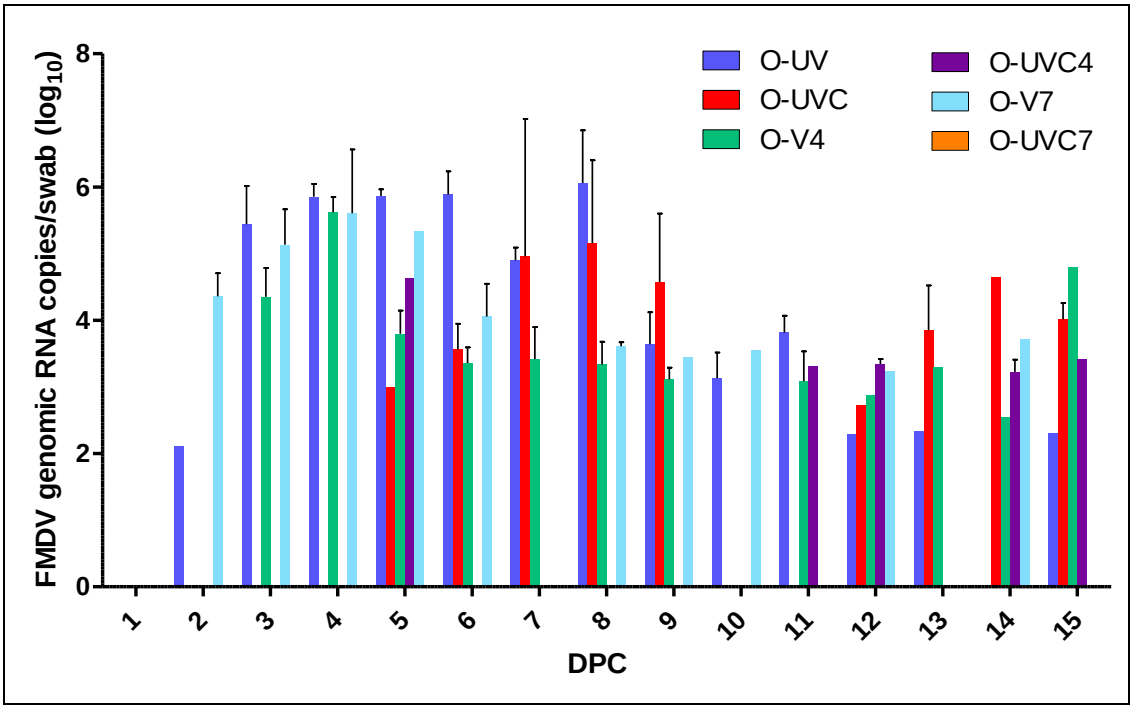

Figure 4.1.2 Mean FMDV RNA copies per group per day in saliva swabs

4.1.4 Antibody response to FMDV structural proteins

Bleeding was staggered between the different groups (see 3.1.2). All the pigs in all groups were sero-negative on the day of challenge, except 2 pigs that were vaccinated 7 days prior to challenge (O-V7) (Table 4.1.1). At 5 dpc, all 5 pigs in O-V7 and O-V4 had sero-converted including 1 pig in group O-UV that had not been vaccinated, but was challenged. By 10 dpc, all the challenged pigs in all 3 groups were sero-positive. The contact animals were bled at 0, 7, 10 and 14 dpc and no sero-conversion was observed until 10 dpc when a single pig in group O-UVC, in contact with the unvaccinated and infected group, had antibodies that lasted to 14 dpc (Table 4.1.1).

4.1.5 Antibody response to FMDV non-structural proteins

None of the pigs had antibodies to the non-structural proteins until 10 dpc when all 4 surviving pigs in group O-UV were positive and remained so until 14 dpc (Table 4.1.1). The contact pig in group O-UVC that had antibodies to the structural proteins, also showed antibodies to the non-structural proteins at 14 dpc. One pig in group O-V4 was sero-positive on both 10 and 14 dpc, while 2 pigs in O-V7 were sero-positive only on 10 dpc.

4.1.6 Conclusions

Upon virulent challenge, 80% of vaccinated pigs in groups O-V7 and 60% of the pigs in group O-V4 were protected. Vaccine-induced antibodies were detected in 2/5 pigs 7 dpv in group O-V7 and in none of the pigs vaccinated 4 days prior to challenge. By 5 dpc, all the vaccinated pigs had sero-converted, indicating an anamnestic response. Transmission did not seem to occur between pigs in groups O-V7/O-UVC7 and O-V4/O-UVC4, respectively, based on clinical evidence that was

confirmed by the absence of antibodies to both the structural and NSP. However, viral RNA could be detected in saliva and nasal swabs of pigs in O-UVC4 between 1 and 4 dpc, but was never detected in group O-V7. Live virus was present in group O-UVC4 between 4 and 14 dpc while no virus was isolated in group O-UVC7. It seems therefore that the contact pigs had been exposed to such low levels of virus that infection did not occur, presumably since the minimum infectious dose was not present. Therefore, vaccination prevented the spread of disease when pigs were kept in close contact with vaccinated and infected pigs presumably by lowering the amount of virus in the room.

One animal in the O-V4 group, that had generalised disease on 3 dpc, had detectable NSP antibodies, confirming infection. Two pigs in group O-V7 had lesions only at the site of inoculation but not generalised disease and were sero-positive for NSP antibodies on one occasion only (10 dpc). This probably indicates a transient viraemia that was not sufficient to induce high levels of antibodies.

Limited transmission occurred between pigs in groups O-UV / O-UVC seemingly only after the partition was breached at 9 dpc and the pigs came in close contact. However, viral RNA could be detected in saliva swabs in group O-UVC from 4 dpc onwards and in nasal swabs only on 14 dpc, indicating that viral RNA was present in the absence of infection. Virus could be consistently isolated from either saliva or nasal swabs 2–13 dpc in group O-UV and intermittently between 4 and 14 dpc in group O-UVC (results not shown) indicating that there was sufficient exposure to the in-contact animals during the peak virus shedding period in the O-UV group. It is therefore not clear whether the infection observed at 13 dpc was due to the close contact or whether the clinical disease was delayed due to low levels of virus present in the O-UVC group. One pig in the O-UVC group, that did not show any clinical disease, was sero-positive on 14 dpc for NSP antibodies and on 10–14 dpc for antibodies against structural proteins.

The RT-qPCR results showed there was comparatively less virus excretion in the vaccine groups (O-V4 and O-V7) when compared to the unvaccinated group O-UV ($P<0.05$). Peak virus excretion ($<10^5$ copy numbers/swab) in saliva was noticed between 2 and 7 dpc in the O-UV group while it was restricted to 2–3 dpc in the O-V4 and O-V7 groups. Similarly, peak RNA excretion ($<10^5$ copy numbers/swab) was detected in nasal secretions between 2 and 6 dpc in the O-UV group while it was restricted to 3 and 10 dpc in O-V4 and on 1 and 2 dpc in O-V7. On all the other days the amount of virus excreted in the challenged group was between 10^3–10^5 copy numbers /swab.

Table 4.1.1 Detection of antibodies to FMDV structural proteins (SP) and non-structural proteins (NSP) by ELISA

Group	Pig ID	SP antibody ELISA					NSP antibody ELISA				
		0 dpc	5 dpc	7 dpc	10 dpc	14 dpc	0 dpc	5 dpc	7 dpc	10 dpc	14 dpc
O-UV	1#1	-	-		Pos	Pos	-	-		Pos	Pos
	2#1	-	Pos		Pos	Pos	-	-		Pos	Pos
	3#2	-	-		Pos	Pos	-	-		Pos	Pos
	4#3	-	-		Pos	Pos	-	-		Pos	Pos
	5#4	-	Dea		Dea	Dea	-	Dea	Dea	Dea	Dea
O-UVC	6#1	-		-	-	-	-		-	-	-
	7#2	-		-	Pos	Pos	-		-	-	Pos
	8#2	-		-	-	-	-		-	-	-
	9#4	-		-	-	-	-		-	-	-
	10#	-		-	NS	-	-		-	NS	-
O-V4	11#	-	Pos		Pos	Pos	-	-		-	-
	12#	-	Pos		Pos	Pos	-	-		Pos	Pos
	13#	-	Pos		Pos	Pos	-	-		-	-
	14#	-	Pos		Pos	Pos	-	-		-	-
	15#	-	Pos		Pos	Pos	-	-		-	-
O-UVC4	16#	-		-	-	-	-		-	-	-
	17#	-		-	-	-	-		-	-	-
	18#	-		-	-	-	-		-	-	-
	19#	-		-	-	-	-		-	-	-
	20#	-		-	-	-	-		-	-	-
O-V7	21#	-	Pos		Pos	Pos	-	-		-	-
	22#	-	Pos		Pos	Pos	-	-		-	-
	23#	Pos	Pos		Pos	Pos	-	-		-	-
	24#	Pos	Pos		Pos	Pos	-	-		Pos	-
	25#	-	Pos		Pos	Pos	-	-		Pos	-
O-UVC7	26#	-		-	-	-	-		-	-	-
	27#	-		-	-	-	-		-	-	-
	28#	-		-	-	-	-		-	-	-
	29#	-		-	-	-	-		-	-	-
	30#	-		-	-	-	-		-	-	-

(-) – Negative; Pos – Positive; NS – not sampled

4.2 Efficacy of high potency A Malaysia 97 monovalent vaccine against heterologous challenge with a A/VIT/2005 SEA-97 FMDV lineage virus in pigs 4 and 7 days post vaccination

4.2.1 Adaptation and titration of A/VIT/2005 virus in pigs

A/VIT/2005 virus was successfully adapted to pigs within 2 passes in pigs. Titration in pigs showed that the titre was $>10^6$ pig ID_{50}/ml.

4.2.2 Vaccine efficacy trial

Seven of the eight unvaccinated and infected pigs in group A-UV showed generalised disease within 72–96 hours post-challenge while one pig showed generalized disease between 9–108 hours post-challenge. All 8 pigs showed lesions on all four feet, snout, lower lip and tongue. None of the five contact animals in group A-UVC showed disease up to 14 days post-exposure.

In the vaccine group A-V7, that was challenged 7 dpv, two animals showed generalised disease 5 and 7 dpc, respectively, with secondary lesions on the coronary band in addition to the site of inoculation. No lesions were observed in the mouth, tongue or snout. The five indirect contact pigs remained normal.

None of the eight challenged pigs in the vaccine group A-V4, challenged 4 dpv showed generalised disease, with lesions only at the site of inoculation. None of the five indirect contact pigs showed lesions or elevated temperatures.

4.2.3 Antibody response against FMDV non-structural proteins

Six of the 8 unvaccinated and infected pigs (A-UV) sero-converted to NSP by 10 dpi, while the remaining 2 pigs remained negative (Table 4.2.1). Two pigs tested positive on 7, 10 and 14 dpc, but 4 pigs had variable results, negative at some time points then returning to positive. None of the in-contact animals in group A-UVC demonstrated detectable antibodies.

In group A-V4, one pig sero-converted at 10 dpc and another at 14 dpc, while their cohorts remained negative. In the contact group A-UVC4, none of the pigs were sero-positive. No pig in either group A-V7 or A-UVC7 showed sero-conversion.

4.2.4 Quantitation of FMDV RNA by RT-qPCR from saliva swab samples

The mean RNA measured by RT-qPCR was determined per group per day after needle challenge. RNA was detected in the saliva samples from the unvaccinated and challenged pigs (A-UV) from 2–8 dpi with a peak at 6 dpi (Figure 4.2.1). RNA was only detected at 3 and 7 dpc in group A-V4 and 3–7 dpc in group A-V7, at significantly lower levels than for group A-UV. No RNA was detected in the contact groups.

Table 4.2.1 Detection of antibodies to FMDV NSPs by ELISA

Groups	Pig ID	0 DPC	3 DPC	5 DPC	7 DPC	10 DPC	14 DPC
A-UV	#6	-	-	-	-	-	-
	#12	-	-	-	Pos	Pos	Pos
	#18	-	-	-	Pos	Pos	-
	#21	-	-	-	Pos	-	Pos
	#23	-	-	-	Pos	-	Pos
	#26	-	-	-	Pos	Pos	Pos
	#32	-	-	-	Pos	-	-
	#39	-	-	-	-	-	-
A-UVC	5 pigs	-	NS	-	-	-	-
A-V4	#9	-	-	-	-	-	-
	#12	-	-	-	-	Pos	Pos
	#39	-	-	-	-	-	Pos
	#42	-	-	-	-	-	-
	#44	-	-	-	-	-	-
	#47	-	-	-	-	-	-
	#56	-	-	-	-	-	-
	#57	-	-	-	-	-	-
A-UVC4	5 pigs	-	NS	-	-	-	-
A-V7	8 pigs	-	-	-	-	-	-
A-UVC7	5 pigs	-	NS	-	-	-	-

(-) – Negative; Pos – Positive; NS – not sampled

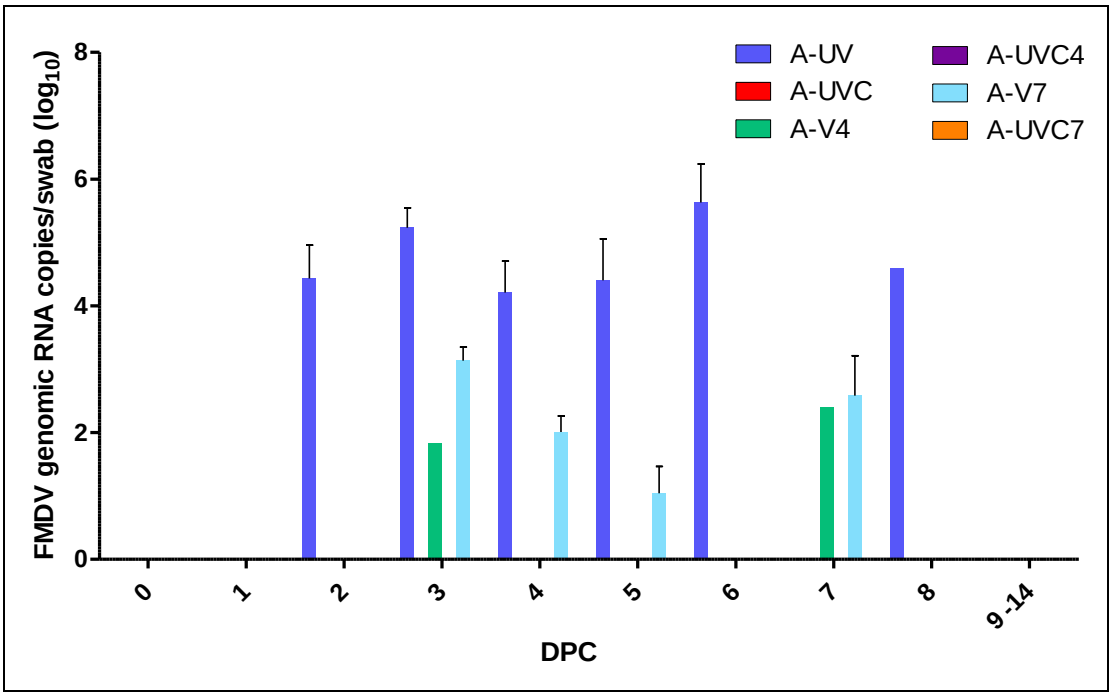

Figure 4.2.1 Mean FMDV RNA copies per group per day in saliva swabs

4.2.5 Quantitation of FMDV RNA by RT-qPCR from nasal swab samples

In group A-UV, RNA was detected in the nasal swabs between 2 and 6 dpc with a peak at 3 dpc and then again at 9 and 14 dpc, but at significantly lower levels compared to saliva samples (Figure 4.2.2). Viral RNA was not detected in any of the contact animals (A-UCV). Low levels of RNA were present at 3 and 4 dpc in group A-V4, while in group A-UVC4 RNA was detected from 3–5, 7–8 and 10 dpc. The animals that were vaccinated 7 days prior to challenge demonstrated RNA 4–8 dpc with a peak at 6 dpc while the contact group A-UVC7 remained negative.

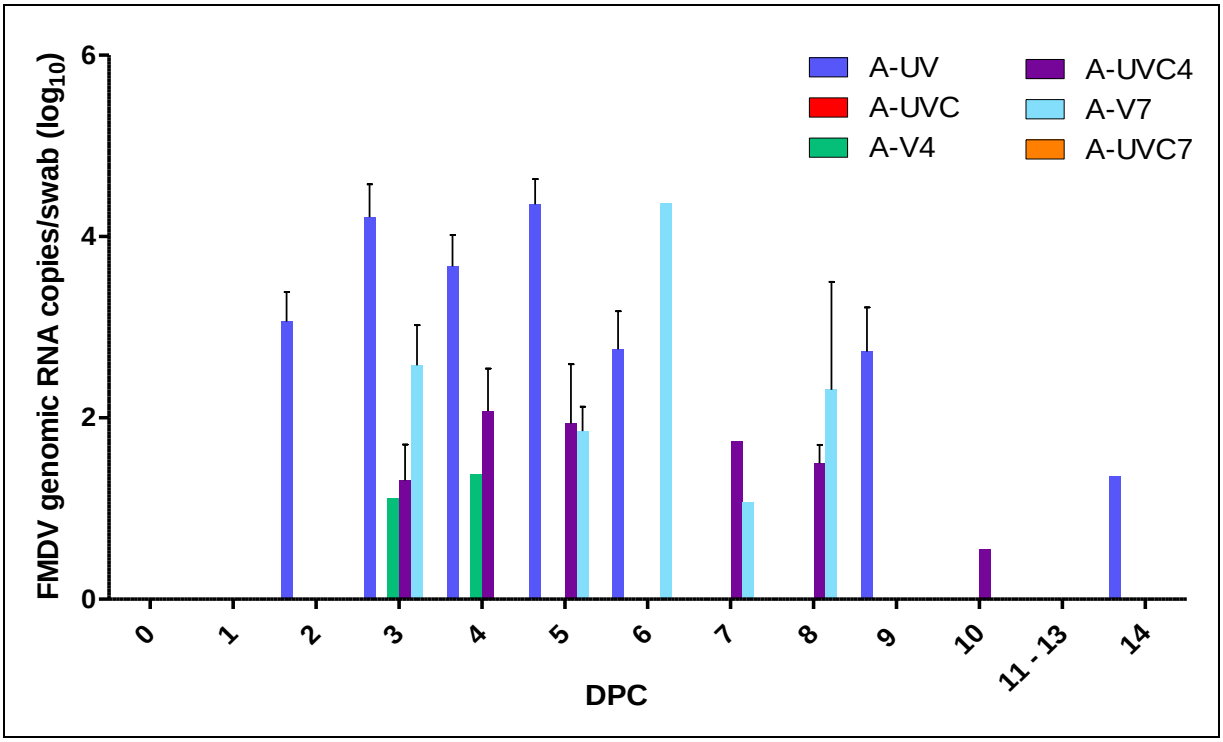

Figure 4.2.2 Mean FMDV RNA copies per group per day in nasal swabs

4.2.6 Quantitation of FMDV RNA by RT-qPCR from faecal swab samples

Very low levels of RNA were detected in the faecal swabs of group A-UV on 2 and 8 dpc only (Figure 4.2.3). The contact animals (A-UCV) did not show any detectable levels of RNA. Low levels of RNA were present between at 3 and 5 dpc in group A-V7, while in groups A-V4, A-UVC4 and A-UVC7 no RNA was detected.

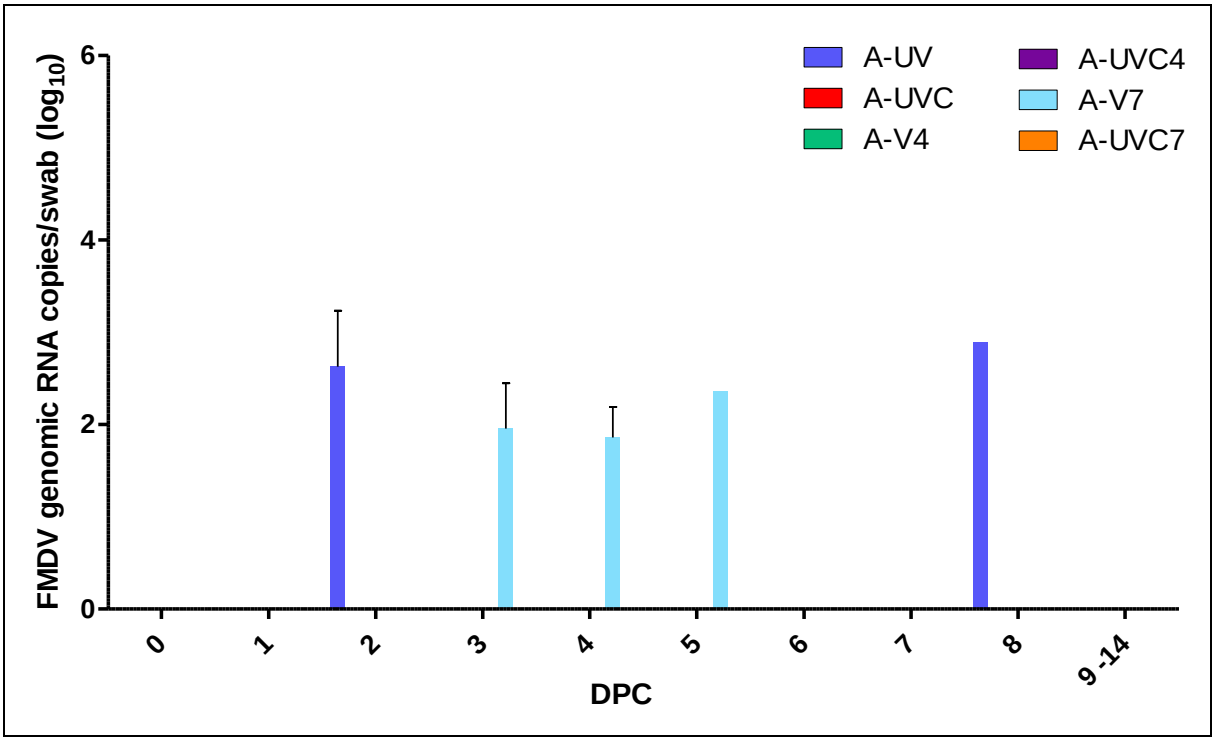

Figure 4.2.3 Mean FMDV RNA copies per group per day in faecal swabs

4.2.7 Conclusions

The vaccine protection was 75% for the pigs that were vaccinated 7 days prior to challenge and 100% in those vaccinated 4 days before challenge. Vaccination significantly decreased the amount of RNA detected in swabs when compared to unvaccinated pigs. The levels of RNA in the blood and virus isolation still need to be performed.

Although no disease was observed in the pigs vaccinated 4 days before challenge, 2 of the pigs sero-converted to NSP at 10 and 14 dpc, respectively. None of the pigs that were challenged at 7 dpv sero-converted, despite the observation of clinical disease in 2 of the pigs. These tests also need to be repeated.

Strict bio-security measures were sufficient to ensure the disease did not spread to the contact animals that shared a room with the challenged animals. There was no clinical disease in any of the contact groups and none of the contact pigs sero-converted to NSP. No RNA could be detected in the saliva samples in any of the contact pigs, but RNA was found in the nasal swabs of group A-UVC4.

4.3 Comparison of the potency of O1 Manisa and O/SKR/2010 high payload vaccines in cattle using O/SKR/2010 as challenge virus

4.3.1 Vaccine potency studies

Cattle were vaccinated with 3 different doses of O/SKR/2010 (Mya98) vaccine and challenged 21 days later with O/SKR/2010 cattle challenge virus. None of the cattle that received a full dose showed any clinical lesions up to 8 dpc, while 2 of those vaccinated with ¼ dose and 3 of those with 1/16 dose demonstrated disseminated disease. This resulted in a PD_{50} = 7.94.

In the heterologous challenge, cattle were vaccinated with O1 Manisa vaccine and challenged with O/SKR/2010. As with the homologous challenge, all 5 cattle that received a full dose were protected, however, 3 of those that received the ¼ and all 5 that received the 1/16 dose had clinical signs indicating FMD infection resulting in a PD_{50} of 3.47.

4.3.2 Serology for antibody response towards FMDV structural proteins

On the day of challenge (21 dpv), the average homologous serum antibody titres (95% CI) by VNT for the O1 Manisa and O/SKR/2010 vaccinated animals were 2.08 (1.81-2.35) and 2.05 (1.61-2.48) for the full dose groups, 1.41 (1.13-1.70),1.80 (1.53-2.07) for the ¼ dose groups and 1.14 (0.87-1.41) and 1.74 (1.54-1.95) for the 1/16 dose groups (Figure 4.3.1). The average heterologous serum antibody titres for the O1 Manisa and O/SKR/2010 vaccinated animals were 1.93 (1.731-2.12) and 1.68 (1.40-1.97) for the full dose groups, 1.35 (1.10-1.60) and 1.35 (1.22-1.48) for the ¼ dose groups and 1.05 (0.89-1.20) and 1.35 (1.03-1.68) for the 1/16 dose groups (Figure 4.3.1).

The overall difference between the homologous and heterologous titres in O1 Manisa vaccinated animals was significant ($P<0.05$; $p=0.02$) and the difference in the O/SKR/2010 vaccinated animals was highly significant ($P<0.01$; $P=0.0006$). However, the homologous and heterologous titres obtained when testing sera obtained from the O1 Manisa vaccinated animals that received a full dose of the vaccine, did not differ significantly ($P>0.05$; $p=0.15$) while the titres of O/SKR/2010 vaccinated animals did ($P<0.05$; $p=0.03$). This indicates that the O1 Manisa vaccine would offer protection in cattle when employed as an emergency vaccine during an O/SKR/2010 virus like outbreaks. The estimated 'r1' values for O/SKR/2010 vs. O1 Manisa vaccine were in the range 0.70 to <1.00 (calculated from the antibody titres in animals that received full dose only).

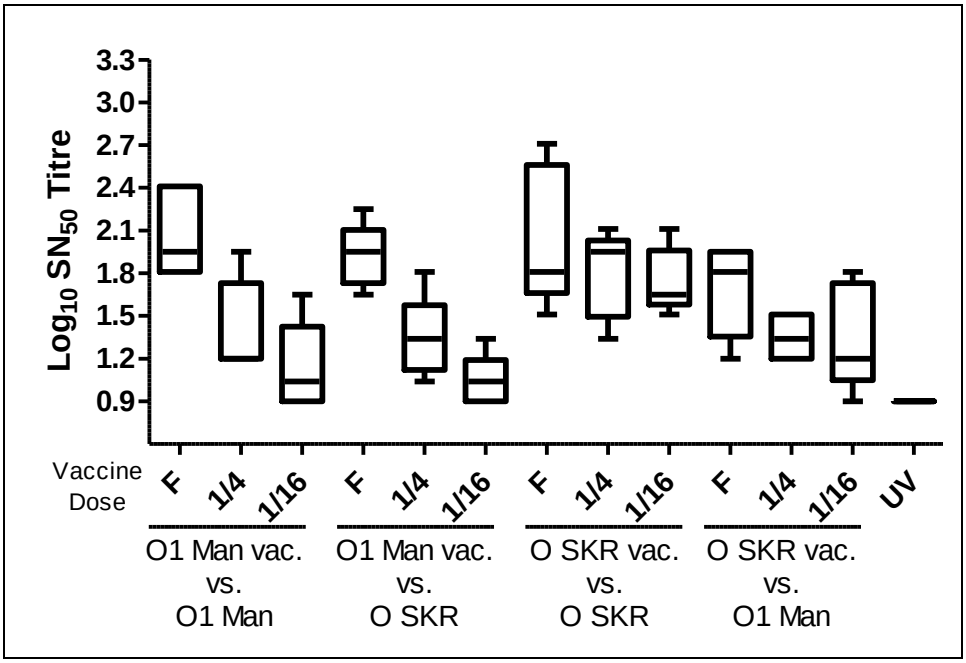

Figure 4.3.1 Average homologous and heterologous serum antibody titres determined by VNT.
F –Full dose; ¼–one-fourth dose; 1/16–one-sixteenth dose; UV–unvaccinated

4.3.3 Antibody response to the FMDV non-structural proteins

Sero-conversion to NSP was observed 5 dpc in one animal vaccinated with a ¼ dose of the O1 Manisa vaccine, and by 6 dpc, a number of cattle in each group demonstrated antibodies; 2 that had received the full dose, 2 of the ¼ dose and 3 of the 1/16 dose groups. All the animals were sero-positive by 7 dpc for the duration of the study (Table 4.3.1).

In the group that was vaccinated with 1/16 dose of the O/SKR/2010 vaccine, 1 animal was sero-positive to NSP 4 dpc followed by its cohorts at 6–8 dpc. The group that had received the full dose of vaccine sero-converted between 6–8 dpc, but one animal was positive only at 8 dpc after which it became negative again, possibly indicating a low level of virus circulation. Of the 5 animals that were vaccinated with ¼ dose, one never sero-converted, while the others were sero-positive between 6-7 dpc. The unvaccinated and challenged cattle sero-converted between 6 and 7 dpc and remained positive until termination.

Table 4.3.1 Results of FMDV NSP antibody testing using the PrioCHECK® FMDV-NS kit

Vaccine	Dose	Animal ID	0 dpv	0 dpc	1–3 dpc	4 dpc	5 dpc	6 dpc	7 dpc	8 dpc	11 dpc	14 dpc
O1 Manisa vaccine	Full Dose	FMD 182	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
		FMD 183	-	-	-	-	-	-	Pos	Pos	Pos	Pos
		FMD 184	-	-	-	-	-	-	Pos	Pos	Pos	Pos
		FMD 185	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
		FMD 186	-	-	-	-	-	-	Pos	Pos	Pos	Pos
	1/4 dose	FMD 187	-	-	-	-	-	Pos	Pos	Pos	Pos	NS
		FMD 188	-	-	-	-	-	-	Pos	Pos	Pos	NS
		FMD 189	-	-	-	-	-	-	Pos	Pos	Pos	NS
		FMD 190	-	-	-	-	Pos	Pos	Pos	Pos	Pos	NS
		FMD 191	-	-	-	-	-	-	Pos	Pos	Pos	NS
	1/16 dose	FMD 192	-	-	-	-	-	-	Pos	Pos	Pos	NS
		FMD 193	-	-	-	-	-	Pos	Pos	Pos	Pos	NS
		FMD 194	-	-	-	-	-	-	Pos	Pos	Pos	NS
		FMD 195	-	-	-	-	-	Pos	Pos	Pos	Pos	NS
		FMD 196	-	-	-	-	-	Pos	Pos	Pos	Pos	NS
O/SKR/ 2010 vaccine	Full Dose	FMD 197	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
		FMD 198	-	-	-	-	-	-	-	Pos	-	-
		FMD 199	-	-	-	-	-	-	Pos	Pos	Pos	Pos
		FMD 200	-	-	-	-	-	-	Pos	Pos	Pos	Pos
		FMD 201	-	-	-	-	-	-	-	Pos	Pos	Pos
	1/4 dose	FMD 202	-	-	-	-	-	Pos	Pos	Pos	Pos	NS
		FMD 203	-	-	-	-	-	Pos	Pos	Pos	Pos	NS
		FMD 204	-	-	-	-	-	-	Pos	Pos	Pos	NS
		FMD 205	-	-	-	-	-	-	Pos	Pos	Pos	NS
		FMD 206	-	-	-	-	-	-	-	-	-	NS
	1/16 dose	FMD 207	-	-	-	-	-	-	Pos	Pos	Pos	NS
		FMD 208	-	-	-	-	-	-	Pos	Pos	Pos	NS

		FMD 209	-	-	-	-	-	-	-	Pos	Pos	NS
		FMD 210	-	-	-	Pos	Pos	Pos	Pos	Pos	Pos	NS
		FMD 211	-	-	-	-	-	Pos	Pos	Pos	Pos	NS
No Vaccine	No dose	FMD 212	-	-	-	-	-	-	Pos	Pos	Pos	Pos
		FMD 213	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
		FMD 214	-	-	-	-	-	-	Pos	Pos	Pos	Pos

(-) – Negative; Pos – Positive; NS – not sampled

4.3.4 Quantitation of FMDV RNA by RT-qPCR in serum samples

No viral RNA was detected post-challenge in the serum samples collected from animals that were administered O1 Manisa neat vaccine. However, RNA was present 1–3 dpc in all cattle that had been vaccinated with 1/16 dose, with RNA detected in one animal until 4 dpc. Three of the cattle that had received ¼ dose had viral RNA in the serum between 1 and 3 dpc and one animal was positive only at 3 dpc.

In contrast, 1 animal that had received the full dose of O/SKR/2010 vaccine had viral RNA present 4 dpc, 1 animal in the ¼ dose was positive on 3–4 dpc and 2 in the 1/16 dose group showed viral RNA 3 dpc. All the unvaccinated controls consistently showed presence of viral RNA from 1 to 3 dpc (Figure 4.3.2).

There was no significant difference between the levels of RNA detected in the serum samples between the two vaccine groups ($P>0.05$) throughout the experiment. However, there was a significant difference between the amounts of viral RNA detected in the serum of the vaccine groups and the control groups ($P<0.05$) on all days of sampling. The levels of virus replication in the ¼ and 1/16 groups, though not significantly different from the full dose groups ($P<0.05$), was sufficient to produce clinical disease in 3 out of 5 animals and 2 out of 5 animals in the O1 Manisa and O/SKR/2010 vaccine groups, respectively.

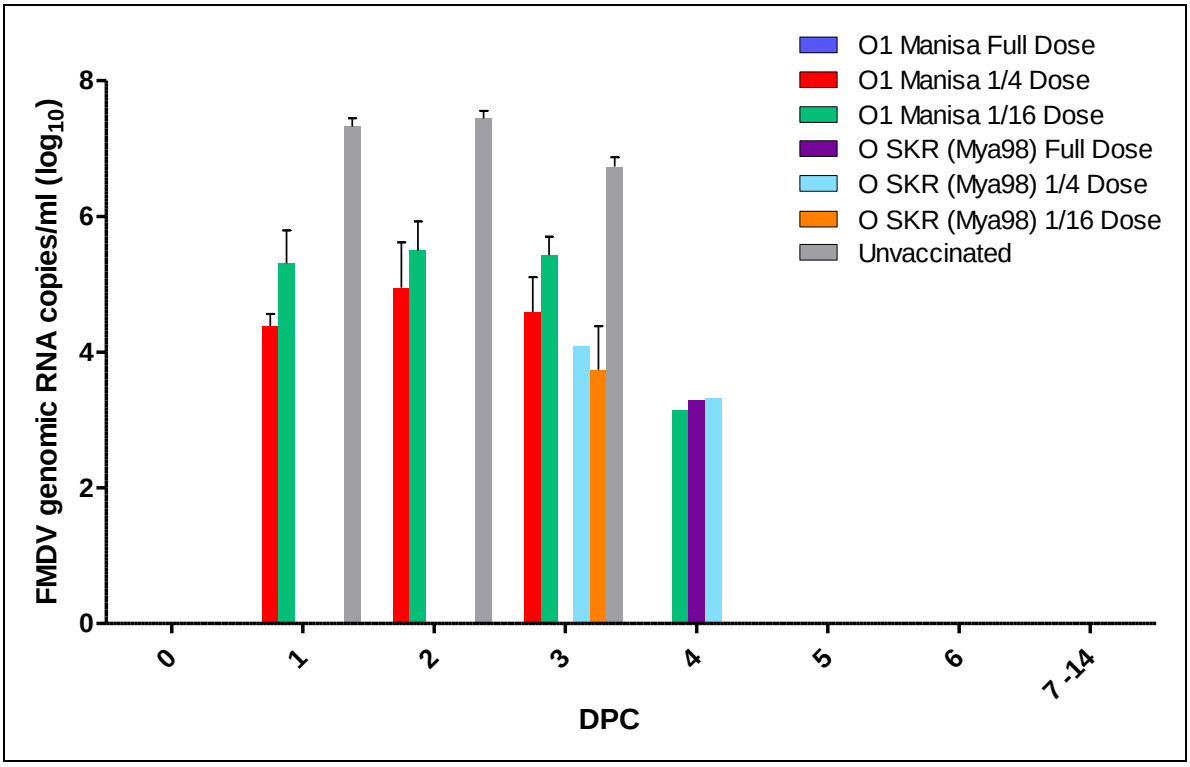

Figure 4.3.2 Mean FMDV RNA in serum (copies per ml) from vaccinated and challenged cattle calves

4.3.5 Quantitation of FMDV RNA by RT-qPCR in saliva swab samples

Most of the cattle vaccinated with different doses of O1 Manisa demonstrated viral RNA consistently between 1 and 7 dpc, with the mean on 1 dpc significantly lower in the O/SKR/2010 full dose group compared to all other groups. Three of the cattle from the 1/16 dose group were negative from 6 dpc. Surprisingly, RNA was less frequently detected in the group that was vaccinated with ¼ dose of O/SKR/2010 with 1 animal showing detectable levels only at 5–6 dpc and one other only at 1, 5 and 6 dpc. All the unvaccinated cattle had viral RNA between 1 and 7 dpc (Figure 4.3.3).

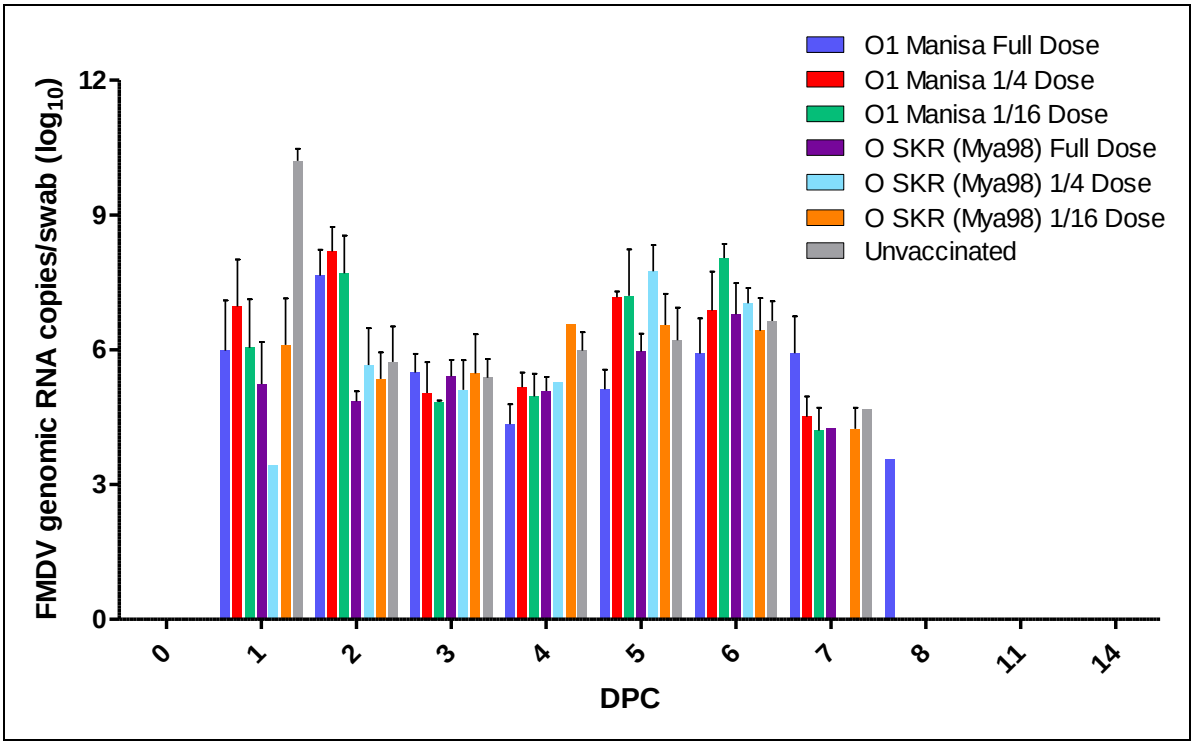

Figure 4.3.3 Mean FMDV RNA in saliva swabs (copies per swab) of vaccinated and challenged cattle calves

4.3.6 Quantitation of FMDV RNA by RT-qPCR in probang samples

Probangs were taken every second day until 8 dpc, then again at 11 and 14 dpc. All the animals showed a decreasing level of viral RNA from 2 dpc onwards where the unvaccinated controls were negative from 8 dpc. Viral RNA was detected in the probangs of the vaccinated and infected cattle between 2 and 6 dpc with intermittent results at 8 dpc, except one animal that had received the full dose of O/SKR/2010 that was negative from 6 dpc. At 8 dpc, viral RNA was found in 10/15 cattle vaccinated with O1 Manisa and 9/15 vaccinated with O/SKR/2010. At 11 and 14 dpc, virus RNA was found in only 1 animal vaccinated with O1 Manisa on each sampling day, while viral RNA was detected in 4 cattle at 11 dpc and 1 at 14 dpc in the groups vaccinated with O/SKR/2010 (Figure 4.3.4).

There was no significant difference between the levels of RNA detected in the probang samples between the two vaccine groups ($P>0.05$) and between the vaccine and the control groups ($P<0.05$) on all days of sampling.

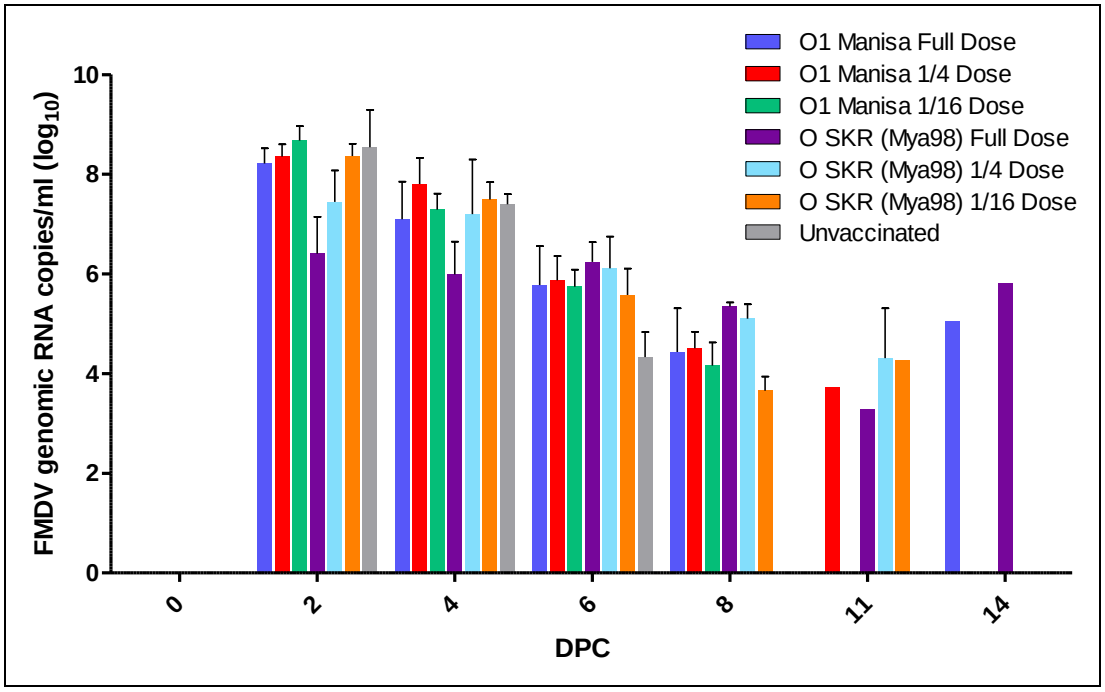

Figure 4.3.4 Mean FMDV RNA in probang samples (copies per ml) of vaccinated and challenged cattle calves

4.3.7 Conclusions

Both vaccines protected all five cattle against challenge at the full dose (>6PD_{50}). The high potency O1 Manisa vaccine was found to be efficacious in the field during the 2010 outbreaks of serotype O in South Korea and assisted in controlling the disease. However, O1 Manisa had a lower potency (PD_{50} = 3.47) compared to the O/SKR/2010 vaccine (7.94), as could be expected from a heterologous vs. homologous challenge. This implies that the new O/SKR/2010 vaccine should be efficacious in South Korea at a lower potency and subsequent cost, but is most likely not cost-effective for vaccine manufacturers due to the cost of up-scaling and registering a new product in the face of an efficacious vaccine.

With the limited analysis done so far, there were no significant differences in the RNA levels in saliva, blood and probangs between the two vaccine groups. However, vaccination significantly lowered the mean viral RNA levels compared to the unvaccinated controls, even in the groups where animals showed clinical disease. It seems therefore that vaccination could reduce the overall viral load and assist with disease control by preventing large-scale contamination of infected premises.

Surprisingly, none of the unvaccinated controls had detectable viral RNA in their probangs 6 dpc, compared to the vaccinated groups where RNA was detected in the groups that had received the full dose of either vaccine. The significance of this finding needs to be clarified by virus isolation to confirm that this is infectious virus.

The homologous titres for both vaccine groups did not show any significant differences. Based on the heterologous titres, the calculated r1-value indicated that O1 Manisa would be protective when challenged with the O/SKR/2010 virus, confirming the results of the efficacy study. In both groups, animals sero-converted to NSP indicating that vaccination did not prevent virus replication, although it did prevent clinical disease in a number of animals. It can be concluded that the high potency O1 Manisa vaccine should be applicable in Australia if a virus related to O/SKR/2010 should be introduced.

4.4 Comparison of the vaccine efficacy of O1 Manisa and O/SKR/2010 in pigs using O/SKR/2010 as challenge virus

4.4.1 Adaptation and titration of virus in pigs

The O/SKR/2010 virus was successfully adapted to pigs after 2 passes and produced generalised disease by 2 dpc in both pigs when used at either 10^4, 10^3 and 10^2 $TCID_{50}$/ml. It was therefore not possible to determine the titre, but due to the high virulence of the virus, it was decided to change the challenge dose from 10^4 $TCID_{50}$/ml (used when pigs were challenged 5 dpv) to 10^3 $TCID_{50}$/ml for the 21 dpv challenge.

4.4.2 Vaccine efficacy trial and revised vaccine efficacy trial

During the initial experiment, 5 pigs each were vaccinated with O1 Manisa and O/SKR/2010 vaccine respectively and challenged 5 dpv with 10^4 $TCID_{50}$/ml of O/SKR/2010. All pigs, including the unvaccinated controls, displayed generalised infection of FMD by 2 dpc and were euthanized.

The study was repeated as before, but pigs were challenged 21 dpv with 10^3 $TCID_{50}$/ml. Two of the 5 pigs vaccinated with O1 Manisa displayed generalised disease by 2 dpc and were removed from the experiment. Of the remaining 3 pigs, 2 displayed generalised disease by 3 dpc while the third did not show any signs of infection, including no signs of FMD at the site of inoculation. According to ethics requirements all 3 pigs were euthanized at 3 dpc.

Two of the 5 pigs vaccinated with the new O/SKR/2010 displayed generalised disease by 3 dpc and were euthanized. Two more showed disease only at the site of inoculation while the third showed no signs of infection, including the site of inoculation. All 3 pigs were monitored and remained healthy for the duration of the experiment until 10 dpc.

One unvaccinated control pig displayed generalised disease by 2 dpc followed by the second pig by 3 dpc when they were both euthanized.

4.4.3 Serological results of pigs challenged 5 and 21 dpv

None of the pigs challenged 5 dpv had measurable titres at the day of challenge (Table 4.4.1). The homologous VNT titres for pigs vaccinated with O/SKR/2010 21 dpv were >2 logs for all pigs, except 1 that showed a titre of 1.52. In contrast, only 2 pigs vaccinated with O1 Manisa had positive titres (1.91 and 1.76 respectively – Table 4.4.1) as determined in a homologous test. It was not clear whether these lower titres could be due to the vaccine formulation of the O1 Manisa vaccine. However, since the heterologous ELISA titres for 4/5 pigs ranged between 2.02 and 3.20 (one animal did not show an ELISA titre) it is also possible that the VNT was sub-optimal (Table 4.4.1). There was good correlation between neutralising titres and clinical outcome. All the pigs were negative for heterologous neutralising antibodies.

Table 4.4.1 Homologous and heterologous VNT titres and ELISA titres of sera from pigs vaccinated with either O1 Manisa or O/SKR/2010 and challenged with O/SKR/2010 5 dpv or 21 dpv

Pig No.	Vaccine	Pigs challenged with O/SKR/2010 5 dpv							
		VNT Titre to:				ELISA titre for Pig anti-FMDV IgG			
		O1 Manisa		O/SKR/2010		O1 Manisa		O/SKR/2010	
		-5 dpc	0 dpc	-5 dpc	0 dpc	-5 dpc	0 dpc	-5 dpc	0 dpc
1	O1 Manisa	<0.90*	<0.90	<0.90	<0.90	<1.20^	<1.20	<1.20	<1.20
2		<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	<1.20	<1.20
3		<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	<1.20	<1.20
4		<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	<1.20	<1.20
5		<0.90	0.90	<0.90	0.90	<1.20	1.20	<1.20	<1.20
6	O/SKR	<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	<1.20	<1.20
7		<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	<1.20	<1.20
8		<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	<1.20	<1.20
9		<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	<1.20	<1.20
10		<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	<1.20	<1.20
11	No Vaccine	<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	1.20	<1.20
12		<0.90	<0.90	<0.90	<0.90	<1.20	<1.20	1.34	<1.20
Pig No.	**Vaccine**	**Pigs challenged with O/SKR/2010 21 dpv**							
		VNT Titre to:				**ELISA titre for Pig anti-FMDV IgG**			
		O1 Manisa		**O/SKR/2010**		**O1 Manisa**		**O/SKR/2010**	
		-21 dpc	**0 dpc**	**-21 dpc**	**0 dpc**	**-21 dpc**	**0 dpc**	**-21 dpc**	**0 dpc**
1	O1 Manisa	<0.90*	1.16	<0.90	1.22	<1.20^	1.16	<0.90	2.02
2		<0.90	1.61	<0.90	0.92	<1.20	1.61	<0.90	2.56
3		<0.90	1.91	<0.90	1.37	<1.20	1.91	<0.90	2.51
4		<0.90	1.46	<0.90	1.22	<1.20	1.46	<0.90	3.20
5		<0.90	1.76	1.22	0.92	<1.20	1.76	1.22	1.62
6	O/SKR	<0.90	1.16	<0.90	2.42	<1.20	1.16	<0.90	2.26
7		<0.90	1.01	<0.90	2.12	<1.20	1.01	<0.90	2.50
8		<0.90	1.16	<0.90	2.27	<1.20	1.16	<0.90	2.16
9		<0.90	1.31	<0.90	2.27	<1.20	1.31	<0.90	2.04
10		<0.90	<0.90	<0.90	1.52	<1.20	<0.90	<0.90	2.04

*Log10 of reciprocal of highest dilution of serum that was able to neutralise either O1 Manisa or O/SKR/2010 virus

^Log10 of reciprocal of highest dilution of serum considered positive in the ELISA

– Positive

4.4.4 Conclusions

The challenge virus was successfully adapted to pigs over 2 passes to present reproducible infection in all pigs when infecting them via the footpad route. The virus

was highly virulent in pigs and caused disease at a dose of 10^2 $TCID_{50}$/ml. It is not clear whether the observed virulence had an impact on the vaccine challenge studies' outcomes.

All animals challenged 5 dpv displayed generalised disease by 2 dpc, suggesting neither vaccine offered protection against pig adapted O/SKR/2010 at 10^4 $TCID_{50}$/ml. As this could be due to the virus challenge dose being too high, it was decided to lower the challenge dose and increase the amount of time post vaccination to allow a comparison between the two vaccines. However, neither vaccine provided full protection with O1 Manisa providing protection to 20% of the pigs and O/SKR/2010 providing 60% protection 21 dpv with 10^3 $TCID_{50}$/ml. It is not clear why the vaccines that provided 100% protection to cattle at full dose (as was used in this study), failed to protect pigs. It seems therefore that the O1 Manisa vaccine will not fully protect pigs if an outbreak of a virus related to O/SKR/2010 should cause an outbreak in Australia.

4.5 Vaccine efficacy trials with high potency O1 Manisa monovalent vaccine against FMDV O/SKR/2010 (Mya-98 strain) in sheep 4 days post vaccination

4.5.1 Clinical signs

Sheep, vaccinated 4 days before challenge, were kept in contact with other sheep (0 dpc) that were infected via the CB 24 hours prior to contact (-1 dpc). All but two of the unvaccinated and infected donor sheep (Groups 1b and 2b) showed multiple lesions in the feet, mouth and tongue as early as 2 dpc up to 14 dpc. This resulted in continued challenge to the vaccine and control groups (1a and 2a). The vaccinated in-contact sheep (Group 1a) were all protected and did not show any FMD-specific lesions throughout the experiment. Of the unvaccinated in-contact sheep (Group 2a), 2/4 control sheep showed FMD lesions on 6 and 9 dpc while two sheep did not show any FMD-specific lesions. In one of the affected animals a lesion was present only in the mouth and no feet lesions were observed.

4.5.2 FMDV structural protein antibody levels

None of the vaccinated sheep (Group1a) showed antibodies 4 dpv, the day of contact (0 dpc; Table 4.5.1). Most of the vaccinated sheep were positive by 3 dpc (7 dpv) with the exception of 1 that became positive at 5 dpc (9 dpv) and 2 others that demonstrated antibodies at 6 dpc (10 dpv). No significant anamnestic response was observed in any of the sheep; however a small secondary increase in antibody levels was seen in 5 sheep after 14 dpc. Of the 4 unvaccinated contact control sheep in Group 2a, 3 had antibodies to the structural proteins from 7 dpc. One sheep tested positive from 14 dpc which suggested this animal was challenged by FMDV, despite the absence of antibodies to the NSP. One sheep never sero-converted. In Groups 1b and 2b, 1 of the unvaccinated and infected donor sheep did not produce antibodies to the structural proteins, suggesting this animal was not infected. The remaining sheep in this group were positive from 3 or 4 dpc and remained positive for the duration of the study.

Table 4.5.1 Detection of antibodies to FMDV structural proteins (SP) by ELISA

Group	Sheep ID	SP antibody ELISA									
		-8–1 dpc	2 dpc	3 dpc	4 dpc	5 dpc	6 dpc	7 dpc	10 dpc	14 dpc	21 dpc
1a VC	#1	-	-	Pos	Pos	Pos	Pos	Pos	Pos	-	-
	#2	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
	#3	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#4	-	-	-	-	Pos	Pos	Pos	Pos	Pos	Pos
	#5	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#6	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#7	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#8	-	-	-	-	-	Pos	Pos	-	-	-
1b UI	#9	-	-	-	-	-	-	-	-	-	-
	#10	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#11	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#12	-	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#13	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#14	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#15	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#16	-	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos
2a UC	#17	-	-	-	-	-	-	-	-	Pos	Pos
	#18	-	-	-	-	-	-	-	-	-	-
	#19	-	-	-	-	-	-	Pos	Pos	Pos	Pos
	#20	-	-	-	-	-	-	-	-	-	Pos
2b UI	#21	-	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#22	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#23	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	#24	-	-	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos

(-) – Negative; Pos – Positive; VC – vaccinated contact; UI – unvaccinated infected

4.5.3 FMDV non-structural protein antibody levels (NSP ELISA)

One sheep of the unvaccinated and infected donor sheep (Groups 1b and 2b) did not produce antibodies to FMDV NSP suggesting infection of this animal was unsuccessful. The remaining sheep were positive from 6 or 7 dpc suggesting infection of 11/12 donor sheep (Table 7.2). None of the animals in the vaccinated in-contact sheep (Group 1a) were FMDV NSP-antibody positive on any of the days tested. In the unvaccinated in-contact sheep (Group 2a) the 2 sheep in one room sero-converted at 14 and 21 dpc, respectively, while the 2 sheep in the other room remained negative (Table 4.5.2).

Table 4.5.2 Detection of antibodies to FMDV non-structural proteins (NSP) by ELISA

Group	Sheep ID	NSP antibody ELISA									
		-8–1 dpc	**2 dpc**	**3 dpc**	**4 dpc**	**5 dpc**	**6 dpc**	**7 dpc**	**10 dpc**	**14 dpc**	**21 dpc**
1a VC	#1	-	-	-	-	-	-	-	-	-	-
	#2	-	-	-	-	-	-	-	-	-	-
	#3	-	-	-	-	-	-	-	-	-	-
	#4	-	-	-	-	-	-	-	-	-	-
	#5	-	-	-	-	-	-	-	-	-	-
	#6	-	-	-	-	-	-	-	-	-	-
	#7	-	-	-	-	-	-	-	-	-	-
	#8	-	-	-	-	-	-	-	-	-	-
1b UI	#9	-	-	-	-	-	-	-	-	-	-
	#10	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
	#11	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
	#12	-	-	-	-	-	-	Pos	Pos	Pos	Pos
	#13	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
	#14	-	-	-	-	-	Pos	Pos	-	-	Pos
	#15	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
	#16	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
2a UC	#17	-	-	-	-	-	-	-	-	-	-
	#18	-	-	-	-	-	-	-	-	-	-
	#19	-	-	-	-	-	-	-	-	Pos	Pos
	#20	-	-	-	-	-	-	-	-	-	Pos
2b UI	#21	-	-	-	-	-	-	Pos	Pos	Pos	Pos
	#22	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
	#23	-	-	-	-	-	Pos	Pos	Pos	Pos	Pos
	#24	-	-	-	-	Pos	Pos	Pos	Pos	Pos	Pos

(-) – Negative; Pos – Positive; VC – vaccinated contact; UI – unvaccinated infected

4.5.4 Sample analysis by RT-qPCR

EDTA whole blood, oro-pharyngeal fluid (probang), nasal, oral and faecal swab samples were tested for the presence of FMDV genome copies by RT-qPCR. The presence of FMDV genome in probang at 28 dpc or later suggests persistent infection, although this still needs to be confirmed by virus isolation.

Unvaccinated and infected donor sheep (Groups 1b and 2b):

EDTA blood: Eight of the 12 donor sheep were viraemic (blood FMDV positive in RT-qPCR) at 0 dpc and a further 2 sheep by 1 dpc. Blood samples from 2 sheep remained negative (Figure 4.5.1).

Probang: Probang samples from 9 sheep were RT-qPCR positive at 7 dpc (8 dpi) and of these, 6 remained positive after 28 dpc, suggesting these animals may be persistently infected (Figure 4.5.2). Two sheep were not positive at any stage.

Nasal Swabs: FMDV RNA was detected in nasal swabs from 9 sheep from as early as 0 dpc. No animals were positive after 5 dpc, with the exception of 1 sheep, which was positive at 10 and 14 dpc. Three sheep never had a positive result (Figure 4.5.3).

Oral Swabs: Eleven of the 12 sheep had FMDV positive oral swabs from as early as 0 dpc, while the last never tested positive. No sheep were positive between 6 dpc and 8 dpc. Swabs tested intermittently positive in 5 sheep up to 28 dpc (Figure 4.5.4).

Faecal Swabs: None of the sheep were positive at any time point.

Vaccinated in-contact sheep (Group 1a):

EDTA blood: None of the animals had FMDV RT-qPCR positive blood samples at any of the days tested.

Probang: None of the animals had RT-qPCR positive probang on any of the days tested.

Nasal Swabs: Of the vaccinated contacts only one sheep was positive at 4 dpc (results not shown).

Oral Swabs: None of the sheep were positive at any time point.

Faecal Swabs None of the sheep were positive at any time point.

Unvaccinated in-contact sheep (Group 2a):

EDTA blood: None of the animals had FMDV RT-qPCR positive blood samples at any of the days tested.

Probang: Two of the 4 sheep never showed RNA in the probangs. One sheep was positive at 7 dpc through to 28 dpc and the other at 14 dpc through to 35 dpc, suggesting one or both of these sheep was persistently infected (Figure 4.5.2).

Nasal Swabs: Nasal swab samples were FMDV RNA positive in 2 of the 4 control sheep with 1 positive at 10 dpc only and another from 3–5 dpc (Figure 4.5.3).

Oral Swabs: Two of the 4 sheep never yielded a positive result, 1 was positive at 6 and 8 dpc and the other at 1, 3, 14 and 28 dpc (Figure 4.5.4).

Faecal Swabs: None of the sheep were positive at any time point.

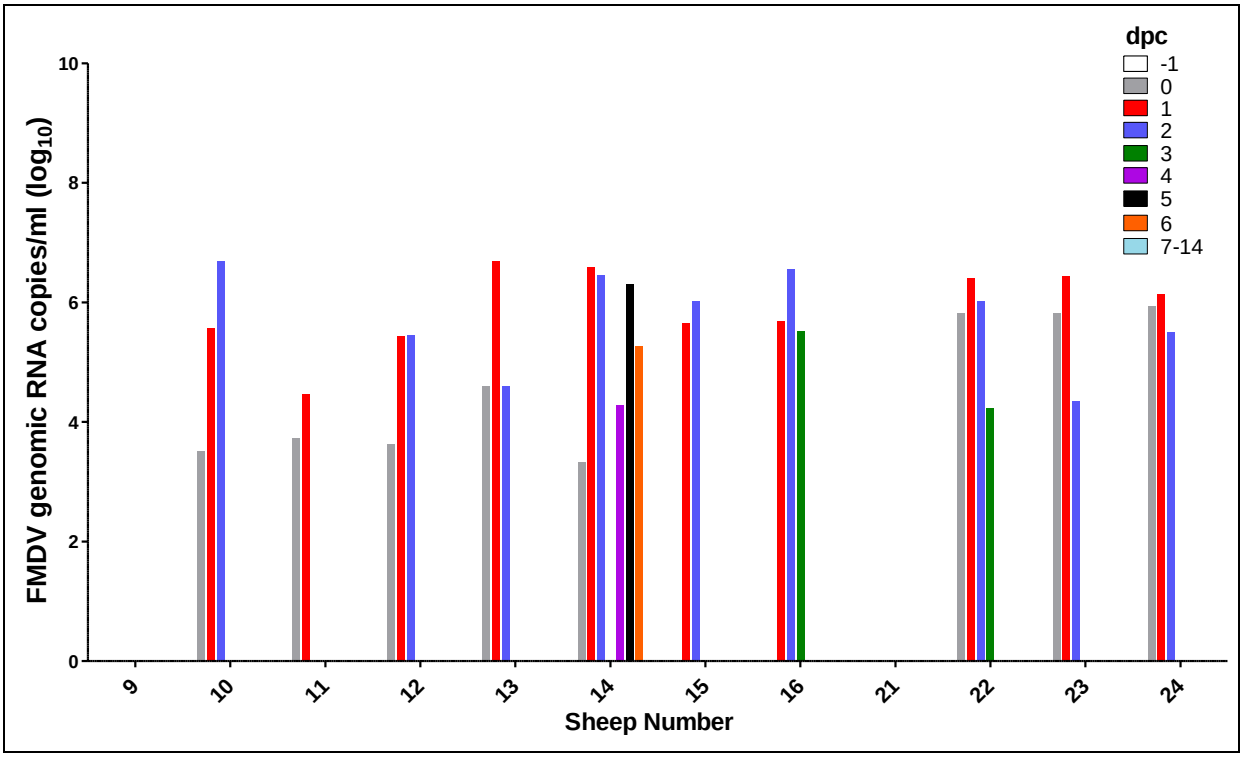

Figure 4.5.1 Levels of FMDV RNA detected in blood (EDTA) from unvaccinated donor sheep challenged by coronary band route

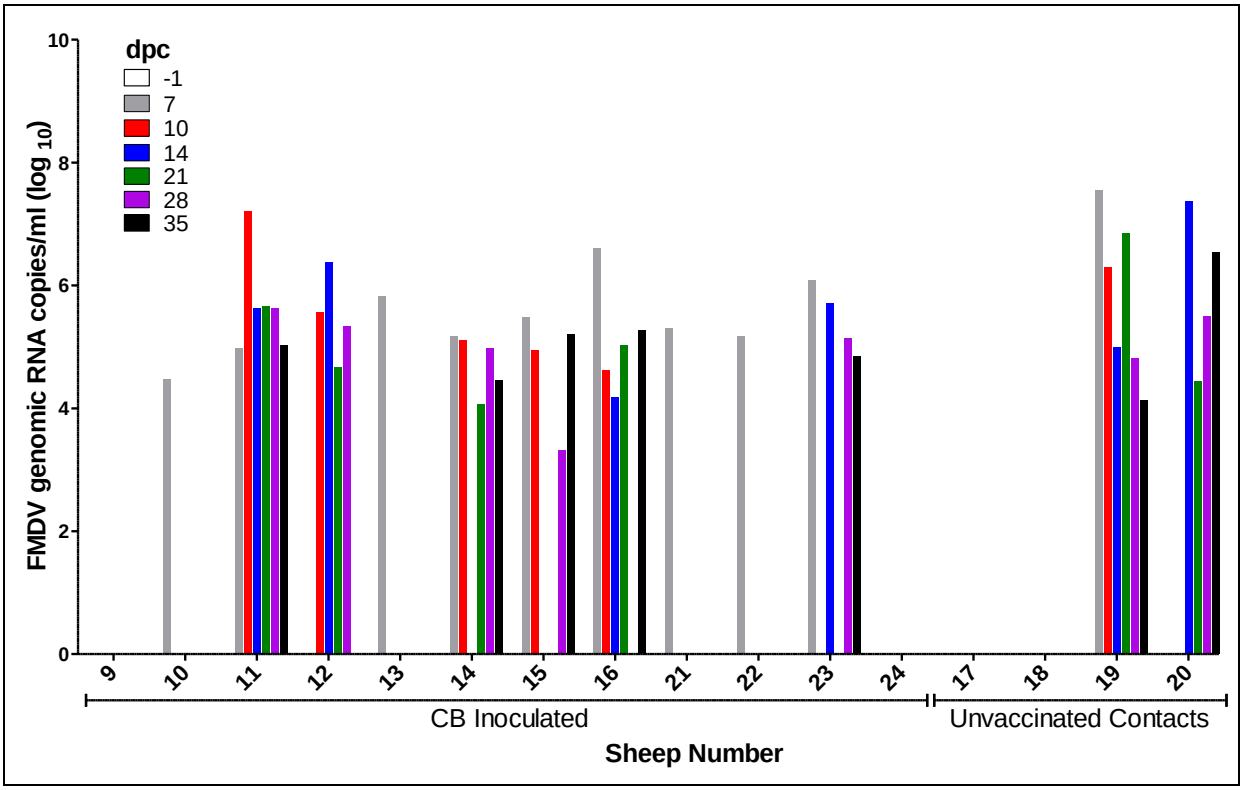

Figure 4.5.2 Levels of FMDV RNA detected in probang samples from unvaccinated donor sheep challenged by coronary band route and unvaccinated and in-contact transmission sheep

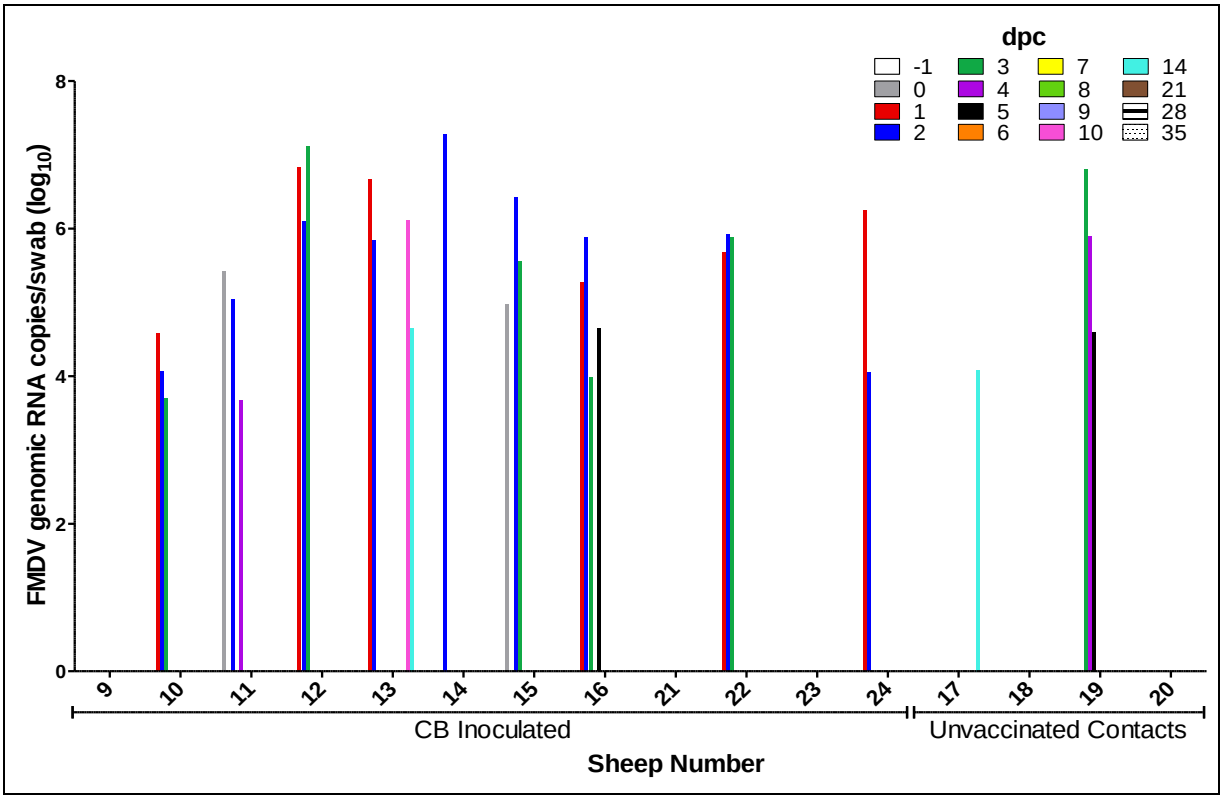

Figure 4.5.3 Levels of FMDV RNA detected in nasal swabs from unvaccinated donor sheep challenged by coronary band route and unvaccinated and in-contact transmission sheep

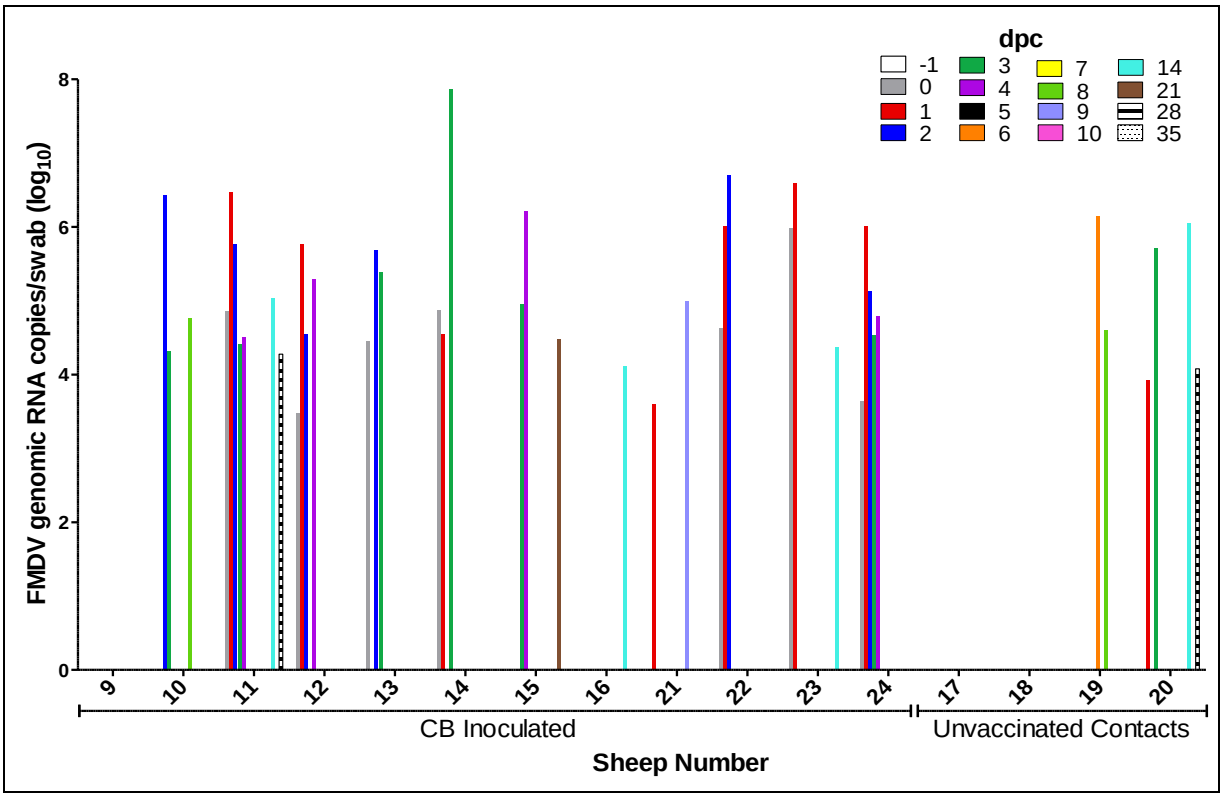

Figure 4.5.4 Levels of FMDV RNA detected in oral swabs from unvaccinated donor sheep challenged by coronary band route and unvaccinated and in-contact transmission sheep

4.5.5 Conclusions

Ten out of 12 CB inoculated sheep showed secondary lesions and provided direct contact challenge to the vaccine and control groups. Virus excretion was confirmed by the detection of FMDV RNA in the probangs, nasal and/or oral swabs from these animals over the sampling period. Of the two animals that did not have lesions, one sero-converted to FMDV NSP and viral RNA was detected in all sample types, except faeces, suggesting that it was sub-clinically infected. This sheep also established a persistent infection. The final CB inoculated sheep was sero-negative and none of the samples or swabs taken from this animal were positive by RT-qPCR, indicating that it was not infected.

Two of the four unvaccinated, contact control sheep (representing one of the two replicate rooms) showed signs of infection and developed disease. In contrast, one of the contact sheep in the second room only sero-converted to FMDV SP and was FMDV RNA positive in a nasal swab on only one occasion. The other contact sheep in this room showed no evidence of infection. Of the two donor sheep that shared this room, the results for one suggest this animal had only a mild infection and was not excreting large amounts of virus, which may account for the fact one of the contact sheep in this room did not become infected.

These results indicate at least two sheep with significant disease and high levels of excretion are required for the transmission of FMDV to unvaccinated in-contact animals. Since only one donor sheep was infected in one room where the contacts were vaccinated, it must be assumed that the contact sheep in this room may not have had significant exposure to cause infection. Despite this, all of the vaccinated sheep were fully protected upon challenge, including those sharing rooms with donors excreting significant amounts of virus (3 of the 4 replicate rooms). This suggests that vaccination with the O1 Manisa monovalent vaccine is effective at protecting sheep from challenge with FMDV O/SKR/2010 as early as 4 dpv.

The RT-qPCR results from probang samples suggest 6 (55%) of the 11 infected donor sheep (as 1 sheep did not become infected) became carriers and both of the infected unvaccinated contact sheep became carriers. One sheep is of particular interest: it had significant levels of FMDV RNA in probang samples up to 35 dpc, despite showing few clinical signs (one small mouth lesion) and having no detectable FMDV RNA in blood or nasal swab samples. Another sheep, that had high levels of viral RNA in blood, probang and nasal and oral swabs, had no clinical disease/lesions.

These results highlight the complex nature of detecting FMDV in sheep and the importance of probang sampling in diagnosis. Faecal swabs do not appear to be a useful sample for detection of FMDV in sheep with this strain of FMDV as all samples were negative. Nasal and oral swabs were both effective for the detection of FMDV early during infection; however, there was significant variation between sheep and at different time points in the positivity of these swabs. Considering the results for all the sheep, viral RNA detection in oral swabs appears to be a slightly more reliable indicator of FMDV status than in nasal swabs.

4.6 Vaccine efficacy trials with O1 Manisa monovalent vaccine against challenge with O/SKR/2010 (Mya-98 strain in sheep)

4.6.1 Determination of the optimal route of infection

4.6.1.1 Experiment 1, phase 1

Intra-naso-pharyngeal inoculation (INP)

Eight sheep were inoculated by the INP route. Two sheep were euthanized after 24 hours and 2 more after 48 hours for pathogenesis studies (results pending). The remaining 4 sheep were kept for a total of 9 days for observation, lesion scoring and sampling. Neither of the animals euthanized at 24 hrs post inoculation showed any generalised lesions and their clinical scores remained 0. One of the 2 animals that were euthanized at 48 hours post inoculation showed generalisation with a clinical score of 3. The 4 remaining animals showed generalisation of disease and reached the score 15 -20 by 6 dpc.

Aerosol Inoculation

Eight sheep were inoculated via aerosol exposure. As before, 2 sheep were euthanized after 24 hours whilst not showing any clinical signs. One out of the 2 animals that were euthanized at 48 hours post inoculation showed generalization with a clinical score of 1. The remaining 4 sheep were kept for a total of 9 days for observation, lesion scoring and sampling and showed generalisation of disease. The clinical scores increased from 2 dpi reaching a maximum at 7–8 dpi. In 2/4 sheep the score did not exceed 5. Viraemia started 1 dpi and lasted to 4 dpi. Viral RNA could be detected in nasal and tonsillar swabs as early as 1 hour post infection and was present until 5–6 dpi. In 2 sheep there was an increase in RNA at 8 and 9 dpi (results not shown).

Coronary band (CB) inoculation

Four sheep were inoculated via the CB. The animals were retained for a total of 9 days for observation, lesion scoring and sampling. Three of the 4 sheep had clinical scores of >10 by 4 dpi. Viraemia lasted between 1 and 5 dpi, while RNA was detected in the swabs until the time of termination (results not shown).

Contact-exposed to sheep infected via CB inoculation

Eight sheep were placed with 4 sheep infected via the CB 48 hours prior to contact. Two sheep in the contact group were euthanized after 48 hours and two more after 72 hours and samples were collected after necropsy for pathogenesis studies (results pending).

All four sheep that were retained until 10 days post contact demonstrated clinical disease 4–5 days post contact. Viraemia occurred between 2 and 6 days post contact and viral RNA could be detected in the nasal and tonsil swabs in some animals as early as 6 hours post contact and lasted until termination (results not shown).

4.6.2 Vaccine efficacy trials

4.6.2.1 Experiment 2, phase 1

Three groups of 7 sheep each were administered with three different volumes of O1 Manisa vaccine in order to study the vaccine dose response at 7 dpv. Four controls were included to ensure the challenge worked (Group 4a). All the animals were challenged on the same day by CB injection with the O/SKR/2010 challenge virus. One sheep in Group 1 and two in Group 3 died due to complications with the anaesthetic. All the challenged animals (except 1 in Group 2) showed generalised signs of FMD by 4 dpc and the experiment was terminated on 6 dpc due to the US government shutdown. As a result the animals could not be retained for 35 days for further sampling to investigate the effect of vaccination on the carrier state.

Serology by virus neutralisation test (VNT)

None of the sheep had antibodies at 7 dpv and there was a good anamnestic response to challenge at 6 dpc except in one sheep that received a full dose of vaccine (Table 4.6.1).

Table 4.6.1 Homologous and heterologous antibody response to vaccination and challenge determined by VNT

Sheep ID	Vaccine Dose	O1 Manisa			O/SKR/2010		
		-7 dpc	0 dpc	6 dpc	-7 dpc	0 dpc	6 dpc
30	1 X	<0.91*	<0.91	Dead	<0.91	<0.91	Dead
31		<0.91	<0.91	2.11	<0.91	<0.91	2.41
32		<0.91	<0.91	2.11	<0.91	<0.91	2.41
33		<0.91	<0.91	2.11	<0.91	<0.91	2.41
34		<0.91	<0.91	2.41	<0.91	<0.91	2.41
35		<0.91	<0.91	2.41	<0.91	<0.91	1.81
36		<0.91	<0.91	<0.91	<0.91	<0.91	1.21
37	½ X	<0.91	<0.91	2.41	<0.91	<0.91	2.41
38		<0.91	<0.91	2.41	<0.91	<0.91	2.11
39		<0.91	<0.91	2.11	<0.91	<0.91	2.41
40		<0.91	<0.91	2.41	<0.91	<0.91	2.41
41		<0.91	<0.91	2.71	<0.91	<0.91	2.41
42		<0.91	<0.91	1.81	<0.91	<0.91	2.11
43		<0.91	<0.91	2.11	<0.91	<0.91	2.41
44	¼ X	<0.91	<0.91	Dead	<0.91	<0.91	Dead
45		<0.91	<0.91	2.11	<0.91	<0.91	2.41
46		<0.91	<0.91	2.11	<0.91	<0.91	2.41
47		<0.91	<0.91	2.41	<0.91	<0.91	2.41
48		<0.91	<0.91	Dead	<0.91	<0.91	Dead
49		<0.91	<0.91	1.81	<0.91	<0.91	2.41
50		<0.91	<0.91	2.41	<0.91	<0.91	2.41
51	Naïve	<0.91	<0.91	2.55	<0.91	<0.91	2.41
52		<0.91	<0.91	2.55	<0.91	<0.91	2.41
53		<0.91	<0.91	2.41	<0.91	<0.91	2.41
54		<0.91	<0.91	2.11	<0.91	<0.91	1.95

*Log_{10} of reciprocal of highest dilution of serum that was able to neutralise either O1 Manisa or O/SKR/2010 virus

- positive

Real-time RT-PCR to detect viral RNA in serum and nasal swabs

Most of the previously vaccinated sheep showed viraemia at 1 dpc with 67% of those that had received a full dose, 86% of the ½ dose and 80% of the ¼ dose testing positive. In contrast, only 25% of the non-vaccinated controls showed viraemia 1 dpc (Table 4.6.2). None of the animals demonstrated detectable levels of RNA at 2 dpc, and at 3 dpc, 75 % of the unvaccinated controls were positive, 17% of the full dose group, 0% of the ½ dose and 40% of the ¼ dose. RNA was detected at 4 and 6 dpc in one sheep that had received the full dose of vaccine, and only 1 of the unvaccinated controls tested positive again at 6 dpc.

One sheep in each of the full and ½ dose groups had viral RNA in their nasal swabs at the day of challenge and needs to be repeated. In all groups animals tested positive between 1 and 6 dpc, with no significant differences between the groups (Table 4.6.3).

Table 4.6.2 Viraemia as determined by detection of FMDV RNA by RT-qPCR in serum samples from vaccinated and unvaccinated sheep challenged with O/SKR/2010 virus

Sheep ID	Vaccine Dose	0 dpc	1 dpc	2 dpc	3 dpc	4 dpc	5 dpc	6 dpc
30	1 X	N*	Animal Dead					
31		N	N	N	N	N	N	N
32		N	P	N	N	N	N	N
33		N	P	N	N	N	N	N
34		N	P	N	N	N	N	N
35		N	P	N	P	N	N	N
36		N	N	N	N	P	N	P
37	½ X	N	P	N	N	N	N	N
38		N	P	N	N	N	N	N
39		N	P	N	N	N	N	N
40		N	N	N	N	N	N	N
41		N	P	N	N	N	N	N
42		N	P	N	N	N	N	N
43		N	P	N	N	N	N	N
44	¼ X	N	Animal Dead					
45		N	P	N	P	N	N	N
46		N	N	N	N	N	N	N
47		N	P	N	P	N	N	N
48		N	Animal Dead					
49		N	P	N	N	N	N	N
50		N	P	N	N	N	N	N
51	Naïve	N	P	N	P	N	N	N
52		N	N	N	N	N	N	P
53		N	N	N	P	N	N	N
54		N	N	N	P	N	N	N

*Samples with Ct values <40 were considered positive; P – positive; N - negative

Table 4.6.3 FMDV RNA detection by RT-qPCR in nasal swab samples collected from vaccinated and unvaccinated sheep challenged with O/SKR/2010 virus

Sheep ID	Vaccine Dose	0 dpc	1 dpc	2 dpc	3 dpc	4 dpc	5 dpc	6 dpc
30	1 X	N*	Animal Dead					
31		P	N	P	P	P	P	P
32		N	P	P	P	N	P	P
33		N	P	P	N	P	N	N
34		N	N	P	P	P	P	P
35		N	P	P	P	P	P	P
36		N	P	N	N	N	P	P
37	½ X	N	P	N	N	P	N	N
38		N	N	P	N	P	P	P
39		N	P	N	N	P	P	N
40		N	P	P	N	P	P	N
41		N	P	P	N	P	P	N
42		P	P	P	P	P	P	P
43		N	P	P	P	P	N	P
44	¼ X	N	Animal Dead					
45		N	P	P	N	N	P	P
46		N	N	P	N	P	P	N
47		N	P	P	N	P	P	N
48		N	Animal Dead					
49		N	P	P	N	P	P	P
50		N	P	N	P	P	P	N
51	Naïve	N	P	P	P	P	N	P
52		N	P	P	P	P	P	P
53		N	N	P	P	P	N	N
54		N	N	N	P	P	P	P

*Samples with Ct values <40 were considered positive; P – positive; N - negative

4.6.2.2 Experiment 2, phase 2

In Experiment 2 phase 2, 1 group of sheep was administered 1 ml of vaccine and challenged at 14 dpv (Group 5) whilst 2 groups of sheep were administered with 2 ml of vaccine and challenged at 14 and 7 dpv (Groups 6 and 7 respectively). Naive controls were included to ensure the challenge worked (Group 4b). The animals were retained until 35 dpc to study virus persistence except Group 1 that was terminated due to the government shutdown (Group 1 from the previous phase acted as the group that received 1ml of vaccine that was challenged at 7 dpv). All the sheep in Group 4b showed generalised signs of FMD by 2 dpc. Four out of 7 sheep were protected in Groups 5 and 6 whereas in Group 7, 3 out of 7 animals were protected. Nearly all the animals showed pyrexia post-challenge.

Serology by virus neutralisation test (VNT)

None of the sheep in Group 5 and 6 had antibodies at 7 dpv however, on the day of challenge at 14 dpv, 6 out of 7 sheep in Group 5 and 5 of the 7 sheep in Group 6 had antibodies, whereas 3 of the 7 animals had responded to vaccination in Group 7 at

14 dpv. In most cases, there was an anamnestic response to challenge at 6 dpc (Table 4.6.4).

Table 4.6.4 Antibody response to vaccination and challenge determined by VNT using O1 Manisa virus

Group	Vaccine and challenge	Sheep ID	0 dpv	7 dpv	0 dpc	6 dpc
Group 5	1ml 14 dpv	55	<0.91*	<0.91	1.51	2.41
		56	<0.91	<0.91	1.51	2.41
		57	<0.91	<0.91	1.81	2.11
		58	<0.91	<0.91	1.51	2.11
		59	<0.91	<0.91	1.21	2.71
		60	<0.91	<0.91	0.91	2.41
		61	<0.91	<0.91	1.81	1.51
Group 6	2ml 14 dpv	62	<0.91	<0.91	1.51	1.51
		63	<0.91	<0.91	0.91	1.81
		64	<0.91	<0.91	1.81	1.81
		65	<0.91	<0.91	<0.91	2.11
		66	<0.91	<0.91	1.21	2.11
		67	<0.91	<0.91	1.51	3.01
		68	<0.91	<0.91	1.51	1.81
Group 7	2ml 7 dpv	69	<0.91		<0.91	3.01
		70	<0.91		1.21	1.81
		71	<0.91		0.91	1.51
		72	<0.91		0.91	2.41
		73	<0.91		1.81	3.01
		74	<0.91		1.51	2.71
		75	<0.91		<0.91	2.71
Group 4b	UV Control	76			<0.91	1.51
		77			<0.91	2.41
		78			<0.91	2.41
		79			<0.91	2.41

*Log_{10} of reciprocal of highest dilution of serum that was able to neutralise O1 Manisa virus

- positive

4.6.3 Conclusions

Both the INP and CB routes of infection caused reproducible disease in sheep with rapid progression to observed clinical signs when infected with O/SKR/2010. With the small numbers in these experiments (only 4 sheep were used), the INP gave marginally better results with higher clinical scores compared the CB route of infection. The in-contact transmission resulted in clinical disease in all 4 contact sheep, but clinical scores were more variable.

The recommended vaccine dose for sheep is half that of cattle (therefore 1ml). Since the vaccine is of high potency, it was decided to test whether lower doses of O1 Manisa vaccine could protect sheep against infection with O/SKR/2010 7 dpv. None of the sheep that received a full and ¼ dose of vaccine were protected and only 1

that received ½ dose did not develop clinical signs. The experiment had to be terminated at 6 dpc, and it was therefore not possible to further compare the different doses. Most sheep had an anamnestic response, but there was no significant difference in the levels of antibodies observed at 6 dpc.

Due to this apparent vaccine failure, it was decided to test the vaccine efficacy at different time points post vaccination and also use different vaccine doses. Two groups of sheep received a full cattle dose (2ml) of vaccine and were challenged 7 and 14 dpv, while another group received a 1ml dose, but was challenged 14 dpv. In both groups that were challenged 14 dpv, 57% of the sheep were protected, compared to the groups that were challenged 7 dpv, where 43% of those that had received 2ml of vaccine, and 0% of those that were vaccinated with 1ml were protected. Antibodies were detected at the time of challenge in a number of the sheep. It is therefore not clear whether the dose of challenge using the CB route of infection was too high to prevent clinical disease.

4.7 Early pathogenesis in pigs infected with O/VIT/2010

4.7.1 Pathogenesis study

From the results of the direct-contact infection model study, it was decided to expose pigs for 2 hours to donors in the pathogenesis study, as this lead to infection and was therefore suitable to study the pathogenesis of the disease. Upon admission of the naive pigs to the room the pigs were fed resulting in close direct contact between the donor and in-contact pigs at the trough. No clinical signs of FMD were visible in any of the contact pigs with the exception of one of the pigs culled at 48 hours post contact, which had mild lesions on the tongue and right fore-foot.

4.7.2 Conclusions

The results from the model study indicated an inconsistency in the development of clinical disease in pigs following heel pad inoculation of O/VIT/2010 at the dose used. Laboratory analysis of samples will shed more light on the infection status of the donor pigs. Only one of two contact pigs developed clinical signs, regardless of the duration of exposure to the donor pigs; however these result did support the fact that 2 hrs was sufficient time for FMDV transmission in pigs. This short period of exposure is beneficial in the study of pathogenesis as there is less variability in the specific time of infection of contact animals, which is important when examining time points early in infection. Consequently, a 2 hr exposure period was employed in Phase 2.

The outcome of the pathogenesis study (Phase 2) will not be known until the analysis of the samples is performed. In all groups at least one donor pig had signs of generalised disease suggesting virus was being excreted in the rooms. The observation of lesions in one of the 48 hours post contact pigs suggests transmission of FMDV during the 2 hr exposure was successful.

4.8 Collection of oral fluids using cotton ropes as a sampling method to detect FMD virus in pigs

4.8.1 Comparison of rope samples and individual saliva swabs

Viral RNA was found in rope samples in all the groups where the animals were exhibiting clinical signs of FMD. In group O-UV, viral RNA was present as early as 1 dpc with Ct values mostly <30 and was detected in each daily sample until termination of the experiment at 14 dpc (Table 4.8.1a). Pigs in group O-V4 initially did not chew the ropes and only individual saliva samples were collected on days 0 and 1; RNA was detected intermediately in rope samples from 2 dpc until 13 dpc. Viral RNA was detected intermittently from Group O-V7 from 1 dpc until 12 dpc. At 6 dpc, RNA was also detected in the contact group, O-UVC, 3 days before they breached the partition. Viral RNA was again detected in this group at 13 dpc when the first clinical signs were observed. None of the other in-contact groups (O-UVC4 and O-UVC7) were positive for viral RNA.

Pigs infected with the A/VIT/2005 virus deposited RNA in the rope samples from 2–3 dpc (A-UV, A-V4 and A-V7) with the shortest duration in Group A-V7 where the pigs were vaccinated 7 days before challenge (2–7 dpc; Table 4.8.1b). In groups A-UV and A-V4, viral RNA was detected up to 11 and 14 dpc respectively. No RNA was detected in rope samples exposed to the contact groups (A-UVC, A-UV7 and A-UV4).

Each pig was individually sampled daily using cotton swabs to collect saliva for comparison with the results from the rope samples. Groups O-UV and O-V7 showed RNA in saliva swabs at 1 dpc, followed by group O-V4 at 2 dpc (Table 4.8.1a). At least 1 pig was positive in Group O-UV for the duration of the experiment, while RNA was also detected in the vaccinated and challenged pigs in Groups O-V4 and O-V7 on most days using swab sampling. At 4 dpc and on most days after that, RNA could be detected in swab samples from the contact Group O-UVC. Although Group O-UVC4 had 1 pig that was positive for RNA 4 dpc, RNA was only detected in swab samples from this group again from 10 dpc. No FMDV RNA was detected in Group O-UVC7 swab samples throughout the experiment.

Group A-UV had FMDV RNA-positive samples from 2–8 dpc, with 7 of 8 pigs being positive at 4 dpc, while the vaccinated and challenged group A-V4 only had 1 of 8 pigs with RNA-positive samples on 3 and 7 dpc. Pigs from Group A-V7 had RNA-positive swab samples between 3 and 7 dpc. Although high numbers of pigs in the latter group were positive on most days, the average RNA copy number was $<10^3$. Groups A-UVC, A-UVC7 and A-UVC4 did not show FMDV RNA in saliva swabs (Table 4.8.1b).

Viral RNA was detected in both rope and individual saliva samples from all groups where the animals were infected. In regard to viral RNA detection, rope sampling had the strongest correlation (99-100% agreement) with saliva swab sampling for the infected groups O-UV and A-V7 and in-contact groups O-UVC7, A-UVC4, A-UVC7 (the latter all negative). Low levels of RNA and variation in days where RNA was detected led to poorer correlation for groups O-V7 (73.3%), O-V4 (58.3%), O-UVC (78.6%), O-UVC4 (73.3%), A-V4 (40.0%) and A-UV (66.7%). For example, in group O-UVC4, RNA was detected in saliva swabs on 4 occasions (4, 10, 11 and 13 dpc), but was not detected in the rope samples (Table 4.8.1a). Given that the pigs in this group never showed clinical disease, it probably indicates that virus excretion was very low. It is therefore possible that when low levels of virus are present, factors that influence RNA recovery such as time at room temperature (the ropes were available to the pigs for up to 30 minutes), proteases and other enzymes could destroy the virus and RNA prior to testing. In contrast, viral RNA was detected in the rope samples of group A-V4, where the individual saliva samples were negative (Table 4.8.1b). This could be as a result of the small amount of material collected with the swabs compared to the rope that was available to pigs for a longer period

where more saliva was collected as a result. More experiments are needed to validate the sensitivity of FMDV RNA detection as well as volumes when using swabs for collection.

Table 4.8.1 Comparison of daily results when sampling pigs using ropes (R) and individual saliva swabs (S) and testing by RT-qPCR. A group of animals were deemed positive for saliva swabs if at least one of the animals in the group was positive on any given day

a) Pigs vaccinated with O1 Manisa and challenged with a serotype O Mya98 virus

Group	Days post-challenge														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
R-O-UV														NS	
S-O-UV														NS	
R-O-UVC														NS	
S-O-UVC															
R-O-V4	NS	NS											NS		
S-O-V4															
R-O-UV4															
S-O-UV4															
R-O-V7															
S-O-V7															
R-O-UVC7															
S-O-UVC7															

b) Pigs vaccinated with A Mal 97 and challenged with a serotype A SEA-97 virus

Group	Days post-challenge														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
R-A-UV															
S-A-UV															
R-A-UVC															
S-A-UVC															
R-A-V4															
S-A-V4															
R-A-UV4															
S-A-UV4															
R-A-V7															
S-A-V7															
R-A-UVC7															
S-A-UVC7															

– Positive; NS – not sampled

The comparative statistical results for sampling performance of the ropes against saliva swabs in detecting the presence of FMDV RNA pre group of animals are shown in Table 4.8.2 The group was considered positive if one animal was positive by saliva and the sensitivity and specificity estimates were calculated using a 2 x 2 table. The sensitivity of viral RNA detection in the rope samples ranged from 0.67 for Expt. 1 to 0.92 for Expt. 2 when compared with the results of the saliva samples. The positive predictive values were 0.94 for Expt. 1 compared to 0.42 for Expt. 2; the latter being lower due to the difference in the number of positive animals between the two experiments. In both the experiments the accuracy was >80% (81%; 75–87%) indicating that rope samples are a good determinant for FMD detection. The Kappa values indicated a moderate level of agreement between the two methods (0.61 for serotype O and 0.48 for serotype A).

Table 4.8.2 Comparative statistics of results obtained from rope samples and individual saliva swabs

Parameters	**O1 Manisa versus O Mya98 (Expt 1)**	**A Malaysia 97 versus A SEA-97 (Expt 2)**	**Overall (Expt 1+Expt 2)**
Positive in Oral Swabs & Rope samples	31	11	42
Positive in Oral Swabs only	15	1	16
Positive in Rope Samples only	2	15	17
Negative in Oral Swabs & Rope samples	37	63	100
Prevalence	0.54 (0.44-0.65)*	0.13 (0.06-0.20)	0.33 (0.26-0.40)
Sensitivity	0.67 (0.57-0.77)	0.92 (0.86-0.97)	0.72(0.66-0.79)
Specificity	0.95 (0.90-1.00)	0.81 (0.73-0.89)	0.85 (0.80-0.91)
Accuracy	0.80 (0.71-0.89)	0.82 (0.74-0.90)	0.81 (0.75-0.87)
Positive Predictive Value	0.94 (0.89-0.99)	0.42 (0.32-0.53)	0.71 (0.64-0.78)
Negative Predictive Value	0.71 (0.62-0.81)	0.98 (0.96-1.01)	0.86 (0.81-0.91)
Kappa (SE=0.085)	0.61 (0.44-0.77)	0.48 (0.26-0.71)	0.58 (0.45-0.71)
Level of Agreement	Moderate	Moderate	Moderate
Odds Ratio	38.23 (8.11-180.26)	46.2 (5.53-386.13)	15.44 (7.14-33.42)

*Values in parentheses are 95% confidence intervals.

4.8.2 Conclusions

Compared to saliva swabbing and visual examinations, rope sampling is far less laborious for people and is stress-free for animals. The ease in which oral fluid samples from ropes were collected and extracted makes rope sampling an extremely useful method of sample collection that may complement FMD monitoring efforts in pig populations. On most days, copious amounts of oral fluids were collected from the ropes; however the method was dependent upon pig behaviour. Pigs were mostly very interested in the ropes but, at times, the clinically affected pigs showed less interest in the ropes, possibly due to effects of the disease (lethargy and lameness). In the experiments described here all pigs had access to the ropes even during periods of disease and diseased pigs often chewed the ropes once their healthy counterparts became bored and lost interest in the ropes. One group of pigs had to be encouraged to accept the rope, but once familiar their interest was maintained throughout the experiment. The ropes were used to distract the pigs

while swab sampling was performed. It is therefore possible that saliva swabs could be positive due to cross contamination of the mouth when pigs were chewing on the ropes. However, since there was a significant number of individual swabs negative on days the ropes were positive, this was not likely to be a problem, but in future experiments, ropes should only be available to the pigs after sampling to avoid this uncertainty.

Overall, cotton rope sampling of oral fluids can be considered a successful method to detect FMDV RNA from pig populations. With further validation of the specificity and sensitivity of detection, this may be a cost effective, non-invasive, sampling tool to detect FMD in a pen considering that susceptible, unvaccinated pigs will rapidly infect each other due to direct contact.

5 Success in achieving objectives

5.1 Summary of vaccine efficacy testing performed during Phase 1 of the project and knowledge gained on FMDV strains that pose a risk to Australia

The initial aim of the project was to test vaccine efficacy using species of cattle and sheep similar to those occurring in Australia in order to provide a close estimation of vaccine and disease behaviour under local conditions. However, this was not always possible due to local circumstances where the work was performed. Nevertheless, with the unvaccinated animals in the study it was possible to observe the clinical signs associated with different isolates from SEA and the development of disease in different species over time when infected with these isolates. These data will be useful in training field staff to recognise FMD in various species.

The O1 Manisa vaccine was effective in protecting a number of pigs against infection with the serotype O isolate that circulated in Vietnam in 2010. Protection was observed as early as 4 (60% protection) and 7 dpv (80% protection). However, the same vaccine was less effective when the pigs were challenged with the O isolate that caused outbreaks in South Korea in 2010. All of the pigs challenged 5 dpv developed disease, and only 20% of those challenged 21 dpv were protected. In both challenge studies a pig derived virus was used, but the experiments were performed at different laboratories. Consequently, it is difficult to directly compare the results, but it may be an indication that O/SKR/2010 is a more virulent virus or is antigenically more diverse from the vaccine strain. The antigenic relationships will be determined in the future, but preliminary studies indicate that O1 Manisa is antigenically related to both viruses and should provide protection with high potency vaccine formulations. The O/SKR/2010 derived vaccine also did not fully protect pigs against infection with the homologous virus at 21 dpv (60% of pigs were protected), implying that the apparent vaccine failure may be due to a highly virulent isolate.

The high potency O1 Manisa vaccine was used with success to control the outbreak in South Korea and was extensively used in pigs. Therefore, despite the apparent failure of the vaccine under experimental conditions, information from the field seems to indicate that other control measures, such as movement control, can be used in addition to vaccination to control an outbreak of O/SKR/2010. In addition, the O1 Manisa vaccine fully protected cattle against challenge when a full dose was used 21 days prior to challenge with O/SKR/2010. Although the homologous O/SKR/2010 vaccine protected the cattle at lower doses (1/4 and 1/16 dose) compared to O1 Manisa, the latter seems to be an effective vaccine when used at high potency and therefore there does not seem to be need for a new vaccine strain in cattle. However, development of such a vaccine against a virulent strain would be useful in

the long run, in case there is a further change in the virus genome and emerging viruses are not related antigenically to O1 Manisa vaccine strain. The sera derived from the full dose vaccine group animals would be a valuable resource for future antigen matching studies with the emerging viruses in SEA.

It has been confirmed that disease transmission between groups of pigs that don't have direct contact is not efficient and strict bio-security can prevent the spread of infection when separate pig pens are present. This information is important for disease control plans and should be emphasised in discussions with decision makers. These experiments were performed with small numbers of pigs and do not represent a farm where the pig density is likely to be much higher with more virus excreted into the environment, including the air. However, our data also indicated that vaccination decreased the amount of RNA in secretions from infected pigs, and therefore could assist in lowering virus load; thus, in conjunction with bio-security measures, vaccination will assist in preventing the spread of disease on pig farms.

For sheep it was necessary to first compare various routes of infection to determine which is the most reproducible in these animals. Both the CB and INP routes infected all sheep and led to the development of significant clinical signs. Although sheep did develop clinical disease by direct contact with infected sheep, the clinical scores were lower compared to the direct methods of infection. While needle inoculation does not represent a natural route of infection, it is often necessary to use this route to increase the chances of a successful challenge. The design of the vaccine efficacy studies varied based on the facilities available (number and size of rooms) and ethics requirements in the various countries. We used various approaches to test the efficacy of O1 Manisa with challenge using O/SKR/2010; one where vaccinated sheep were infected directly in the CB and the other where infected sheep were used as donors to challenge vaccinated sheep. The outcomes were very different. The vaccinated sheep that were exposed to infected sheep were fully protected when challenged 4 dpv, while those that were challenged by the CB were only partially protected 14 dpv, even with a double dose of vaccine. It is possible that the dose of challenge virus when using the CB route in was too high and is not representative of a natural challenge, as would occur during an outbreak in sheep. The apparent vaccine failure should therefore be interpreted with care.

The vaccine that was used in the direct CB challenge in sheep accidentally warmed up during transit between the vaccine manufacturer and the laboratory. The data logger showed that the vaccine was exposed to 18^{o}C for approximately 18 hours. After discussions with the manufacturer, it was decided that this should not affect the efficacy of the vaccine and that a short period of warming up could mimic what is likely to happen when vaccine is deployed in the field. However, given that the vaccine partially failed with the direct inoculation challenge, it is not clear whether or not the warming up decreased the vaccine efficacy. None of the 7 sheep that received a full dose of vaccine had antibodies 7 dpv, while 3/7 that were vaccinated with a double dose (2 ml) were sero-positive. At 14 dpv, 6/7 and 5/7 of the sheep that have been vaccinated with 2 ml and 1 ml, respectively, had antibodies. It seems therefore that the vaccine was immunogenic but did not afford full protection. These data emphasise the need to ensure that the cold chain is maintained during vaccination campaigns; an aspect that should be discussed further with the relevant stakeholders.

Although it is unlikely that emergency vaccines will be stored for long periods of time, it was encouraging to note that the A Malaysia 97 vaccine used in the pig challenge experiment, which was stored for 12 months prior to the challenge experiment, still performed well. It indicates that, given the vaccine is stored as recommended, the shelf life is as claimed by the manufacturer.

As a whole, the experiments performed to date indicate that the vaccine strains in the Australian vaccine bank are likely to protect cattle, sheep and pigs against infection (Table 5.1). However, there were differences in the level of protection observed and due to the apparent virulence of some isolates, focus should also be on other control measures and not rely solely on vaccination. The viruses are constantly evolving in the field and novel viruses may be introduced from other regions of the world. It is therefore necessary to constantly monitor isolates by *in vivo* methods like vaccine matching studies using homologous reagents and also perform challenge studies to ensure the vaccines in the bank will be efficacious.

Table 5.1 Summary of the vaccine efficacy experiments performed during Phase 1

Collaborator	Species	Objectives	Outcomes	AEC number
NAVETCO, RAHO6	Pigs	Adapt O/VIT/2010 to pigs	Virus adapted in 3 passes	AEC1465
	Pigs	Test the early protection of the O1 Manisa high potency vaccine against heterologous challenge with O/VIT/2010. Test whether vaccination prevents spread to pigs in the same room but with no direct contact.	60% protection at 4 days,80% protection at 7 days Strict bio-security is sufficient to prevent spread to nearby pigs.	AEC1497
	Pigs	Adapt A/VIT/2005 to pigs	Virus adapted in 3 passes	AEC1514
	Pigs	Test the early protection of the high potency A Malaysia vaccine against heterologous challenge with A/VIT/2005. Test whether vaccination prevents spread to pigs in the same room but with no direct contact.	100% protection at 4 days,75% protection at 7 days Strict bio-security is sufficient to prevent spread to nearby pigs.	AEC1571
Pirbright Institute, UK	Cattle	Compare O1 Manisa high potency vaccine to the newly developed O/SKR/2010 vaccine strain in challenge exps using homologous virus to O/SKR/2010	O1 Manisa against heterologous challenge PD50 = 3.47, O/SKR/2010 with homologous challenge PD50 = 7.94, all cattle that received a full dose were protected	AEC1570
	Pigs	Compare O1 Manisa high potency vaccine to the newly developed O/SKR/2010 vaccine strain in challenge exps using homologous virus to O/SKR/2010; 5 dpv	All pigs showed disease 2-3 days post challenge and were euthanized	AEC1595
	Pigs	Compare O1 Manisa high potency vaccine to the newly developed O/SKR/2010 vaccine strain in challenge exps using homologous virus to O/SKR/2010; 21 dpv	O1 Manisa protected 20% and O/SKR/2010 60% of pigs	AEC1632
PIADC, USA	Sheep	Compare different routes of challenge	INP and CB infection provided most reproducible results	AEC1636
		Compare different doses of vaccine (full, 1/2 and 1/4 dose) challenged 7 dpv via CB infection	All sheep (except 1 that received a 1/2 dose) showed generalised disease by 4 dpc; exp terminated at 6 dpc due to government shutdown	AEC1636
		Compare different doses of vaccine (1ml and 2 ml) and challenge 7 and 14 dpv via CB infection	1 ml vaccine challenged 14 dpv = 4/7 protected; 2ml vaccine challenged 14 dpv = 4/7 protected; 2ml vaccine challenged 7 dpv = 3/7 protected	AEC1636
NAVETCO, RAHO6	Pigs	Pathogenicity study in pigs using O/VIT/2010	Performed and samples collected	AEC1647
NCFAD, Canada	Sheep	Test the early protection of the O1 Manisa vaccine against heterologous challenge with O/SKR/2010 when vaccinated sheep have contact with infected sheep	All vaccinated contact sheep were protected	AEC1637

5.2 Summary of other objectives

5.2.1 Improve laboratory diagnostic capability and store reagents

Until recently we were unable to obtain import permits to allow serum and genomic material (cDNA and PCR products) collected from our studies in other countries into Australia. Due to this we have not been able to establish full-genome sequencing techniques at AAHL. However, we have set up collaboration with Duke-NUS Graduate Medical School, Singapore, who have been developing techniques to do deep sequencing on RNA extracted from the FMDV isolates obtained during the pig experiments in Vietnam. Once these techniques are established we will attempt direct sequencing from viral RNA on swabs and so avoid any sequence bias as a result of cell culture adaption. We will also ensure AAHL staff receive training and establish local capability. In addition, we have agreed with the Pirbright Institute to perform deep sequencing on swabs obtained during the pig and cattle experiments performed at their facility.

We have been able to transport sera obtained from infected sheep collected during the experiment conducted at NCFAD to AAHL and these will be used in the future to validate serological assays including DIVA. In the meantime, AAHL reagents have been used in Vietnam to test the pig sera, thereby adding to our knowledge on how the tests perform. Bulk sera are also available to send to other diagnostic laboratories in Australia under the LEADDR agreement and will be used in future proficiency testing (PT) rounds.

A permit has been issued to import cDNA and PCR products from Vietnam and these will be used to validate the molecular diagnostic assays and in PT rounds provided by AAHL to regional and local labs. We still need permits to do the same from other labs where we have performed experiments.

Various cell lines are available to isolate FMDV from clinical material, and these are tested as they become available. Two new cell lines (the ZZR line from Friedrich-Loeffler-Institut, Germany and the LFBK cell line expressing the bovine FMD integrin receptor from PIADC) have been imported to AAHL and these will be tested in Vietnam to determine whether they are suitable for primary diagnosis. A recommendation will be made to the Diagnostic, Surveillance and Response Theme at AAHL as results are generated.

The project has furthermore demonstrated the success of using swabs and probangs as diagnostic tools in cattle, sheep and pigs. Often clinical material such as vesicular fluid or epithelial flaps are not available, either because the animals are in the incubation phase and are yet to develop lesions or the lesions have started healing. In addition, clinical signs in sheep and goats are frequently mild or inapparent, and lesions on the coronary band can be obscured by wool/hair. Virus can be found in excretions both before and after clinical signs are observed and swabs or probangs can be recommended for diagnostic use in the face of an outbreak (Table 5.2).

Table 5.2 provides a summary of the first and last day samples tested positive from infected or exposed animals. Often only 1 animal tested positive on these days and the table therefore does not represent the optimal time where most animals tested positive. In some cases there were also days between these time points where no animals tested positive. However, it does provide some guidance as to the time period swabs can be used for FMD diagnosis compared to clinical disease. All the data are not available yet and the table will be updated over time.

There were differences between the serotype O and A isolates in pigs where both nasal and saliva swabs from the serotype O infection tested positive until 14 dpc, when the experiment was terminated. In contrast, the swabs collected from pigs

infected with the serotype A isolate were positive between 2 and 9 dpc. It seems therefore that strain specific differences in the duration of excretion may occur. There were no significant differences between nasal and saliva swabs in pigs and both samples are suitable to detect disease.

Table 5.2 Summary of the first and last days that samples tested positive for viral RNA

		Days post challenge					
Experiment	**Species**	**Clinical signs**	**Nasal Swabs**	**Saliva Swabs**	**Faecal Swabs**	**Blood/ Serum**	**Probang**
O1 Manisa vaccine; O/VIT/2010 challenge NAVETCO	Vaccinated pigs	2 to 4*	1 to 14	2 to 14	1 to 10	NT	ND
	Unvaccinated pigs	2 to 3	1 to 14	1 to 14	1 to 9	NT	ND
	Contact pigs	13	5 to 14	4 to 14	2 to 14	NT	ND
A Malaysia 97 vaccine; O/VIT/2005 challenge NAVETCO	Vaccinated pigs	5 to 7	3 to 8	3 to 7	3 to 8	NT	ND
	Unvaccinated pigs	2 to 7	2 to 9	2 to 8	2 to 8	NT	ND
	Contact pigs	Neg	Neg	Neg	Neg	NT	ND
O1 Manisa vaccine; O/SKR/2010 challenge Pirbright	Vaccinated cattle (full dose)	Neg	ND	1 to 8	ND	Neg	2 to 14
	Vaccinated cattle (1/4 dose)	4 to 8	ND	1 to 7	ND	1 to 3	2 to 11
	Vaccinated cattle (1/16 dose)	4 to 8	ND	1 to 7	ND	1 to 3	1 to 8
O/SKR/2010 vaccine; O/SKR/2010 challenge Pirbright	Vaccinated cattle (full dose)	Neg	ND	1 to 7	ND	4	2 to 8
	Vaccinated cattle (1/4 dose)	4 to 8	ND	1 to 6	ND	3 to 4	2 to 14
	Vaccinated cattle (1/16 dose)	4 to 8	ND	1 to 7	ND	3	2 to 8
	Unvaccinated	2 to 3	ND	1 to 7	ND	1 to 3	2 to 6
O1 Manisa vaccine; O/SKR/2010 challenge Pirbright	Vaccinated pigs	2	NT	NT	NT	ND	ND
O/SKR/2010 vaccine; O/SKR/2010 challenge Pirbright	Vaccinated pigs	2 to 3	NT	NT	NT	NT	ND
	Unvaccinated pigs	2 to 3	NT	NT	NT	NT	ND
O1 Manisa vaccine; O/SKR/2010 challenge NCFAD	Vaccinated sheep	Neg	4	Neg	ND	Neg	Neg
	Infected sheep	2	1 to 6	1 to 28	ND	1 to 2	8 to 28
	Unvaccinated contact sheep	6 to 9	3 to 10	6 to 28	ND	Neg	7 to 35
O1 Manisa vaccine; O/SKR/2010 challenge PIADC	Vaccinated sheep	2 to 4	NT	NT	ND	NT	NT
	Control sheep	2	NT	NT	ND	NT	NT
	Infected via INP	2 to 3	0.1 h to 5	ND	ND	1 to 4	ND
	Infected via aerosol	3 to 4	0.1 h to 5	ND	ND	1 to 4	ND
	Infected via CB	1 to 2	1 to 9	ND	ND	2 to 5	ND
	Infected via direct contact	4 to 5	1 to 9	ND	ND	2 to 6	ND

*first day a sample tested positive for FMD viral RNA until the last day a sample was found positive

ND – sample not collected; NT – sample not tested

Viral RNA was found in the faeces of pigs, but not in sheep. Although faecal swabs are not a sample of choice for diagnostics due to lower sensitivity observed in pigs

compared to nasal and saliva swabs, these results indicate that environmental viral contamination from faeces may be more prevalent when pigs are infected compared to sheep. However, more data need to be collected to verify this conclusion.

Unexpectedly, the saliva swabs in sheep infected via the CB were positive until 35 dpc, the same duration as the probang samples. In contrast, the nasal swabs were positive up to 10 dpi. More work needs to be done with other virus isolates to determine whether this long period of excretion is a strain specific characteristic.

In all species, swabs tested positive for viral RNA before clinical signs were observed. Saliva swabs are easier to take and collect more material due to the amount of saliva present. In contrast, nasal swabs often collect less material and animals resist nasal sampling more than saliva swabs. More data are needed on probangs, but with the limited information in sheep, probangs were positive until 35 dpc in approximately 50% of the infected sheep.

5.2.2 Assist with the design of FMD control strategies

The project focussed not only on the improvement of laboratory assays and sampling during an outbreak, but also on other non-invasive, cost effective methods for field diagnostics – in this case, the use of ropes as surveillance tools in piggeries. The ropes were found to be effective for detecting FMDV infection and can be recommended to decision makers as a surveillance tool (3.8 and 4.8).

It has been confirmed that aerosol transmission between separate groups of pigs is not efficient and strict bio-security can prevent the spread of infection (see 4.1 and 4.2). This information is important in the design of disease control plans.

The data have shown that high potency vaccines can protect domestic animals with a heterologous challenge, supporting AUSVETPLAN's inclusion of vaccination as part of outbreak control.

To date only one experiment has data on the carrier state. Up to 50% of the CB infected and unvaccinated contact sheep had viral RNA in their probangs 35 dpc. None of the vaccinated sheep were infected, and therefore their probangs were negative. However, it has not been confirmed whether these samples contain live virus. It is also now recognised that carriers may not play an epidemiologically significant role in the spread of FMD, but this information adds to our knowledge on the duration of the carrier state. In future, more experiments will investigate the carrier state in sheep and cattle, and combined, these data could be used to inform control plans.

5.2.3 Provide scientific data for disease dispersion models

In all the experiments swabs were taken from unvaccinated and vaccinated infected animals and the amount of viral RNA quantified. These data will be essential to ensure dispersion models provide accurate predictions when used during an outbreak. The amount of RNA found in unvaccinated and vaccinated animals was quantified and will therefore provide insight in whether vaccination of different species will lower the amount of virus excreted in the environment, be cost effective and assist in preventing widespread outbreaks.

5.2.4 Capacity building in SEA

One of the vulnerabilities identified and addressed as part of the project is lack of capacity in SEA to diagnose FMD and perform genetic and antigenic characterisation

on isolates from the region. It is important that more outbreak isolates are characterised to ensure we know whether variants are emerging or whether new introductions have occurred. This knowledge will not only assist the region in their choice of vaccine strains, but will also provide epidemiological information on routes of transmission. In addition, it will provide Australia with intelligence on perceived risks from the region.

Since the start of the project, we have actively engaged the OIE FMD Regional Reference Laboratory, based in Pakchong, Thailand to collaborate on training and capacity building in Pakchong, focusing on specific areas outlined below. A contract still needs to be signed.

- Develop P1 and full-genome sequencing and analytical methods to assist in better understanding the molecular epidemiology of FMDV in the SEA region and motifs related to cell receptor recognition, cell culture adaptation and pathogenesis.
- Vaccine matching using r-values and comparison of results obtained using VNT and ELISA. AAHL and RRL have access to different sets of reagents and combining the results would provide a better indication of potential vaccine efficacy in the region and assist in the selection of vaccine strains.
- Testing the sensitivity of various different cell lines for the isolation and propagation of FMDV for research and diagnostics, which could lead to an increase in the number of viruses isolated and improve virus banks.
- RRL is responsible for setting up PT rounds for the SEA regional labs and AAHL can provide training as they are managing their PT provision under NATA accreditation.
- Upon the recommendation of their department (DLD), the project would facilitate at least one PhD study for a RRL staff member. The AAHL project operational budget would be utilised to facilitate such post graduate studies, excluding the student registration fees. AAHL endeavours to assist in obtaining a scholarship from AusAid or any other funding body to cover registration fees.
- To ensure that the RRL BSL-3 laboratory operates according to bio-security standards, AAHL will either provide an expert on bio-containment and biosafety to perform an audit of the RRL building and procedures and make recommendations as needed, or provide training for RRL staff at AAHL. The project will cover these costs.

Whilst performing the animal experiments in Vietnam, training was provided to Vietnamese staff in bio-security. In addition, AAHL staff wrote all the SOPs to work in a high containment animal facility and these were tested and improved as a collaborative project between the various role players. In addition, the collaborators in Vietnam received training in the execution of animal experiments and exposure to Australian animal ethics regulations. AAHL staff also provided on-the-job training to scientists in the laboratories whilst working there.

A training workshop for six Vietnamese early career scientists was held at AAHL from 11–30 August 2013 where they received training in various aspects related to FMD diagnosis, cell culture, and also visited the Large Animal Facility at AAHL. NAVETCO has subsequently bought FMD ELISA reagents and is in a position to do their own serological testing, thereby increasing capacity for FMD diagnostics in Vietnam.

One person each from NAVETCO and RAHO6 were sponsored to attend the Global FMD Research Alliance (GFRA) meeting in South Africa, 17–19 April 2012.

5.2.5 Capacity building in Australia

The project has appointed two research scientists and one research assistant. The scientists both had prior experience in FMD research but have gained significantly more since working on the project. CSIRO has offered one person an indefinite position, thereby ensuring the capacity will be retained.

Several AAHL staff have also had the opportunity to participate in the animal and laboratory experiments, thereby ensuring more staff are familiar with clinical FMD and laboratory assays. In addition, one staff member from the Department of Primary Industries in Western Australia also participated in an animal trial.

6 Impact on meat and livestock industry – Now and in five years time

Foot-and-mouth disease continues to be a serious threat to the meat and livestock industries in Australia. It is of high importance to continue research and capacity building both here and in neighbouring countries to improve diagnostic capability and increase our understanding of the epidemiology and pathogenesis of FMDV strains in the region. It is also essential that vaccine efficacy studies in relevant animal species are ongoing, ensuring protection against newly emerging strains of this continually evolving the virus. These actions, as implemented in the first phase of this project, will help to ensure a high level of preparedness and an effective response in the face of an incursion.

The knowledge gained in this project will in future directly impact on the choice of FMDV vaccine strains to be kept in the Australian vaccine bank. It also provides the country with the opportunity to appropriately address issues regarding newly emerged strains, be better prepared to control an outbreak and use vaccines in a cost effective manner.

The Australian industries will have firsthand knowledge on strains that are potential risks and be in a position to respond by either updating the vaccine bank if needed, or engaging with vaccine manufacturers to develop new vaccine strains. The industries can therefore be proactive, rather than reactive.

Improved diagnostics methods, availability of diagnostic reagents, roll out of these to state labs and experienced staff increase the laboratory capacity in the country and ensure that an outbreak can be rapidly and accurately diagnosed. Knowledge of the correct samples to take for testing will also further increase the country's capacity for an early response and protect the livestock industries.

The data from the project that could be used in dispersion models will ensure more accurate predictions are made in the face of an outbreak, and that control measures can be targeted where most needed. This could decrease the amount of time it takes to control an outbreak and lead to more cost effective control measures.

The outcome of the project is that Australian livestock industries, and the Australian community, will be better prepared to minimise the inevitable disruption to trade and domestic livestock markets that would be caused by an outbreak of FMD.

7 Conclusions and recommendations

The project has enabled Australia to make a significant contribution to FMD research, not only locally, but internationally. Australia now has a significant position in the

Global FMD Research Alliance by virtue of its international collaborations and scientific contribution. The collaboration with various prestigious research groups puts the country in a very strong position regarding access to expertise not locally available. Decisions regarding the vaccine bank will be influenced by the outcome of the project and information will be incorporated into contingency plans in the case of an outbreak.

The project has now entered Phase 2 and will address the emergence of a new serotype A virus in SEA that may be antigenically different to the vaccine strains, leading to reports of vaccine failure. However, it is probable that such variants will arise in the future and therefore the possibility of future funding should be considered.

8 Acknowledgements

Funding for this work was provided through the Meat and Livestock Australia Donor Company matching arrangements with the Australian Government. Animal Health Australia provides a project management service for all relevant industries. The active involvement of Johann Schroder (MLA) and Kevin de Witte (AHA) is highly appreciated. The livestock industries are acknowledged for their financial support of the project.

Funding agencies are:

- Cattle Council of Australia (CCA Inc)
- Australian Dairy Farmers (ADF)
- Australian Lot Feeders' Association (ALFA)
- Sheepmeat Council of Australia (SCA)
- Wool Producers Australia (WPA)
- Australian Pork Limited (APL)
- Goat Industry Council of Australia (GICA)

All the collaborators are recognized for their involvement, assistance and scientific inputs. In all cases the in-kind contributions by the collaborators have added to the success of the project.

- National Veterinary Company, Ho Chi Minh City, Vietnam
- Regional Animal Health Office 6, Ho Chi Minh City, Vietnam
- Pirbright Laboratories, United Kingdom
- Plum Island Animal Diseases Centre, USA
- National Centre for Foreign Animal Diseases, Canada
- Merial
- Global FMD Research Alliance

The contribution of the external scientific evaluators is highly appreciated

- Aldo Dekker, Central Veterinary Institute, Wageningen UR, the Netherlands
- Luis Rodriquez, Plum Island Animal Diseases Centre, USA