Project Title: Livestock Vaccines Against Viral Diseases for Developing Farmers in sub-Saharan Africa

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Research Organizations Involved in the Study:

Agricultural Research Council-Onderstepoort Veterinary Institute
University of Alberta
National Centre for Foreign Animal Disease
Vaccine and Infectious Disease Organisation – International Vaccine Centre

Location of Study: South Africa
Canada

By: Lorne Babiuk, University of Alberta
David Wallace, ARC-OVI

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***Abstract:** *Research outputs should include an abstract of 150-200 words specifying the issue under investigation, the methodology, major findings, and overall impact.*

Infectious diseases cause significant economic losses especially in Africa where many diseases are endemic and not effectively controlled. This project focused on the development of a thermo-stable single dose multivalent vaccine offering protection against Rift Valley fever (RVF), peste des petits ruminants (PPR), and capripox diseases. To achieve this, an attenuated Lumpy Skin Disease Virus (LSDV) vector has been developed and demonstrated to be safe and effective in sheep and goats. This vector was then used to generate a multivalent vaccine construct by inserting protective antigen genes from RVF and PPR viruses. Expression of the antigens has been demonstrated in the vaccine vector construct in cell culture using Western blotting and/or mass spectrometry. The vaccine construct is currently being evaluated for efficacy in sheep and goats against capripox, RVF and PPR viruses. In addition, African swine fever vaccine candidates were developed using porcine adenovirus as a vaccine vector and have been evaluated in swine for immunogenicity. The successful demonstration of the efficacy of these vaccines will be followed by field trials to demonstrate efficacy in the field, pending regulatory approval. In order to encourage uptake of the vaccines once they are available, livestock farmer education, pilot studies on gender roles in livestock owner communities and economic impact studies of two of these diseases were conducted in South Africa.

***Keywords:** Vaccine, lumpy skin disease, sheep and goat pox, peste des petits ruminants, Rift valley fever, African swine fever, food security

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EXECUTIVE SUMMARY

This project was aimed at the development of two vaccines: a single vaccine product offering protection against multiple viral diseases of significant regional and global economic importance: Rift Valley fever (RVF), peste des petits ruminants (PPR), sheep pox (SP), goat pox (GP), and lumpy skin disease (LSD), as well as a second vectored-vaccine against African swine fever (ASF). These products offer significant benefits – thermostability, single-dose administration, single immunization-multiplex protection - in comparison to existing vaccines or, as it is in the case with ASF, protection against a disease with no currently existing vaccine. The project will contribute significantly to food security for smallholder (emerging) farmers, with impact on major species of farm animals: cattle, sheep, goats and pigs. The results of effective prevention of the diseases described above will have positive impacts on the lives and security of women, which are often in charge of livestock on smallholder farms in parts of Africa.

A LSDV knockout (KO) virus, being developed as a vaccine vector was demonstrated to be attenuated in cattle, however the KO construct induces an injection site reaction that is not acceptable for its use as a vaccine in cattle. Further attenuation of the construct will be performed by knocking out additional genes responsible for this reaction. The LSDV KO virus was demonstrated to be safe in sheep and goats at 300 plaque forming units and did not cause any unacceptable injection site reactions. The vaccine did not cause viremia as well as viral shedding in oral and nasal secretions following vaccination indicating that the vaccine did not replicate beyond the injection site. This vaccine was able to protect sheep and goats against sheep pox and goat pox challenge. This LSDV KO virus was used to construct two multivalent vaccine constructs with RVF and PPR protective antigens expressed in both secreted and non-secreted forms. Expression of the RVFV glycoproteins and PPRV fusion protein were demonstrated from both these vaccine constructs using Western blot analysis and/or mass spectrometry. We are currently evaluating both constructs in sheep and goats for protection against PPR and sheep and goat pox, as well as the generation of RVFV antibodies.

Similar requirements were pursued for the vectored vaccine for African swine fever. Currently 6 adenoviral vectors are complete and have been tested in clinical trials.

A plan for training veterinarians and animal health technicians was developed and implemented with training sessions taking place in May 2013 and February 2014 and with information media developed and distributed. A socio-economic analysis was conducted in collaboration with the ARC and Human Sciences Research Council (HSRC) in South Africa, and Lethbridge University in Canada.

Rift Valley fever (RVF) and lumpy skin disease (LSD) outbreaks have been experienced in in different parts of South Africa in recent years. Whilst RVF is characterized by high rates of abortion and neonatal mortality, LSD is deemed less serious by respondents to the economic impact study. Surveys were conducted to estimate the extent of expenditures and losses to livestock

farmers in South Africa, related to the two diseases. As LSD is not a controlled disease, it is difficult to enforce surveillance and vaccination. Although it does not cause major mortalities, farmers are keen to vaccinate if informed of a likely outbreak, to limit production losses. Most of the 150 farmers surveyed in the most affected areas of SA vaccinated against RVF, but roughly a third (31.3%) still incurred animal losses; with an equal spread across provinces and type of farm. Sheep were the most vulnerable livestock species with the most impact to farmers with large herds. Communal farmers, who mostly keep cattle and goats, and where general State sponsored vaccination took place, were far less affected. The survey revealed 4 783 animal mortalities and 6 460 abortions due to RVF, much higher than indicated by official notifications. At farm level economic costs to RVF were estimated using deterministic model. Since the main emphasis of the survey was to estimate economic costs of those farmers who suffered from RVF, the survey was deliberately conducted in areas where it was known that a high rate of disease incidence was incurred. While this method enhanced the collection of cost and loss data, it could not be used to estimate the overall financial impacts on the livestock industry at the national level. Hence, the farm level results were scaled up to estimate national losses using two conversion methods; Scalar A (484/32 = 15.125) treating all affected farms as separate outbreaks and Scalar B (484/10 = 48.4) treating the geographic location of the farm as 1 outbreak. These conversion methods were based on the reported number (484) of outbreaks by Pienaar and Thompson (2013). A conservative scaling up of the results to national level resulted in an estimated loss of R66.7 million (81.6 million in 2014 Rand; C$8.2 million) whilst a more realistic scaling factor resulted in an estimated total national production loss in 2010 of R213.6 million (261.2 million in 2014 Rand; C$26.1 million). These are based on losses due to mortality and reductions in milk produced and exclude losses in production of other animal products for which data were not available. The sporadic nature of RVF outbreaks results in inconsistent vaccination practices, but vaccination is effective and the development of vaccines is a priority, as RVF is of significant economic importance.

Two PhD students in ARC-OVI and 5 Post-doctoral fellows in Canada participated in this project. Courses for farmers and animal health practitioners were offered in South Africa to 120 trainees. Results were published in two peer-reviewed articles and were presented in scientific and policy forums in South Africa, Canada and other countries.

The project was in close collaboration with project 106929 (Development of a Vaccine to Eradicate Contagious Bovine Pleuropneumonia in Africa).

The data obtained during the project has potential commercial value and therefore, publication has been delayed until the evaluation of all details related to protection of intellectual property has been finalized. Overall the project milestones have been achieved.

THE RESEARCH PROBLEM

The African continent is the home of 12 of the 16 most devastating animal diseases; and eight of these are present in South Africa. Many of these are viral diseases responsible for impaired agricultural development. In addition, new pathogens and new diseases, several of which are vector-borne are emerging, at a relatively predictable rate based upon historic trends.

The proposal addresses five different diseases listed by World Organization for Animal Health (OIE): Rift Valley fever (RVF), peste des petits ruminants (PPR), lumpy skin disease (LSD), sheep (SP) and goat pox (GP) and African swine fever (ASF) which are of importance to smallholder farmers in Africa. These diseases were chosen for development of a strategy for prevention/eradication by vaccination.

The novel vaccine approaches under development in this project are platform technologies, which can be applied to other diseases of interest, including those which emerge in the future. Control of these five diseases is also significant from the perspective of international trade, as the presence of the named diseases represents a significant barrier to the movement of animals and their products, a restriction that is not limited to Africa. The research will also contribute to the development of manufacturing capacity of the African biotechnology sector through new products for international distribution.

The research process for the project had two main directions of research:

- Development of a single multivalent vaccine against RVF, PPR, LSD and sheep and goat pox, using an LSDV vector.
- Development of a vaccine against ASF, using an adenoviral vector.

The overall progress of the project is on track towards the development stage for these vaccines. The project rationale and the PPR infection model have been written as scientific articles to contribute to scientific knowledge that may influence policy.

PROGRESS TOWARDS MILESTONES

Twelve Month Milestones:

Milestone 1. Inception workshop and development of a detailed work plan: The inception workshop took place in Jacaranda Hotel in Nairobi, Kenya on July 1-2, 2012.

Milestone 2. Hiring staff and purchasing of equipment: Staff was hired at ARC-OVI (Dr. Thireshni Chetty, Tinyiko Fanti, Dr. Ronica Ramsout and Sharon Lerooibaaki, Donald Makgholo and Derio Makgoale) Note: Replacement of staff: Due to resignation of staff members, additional staff were hired at ARC-OVI (resignations: Donald Makgholo and Ronica Ramsout; hired staff: Faith Nkosi and Kgabo Motona and Ntabiseng Dujta [student]), University of Alberta (Dr. Thang Truong and Dr. Hani Boshra), NCFAD (Dr. Charles Nfon) and VIDO-InterVac (Kyle Brown, Robert Brownlie, Wayne Connor, Pankaj Kumar, Mario Ortega). Equipment was purchased at ARC-OVI**.**

Milestone 3. Assembly of Scientific Advisory Board: the board was assembled, including Dr. Joseph Musaa (Ministry of Livestock and Fisheries Development, Department of Veterinary Services, Kabete, Kenya), Dr. Willie Donahie (Moredun Research Institure, Penicuik, Edinburgh EH530QA), Dr. Adrian Hill (Jenner Institute, Oxford 0X3 7DQ, UK), Dr. Robin Nicholas (Animal Health and Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB UK) and Dr. Dieter Schillinger (Animal Health Consultancy, D-81247 Munich, Germany).

Milestone 4. Identification of ASF genes with protective potential: The ARC-OVI team led by Dr. Livio Heath, has selected five genes as candidates for vaccine development: p54, p30, p220, p70 and CD2-like protein – all from a Southern European isolate of ASFV. All but p220 were cloned and sent to VIDO-InterVac for insertion into porcine adenovirus.

Milestone 5. Evaluation of the LSDV (LSDV KO_1 and LSDV KO_2) knockouts to determine the optimal LSDV vector: Since rabbits were not found to be a suitable small animal model for LSD, further evaluation was conducted in cattle. The results of these trials determined the optimal LSDV vector to be used for the construction of the multivalent vaccine. LSDV KO_1 was selected as the vaccine vector construct.

Milestone 6. Expression of ASF genes with protective potential: Protein specific antibodies to detect expression of recombinant proteins were generated, and recombinant (PAdV-3) expressing ASFV vaccine antigens constructed using codon-optimized AFSV genes.

Milestone 7. BAC-LSDV generation: Due to problems encountered with antibiotic selection the generation and selection of recombinants using a BAC system was not viable and thus a more conventional method was used for vaccine construction.

Milestone 8. Utilize BAC-LSDV construct to optimize the LSDV vector to elicit improved immune responses to expressed antigens (ARC and NCFAD): Unfortunately the BAC-LSDV construct did not work since antibiotic selection was problematic. As suggested in the grant proposal this task was accomplished by reverting back to conventional methods.

Milestone 9. Communication and vaccine delivery systems strategy (including plans to train farmers) developed: The first training workshop was organised at the end of May 2013, targeting participants from the Southern African Developing Countries (SADC), and covering RVF, LSD, PPR, ASF, SP and GP. Information pamphlets/brochures were developed.
During a meeting with communal farmers, it was agreed that they would follow a 2 year animal health programme and track changes in their animal productivity and general health. If they found the programme helpful (losing less animals, increased milk production, increased income for meat sold, etc) then the programme will become permanent and the ARC-OVI would re-evaluate on a biannual basis.
Training programmes were designed for animal extension officers as well as state veterinarians so that they are better equipped to assist rural developing farmers using whatever resources these farmers have available to them.

Milestone 10. Review article describing the project prepared for a peer reviewed international journal: The article has been accepted in Journal of Antiviral Research. An additional manuscript is being written on the peste des petits ruminants challenge model in sheep and goats that was developed for this project.

Twenty-Four Month Milestones:

Milestone 1. Organize and conduct a Midterm meeting: All team members, the Scientific Advisory Board, and the team of CIFSRF project 106929 had a joint mid-term meeting in Banff, Alberta, from May 01-03 2013. Progress to date and the way forward were discussed, with attention directed towards future synergy of the efforts in Kenya and South Africa. The need for such synergy was confirmed by all participants specifically when looking towards the transition from research/development to field testing and pre-production of the vaccines being developed by both project teams. Meeting participants recommended that the time and effort dedicated to the socio-economic objectives of the projects be increased. As a result significant time and effort were directed towards the socio-economic component of the project and an agreement was signed with the South Afrcian Human Sciences Research Council (HSRC), in collaboration with the ARC Economic and Biometrical Services.

Milestone 2. Conduct training for veterinarians on new diagnostic techniques and prevention of ASF, RVF, PPR and LSD: Training for veterinarians was provided on four separate occasions in Pretoria, Soweto, Brits and Kyalami; the first three events took place in May, August and September 2013, and the last in February 2014.

Also a leaflet and two posters were prepared and printed for participants, with posters being laminated for multiple uses in planning animal care work. Training was focused on realistic conditions, like problem identification, biosecurity and disease control in no-equipment field conditions, sample collection and diagnostics. Team members also participated in ARC-organised 2-week training courses for animal technicians.

Milestone 3. Optimize the LSDV vector to elicit improved immune responses to expressed antigens: The LSDV vector has undergone the necessary genetic engineering and different promoters were evaluated using luciferase and GFP reporter genes to identify the optimal promoters for the RVF and PPR virus antigens. In addition, antigen secretion signals were used for the RVF and PPR virus antigen genes as it may be possible to use this strategy to enhance antibody responses to these antigens in target animals. One LSDV KO virus was demonstrated to be attenuated in cattle, however, it caused a local injection site reaction that is not acceptable for its use as a vaccine. Further attenuation of the construct will be performed by knocking out additional genes which may be responsible for causing this reaction. The LSDV KO virus was demonstrated to be safe in sheep and goats at 300 plaque forming units and did not cause any unacceptable injection site reactions. The vaccine did not cause viremia, nor viral shedding in oral or nasal secretions following vaccination, indicating that the vaccine did not replicate beyond the injection site. This vaccine was able to protect sheep and goats against sheep pox and goat pox challenges respectively.

Milestone 4. Generate LSDV-vectored constructs expressing protective antigens for RVF and PPR: The LSDV KO virus was used to construct two multivalent vaccine constructs with RVF and PPR virus protective antigens expressed in both secreted and non-secreted forms. Expression of the RVF virus glycoproteins (GPs) and PPR virus fusion (F) protein have been demonstrated from both these vaccine constructs using Western blot analysis and/or mass spectrometry. We are currently evaluating these vaccine constructs in sheep and goats for protection against PPR and sheep and goat pox, as well as the generation of RVFV antibodies.

Milestone 5. Develop adenovirus-vectored constructs containing ASF antigen genes: Recombinant porcine adenovirus constructs were made for codon-optimized ASF virus genes p30, p54, CD2V, p220, p72, D117 and EP152R.

Milestone 6. Evaluate the adenovirus-ASF constructs in swine for immunogenicity to ASF: Rabbit antisera were produced for p30, p54, CD2V and p72 ASF virus genes and a peptide library was synthesized for measuring T-cell responses. The induction of immune response was analyzed in pigs immunized with recombinant PAdV-3 constructs expressing individual candidate vaccine antigens of ASFV.

Thirty Month Milestones:

Milestone 1. Evaluate the LSD vaccine construct for protection to capripox, RVF and PPR. Protection against virulent PPRV challenge in sheep and goats has been demonstrated. Protection against RVFV challenge has been delayed as a result of regulatory issues pertaining to the trial and national disease priorities requiring use of the animal facility. Protection against capripox challenge is underway.

Milestone 2. Evaluate the adenovirus vaccine constructs for protection to ASF in swine. Adenoviral ASF constructs' immunogenicity in pigs was finalized and analyzed. Evaluation of protection is scheduled to start in the Canadian fall to determine the efficacy of the vaccine constructs.

Milestone 3. Effective LSDV vector-based vaccine for capripox, PPR and RVF. The trials currently underway on the LSDV-RVF-PPR construct will determine if we have a vaccine that is suitable for evaluation in field trials, pending regulatory approval.

Milestone 4. Testing an adenovirus-vectored ASF vaccine. Some of the ASF adnovirus vaccine constructs have been evaluated for immunogenicity in swine specifically to evaluate ASF-specific cell mediated immune responses.

Milestone 5. Develop an implementation plan and rollout strategy for South Africa and elsewhere. The project was granted funding for a second, developmental phase. Therefore we have re-directed both the implementation plan and rollout strategy development towards Phase 2, when socio-economic data and dialogue with manufacturers will greatly strengthen the plan.

Milestone 6. Publication of results in scientific journals and presentations at conferences/meetings. The work in this project has been presented at a number of conferences, including the Prairie Infectious Immunology Network Conference 2012, Canadian Animal Health Laboratorians Network 11th Annual Meeting-2013, Agri Youth Indaba-2013, Agricultural colleges Educators' workshop-2013, International Food Security Dialogue 2014 in Edmonton, International Food Security Dialogue 2014 in Addis and Agricultural Research for Development Impacts – CIFSRF Africa Symposium Kenya. Two publications have resulted: an article on PPR in 2014 (http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0087145) and another article on capripox-vectored vaccines was published in "Journal of Antiviral Research" in 2013.

Milestone 7. Preparation of analytical technical report for publication. This current report.

Milestone 8. Prepare a set of policy briefs and research briefs for publication. Research briefs have been published in the newspaper "Business Times" - South Africa, and on ARC and IDRC

websites. Publications and policy brief/s resulting from the socio-economic and economic impact studies are also being prepared.

Milestone 9. Prepare two drafts for publication in peer-reviewed international journals. A manuscript describing the attenuation of the LSDV KO in cattle is being written. In addition, a manuscript on the use of the LDSV KO in sheep and goats to protect against sheep and goat pox has been written. Both these papers will be submitted following the filing of a provisional patent on the LSDV KO construct for use as a vaccine vector. The application for this provisional patent is already in progress.

SYNTHESIS OF RESEARCH ACTIVITIES AND RESULTS

Below are the objectives as listed in the proposal:

i) Identify novel antigens that can be used as vaccine candidates for ASF.

The ARC-OVI team, led by Dr. Livio Heath, has selected five genes as candidates for a vaccine: p54, p30, p220, p72 and CD2-like protein – all from a southern European isolate of ASFV.
The genes were selected based on previous data relating to p30, p54, and p72 immunogenicity. Sera generated against p54 and p72 are known to prevent virus attachment, while sera against p30 inhibit virus internalization. Protective efficacy in animals injected with the proteins was only demonstrated by delays in clinical manifestation and decreased viremia. However, since our project is focused on delivering the candidate vaccine by adenoviral vector we need to evaluate the type of immune response, which can have significantly different protective effects. A second set of genes of interest was identified, consisting of D117 and EP152R, both expressed early in infection. Expression of these genes was found to be associated with IL8, IL12, IL1a, IL4 and TNF expression in macrophages.

ii) Develop an improved LSD vector vaccine containing RVF and PPR genes encoding immuno-protective antigens.

Previously the group of Drs. Wallace and Mather generated a number of LSDV recombinant viruses in order to produce a LSD vaccine with improved protective efficacy. These contain knockout mutations targeting putative immunomodulatory genes – LSDV KO_1 and LSDV KO_2.
The LSDV Warmbaths field isolate and the OBP LSD vaccine were tested in rabbits to evaluate their suitability as a small animal model for LSD, but no measurable immune responses. Since the rabbit model has limitations, further evaluation was confirmed in cattle, the host species. The two LSDV knockout constructs (LSDV KO_1 and LSDV KO_2), using the Warmbaths field strain of LSDV as parental virus, were evaluated in cattle for residual virulence (using a high vaccine dose)

and ability to protect against challenge (Figure 1). Animals were vaccinated and challenged 4 weeks later.

Most vaccinated cattle showed marked local reactions at the vaccination sites, with some fever, and virus was isolated from 3 out of 5 cattle inoculated with LSDV KO_1 construct but not from the cattle in the LSDV KO_1 group. A number of animals inoculated with the LSDV KO_1 construct showed generalised lesions and were euthanazed. Immune responses (humoral and cellular) were induced in the remaining animals (group LSDV KO_1) and they were protected from challenge.

The LSDV KO_1 construct was then evaluated in sheep and goats for safety and immunogenicity. Sheep and goats were vaccinated with $1 \times 10^{2.3}$ pfu using intradermal injection and were monitored for clinical disease as well as for viral replication in blood and mucosal secretions. Following vaccination sheep and goats, did not develop clinical disease and no capripoxirus-specific viral DNA was detected in blood or mucosal secretions using real-time PCR. Following challenge with virulent sheep and goat pox viruses, all vaccinated sheep and goats were completely protected, whereas all non-vaccinated control animals developed clinical disease, indicating the vaccine is safe and effective in sheep and goats (Figure 2). Antibodies specific for capripoxvirus were evaluated at different time points following vaccination and challenge and neutralizing antibodies were detected in vaccinated animals prior to challenge and in all animals following challenge (Figure 3).

Following these results the LSDV KO_1 construct was chosen as the vector for expression of protective antigens of RVF and PPR viruses, although further attenuation is required for its use in cattle.

For the purpose of improving the immune responses induced to the LSDV-vectored protective antigens, a tissue plasminogen activator (tPA) leader sequence was synthesized and inserted in frame with the RVFV glycoprotein Gn and Gc genes and the luciferase leader sequence in frame with the PPRV F gene. The rationale for this approach is that improved Gn, Gc and F protein secretion *in vitro* will lead to the induction of improved immune responses in the target animals.

In order to select the best promoters to drive expression of the RVFV and PPRV immunogens, putative LSDV gene promoters were identified and aligned with a poxvirus synthetic early-late (pSEL), fowlpox virus early-late and the vaccinia virus (VV) P7.5 early-late promoters. Two of the LSDV early-late promoters identified were then selected for further evaluation. In addition, capripoxvirus early, intermediate and late, and the VV P7.5 early-late promoter were evaluated in a luciferase assay system at both NCFAD and ARC-OVI to determine and compare the strengths of the individual promoters. The results from this experiment determined that the VV P7.5 promoter was the strongest and most suitable for driving expression of the RVF and PPR virus protective antigens.

The RVFV GP genes and the PPRV F gene in both the native and secreted forms were cloned and inserted into the LSDV transfer vector to be used for generating the final vaccine constructs (Figure 4). The two LSDV vaccine constructs, containing the RVF GP and PPR F protective antigen genes, were generated using homologous recombination, followed by selection to homogeneity. The selected vaccine constructs were then evaluated for the expression of the RVF and PPR viral

proteins. This was achieved using Western blot analysis to demonstrate the RVFV GP expression and mass spectrometry to demonstrate the PPR F protein expression (Figure 5). The selectable marker genes were then removed from the vaccine constructs and they are currently being re-evaluated for expression of the RVF and PPR viral proteins.

iii) Develop an adenovirus-vectored vaccine containing ASF antigen genes.

The selected seven genes for vectored ASF vaccine candidate were: p54, p30, p220, p72, CD2-like protein, D117, and EP152R – all from MAL 2011/2, Genotype II of ASFV. All were cloned and sent to VIDO-InterVac for insertion into porcine adenovirus (Figure 6).

The following steps were taken at VIDO-InterVac towards the development of adenovirus-vectored ASF vaccine candidates:

Protein-Specific Antibody Production

To generate protein-specific antibodies to detect expression of recombinant proteins, antigenic peptides were predicted using the online software "Predicted antigen peptides" of Immunomedicine (http://imed.med.ucm.es/Tools/antigenic.pl). Three peptides were chosen for each protein which were located centrally and at the carboxyl and amino terminal ends. The peptides were conjugated with BSA or KLH. Rabbits were immunized with conjugated peptides (500µg/rabbit) emulsified with Freund's Complete Adjuvant (FCA), followed by two injections (conjugated peptide, 250 µg/rabbit) in Freund's incomplete adjuvant (FIA) four weeks apart. Serum was collected twelve days after the third injection to test for protein specific antibodies. Western blot analysis of cell lysates from cells transfected with individual HA tagged ASFV proteins suggested that only p72 specific peptides produced protein-specific antisera.

Recombinant PAdV-3 Expressing ASFV Vaccine Antigens

a) Replication-defective PAdV-3:

A cassette containing CMV promoter and BGH polyA termination signals was PCR amplified from an expression vector (polyCMV.BghpolyA) and cloned into the *Srf*I site of pPAV228 (PAdV-3 E1 transfer vector), which allows integration into the E1 region, to give pPAV228-CMV. The open reading frames of ASFV proteins P30, P54, P72 and CD2V were amplified by PCR using gene specific primers and cloned downstream of the CMV promoter within pPAV228-CMV, creating plasmids p228.p30, p228.p54, p228.72 and p228.CD2V. Full length recombinant plasmids were generated by recombining *Pme*I digested Individual recombinant transfer plasmid with *Pac*I digested plasmid pFPAV228 (E1 deleted full length PAdV-3 genomic DNA in a plasmid) DNA in *E. coli* BJ5183.

The VR1BL cells were transfected with 5-10µg of individual *Pac*I digested plasmid DNAs. The cells showing cytopathic effects in 7-10 days were collected freeze thawed and recombinant viruses were expanded.

b) Replication-competent PAdV-3:

The smaller version of CMV promoter (CMV462) was cloned into *SnaB*I site of pPAV300 (E3 transfer vector) creating plasmid pPAV300-CMV462. The open reading frames of ASFV proteins P30, P54, P72, CD2V, D117 and E152R were amplified by PCR using gene specific primers and cloned downstream of the CMV promoter within pPAV300-CMV462, creating plasmids p.300.p30, p300.p54, p300.p72 and p300.pCD2V, p300.D117 and p300.E152R. Since p220 gene is long, we PCR amplified six fragments (each containing an ATG and a stop codon) covering the whole p220 gene. The individual ORFs were clones downstream of CMV promoter within pPAV300-CMV462 creating plasmids p300.220a, p300.220b, p300.200c, p300.220d and p300.220e. Full length recombinant plasmids were generated by recombining individual DNA fragment I (4-5kb *EcoR*I-*Not*I fragment containing specific gene cassette) with *SnaB*I digested plasmid pFPAV300 (E3 deleted full length PAdV-3 genomic DNA in a plasmid) DNA in E. coli BJ5183.

The VIDO R1/VR1BL cells were transfected with 5-10μg of individual *Pac*I digested plasmid DNAs. The cells showing cytopathic effects in 7-10 days were collected freeze thawed and recombinant viruses were expanded. The identity of each recombinant virus was confirmed by restriction enzyme analysis of virion DNA, RT-PCR analysis of gene specific mRNA\Western blot analysis of recombinant protein (Figure 7).

c) Synthesizing codon optimized ASFV genes:

To further optimize the expression of ASFV genes in recombinant PAdV-3, we have also synthesized (GenScript) codon optimized (porcine species) ASFV genes (p30, p54, p72, CDV2, D117 and E157R containing Kozak sequence and flanked by *Nhe*I-*Kpn*I restriction enzyme sites. The blunt end repaired Nhe1-KpnI fragment of individual genes was cloned downstream of the CMV promoter within pPAV300-CMV462 creating plasmid p.300.p30s, p300.p54s, p300.p72s p300.pCD2Vs, p300.D117s and p300.E157Rs. Using approach described earlier (Section b), we constructed full length genomic clones of PAdV-3 containing individual ORFs inserted in the E3 region. Transfection of VIDO R1\VR1BLcells with individual recombinant genomic DNA produced cytopathic effects. The infected cells were collected, freeze-thawed and recombinant viruses were expanded. The identity of each recombinant virus was confirmed by restriction enzyme analysis of virion DNA, RT-PCR analysis of gene specific mRNA\Western blot analysis of recombinant proteins (Figure 7, 8, 9 and 10).

iv) Evaluate the LSDV-vectored vaccine constructs for immunogenicity in cattle, sheep and goats to capripoxvirus, RVFV and PPRV.

The LSDV KO constructs expressing the RVF and PPR viral antigens in both their native and secreted forms are currently being evaluated in sheep and goats. Sera from various time points following infection will be collected and evaluated for antibodies to sheep and goat pox viruses, PPRv F and RVFV PG using ELISAs, as well as virus neutralization testing. The necessary

serological tests have been developed for this project. In addition, cell-mediated immune responses to the antigens will be evaluated following vaccination.
The vaccine constructs will not be evaluated in cattle at this stage as further attenuation of the vaccine vector for use in cattle is required.

v) Evaluate the adenovirus-ASFV constructs in swine for immunogenicity to ASFV.

To evaluate the immunogenicity of recombinant PAdV-3 constructs expressing ASFV candidate vaccine antigens, three-four week old pigs (6/group) were immunized intramuscularly (I/M) with 10^6 $TCID_{50}$/pig or $2X10^7$ $TCID_{50}$/pig twice at day 1 and day 21 with individual replication-competent recombinant PAdV-3 constructs expressing individual candidate vaccine antigens. A third group (6 pigs/group) acted as controls. For each animal, blood was collected for sera at 0, 15 and 30-32 days post first immunization. EDTA blood was collected at day 30-32 post first immunization for analysis of PBMCs. A15-mer peptide library containing 5-mer overlap representing all vaccine antigens (p30, p72, p54, D117, E157R, p220, pCDV2) was synthesized and used as antigen for PBMC stimulation (Figure 11). Analysis of PBMCs using the peptide library indicated p72 contains epitopes recognized by $CD4^+$ and gD-T cells. Moreover, analysis of PBMCs using an Interferon γ Elispot assay demonstrated that p72 contains potential epitopes involved in the induction of interferon γ suggesting that p72 may be involved in inducing cellular immunity (Figure 12). Presently, PBMCs stimulated with overlapping peptide library are being analysed by ELISAs (supernatants) for cytokine profiles and RT-qPCR (cells) for cytokine specific mRNA. In addition, sera collected from various time points following immunization is being evaluated for induction of antibody responses.

vi) Evaluate the LSDV vaccine construct for protection to RVF, PPR and capripox in cattle, sheep, and goats.

The LSDV KO vaccine constructs expressing the RVF and PPR viral antigens in both their native and secreted forms are currently being evaluated in sheep and goats. Following vaccination sheep and goats will be challenged with PPRV. A relevant PPR virus challenge model has been developed in both sheep and goats, and appropriate diagnostic tests, including real-time RT-PCR, whole virus ELISA, virus neutralization assays, histology and immunohistochemistry, have been developed to compare and differentiate vaccinated and non-vaccinated sheep and goats. Sheep and goat pox, and RVF, challenge models and diagnostic tests have previously been developed. Therefore all the necessary challenge models are available for use to evaluate the LSDV-vectored vaccine constructs. Sheep and goats will be monitored for clinical disease and swabs and blood will be collected from both vaccinated and control animals to compare PPR viral replication. Following the PPR challenge, the vaccinated sheep and goats will be challenged with sheep and goat pox to demonstrate the continued ability of the modified LSDV KO vaccine constructs, now containing the protective antigens of RVF and PPR viruses, to protect against sheep and goat pox. The necessary

permission is currently being sought from the regulatory authorities to evaluate the vaccine constructs in sheep and goats for protection against RVF challenge.

vii) Evaluate the vaccine constructs for protection to ASF in swine.

An ASF challenge model has been developed in swine using ASF infected pig blood delivered by oral and intranasal administration that results in pigs developing ASF clinical signs of disease and death 6-9 days following infection. This model was used to evaluate a DNA vaccine for ASF with and without electroporation. Unfortunately this DNA vaccine did not prevent death in pigs, but was able to reduce time to death and viral loads in blood compared to non-vaccinated pigs. The evaluation of the adenovirus ASF vaccine constructs is ready and will be started in the fall.

viii) Educate developing farmers through training and information dissemination relating to the specified diseases (RVF, PPR, capripox and ASF), and their control.

The first training workshop was organised, involving participants from the Southern African Developing Countries (SADC). It took place at the ARC-OVI at the end of May 2013. It covered RVF, LSD, PPR, ASF and S&GP. Information pamphlets/brochures were also developed, utilising the information gleamed from two field trips to Bultfontein, to meet the specific needs of veterinary extension officers and developing farmers.
In addition, more training sessions were conducted: In August 2013 in Soweto with Agri Youth; in September 2013 in Brits at the Agricultural colleges Educators workshop and in February 2014 in Kyalami at the Veterinarians Journal Club and in August 2014 at the ARC-OVI.

In fulfilling the IDRC's directive the South African Co-PI's and key team members participated in a gender awareness workshop in April 2013 at Glenburn Lodge (Krugersdorp) and in a communication and policy brief development workshop in November 2013 at Roodevallei Lodge (Pretoria).
After signing an Amendment to the Memorandum of Grant Conditions (attached) two new objectives were added:

ix) To establish the economic impacts of the effects of Rift Valley fever and lumpy skin disease and the benefits of vaccination in South Africa

Rift Valley fever (RVF) and lumpy skin disease (LSD) outbreaks have been experienced in different parts of South Africa in recent years. Both are spread by insects (mosquitoes and biting flies respectively). Whilst RVF is characterized by high rates of abortion and neonatal mortality, LSD is deemed less serious by survey respondents, although mortality was reported. Two surveys were conducted to estimate the extent of expenditures and losses to livestock farmers in South Africa, by the two diseases:
The first in early 2013 gauged losses to LSD and was conducted in 12 villages in Marble Hall in a district of Limpopo where isolated incidents of LSD were experienced between 2010 and 2012. A

total of 217 individuals were interviewed. Nearly half (43%) of the 217 sampled farmers were female. The 217 respondents collectively owned 2,448 cattle, 58 sheep and 871 goats. About 50% of the respondents owned between 0-8 head of cattle, 29% between 9-16 cattle, while 21% owned more than 16 cattle (Table 1). Women owned about 88% of all sheep and 41% of goats.

Table 1: Distribution of cattle numbers

Number of cattle	Female	Male	Total
0-8	49 (45%)	59 (55%)	108 (50%)
9-16	26 (41%)	38 (59%)	64 (29%)
>16	18 (40%)	27 (60%)	45 (21%)

Source: Survey results

About 31% of respondents indicated that they did not spend any resources on vaccines, while 69% indicated that they incur annual expenses to ensure good health for their livestock (Table 2). The common practice in this area is that farmers in each village contribute a fixed monthly amount towards the purchase of selected vaccines by the farmers group. Farmers are individually responsible for the purchase of any vaccine that is not on the selected list. During the LSD outbreaks farmers were advised to vaccinate and a total of 2,891 cattle were vaccinated in the 12 villages at an estimated cost of R48, 453 (4.8C$).

Table 2: Annual expenditure on vaccines

Expenditure (R)	Female	Male	Total
Free	36 (58%)	31 (46%)	67 (31%)
100- 250	16 (36%)	28 (64%)	44 (20%)
251-500	26 (43%)	34 (57%)	60 (28%)
>500	15 (32%)	31 (67%)	46 (21%)

Source: Survey results

During the survey a loss of 68 cattle to LSD was reported resulting in a revenue loss of R442, 000 (44.2 000 C$). Data on losses in other areas of SA were not available. As LSD is not a controlled disease, it is difficult to enforce surveillance and vaccination. Respondents were however eager to protect their livestock and although LSD does not cause major mortalities, farmers are keen to vaccinate if informed of a likely outbreak, to limit production losses.

A total of 150 livestock farmers were interviewed to determine socio – economic impacts of RVF. A total of 115 farmers were black and 95% of them operated in communal land. Most (77%) of the 150 livestock farmers indicated that they vaccinated against RVF and most often their entire herds, especially in 2010 when the disease hit hardest. The vast majority (93%) of black farmers indicated that the state vaccinated all their animals against RVF, while only 54% of white farmers vaccinated all their livestock against RVF. Consequently, 71% of white commercial farmers were affected by the outbreak compared to 19% of black farmers. The majority (58%) of farmers who did not vaccinate all their livestock incurred animal losses compared to 31% of farmers who vaccinated all their livestock against RVF.

Roughly a third (31.3%) of farmers incurred animal losses - equally spread across provinces and type of farm, but sheep appeared most vulnerable as were farmers with large herds. Communal farmers, who mostly kept cattle and goats, and where general State sponsored vaccination took place, were far less affected. The survey revealed a high rate of animal mortalities and abortions, much higher than indicated by official notifications of the disease. Pienaar and Thompson (2013) indicated that in 2010, "484 outbreaks were reported, with 13,342 animal cases and 8,877 animal deaths. "The 150 farmers in the survey reported 4,783 animal deaths, more than half of all mortalities reported for the whole country. In addition, 6,460 abortions were reported in the survey.

Two methods were used to scale the 2010 survey results to the national level. The first was extremely conservative and resulted in an estimated loss at the national level of R66.7 million (81.6 million in 2014 Rand; C$8.2 million). A more realistic scaling factor resulted in an estimated total national production loss in 2010 of R213.6 million (261.2 million in 2014 Rand; C$26.1 million). These estimates are based on losses reported by farmers in the survey of five types: deaths of pregnant ewes/cows, deaths of non-pregnant ewes/cows, deaths of suckling animals, abortions, and reductions in milk produced. These estimates do not include losses in production of other animal products, such as hides, wool, and mohair, nor do they account for possible reductions in prices of animals or animal products resulting from trade restrictions during the outbreak. Data were not available to assess the extent, if any, of these types of losses. Nevertheless, the survey results reveal a substantial loss in revenues to the livestock industry in South Africa as a result of RVF in 2008, 2009, and, especially, in 2010.

Vaccination for RVF increased from 4% national coverage in 2008 to 87.33% in 2010, decreasing again to 38.67% in 2012. This is likely linked to RVF's sporadic outbreak nature resulting in inconsistent vaccination practices. However, vaccination is an effective strategy in preventing the disease and the development of effective vaccines is a policy priority, also as RVF is of significant economic importance and deserves research attention.

x) To determine small scale farmers' experiences (behaviour patterns, usage, uptake, knowledge, attitudes) in relation to the value of vaccines and their potential benefits related to access, challenges, opportunities, social and economic benefits, and contributions to food security.

To determine small scale farmers' experiences in relation to vaccines and their potential benefits the HSRC conducted a comprehensive study, albeit limited to the cross-sectional perspective of two sites/communities in two adjacent provinces in South Africa. The study provided important and valuable insights into a number of areas that need to be addressed.
The effective use of novel vaccines requires a context in which knowledge and understanding of diseases (causes, symptoms, treatments, prevention, etc.) and vaccines (their purpose, which vaccines are used to prevent which diseases, at which time of year these should be administered etc.) is sufficiently strong, and that other factors such as a strong system, knowledge and practice of preventative animal health is in place. The study revealed the following:

1) Livestock keepers see the need for more effective knowledge and training, with 58% requesting training in understanding disease symptoms, and 57% requesting training vaccination practices.

2) Primary animal healthcare knowledge is generally poor among both female and male livestock keepers. While livestock keepers may be able to identify symptoms, they cannot necessarily link these either to a specific disease, or to its treatment. For instance, few livestock keepers knew that diseases can be tick-borne (36%), and which diseases are tick-borne [page 131 *of the Final HSRC Technical report*]. Most respondents did not know which diseases are zoonotic (87%) [*See pages 83-84 of the Final HSRC Technical report* for qualitative examples and quotes around farmers' understanding of diseases and disease symptoms, and an inability to generally link the two].

Figure 13: *Measuring differences in gender knowledge relating to tick-borne diseases*

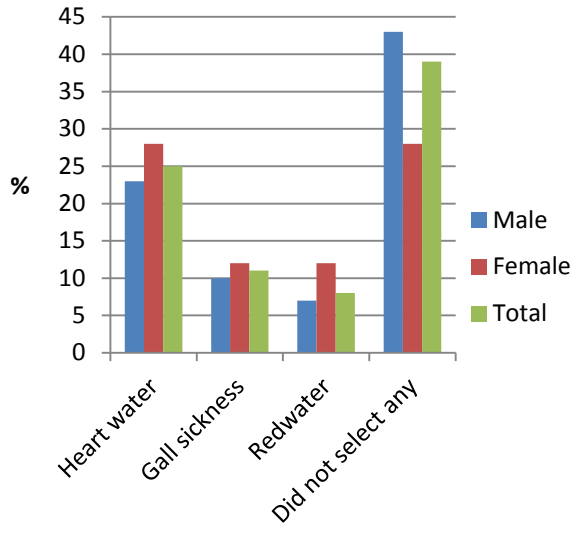
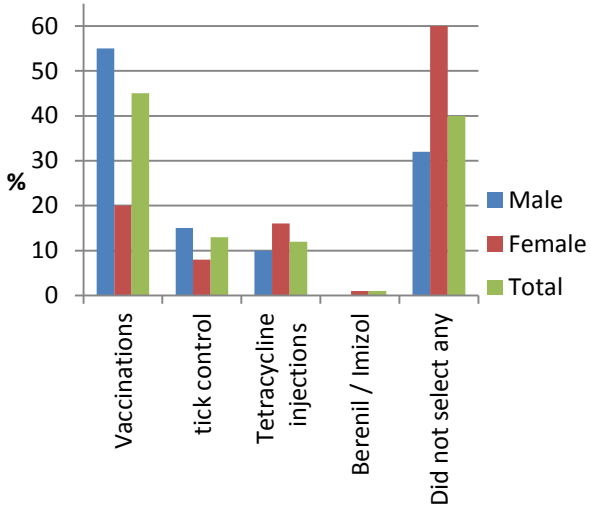


Figure 14: *Measuring male and female knowledge relating to practices for preventing tick-borne diseases*



3) Knowledge of vaccines is limited, including the difference between medicines and vaccines (43% of those surveyed did not think there is any difference between medicines and vaccines, while 23% understood the difference to be that vaccines are preventative while medicines are used to treat diseases), and the diseases for which vaccines can be used as a preventative measure.

4) Knowledge and understanding about how and when diseases are treated, including through the use of vaccines, is captured in the words of a male farmer, which shows that there is some understanding, though this is limited (so for instance, the farmer understands the importance of timing of vaccinations, but perhaps less clearly its preventative purposes):
The doctors know at a certain time they vaccinate for a certain disease, and so on. They set a time table so that they know in which season they are coming. Sometimes they vaccinate for miscarriages, foot and mouth diseases and at times they vaccinate for dry skin diseases. If the disease is too strong for them to use this medication, they send you to take your cow to the doctor […] Sometimes we have diseases like dry skin or sweat. The doctors can say this disease is rife and we need to vaccinate so it won't infect other cows. Sometimes you find that the cow has a foot disease where its foot gets cracks underneath, others get miscarriages. They know when it's time for all of these diseases in order for them to vaccinate.[pages 84-85 of the Final HSRC Technical report]

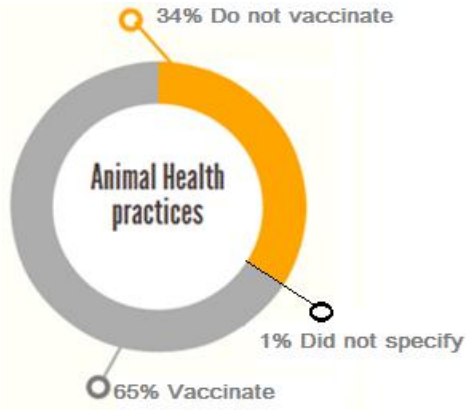
5) Some mistrust exists around vaccines and their use, particularly the idea that vaccines are harmful. For instance, this is the testimony from one animal health practitioner:
"[T]here's this allegation […] let's say maybe [the AHT] has gone to vaccinate some of the cows then you will find out that there are some that would be dying but not due to [the AHT] but due to certain diseases then they associate and say the vet person has been here he's killing our cattle that's why some of them are adamant not to come [for assistance]". [page 17 of the Final HSRC Technical report].

Accordingly, and as a consequence of the general lack of knowledge, the study found that animal health practices among farmers were mostly focused on and limited to two essential practices: 1) the use of Terramycin and 2) dipping their cattle (while de-worming was perhaps a third practice that many – 36% – of farmers relied on). The most commonly used medicine among small-scale livestock keepers is Terramycin – we found that it was often viewed as a cure-all medication and used for any and every disease [page 85 of the Final HSRC Technical report]. The most common disease preventative health practice was dipping, which 97% of farmers engage in [page 130 of the Final HSRC Technical report]. Very few livestock keepers engaged in disease preventative animal health practices beyond dipping, including vaccine use.
While knowledge is a key factor in limiting the use of other disease preventative practices, other factors do play a key role, including, 1) mistrust of state animal health services, and 2) unaffordability of medicines and vaccines, and therefore reliance on cheaper alternatives (including high transport costs limiting access to medication/vaccines, which the state does not provide, and the lack of refrigerators to store them).

Vaccination practices:

1) Two-thirds (N=55, 65%) of households reported vaccinating their livestock and twenty-nine households (34%) reported not vaccinating their livestock. Slightly more male-headed households (N=44, 68%) reported vaccinating their livestock compared to female-headed households (N=11, 60%). [page 133 of the Final HSRC Technical report]

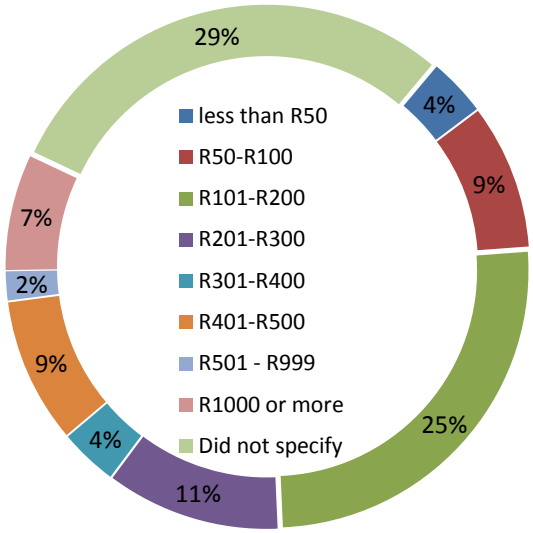
Figure 15: *Measuring vaccination practices.*



2) When asked about the reasons people vaccinate, the statement selected by most respondents was that they believe vaccines to cure animal diseases (N=22, 40%). For those households that do vaccinate their livestock, administration of vaccines are primarily done by an animal health technician (N=26, 47%), and secondarily by the farmer him/herself (N=17, 31%). [page 133 of the Final HSRC Technical report].

3) A quarter (N=14) of households that vaccinate (N=55) reported spending between R101 and R200 on average on vaccines per purchase event. Seven households report paying less than R100 on vaccines per purchasing event, while four indicated they pay R1000 or more for a vaccine on average.

Figure 16: *Average expenditure of households on vaccines per purchasing event*



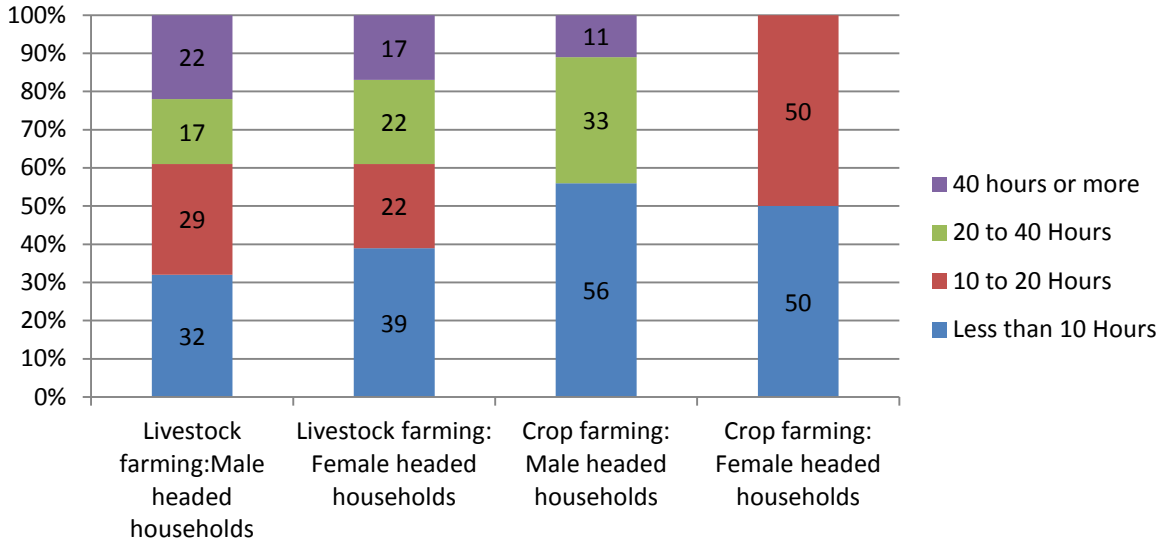
4) Most households (N= 62, 73%) had an estimated monthly income of less than R4000 a month (that equals seven in ten households). Two-fifths (40%) of female-headed households indicated a monthly household income of less than R2000 per month compared to nearly two-fifths (39%) of the male-headed household sample that indicated a monthly household income of between R2000 and R4000 (Table 18 of the Final HSRC Technical report). This is noteworthy as it indicates that female-headed households are generally poorer. Even though gender is related to household income, it should be noted that all households in the sample are generally poor [page 126 of the Final HSRC Technical report].

5) Livestock farming is mainly a male activity whereas crop farming is mainly a female activity. For those involved in mixed farming, males are more likely to spend more time in the livestock component compared to females. [page 129 of the Final HSRC Technical report]

6) Women become involved in livestock keeping primarily due to death of a spouse (which results in the woman becoming the caretaker of the deceased husband's livestock) [page 76 of the Final HSRC Technical report], due to lobola (bride price) [page 77 of the Final HSRC Technical report], or due to inheritance from her parents.

7) Where women are engaged in livestock farming, many do so with poultry and pigs in the areas where the study took place [page 78 of the Final HSRC Technical report].

Figure 17: Time and gender of household heads engaging in farming activities



8) Some women farmers claimed that women are more responsible and reliable in livestock keeping and farming than men: "[Res1:] It is much better when women are farming because they [are] able to make a living [through] that. [Res2:] Women are better than men [when it comes to] farming". When probed further on the reasons for this, the second respondent states: "[…] you should know when a woman is farming if she can sell a cow then that money will come straight home [laughter] if the man can sell a cow you will never see that money […]. [page 77 of the Final HSRC Technical report]

9) Male household heads and male respondents are more likely to manage the day to day activities themselves (63%), compared to female household heads and respondents who primarily hire someone to run the day to day farming activities (59%) (Table 6) [Page 111 of the Final HSRC Technical report].

10) Animal healthcare is seen as a primarily male domain. While women work as animal healthcare workers (Animal Healthcare Technicians) and as State Veterinarians, these women state the difficulty of working with women livestock keepers who they suggest are not as capable as men in caring for their animals.

11) One of the continuing barriers to women's involvement in livestock keeping, particularly of cattle, is that women are in some cases still not allowed to enter kraals [page 78/ 79 of the of the Final HSRC Technical report]. However the taboos around women's bodies and presence have lessened over the years: During a focus group discussion a male farmer in Marble Hall says that while in the past a "girl couldn't get in[to] the kraal but a boy [could], but now they can if a calf is sick [women] can give it medication".

12) Restrictions and fears about women's bodies and presence around kraals and pregnant cows previously affected women's abilities to engage in animal healthcare, as another (female) farmer confirms:
There was a saying then that if a woman walk[s] between the animals when they are pregnant they are going to miscarry the calf, but I think now it's a little bit better. And then they didn't want us women to inject the cattle because if it dies they were going to blame you and say it is dead because of you, but now we touch them anyhow, yes things have changed a lot. [pages 82-83 of the Final HSRC Technical report]

13) The qualitative testimony of animal health practitioners suggests that women are faced with competing challenges in terms of a) physical strength and b) time constraints where women's household activities prevent them from having time to regularly oversee their animal's wellbeing: "they won't find time to check after the animals to see which animals are sick so they only depending from the headbo[ys] to see which animal is sick". [page 16 of the Final HSRC Technical report]

14) Animal health practitioners express frustration when having to deal with female farmers: "They know less […] With livestock they don't know much […] when you find a woman that is doing farming you know you have to do everything even if you can explain to her but they don't have those guts to assist." [page 81 Final HSRC Technical report]

15) Women are mostly 'invisible' at the most frequent point of contact between livestock keepers and state animal health services, the dip tank. As this AHT attests:
In all the dipping I have been to, I haven't seen them, in dipping they don't come, I don't know maybe it is because men are saying they must stay behind or what and then maybe they're not interested. And again those I have met when they come to ask about an animal it is because the husband is not there or maybe he passed away or there is no one who can come. So in most cases we [don't] meet so many women in dipping […] [page 76 of Final HSRC Technical report].

SYNTHESIS OF RESULTS TOWARDS AFS OUTCOMES

1. New technologies and/or farming systems and practices. By the end of the project two new vaccine candidates have been evaluated in experimental settings. The results from these trials will determine if they are suitable candidates for field trials or still require more development prior to field trials. We have utilized new technology that will benefit farmers, the economy and the private sector, this technology is unique allows us to overcome a big challenge of thermal stability - deliver vaccines to remote areas.

2. Dietary diversity & nutrition. Not applicable

3. Engagement of Canadian researchers with Southern researcher organizations (for CIFSRF-funded projects only). Canadian expertise is increased by first-hand knowledge and experience in prevention of foreign animal diseases. Canadian knowledge about the diagnosis and control of foreign animal diseases is also being increased. South African expertise in ASF, LSD and RVF diseases, which are endemic in South Africa, have greatly enhanced collaboration between Canadian and South African researchers.

4. Research groups. The project is intensifying connections and collaboration between Canadian and African research centres, thus improving the capacity of both sides to prevent and/or control outbreaks of economically important infectious livestock diseases. Prevention and/or control of infectious diseases is critical to help maintain and improve food security. We have added to capacity building, trained 5 young scientists, and built a cohesive research team with value added to all the national teams.

5. Food distribution. Not applicable.

6. Food processing and storage. Not applicable to this project.

7. Risk-mitigation. Not applicable to this project stage.

8. Access to resources. Not applicable.

9. Income generation. Not relevant at this stage of the project.

10. Policy options. The primary project outcomes could provide the opportunity for new vaccination campaigns against a number of important animal diseases with envisaged significant positive impacts on food security. A policy brief from HSRC may impact policymakers in South Africa.

11. Information and Communication Technologies (ICTs). The project has no direct impact on communication technologies.

12. Gender. Many of the smallholder farmers in sub-Saharan Africa are women, and thus improving animal health has the potential for direct beneficial effects on multiple aspects of their lives and status. However, the role of gender in livestock farming in South Africa has not been defined. A study was conducted by the HSRC to address this. Some of the key findings are:

13. Environment. Not applicable.

PROBLEMS AND CHALLENGES

Revised national and international policies pertaining to the prevention and control of foreign animal diseases have required some changes be made to the multivalent vaccine development aspects of the project. As southern Africa is free from PPR, no vaccine containing PPRV antigens will be granted a licence for manufacture or use in South Africa. To solve this issue a LSDV-RVF construct will be generated for potential use in South Africa and other African countries free of PPR, but endemic for RVF and capripox in sheep, goats and/or cattle. The LSD-RVF-PPR vaccine construct will still be useful for the rest of Africa in sheep and goats, and possibly cattle, where all the diseases are endemic. The main challenge has been the added load in managing the socio-economic studies. While this was resolved for the current phase of the project, we plan to delegate the coordination of socio-economic, biomedical and regulatory aspects of Phase Two work to a separate person/s in order to optimize the usage of expertise from all team members. The ARC will establish a local project-management committee with each member leading individual work-package project components. The committee chairperson will interact regularly with the phase two project manager and all other project collaborators (Canada, Kenya etc.).

RECOMMENDATIONS

This project is on a tight timeline; however most of the prescribed milestones will still be met. At, or soon after, the end of the project the deliverable to determine the efficacy of the multivalent LSDV vaccine against capripox, PPR and RVF should be achieved. For the efficacy of the ASF vaccine constructs this will take some additional time as these trials will commence in the fall. The efficacy evaluations will be completed under experimental conditions, therefore at the end of the project the data generated will determine if the vaccines may be good candidates to evaluate in field trials, pending regulatory approval. The project scientific advisory board recommends that if the vaccines are demonstrated to be effective under experimental conditions they should be evaluated under field conditions. This will require additional resources and a new extension to the project to have the vaccines fully evaluated, manufactured and licensed for use in the field. The continued support of this project to enable full evaluation of the vaccine constructs, perform field trials and manufacture and distribute them, leads us to believe that these vaccines have the potential to be beneficial to the economies of countries and farmers in Africa. There is a need to work with regulatory agencies throughout Africa to embrace safe genetically modified vaccines, including the education of governments and publics about the benefits of this technology. Given the existing animal health practices and feasibility of countermeasures, the vectored vaccines remain the only approach for disease control with short research-and-development timeline, cheap production and no-cold-chain distribution. A success story involving vectored vaccines has the potential to significantly facilitate the further application of this powerful disease control tool, thus resulting in truly global downstream benefits.

Recommendations resulting from the gender study:

To ensure effective use and uptake of the vaccine the social dynamics at play in two South African communities was investigated. The recommendations from this study are:

1) If vaccination programmes are to be implemented in the long-term, significant improvements are required among rural livestock keeping communities in terms of general primary animal healthcare knowledge, practices and understandings.

2) The general primary animal healthcare needs of livestock keepers need to be met before new vaccines can be introduced and made effective within the farming practices of livestock keeping communities.

3) A better understanding of the state's long term plans, policies and budgetary allocations in terms of provision of vaccines and animal health services needs to be ascertained.

4) The gender implications of new vaccine development need to be thought through more carefully for Phase 2.

5) The relationship between state animal health practitioners and livestock keepers needs to be improved in order to improve knowledge uptake in relation to vaccines and other preventative health practices.

6) Besides making farmers aware of the necessity of vaccines to prevent diseases, the cost of vaccines and their accessibility needs to be addressed in order to incentivize farmers not to rely on cheaper and retrospective methods of disease treatment.

7) The development of new vaccines needs to take into account both the socioeconomic contexts which provides significant barriers to uptake of vaccines, as well as the policy environments within which animal healthcare is administered by the state (and how the relationship between this environment and livestock keepers impacts on vaccine uptake).

8) The production of teaching materials and training programmes about primary animal healthcare, which is also sustainable (cost effective) and consistent, and deeply integrated into the everyday practices of livestock keepers would be an essential component in improving the use of vaccines among poor livestock keepers.

9) There is a need for a more comprehensive, large scale study (using a combination of quantitative and qualitative methodologies) on farming practices and knowledge within rural contexts across South Africa (that draw on comparative insights as well), in order to make stronger recommendations for long term national strategies on improving animal healthcare, including through the development of new vaccines.

APPENDIX: Figures

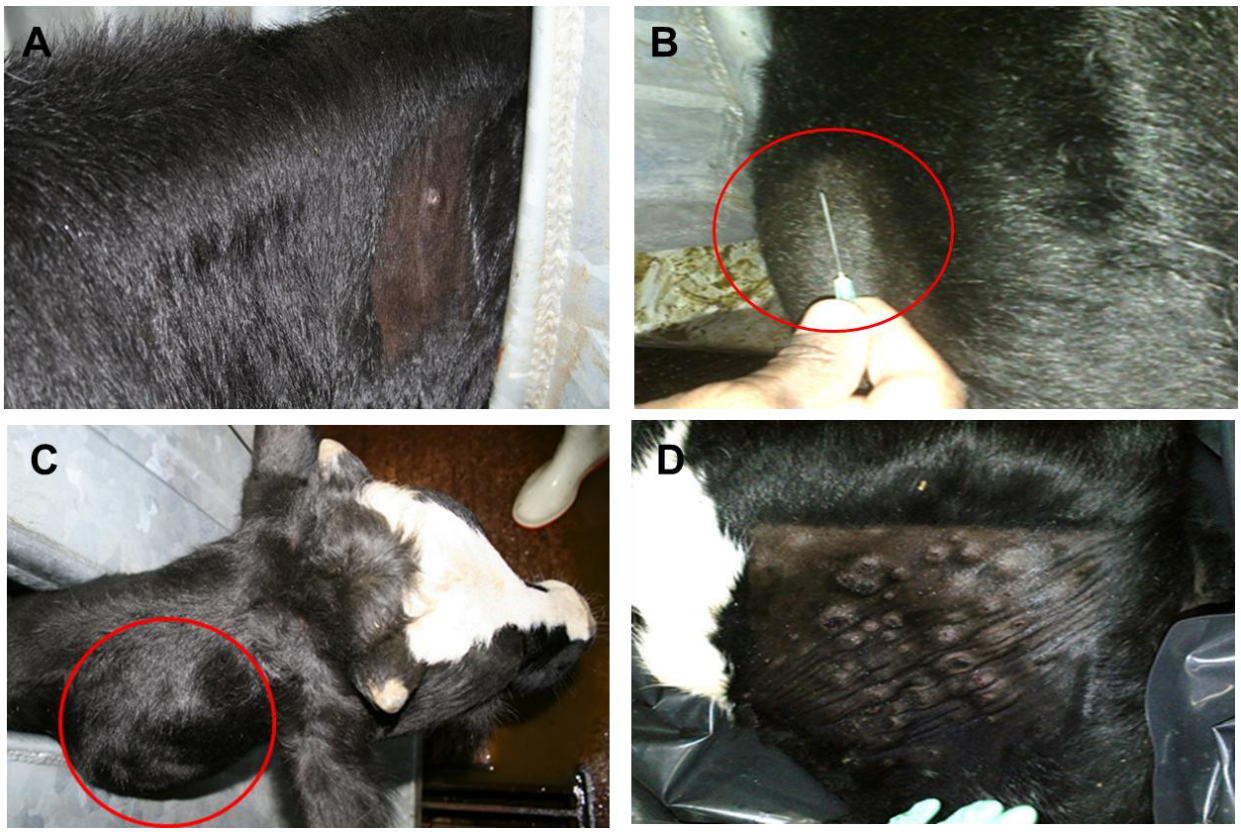


Figure 1: Clinical signs in cattle following vaccination with (A) LSDV commercial vaccine, (B) KO_1 and (C and D) KO_2. Animals vaccinated with KO_1 and KO_2 developed large lesions at the inoculation sites (circled). In addition, one of the animals vaccinated with KO_2 developed generalised LSD lesions. The KO_2 was thus not evaluated as a potential vaccine vector due to these adverse clinical signs.

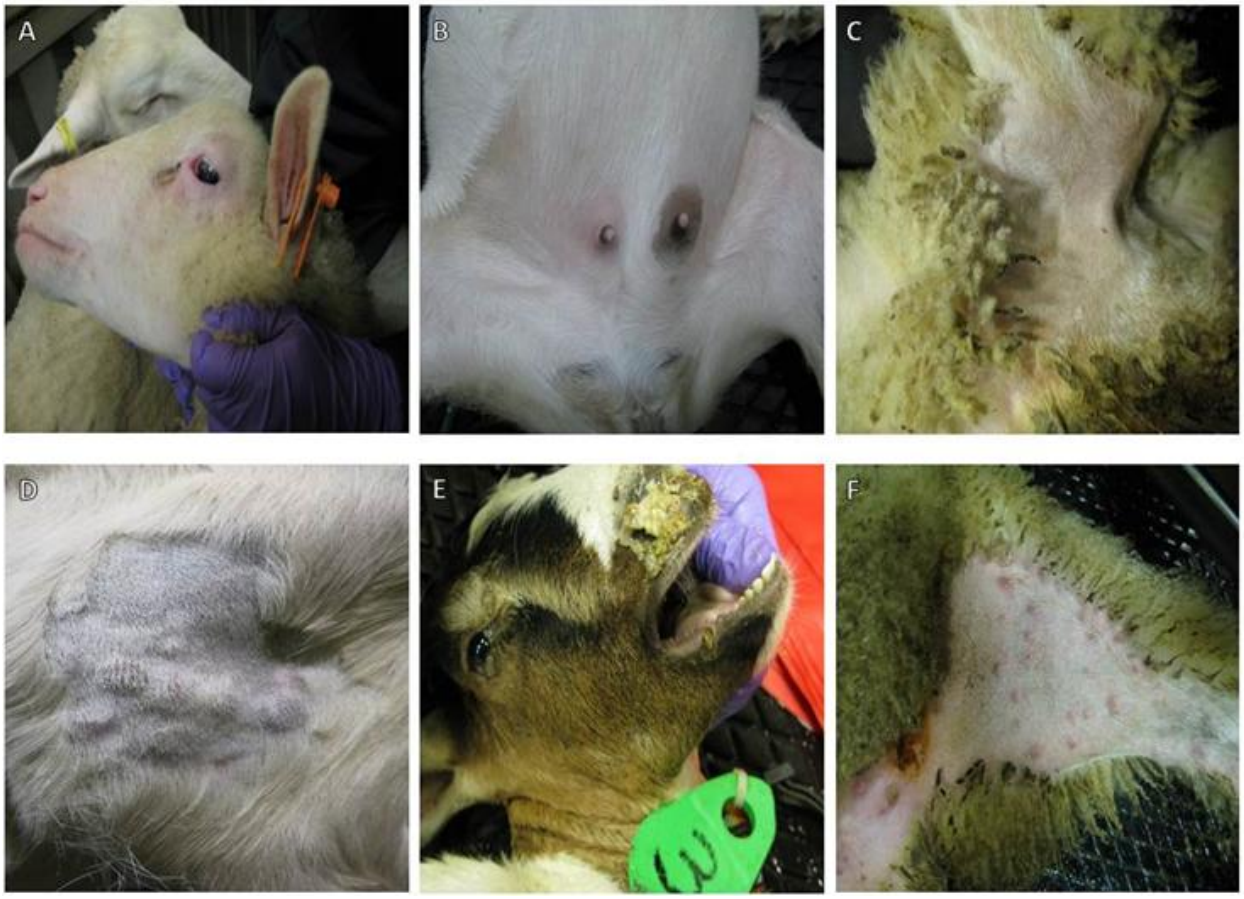


Figure 2: Clinical signs and gross pathology following vaccination with LSDV KO_1 (1 x 102.3 pfu) and challenge in sheep and goats at 10 days post-challenge. (A) Conjunctivitis in unvaccinated sheep; (B and C) lack of pox lesions in a vaccinated goat and sheep, respectively, are in contrast to pox lesions seen in unvaccinated goats (D) and sheep (F) at the same time point. Nasal and mucosal discharges were also observed in unvaccinated animals (E).

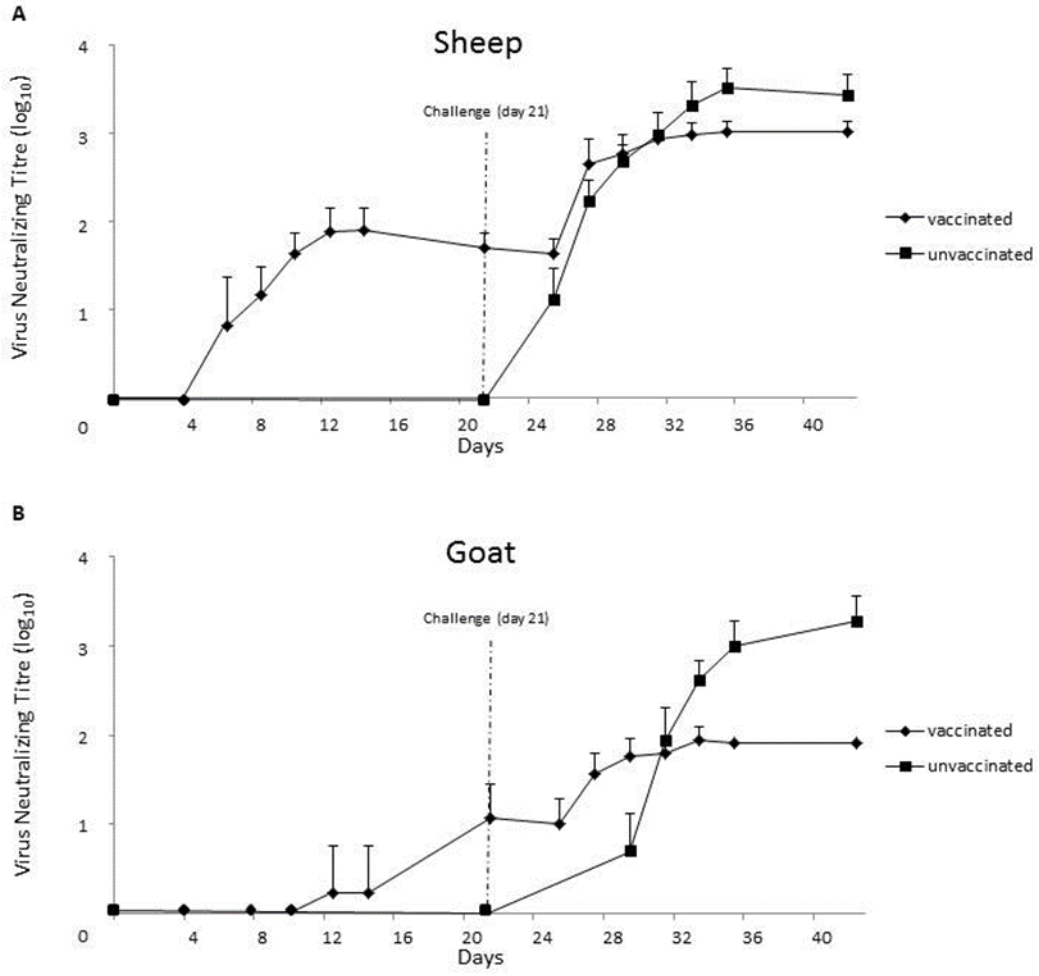


Figure 3: Serum neutralization (SN) testing following vaccination with KO_1 (1 x 102.3 pfu) and challenge with virulent capripoxvirus in sheep (A) and goats (B). SN titres are presented as mean values with standard deviations at each time point. Sheep developed SN antibody titres within 4 days post-vaccination (A), whereas responses in goats were slower. Vaccinated sheep and goats were protected post-challenge.

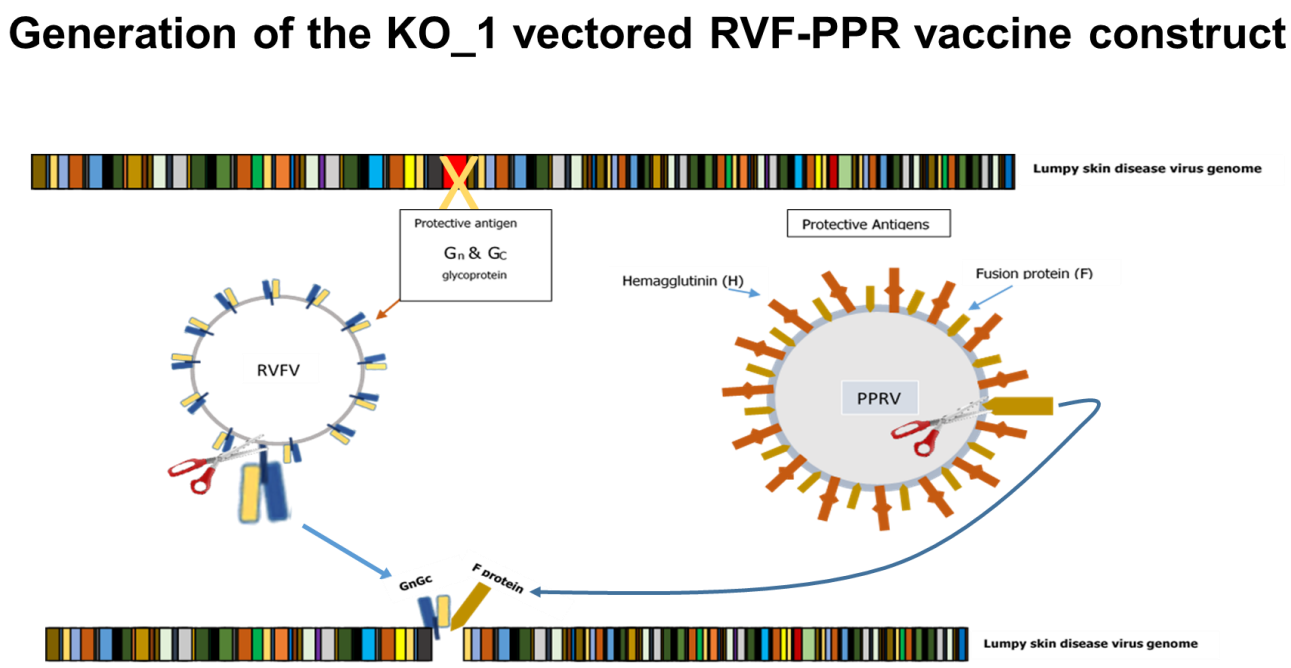


Figure 4: Schematic showing the generation of the KO_1 vaccine construct containing protective antigen genes of RVFV and PPRV. The protective glycoproteins (Gn and Gc) of RVFV and the fusion (F) protein of PPRV were inserted into the selected target site of the KO_1 genome.

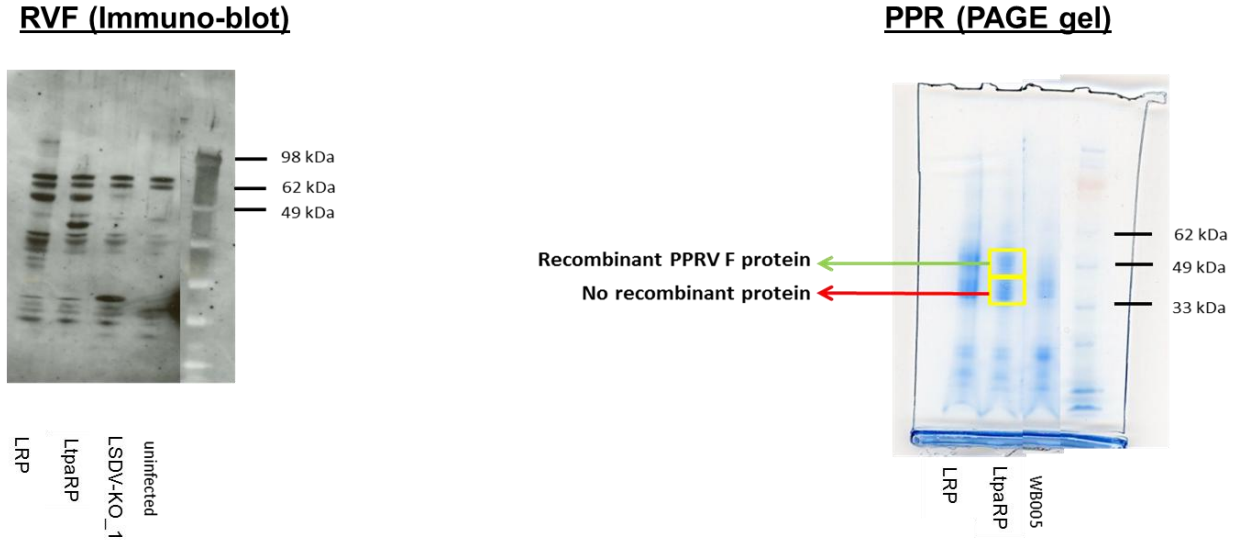


Figure 5: Expression of the RVF and PPR viral antigens by the KO_1 vectored vaccine constructs in cell culture. Mass spectrometry confirmed the identity of the PPRV F protein from the cut out band from the gel.

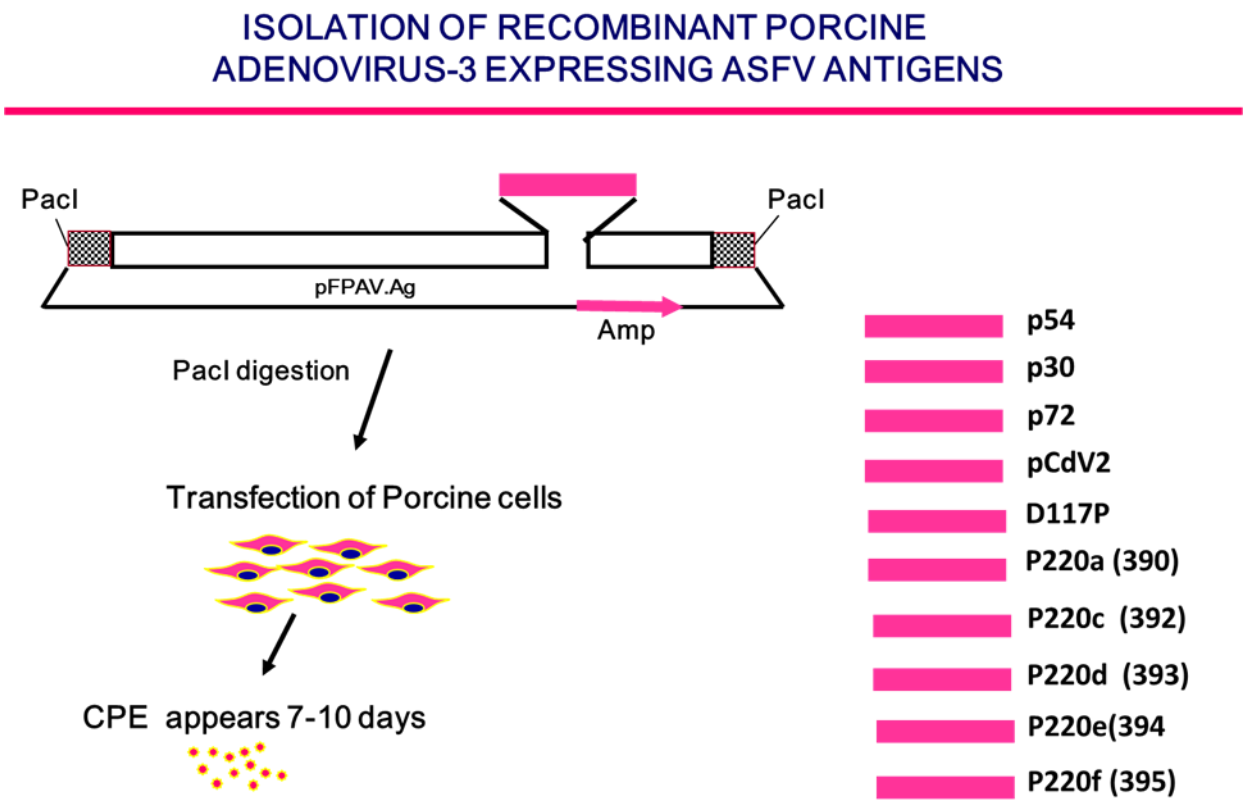


Figure 6: Construction of recombinant porcine adenovirus ASF vaccines.

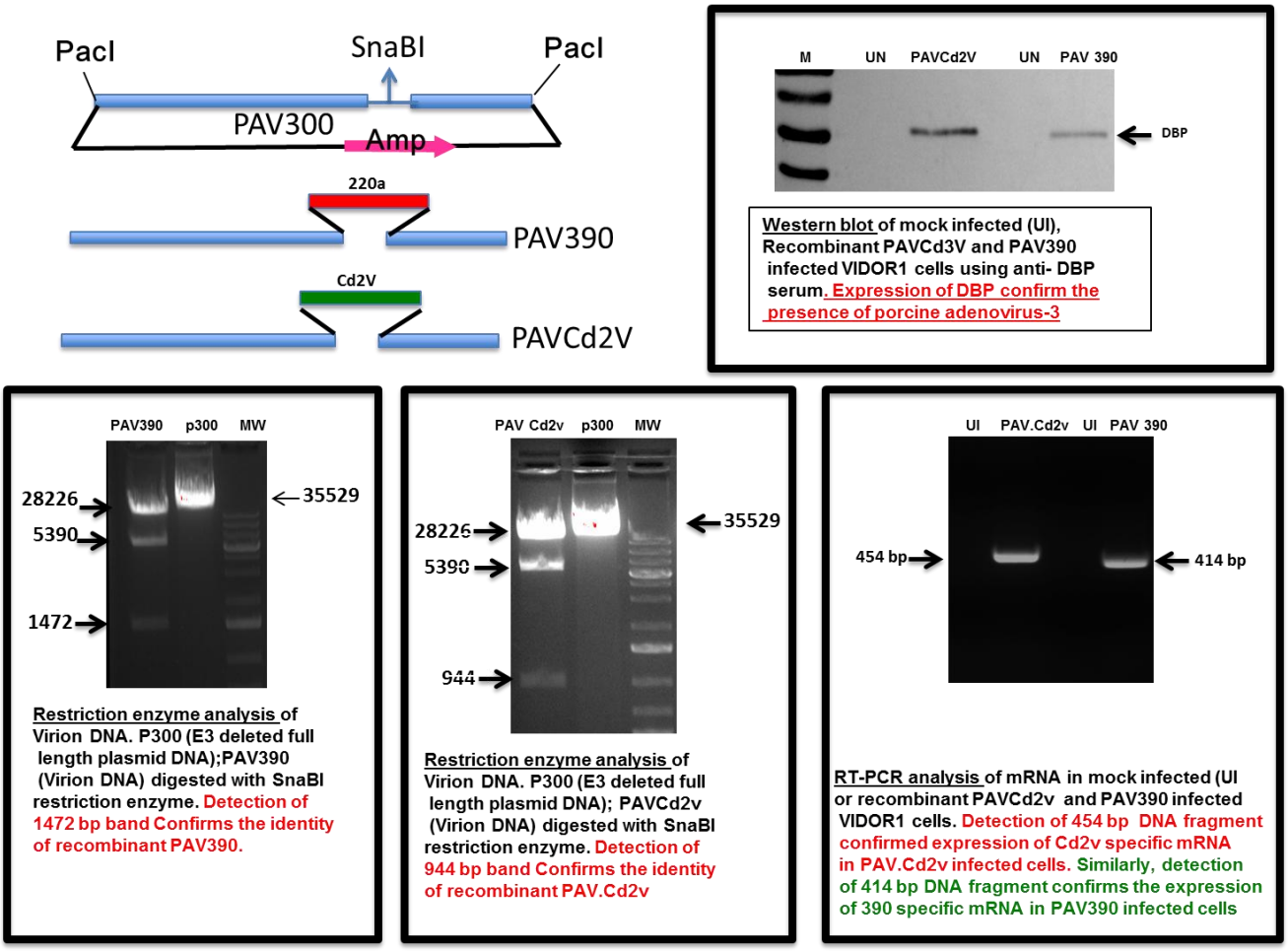


Figure 7: Confirmation of ASFV antigens using restriction enzyme analysis and Western blot analysis.

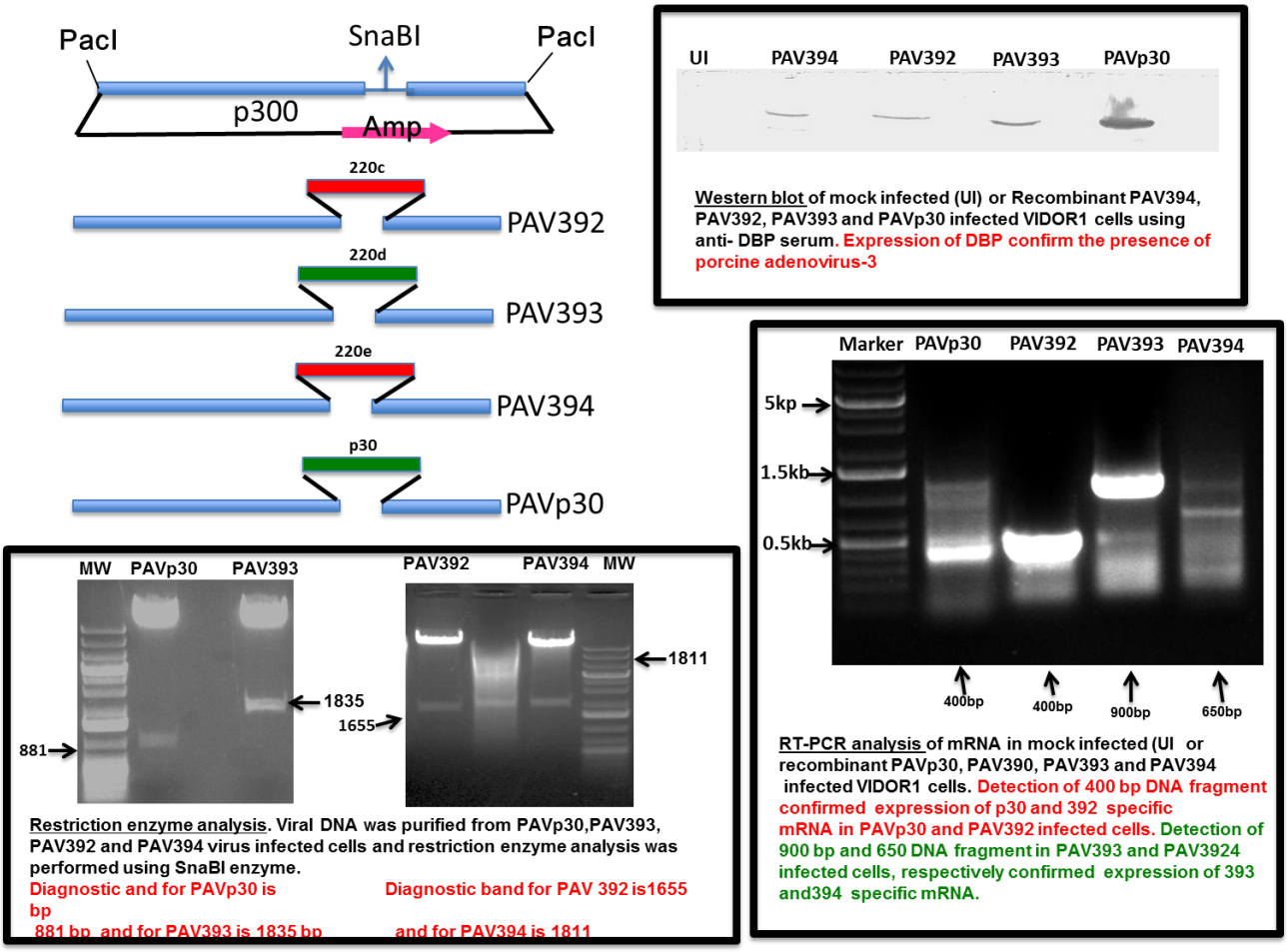


Figure 8: Confirmation of ASFV antigens using restriction enzyme analysis and Western blot analysis.

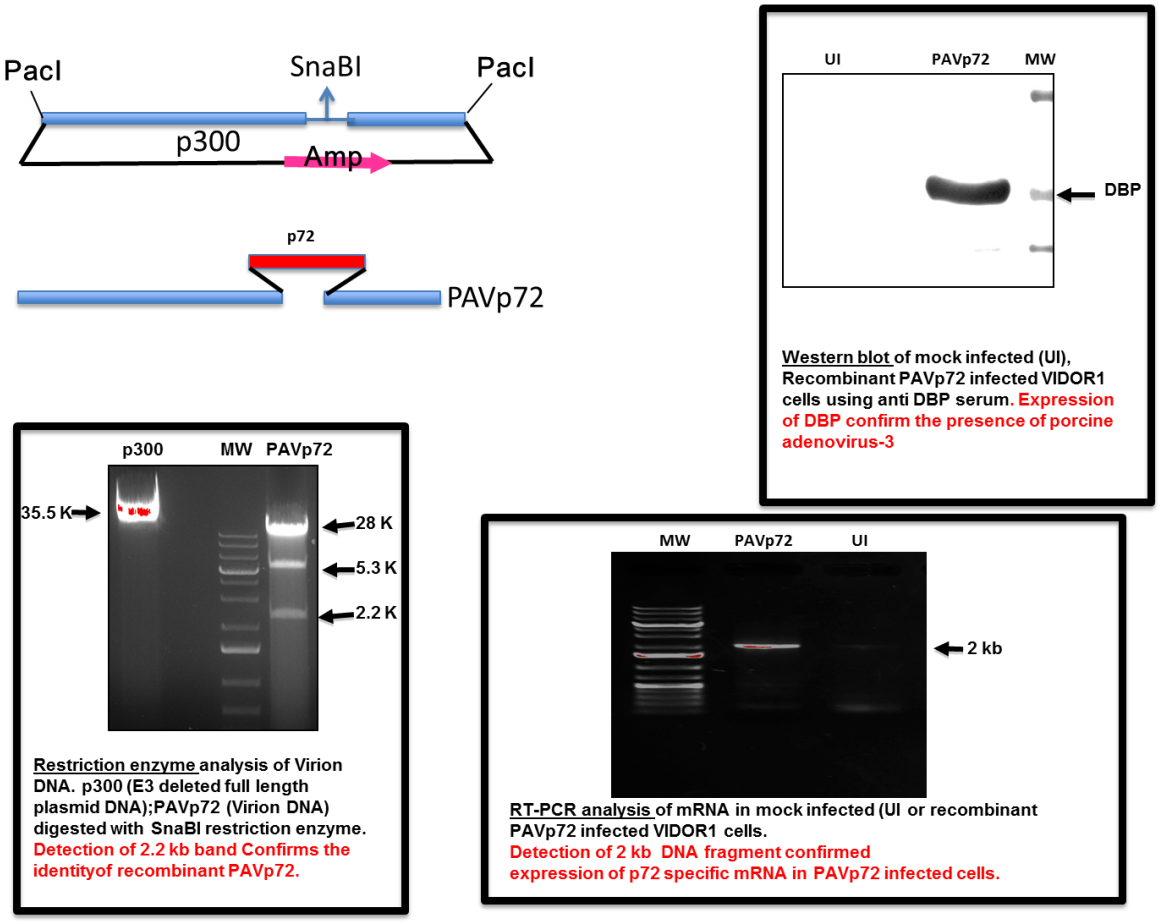


Figure 9: Confirmation of ASFV antigens using restriction enzyme analysis and Western blot analysis.

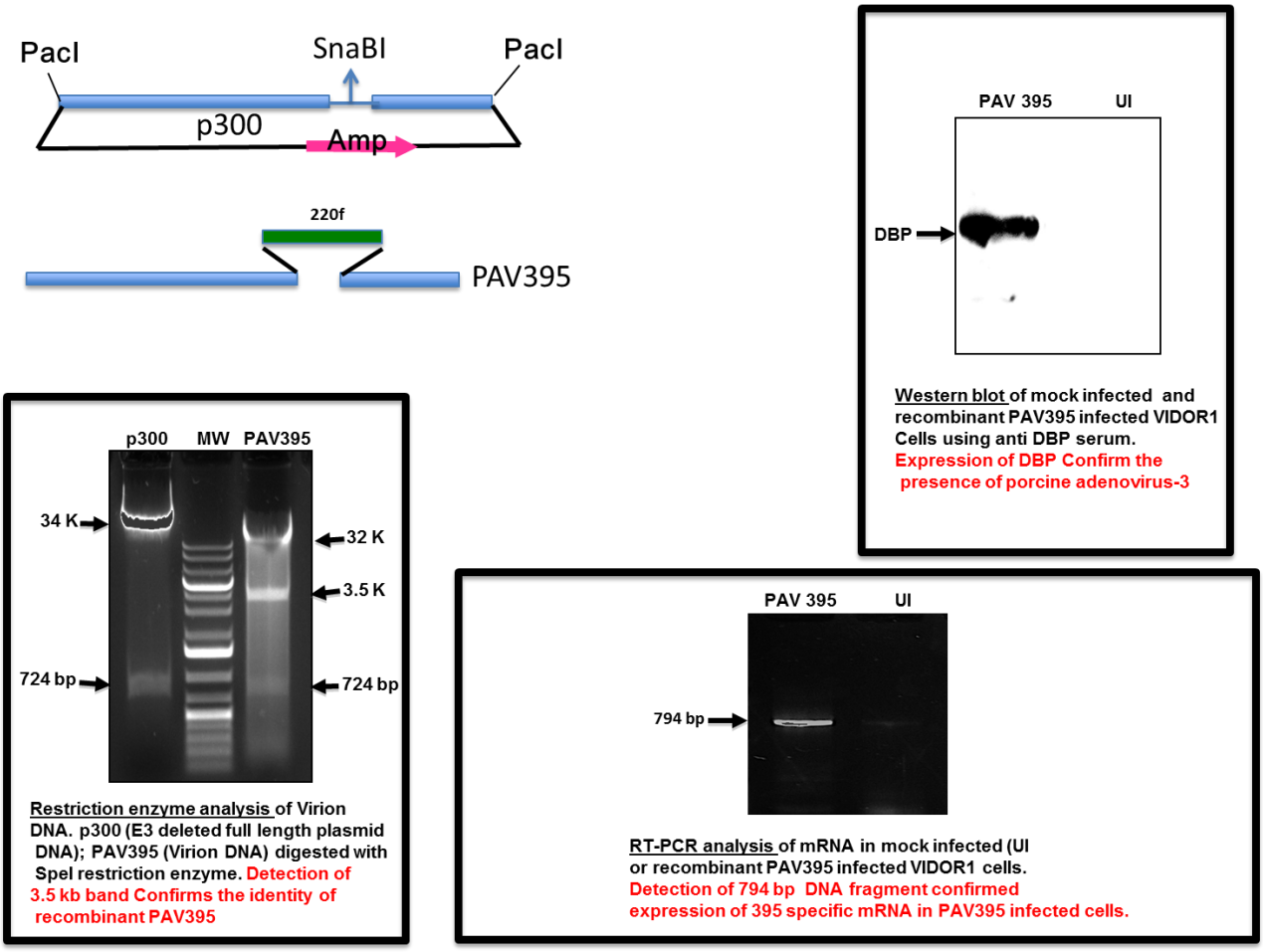
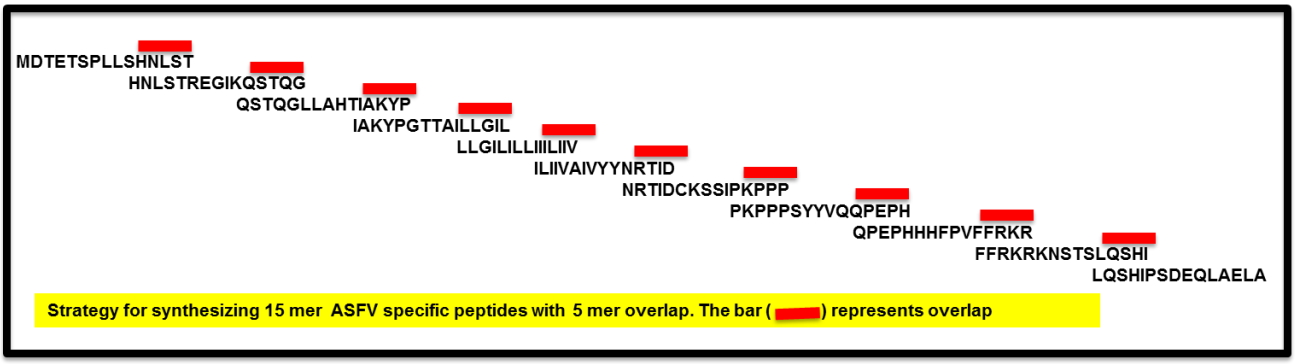


Figure 10: Confirmation of ASFV antigens using restriction enzyme analysis and Western blot analysis.



Peptide stimulation

a) PBMC stimulated with individual peptide groups and supernatant collected at 39 hr and 43 hr
b) Cells collected at 15 h and 19 h post stimulation

Proliferation:

Supernatant collected at 5 day

Samples will be analyzed by

a. **ELISA**
Using BioPlex (IL12, IL13, IL17a, IL10, IFNg) we will assess soon the PBMC production of several cytokines in response to the peptides.. As soon as available
the culture supernatant will be tested

b. **RT-qPCR**
Based on the other results we will perform some qPCR on the cDNA produced from stimulated PBMC to assess several cytokines at the transcript level.

ELISA sera

The sera collected at different times have been sent to Winnipeg for the antibody detection as we do not have killed ASFV here so far.

Figure 11: Strategy for evaluating cell mediated immunity using peptides for ASF antigens.

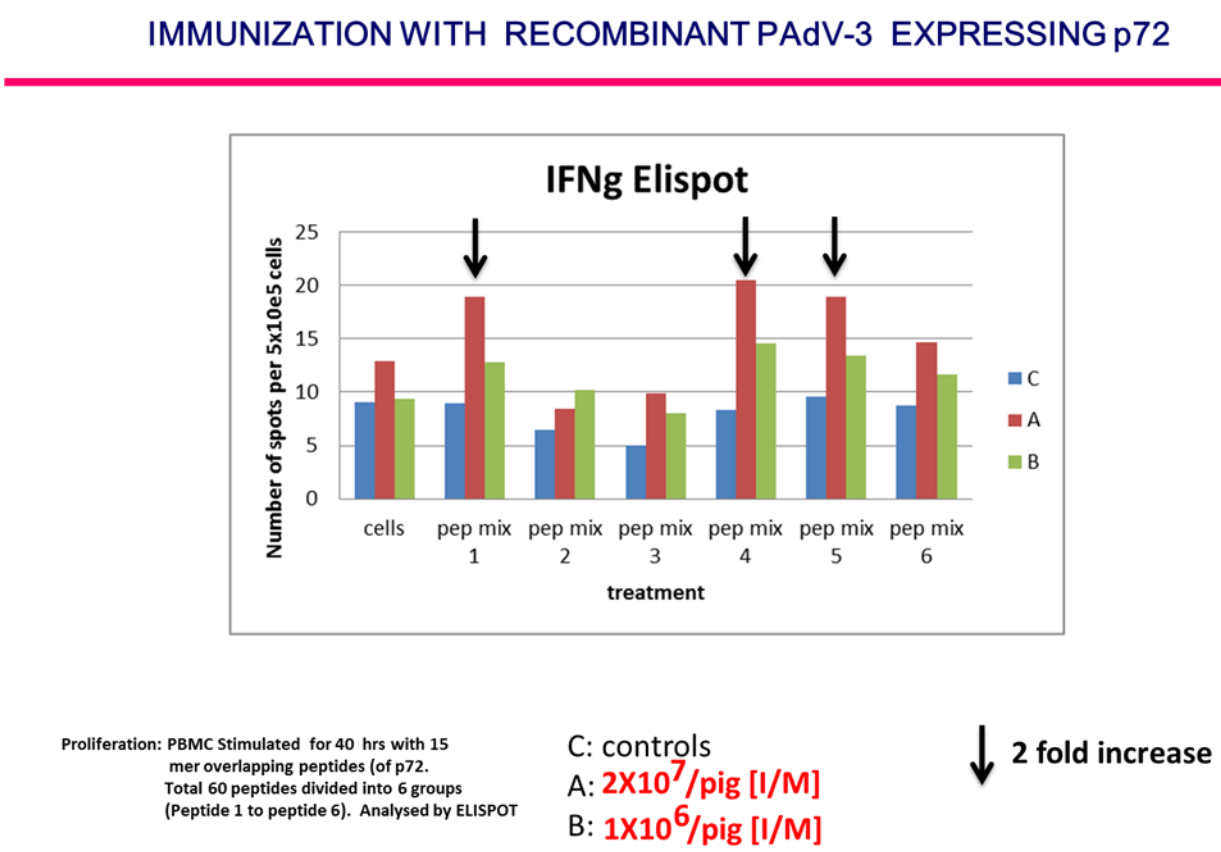


Figure 12: Cell mediated immunity following immunization with recombinant PAdV-3 expressing p72.