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Improved surveillance, preparedness and return to trade for emergency animal disease incursions using foot-and-mouth disease as a model: The FMD Ready Project

Final Report

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Executive summary

The future of biosecurity, from an understanding of the risks posed by diseases and pests, to strong surveillance and implementation of effective systems control, involves questions touching multiple disciplines of natural and social sciences and strong partnerships with stakeholders.

This report provides an overview of the work performed by the FMD Ready project: *Improved surveillance, preparedness and return to trade for emergency animal disease (EAD) incursions using foot-and-mouth disease (FMD) as a model*.

The FMD Ready Project aimed to enhance Australia's preparedness and facilitate a rapid return to trade in the event of an emergency animal disease (EAD) outbreak:

- Assurance that Australia continues to have a fit-for-purpose FMD vaccine bank effective against the highest risk FMD viral strains for Australia, and rapid diagnostic tests suitable for testing strains pre-, post- and during an outbreak.
- A new model for a producer-led emergency animal disease (EAD) surveillance system for consideration by the jurisdictions.
- An integrated EAD outbreak management decision support system to allow response scenarios to be rapidly tested and costed before and during outbreaks.
- Meteorological, pathway and molecular diagnostic based tools to rapidly characterise and map outbreak pathogen spread and provide animal biosecurity response intelligence.

The FMD Ready project achieved these broad goals. Some examples of achievements include:

- Contributing scientific input to decision-making on which antigens should be included when renewing Australia's FMD vaccine bank (2020-2025)
- Improving the availability, accuracy and efficiency of diagnostic tests for use in detection of an FMD incursion to ensure accurate diagnosis and surveillance
- Improving the participation of primary producers in biosecurity and surveillance networks, resulting in increased awareness of how to recognise and report emergency animal diseases, and helping to build trust in local networks between primary producers and government agencies
- Increasing the interest of government agencies in working with livestock industry networks to improve biosecurity and surveillance outcomes
- Development of biosecurity communication tools by producers for producers
- Updating and expanding the Australian Animal Disease spread model (AADIS) as a decision support tool and integrating it with improved economic modelling tools to inform disease

control strategies, including vaccination and the use of trading zones to support earlier return to trade.

- Development of an application (SPREAD) to incorporate big data into the real-time modelling of disease spread during an EAD outbreak. This application could be used to determine how virus spreads from farm to farm in an outbreak, which would directly benefit affected and neighbouring primary producers, as well as agencies responding to the outbreak.

The transdisciplinary nature of the FMD Ready project has demonstrated that collaboration between different research disciplines (e.g. modelling, economics, science) combined with direct interaction and collaboration with livestock industries, governments, RDCs and other agencies, can deliver solutions to complex problems that may not be possible with less diverse research teams.

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1 Background

1.1 Foot-and-mouth disease

Foot-and-mouth disease (FMD) is an infectious viral disease affecting cloven-hoofed livestock, including cattle, sheep and pigs. While the disease does not usually cause mortality in adult animals, it can cause death in young animals and the production losses and economic impacts due to trade embargoes can be severe. A nationwide outbreak of FMD would devastate Australia's agricultural industry and economy, with estimated direct and indirect costs of up to AU$50 billion dollars due to lost trade, response management and social and environmental impacts over a 10 year period[1].

Although FMD is endemic in many parts of the world, disease-free countries and zones exist and are maintained through costly biosecurity measures. As a result, trade restrictions apply to susceptible animals and animal products from countries with FMD. There has been no recorded outbreak of FMD in Austalia since 1872[2] and this disease-free status, together with the absence of various other infectious diseases of livestock, provides Australia access to lucrative export markets.

Reducing Australia's vulnerability to emergency animal disease (EAD) incursions will increase the security and growth prospects of a wide range of livestock production and associated industries. Easy and prompt reporting, containment and eradication will enable industry recovery through rapid resumption of trade and exports.

1.2 FMD Risk Management Project Phases One and Two

The FMD Risk Management Project (FMD-RMP) was originally identified as being of benefit to the nation following consultations between government, relevant livestock industries and CSIRO. The Project proposal, developed by AHA and CSIRO-Australian Centre for Disease Preparedness (ACDP), was supported through seed-funding from susceptible livestock industries represented by the Cattle Council of Australia, Australian Lot Feeders Association, Australian Dairy Farmers, WoolProducers Australia, SheepMeat Council of Australia (now Sheep Producers Australia), Australian Pork Limited and the Goat Industry Council of Australia.

The first three years of the project (Phase One; 2011-2013) contributed significantly to Australia's FMD preparedness in the areas of:

- vaccine matching and efficacy
- disease pathogenesis
- diagnostic capability

[1] Buetre B, Wicks S, Kruger H, Millist N, Yainshet A, Garner G, Duncan A, Abdalla A, Trestrail C, Hatt M, Thompson L-J and Symes M. 1993. Potential socio-economic impacts of an outbreak of foot-and-mouth disease in Australia. ABARES report (https://www.agriculture.gov.au/sites/default/files/abares/documents/RR13.11PotSocEcoImpctOfFMD_v1.0.0.pdf)

[2] Bunn CM, Garner MG, Cannon RM (1998) The 1872 outbreak of foot-and-mouth disease in Australia--why didn't it become established? .Aust Vet J. 76(4):262-9.

- virus evolution and trends
- capacity building in Australia and South-East Asia (SEA).

Phase Two of the project (2013 – 2016) focused largely on assessing the effectiveness of the vaccines held in the Australian Vaccine Bank against viruses currently circulating in SEA. A number of studies in target species were performed to ascertain whether the FMD vaccines would protect cattle, sheep and pigs against clinical disease post-challenge and to measure the immune response pre- and post- FMD virus challenge. Additionally, studies were conducted to research the course of disease through viraemia (virus in the blood), the amount of virus excreted in secretions such as saliva and nasal fluids, persistence in the oro-pharynx (using oro-pharyngeal scrapings via probang sampling), and demonstrate the benefits of vaccination on reducing viral excretion, as well as providing material for validation of diagnostic tests. The project also addressed other objectives including capacity building and networking with laboratories in SEA and further abroad to improve Australia's diagnostic capability and surveillance.

Phases One and Two of the FMD-RMP focussed on the laboratory aspects of FMD preparedness, and were not designed to address either vaccination-based strategy for FMD control, nor optimal farm-based surveillance or decision support for EAD outbreak resolution more generally.

The FMD Ready project (2016 -2020; extended up to 2022):

- builds on past work from the FMD-RMP (Phases One and Two) to provide advances in integrated disease outbreak detection and response systems that will be relevant for not only FMD, but other EADs
- is a holistic project that provides information about risks from FMD endemic areas, vaccine protection and improved post-outbreak surveillance
- enables socially accepted surveillance systems to be put in place that enables and encourages early detection (a critical element to any outbreak identification and management)
- provides decision support tools and systems based on linking existing epidemiological models with new economic modules and scenario testing interfaces, by allowing risk- and cost-based decisions to be made about appropriate vaccination with other response strategy scenarios, and
- develops a framework to maximise the use of large, existing datasets (e.g. climate and sequencing data) to provide enhanced disease investigations to assist veterinary services to better determine how, and from where, farms became infected. Such enhanced investigations supported by Big Data are valuable and applicable at the tail-end of the epidemic, where the dollar-cost of each prolonged outbreak can run into millions, delaying the required proof of freedom survey that will enable an early return to trade.

1.3 Transdisciplinary teams approach

The project took an integrated approach across the biosecurity continuum, including in preparedness planning and tools, surveillance systems, incursion response, and return to market. This included developments in diagnostic assays, Big Data analytics, disease pathway analysis and stakeholder engagement.

The need for further collaborative research became apparent during Phase Two of the FMD-RMP. Thus, the FMD Ready Project took an integrated approach with the aim to achieve substantial improvements across the biosecurity continuum, including in:

- Preparedness planning – FMD is caused by an RNA virus that is constantly evolving. To increase Australia's preparedness for FMD outbreaks, it is necessary to work with FMD virus in SEA and other countries with suitable containment facilities, improve relevant laboratory tests and undertake vaccine matching against the Australian FMD Vaccine Bank. Australia does not permit the entry of live FMD virus for research, so studies are undertaken in collaborating countries.
- Surveillance systems – the sooner the disease is reported, the faster it can be contained and eradicated with less impact on the economy. A new social-based approach built around producer values formed the basis to form an effective surveillance system for EADs to rely on bottom-up behaviours and a values-based approach.
- Incursion response – it is critical that the socio-economic consequences of alternative management options are considered when implementing an EAD response. This project incorporated vaccination scenarios into an existing model and integrated the results with an economic model to allow testing of economic impacts of alternative management options for EAD response.
- Return to market – delays in return to normal business lead to substantial economic and social costs. The project examined several ways of facilitating a quicker return to market by combining laboratory diagnostic and genomic-level bioinformatics advances into epidemiological and economic analyses. These approaches demonstrated benefits to:
    - more quickly spatially analyse how, and by what means, animal diseases spread through the landscape;
    - implement new faster and cheaper sampling strategies and tools meeting post-outbreak surveillance requirements; and
    - establish trading zones which assist unaffected areas in resuming trade sooner.

  Together the tools deliver strategic and near real-time tactical guidance for incursion response decisions with reduced uncertainty.

This is a truly multidisciplinary project for an EAD. The development and utilisation of world leading technologies to optimise all phases of an EAD response is an exciting advancement in national biosecurity.

A list of the researchers who participated in the project is provided in Appendix 1.1.

2 Project objectives

The FMD Ready Project aimed to enhance Australia's preparedness and facilitate a rapid return to trade in the event of an emergency animal disease (EAD) outbreak through the development of generic processes and support tools, using FMD as a model, through:

- Assurance that Australia continues to have a fit-for-purpose FMD vaccine bank effective against the highest risk FMD viral strains for Australia and rapid diagnostic tests suitable for testing strains pre-, post- and during an outbreak.
- A new model for producer-led (EAD) surveillance systems for consideration by the jurisdictions.
- An integrated EAD outbreak management decision support system to allow response scenarios to be rapidly tested and costed before outbreaks.
- Meteorological, pathway and next generation sequencing based tools to rapidly characterise and map outbreak pathogen spread and provide animal biosecurity response intelligence.

To achieve this, the research was conducted in the following 4 sub-projects (SP), with collaboration between the research teams where relevant:

SP1: RAPID DIAGNOSTICS AND VACCINATION STRATEGY PREPAREDNESS

SP2: FARMER-LED SURVEILLANCE SYSTEMS

SP3: DECISION SUPPORT TOOLS FOR DECISION-MAKING FOR OUTBREAK MANAGEMENT

SP4: ANALYTICAL TOOLS TO DETERMINE THE PATH OF FARM-TO-FARM DISEASE TRANSMISSION

3 Enhancing Australia's preparedness for a FMD incursion: rapid diagnostic tests and an effective national FMD vaccine bank

3.1 South East Asia FMDV strain evolution and antigen matching

3.1.1 *Introduction*

In vitro antigen matching assays are important predictors of serological cross-reactivity between field and commercial vaccine strains and can be used to indicate whether or not the strains in the Australian Vaccine Bank (AVB) will protect against viruses currently circulating in South East Asia (SEA). Although studies have shown that r_1-values (the antigenic relationship between the vaccine strain and field virus) can predict likely vaccine efficacy, the correlation may not be as definitive for emergency, high payload vaccines (>6 PD_{50}). However, as *in vivo* challenge studies to estimate the true potency of emergency vaccines are limited due to high costs, animal ethics considerations, and the time it takes to perform these studies, the *in vitro* results can be used to determine when live animal vaccine efficacy studies are required to demonstrate efficacy.

As part of the previously funded FMD Risk Management project, Phase 2, antigen matching and molecular epidemiology studies were initiated in 2013 at the OIE-Regional Reference Lab (RRL) for FMD in SEA, Pakchong, Thailand, to measure the antigenic relationships of FMD viruses from SEA with the commercial vaccine strains in the AVB. In addition to working with RRL, we had expanded our collaboration to include the Centre for Veterinary Diagnostics in the Regional Animal Health Office 6 (RAHO6) - Ho Chi Minh City, Vietnam, to give us access to more virus isolates from that country.

Our previous results from antigen matching studies with **serotype A** isolates from SEA, carried out between 2013 and 2016, provided evidence for the antigenic drift from the commercial vaccine strains VA1 and VA2 since 2011, and the relative increase in number of isolates that show heterologous or intermediary matching to the commercial vaccine strains. Phylogenetic analysis also supported the hypothesis of genetic drift since 2004 and emergence of a new serotype A variant strain during 2012–2014. By 2016, three main variants of the A/SEA-97 lineage (2004–2008, 2010–2012 and 2014–2015), encompassing the majority of the field isolates tested, were identified. These isolates did not antigenically relate to the commercial vaccine strains, VA1 and VA2, or to the Thai vaccine strain, A/TAI/Sukhon Nakhon 97. A new vaccine strain, A/TAI/Lop Buri/2012, was introduced by the vaccine manufacturer in Thailand during 2013 and most of the viruses during that period were antigenically related to this new strain. The serotype A viruses that emerged during 2014–2016 matched better with VA2 compared to VA1.

The **serotype O** isolates in SEA demonstrated either intermediate or heterologous matching to VO1, indicating that this vaccine strain was perhaps not suitable for use in SEA as a homologous vaccine. In contrast, studies with VO2 suggested most of the viruses were homologous and a few were intermediary to this strain, and VO2 was likely to offer better protection and should be the vaccine strain of choice for this region confirming the use of a bivalent VO1/VO2 vaccine. Phylogenetic analyses using the sequence encoding the VP1 protein, revealed two sublineages of O/SEA/MYA98 and few outbreaks due to PanAsia strains.

This is an ongoing study due to the constantly changing nature of FMD viruses, and in this report, we are presenting the results of vaccine matching studies of viruses isolated between 2015–2018

against the commercial vaccine strains VO1, VO2 and VA2. Due to travel restrictions, we have not been able to perform vaccine matching studies on viruses isolated between 2019 and 2022.

3.1.2 *Method*

Homologous reagents (vaccinated bovine sera and immunised rabbit and guinea pig sera) of three three commercial vaccine strains, VA2, VO1 and VO2 were used in liquid-phase blocking ELISA for vaccine matching studies (Hamblin et al., 1984). Serotype A (n=75) and serotype O (n=58) cell culture adapted field viruses, isolated during 2015–2017 from Thailand, Laos and Vietnam, were used for antigen matching. The 'r_1' value, was derived as the antilog of the negative value of the log differential of homologous and heterologous titre, and was interpreted according to Samuels et al. (1990) where an r_1 value > 0.39 was interpreted as homologous (vaccine will provide protection), between 0.19 and 0.39 as intermediate (a new/alternate vaccine may be required) and <0.19 as heterologous (a new/alternate vaccine is required).

3.1.3 *Results*

Of the 75 serotype A isolates tested, 70 had homologous (93%), 2 had heterologous (3%) and 3 had intermediate (4%) r_1 values with **VA2** vaccine strain.

The most recent serotype O field isolates matched poorly with the **VO1** vaccine strain. Only 15 of the 58 isolates (25.9%) were homologous while 6 were intermediary (10.3%) and 13 were heterologous (22.4%). Twenty-four isolates (41.4%) did not bind with VO1 reagents, indicating a complete difference between the viruses and vaccine strain.

The vaccine strain, **VO2**, provided a better match compared to the VO1. Of the 58 isolates tested, 47 were homologous (81.0%), 4 were intermediary (6.9%) and only 2 were heterologous (3.45%). Five isolates (8.6%) showed poor or no binding with VO1 reagents.

3.1.4 *Discussion*

Vaccine matching studies with **serotype A** isolates from SEA indicated that the isolates from 2015–2017 have homologous r_1 values with the VA2 vaccine strain. This is a major shift in relative homology since 2014. In our earlier reports (2015 & 2016) we showed that a number of field isolates from SEA, collected during 2012–2014, had heterologous or intermediate r_1 values to the serotype A commercial vaccine strains (VA1 and VA2). Since 2014 there was a trend towards homology to VA2 and heterology to VA1. The current *in vitro* study establishes that VA2 continues to be the most suitable vaccine strain against serotype A viruses for this region.

VO1 is an established vaccine strain that is available in all vaccine banks and has been used in commercial vaccines for SEA, except in Thailand. The data for **serotype O** revealed that the isolates in SEA that belonged to O/Mya98 and Panasia lineages were generally intermediate or heterologous to VO1, but with varying degrees of homology towards the O/ME-SA/Ind-2001d lineage, and this vaccine strain would be less effective against the strains currently circulating in SEA. However, the alternative vaccine strain for this region would be VO2, which offers better coverage against all the lineages in SEA (except O/Cathay topotype). We suggest that VO2 should be the vaccine strain of choice for this region or any other equivalent strain that is similar to VO2. The results of our *in vivo* challenge studies using VO2 in combination with VO1 provide evidence to support this (see list of references summarising studies from the FMD Risk Management and FMD Ready projects below).

Despite the introduction of new strains of viruses such as O/ME-SA/Ind/2001d into the SEA region, the commercial commercial vaccine strains such as VA2 and VO1 have been shown to provide protection against clinical disease *in vivo*. In addition, high payload vaccines such as those that will be used in Australia during an outbreak, will provide protection against clinical disease but not protect all animals against infection. However, the use of these vaccines will also lower virus excretion, thereby assisting in control efforts (see list of references below).

Continued, real-time monitoring for the emergence of variant strains in SEA by both vaccine matching studies and phylogenetic analysis is required for effective control of the disease in this region. All field strains that are isolated must be simultaneously matched with commercial vaccine strains and sequenced for their genetic lineages. This will provide valuable information on how the viruses of a particular lineage evolve and match with commercial vaccine strains.

Australia is committed to working closely with the countries in the region for management and control of FMD. By working closely with the national and reference laboratories for FMD in SEA, Australian scientists can obtain firsthand information on the emerging FMD viruses and their relationships with the strains in the AVB. The information gained in these studies will assist Australia in making informed decisions on the antigen bank and improve preparedness for FMD management and disease control, as well as modelling spread with and without vaccination.

This work needs to be continued to monitor more recent isolates to ensure Australia remain prepared for potential incursions.

3.1.5 *Reference Materials*

Hamblin et al (1984). A rapid enzyme-linked immunosorbent assay for the detection of foot-and-mouth disease virus in epithelial tissues. Veterinary Microbiology, 9, 435-443.

Samuels et al (1990). Antigenic analysis of serotype O foot-and-mouth disease virus isolates from the Middle East, 1981 to 1988. Vaccine, 8, 390-396.

3.2 *In vivo* vaccine efficacy testing

3.2.1 *A Malaysia 97 emergency vaccine against an isolate belonging to A/Asia/G-VII Lineage*

3.2.1.1 *Introduction*

Since 2015, a new lineage of foot-and-mouth disease (FMD) virus, A/ASIA/G-VII has emerged and caused major outbreaks in the Middle East. *In vitro* vaccine matching data indicated that viruses belonging to this lineage poorly matched (low r_1-value) most commercially available vaccine strains (Bachanek-Bankowska et al 2018), including those in the Australian Vaccine Bank and those being used in the South East Asian (SEA) region, the highest risk for introduction to Australia. Although this new lineage has not been identified in SEA, the recent introduction of a novel serotype O lineage virus (O/Ind/2001d/e) into the region serves as a reminder that new introductions are possible, and that Australia needs to be prepared.

The aim of this study was to assess the performance of two serotype A vaccine strains in the Australian Vaccine Bank, A22/IRQ/64 or A/MAY/97, against challenge with a representative field virus from the A/ASIA/G-VII lineage (A/IRN/22/2015) to determine if the high payload vaccines will provide protection *in vivo* despite the *in vitro* data (low r_1 value). It has been shown that such high payload vaccines can provide cross protection and reduce clinical severity of disease and decrease virus excretion, both factors that could assist with lowering the risk of spread and assisting with control programs.

3.2.1.2 *Method*

Two monovalent emergency vaccines, A22/IRQ/64 and A/MAY/97 were formulated from inactivated vaccine antigens that are held by the Australian Vaccine Bank, at the antigen payload that will be used in an emergency in Australia. The vaccines were formulated as a double oil emulsion by Boehringer-Ingelheim, Pirbright and shipped to Wageningen Bioveterinary Research (WBVR) in Lelystad, The Netherlands, where the studies were performed as part of a cost share and scientific collaboration. Each vaccine was administered intramuscularly in the neck of groups of cattle at the recommended dose. The study was carried out in two phases.

In Phase 1, a full dose protection test using A/MAY/97 and A22/IRQ/64 was assessed using 5 mixed Dutch dairy breed heifers each. At 3 weeks post-vaccination, the vaccinated and two control cattle were challenged by intra-dermo-lingual route with a $10^{3.6}$ PFU/mL of the original virus suspension of FMDV isolate A/IRN/22/2015, injecting 0.1 mL at 2 sites.

Based on the results from Phase 1, the Phase 2 study was performed as a heterologous potency test (PD_{50}) with A/MAY/97 vaccine. A total of 18 cattle was randomly assigned to 4 groups, 3 groups of 5 vaccinated cattle and 1 group of 3 unvaccinated control cattle. The groups received either a full dose, one-third dose and one-ninth dose. At 3 weeks post-vaccination, the vaccinated and control cattle were challenged by intra-dermo-lingual route with a $10^{3.6}$ PFU/mL of the original virus suspension of FMDV isolate A/IRN/22/2015, injecting 0.1 mL at 2 sites.

In both phases, animals were observed for clinical signs of FMD and lesions on the feet at 4- and 8-days post challenge (dpc) with protection scored as an absence of lesions on the feet. Antibody responses post-vaccination and post-challenge were measured along with viraemia and virus shedding in the nasal and oral fluids, both by virus titration on cell cultures and genome detecting

real-time RT-PCR. Statistical analyses of the data were performed using Fischer's exact test and linear mixed effects models. The potency of the A /MAY/97 vaccine was calculated by the method of Spearman-Karber (Darling et al 1998) and by logistic regression.

3.2.1.3 *Results*

The results from the initial full dose protection study in Phase 1 indicated that two of the cattle vaccinated with A/MAY/97 had a doubtful lesion at one of the feet with no sign of lameness. No samples were taken as previous studies have shown that virus can usually not be isolated at 8 dpc. When the doubtful lesions in the vaccinated cattle were considered negative for FMDV infection, all the cattle vaccinated with A/MAY/97 were protected compared to only 2 of the 7 (29%) cattle vaccinated with A22/IRQ/64, resulting in a significant difference between A22/IRQ/64 and A/MAY/97 ($p = 0.02$, Fischer exact test). However, when the doubtful feet lesions were considered positive, 5 of the 7 (71%) A/MAY/97 cattle were protected and the difference between the 3 groups were not significant ($p = 0.19$, Fisher exact test).

Viraemia, defined as isolation of infectious virus from serum, was not observed in any of the vaccinated cattle. In one of the cattle vaccinated with A22/IRQ/64, FMDV genome could be detected by RT-PCR for 1 day, indicating that viraemia did occur, but was transient. Only control cattle developed viraemia, therefore statistical analysis of viraemia was not considered relevant.

Live virus could be detected in mouth swabs of all cattle (as expected with intradermalingual challenge) and the nose swabs of 16 out of 17 cattle, regardless of vaccination. The duration of FMDV detection in nose swabs and mouth swabs was significantly different between groups (Linear Mixed Effects Model; see Appendix 3.1). A significant difference between A/MAY/97 vaccinated cattle and the control group ($p < 0.01$ for mouth swabs, $p = 0.02$ for nose swabs) as well as between the A22/IRQ/64 vaccinated cattle and the control cattle ($p < 0.01$ for mouth swabs, $p = 0.03$ for nose swabs) was observed, but no difference between both groups of vaccinated cattle (pairwise t-test). The maximum virus titre observed in the A/MAY/97 and A22/IRQ/64 vaccinated cattle was significantly lower in nose swabs (ANOVA $p < 0.01$, $p < 0.01$ for A/MAY/97 versus controls, and $p = 0.02$ for A22/IRQ/64 versus controls) compared to the control cattle, but no difference was found between the vaccine groups (pairwise t-test). No difference in maximum virus titre in the mouth swabs was observed between the groups (ANOVA $p = 0.07$).

In view of these promising results, A/MAY/97 vaccine was tested in a potency test in Phase 2. In the heterologous potency test, using the A/MAY/97 vaccine, all 5 cattle receiving a full vaccine dose, 4 out of 5 cattle receiving a 1/3 dose and 2 out of 5 cattle receiving a 1/9 dose were protected from FMD generalisation. Based on this result, a heterologous potency of 6.5 PD_{50}/dose (95% CI <3, 13>) was calculated. Using logistic regression, with the slope of previous experiments as offset, a slightly higher potency with a higher upper limit of the 95% confidence interval was calculated (11 PD_{50}/dose, 95% CI <3, 38>).

Viraemia was not detected in any vaccinated animals, but FMDV genome was detected in 4 of the vaccinated cattle (1 vaccinated with a 1/3 dose and 3 with 1/9 dose). In all cattle, FMDV was detected in the nose and mouth swabs. The duration of virus detection was significantly different between the 4 groups in both serum ($p \leq 0.01$) and nose swabs ($p = 0.02$). Also, the maximum amount of virus detected in serum ($p \leq 0.01$) and nose swabs ($p = 0.03$), was different between groups. However, the duration of virus detection as well as the maximum amount of virus, in one or all vaccinated groups differed significantly from the control group (pairwise t-test), but there was no

significant difference between the different dose groups. In the mouth samples, no significant differences were seen in the duration and maximum amount of virus detection between the groups ($p = 0.3$). Similar results were obtained when analysing FMDV genome detection; with only significant differences in duration and the Ct of genome detection as well as the minimal Ct (highest amount of genome) in serum and nose swabs between vaccinated and control cattle, but not between the different doses of vaccine applied. Groups that received a higher vaccine dose developed significantly higher neutralising antibody titres in both the homologous A/MAY/97 and A/IRN/22/2015 (linear mixed effects model).

3.2.1.4 *Discussion*

Our studies showed that FMDV emergency vaccine A/MAY/97 can protect against challenge with an FMDV field isolate belonging to the A/ASIA/G-VII lineage with a suitable heterologous potency of 6.5 PD_{50}/dose. Both the A/MAY/97 and A22/IRQ/64 vaccines shortened the duration of viraemia and virus excretions in nose and mouth swabs and decreased the maximum amount of virus detected. Although the A22/IRQ/64 vaccine did not provide full protection, it could still assist in controlling outbreaks by decreasing the amount of virus present in the environment. These data support previous studies showing that a high potency emergency vaccine can protect against clinical disease when challenged with a heterologous strain of the same serotype, indicating that not only the r_1-value of the vaccine, but also the homologous potency of a vaccine should be considered when advising on vaccines to control an outbreak.

The experiments were done in collaboration with European laboratories to also inform the European vaccine banks. The Australian Vaccine Bank received confirmation that the current strains in the bank would be suitable to use in an emergency with a preference for A/MAY/97, and there is a recommendation to the European banks to consider inclusion of A/MAY/97. Since this study has been completed, tailored A/ASIA/G-VII vaccines have become available from international suppliers, providing more options (see Section 3.3).

3.2.1.5 *Reference Materials*

Bachanek-Bankowska, K.; Di Nardo, A.; Wadsworth, J.; Henry, E.K.M.; Parlak, Ü.; Timina, A.; Mischenko, A.; Qasim, I.A.; Abdollahi, D.; Sultana, M.; et al. Foot-and-Mouth Disease in the Middle East Caused by an A/ASIA/G-VII Virus Lineage, 2015–2016. Emerg. Infect. Dis. 2018, 24, 1073–1078.

Darling, A.J.; Boose, J.A.; Spaltro, J. Virus Assay Methods: Accuracy and Validation. Biologicals 1998, 26, 105–110.

Appendix

The publication is available as Appendix 3.1: Cross-Protection Induced by a A/MAY/97 Emergency Vaccine Against Intra-Serotype Heterologous Challenge with a Foot-and-Mouth Disease Virus from the A/ASIA/G-VII Lineage. Vaccines 2020, 8, 24; https://doi.org/10.3390/vaccines8010024

3.3 New serotype A/Asia/G-VII lineage emergency vaccine against A/Asia/Iran-05 lineage strain

3.3.1 *Introduction*

Most vaccine banks have a limited number of vaccine strains due to cost and other considerations; therefore, if a new commercial vaccine strain becomes available, it is reasonable to assess if this new vaccine strain can replace another strain in the vaccine bank. We posed the question if the newly developed A/ASIA/G-VII vaccine can replace the A/IRN-05 vaccine or if both the strains need to be present in the vaccine banks. To quantitatively answer this question, we performed a full heterologous potency test in cattle according to the European Pharmacopoeia using an A/ASIA/G-VII emergency vaccine and challenge with A/ASIA/IRN-05 lineage strain, 3 weeks post-vaccination in a cost share collaboration with the Wageningen Bioveterinary Research (WBVR), The Netherlands, and The Pirbright Institute, United Kingdom.

3.3.2 *Method*

Monovalent double oil emulsion vaccine with A/ASIA/G-VII at an antigen payload that would result in a potency value of >6 PD_{50}/dose, was prepared by M/s Boehringer Ingelheim (formerly M/s. Merial Company Limited), United Kingdom. A full heterologous potency was performed with three groups of 5 cattle vaccinated intramuscularly in the neck with either a full dose, 1/4 or 1/16 dose of vaccine as described in the European Pharmacopoeia at WBVR. At 3 weeks post-vaccination, the vaccinated and three control cattle were challenged by intra-dermo-lingual route with a $10^{3.6}$ PFU/mL of the original virus suspension of FMDV isolate A/IRN/10/2018, injecting 0.1 mL at 2 sites. Cattle were inspected for lesions in the mouth and feet at 4- and 8- days post challenge (dpc). Protection is indicated by an absence of lesions on the feet. Antibody responses post-vaccination and post-challenge were measured along with viraemia and virus shedding in the nasal and oral fluids, both by virus titration on cell cultures and genome detecting RT-PCRs. Statistical analyses of the data were performed using Fischer's exact test and linear mixed effects models. The potency of the A/ASIA/G-VII vaccine was calculated by the method of Spearman-Karber (Darling et al 1998) and by logistic regression.

3.3.3 *Results*

All three unvaccinated controls developed clinical signs of FMD and showed generalised disease by 4 dpc. Three out of five cattle that received a full dose, and one cattle each in the one-fourth and one-sixteenth dose were protected from clinical disease and generalisation. The estimated heterologous potency was 2 PD_{50}/dose (CI: 0.5 - 6.0). The challenge results matched the very low r1 value results (0.0) between A/ASIA/G-VII vaccine strain and viruses from the A/IRN-05 lineage in the *in vitro* vaccine matching studies.

Homologous neutralising antibodies (>1.20 $\log_{10}$) were observed in 7 out of 15 vaccinated animals on day 21 post vaccination. None of the vaccinated animals had any detectable cross-neutralising antibody titres (>1.20 $\log_{10}$) against the heterologous challenge virus A/IRN/10/2018 on the day of the challenge. Anamnestic responses to the vaccine strain and the challenge virus could be observed in all the vaccinated groups by 5 dpc. When comparing the responses in different groups, there was not a significant difference (P>0.05) in the anamnestic response post-challenge.

Viraemia was observed in all control animals between 1 and 3 dpc, and from one animal each in 1/4 and 1/16 groups but not from any animals in the full-dose group. There was a highly significant difference in viraemia between the vaccinated and the unvaccinated control cattle ($P<0.001$). FMDV was isolated from the nasal swabs of most animals between 1 and 5 dpc. Virus excretion in the nasal secretions was limited to only one day in most of the animals in the different vaccine groups. However, two animals showed virus excretion for more than one day (1–3 dpc). The unvaccinated controls showed virus excretion at least up to 4 dpc. There was a highly significant difference in the excretion levels between the vaccine groups and unvaccinated control group ($P<0.05$). Infectious virus was isolated and titrated from the oral swabs across all the vaccine groups between 1 and 5 dpc and up to 8 dpc in the unvaccinated control groups. There was no difference in the excretion levels between the different groups ($P>0.05$) except for the duration of excretion (See Appendix 3.2).

3.3.4 *Discussion*

Vaccine strains selected for banks should preferably have a broad antigenic cover to also provide protection against heterologous strains and decrease virus excretion. This study demonstrated the low cross protection of the new vaccine strain belonging to A/ASIA/G-VII lineage against a commonly circulating lineage of FMDV belonging to A/ASIA/IRN-05 lineage. Therefore, replacing the A/ASIA/IRN-05 vaccine strain with A/ASIA/G-VII vaccine strain in the FMDV vaccine banks is not preferable as the former strain has a broader cover and since it has been used in the field for many years, also has more empirical information on its effectiveness. The recommendation to the Australian Vaccine Bank was therefore to continue including the A/MAY/97 strain for use against the A/G-VII lineage and rather retain the A/IRN-05 lineage strain. Despite the poor protection, vaccination still decreased virus excretion, indicting the positive impact vaccination will have during control options.

3.3.5 *Reference Materials*

Darling, A.J.; Boose, J.A.; Spaltro, J. Virus Assay Methods: Accuracy and Validation. Biologicals 1998, 26, 105–110.

Appendix

The publication is available as Appendix 3.2: The new FMD serotype A/Asia/GVII lineage emergency vaccine offers low levels of protection against circulating FMDV A/Asia/Iran-05 lineage strains. Viruses, 14, 97. https://doi.org/10.3390/v14010097

List of studies supported by the FMD Risk Management and FMD Ready projects to determine *in vivo* vaccine efficacy

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Vosloo Wilna, Nguyen Thi Thu Hong, Fosgate T Geoffrey, Morris Michelle Jacqueline, Wang Jianning, Kim Van Phuc, Quach Vo Ngon, Le Thi Thu Phoung, Dang Hung, Tran Xuan Hanh, Vo Van Hung, Le Thi Quynh Anh, Mai Thi My Tien, Le Tin Vinh Quang, Ngo Thanh Long, Singanallur Balasubramanian Nagendrakumar. 2015. 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. Vaccine 33: 2778-2785.

Horsington J, Singanallur NB, Nfon C, Bittner H, Vosloo W. 2022. Investigation into the protective ability of monovalent and bivalent A Malaysia 97 and A22 Iraq 64 vaccine strains against infectiou with an A/Asia/SEA-97 variant in pigs. (Manuscript under preparation for Frontiers in Veterinary Science – Special Issue on Foot-And-Mouth Disease Epidemiiology, Vaccines and Vaccination: Moving Forward).

3.4 FMD virus assay improvements documented and validated

The improvement of diagnostic assays has been addressed in several different activities and is presented as:

- Evaluation of reagents for serotyping of FMDV isolates and suspected samples (3.4.1)
- Potential of reduction of AgPath reagent used in FMDV Real-Time TaqMan assay (3.4.2)
- Evaluation of sensitivity of commercial ELISA kits for sero-surveillance of FMD in goats of Lao PDR (3.4.3)
- Developing next generation sequencing protocols for full FMDV genome determination from low concentration samples (3.4.4)

The COVID-19 pandemic has impacted on the planned validation of a fast format serotype specific real time PCR that can determine the serotype of an isolate. This work needs to be performed at the Regional Reference Laboratory, Pakchong, Thailand where we have access to live virus samples and will be done at a later stage.

3.4.1 *Evaluation of reagents for serotyping of FMDV isolates and suspected samples*

3.4.1.1 *Introduction*

Reports emerged from various laboratories in South East Asia (SEA) that the reagents in the antigen ELISA, used for serotyping of FMD viruses from SEA, were not able to identify FMD virus serotypes from many clinical samples that returned a positive result indicative of the presence of FMD virus in a molecular diagnostic test. In addition, during recent participation in the Pirbright Proficiency Testing (PT), the diagnostics section at the Australian Centre for Disease Preparedness (ACDP) failed to determine the serotype in some samples present in the panel using the reagents for antigen ELISA. The antigen ELISA is based on a rabbit antibody that is serotype specific and is coated onto an ELISA plate. It traps the virus if present in the sample suspension and is detected by using a serotype specific guinea pig antibody. The sensitivity of the assay depends on how broad the reactive spectrum is for the trapping and detecting antibodies as well as their ability to trap and detect newly emerging virus strains. Since these diagnostic reagents are made from historical viruses mostly used as vaccine strains, constant monitoring and recommending change of reagents, if required, is an important task of a diagnostic laboratory, especially the OIE Reference Laboratories. We evaluated the ACDP reagents on FMD samples received at the OIE-Regional Reference Laboratory (RRL) for FMD in SEA (Pakchong, Thailand) during a visit to their facility. The objectives were to perform the ACDP serotyping antigen ELISA on tongue epithelial samples and live virus isolates, and to identify suitable reagents or a combination of reagents for antigen ELISA to recommend for use at ACDP.

3.4.1.2 *Method*

An antigen ELISA was performed at RRL using the routine reagents from ACDP to determine the serotype of FMD virus in clinical samples and cell culture supernatants. For serotype O, the antigen ELISA was performed using rabbit and guinea pig antibodies derived from either O1 BFS or VO1 vaccine strains and for serotype A, a combination of A5, A22 and A24 strains. In addition, we evaluated additional reagents derived from the VO2 (serotype O) and VA1 and VA2 vaccine strains (serotype A). These three strains are known to have broad spectrum in identifying FMD viruses emerging from SEA. Antigen detection and serotyping were performed both on a 10% tissue suspension of the clinical sample (representing the primary detection in the laboratory) as well as on

tissue culture supernatant after the virus was isolated on either primary bovine thyroid or BHK-21 cell lines often resulting in higher concentrations of virus and improved detection (secondary detection).

The number of samples tested are furnished below:

- 10% suspension of Tongue Epithelium
    - Serotype O – 25 (2014 – 11 samples; 2015 – 14 samples)
    - Serotype A – 25 (2014 – 25 samples)
- Tissue culture adapted isolates)
    - Serotype O – 25 (2014 – 20 isolates; 2015 – 5 isolates)
    - Serotype A – 25 (2013 – 11 isolates; 2015 – 14 isolates)

3.4.1.3 *Results*

Serotype O

When using homologous reagents, where the rabbit and guinea pig antibodies were prepared against the same virus for either VO1 or VO2, the VO1 assay failed to detect FMD virus in several samples whereas the VO2 assay had superior detection. A combination of the antibodies against VO1 and VO2 in one test resulted in an improved assay (Table 1).

Serotype A

Antigen ELISA performed with the combined antibodies derived from three different serotype A viruses showed good reactivity against most field samples, indicating the antibodies used for detection of serotype A should be sufficient to detect most serotype A viruses from SEA (see Table 1). The antibodies prepared using VA1 and VA2 showed fair to good reactivity indicating that if needed, these reagents can be used as a backup for identifying serotype A viruses at ACDP.

Table 3-1 Summary of results when comparing different reagent combinations for the antigen detection ELISA for serotypes O and A

Reagent	10% Tissue Suspension	Cell culture supernatant
Serotype O		
VO1 & VO2 combo	96%	100%
VO1	36%	92%
VO2	96%	100%
O TAI 189/87 (Udon Thani)	92%	100%
Serotype A		
A Combo (3 viruses)	92%	100%
VA1 & VA2	92%	100%
VA1 & VA2	96%	100%
A/TAI/2012 (Lopburi)	84%	100%
VA2*†	92%	88%
VA1*‡	88%	68%

* Two samples were not tested due to insufficient volume

† Three samples were not tested due to insufficient volume

‡Eight samples were not tested due to insufficient volume

3.4.1.4 *Discussion*

Based on the results, it was recommended that the ELISA typing reagents for identification of serotype O isolates at ACDP should be based on a combination of reagents to VO1 and VO2 to increase the sensitivity of the antigen ELISA.

It was found that the A combination of reagents was able to identify all serotype A isolates from SEA tested in this study (all samples tested were detected following propagation of isolates in tissue culture) with no need to adjust the reagents.

ACDP have incorporated VO2 antibodies in the antigen ELISA protocols. At the RRL Pakchong, the existing reagents were found to be sensitive. However, the recommended changes have not been adopted by all testing laboratories in SEA due to a lack of availability of these reagents.

Due to the high mutation rate of FMD virus, studies such as these are recommended at least once every two years to ensure diagnostic tests will be suitable to detect viruses that are circulating in high risk areas.

3.4.2 *Potential of Reduction of AgPath Reagent used in FMDV Real-Time Taqman assay*

3.4.2.1 *Introduction*

Real-Time TaqMan PCR is a frontline test for the molecular diagnostic service at ACDP. Trends indicate that there will be an increasing number of tests in the future. For example, there was 56.8% increase in the number of TaqMan reactions for various diseases in 2017 (48,115 reactions) compared to 2014 (30,718 reactions). There is potential to significantly reduce the cost of testing by decreasing the reaction volume of the real-time TaqMan assays. Decreasing the volume without loss of test sensitivity would increase the number of available reactions/kits from the same budget allocation. This would provide benefits to ACDP, the regional program and research projects. However, ACDP is accredited by the National Association of Testing Authories (NATA) and any adjustment to assays needs to be fully validated before they can be implemented. To determine if a reduction in the assay volume impacts on the test sensitivity for the FMDV assay, we visited the FMD OIE Regional Reference Laboratory in Pak Chong, Thailand where clinical FMDV samples are available.

3.4.2.2 *Method*

The real-time RT-PCR implemented at ACDP for FMDV diagnosis was used to compare the 1.0X and 0.6X reaction volumes on seventy-seven FMDV infected tissue samples in a 10% suspension in phosphate buffer saline. The viral genome (RNA) was extracted using the RNeasy extraction kit (Qiagen, USA). TaqMan assays were conducted in duplicate (except for limit of detection assays which were conducted in triplicate). TaqMan PCR reaction mixtures, Primer-Probe Mix, plasmid positive controls and PCR cycle parameters were identical to those used at ACDP except that the PCR instrument was a Roche LightCycler 480ii rather than the ABI 7500 FAST. The assays were compared for limits of detection (LOD), repeatability and test agreement.

3.4.2.3 *Results*

Comparison of Ct values of the 1X and 0.6X FMDV assay indicated that the maximum within-sample variation was 1.42 Ct. Compared to the standard 1.0X FMDV assay, the Ct values (mean of duplicate samples) obtained using the 0.6X FMDV assay were marginally lower for 75 samples and marginally higher for only 2 samples. Overall, the data indicated that the assays were highly comparable in sensitivity using the 0.6X FMDV assay. The 0.6X assay had high repeatability estimate and the LOD was similar to the standard 1.0X assay.

3.4.2.4 *Discussion*

Comparative testing of known FMDV-positive field samples indicated that, in almost all cases, Ct values were comparable to the reduced volume (0.6X) method, indicating no loss in sensitivity compared to the standard FMDV assay. The statistical significance of the data was evaluated, and it is concluded that the 0.6X method, using a total volume of 15 µL, is comparable with our current method using a total volume of 25 µL. With the reduced volume method, we have an approximately 40% cost savings of reagents and primers/probe used in the TaqMan reaction. As a result, the reduced volume (0.6X) has been recommended to replace a standard 25 µL volume for FMDV real-time TaqMan testing at AAHL and other TaqMan assays such as internal control, influenza, bluetongue, classical swine fever and African swine fever.

Appendix

The complete report is available as Appendix 3.3: Potential of Reduction of AgPath Reagent used in FMDV Real-Time TaqMan assay.

3.4.3 *Evaluation of sensitivity of commercial ELISA kits for sero-surveillance of FMD in goats of Lao PDR*

3.4.3.1 *Introduction*

Once an outbreak of FMD has been eradicated, surveillance will be required to provide proof that the disease is no longer circulating amongst livestock. Serological assays that detect antibodies to the virus will be an important aspect of such post outbreak surveillance. One of the tests that will be used, especially if vaccination was part of the control plan, is an antibody test that detects antibodies to the non-structural proteins (NSPs) of the virus. These antibodies are only produced when the virus replicates. The FMD vaccine is an inactivated vaccine, therefore no virus replication happens, and no antibodies are developed to the NSPs. Antibodies will be formed to the structural proteins (SPs), but this could be due to infection and vaccination. The NSP ELISA can therefore be used to distinguish between vaccinated and infected animals.

The test has been validated for use with cattle and pig sera, but very little information is available on the use of this test, as well as those that detect antibodies to the SPs, in goats. We identified an opportunity in SEA where we could apply both antibody tests and gain more information on their use, whilst also gaining more knowledge on the epidemiology of FMD in the region. Sheep and goats do not show overt clinical disease, therefore serological data will be crucial to proof they had not been infected.

FMD causes significant economic loss in Lao PDR (Laos) and perpetuates the cycle of smallholder poverty mainly through large ruminant productivity losses, increased costs of production and potential limitations to market access for trade in livestock and their products. Goats are emerging as an important livestock species in Laos, and there is an increasing trend in the number of households with goats, often farmed alongside cattle and buffalo. Although an FMD susceptible species, very little is known about the role of goats in the epidemiology of the disease in Laos. A cross-sectional seroprevalence study was conducted by detecting antibodies to the NSPs, an indication of a previous infection, and serotype-specific SPs that could be due to vaccination or infection. New commercial ELISA kits were made available for use in this project and we ascertained their application for sero-surveillance in goats.

3.4.3.2 *Method*

Laos has seventeen provinces; each further subdivided into districts with many villages. The study was conducted between September 2017 and March 2018 in eight provinces of Laos. Five of the selected provinces were involved in FMD vaccination campaigns through the Australian funded STANDZ program in the northern and central provinces between 2012 and 2016 (Nampanya et al 2018) and the central and southern provinces since 2016 funded through the New Zealand FMD control program (Mcfadden et al 2019). The provinces in the north were Borkeo, Luang Namtha , Luang Prabang, and Xayabouli; central provinces included Xieng Khouang, Khoummoune, and Savannakhet and one southern Province, Champasak.

Blood samples were collected by jugular venepuncture, and all samples were shipped on dry ice to the National Animal Health Laboratory (NAHL), Vientiane. In each province, data were collected on animal related variables including age (in groups of <12, 12–24, and >24 months), body weight (kg) and sex (male/female) as well as grazing practices (free/forage/stall), co-grazing (yes/no); and occurrence of FMD and Orf in the last 2 years. There were no official records for vaccination of goats in any of the districts in the study area.

Serological assays for antibodies to the NSP and SP of FMDV were performed using Prionics kits (NS ELISA Kit and serotype O, A, and Asia1 specific cELISA kits supplied in kind by M/s. Thermofisher Scientific, Australia) at the NAHL in Vientiane. All the assays were performed according to the manufacturer's instructions, and the samples declared as positive or negative based on the %

inhibition (PI) values (PI > 50% was positive), for the NSP and serotype specific SP assays. Animals were classified as infected solely on the NSP result obtained; positive (1) or negative (0). The SP results were not considered for this classification due to the possibility that antibodies may be due to vaccination and not natural exposure. A binomial logistic linear mixed model (LMM) was fitted for the multivariable analysis. Farmer, village, district and province were included as random effects to account for clustering, and the intraclass correlation (ICC) coefficient for each of these random terms was calculated based on the methodology described for ICC estimation from the random intercept logistic model. Clustering was deemed high for random effects that had an ICC greater the 0.3. A backwards stepwise elimination approach was used until all variables had a p-value of <0.05 and were considered significantly associated with the outcome variable. Goodness-of-fit of the final regression model was assessed by calculating conditional R2 for the final model (R2 GLMM(c)) and the amount of variation in the data explained by the fixed effects was determined by calculating marginal R2 for the fixed effects (R2 GLMM(m)). Estimated prevalence and confidence intervals were calculated using the prevalence package (v0.4.0) in R.

3.4.3.3 *Results*

The NSP seroprevalence in the provinces of Borkeo and Xayabouli in the north was 42 and 8%, respectively and in Khammoune in the centre, it was 20%. In the other five provinces, Luang Namtha and Luang Prabang (northern Laos), Xieng Khouang and Savannaket (central Laos), and Champasak (southern Laos), the seroprevalence was close to zero. The multivariable analysis indicated that age ($p < 0.001$) was positively associated with animal-level seropositivity and males were less likely to be seropositive than females (OR: 0.29; 95%CI: 0.10–0.83; $p = 0.017$).

Some goats did not have antibodies to NSP but were positive for antibodies only to SP (2.5–18.7%) and in some cases, to more than one serotype. In northern Laos, the province of Xayabouli had the highest percentage of animals showing antibodies to serotype O (18.7%) with another 8% animals positive to both serotypes O and A. Goats with antibodies to serotype O were found in all provinces. Antibodies to serotype Asia1 only were detected in Champasak (1.3%) and together with serotypes O and A in Luang Namtha (1.3%) and Luang Prabang (1.3%) provinces (See Appendix 3.4).

3.4.3.4 *Discussion*

Seroprevalence to both NSP and serotype O in Borkeo, Xayabouli and Khammoune indicated the likelihood of FMDV transmission and raising the possibility that caprine outbreaks occurred and were unrecognized. In the other provinces, the seroprevalence was close to zero, and a careful analysis of the results showed that the sera that tested NSP positive were close to the cut-off value, suggesting these may be non-specific reactions. Based on these results, and in the absence of reported clinical disease, and vaccination in goats, we conclude that at least two provinces in the north and one in the centre had FMDV infection in goats in the recent past.

The study confirmed the utility of the NSP antibody kits and other serological kits to detect antibodies against serotype O, A and Asia1 viruses in goats, and are valuable additions for FMD sero-surveillance in this region. It should be mandatory to include goats in sero-surveillance activities for FMD in Laos and presumably other countries in the region, particularly where large-scale vaccination strategies in large ruminants are planned for FMD control and establishment of FMD free zones by vaccination in South East Asia.

With the increase in number of goat farms in Australia, especially Victoria catering to a niche market with goat milk, sero-surveillance for FMD in goats will be vital during the recovery and ‘proof-of-freedom’ stage if an outbreak occurs.

3.4.3.5 *Reference Materials*

Mcfadden et al (2019) The New Zealand OIE foot-and-mouth disease control programme in southeast Asia. Surveillance. (2019) 46:12–4. Available online at: http://www.sciquest.org.nz/node/153398

Nampanya et al (2018) Implementing large foot and mouth disease vaccination programmes for smallholder farmers: lessons from Lao PDR. Epidemiol Infect. 146:2086–95. doi: 10.1017/S0950268818002443

Appendix

Appendix 3.4: Singanallur et al 2020; Serological Evidence of Foot-and-Mouth Disease Infection in Goats in Lao PDR. Frontiers in Veterinary Sciences, 7: 544. https://doi.org/10.3389/fvets.2020.00544

3.4.4 *Developing next generation sequencing protocols for full FMDV genome determination from low concentration samples*

3.4.4.1 *Introduction*

Tracing and monitoring the transboundary movements of FMDV have been successfully achieved using consensus sequences of the 1D coding region of the viral genome (Kasambula et al., 2012; Knowles and Samuel, 2003; Samuel and Knowles, 2001). However, over shorter epidemic time scales, where viral populations have not substantially diverged, 1D sequencing alone cannot provide the required resolution to discriminate between viruses in field samples collected from neighbouring farms within outbreak clusters (Cottam et al., 2009). Consensus sequencing using the Sanger method identifies the predominant or major viral sequence in a sample but is uninformative about minority variants that are present. Evidence for population heterogeneity, where individual sequences differ from the consensus sequence, was obtained routinely using cloning approaches (Airaksinen et al., 2003; Cottam et al., 2009), providing insights into the evolutionary processes that shape viral populations. Unfortunately, these cloning processes are laborious and usually provide only a limited resolution of the mutant spectrum within a sample. Whole genome sequencing at the consensus level has proven to be a powerful tool for the reconstruction of virus transmission trees (Valdazo-Gonzalez et al., 2012). Next-generation sequencing (NGS) techniques offer a “step-change” increase in the amount of sequence data that can be rapidly generated from a sample and can be applied to sequence viral genomes to obtain ultra-deep coverage (Wright et al., 2011).

Conventional sequencing protocols are subject to biases such as those encountered during PCR amplification and propagation in cell culture, are restricted by the need for large quantities of starting material, and do not convey sufficient information on how the virus is evolving within the host before transmitting to other susceptible species (Logan et al., 2014). NGS techniques create massive amounts of sequence data in parallel and can be used to produce a snapshot of genetic diversity of the FMDV polyprotein coding region, at both intra-host and intra-herd levels (Radford et al., 2012). This resolution enables monitoring of the entire sequence swarm that exists within FMDV samples and can be employed to assess the impact of transmission within and between hosts upon sub-consensus polymorphisms (Paton, Gubbins, and King, 2018). A new method for deep sequencing of FMDV genomes using an enrichment method with biotinylated oligonucleotide baits targeting the FMDV genome is described to allow data to be generated for the SPREAD application developed in Subproject 4.

3.4.4.2 *Method*

Oral and nasal fluids, and rectal samples were collected from pigs that were recruited for two independent vaccine efficacy studies for serotypes O and A (Nagendrakumar et al., 2015; Vosloo et al., 2015; Wilna et al., 2015) and one pig pathogenesis trial (unpublished) using dry cotton swabs. Total RNA was extracted from these samples and Illumina libraries constructed. Size selection was performed to obtain a library size between 400 and 600 nt and libraries were purified and quantified.

Biotinylated DNA baits that were complementary to the FMDV genome, and 120 nt in length, were designed such that they were located at least 500 nt apart on the FMDV genome, assuming an average deep sequencing library size of the sample would be approximately 300 nt, following protocols described earlier (Kamaraj et al., 2019). Following this approach, the FMDV genomic RNA (after its conversion to cDNA) was captured with 18 oligonucleotide baits targeting the highly conserved regions of the 8.3 kb FMDV genome of which 14 baits were common to serotypes O and A while, 2 baits each were specific to serotype O and serotype A. The enriched libraries were purified and quantified.

For genome assembly and variant calling, sequencing reads were subject to processing to trim the adapters and host reads were removed. The remaining non-host reads were assembled into contigs and expanded by incorporating unassembled viral reads through an iterative mapping process until no further viral reads were incorporated.

Exploratory data analysis was performed and the location and frequency of single nucleotide polymorphisms (SNPs) along the FMDV genome were determined and, to ensure the positions were correctly aligned, the coordinates were adjusted relative to a multiple sequence alignment (MSA) for that serotype. Venn diagrams showing the correspondence of SNP locations between different groups of samples were constructed for comparison. Only the positions of SNPs and no indel variants were considered for the data analysis.

3.4.4.3 *Results*

To create a virus capture panel targeting serotype O and A simultaneously, 18 baits targeting the highly conserved regions of the 8.3 kb FMDV genome were synthesised, with 14 common to both serotypes and 2 baits each specific to serotype O and serotype A.

The resulting reads from both unenriched and enriched libraries were mapped against specific FMDV serotype O and A isolates and post enrichment mapping achieved 93.70–96.25% genome coverage compared to <10% without prior enrichment in samples with high concentrations of virus. The specificity of the approach was confirmed using samples from uninfected pigs. To measure the efficacy of the bait panel in clinical samples, the enrichment protocol was tested on thirty oral and nasal swabs. The FMDV genome composition to the host genome for different samples varied from 0.10% to 95% before enrichment. After enrichment, the FMDV component was between 94% and 99.6% for both serotypes tested, and the number of FMDV-specific reads increased almost 3000-fold to 95%. Full-length consensus genome sequences were obtained for most oral/nasal swabs from both serotypes.

The minimum frequency of reads mismatched to the consensus at a given position was initially set to 0.5% (1 in 200 reads). Changing the threshold for SNP inclusion greatly influenced the number of variable positions. By plotting SNPs relative to their coordinate location in the FMDV genome, the density of variable positions was examined. Three positions (3833, 5261, 7599) stood out as being more frequently variable in type A viruses, while clusters of SNPs were apparent for both type A and type O viruses. The majority of observed variants were unique to single samples. However, a small number were identified in multiple samples. Of these, a subset was shared between the different treatment groups (See Appendix 3.5 for full paper).

3.4.4.4 *Discussion*

Reconstruction of viral transmission pathways relies on phylogenetic analysis of viral sequences recovered from field samples. Molecular sequence data are an important component of FMD control strategies, allowing the generation of phylogenetic trees (Knowles and Samuel, 2003). Whole genome sequencing is a powerful tool for reconstructing fine-scale transmission pathways due to the increased resolution of genomic information (Cottam et al., 2008; Wright et al., 2013). Some previously described methods are biased by amplification steps whereby less common sequences will not be represented or detectable following amplification and therefore will be missed, even with NGS.

We demonstrated proof of concept for obtaining high-quality full-length FMDV genome sequence data directly from clinical samples, including those containing very low concentrations of FMDV RNA, without isolation and propagation of the virus in cell culture. Several samples yielded full-genome sequence even when the RNA concentration was at or below the limit of detection using RT-qPCR. In fact, genome coverage of >80% was obtained from every sample tested, demonstrating the power of the enrichment technique for obtaining viral genome sequence directly from clinical samples. To ensure the method can be used for all serotypes and variants within serotypes, it may be necessary to design more baits.

Next-generation sequencing reveals the fine polymorphic substructure of the viral population, from nucleotide variants present at just below 50% frequency to those present at fractions of 1%. Using variant call analysis, 2200 and 1861 SNPs were identified for serotype O and A in this study, respectively, at a set threshold of 0.5 %. Changing the threshold for SNP inclusion greatly influenced the number of variable positions. As the threshold increased, the number of samples that were variant at a given position also changed. Therefore, the threshold percentage setting is very important in calling SNPs and variants. This analysis, while basic, shows that the enrichment method has great potential for facilitating advanced SNP analysis. While not explored in great depth here, it clearly illustrates the high-resolution SNP data that can be expected due to increasing the depth of coverage via enrichment.

This technology has the potential to facilitate an in-depth study of the intra-host genetic diversity during FMDV infection, with or without vaccination, thereby revealing the location and frequency of SNPs within specific viral populations under different selection criteria. Such data, which are currently being evaluated for the genome sequences reported herein, will provide further insights into, and enhance our understanding of, the infection dynamics and evolutionary processes of this highly varied and complex viral pathogenHaving access to high quality genome sequence data is crucial for the SPREAD application described for Subproject 4. The SPREAD application developed the pipeline to perform all the bioinformatics steps to work the raw data into a format that is subsequently applied to forensically trace the movement of viruses between premises. Such collaboration across subproject research teams was integral to the success of the project.

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Appendix

The full paper is available as Appendix 3.5: Singanallur et al (2019) Probe capture enrichment next-generation sequencing of complete foot-and-mouth disease virus genomes in clinical samples. J Virol Met 272: 113703. https://doi.org/10.1016/j.jviromet.2019.113703

3.5 Novel methods for FMD virus inactivation in samples for safe transport

3.5.1 *Introduction*

In FMD-free countries like Australia, initial outbreak identification will be performed by high-containment national reference laboratories. Molecular tests to identify the virus genome will be deployed to confirm the outbreak and also during continued surveillance. Once the disease incursion is confirmed, subsequent continued diagnostic surveillance, and post outbreak surveillance may be performed in state laboratories using molecular tests. Virus is easily inactivated in "simple" sample types like swabs and lateral flow devices (Romey et al., 2017) by treatment with the guanidium isothiocyanate based buffers that are commonly used for preserving viral genome, or citric acid. However, complex matrix samples like epithelium tissues might hinder virus inactivation, particular if large pieces are collected and virus is contained deep within the samples. Such tissues from infected animals could potentially contaminate the state laboratories, leading to their quarantine. It is essential to have biosafety protocols in place to prevent this (FAO, 2013). Tissue samples must be treated with suitable reagents upon collection to inactivate residual virus during transport and before further processing. Importantly, these reagents must also preserve the viral RNA genome to allow identification and characterisation of the virus, should it be present.

The FMD viral genome is a positive sense RNA embedded within a non-enveloped protein capsid and once the genome is released from the capsid, can easily be destroyed. Commercial reagents are available to preserve nucleic acid in tissue samples, allowing storage at ambient temperatures for up to 30 days without loss of RNA integrity. In addition to the extensively used phenol- and guanidinium- based reagents that carry operator safety risks, reagents that are safer to handle and not light-sensitive are available and need to be evaluated for the purpose of inactivating FMDV in tissue samples.

The objectives of the study were to test the suitability of RNA preservation reagents to inactivate FMDV in epithelium samples (similar to what is expected during an outbreak), measure the time taken for complete inactivation of virus in these tissues, and to explore the suitability of RNA extracted from the treated tissue samples for molecular methods such as real-time RT-PCR (RT-qPCR), sequencing and for recovery of infectious virus by transfection of viral RNA.

3.5.2 *Method*

Two separate studies were performed: an initial study that evaluated a set of reagents, and a follow-up study that evaluated a second set of reagents for FMDV inactivation in cattle epithelium tissue. Animal and laboratory work were performed at the BSL3-Ag containment facility of the Friedrich-Loeffler-Institut in Riems, Germany. For the initial study, four cattle were inoculated into the epithelium of the tongue with either FMDV O/ALG/3/2014 (two cattle) or FMDV A/IRN/22/2015 (two cattle). One day after inoculation, the cattle were euthanised and epithelial flaps collected from the tongues. For the follow-up study, eight cattle were inoculated as described for the initial study with four cattle in the O/ALG/3/2014 group and four in the A/IRN/22/2015 group. Two days after inoculation, the cattle were euthanised and epithelial flaps collected from the tongues.

Epithelium tissue samples were fully immersed in inactivation buffers for the initial study (RNAlater, RNA Shield or PBS) and follow-up study (PAXgene Tissue System Fixative and Stabiliser respectively, McIlvaine's citrate-phosphate buffer and PBS) and incubated at room temperature for 2, 6, 24 or 48 h. The follow-up study included an additional processing step for a subset of each virus-buffer-timepoint combination, which comprised manual homogenisation of the tissue fragment in the relevant buffer before the incubation period. After incubation, the tissue pieces were rinsed with PBS, homogenised, and clarified by centrifugation. Supernatants were used for virus isolation and RNA extraction. Homogenates were tested for the presence of infectious FMDV by infecting monolayers of suitable cells, followed by monitoring for cytopathic effects and indirect double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to detect viral antigen. Virus titrations were performed in 96-well microtitre trays.

The amount of viral RNA extracted from the epithelium homogenates was quantified by RT-qPCR targeting the $3D^{pol}$ coding region of the FMDV genome. The genome region coding for the VP1 capsid protein was amplified by RT-PCR and sequenced to evaluate nucleic acid preservation.

Transfection of cells with extracted FMDV RNA was performed to further evaluate RNA stability and preservation. Transfected cells were observed for CPE and presence of FMDV antigen confirmed by FMDV antigen ELISA.

3.5.3 *Results*

For detailed results, figures and tables, see the two published paper related to this study, in Appendix 3.6 and Appendix 3.7.

RNAlater was effective at inactivating virus in only one of four O/ALG/3/2014 replicates after 24 h incubation. With A/IRN/22/2015, a gradual reduction in titre was observed with increasing incubation times in RNAlater, however 48 h was required for complete inactivation. After 48 h incubation all replicates of O/ALG/3/2014 and A/IRN/22/2015 were inactivated.

Of the RNA Shield samples, one 2 h and one 6 h homogenate were positive for CPE after 48 h incubation, whereas both 24 h homogenates were negative. These results indicate the RNA Shield was effective at inactivating all FMDV after 24 h, and substantially reduced the level of infectious virus after 2 or 6 h.

PAXgene Tissue System Fixative was effective at inactivating O/ALG/3/2014 in all 8 replicates with 2 hours of incubation; one replicate of A/IRN/22/2015 after 2 hours incubation was positive by virus isolation, but the titre was below the limit of detection of the titration assay. The PAXgene Tissue

System Stabiliser was unable to completely inactivate A/IRN/22/2015 in all replicates after up to 48 hours of incubation, although an inactivation effect was noted from 24 hours onwards as demonstrated by a drop in median titre. The Stabiliser was able to inactivate O/ALG/3/2014 in 6 of 8 replicates after 2 hours incubation and resulted in reduction of virus titre in the two remaining positive replicates below the limit of detection of the virus titration. McIlvaine's citrate-phosphate buffer was effective in completely inactivating virus in all replicates after 2 hours of incubation.

The manual homogenisation of tissue before immersion in buffer, performed on a subset of samples in the follow-up experiment, did not have any discernable effect on sample inactivation compared to immersion without manual homogenisation. Therefore the subset of manually homogenised replicate samples from each respective time point and buffer treatment, and the 4 matching replicate samples that were immersed in buffer immediately, were combined into a single group each for the analyses described here.

Cytopathic effect caused by virus replication was observed with all PBS-treated samples.

A one to two $\log_{10}$ reduction in viral RNA, compared to the PBS controls, was observed in O/ALG/3/2014 samples treated with RNAlater, regardless of incubation time, whereas no reduction was observed with the A/IRN/22/2105 samples. In contrast, for both virus strains, a notable reduction of detectable viral RNA was observed in samples after 2 or 6 h incubation with RNA Shield, but not after >24 h incubation. Apart from an apparent reduction in viral RNA in O/ALG/3/2014 samples treated with PAXgene Tissue System Stabiliser at all time points compared to all other samples, there was no drastic effect on RT-qPCR RNA detection following incubation with the PAXgene Tissue System Fixative or McIlvaine's citrate-phosphate buffer, up to at least 48 hours.

Sequence reads of 500 to 900 bases matching input viruses were obtained, indicating that the integrity of the viral RNA was sufficiently maintained with all the reagents tested.

Recovery of infectious virus (confirmed by antigen ELISA) following transfection of the extracted FMDV RNA was achieved with both virus strains, regardless of reagent used or inactivation period.

3.5.4 *Discussion*

Of the two reagents tested under the conditions of the initial study, RNA Shield yielded more promising results for inactivation of FMDV in tissue samples. While 24 h incubation is advised by the manufacturer to ensure virus inactivation and preservation of the viral RNA, our results suggest this reagent can reduce the level of infectious virus considerably after just 2 h. Further analysis and validation of this method is required. The incubation times needed to inactivate the FMDV in the preliminary study were found to be too long to be practical, especially during an outbreak in Australia where the sample needs to arrive at a laboratory for exclusion testing within <24 hours.

We therefore perfomed a follow-up experiment with additional inactivation and preservation buffers. Sufficient penetration of inactivating agents into submerged tissue pieces is a concern for complete inactivation. We found that manual homogenisation of infected epithelial tissue, before incubation in the buffers tested here, did not have any noticeable effect on the ability of the buffers to inactivate the two viruses in the study. However, such an approach might be advantageous when using different inactivation preservation buffers, and when using larger pieces of tissue, but this needs to be further investigated. Of the three buffers tested in the follow-up experiment, the inexpensive and easy-to-prepare McIlvaine's citrate-phosphate buffer was found to be the most promising candidate buffer for future use. McIlvaine's citrate-phosphate buffer was effective at

inactivating both viruses within 2 hours in epithelial tissue. In addition, viral nucleic acid was adequately preserved to allow detection by RT-qPCR after up to 48 hours of incubation, as well as successful VP1 sequencing and virus rescue by transfection after 24 hours of incubation. The buffer is cheap and easy to produce and does not present a health risk to operators when preparing or handling it. The buffering component should also render it stable for storage by users in between farm visits.

Virus inactivation will permit safe molecular testing of virus in suspect lesion samples in BSL2 laboratories that are not certified to handle infectious FMDV in a free country. Additionally, in high-containment laboratories, the ability to successfully recover infectious virus following transfection of RNA extracted from inactivated samples submitted from BSL2 laboratories will be beneficial for characterisation of the virus during an outbreak.

3.5.5 *Reference Materials*

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Appendix

The publication is available as Appendix 3.6: Horsington et al (2020) Inactivation of foot-and-mouth disease virus in epithelium samples for safe transport and processing in low-containment laboratories. J Virol Met 276: 113770. https://doi.org/10.1016/j.jviromet.2019.113770

The publication is available as Appendix 3.7: Jansen van Vuren et al (2022) Chemical inactivation of foot-and-mouth disease virus in bovine tongue epithelium for safe transport and downstream processing. J Virol Met 305: 114539. https://doi.org/10.1016/j.jviromet.2022.114539

3.6 Virus inactivation at different temperatures and humidity for FMD disease spread models

A nationwide outbreak of foot-and-mouth disease (FMD) would devastate Australia's agricultural industry and economy resulting in losses in excess of $50 billion dollars over 10 years. These impacts could be similar with other emergency animal diseases (EADs) such as African swine fever, lumpy skin disease and African horse sickness posing an imminent threat. Rapid reporting, containment and eradication will expedite industry continuity through quick resumption of trade and exports. Many natural and artificial routes have been implicated in spread of EADs. In some historical FMD outbreaks, wind dispersion, under very specific climatic conditions, has been implicated in the transboundary spread of FMD virus (FMDV). However, in Australia the conditions are highly variable in different regions and often seen as not conducive to long distance wind dispersion. We are interested in short distance spread where local climatic conditions could be favourable for spread between farms. It is therefore necessary to consider temperature extremes and varying relative humidity levels in modelling virus survival. Current data are not sufficient to ensure accurate modelling of between farm spread and new data are needed to understand the inactivation kinetics of different viruses. Combining this information with other epidemiological factors will assist in modelling disease transmission pathways and designing tailor made control measures.

We used FMDV as a model to map the inactivation kinetics of different serotypes of the virus to study the influence of temperature and relative humidity on virus survival over time to use in current disease dispersal models. Six virus strains from three different serotypes of FMD virus were tested using nine different temperature/humidity combinations and incubated for ten different time intervals to determine virus survival. The impact of increasing temperatures and humidity on virus survival was modelled using linear and non-linear regression analysis models. Within and between strain comparisons were performed. The data generated suggest that temperature, rather than humidity, plays a major role in the survival of FMD virus, and that there are distinct differences in the sensitivity of different viruses to these environmental conditions. Viruses are rapidly inactivated at temperatures above 20°C, whereas survival at 10°C could be up to 4 or 5 days.

This study provides valuable data for modelling long distance wind dispersal of FMD virus by using historical isolates linked to potential spread by this route. Data generated with contemporary isolates, on the other hand, helps modellers to study the potential risk of long-distance wind dispersion of viruses currently circulating in SEA Asia and presenting a potential risk of introduction to Australia. The design and data from this study will provide a framework for similar studies on other viruses where wind dispersion is thought to play a role, to obtain scientific data to support dispersion model development.

4 A new model for a producer-led animal health surveillance system

4.1 Introduction

Subproject 2 activities contributed to the development of a new model for producer-led animal health surveillance system for consideration by the jurisdictions, using an integrated bioscience and social science approaches to achieve the following two specific objectives:

- Increased partnerships among livestock farmers and other animal health stakeholders
- Improved general surveillance for early detection of disease and spread leading to fewer, less impactful and more readily controlled, outbreaks.

This subproject used three complementary and integrated approaches:

1. a systems approach for integrated social and biosciences research
2. a risk-based approach for prioritisation of emergency animal disease (EAD) vulnerability among livestock producers, and
3. a partnership approach to create platforms for interaction among a network of value chain actors to devise innovative solutions to structural and behavioural challenges to improve animal health surveillance.

Subproject 2 was designed using a systems-thinking approach (Sterman 2000, Maru and Woodford 2001). This approach was used to promote transdiciplinary research for a more comprehensive understanding of challenges and opportunities for improving partnerships among farmers and animal health stakeholders and strengthening general surveillance. The approach provided special emphasis on integrating social and behavioural studies (the use of which has been limited in previous animal health research) with biosciences to understand the drivers, motivations, barriers and capacities that determine current levels of engagement with and adoption of on-farm biosecurity measures and general surveillance. This is consistent with recent international literature which recommends moving from universal and highly technical biosecurity guidelines and awareness communication strategies to more localised and collaborative approaches informed by social science (Enticott, 2014, Hinchliffe and Ward, 2014, Pfeiffer, 2013). A systems approach recognises that structures such as institutions, organisations, and networks generate system behaviour. Current biosecurity and surveillance behaviours among livestock producers and other stakeholders are primarily driven by institutional and social structures such as regulations, policies, incentives, shared values and knowledge systems. It is essential to understand current biosecurity and surveillance behaviour among stakeholders within the systemic influence of such system structures.

Animal health surveillance is an essential but costly exercise. Robust science and evidence-based risk prioritisation are prerequisites for efficient allocation and use of limited resources to achieve effective outcomes. Consistent with recent biosecurity direction (Australian Government, 2015) and research efforts (East et al., 2013, Martin et al., 2015) the research in this sub-project has used risk-based approaches for better targeting selection of pilot groups. This component of the subproject complements research on EAD epidemiological risk analysis and mapping, adding social risk-based

analysis to ensure that resources are directed towards where both, biological and behavioural risks are.

In the past two decades, agricultural extension has seen a shift from promoting adoption of practices through persuasion to local innovation through partnership between public and private sectors and other stakeholders (Leeuwis, 2004b). This has resulted in a move away from traditional linear research-extension-adoption models that have been shown not to be effective in addressing complex issues that involve multiple stakeholders (Spielman, 2005). Instead, an agricultural innovations systems approach that promotes innovation platforms has been adopted based on a realisation that research is not the only source of innovation. Close interactions and partnerships among stakeholders (farmers, industry representatives, government officers, rural livestock agents and researchers) have been found to have a greater chance of leading to innovative and sustainable solutions (Hall, 2007b, Edwards et al., 2013). A partnership approach for innovation within the sub-project contributed to a practice of building trusting relationships for improved surveillance that could lead to early detection of disease and effective responses.

Using these three approaches this subproject conducted four key research activities, these being:

1. A synthesis report describing the current state and trends of animal surveillance systems in Australia
2. The development of an EAD vulnerability prioritization of livestock producers
3. The use of an Agricultural Innovation Systems approach to develop farmer-led innovation pilot groups and
4. The development of a scaling up strategy.

4.2 A synthesis of current state of animal surveillance systems in Australia

4.2.1 Introduction

The objectives of this component of the subproject were to:

1. Understand the state of the current livestock disease surveillance system and its successes and challenges
2. Learn from, build on and complement initiatives for improved surveillance
3. Identify factors that promote or constrain partnerships among livestock stakeholders for improved surveillance and
4. Identify industry segments, groups and networks of producers more vulnerable to incursion and spread of diseases, and the key social, cultural, behavioural and institutional characteristics for such vulnerability, according to previous studies and stakeholder perspectives.

4.2.2 Methods

To achieve the objectives of this component of subproject 2, a review of scientific literature, including published manuscripts and research reports, and government and industry documentation, in relation to animal health disease surveillance in Australia was initially conducted. To complement the information gathered during this review, a total of 18 interviews and three sets of focus groups were

conducted. The interviews conducted were with government organisations (n = 7; Commonwealth and state/territory jurisdiction veterinary and animal health, biosecurity and surveillance officers and managers), industry stakeholders or industry/producer-led initiatives (n = 10) and Animal Health Australia. Two focus groups were conducted with industry representatives and one with state/territory jurisdiction representatives. Three different semi-structured interviews were designed, with the aim of gathering information on current animal health surveillance activities and programs, including producer and industry-led initiatives, and the perceived challenges in disease surveillance, and how partnerships among livestock producers and other stakeholders could be enhanced to improve surveillance.

4.2.3 Results

According to the outcomes of this review, the current animal health surveillance system in Australia can be improved. Areas for improvement to general surveillance include the need for well-informed and increased number of notifications of suspect significant diseases. Ideas, suggested by interviewees as solutions, span addressing behavioural and structural issues as well as their interactions. These include increasing producer awareness of requirements for notification, and the ability and motivation of producers and animal owners to recognise and report suspect significant disease events. However, despite differences across jurisdictions, institutional responses are also required to address structural issues, for example the cost of investigating animal health incidents, accessing veterinarians and the low level of trust with government agencies, still identified as key challenges for general surveillance. Other challenges for improving surveillance requiring institutional response include perceived top-down orientation of the surveillance system and the lack of nationally consistent and harmonised surveillance data management and reporting that is responsive, efficient and effective in delivering useful outputs to producers and other stakeholders.

All these issues require government working closely with animal owners, producers and value chain actors. Lessons from past and present partnership initiatives show that clear focus, compelling drivers, clear benefits, and enforceable penalties and trusting relationships are key factors for success. The sub-project considered these lessons when establishing and running pilot groups, who share similar challenges with respect to surveillance and other relevant characteristics. The sub-project studied the values and needs of producers in these groups and piloted partnerships that address behavioural and structural issues relevant to these groups. While pilots were important to find out what partnerships for improved surveillance may look like in different contexts at the local level, concurrent engagement and dialogue were also essential at jurisdictional and Commonwealth levels to address the cross-scale structural issues raised.

4.3 A risk-based approach for prioritisation of EAD vulnerability among livestock producers

4.3.1 Introduction

The risk-based approach adopted in the producer-led surveillance subproject has involved developing risk characterisations of producers based on behavioural and structural (social, institutional, biophysical, technological) variables. A characterisation or typology is an ordering or a

systematic classification of units of interest based on the similarity/dissimilarity of their states, characteristics, properties or behaviours (Collier et al., 2012, Maru et al., 2012). A typology refers both, to the process of classification and to the set of output types or categories that emerge from the process (Bailey, 1994, Nelson et al., 2010b). Typologies assist with reducing complexity, detecting patterns and groups, and prioritizing resource allocation and interventions (Emtage et al., 2006). Typologies can drive successful engagement with stakeholders and the development of programs of interest to the stakeholders (Aslin et al., 2004 in Emtage et al., 2006).

The application of typology in this subproject had the main objective to enhance our current understanding of producer characteristics, practices, and context that define their exposure to the risk of emergency animal disease introduction and delayed detection and reporting, across producers within the beef and dairy cattle, sheep, goat and pork industries. The outcomes of the typology component will complement recent EAD epidemiological risk analysis and mapping based on biological risks (Martin et al 2015).

The component of the subproject involved three core activities:

1. Development of a typology framework based on EAD vulnerability
2. Cross-sectional study among livestock producers
3. Development of Bayesian Network Models

4.3.2 Vulnerability framework

Robust typology requires a clear overarching concept with appropriate and sufficient dimensions to guide the identification of valid variables/indicators. These variables reflect each dimension and inform the collection of quantitative and/qualitative data and subsequent data analyses.

The overarching framework adopted for characterising risk for producers in the face of an EAD (FMD) incursion involved discussions around which approaches to risk characterisation are the most applicable in the Australian primary industry producer context. Given risk management involves risk mitigation, considering risk in relation to a specific threat and vulnerability of subjects to that threat results in multiple opportunities for potential risk reduction through modification of either variables around the threat or vulnerability (Nelson et al., 2010a).

Vulnerability as an overarching concept to inform typology: While modifying the threat – introduction of EAD (in our case FMD as a model) is beyond the scope of the subproject, reduction of vulnerability of Australia's livestock industries to harm from exposure to and spread of EADs (as a result of livestock producers failing to inspect, detect and report suspicious signs) is a central focus. Vulnerability, broadly defined as the susceptibility of a system of interest to threats is determined by level of exposure to the threat(s), sensitivity to the threat(s), and the adaptive capacity -to the threats (Nelson et al., 2010a, Adger, 2006, Gallopín, 2006). In this study, a key part of Australia's adaptive capacity following an EAD outbreak is its ability to quickly and effectively respond to and control an EAD outbreak. This is highly dependent on livestock producers' response capacity by means of inspecting, detecting and reporting suspicious symptoms. The vulnerability of Australia's

livestock industries to an EAD is therefore related to producers' on-farm biosecurity practices, or lack thereof. Exposure refers to the degree, duration, and/or extent in which the system is in contact with, or subject to, the threat (Gallopín, 2006). Adaptive capacity refers to the system's ability to respond to the threat and moderate potential damage (Adger, 2006).

Different constituent concepts that contribute to vulnerability can be illustrated with a simplified example in relation to introduction of FMD at the producer level. Level of adherence to strict biosecurity, proximity to other farms, and route of introduction will have effects on the degree to which a farm will come in contact with or be susceptible to FMD virus (exposure) once introduced. The mix of animal types held and the biosecurity measures in place (e.g. isolate sick animals) will also determine the degree to which the disease affects and spread (sensitivity). The ability and willingness to inspect regularly, competence in detecting the disease, reporting early, and adopting response measures will form part of producers' response capacity.

For this subproject the concept of vulnerability has composite dimensions of exposure and response capacity that form a matrix which groups the unit of interest as high, moderate, or low vulnerable to FMD threat. Figure 4-1 shows the vulnerability matrix with three levels of categorisation of vulnerability based on the interaction of the dimensions.

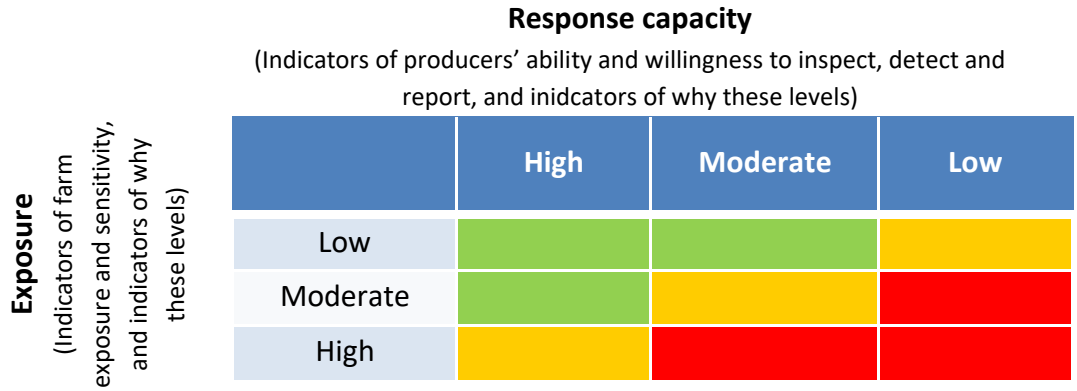

Fig. 4-1 Mapping vulnerability as the intersection of exposure and response capacity green = low vulnerability, orange = moderate vulnerability red = high vulnerability (adapted fromNelson et al., 2010a)

4.3.3 Cross-sectional study among livestock producers

4.3.3.1 *Methods*

An epidemiological cross-sectional survey was developed to gather quantitative data to build vulnerability-based typologies in the five industries susceptible to FMD (beef and dairy cattle, sheep, goats and pigs). The dimensions of livestock operations' FMD vulnerability, the framework developed and evidence from existing epidemiological, behavioural and social science research informed the design of the survey tool. The survey tool measured variables that reflect exposure and response capacity of producers related to vulnerability to FMD.

Development and distribution of the questionnaire: The questionnaire was developed considering questions reflecting exposure and response capacity, as well as demographic and farm

characteristics. In brief, the EAD exposure indicators included in the survey where variables in relation to the following characteristics:

- producers' level of understanding laws prohibiting swill feed amongst pork producers
- swill feeding practice amongst pork producers
- on-farm record keeping
- mix of other animals on farm
- extent of animal movement
- adherence to farm biosecurity measures (*disinfecting, restricting visitor or vehicle movements, ensuring all machinery brought onto the property is cleaned, ensuring purchases are from reliable sources, quarantining new stock or sick animals, selling in designated areas with regular inspection*)
- proximity to other farms
- proximity to feral pigs.

In relation to indicators for ability/willingness, as a reflection of response capacity, the following characteristics were considered in the study:

- perception of FMD risk level (or EAD in general)
- knowledge of the disease and signs
- access to veterinarians
- relationship with vet
- number of visits to veterinarians
- financial investment capacity
- trust in animal health authorities
- number of suspected disease reports.

The questionnaire consisted of 61 open and closed questions, with a set of core questions that were maintained across each of the five industries studied, as well as industry-specific topics. The questions covered four main areas: Demographics and husbandry practises (24 questions), Biosecurity practices and beliefs (7), Animal health management practices (21) and Networks, trust and relationships (9). Questions included perceptions of risk from an FMD incursion, stock selling, buying and monitoring practices and trusted information sources, as well as an opportunity to identify practices that respondents thought increased the risk of and FMD incursion. The questionnaire was designed to be distributed online and posted and distribution was driven by the available registers and support provided by industry and government agencies were approached to assist in the distribution of the survey.

Data analysis: Data from the online or postal questionnaires was downloaded or entered, respectively, in Excel (PC/Windows XP, 2007) and checked for data entry errors. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. was used for the statistical analyses. Descriptive statistics were initially used to obtain a description of characteristics and practices of producers. Subsequently, univariable and multivariable logistic regression analyses were conducted to investigate associations between producer socio-demographic characteristics and practices and those variables related to vulnerability to an EAD outbreak including producers' behaviours and

attitudes in relation to animal health surveillance. Due to the high number of variables related to exposure and response capacity included in the questionnaire, analysis of associations of producer socio-demographic characteristics and individual variables through logistic regression, was not sufficient for developing risk-based typology groupings of producers. As such, a Bayesian Network approach was used to analyse the data, in order to capture the nuances of the complex relationships between the variables, facilitating a more focused risk-based approach to on-farm surveillance (Brugere et al., 2017, Halliday et al., 2012, Lupo et al., 2014). An interpretative coding schema was used to qualitatively analyse open-ended questions and identify thematic categories.

4.3.3.2 *Results*

A summary of the responses to the questionnaire is provided in Table 4-1, and descriptive results for key demographic characteristics of participant producers from the three completed surveys are summarised in Table 4-2.

Table 4-1 Postal and online distribution of FMD Ready surveillance survey

Distribution method	Sheep	Beef	Goats	Pork*	Dairy
Postal distribution	1121	1118	66	500	-
Postal response	136	142	13	10	-
Online response	361	277	118	90	56
Total	497	419	131	100	56

Table 4-2 Descriptive statistics from sheep, beef and goat producer surveys

Variables		Sheep (%)	Beef (%)	Goats (%)
Location	VIC	206 (46)	111 (30)	28 (25)
	NSW	197 (44)	211 (56)	45 (40)
	QLD	9 (2)	39 (10)	25 (22)
	TAS	-	3 (1)	2 (2)
	SA	8(2)	1 (0.5)	1 (1)
	WA	21 (5)	2 (0.5)	5 (4)
	NT	-	4 (1)	-
	ACT	-	1(0.5)	-
Years farming	<5	51 (11)	15 (4)	36 (32)
	5-10	50 (11)	34 (9)	29 (28)
	11-20	64 (14)	63 (17)	23 (20)
	>20	279 (62)	260 (69)	22 (19)
Gender	Male	331 (74)	249 (66)	39 (35)
	Female	114 (25)	126 (34)	69 (62)

Age	18-25	7 (2)	5 (1)	8 (7)
	26-35	51 (11)	18 (5)	6 (5)
	36-50	121 (27)	78 (21)	41 (36)
	51-65	181 (40)	164 (44)	30 (27)
	66-80	81 (18)	99 (26)	22 (20)
	>80	6 (1)	11 (3)	2 (2)

Sheep industry: Over half of respondents reported that there was no likelihood of an FMD outbreak occurring on their property (56.0%), and approximately a third (37.5%) considered an outbreak in Australia as moderately likely. The majority of respondents considered that an outbreak on their property, in their region or somewhere in Australia would be extremely severe (78.3%, 73.6%, 65.8% respectively). When asked to consider how concerned they felt about an FMD outbreak, and how confident they were that they could correctly identify signs of FMD in their sheep, most reported that they were not concerned or a little concerned (49.3%) and had little or no confidence (67.5%). Of those producers that brought stock in, most reported always doing a prepurchase examination if they purchased in stock (71.3%) and used a National Vendor Declaration (NVD) or Sheep Health Statement (SHS) (75.4%). The majority of respondents (84.1%) reported always checking new stock for signs of disease and 64.9% isolating them. When considering frequency of undertaking biosecurity actions around visitors on their property, 52.8% of respondents reported that they never/rarely restricted visitor/vehicle/farm equipment access to their property, while 72.4% reported that they never/rarely required their visitors to undergo specific biosecurity procedures. The majority of respondents (70.3%) reported always/most of the time took action to control feral animals. When considering who is responsible for inspecting their animals, detecting unusual signs of disease and reporting the signs, most respondents identified themselves or their staff (95.7%, 80.2%, 91.5% respectively).

Beef industry: Approximately half of respondents (49.4%) thought that the likelihood of an FMD outbreak occurring on their own property was negligible; however, this perception was different when considering the likelihood of an outbreak at the region or country level. Over half of respondents (52.4%) thought an outbreak to be a little likely in their region, and a third believed an outbreak to be moderately likely somewhere in Australia (31.5%). Despite these differences, most respondents agreed that an outbreak of FMD on their property (84.9%), region (82.1%) or in Australia (79.6%) would be extremely serious. When asked to consider how concerned they felt about FMD, 19.6% of respondents reported no concern, while 28.8% reported extreme levels of concern. Regarding confidence in being able to correctly identify signs of FMD, 18.0% of respondents reported high levels of confidence (very, extreme), with over half reporting little or no confidence (54.8%). In general, producers implemented appropriate biosecurity practices in relation to incoming stock. When asked about the last 12 months, the majority of respondents reported that they had inspected stock before buying them (76.6%), with 78.5% using a National Vendor Declaration or Cattle Health Statement when buying new stock in. The majority of respondents reported isolating new stock (72.0%) and always checking the new stock for disease (88.7%). In relation to other biosecurity measures, implementation was limited. Only a quarter (25.4%) of respondents reported that they always restricted visitor access, including vehicles and farm equipment to their farm, while another quarter (23.1%) never did. Around 40% of respondents

(39.3%) reported never requiring visitors to undergo specific biosecurity practices, with 18.7% never, rarely or occasionally taking action to control feral animals on their farm. When asked to consider who they think is responsible for observing, detecting and reporting unusual signs of disease in their animals, while the majority of respondents identified themselves as responsible for the three tasks (95.5%, 80.7% and 81.6%, respectively), a lower proportion of producers considered themselves responsible for detecting and reporting compared with observing.

Goat producers: While the majority of respondents reported that there was no likelihood of an FMD outbreak occurring on their property (68.4%), 35.5% considered an outbreak in Australia as moderately likely. Most respondents considered that an outbreak on their property, in their region or somewhere in Australia would be extremely severe (84.4%, 79.5%, 75.6%, respectively). When asked to consider how concerned they felt about an FMD outbreak, and how confident they were that they could correctly identify signs of FMD in their goats, most reported that they were not concerned or a little concerned (59.7%) and had little or no confidence (64.0%). The majority of respondents did not employ workers from overseas (92.4%) and were part of a quality assurance program in their industry (81.1%). Most reported always doing a prepurchase examination if they purchased in stock (80.6%) and used a National Vendor Declaration (NVD) or Goat Health Statement (GHS) when buying stock in (74.7%). The vast majority (89.5%) of respondents always checked new stock for signs of disease and isolated them (73.1%). When considering frequency of undertaking biosecurity actions around visitors on their property, 53 respondents (54.7%) reported that they always or most of the time restricted visitor/vehicle/farm equipment access to their property, while 39.2% reported that they frequently (always/most of the time) required their visitors to undergo specific biosecurity procedures. A third (34%) of respondents never required this of visitors. The majority of respondents (42.9%) always took action to control feral animals. When considering who is responsible for inspecting their animals, detecting unusual signs of disease and reporting the signs, most respondents identified themselves (94.6%; 85.1%; 88.0%, respectively).

Dairy industry: Most respondents (57.8%) reported that there was no likelihood of an FMD outbreak occurring on their property, and considered an outbreak in Australia as a little likely (39.1% of respondents). The majority of respondents considered that an outbreak on their property, in their region or somewhere in Australia would be extremely severe (87.0%, 82.6%, 73.9% respectively). When asked to consider how concerned they felt about an FMD outbreak, and how confident they were that they could correctly identify signs of FMD in their cattle, most (54.5%) reported that they were not concerned or a little concerned and had little or no confidence (64.8% of respondents).The majority of respondents did not employ workers from overseas (57.6%) and were part of a quality assurance program in their industry (94.3%). Of those producers that brought stock in, most reported always doing a prepurchase examination if they purchased in stock (87.5%) and used a National Vendor Declaration (NVD) or Cattle Health Statement (CHS) (80.0%). The majority (85.7%) of respondents who ran open herds reported always checking new stock for signs of disease and isolating them (58.1%). When considering frequency of undertaking biosecurity actions around visitors on their property, 29 respondents (56.8%) reported that they never/rarely restricted visitor/vehicle/farm equipment access to their property, while 72.6% reported that they never/rarely required their visitors to undergo specific biosecurity procedures. The majority of respondents (33.3%) reported always took action to control feral animals, with 29.4% reporting doing this occasionally. When considering who is responsible for inspecting their animals, detecting

unusual signs of disease and reporting the signs, most respondents identified themselves or their staff (86.4%, 70.4%, 76.8% respectively).

Pork industry: When asked to consider the likelihood of an FMD outbreak occurring on their property most respondents reported no likelihood (64.6%), and considered an outbreak in Australia as moderately likely (43.8%). The majority of respondents considered that an outbreak on their property, in their region or somewhere in Australia would be extremely severe (75.0%, 76.1%, 72.3% respectively). When asked to consider how concerned they felt about an FMD outbreak, approximately a third of participants (31.8%) reported that they were not concerned with 25.0% reporting extreme levels of concern. Over one quarter of respondents (26.3 %) reported moderate confidence in being able to correctly identify signs of FMD in their pigs, with 21.1 % reporting extreme confidence and 18.4% reporting both no confidence and little confidence. The majority of respondents reported employing workers from overseas (61.2%) and were part of a quality assurance program in their industry (98.3%). Of those producers that brought stock in, most reported always doing a prepurchase examination if they purchased in stock (88.0%) and all used a PigPass National Vendor Declaration (PigPass NVD). All respondents who ran open herds reported always checking new stock for signs of disease and the majority reported always isolating them (86.7%). When considering frequency of undertaking biosecurity actions around visitors on their property, 22 respondents (44.9%) reported that they never/rarely restricted vehicle access to their property, while 46.9% reported always doing this. The majority of respondents (61.1%) reported that they always required their visitors to undergo specific biosecurity procedures. The majority of respondents (78.9%) reported always/most of the time took action to control feral animals. When considering who is responsible for inspecting their animals, detecting unusual signs of disease and reporting the signs, most respondents identified themselves or their staff (87.2%, 87.6%, 81.1% respectively).

4.3.4 Development of Bayesian Network Models

4.3.4.1 *Introduction*

Data from the cross-sectional studies of beef, goat, and sheep producers across Australia were used to develop vulnerability typologies using a Bayesian Network (BN) (also known as Bayesian Belief Network) analysis (Australian Government, 2009; Kjærulff, 2013). The conceptual model used to develop the BN is found in Figure 4-2. This section of the report provides a background to the BN technique, the methods and results from these analyses.

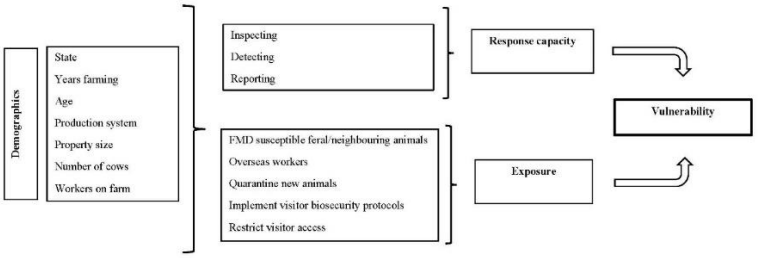

Fig. 4-2 Bayesian network conceptual model for developing Australian livestock producers' typology around vulnerability to FMD

As previously identified, producer vulnerability was defined/assessed by the intersection of exposure and response capacity (Nelson et al., 2010) as seen in Figure 1. One of the subproject goals was to develop a FMD risk management framework based on producer vulnerability typologies. The BN approach was adopted for data modelling and analysis so that the nuances of the complex interrelationships between the variables associated with vulnerability could be captured.
Originally developed as a modelling tool from artificial intelligence in the late 1980s, BNs have more recently been used across scientific, industrial and government organizations (Pearl, 1988; Kjærulff and Madsen, 2008a). As probabilistic graphical models, BNs allow for effective modelling of physical, biological and social systems operating under uncertainty (Kjærulff and Madsen, 2008a; Korb and Nicholson, 2011). More specifically, a BN approach is suitable for developing a risk management framework involving a large number of different types of data, e.g., categorical, numeric, and hidden (not observable) variables with complex interrelationships (Australian Government, 2009; Norsys software Corp., 2018b).

Formally, a BN model is a graphical representation of a joint probability distribution of a set of random variables in which each variable is represented by a node and the dependency relationship is represented by a link/edge for two associated variables (Pearl, 1988; Kjærulff and Madsen, 2008a). BNs provide a mathematically correct way of assessing the effects of different variables on each other. These assessments can be made in either direction: computing the most likely effects given the values of certain causes, but also determining the most likely causes of observed events.
Since a BN model represents the joint distribution of all variables included in the model, any one variable may be selected as a target variable (equivalent to the 'response' variable in a regression model) and then to perform various inferential analysis by assuming different scenarios in terms of the 'findings' of other variables. For example, by fixing the values of other variables, the distribution of the target variable values can be predicted. In this study we used BN software Netica (version 6.05) (Norsys software Corp., 2018a) to develop BN models for sheep, beef and goat producers.

4.3.4.2 *Methods*

The top level of the model is the Vulnerability node which is defined by the two hidden variables of Response Capacity and Exposure Level. The Exposure Level is characterised/defined by observed data variables and demographic variables. The Response Capacity is characterised/defined by three sub-level hidden variables (Inspection, Recognising, and Reporting) and demographic variables. The sub-level hidden variables are further broken down to lower-level hidden variables (practice or attitude) or directly linked to/defined by observed variables. The model structure is completed by determining the optimal way to identify/classify the possible meaningful categorical groups with each hidden variable in the model.

With the completed BN model, various statistical analyses can be performed with respect to FMD risk management. The primary statistical inference analysis using a BN model was to find out how other variables/nodes changed given an observation of a selected variable/node. This enabled us to investigate the interrelationships between the vulnerability levels and the producers' profile variables, with the results outlined below.

4.3.4.3 *Results*

Beef: The beef BN model and the results from the analysis can be found in Appendix 4.1 or in the Preventive Veterinary Medicine Journal, here https://pubmed.ncbi.nlm.nih.gov/31981953/. A summary description of the typology has been given in previous milestone reports.

Sheep: The Sheep BN model and the results from analysis can be found in Appendix 4.2.
A narrative describing these typologies is provided below (please note that a producer is referred as 'he' in the descriptions). The sheep typology paper can be found in the frontiers in Veterinary Science, here *https://www.frontiersin.org/articles/10.3389/fvets.2021.668679/full).*

Low vulnerability

a. High response capacity/low exposure: He is equally likely to be from NSW and Victoria. He is likely to have a mixed livestock enterprise of moderate to large size (501-3000ha), with high stock numbers (1001-5000 ewes). He is likely to be a third generation farmer with more than 20 years' experience. Sheep production is not his primary source of income and he is likely to have 1-5 employees. He is likely to be between 26 and 50 years of age and not likely to seek biosecurity information.

b. High response capacity/moderate exposure: He is likely to be from Victoria, have a mixed livestock enterprise, have a moderate sized farm (101-500 ha) and between 301-1000 ewes. He is likely to be a very experienced farmer (more than 20 years) and be third generation. He is likely to employ between 1-5 workers on the property, have an alternate primary source of income, and be between 51-65 years of age. He is very unlikely to seek out biosecurity information.

Moderate Vulnerability

a. High response capacity/high exposure: He is very likely to be from Victoria, have a mixed livestock enterprise, with a small property (1-100 ha) and 1-100 ewes. He is likely to be a first generation farmer but with more than 20 years' experience. He is likely to employ between 1-5 workers on the property, have an alternate primary source of income and be between 66-80 years of age. He is very unlikely to seek out biosecurity information.

b. Low response capacity/low exposure: He is likely to be from NSW, with a small to moderate sized property (101-500 ha) and between 101-300 ewes. He is a very experienced farmer (more than 20 years) and third generation. He is likely to employ between 1-5 workers on the property, have an alternate primary source of income, and be between 51-65 years of age. He is likely to seek out biosecurity information.

High vulnerability

a. Low response capacity/moderate exposure: He is likely to be from NSW, have a mixed sheep and cropping enterprise of moderate size (501-3000 ha) with between 1001 and 5000 ewes. He is likely to be a third generation farmer with more than 20 years' experience. He is likely to employ between 1-5 workers on the property, have an alternate primary source of income, and be between 51-65 years of age. He is unlikely to seek out biosecurity information.

b. Low response capacity/high exposure: She is likely to be from Victoria, have a small mixed livestock enterprise (1-100 ha) with between 1-100 ewes. She is likely to be a first generation farmer with less than 5 years' experience. She is likely to employ between 1-5 workers on the property,

have an alternate primary source of income, and be between 36-50 years of age. She is unlikely to seek out biosecurity information.

Goat: The Goat BN model and the results from analysis can be found in Appendix 4.3 or in the Preventative Veterinary Medicine Journal, here https://pubmed.ncbi.nlm.nih.gov/33385617.
A narrative describing these typologies is provided below (please note that a producer is referred as 'she' in the descriptions).

Low vulnerability

a. High response capacity/low exposure: She is likely to be from Victoria and to have a small goat only enterprise (1-100 ha), with between one and 50 does. She is highly unlikely to have any rangeland animals. She is likely to be a first generation farmer with less than five years' experience. Goat production is not her primary source of income and she is likely to have between 1-5 employees. She is likely to be between 66-80 years of age and be moderately active in seeking biosecurity information.

b. High response capacity/moderate exposure: She is likely to be from Victoria, have a small goat only enterprise (1-100 ha), with between one and 50 does, with no rangeland animals. She is likely to have less than 5 years' experience and be first generation. She is likely to employ between 1-5 workers on the property, have an alternate primary source of income, and be between 36-50 years of age. She is likely to be moderately active in seeking biosecurity information.

Moderate Vulnerability

a. High response capacity/high exposure: She is likely to be from Victoria with a small goat-only enterprise (1-100 ha) and between one and 50 does and no rangeland animals. She is likely to be a first generation farmer with between five and 10 years' experience. She is likely to employ between 1-5 workers on the property, have an alternate primary source of income and be between 51-65 years of age. He is likely to be extremely active in seeking out biosecurity information.

b. Low response capacity/low exposure: She is likely to be from QLD, and to have a small goat-only enterprise (1-100 ha), with between one and 50 does. She is unlikely to have any rangeland animals. She is likely to be a first generation farmer with less than five years' experience. Goat production is not her primary source of income and she is likely to have between 1-5 employees. She is likely to be between 36-50 years of age and be very active in seeking biosecurity information.

High vulnerability

a. Low response capacity/moderate exposure: She is likely to be from NSW, have a small goat-only enterprise of moderate size (1-100 ha) with between one and 50 does. She is unlikely to have rangeland animals. She is likely to be a first generation farmer with between five and 10 years' experience. She is likely to have no employees on the property, very likely to have an alternate primary source of income, and be between 36-50 years of age. She is likely to be very active in seeking out biosecurity information.

b. Low response capacity/high exposure: She is likely to be from NSW, have a large mixed livestock enterprise (501-3000 ha) with between 51-300 does and no rangeland animals. She is likely to be a third generation farmer with between 11-20 years' experience. She is likely to employ between 1-5 workers on the property, have an alternate primary source of income, and be between 36-50 years of age. She is likely to be a little active in seeking out biosecurity information.

4.4 Agricultural Innovation Systems approach to develop farmer-led innovation pilot groups

4.4.1 Introduction

The subproject applied an agricultural innovations systems (AIS) approach to establish farmer-led partnership models for improved surveillance. In the last two decades, agricultural extension has seen a shift from promoting adoption of technologies, policies and practices through persuasion to local innovation through partnership between public and private sectors and other stakeholders (Hall et al., 2001, Leeuwis, 2004a). This has resulted in the international agricultural research and development experts moving away from traditional linear research-extension-adoption models that have been shown not to be effective in addressing complex issues such as general surveillance that involve multiple stakeholders (Spielman, 2005). There has also been a realisation among international agricultural research and development community that research, technology development or policy prescription each in isolation are inadequate at solving complex problems. Instead, AIS approach has been developed to promote multi-stakeholder platforms at different scales to come up with innovative solutions that are fit-for purpose and the local contexts and simultaneously address technical, social and institutional issues (Maru 2018). Close interactions and partnerships among stakeholders have been found to have a greater chance of leading to innovative and sustainable solutions (Spielman, 2005, Edwards et al., 2013, Hall, 2007a).

AIS uses innovation platforms (IP), sometimes also known as public-private partnerships, value chain alliances, multi-stakeholder platforms or innovation networks. IPs are forums that operationalise an AIS approach. Innovation platform are arenas for effective interaction among stakeholders with shared issues of interest, in this case general on-farm surveillance, to share knowledge, learn together, promote trust and coordinate efforts at different scales for innovation that is fit for purpose and context (Makelo et al., 2014, Adekunle and Fatunbi, 2012).

The approach highlights that the capacity of the surveillance system depends not only on the strength of individual actors' knowledge and contribution, but also on the links or relationships between the actors and their complementary roles. The underlying idea is that increased interaction, negotiation and learning between stakeholders in IPs can facilitate integration of local knowledge and scientific knowledge to achieve great synergies that leads to technical, behavioural and institutional changes and support system innovations (Hall et al., 2003, Hawkins et al., 2009, Leeuwis, 2013).

In Subproject 2, innovation platforms have been arenas for partnerships and among producers and other animal health stakeholders with an intent to introduce a bottom-up producer-led surveillance model that improves trust and partnerships and complements existing animal health surveillance systems.

The AIS approach encourages having innovation platforms at the local level to address practical and operational issues and at higher scales to address structural and strategic issues. Forming innovation platforms at different levels: local and higher scales is essential given the interacting nature of the factors constraining general surveillance.

4.4.2 Methods

Local innovation platforms

To establish and run local innovation platforms, the following steps were undertaken:

- The research team, in consultation with industry and government stakeholders, selected five pilot areas based on criteria of areas of potential risk for FMD introduction, intensity of livestock sector activity and geographical and industry distribution – resulting in a pilot group per livestock sector in five states.

  In each selected local area, the subproject team first facilitated meetings to provide information on the project and invite producers and other animal health stakeholders in the pilot area to be members of local innovation pilot groups. This then was followed by innovation pilot group meetings of each of the five pilots, during which initial activities that formed part of their action plan were drafted. This action plan was developed considering that the aim of the group was to work together with the project team to understand group members' specific needs, motivations and challenges around

  - monitoring animal health problems
  - seeking advice and
  - reporting suspected signs of EAD in a timely manner to veterinarians or other authorities.

- Five local innovation pilot groups were established in different states across Australia.
- Semi-structured interviews were conducted with members who consented to join the innovation pilot groups. The interview was to identify local needs, priorities, challenges and opportunities for improving surveillance and networks that then informed discussions innovation pilot group meetings.
- Information gathered through the interviews was used to conduct a stakeholder analysis for two of the pilot groups, to better understand current stakeholder networks and influences and interests on on-farm surveillance.

National Platforms

The research team used the FMD Ready Stakeholder annual forum and approached the National Animal Health Surveillance and Diagnostic (NAHSD) 2016-2019 Business Plan Implementation Group to act as national innovation platforms.

The research team also conducted a cross-pilot workshop that brought together pilot group farmers, other local animal health stakeholders, representatives of livestock industry peak bodies, animal health stakeholders from the Department of Agriculture, Water and the Environment as well as chief veterinary officers and other personnel from states and territories.

Evaluation

To evaluate the outcomes of the partnerships pilot groups two methods were used.

Baseline and endline surveys – these surveys were conducted with producers before commencing and at the end of the pilot group activities respectively. The surveys focused on the level and change in producers' relationships and trust on different components of the surveillance system; their knowledge and engagement with surveillance and biosecurity, including reporting.

Significant change stories – this method involved interviews with producers, and other animal health stakeholders from each of the pilot groups as well as government, industry and non-government stakeholders who had been involved with the FMD Ready project. The interview focused on whether participants observed or/and experienced any change (positive, negative or no change) as a result of subproject 2. This allowed us to capture intended and unintended changes that could have been triggered across different domains such as: capacity (changes in perspective, knowledge and skills), practice, networks and institutions.

4.4.3 Results

Local Innovation Platforms

Five local innovation pilot groups were established in 2018 at different state jurisdictions:

- Dairy - Maffra, Victoria
- Beef - Durong, Queensland
- Sheep - Esperance, Western Australia
- Pork – TasmaniaGoat - South Australia

The dairy, beef, sheep and pork innovation pilot groups run for 26 months and the goat pilot group which started later, for 20 months. Each pilot group has met regularly, approximately every 3 months for the duration of the project (Table 4-3).

Table 4-3 Innovation Pilot group members and meetings

Innovation Pilot group	Pork	Sheep	Goat	Beef	Dairy
Members	12	13	20	10	10
Number of Pilot Meetings	9	7	5	7	9
Training activities	3		2	2	

Products

Pilot groups have developed new fit-for-purpose well-tailored surveillance guides and media articles on the impact of potential introduction of EADs and the need to improve on-farm biosecurity and surveillance. These include:

1. A biosecurity and surveillance Chain of Response (CoR) developed by the Beef Innovation Pilot group for Queensland farmers (see Appendix 4.4). A national version has been developed for farmers across the country. This product provides easy and accessible step-

by-step guide for farmers to follow when they face an animal health incident. This product is now supported by the Queensland Chief Veterinary Officer and distributed to 116 farmers. New South Wales and Tasmania Chief Veterinary Officers has expressed their interest for having the product for distribution to their state livestock farmers.

2. LIVE (Look, Investigate, Verify and Evaluate) developed by the Sheep innovation pilot group is a simple surveillance guide consistent with official requirements (see Appendix 4.5). The aim of the guide is to have a simple accessible reminder to farmers on the importance of regularly checking their animals and immediately reporting unusual signs of disease for investigation. This guide has been published in ASHEEP Group Newsletter.

3. *My pig is sick – what should I do?* Initiated by Tasmanian Government Department of Primary Industries, Parks, Water and Environment, in the context of threats of African Swine fever, the pork innovation pilot group provided critical input to make it relevant and accessible to farmers (see Appendix 4.6). This resource has been distributed through a small Farm Living Field Day in October 2019 and through the Pork Innovation Pilot Group Network Facebook group.

4. National Livestock Identification System (NLIS) flyers - developed by both the Sheep and Dairy innovation pilot groups (see Appendix 4.7). This simple and easy to follow flyer tailored to smallholder farmers is on the requirements and processes to comply with NLIS requirements. The NLIS flyer from the Sheep Pilot group was distributed in collaboration with the ASHEEP group while the version from the Dairy Pilot group is distributed in collaboration with the Maffra Local Council.

5. Goat Diseases – The Farmer's Guide. This guide was developed based on an existing *Sheep Diseases – The Farmer's guide.* (See Appendix 4.8 or go to the the MLA website (https://www.mla.com.au/globalassets/mla-corporate/news-and-events/documents/21-00248_lw_report_goatdiseaseguide_updated.pdf)). It was initiated by the innovation pilot group and developed in collaboration with MLA, this product will inform goat farmers to report when they encounter unusual signs and implement good biosecurity practices.

6. Development of new communication materials and signage to build producer awareness on key animal health and biosecurity issues. These include a media campaign in the GippsDairy publication, initiated and developed by the Dairy innovation pilot group and in the ASHEEP website and biosecurity signage in roads, initiated and developed by the Sheep innovation pilot group. Other examples include a flyer for producers to help improve awareness about FMD risks associated with seasonal workers and connected with emerging ecotourism industry in the area.

National innovation platform

The research team presented issues and opportunities raised across the local innovation pilot groups that require national attention in the annual FMD Ready project forums, and the team was invited

twice to make presentations to the National Animal Health Surveillance and Diagnostic Business Plan 2016-2019 Implementation group. The research team articulated how the objective of the subproject - building partnership and trust among producers and animal health stakeholders to improve surveillance - is closely aligned with the objectives of the business plan, and lessons learnt from the subproject can contribute to the design of a new 2020-2024 business plan. Invited by Dr Sarah Britton, Dr Yiheyis Maru and Associate Professor Marta Hernandez-Jover presented the learnings from the approach, process and achievement of the partnership pilot groups and the vulnerability typology in the Transformational Change to National Animal Health Surveillance Workshop on 16 June 2020. Dr Heleen Kruger, member of the subproject team, was also invited to attend the workshop. The workshop was organized by the Task Group of the National Animal Health Surveillance and Diagnostics (NAHSD) Business Plan 2016-2019 and aimed at providing an input for the design of a new business plan 2020-2024.

Dr Yiheyis Maru and Associate Professor Marta Hernandez-Jover from the subproject team were subsequently invited to take part in the design of the new National Animal Health Surveillance Business Plan 2020-2024.

Cross-Pilot Workshop

A total of 45 participants attended the Cross-pilot workshop in February 2020. Participants a) discussed achievements of each innovation pilot group and the subproject as a whole, b) summarised lessons learned, c) deliberated on the sustainability of the innovation pilot groups and/or the functions that they have been playing, and d) explored pathways for scaling of what was learned from each pilot and the overall project. A summary of results of the workshop is presented here.

In the cross-pilot workshop, members from across the five innovation pilot groups, industry representatives, and state and federal animal health authorities, noted the stronger partnerships and trust between farmers and diverse animal health stakeholders achieved, which would have not been possible in the absence of the innovation pilot groups.

Achievements

- Development of new communication materials to build producer awareness on key animal health and biosecurity issues
- Delivery of training workshops to share knowledge and attract new producer members.
- Development of new fit-for-purpose and simple-to-use surveillance guides
- Establishment of stronger connections between animal health stakeholders
- Commitment from pilot group members to use their influence within their networks beyond the group.

Learnings from the pilot groups: During the cross-pilot meeting, the groups described five common foundational elements they learnt while being part of the project, which they will take forward as they transition beyond the project:

1. Setting the group goals early to maintain interest and engagement. To stay true to the co-design principle of the AIS approach, the innovation pilot groups used the information meeting and the first pilot meetings to understand the challenges and opportunities, define what they would like to achieve that promotes partnerships and improves surveillance. Cross-pilot workshop participants observed that this process could have been done more quickly to maintain a high level of interest and engagement of pilot group participants.
2. Expanding the groups' focus beyond animal health surveillance and biosecurity to make the pilot group address the needs and priorities of farmers, in addition to animal health, and to make the groups attractive for more farmers to join.
3. The incorporation of online communication tools and technology to help break down barriers to engagement and attract more producers and other animal health stakeholders.
4. The importance of continuing the established innovation pilot groups with representation from diverse sectors, including producers, government and private veterinarians and/or government biosecurity officers, industry representatives, stock agents, local government, and Natural Resource Management, to continue delivering impact on surveillance and biosecurity.
5. Leveraging existing established organisations or initiatives, e.g. producer groups, industry support programs etc. where possible to build on a diverse animal health stakeholder group and ensure sustainability.

Stakeholder perspectives on changes due to the farmer-led surveillance project

The Subproject team conducted interviews with 30 pilot members and and 13 FMD Ready project Stakeholders. Results of the analyses of the interviews show five types of positive changes as a result of the sub-project.

1. Increased awareness and knowledge that led to improved surveillance and biosecurity practices

I think, you'd have to say awareness about animal health surveillance, …I think that has improved because we've seen disease reports … I can think off the top of my head of three disease investigations that were directly or indirectly related to this group. (Veterinary Officer 1)

I am isolating animals quicker than I was before, if I think there's an issue, …a few other people that have got on and contacted their vet more often than they have done in the past (Producer 1)

Since this project has started, we've certainly done two or three investigations. (Producer 2)

2. Created strong connections and relationships that led to trust and health issue reporting

being able to put faces to names and contacts and to meet people definitely made a big difference for us. It will make a difference going forward (Producer 3).

*Opportunity for coming together of disparate groups in the goat industry in South Australia as well as a connection and collaboration with the vet profession to building trust (*Producer 4).

one of the group members had established a good relationship with a vet. So that is positive. That is a massive step forward with everything. For building up trust, for asking for advice (Private Vet 1).

I think the most significant change that I would attribute to these pilots is the allaying of producers fear that recording or reporting, surveillance of and reporting of a disease could have punitive consequences is a massive advance (Industry Representative 1).

3. Opportunity for producers and diverse stakeholders to form new networks for different purposes

Piggy backing on this project has helped the goat industry to get together and we look like developing a group, a goat group that will sit under Livestock SA (Policy Officer 1).

I think it's been really useful having a good cross-section of – of people and industry, which isn't the norm for producers. But working with agents and stock – stockyards, saleyard people, and private vets, and council workers (Private Vet 2).

I think the networking has probably been one of the most significant changes throughout the project and I think towards the end of the project we really did have those key messages and actions happening a lot more …the group started working really well together and actually thinking of it more as a state wide biosecurity role rather than just a pilot group (NRM Officer 1).

4. Learning from different perspectives, each other and resolving issues quickly

Where all the groups came together. What I learnt there was farmers learnt from farmers and we need a lot of these pilot groups - we don't need a lot of funding – but we need these groups so that they can communicate it to better the industry and learn off of each other (Producer 5).

So, it didn't matter whether it was industry policy, whether it was South Australian policy, whether it was legislation, was it difficult, they were all there. I think there was very few times, in fact near on zero, where we had to take a question on notice, so I think that was an absolute positive, yeah (Private Vet 2).

Getting the insights directly from like the stakeholders being stock agents, farmers, service providers and how they played off and interacted with each other. So, it's not easy to get that information sort of critiqued so instantaneously at the grassroots level (Veterinary Officer 2).

5. Valuing more the science of integration and the partnership approach

At the beginning, I wasn't sure that our group of stakeholders would understand what you were doing let alone embrace it and I've been really impressed with not only them because I think they have embraced it but also Government and, and me. I've gone from being cynic to believing that what you were delivering is actually really valuable (Other 1).

Doing social behavioural analysis ..is key for surveillance. It has been overlooked a lot in the past and I think we need to be bringing that more and more into what we are doing …if we really do want to improve surveillance reporting that we get (CVO 1).

…an interesting legacy for this work, is not even expanding what happens with each of the networks, but changing decision makers' minds about, well actually, we can either print a thousand brochures and mail them out to people, or we can try and spend a bit more effort trying to engage with people and see what they need, and work with them in more of a co-design or a peer to peer approach (Animal Health Officer 1).

Project Close Stakeholder Workshop

In November 2021, the project held a virtual project closure meeting with more than 36 stakeholders from the pilot groups, industry representatives government animal health officers. Participants at the workshop shared their perspectives on scaling up the approach and outcomes of the subproject, the usefulness of the different knowledge products produced by the pilot groups, and the sustainability of the pilot groups. Of note participants, requested communication products, like the Chain of Response (see Appendix 4.4) to be distributed and accessible online, suggested meeting to promote sustainability of the beef and pig pilot groups. Following this workshop the Chain of Response product was distributed to to industry leaders. For scaling the project approaches and outcomes, participants suggested developing proposals for funding that build on the results of the sub-project[3]. In addition, the sub-project 2 team facilitated a follow-up meeting between the Queensland Government, the South Burnett Graziers Network and other key stakeholders including the Cattle Council of Australia to explore funding to continue the pilot going forward.

4.4.4 Discussion

The AIS promotes partnership for innovation to address complex problems (Maru et al., 2016): in the case of this subproject, improving animal health surveillance. Innovation to address complex problems requires not only technological/ technical innovation but also changes in behaviours and structures such as institutions (rules, policies and programs), capacities, trust and relationships.

AIS approach has been implemented in the subproject to achieve two objectives: a) increase partnerships and trust among livestock farmers and other animal health stakeholders and b) improve general surveillance for early detection of disease and spread leading to fewer, less impactful and more readily controlled disease outbreaks.

Analyses of the interviews of participants from the significant change stories, the baseline and endline surveys and feedback from the cross-pilot workshops show that better relationships and improved trust were achieved participants in the innovation pilot groups. Through working together in regular meetings, the pilot groups a) generated tailored products and initiatives that improved EAD awareness and awareness of the importance of on-farm surveillance and biosecurity among producers and other animal health stakeholders; b)organised information and training sessions addressing producers' issues and needs thereby building animal health knowledge and skills; c)

[3] The funding proposals included submissions to the Smart Farming initiative and the CSIRO Biosecurity mission.

Initiated conversations on balancing animal health officers' focus on enforcing regulations with building rapport and relationships with producers and d) made existing producer networks stronger by adding diverse animal health stakeholders and formed new animal health stakeholder networks.

Participation in the innovation pilots substantially increased pilot group producers':

- animal health advice/help seeking behaviour
- regular practices of visual, detailed health checking and veterinarian inspection of their animals and
- reporting animal health incidents and disease investigations.

This meets the second objective of the subproject.

Close cross-scale interactions of pilot groups with state and federal government animal health officers and industry representatives also led the latter to realize the importance of the bottom -up partnership approach and the complementary role the local innovation pilot groups could play in improving surveillance and biosecurity. As a result, some of the pilot groups will continue, because they were built on an existing network (ASHEEP for the sheep innovation pilot group), or because they formed a strong new network (the Goat innovation pilot group), are supported and will be facilitated by industries (Dairy Australia – Dairy innovation pilot group; Australian Pork Limited – Pork innovation pilot group) or government (Queensland Chief Veterinary office -Beef innovation pilot group).

The subproject integrated social sciences with animal health sciences to understand the values and needs of farmers, risk factors that make them vulnerable to EAD, and behavioural and institutional barriers to adoption of improved surveillance and biosecurity practices. The partnership approach introduced through this project requires funding agencies and researchers to relinquish some control of projects to allow co-ownership and co-design with stakeholders for impactful research that delivers impact. However, this is often counter-intuitive to commonly held beliefs about research, and the desire to undertake research under a controlled environment for rigorous research outputs.

A principled application the Agricultural Innovation System Approach of the sub-project has given FMD Ready stakeholders some ownership of EAD preparedness, by listening and valuing their perspectives and sharing with them the responsibility of co-design and implementation of effective solutions.

Limitations and challenges

A significant barrier found across the pilots was the focus of the subproject being animal health surveillance. This topic was identified not to be the highest priority among the multiple and varied issues farmers deal with day to day, such as nutrition, reproduction, trade, etc. In the process, the subproject adapted within its scope to accommodate topics of interest to farmers for example, by organising training on herd health and nutrition. However, the lesson learned is that for any future project, the design phase should consider farmers' priorities from the start, so that they can be dealt with as a package with greater scope for adoption.

4.4.5 Reference materials

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Appendixes

Appendix 4.1 Manyweathers et al (2020) Understanding the vulnerability of beef producers in Australia to an FMD outbreak using a Bayesian Network predictive model. Prev Vet Med 175: 104872. https://doi.org/10.1016/j.prevetmed.2019.104872

Appendix 4.2 Manyweathers et al (2021) Using a Bayesian Network Predictive Model to Understand Vulnerability of Australian Sheep Producers to a Foot and Mouth Disease Outbreak. Frontiers in Veterinary Science 8: 668679. https://doi.org/10.3389/fvets.2021.668679

Appendix 4.3 Manyweathers et al (2021) The goat industry in Australia: Using Bayesian network analysis to understand vulnerability to a foot and mouth disease outbreak. Prev Vet Med 187: 105236. https://doi.org/10.1016/j.prevetmed.2020.105236

Appendix 4.4 Biosecurity and surveillance chain of response

Appendix 4.5 Autum Field Day Review. In ASHEEP News, Newsletter #53-May 2019

Appendix 4.6 My pig is sick – what should I do? In Biosecurity Fact Sheet-November 2019

Appendix 4.7 So you keep animals?

Appendix 4.8 Goat Diseases: The Farmer's guide – September 2021. Department of Primary Industries and Regions, Government of South Australia

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5 An integrated EAD outbreak management decision support system

5.1 Introduction

Subproject 3 of the FMD Ready project is intended to help decision-makers understand the consequences and social and economic costs and benefits of the alternative response strategies during and after an outbreak of FMD. The project has further developed the Australian Animal Disease Spread (AADIS) model (Bradhurst et al. 2015, 2016) to provide improved capacity for decision support and simulation and scenario analysis for government and industry stakeholders. The results demonstrate how vaccination might be beneficial in a control strategy for an FMD outbreak under some circumstances. The project also looked at the consequences of using vaccination on proof-of-freedom surveillance and recommencing livestock trade and exports. Vaccination is increasingly recognised as a potentially useful tool to assist in containing and eradicating FMD outbreaks. However, while vaccination can positively contribute to earlier eradication of the disease, it will be associated with additional costs. Keeping vaccinated animals in the population will delay the interval until FMD-free status can be regained under the World Organization for Animal Health guidelines (OIE, 2019) and add complexity to the post-outbreak surveillance programs aimed at supporting the re-establishment of FMD-free status. These issues are of particular concern for countries with significant exports of livestock and livestock products as, under current international guidelines, the presence of FMD-vaccinated animals in the population could be expected to cause market access difficulties.

Decision support tools, including disease models, can offer valuable insights into the effectiveness of different control measures (Garner and Hamilton, 2011). In particular, vaccinating early in an outbreak is likely to be more effective than delaying vaccination (Roche et al., 2014b). Not using vaccination in some situations may lead to larger and longer outbreaks, increased control costs and greater on-going impacts on industry and local communities.

The first phase of sub-project 3 provided a more thorough investigation into possible incursion scenarios and control options available to manage an FMD outbreak, with a focus on vaccination as a disease control option. In consultation with State and Territory animal health agencies, thirteen incursion scenarios and nine control strategies were identified. The size and duration of outbreaks were compared in terms of the total number of infected premises and the duration of the control stage of an FMD outbreak. The second phase of sub-project 3 investigated the implications of vaccination for the post-outbreak management of vaccinated animals and surveillance to demonstrate disease freedom.

Since Australia has not had an outbreak of FMD since 1872 (Bunn et al., 1998) epidemiological modelling was used to evaluate different control strategies and compare different approaches to post-outbreak management of vaccinated animals and surveillance to substantiate FMD-freedom. The Australian Animal Disease (AADIS) model (Bradhurst et al. 2015, 2016) was used to simulate FMD outbreaks in Australia that were controlled with and without vaccination as part of the response. In both Phase 1 and Phase 2 of the project, economic analysis of the simulation scenarios

helped us better understand how the choice of control strategy affects producer returns and whether trade losses might be mitigated using trading zones.

5.2 Method

Sub-project 3 used a combination of methods, including stakeholder consultation, literature review, disease spread modelling using the AADIS model, economic modelling, and statistical analyses. The following sections describe these methods.

5.2.1 Stakeholder consultation

A mix of stakeholder workshops and questionnaires was used to consult and involve stakeholders in scenario analysis[4]. Inputs from State and Territory jurisdictional stakeholders were collected through workshops and surveys. These inputs were used to identify key commonalities and uncertainties that affect the use of vaccination as an FMD response strategy and to inform the selection of incursion scenarios, control strategies, and the parameterisation of the costs and resourcing of these strategies. For example, a questionnaire sent to all State and Territory jurisdictions requested information about two or three incursion scenarios of interest based on the most likely or important scenarios for FMD introduction for their jurisdiction. It requested information about the method of FMD introduction, type of farm and when FMD was introduced, time until detection, and the reason for selection.

5.2.2 The AADIS model

Simulations were conducted using the AADIS model. The AADIS model is a national-scale model of livestock disease spread and control that can be used to simulate alternative spread scenarios and control strategies. AADIS constrains disease control according to the availability of resources such as personnel and vaccines and models the suite of control measures prescribed in Australia's response strategy for FMD, AUSVETPLAN (Animal Health Australia, 2014).

AADIS is a national-scale epidemiological model used by animal health authorities in Australia to support FMD planning and preparedness. It is a spatiotemporal agent-based simulation of the spread and control of an emergency animal disease. The herd is the epidemiological unit, where a herd is defined as a group of co-mingling animals of the same species. A farm may have one or more herds (e.g. a mixed beef-sheep farm would be made up of a beef cattle herd and a sheep herd). Each herd agent has an embedded set of differential equations that model the herd's infected, infectious, serological and clinical prevalence over time, taking into account species, production system and virus strain.

The agents interact in a model environment that stochastically spreads disease across multiple spread pathways (direct contacts, indirect contacts, saleyard spread, airborne transmission and local spread). Control measures (stamping out, surveillance, tracing, movement restrictions and vaccination), are based on the Australian Veterinary Emergency Plan (AUSVETPLAN) for FMD (Animal Health Australia 2014). Similar to an actual outbreak response, the simulated control measures are dynamically constrained by the available resources (staff and consumables such as vaccine), the accuracy of reports of clinical disease, inefficiencies in tracing systems, and non-compliance with

[4] This research received ethics approval from the CSIRO Human Ethics research committee.

movement restrictions. Bradhurst et al. (2019) have described recent model enhancements to assist with the evaluation of different post-outbreak management strategies to support recovery of FMD-free status and return to trade.

5.2.3 Phase 1 simulation study design

To examine the effectiveness of alternative approaches to incorporating vaccination into a control strategy for FMD across a range of starting conditions, we simulated nine control strategies for thirteen incursion scenarios:

- Seven control strategies incorporate vaccination.
- 500 runs of the simulation for each combination in the baseline were run.
- Additional simulations were runs for the sensitivity analyses, such as varying the vaccination zone size and the vaccination start day.

To characterise the incursion scenarios and control strategies, the AADIS model was parameterised using a combination of values estimated for previous studies and in consultation with government and industry stakeholders.

Incursion scenarios

Using inputs from jurisdictional stakeholders, seed herds and snapshots were selected to represent key features of the incursion scenarios of interest to stakeholders whilst standardising some factors to facilitate comparison, such as the time until detection. The seed herd is the herd where the simulated incursion is started, and the snapshot is the state of disease spread after 21 days of silent spread (before detection and any control begins). A fixed silent spread phase of 21 days was assumed for this study based on recent studies in Australia (Martin et al 2015, East et al. 2016, Garner et al. 2016). Figure 5-1 shows the locations of the seed herds for each of the 13 incursion scenarios.

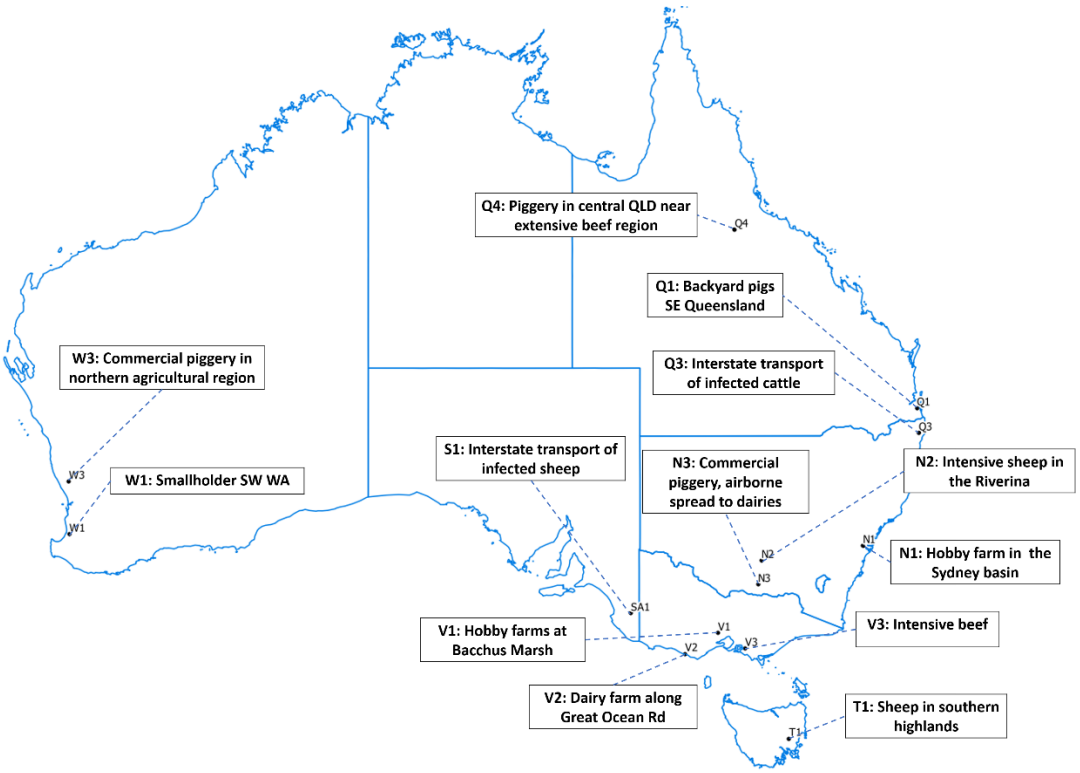

Figure 5-1 Locations of seed herds for each incursion scenario

Representative runs were selected for each of the incursion scenarios so that alternative control strategies could be compared from the time when the disease is first detected and control commences. Table 5-1 describes the starting conditions of each incursion scenario.

Table 5-1 Starting conditions for simulation study FMD incursion scenarios: seed herds and snapshots

Incursion scenario	ID	Scenario description	Seed herd		Snapshot
			Operation type	# animals	# infected herds at detection
New South Wales	N1	Hobby farm in the Sydney basin	Smallholder	8	6
	N2	Intensive sheep in the Riverina	Sheep farm	1210	2
	N3	Commercial piggery, airborne spread to dairies	Commercial piggery	4643	**9**
Queensland	Q1	Backyard pigs SE Queensland	Smallholder	9	4
	Q3	Interstate transport of infected cattle	Intensive beef	109	13
	Q4	Piggery in central QLD near extensive beef region	Small pig farm	363	2
South Australia	S1	Interstate transport of infected sheep	Mixed sheep/beef	3271	3
Tasmania	T1	Sheep in southern highlands	Sheep farm	1418	2
Victoria	V1	Hobby farms at Bacchus Marsh	Smallholder	12	3
	V2	Dairy farm along Great Ocean Rd	Dairy herd	516	44
	V3	Intensive beef	Intensive beef	89	16
Western Australia	W1	Smallholder SW WA	Smallholder	7	10
	W3	Commercial piggery in northern agricultural region	Commercial piggery	10836	10

Control strategies

Control strategies were selected to provide a comparison between alternative approaches using vaccination and benchmark control strategies without vaccination as shown in Table 5-2.

Table 5-2 Control strategies

Control strategy ID	Stamping out	Pre-emptive culling of DCPs	Vaccination[5]	Targeting of vaccination		
				Animals/operations	Ring or Annulus	Area
SO1	Yes	No	No	-	-	-
SO2	Yes	Yes	No	-	-	-
SO3	Yes	No	Yes	All species*	5km ring	All
SO4	Yes	No	Yes	All species	5km ring	High risk area
SO5	Yes	No	Yes	All species except pigs and smallholders	5km ring	All
SO6	Yes	No	Yes	Vaccination of specialist cattle producers^	5km ring	All
SO7	Yes	No	Yes	Vaccination of specialist cattle producers^	5km annulus, 5km from IPs (out-in)	High risk area
SO8	Yes	No	Yes	Feedlots and large dairy farms >500 head	5km annulus, 5km from IPs (out-in)	All
SO9	Yes	Yes	Yes	All species	5km ring	All

* Beef cattle on extensive properties were not targeted for vaccination in any control strategy. ^ Including feedlots, dairy and intensive beef farms, but excluding extensive beef and mixed beef-sheep farms to avoid including large numbers of sheep on mixed farms in the vaccination program.

Alternative approaches to vaccination differed in terms of the animal species/types of operation targeted, whether ring vaccination (5km ring around an IP) or an annulus (vaccinated from the outside-in within a 5km wide annulus starting 5km away from an IP), and whether all areas were targeted or only herds in pre-identified high risk, livestock dense areas were targeted for vaccination. Estimates of resource teams available to undertake control activities were provided by jurisdictional animal health staff and considered the availability of resources from both the public and private sectors. Sensitivity analyses were conducted to test the effect of changes in key parameters – resources, duration of the national livestock standstill period, vaccination start day and vaccination ring size – on the size and duration of an outbreak. We tested whether there are differences in the mean number of IPs and the last day of control for each of the control strategies for each of the starting locations using Dunn Tests (Dunn, 1964) and a Bonferroni adjustment.

5.2.4 Phase 2 simulation study design

Following an outbreak of FMD, surveillance will be required to demonstrate that FMD eradication has been successful. The objective of this phase of the study was to test the hypothesis that new

[5] For all vaccination strategies, AADIS was set up so that vaccination was triggered on Day 14 of the control phase if there were five or more infected premises.

technologies, such as real-time reverse transcription polymerase chain reaction (RT-qPCR) tests and sampling techniques such as bulk milk testing (BMT) of dairy cattle, oral swabs, and saliva collection with rope tethers in piggeries, could enable the post-outbreak surveillance more efficiently than currently recommended methods based on serological surveillance (Annex III in the European FMD directive 2003/85/EC (European Union 2003, OIE 2019).

The AADIS model was used to simulate FMD outbreaks, with and without emergency vaccination as part of the response. A sub-set of the outbreaks in WA, QLD, NSW, and VIC used for Phase 1 were selected for the Phase 2 simulations. Baseline post-outbreak surveillance approaches for unvaccinated and vaccinated animals based on the European FMD directive 2003/85/EC (European union, 2003) were compared with alternate approaches in which the sampling regime, sampling approaches and/or the diagnostic tests used were varied. The alternative surveillance approaches were identified through a meeting of epidemiologists, FMD researchers and diagnosticians held in November 2018 in Canberra (Anon. 2018). One alternative surveillance approach for non-vaccinated animals and three alternatives for vaccinated animals were identified. The study design is shown in Figure 5-2.

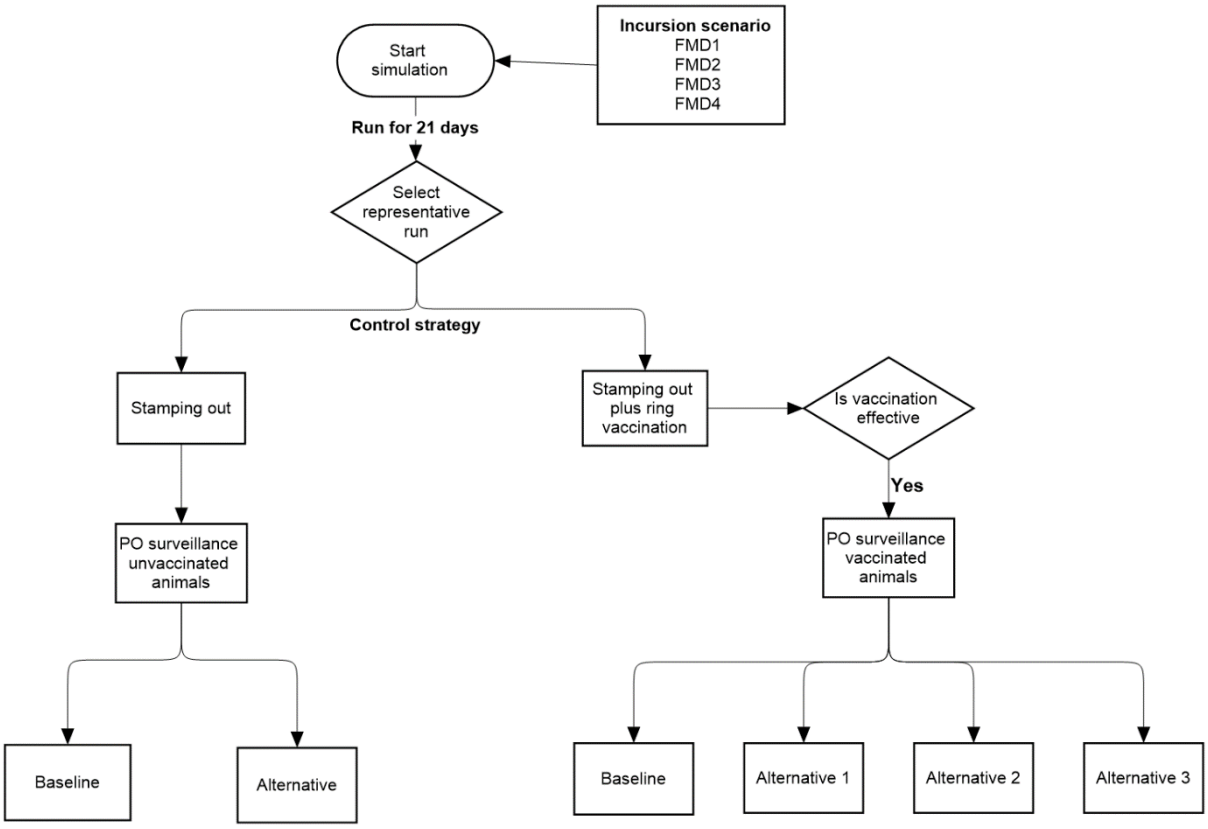

Figure 5-2 Post-outbreak surveillance study design (Source: Garner et al., 2021)

The alternative approach for non-vaccinated animals replaces on-farm clinical inspection of dairy herds with bulk milk testing (BMT), blood sampling of sheep with oral swabs and on-farm clinical inspection of pigs with saliva collection using rope tethers. Serology is replaced by RT-qPCR testing of the milk or oral fluids. The three alternate surveillance approaches for vaccinated herds are:

1. Alternative 1 (Alt 1) continued to use clinical inspection plus serology but with a reduced sampling intensity. Instead of testing all vaccinated animals a 95:1 herd sampling and 95:5 within-herd sampling regime was used.
2. Alternative 2 (Alt 2) replaces serology with oral swabs and pooled RT-qPCR.
3. Alternative 3 (Alt 3) is the same as for Alt 2 but replaces on-farm visits and sample collection for dairy herds with BMT and for pig herds with saliva collection using ropes.

For RT-qPCR testing, pooling of samples (n=5) at the laboratory was assumed (Kirkland 2016). Based on experimental findings, (Horsington et al., 2017, 2018a and b; Parthiban et al., 2015; Singanallur et al., 2015, 2017, Vosloo et al., 2015) it was assumed that infected animals would test positive for up to 14 days after development of clinical signs.

The approaches were compared in terms of the resources, time taken, cost, and effectiveness (i.e. ability of the surveillance regime to correctly identify the infection status of herds). Pairwise comparisons were used to test the effectiveness of different post-outbreak surveillance approaches under different potential FMD outbreak scenarios locations. The variables of interest include clinical inspections, herds sampled, total tests, time to complete surveillance (days), cost, false positives, true negatives, and false negatives. A Dunn Test statistic was chosen as the appropriate non-parametric pairwise multiple comparison test due to the data being non-normally distributed.

5.2.5 *Economic analysis*

Just as the analysis of new surveillance tools and sampling strategies showed promise in concentrating efforts based on virus and animal behaviour, economic analysis indicates value in targeting of both vaccination and management of international trade restrictions. Vaccination control strategies targeted by species and region are combined with a hybrid of bilaterally established trade zones and the OIE containment zone approaches. A decision was made to examine the results at the 75th percentile in order to capture a higher end of potential outbreaks' economic impacts. The numbers and types of animals culled as estimated in AADIS from the above-described Australian incursion and control strategies were used to determine the sudden reductions, or shocks, to livestock and livestock product supply.

The extent of geographic spread as well as outbreak duration from the AADIS work are used as the basis to design state level trading zones which Australia could consider in the event of an FMD outbreak. The pattern of trade recovery after historical outbreaks of FMD in other exporting countries was examined by calculating the number of months before individual importing countries' value of trade in FMD susceptible livestock and their products reached average pre-outbreak levels. This information was used to estimate the influence of outbreak length on the length of trade embargoes, which are an official ban on trade by one country affecting another country's trade. Embargoes due to livestock disease outbreaks are put in place by both exporting and importing countries and do occur simultaneously. These were in turn applied to an average of 2016 to 2018 historical trade patterns to determine the percentage reduction in, or trade shock to, Australian exports of susceptible livestock and their products.

The calculated supply and trade shocks were fed into the ABARES Agricultural sector partial equilibrium model (AgEmission) of Australian feed and livestock markets (Buetre et al., 2013) in order to estimate the potential declines in gross value product – producer revenues adjusted for

compensation payments – of the industries due to an FMD outbreak with a 2017/2018 base year of the annual model. In effect, the national effects estimated measure financial impacts on producers of large quantities of previously exported products remaining in the domestic market, markedly depressing domestic prices, combined with the loss of animals due to disease under the stamping out and vaccination strategies described above.

5.3 Results

In this section we present the evidence to support each of the key lessons from sub-project 3.

5.3.1 *Smaller outbreaks can be effectively managed by stamping out without vaccination*

The Phase 1 simulations examined alternative vaccination strategies across a wide range of incursion scenarios across Australia. Using these results, we compared alternative disease control strategies that incorporate vaccination with benchmark control strategies with stamping out only.

Benchmark strategies for all incursion scenarios

The two benchmark strategies were stamping out (SO1) and stamping out with pre-emptive culling of Dangerous Contact Premises (DCPs) (SO2). Stamping out is the default approach for controlling an outbreak of FMD and aims to ensure Infected Premises (IPs) are quarantined and that susceptible animals are destroyed to limit virus spread (Animal Health Australia, 2014). Table 5-3 presents descriptive statistics for all incursion scenarios for the benchmark SO1 control strategy for the 'total number of IPs' and the 'last day of control'.

Table 5-3 Descriptive statistics for the SO1 benchmark control strategy for all incursion scenarios

Variable	Scenario	N1	N2	N3	Q1	Q3	Q4	S1	T1	V1	V2	V3	W1	W3
Total number of IPs	Mean	10	2	12	5	36	2	5	2	2	872	128	23	15
	Median	9	2	11	5	36	2	5	2	2	734	91	21	14
	Std. dev.	3	0	4	1	8	6	1	1	0	690	225	9	3
Last day of control	Mean	47	41	51	48	62	36	48	47	39	223	124	64	51
	Median	46	40	49	48	57	32	45	46	39	207	109	58	50
	Std. dev.	5	3	6	3	12	21	6	3	0	82	61	21	4

The 'last day of control' measures the number of days of disease control as the number of days of culling plus two incubation periods (28 days). For many of the incursion scenarios, the outbreaks were small and controlled relatively quickly. The Victorian scenarios V2 and V3 were the largest, followed by W1. In particular, the V2 outbreak could become very large and potentially last more than 12 months.

Control strategy SO2 is the same as SO1 but also includes the pre-emptive culling of Dangerous Contact Premises (DCPs). The pre-emptive culling of Dangerous Contact Premises (DCPs) is an

additional control measure that would be considered to help contain and manage the outbreak[6]. A DCP is designated when tracing identifies premises that are considered to have a high risk of being infected; in particular, premises of a type that could have been a source or destination of movements of concern (Bradhurst, 2016). Control strategy SO2 serves as a benchmark for comparison with the strategy SO9, which combines vaccination with the pre-emptive culling of DCPs.

Comparing the two benchmarks for stamping out (SO1) and stamping out with pre-emptive culling of DCPs (SO2) using Dunn tests, we found that there were statistically significant differences in the total number of IPs for incursion scenarios N1, W1 and W3 and in the last day of control for N1 and Q3. The differences between the medians, however, were small and did not appear important for disease control. Notably, no statistically significant differences were found between SO1 and SO2 for the two incursion scenarios with large outbreaks, i.e. V2 and V3.

The effect of vaccination on outbreak size and duration

Descriptive statistics and Dunn-Test statistics were used to compare the effect of the vaccination strategy across all thirteen incursion scenarios by comparing a strategy with stamping out only (SO1) with a comprehensive vaccination strategy (SO3), which involves vaccinating all species in a 5km ring around each infected premises. Figure 5-3Figure 5-3 presents boxplots of the distributions of (a) the total number of IPs and (b) the last day of control across all 500 iterations for strategies SO1 and SO3. For each incursion scenario, N1 to W3, Figure 5-3 shows boxplots SO1 on the left and SO3 on the right. The lower and upper whiskers of the boxplots represent the 25th and 75th percentiles, respectively, with values outside these bounds not shown.

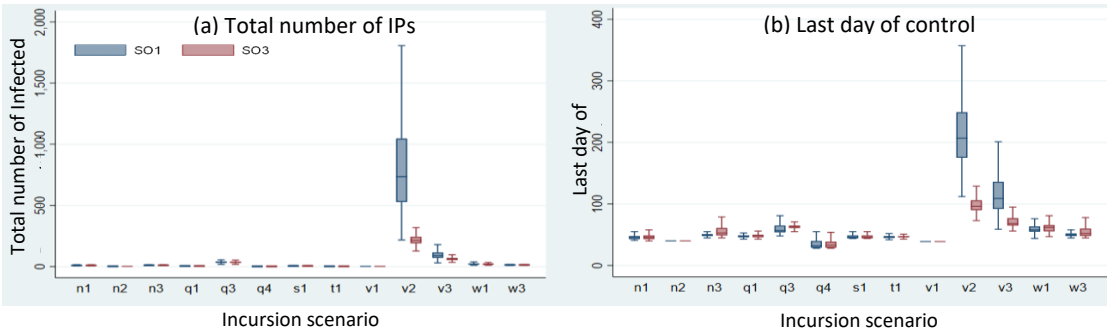

Figure 5-3 Boxplots of (a) total number of IPs and (b) last day of control for the SO1 stamping out strategy (blue) and the SO3 vaccination strategy (red) for each incursion scenario (Source: Capon et al., 2021 Appendix 5.1)

In Figure 5-3, most of the incursion scenarios led to small outbreaks which were controlled relatively quickly under the baseline SO1 strategy. Vaccination (strategy SO3) offered no benefits in terms of reducing the size of the outbreak (number of IPs) or duration. However, in the case of Victorian scenarios (V2 and V3) the outbreaks were larger. In these cases, vaccination was effective in reducing the size and duration of the outbreaks. There was a marked contrast between the median of 734 for the total number of IPs for the V2-SO1 stamping out strategy and the median of 214 for the V2-SO3 vaccination strategy. The same pattern held for V2, with a median of 91 IPs for V3-SO1

[6] A DCP is "a premises that, based on a risk assessment, is considered highly likely to contain an FMD-infected animal(s) or contaminated animal products, wastes or things." (Animal Health Australia, 2014).

compared with 61 for V3-SO1. Although only vaccination strategy SO3 is presented in Figure 5-3, Dunn tests comparing every vaccination strategy (SO3 to SO9) with SO1 showed similar effects. A sensitivity analysis around the assumptions of the Vacc3km, Vaccday10, and Vaccday21 suggests that results are robust to changes in the size of a vaccination ring and the start day for vaccination.

5.3.2 *The size and duration of larger outbreaks can be significantly reduced when vaccination is used*

For this comparison, we focus on comparing the seven alternative approaches to vaccination (SO3 to SO9) with the benchmark stamping out approaches (SO1 and SO2) for the two incursion scenarios in Victoria, V2 and V3, which were associated with larger outbreak sizes and for which vaccination was shown to be very effective in reducing size and duration of the outbreaks. V2 is an incursion scenario that begins in a dairy herd along the Great Ocean Rd and V3 begins in smallholder property at Bacchus Marsh. Figure 5-4Figure 5-4 compares the effect of the different vaccination strategies on outbreak size and duration.

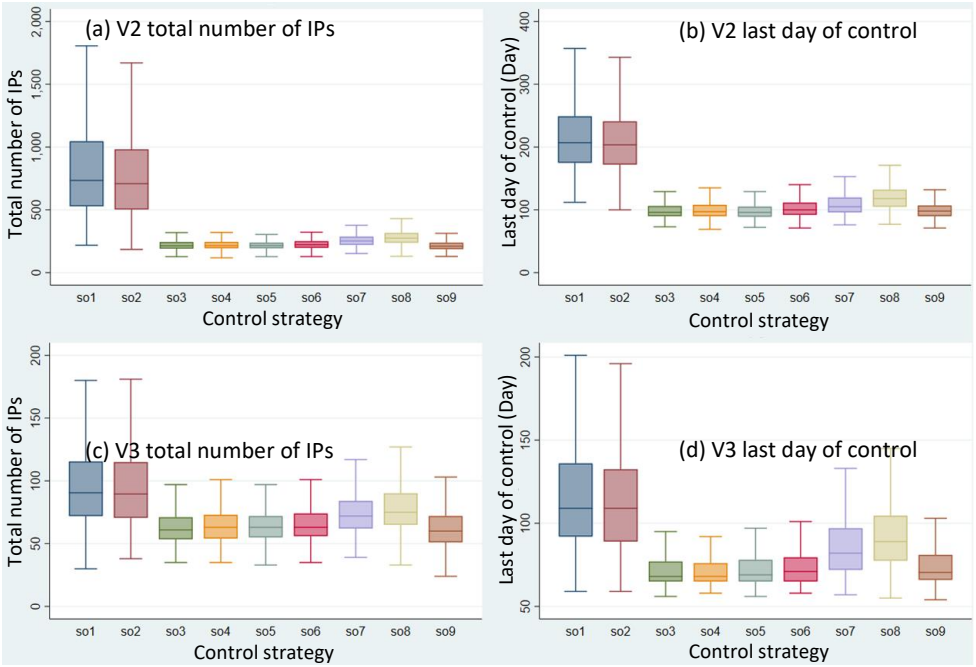

Figure 5-4 Boxplots of (a) V2 total number of IPs, (b) V2 last day of control, (c) V3 total number of IPs, and (d) V3 last day of control for each control strategy, SO1 to SO9. (Source: Capon et al., 2021 Appendix 5.1)

All vaccination strategies were effective in reducing outbreak size and duration. However, strategies SO7 and SO8 (the annulus or 'donut' strategies) were less effective than the ring vaccination strategies.

Appendix

Appendix 5.1: Capon TR, Garner MG, Tapsuwan S, Roche S, Breed AC, Liu S, Miller C, Bradhurst R, Hamilton S. 2021. A simulation study of the use of vaccination to control foot-and-mouth disease outbreaks across Australia. Front. Vet. Sci. 8:648003. doi: 10.3389/fvets.2021.648003.

5.3.3 New technologies for FMD post-outbreak surveillance based on non-invasive sampling methods and RT-qPCR tests can substitute FMD freedom faster and cheaper than traditional approaches based on serological surveys

New technologies for FMD post-outbreak surveillance were compared with traditional approaches based on serological testing in unvaccinated and vaccinated populations (Garner et al., 2021 – Appendix 5.2).

5.3.3.1 *Non-vaccination (Strategy SO)*

The alternate surveillance took less time to complete and cost less. With clinical inspection replaced with BMT sampling for dairy herds and saliva collection using ropes and RT-qPCR testing for pig herds, there were fewer clinical inspections, but more herds sampled. In addition, pooling of samples for testing meant the total number of tests was lower. There was no remaining infection in the population at the end of the control program and both surveillance strategies correctly found no infected herds. While the risk of missing infected herds (false negatives) was low with both surveillance approaches, the alternative surveillance significantly reduced this risk in the larger Victorian outbreak (V2). In three out of the four scenarios the number of false positive results was also significantly less under the alternate surveillance approach. The greatest benefits of the alternate surveillance approach were seen in the largest outbreak in Victoria where on average the time to complete the surveillance was reduced by 12 days and the average cost of the surveillance program fell from AUD15.8 million to AUD11.5 million.

5.3.3.2 *Vaccination (Strategy SORV)*

Of the incursion scenarios selected for Phase 2 simulations, vaccination was only shown to be effective in reducing outbreak size in the large Victorian outbreak (V2). Under a vaccinate-and-remove policy, vaccinated animals are removed from the population during or after the outbreak Only surveillance of non-vaccinated animals in previously infected areas is required. Similar results were found as when vaccination is not used. That is, the alternate surveillance approach was effective in significantly reducing the time required and costs of the surveillance without reducing effectiveness.

Under the vaccinate-and-retain policy, vaccinated animals are retained in the population and are subject to post-outbreak surveillance. Compared to the baseline, the alternate surveillance approaches significantly reduced the numbers of herds sampled, the total tests done and costs of the post-outbreak surveillance. Reductions in time to complete the surveillance were modest. The alternate surveillance approaches produced small reductions in the number of false positive results with vaccination, it was possible to get false negative herds. The alternative surveillance significantly reduced the number of false negative results. They also took less time and were less expensive than the default approach. The Alt 3 approach consistently outperformed the other approaches in this study.

Under a vaccinate-and-retain policy there will be both unvaccinated and vaccinated animals subject to surveillance. The greatest benefits will be realised when the alternate surveillance approaches for unvaccinated animals and for vaccinated animals are combined. For the Victorian V2 outbreak, on

average, the combined alternative surveillance approach reduced time to complete surveillance by 12% (Figure 5-5) and surveillance cost by 27% (Figure 5-6).

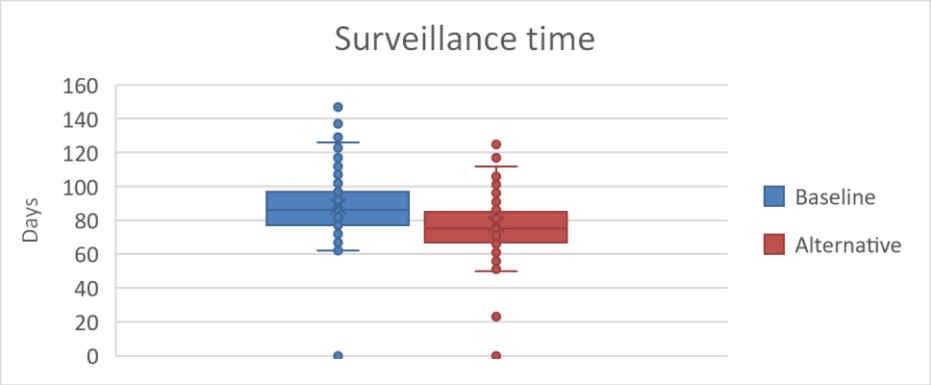

Figure 5-5 Comparison of baseline and combined alternate surveillance approaches on surveillance time

Figure 5-6 Comparison of baseline and combined alternate surveillance on surveillance cost

Appendix

Appendix 5.2: Garner G, Vosloo W, Tapsuwan S, Bradhurst R, Hillberg Seitzinger A, Breed AC, Capon T. 2021. Comparing surveillance approaches to support regaining free status after a foot-and-mouth disease outbreak. Prev Vet Med, vol 194, 105441. https://doi.org/10.1016/j.prevetmed.2021.105441

5.3.4 *In smaller outbreaks, large reductions in losses to producers from an FMD outbreak are possible if trading zones are used to maintain trade from uninfected parts of Australia*

Trading zones for the eleven smaller outbreak simulations were modelled using the border of an individual state, except for the incursion scenario Q3 which also affected NSW. Prior to applying the trading zones at state levels in the economic simulations, a 3-day national standstill of susceptible livestock as described in AUSVETPLAN is assumed along with a 14-day national embargo on exports of susceptible livestock and livestock products. From day 15 of the outbreak, the embargoes on Australian exports are removed with trading zones formed by the unaffected state(s) resuming exports. The embargoes on affected state(s) remain in place until the last day of cull plus a 90-day waiting period for return to disease freedom based on OIE guidelines (OIE Guidelines, 2020). No additional time was added to export recovery for the small FMD outbreaks found in the eleven incursion scenarios analysed for this study based on an examination of export recovery from historical outbreaks of FMD. A decision was made to use the OIE guideline of 3 months after the last affected animal is culled for resumption of trade with 100 percent of export trade assumed to be embargoed from the infected states during the 90-day period in order to again remain on the conservative side for extent of decline in the trade. Finally, it must be noted that the trade from uninfected states is assumed to be able to access current or alternative ports for shipment of their product.

The calculated length of embargoes under trading zones are applied to average quarterly Australian state level exports from 2016 to 2018 to determine the percent of national trade that occurs from each state to each importing country (Source: Trade Data Monitor). Using quarterly data allows some recognition of regional and seasonal patterns in trade specific to the quarter in which the incursion scenario is set to occur. However, the sudden nature of a livestock disease outbreak is dampened with the introduction of the trade shocks into the economics model which has an annual time step. Potential reductions in the quantities of livestock and livestock products embargoed should Australia effectively implement trading zones in this manner range from approximately 20 to 80 percent compared to a national embargo.

The percentage reductions in exports by livestock and livestock product category are used as the export demand disruption, or shock, in the Agricultural sector partial equilibrium model (AgEmission) of Australian feed and livestock markets (Buetre et al., 2013) in order to estimate the potential national declines in producer revenues adjusted for compensation of the industries due to an FMD outbreak. This contrasts to other measures of impact which are calculated from changes in export revenues (Bradhurst et al., 2019).

Where producer revenue losses are between $7 billion and $13 billion under a national embargo, they are reduced to between $3 billion and $9 billion under trading zones (Figure 5-7Figure 5-7). This is a reduction in lost revenue ranging between 21 and 80 percent for the eleven incursion scenarios and five livestock products. The average reduction in losses is highest for dairy at 72 percent, followed by beef at 63 percent. Pig meat's average potential losses fall by 64 percent with sheep meat declining 49 percent and wool 38 percent. An outbreak in New South Wales and one in Victoria have smaller potential national benefits from trading zones in percent terms from their incursion

scenarios than other states incursion scenarios due to very limited disease spread (Hafi et al., 2022 – Appendix 5.3).

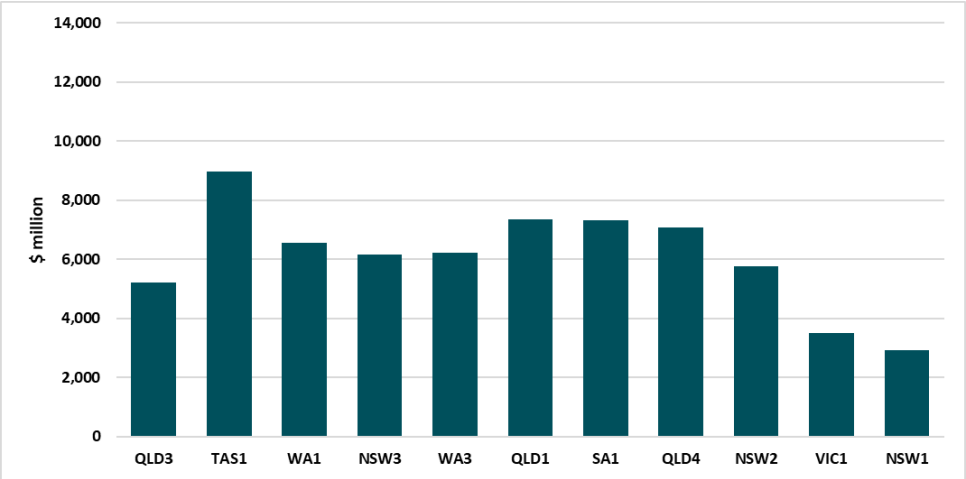

Figure 5-7 Change in Gross Value Product impact as a measure of benefits from trading zones during small Australian livestock disease outbreaks

Appendix

Appendix 5.3: Hafi A, Addai D, Breed AC, Bradhusrt R, Capon T, Garner MG, Miller C, Pinol J, Seitzinger AH, Tapsuwan S. 2022. Economic benefits of implementing trading zones for Australian livestock disease outbreaks of limited duration. Aus Vet J, 100 (4); doi: 10.1111/avj.13141.

5.3.5 The reduction in the geographic extent of a large outbreak due to vaccination can be enough to overcome the costs of a longer delay in return to disease freedom under vaccination

Several studies have found disease control and economic benefits from vaccination-to-remove applied to large outbreaks in formerly FMD-free countries (Schroeder et al., 2015; Feng et al., 2017; Porphyre et al., 2018; Bradhurst et al., 2019; Miller et al., 2019). These economic results relied on the OIE Terrestrial Animal Health Code guidelines for declaration of a return to disease freedom after FMD outbreaks as a proxy for export recovery. Using OIE guidelines constrained results by establishing the time to recognition of disease freedom in set periods relative to outbreak control events. Examinations of multiple historical livestock disease outbreaks' export recovery patterns question use of these fixed three- and six-month assumptions based on OIE declaration of disease freedom guidelines (Johnson et al., 2011a and 2011b, Johnson et al., 2015, Thompson, 2018).

Economic modelling was used together with the AADIS outputs to further analyse export recovery assumptions for large outbreaks different from the application of the OIE declaration-of-freedom guidelines described above and with trading zones applied. Historical trade patterns of export recovery following FMD outbreaks controlled by stamping out alone were employed to estimate trade recovery as a function of outbreak length. These estimates were combined with embargoes beginning at the national level and transitioned into state level trading zone configurations as the AADIS simulations' outbreak end dates were reached. Economic analyses of targeted vaccinate-to-

remove and vaccinate-to-retain control policies were undertaken to estimate the additional time trade could be restricted under vaccination and still have an equivalent economic impact to stamping out only with trading zones. Sensitivity analyses also occurred for potential negative consumer reactions to the presence of livestock disease and for the possibility of implementing FMD virus inactivation measures to wool allowing earlier resumption of the wool export trade than for other livestock products.

For the V2 simulated outbreak, where vaccinate-to-remove was utilized along with trading zones, producer losses were reduced by AUD 4 billion in present value terms over 10 years estimated at a 7% discount rate (PV10,7%) compared to an outbreak where stamping out alone is applied with trading zones. Introducing FMD virus risk mitigation measures for wool to further target trading zones reduced the economic impacts by an additional AUD 3.6 billion (PV10,7%). Time to export recovery simulated for the stamping out alone with trading zones and vaccination control options with trading zones was 7 to 11 months beyond completion of post-outbreak surveillance activities and eligibility for an OIE declaration of return to disease freedom.

By extending the export shocks applied in AgEmissions under the V2 vaccination control options, it was found that four additional months of trade restrictions due to vaccination under trading zones would lead to GVP reductions equivalent to those for stamping out alone with trading zones. In terms of the outbreak control measures, this would be 18 months beyond when the last vaccine was administered and the last vaccinate was removed. Outbreak response cost savings and additional potential costs under vaccinate-to-retain with trading zones were also compared to the vaccinate-to-remove control with trading zones. (Seitzinger, et al., 2022a; Appendix 5.4)

Simulation modelling further examined targeted use of limited vaccine supplies in combination with varying surveillance resources, also using the V2 incursion scenario. Adding to the pool of outbreak surveillance resources available by 50 percent in combination with vaccination decreased outbreak duration and outbreak response costs. The median duration was reduced by an additional 13 percent and response costs declined by an additional 8 percent. Pooling of vaccine resources overcame the very early binding constraints under proportional allocation of vaccine to individual states with similar reductions in outbreak duration to those with additional surveillance resources. However, government costs rose substantially by over 40 percent and introduced additional risk of a negative consumer response (Seitzinger et al., 2022b).

Appendixes

Appendix 5.4: Seitzinger AH, Hafi A, Addai D, Garner G, Bradhurst R, Breed AC, Capon T, Miller C, Pinol J, Tapsuwan S. The economic benefits of targeted response strategies against foot-and-mouth disease in Australia. Prev Vet Med. 2022;204:105636. doi: 10.1016/j.prevetmed.2022.105636.

5.4 Discussion

Subproject 3 has investigated the potential for vaccination to be incorporated into control strategies for FMD and the consequences for the post-outbreak surveillance and management phase of an outbreak.

In this section of the report, we have summarized the evidence from Subproject 3 that support the following key results:

1. Smaller outbreaks can be effectively managed by stamping out without vaccination
2. The size and duration of larger outbreaks can be significantly reduced when vaccination is used
3. New technologies for FMD post-outbreak surveillance based on non-invasive sampling methods and RT-qPCR tests can substantiate FMD freedom faster and more cheaply than traditional approaches based on serological surveys
4. In smaller outbreaks, large reductions in losses to producers from an FMD outbreak are possible if trading zones are used to maintain trade from uninfected parts of Australia
5. The reduction in the extent of a large outbreak due to vaccination can be sufficient to overcome the possible delay in return to 'disease freedom' status particularly if used with trading zones
6. Business continuity planning, including consideration of permitted movement needs for negligible risk product, effective stocks management, and virus risk mitigation measures, would support maintaining market access to the extent possible during an outbreak and recovering market access after an outbreak.
7. National losses to producers from small highly infectious livestock disease outbreaks could be reduced by 20 percent or greater with the implementation of trading zones, allowing uninfected regions of the country to return to trade as the outbreak is controlled.
8. Targeted vaccination and trading zones reduced national producer losses by 12.6 percent for a large outbreak simulated in Victoria.

Subproject 3 has investigated alternative control strategies for incorporating vaccination into control for FMD across Australia, including areas considered to be at lower risk for introduction and spread of FMD. It is reassuring that for most scenarios examined in this project, FMD outbreaks were small and readily able to be controlled with available resources and control strategies without vaccination. In these scenarios, the trigger of five or more infected premises on Day 14 of the control phase for vaccination to be implemented was also less likely to be met. For these smaller outbreaks, economic analysis of the application of trading zones allowing uninfected portions of the country to continue trading internationally provide large potential producer revenue gains to weigh against the implementation costs of such areas.

In some areas, however, there is potential for large, long outbreaks to occur. For these areas, the use of vaccination can reduce the size and duration of an FMD outbreak. We have identified scenarios where outbreak duration is significantly reduced when vaccination is used for the larger outbreaks. In particular, the largest simulated outbreaks were observed for two of the Victorian incursion scenarios (V2 and V3) where FMD incursion is assumed to occur in dairy farms and intensive beef farms, respectively. These results are consistent with the observation that Victoria is

considered a higher risk area of Australia for FMD introduction, establishment and spread because of its geographic conditions, including its relatively high human population and its higher stocking rates. For these types of large outbreaks that are possible in Victoria, vaccination can also reduce the probability of observing a very large, long outbreak. Companion work on the economic implications of combining vaccination strategies used against large outbreaks with trading zones further supports targeted use of vaccination in combination with increasing surveillance resources.

Notwithstanding the effectiveness of vaccination to reduce the size and duration of large outbreaks, under current international guideline (OIE) there remains a strong belief that a vaccinate-and-retain policy will always result in the longest return to markets for exports of susceptible livestock and their products. This is because of the extended delay in OIE guidelines to regaining FMD-freedom from disease recognition when vaccinated animals are retained compared to if they are removed (6 months versus 3 months). The differential time periods are being challenged and new diagnostic approaches offer the potential that surveillance might be able to provide acceptable levels of confidence in the infection status of vaccinated populations in the future. In addition, while the declaration of disease freedom influences trade recovery, it is increasingly recognised to be only one of many factors affecting the return to export markets.

Our work builds on previous modelling studies in Australia (Abdalla et al., 2005, Roche et al., 2014b, Garner et al., 2016) and overseas (Tomassen et al., 2002, Keeling et al., 2003, Roche et al., 2014a) that have shown that vaccination is effective in reducing the duration and/or size of outbreaks where disease is widespread occurring at a high rate of spread, or resources for stamping out are limited. By examining a broader range of incursion scenarios across Australia, our results provide further information about where vaccine supplies and surveillance resources could be effective and can help disease managers gain a clearer understanding of how vaccination could help manage an FMD outbreak under Australian conditions.

Subproject 3 has investigated the potential for new approaches to post-outbreak surveillance. Following an FMD eradication program, surveillance will be required to demonstrate that infection has been eradicated from the population. Substantiating freedom from infection after an outbreak of FMD is an essential component of a disease control program and a necessary step in regaining FMD-free status. Newer diagnostic methods and innovative ways of sample collection offer the potential for more efficient approaches to post-outbreak surveillance. This study confirmed that alternate approaches to FMD surveillance based on non-invasive sampling methods and RT-qPCR tests have the potential to enable post outbreak surveillance substantiating FMD freedom to be done more quickly and less expensively than traditional approaches based on serological surveys.

Whilst the largest economic impacts of an FMD outbreak are caused by trade losses, work in sub-project 3 has also started developing methods to estimate the impact of movement controls on uninfected producers within the affected areas. For example, the costs to the dairy industry can be estimated in terms of farm costs for maintaining dairy cows and bobby calves that cannot be moved to slaughter, and forgone milk sales revenue. This type of approach can be used in future to investigate the potential for mitigating risks to business continuity using measures such as permitted movements. This type of system has the potential to reduce consequential losses to uninfected producers due to movement restrictions.

Acknowledgements

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6 Tools to rapidly characterise and map outbreak pathogen spread

6.1 Introduction

The biosecurity problem Subproject 4 addresses is the management of FMD and other emergency animal disease (EAD) incidents at the local level. For this, the operational unit is the Infected Premises (IP), and a fundamental question for the management of an IP is how it became infected, as this informs potential lapses in biosecurity which must be addressed for effective control of the incident. Traditionally, informing the pathway by which the IP became infected has been determined by a administering a questionnaire to property managers during an outbreak investigation visit about management practices, including potential movements of animals, people and products onto and off the IP. Subproject 4 aimed to improve on this by developing a Big data decision support system (DSS) collating data related to environmental and managerial practices into a user-friendly, web-accessible application. This application was named *SPREAD* ("System for Preparedness and Response for Emergency Animal Diseases").

The main pathways of transmission of FMD virus from farm to farm ("F2F") are known, but their importance varies through the course of an outbreak. Initially – before the outbreak is detected – animal and production movements like milk collection may be the main route of spread of the virus. However, following detection, these pathways are of less importance as standard EAD management mandates they be stopped. Thereafter the pathway of subsequent transmission is less defined, and analysis of large outbreaks in Europe has determined that 'local' spread can account for 80% of the transmission pathway. Local spread has been hypothesised to be due to wind-borne dispersion of the virus, but objective evidence for this is currently lacking.

The objective of the Subproject 4 was thus to develop an online system to enable the risk of local windborne dispersion to be assessed in near real-time, and transmission confirmed by using next generation sequencing (NGS) of viruses collected from infected animals on the IPs. Due to the size and complexity of the assessment and confirmation, involving large amounts of data and its processing, we adopted a Big Data approach. In adopting this approach, we implemented an IT architecture for SPREAD which will permit the incorporation of other disease transmission pathways such as animal movements, as well as to potentially extend it to other emergency animal diseases.

6.2 Method

For the IT development of *SPREAD*, we used an architecture centred on the use of web Application Programming Interfaces (APIs), which acts as intermediate software between the user interface running within a browser and the data processing and modelling which occurs within a cloud computing environment. This use of web APIs had previously been successfully applied in a collaborative project between CSIRO and BOM to develop an application ('*TAPPAS'*) enabling the wind dispersion modelling of *Culicoides* midges (Durr, Graham, & van Klinken, 2017). However, *TAPPAS* was a relatively simple application, only attempting to model the dispersion part of the transmission pathway, while a more complete risk assessment requires also the modelling of the pathogen emission from the source IP as well as the infection process at downwind premises (Schley, Burgin, & Gloster, 2009). Furthermore, we needed to implement a whole bioinformatics

module to enable the construction of genomic networks to assess the likelihood that the hypothesised source IP was the actual infecting IP.

Implementing all these required careful design and co-ordination, which involved developing or modifying existing models (e.g. the *TAPM* model for atmospheric dispersion), linking these models with data (e.g. the meteorological data sourced from BOM or sequence data from Genbank), developing Web APIs to manage user requests and output, and a user interface to make these requests and to visualise the model outputs. The multidisciplinary team which successfully delivered this involved staff from CSIRO Australian Animal Health Laboratory (project co-ordination and data management), CSIRO Oceans and Atmosphere (wind dispersion modelling), CSIRO Health and Biosecurity (for bioinformatics), the Bureau of Meteorology (for Web API development and meteorological data), a private software development company (NewtonGreen Technologies) and RMIT University (for mathematical infectious disease modelling).

The *TAPM* model was developed for pollutant atmospheric dispersion, and to make it relevant for epidemiological investigations required that we add functionality by undertaking specific research. Most obvious is the need to allow for the dispersed virus being inactivated in the atmosphere, the rate of which would be expected to depend upon temperature and humidity. To quantify this, we collaborated with SubProject 1 researchers to design a series of inactivation experiments in which varying temperature and humidity were simulated in an environmental chamber. These experiments – which were undertaken at FLI, Germany - showed the overwhelming importance of temperature for virus inactivation, which was a few hours at 30 °C and several days at 10 °C. A second research activity to make *SPREAD* more epidemiologically realistic was to allow for time-varying excretion of the virus at the farm level, which occurs when the number of excreting animals increases as the disease spreads amongst a herd. To model this, we worked with a team of mathematical modellers at RMIT University.

A challenge with Big data applications like *SPREAD*, which combine large datasets with relatively complex models, is that technical errors may occur which go unrecognised. To avoid this, we undertook model 'verification' by collating datasets of peer-reviewed publications where FMD wind dispersion and/or construction of FMDV genomic networks had been undertaken. These verification case studies were all from the UK, reflecting this countri's lead role in FMD research and a general willingness to share data. In total, we undertook comparisons for 5 outbreaks where wind dispersion modelling had been undertaken, one from the 1967-68 FMD epidemic (Hampshire), three from the 2001 epidemic (Northumberland, Durham and Cumbria) and one from the 2007 Surrey outbreak. For the genomic network, we used published sequence data from a cluster in Cumbria in 2001 and the Surrey outbreak in 2007.

SPREAD is intended to be used by veterinary epidemiologists and laboratory sequencing staff, neither of whom can be assumed to have advanced IT skills. Furthermore, as it will used in an emergency, there is little time for users to undergo extensive training in its use. To overcome this, much effort was expended in developing a user-friendly graphical user interface (GUI) running within a browser. To assess whether this GUI design was successful, we ran two 'user acceptance testing' (UAT) workshops in August and October 2019, the first focussed on the design on the epidemic visualisation module and the second on the wind dispersion module. Further UAT workshops were planned for early 2020 but were cancelled following the disruptions following the COVID-19

epidemic. In their place, live demonstrations of *SPREAD* are being undertaken, and have already been successfully completed for Western Australia and Queensland.

6.3 Results

Although the only visible result of SP4 to users is the *SPREAD* web interface, this belies an immense amount of IT and scientific work required to achieve a working application. In total approximately 16 other tasks were undertaken, which are summarised in Table 10.

Table 6-1 Summary of IT and scientific tasks undertaken to achieve working SPREAD application

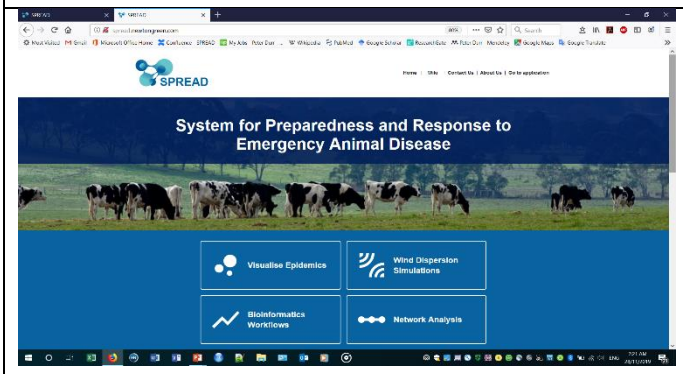
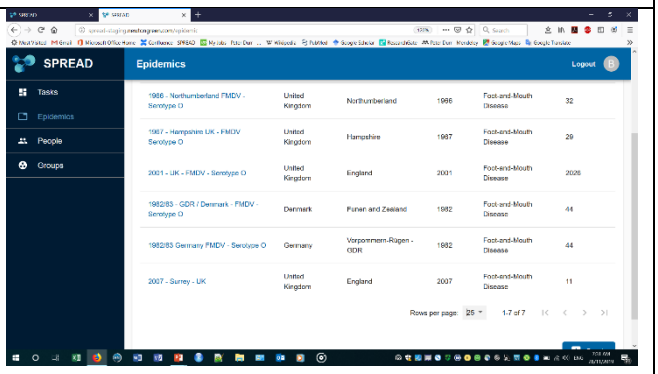
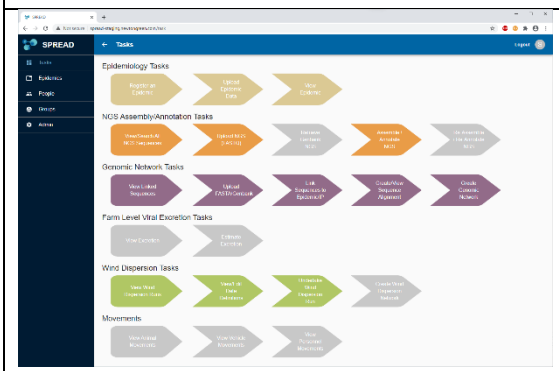
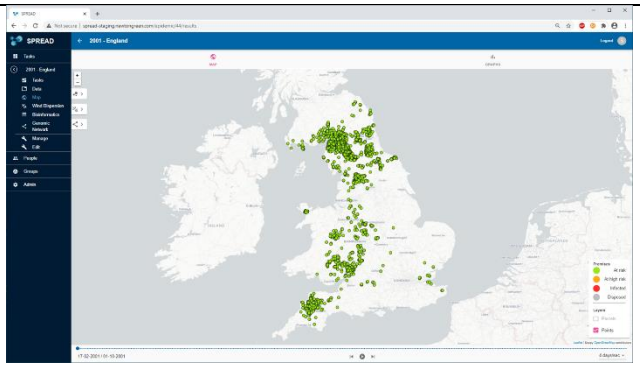
Task	**Prinicipal organisation**	**Timeline (approximate)**	**Output**
Customising *TAPM* for F2F dispersion modelling	CSIRO Oceans & Atmosphere	Sep 2016 – Dec 2017	Version of *TAPM* ready for loading onto server plus documentation
Preparing a virtual Server Environment for *SPREAD*	CSIRO AAHL / CSIRO IM&T	Aug 2017 – Sep 2017 Oct 2018	Deployment of a suitably sized server for hosting the *SPREAD* environment. This server was revised and now holds the API Docker containers and output files.
Deploying a PostgreSQL database for *SPREAD*	CSIRO AAHL	Feb 2017 – Jun 2018	PostgreSQL production database is supported and maintained by CSIRO IM&T. The BioSQL database housing all genomic data and an internal PHP website for maintenance of records.
Developing Web APIs for the *SPREAD* models and data management	BOM	Oct 2017 - Jul 2020	14 APIs were built in total, for retrieving Genbank and SRA data from NCBI, adding records for SPREAD Genomic database, assembly and annotation of FASTQ data, creating and rebuilding the classifier, retrieval of genomic records in specific file formats, uploading user defined files, creating

			multi-sequence alignments, running SCOTTI genomic networks, intra-herd modelling with viral excretion estimates and undertaking TAPM wind dispersion models.
Enabling access to current climate datasets and compilation of historical ones	BOM	Oct 2017 - Aug 2020	Access to global and regional forecast and reanalysis data (Jul 2020-Sep 2020) Specific historical datasets for case studies were acquired from NCEP and ERAI for the specific time periods.
Undertaking FMDV genome analyses to establish reference genomes (for NGS assembly)	CSIRO-AAHL	Jun 2017 – Dec 2017	Development of an *in silico* genotyping system for FMDV
Developing bioinformatic scripts to enable NGS assembly and construction of genomic networks	CSIRO-AAHL / CSIRO H&B	May 2018 – Jun 2020	Each workflow API is run from multiple Python scripts. These are all maintained within a CSIRO Bitbucket repository.
Collation of the historic FMD case study data	CSIRO-AAHL	Feb 2017 – Mar 2020	Collation of the outbreak (and genomic) data for 10 FMD outbreaks in Europe
Undertaking FMD virus survival experiments	CSIRO-AAHL / CSIRO H&B / FLI / CSIRO O&A	Jun 2017 – Sep 2019	Survival curves (and functions) for FMDV at temperature / humidity combinations
Development of intra-herd FMDV transmission models	RMIT University / CSIRO-AAHL / CSIRO H&B	Jan 2019 – Jun 2020	4 intra-herd models were created for use dependant on available epidemic data. These have been implemented within

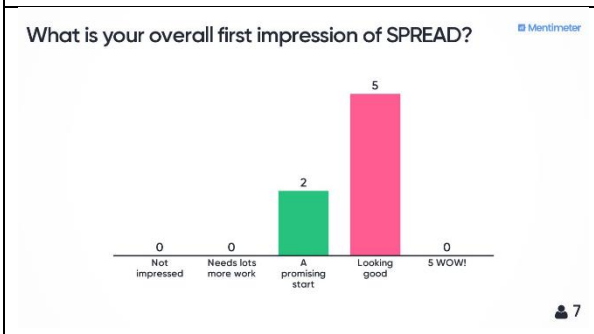
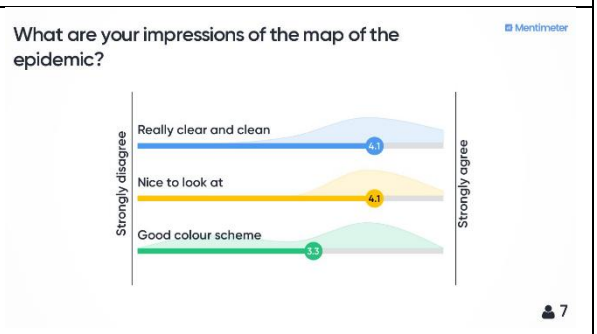
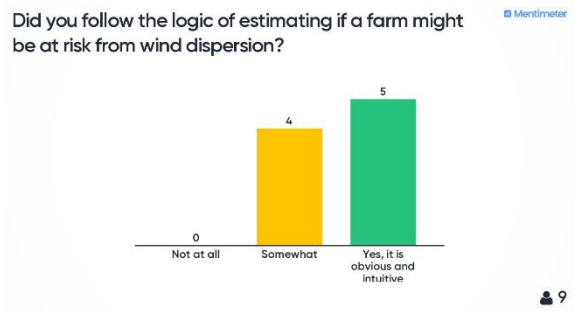
			Python and have associated API's
Designing the *SPREAD* web interface	CSIRO-AAHL	Dec 2017 – Feb 2018 Apr 2019 – Jun 2019	Wireframes were created for each web page, defining the layout, data input and methods of visualisation. The basis of these were used for the application development.
Undertaking the initial development of *SPREAD's* wind dispersion module	Intersect Pty Ltd / CSIRO AAHL	Jan 2017 – May 2018	Beta development of the wind dispersion module.
Developing the beta and release versions of the *SPREAD* application	NewtonGreen Technologies / CSIRO-AAHL	Feb 2019 – Oct 2020	A working *SPREAD* application was delivered by NewtonGreen Technologies. A user can create epidemics, visualise with a map, upload and edit data, run wind dispersion and review results, assign sequences to farms, upload genomic data, create a multi sequence alignment, and genomic network.
Enabling network access and implementing Docker containerisation	CSIRO-AAHL / CSIRO H&B / CSIRO IM&T	Oct 2019 – Jun 2020	All API's run within spawned Docker containers. This architecture will allow for easy deployment to various environments and is non-specific for the operating system. External access to internal CSIRO server was implemented and passed security testing.

Undertaking the *SPREAD* UAT workshops / live demonstrations	CSIRO-AAHL / NewtonGreen Technologies	Aug, Oct 2019 Oct 2020	Visualisation and wind dispersion UAT workshops were delivered in August and October 2019. Recently *SPREAD* demonstrations have been given to WA and QLD.
Performing the *SPREAD* verification using historical FMD case studies	CSIRO-AAHL	Oct - Dec 2020	Draft paper ready for submission to a journal

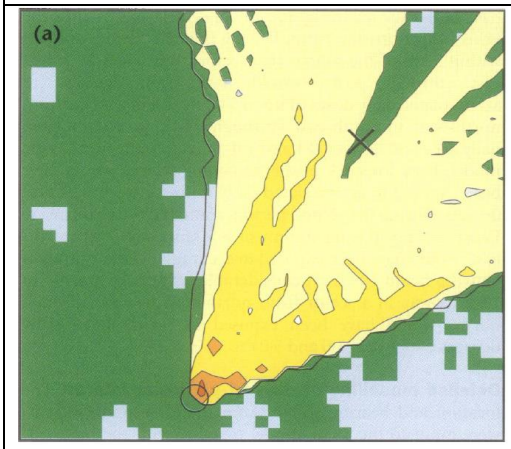
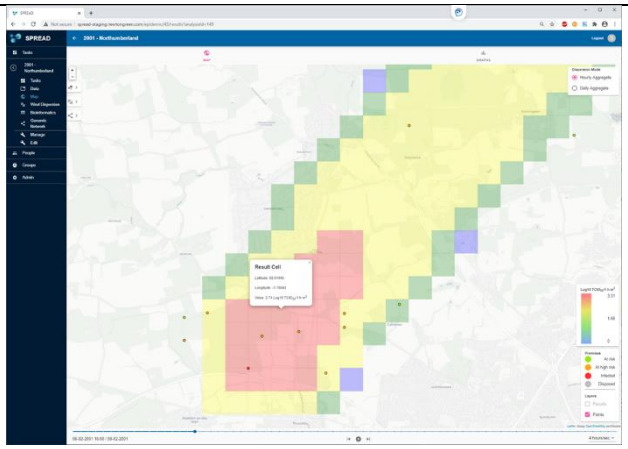
Regarding the web interface of the *SPREAD* application, the actual build by the IT company NewtonGreen Technologies started in February 2019, and was completed in stages, with the current release version delivered in October 2020. Screenshots of SPREAD are shown below.

The *SPREAD* Home page	A listing of FMD historical epidemics that can be visualised within *SPREAD*;
	
The tasks that *SPREAD* can perform, organised into workflows	A visualisation of an FMDV epidemic, in this instance within the UK in 2001, each green dot referring to an infected premise (IP).
	

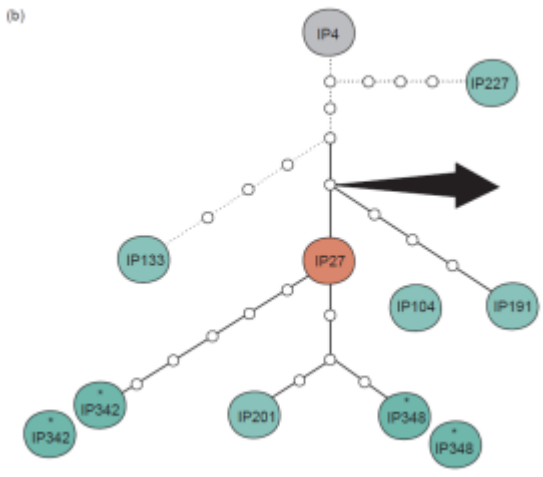
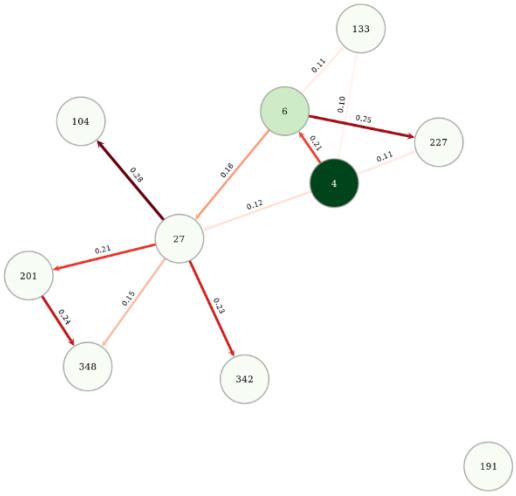
An important objective of the *SPREAD* interface was that it was user-friendly and intuitive, and this was assessed by two UAT workshops, with the first focused on the visualisation of epidemics and the second on the wind dispersion. In general, users found the design attractive and intuitive, as judged by their immediate responses using an online survey tool. Most encouragingly, users at the second UAT when asked for terms that come to mind when considering *SPREAD* suggested 'potential' and 'useful' as the commonest words.

UAT workshop 1 – Q1 – assessing the look & feel of *SPREAD*	UAT workshop 1 – Q6 – Assessing the epidemic mapping
	
UAT workshop 2 – Q5 – assessing how *SPREAD* delivers as a risk assessment tool	UAT workshop- Q7 – word cloud of *SPREAD* (the application)
	

The scientific assessment of *SPREAD* was via the verification exercises comparing the output of *SPREAD* to published case studies. Thus it can be seen that the trajectory of the modelled plume for the index IP for the UK 2001 epidemic ("Burnside Farm") obtained using *SPREAD* is comparable to that published by Gloster et al. (2003).

Published trajectory of modelled FMDV from the index farm of the UK 2001 epidemic	Trajectory of the same IP as modelled using *TAPM* within *SPREAD*
	

For the genomic network, *SPREAD* implements a more sophisticated Bayesian approach to its construction than the original non-parametric TCS method described by Cottam et al. (2008). SCOTTI improves on the TCS method, by assigning probabilities that the genome from the source IP as an indirect ancestor of that in all the potential infected IPs, and thus allows the user to assess competing hypotheses as to the IP-IP infection relationship (De Maio, Wu, & Wilson, 2016).

A published genomic network of the Longtown cluster of the UK 2001 outbreak as determined using TCS (Konig et al., 2009)	The *SPREAD* genomic network for the same Longtown cluster as implemented using SCOTTI.
	

Whilst the focus of SP4 was the design, development and deployment of the *SPREAD* application, it is important to note that this involved three original scientific research activities:

1. The design of the FMD virus inactivation study, which was implemented by members of SP1. This is the first time the inactivation of the virus under controlled experimental conditions of both temperature and humidity has been undertaken.
2. The implementation of a method to estimate time-varying FMDV excretion from IPs, including an original model (developed by RMIT University) to allow for livestock being at pasture.
3. The development of a novel sequence genotyping method for FMDV based on the amino acid motifs at the beginning and ending of the viral proteins, and has the potential to replace the current typing system ("topotyping") based on PCR.

6.4 Discussion

The concept of using IT systems to help guide FMD outbreak management at the local level was proposed over 20 years ago by a team at Massey University in New Zealand (Sanson, Morris, & Stern, 1999). Whilst successfully used for FMD preparedness in NZ, the EpiMan system was highly tailored to the NZ situation and somewhat inflexible in its data requirements. This meant that it was not able to be transferred as working software to other countries nor was it able to be used for other EADs. Thus, when NZ was faced with eradicating *Mycoplasma bovis* from its cattle herd in 2017, EpiMan has not been used, and instead new software systems have needed to be built.

The progressive development of web technologies – and particularly the introduction of Web APIs - has enabled *SPREAD* to adopt a highly flexible architecture. The most important feature is that the models and the databases are decoupled from the user interface. Thus, new epidemiological and bioinformatic models can be developed and deployed onto the server-side of *SPREAD* relatively rapidly and then accessed programmatically via the Web API. This means that *SPREAD* can readily adapt to new EADs and deliver outputs within weeks.

SPREAD is built to be used by veterinary services in the jurisdictions and is not intended to be used directly by producers and industry. The ultimate beneficiaries however are producers and the livestock industry, due to the impact FMD and other EADs can directly have on livelihoods if they are infected or else indirectly due to the effect downward impact on produce prices arising from export bans and potential loss of consumer confidence.

As the principal users of *SPREAD* are the jurisdictional veterinary services, the most pressing follow-on activity is to provide for them access and training in *SPREAD's* use. Currently jurisdictions can use *SPREAD* by uploading premises (and potential IP) data and running wind dispersions through the web interface. However, this is not optimal, as this will involve handling multiple files and require that the user adds data about these files to ensure that they be inserted into *SPREAD's* database. Technically this is a common Big data problem, for which there are several solutions, the challenge being to find the one that works best for each jurisdiction's IT network and security. Already there is a follow-on activity funded by the WA Government for 2020-21 which will be exploring seamless *SPREAD* access options, focusing on the use of MAX - WA's emergency response software – as an intermediary system to deliver relevant data to *SPREAD*. If this activity proves successful, then an additional activity might explore the possibility of using MAX to deliver animal movement data,

which can be then be analysed by *SPREAD*. Once enabled, this will allow *SPREAD* to analyse diseases where non-local farm-to-farm transmission via animal movements is the predominant method of transmission.

Although the compilation of the historical case studies was primarily to undertake verification of the wind dispersion modelling, there is now considerable potential to use these to better define the role of wind dispersion as the pathway of local spread of FMD. Thus, a suggested follow-on research activity will be to systematically undertake wind dispersion runs from each IP in some of the clusters of the 2001 UK epidemic and assess whether wind dispersion was possible, plausible or probable as a means of transmission. If successful, this may assist in resolving a long standing debate in the FMD community about the importance of aerosols for transmission of the virus.

Overall the *SPREAD* was an extremely challenging project, tackling a difficult problem and requiring the integration of a range of scientific disciplines and IT technologies. Through the project's course there were many challenges, each of which was worked through and overcome. However, it needs to be recognised that this would not have been possible if a shorter timeline had been imposed on the project. Thus, a key learning for project delivery is that complex projects – which undoubtedly includes the development of *SPREAD* – require realistic timelines for delivery, and it a credit to the RRD4P program that this was appreciated.

6.5 Reference materials

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7 Conclusion

7.1 Key findings

7.1.1. General

- The transdisciplinary approach to address various facets of the FMD Ready project has demonstrated that collaboration between different research disciplines (e.g. modelling, economics, science), combined with direct interaction and participation of livestock industries, governments, RDCs and other agencies, can deliver solutions to complex problems that may not be possible with less diverse research teams.
- Australia is better prepared for an EAD incursion with pre-border knowledge of FMD threats, tools that can be used prior and during an outbreak and a better understanding of control options and their costs.

7.1.2 Subproject 1

- Accredited diagnostic support is crucial for EAD control during and after an outbreak when surveillance is required to prove successful disease eradication and freedom re-established. Additionally, accurate diagnostics is crucial for EAD confirmation, especially with high impact diseases.
- Scientific data on circulating and emerging virus strains are essential to ensure the investment in the FMD vaccine bank will provide the best outcome should vaccines be required to control an outbreak.
- Access to research funding to test vaccine against new emerging viruses *in vivo* has impact on decisions regarding vaccine banks and choice of vaccine to use in an emergency.
- Methods to ensure samples can be transported in a safe manner, during and after an outbreak, should be tested and validated and be available when required.
- Laboratory results are essential to support models and the SPREAD application and ensure their accuracy.

7.1.3 Subproject 2

- A partnership approach to understand problems and co-design solutions will have more traction than trying to persuade producers to adopt practices through sequence of research and extension.
- Understanding farmers' vulnerability to emergency animals' disease, based on their behaviours, practices and farming characteristics, can inform government and industry risk-based approach to disease preparedness.
- A systems approach is effective for understanding both behavioural and structural barriers to improving on-farm animal health surveillance and biosecurity
- The partnerships pilots provided producers and other local animal health stakeholders ownership of EAD preparedness by listening and valuing their perspectives and sharing with them the responsibility of co-design and implementation effective solution.

7.1.4 Subproject 3

- Smaller outbreaks can be effectively managed by stamping out without vaccination
- The size and duration of larger outbreaks can be significantly reduced when vaccination is used
- Vaccination can reduce the probability of extremely large and long outbreaks
- Mapping the risk of disease spread helps identify regions where vaccination might have a useful role
- New technologies for FMD post-outbreak surveillance based on non-invasive sampling methods and RT-qPCR tests can substantiate FMD freedom faster and more cheaply than traditional approaches based on serological surveys
- In smaller outbreaks, large reductions in losses to producers from an FMD outbreak are possible if trading zones are used to maintain trade from uninfected parts of Australia
- The reduction in the extent of a large outbreak due to vaccination can be sufficient to overcome the possible delay in return to 'disease freedom' status if used with trading zones
- Business continuity planning, including consideration of permitted movement needs for negligible risk product, effective stocks management, and virus risk mitigation measures, would support maintaining market access to the extent possible during an outbreak and recovering market access after an outbreak.
- National losses to producers from small highly infectious livestock disease outbreaks could be reduced by 20 percent or greater with the implementation of trading zones, allowing uninfected regions of the country to return to trade as the outbreak is controlled.
- Targeted vaccination and trading zones reduced national producer losses by 12.6 percent for a large outbreak simulated in Victoria.

7.1.5 Subproject 4

- The collation of the data from historical outbreaks of FMD in disease free countries in Europe and Asia confirmed the importance – and the challenges – of effectively managing local spread in the control of large FMD outbreaks
- The complexity of local spread – with various direct and indirect pathways – indicates the need for a decision support system (DSS) to assist with its management
- Advances in atmospheric dispersion and genomic network modelling suggest that combing these might be the basis for such a DSS – but this presents challenges for data management and processing
- *SPREAD* explicitily overcomes this challenge by adopting a Big data approach whereby all data processing is run within cloud-based high performance computing. As such it is now possible to do complex atmospheric dispersion runs and the analysis of large ("next gen") sequence data files within relatively short time frames
- The development of *SPREAD* has therefore provided Australia with a state-of-the-art application for managing local FMD transmission. Furthermore, it is also readily extendable to other pathways such as animal movements and other EADs, such as highly pathogenic avian influenza (HPAI).Further investment is needed to enable real-time linkages with the

jurisdictional databases. This has commenced with WA Department of Primary Industries and Regional Development.

7.2 Benefits to Industry

General

By applying a transdisciplinary approach to EAD preparedness, the FMD Ready Project (the Project) has transformed the way multiple research teams with different capabilities address complex problems. The Project was managed as different subprojects (SP 1-4), but the research teams interacted regularly with inputs from different teams and presented a suite of tools to ensure Australia is better prepared for an EAD incursion and as a result provide protection to our livestock industries and lessen disease impact.

Early detection of an EAD considerably reduces the size of an outbreak: the earlier it is recognised, the less chance there is for a widespread outbreak, and this will limit the potential impact. The Project investigated the risk profiles of various livestock industries and designed approaches to improve on farm surveillance and reporting of unusual events.

Part of preparedness is to simulate outbreaks in different sectors of the livestock industry and test a range of approaches to control EADs. Due to the infrequent nature of these events, and lack of true data (for Australia), the Project used simulations to model outbreaks, how best to control these modelled outbreaks and measure the financial impact of the different control approaches. The Project also designed options to improve post outbreak surveillance to provide proof of disease freedom and help resume trade.

Naturally, for important EADs we need to accurately diagnose them, and the Project ensured that the assays for detection and confirmation of FMD are validated and provided tools to trace the virus during outbreaks. The Project also investigated ways to improve the safe transport of biological samples collected from potentially infected animals, thereby improving biosecurity during the diagnostic and surveillance process. Offshore surveillance was done to gather intelligence on the FMD viruses circulating in the SEA region and testing the vaccines in the Australian Vaccine Bank to ensure our livestock industries will have access to the best vaccines.

Finally, the Project developed a tool called SPREAD, that can be used during an outbreak to trace virus movement, by understanding the virus survival influenced by various climatic factors and help with decisions for control of EAD.

Through this Project, the industries now have access to a package of tools to prepare the country for an EAD incursion.

Subproject 1 focused on laboratory-based diagnosis and confirmation of FMD as well as ensuring Australia has information related to vaccines that will provide protection against important circulating viruses.

Better preparedness for an FMD incursion by:

Supporting the FMD Australian Vaccine Bank - The Bank is renewed every five years and the Project provided scientific data to ensure the correct vaccine strains are included that will provide protection to viruses circulating globally but relevant to Australia, with particular focus on SEA. This ensures the livestock industries will have access to epidemiologically suitable vaccines if an incursion of FMD occurs.

Improving diagnosis for FMD - Due to the impact of an FMD incursion, confirmation or rejection that clinical symptoms are possibly due to FMD infection, is essential. It is therefore crucial to ensure laboratories have access to rapid, and accurate assays. The Project assisted in modifying existing testing protocols to decrease cost of testing, ensuring sequence data can be obtained from samples that have low levels of virus/viral genome to help determine the possible international origin of the virus, but more importantly, to trace virus movements during an outbreak.

Improved biosafety when transporting diagnostic samples - FMD virus is highly contagious and it is important to ensure transport and testing of samples to diagnostic laboratories will not pose a biosafety risk. The Project identified an inexpensive inactivation buffer that can be easily prepared and inactivates FMD virus rapidly but allows virus to be recovered by additional molecular methods, of further studies are needed.

Better response during an outbreak by providing accurate scientific data that are needed to model and trace virus spread during an outbreak. This will allow better allocation of resources to areas of high risk and limit further spread of the infection.

Subproject 2 applied a systems based transdisciplinary approach in partnership with producers, industries and governments. The subproject delivered extensive producer surveys to understand their vulnerability to EAD and trailed innovation pilot groups in five states and five different livestock species to develop a producer-led partnership model that enhances trust, reporting, and animal health surveillance. The resulting benefits and implications for industry include:

A better understanding of producer vulnerability to EADs for appropriate resource allocation: Current approaches are highly focused on disease agent characteristics, introduction and spread pathways and the biophysical context of farms. The producer-centred vulnerability models are based on producers' behaviours, beliefs, practices and faming characteristics, which have the potential to provide more evidence for targeted industry support to producer types and better preparedness efforts. Industry and government use risk-based approaches to disease preparedness.

Effective animal health surveillance and on-farm biosecurity products: The distribution of the communication products developed by producers, for producers will benefit industries. This include the following industry first products developed; a Goat Diseases- Farmers' Guide, a Chain of Response to biosecurity incidents, and a NLIS flyers tailored for smallholder farmers and distributed with help from local council.

Shared responsibility for animal health surveillance and on-farm biosecurity helping to drive improvement in practices at a local scale: Increases in trusted relationships between farmers and

other animal heath stakeholders, especially producers and Government animal health and biosecurity officers helped increase trust which contributed to improve reporting, disease investigation and health advice seeking and more biosecurity measures in practice at local scales.

Producer-centred animal health networks with capacity to continue delivering and improving surveillance, on-farm biosecurity and more: The five partnership pilot groups established were either an enhancement with an existing group or completely new. For example, the new goat pilot provided a foundation for the establishment a goat group in South Australia to pursue animal health and other interests of the producers. The subproject has also fostered partnerships of producers with state and national livestock industry bodies and state governments.

More evidence and support for strong involvement of industries in shaping further national and animal health and biosecurity strategies: The results of the innovation system and partnership approach by the subproject have provided evidence and examples for industries to propose strategies as part of the new National Animal Health Surveillance Business Plan 2021-2026.

Subproject 3 has linked disease spread modelling and economic analysis to better understand the consequences and social and economic costs and benefits of the alternative response strategies during and after an outbreak of FMD. The benefits and implications for the red meat industry because of this project include:

Better evidence to support strategic decision-making about emergency animal disease preparedness and response - Australian Animal Disease Spread (AADIS) simulations have provided evidence-based support to increase confidence in decisions about whether to use vaccination as part of control strategies for an FMD outbreak. In particular, the project provides evidence of the types of smaller outbreaks that can be effectively managed by stamping out without vaccination and evidence of the types of larger outbreak whose size and duration can be significantly reduced when vaccination is used.

Better decision support tools - The project has further developed the Australian Animal Disease Spread (AADIS) model (Bradhurst et al. 2015, 2016) to provide improved capacity for decision support and simulation and scenario analysis for government and industry stakeholders. This work has further increased the capacity of the AADIS model to investigate alternative approaches to post-outbreak surveillance and management. These findings support more cost-effective disease management responses, e.g., where new technologies for FMD post-outbreak surveillance based on non-invasive sampling methods and RT-qPCR tests can substantiate FMD freedom faster and more cheaply than traditional approaches based on serological surveys.

Faster return to trade and increased business continuity - The project has identified disease management strategies that can reduce export market losses and strategies that can increase business continuity during an emergency animal disease outbreak. This project has linked disease spread modelling with economic analysis to demonstrate that in smaller FMD outbreaks, large reductions in producer losses are possible if trading zones are used to maintain trade from uninfected parts of Australia. For large outbreaks, the reduction in the geographic extent of a large outbreak due to vaccination can be enough to overcome the costs of a longer delay in return to disease freedom under vaccination.

7.3 Report on Communications and Extension activities

A full report on Communications and Extension activities to December 2020 has been submitted (Appendix 8.1).

7.4 Monitoring and evaluation report

Monitoring and evaluation (M&E) for the FMD Ready project took place throughout the project, with research project leaders collecting data on the key evaluation questions on a regular basis. A final M&E report was developed by Animal Health Australia in consultation with, and on behalf of, the research partners for this project in December 2020 (Appendix 8.2).

Appendix:

Appendix 8.1: FMD Ready Project: Communications, Engagement and Extension Report

Appendix 8.2: FMD ready Project: Monitoring and Evaluation Report

7.5 Project outputs

A list of all the project publications, conference and meeting presentations and other outputs are provided in Appendix 8.3

8 Conclusions

8.1 Insights and recommendations

There are a number of important learnings for transdisciplinary research teams from the FMD Ready Project:

- Research team interaction
    - When a research team is diverse and located in different institutions and geographical locations, an early attempt should be made to bring the team together to get to know each other, to build trust and cohesion and to build a level of understanding of each others' fields of discipline.
    - Early interaction helps the team members to understand each others' skill sets and how they all contribute to achieving the overall project objectives. It is also critical to integrate the findings of the different subprojects to ensure a holistic message is provided to producers and industry.
    - Building the team takes time and effort but the FMD Ready researchers agreed that it was key to achieving cross-collaboration and integration of research across the different subprojects.
    - Regular formal and informal meetings between team leaders are essential to build trust and ensure progress
- Stakeholder engagement

    - Meet with as many potential role players as possible before the project proposal is submitted (preferably F2F) to clarify the scope and understand their priorities and ideas.
    - Communication is key! This can only happen with regular meetings/contact.
- Crossing research barriers
    - There needs to be an overall, agreed goal/objective and collaboration between parties when defining objectives.
    - Team members should have an interest in the other disciplines and understand what others can deliver to the overall goals.
- Future scoping
    - Early attempts should be made to identify future research objectives, especially before the team may disperse at the end of the current project.
- Governance
    - Formal governance of large projects is essential. The creation of a steering and governance committee with specific terms of reference played and important role in ensuring the success of the project.
    - Having a strong project manager (in this project Animal Health Australia performed this role) is critical for the performance of a large, transdisciplinary project.

8.2 Future research and recommendations

The following areas for future R&D were identified by the researchers and stakeholders at the 2020 Virtual annual stakeholder workshop and upon more recent reflection:

Subproject 1:

- The need to continually monitor FMD strains and other EADs circulating in the world, and particularly in South East Asia, to inform Australia's preparedness, including FMD vaccine bank composition and recommended vaccines for other EADs. This requires continued involvement of the research team in South East Asia
- Explore and adapt new methods for virus sequence analysis using alignment-free methods such as K-mer based approaches (basic evolutionary research)
- Developing/adaptation of diagnostic platforms and bulk sampling techniques for EADs and validation for lab/field use both for primary diagnosis and surveillance (both basic and applied research). These platforms need to address different analytes available for diagnosis and be able to detect the infectious agents at low concentrations, serological tests that can detect numerous diseases in one test and host responses to different infectious diseases
- Work towards point of care detection without the need to complex sampling handling and amplification
- Address policy and social issues around the use of point of care diagnostics
- Further work on intradermal vaccination to improve efficacy and workplace health and safety (applied research)

- Further develop systems immunology platforms for FMD and other EADs to better understand and modulate immune responses to vaccination and improve protection (basic and applied research)
- Research on inactivation of FMD virus and other EADs in different export commodities to facilitate trade during and after an outbreak (applied research)
- Improvement of forensic tracing methods for EADs, based on DNA sequencing technologies, and seamless integration with the *SPREAD* application from SP4 to assist with outbreak modelling and prediction (applied research)

Subproject 2:

Partner with government, industry, MLA and AHA to:

- Scale out, up and deep the learnings from partnership pilots. Scaling out involves supporting the five pilot groups to increase number of the partnership group members, approaching existing farmer groups to include diverse animal health stakeholders to discuss and work on improving animal health and biosecurity. Scaling up involves industries and government to incorporate the learnings into their policies and programs. Scaling deep involves taking this bottom-up approach to surveillance and focus on building trusting partnership into the culture of the animal health surveillance system.
- Further develop vulnerability mapping BBN model for policy- makers and practitioners for decision making. The dynamic nature of the Bayesian model developed allows for the incorporation of new data to improve accuracy of assessed vulnerability based on farmers' practices and behaviours, which can then be used for more targeted decision making on practising groups and resource allocation for improving surveillance and biosecurity .
- Build livestock systems resilience to disease, drought and other disruptions. Animal diseases interact with increasing impacts of drought and other climatic and non-climatic disruptions on livestock farmers. Preparedness to these interacting challenges requires building resilience in the livestock farming system. Building on the vulnerability framework developed in this project, this research proposal is to partner with farmers to develop evidence-based resilience options and pathways for livestock farmers and value chains.
    - Develop a transdisciplinary approach to feral pig control and African Swine Fever (ASF) preparedness. This transdisciplinary approach could include: Investigate and map trade, customary and social network of potential pathways for introduction and spread of ASF to domestic and feral pig population
    - Assess the social, behavioural and structural vulnerability profiles of high-risk smallholder pig farmers, and communities to ASF to assist with effective communication and trusted partnership for improved surveillance and biosecurity practices
    - Study the implication of complementary and different values assigned to feral pigs by different stakeholders and their implication on effective feral pig management.
    - Explore the potential to expand the role of indigenous communities to assist with risk- and value-driven feral pig management and community-based animal health surveillance and biosecurity

- Develop a monitoring, evaluation and learning (MEL) framework to assess the effectiveness of current activities to mitigate the risks of introduction, establishment and spread of ASF and assist with developing active adaptive management

Subproject 3:

- Update demographic and movement data in AADIS in collaboration with jurisdictional and national veterinary staff
- Application of new AADIS modules to other EADs to support policy development
- Communication of FMD Ready research results to influence revision of international standards and guidelines on efficient and effective post-outbreak surveillance for FMD for earlier return to trade.
- Exploration of implementing surveillance and business continuity measures necessary to improving and defending uninfected areas' market access.
- Determination of economic trade-offs among emergency response resources modelled in AADIS to address questions on what resources are best deployed when and how.
- Potential to further ground-truth the results for additional incursion scenarios, building on existing risk mapping to investigate the potential for the attributes of starting conditions (e.g. seed herd type and size, farm densities) to explain the risks of disease spread. This work would help disease managers better understand how geographic factors affect the risk of disease spread for a larger range of starting conditions and to understand how geographic factors affect the vulnerability of some areas to disease spread.
- Understanding of how we will know when an FMD outbreak is truly over; that is, when it is safe to lift restrictions. Keeping restrictions on longer than necessary will increase costs to industry, while lifting them too early (i.e. before the disease has been truly controlled) runs the risk of new outbreaks. Simulation modelling could be used to explore this issue and support preparation and planning by identifying objective decision-support criteria.
- Build on producer surveillance findings from subproject 2 to more effectively communicate and respond to epidemiological and economic modelling results.

Subproject 4:

- Implement *SPREAD* in the jurisdictions, beginning with WA, including development of a training module, incorporation of property boundary data and its seamless transfer between WA DPIRD and CSIRO networks.
- Extend *SPREAD* to other EADs, especially those which intermittently reoccur in Australia, e.g. highly pathogenic avian influenza, and those which are exotic but which are currently of high risk of incursion e.g. African swine fever and African horse sickness.

9 List of Appendix Files

9.1 Subproject 1

Appendix 3.1: Cross-Protection Induced by a A/MAY/97 Emergency Vaccine Against Intra-Serotype Heterologous Challenge with a Foot-and-Mouth Disease Virus from the A/ASIA/G-VII Lineage.
Appendix 3.2: The new FMD serotype A/Asia/GVII lineage emergency vaccine offers low levels of protection against circulating FMDV A/Asia/Iran-05 lineage strains.
Appendix 3.3: Potential of reduction of AgPath reagent used in FMDV real-time TaqMan assay.
Appendix 3.4: Serological evidence of foot-and-mouth disease infection in goats in Lao PDR
Appendix 3.5: Probe capture enrichment next-generation sequencing of complete foot-and-mouth disease virus genomes in clinical samples.
Appendix 3.6: Inactivation of foot-and-mouth disease virus in epithelium samples for safe transport and processing in low-containment laboratories.
Appendix 3.7: Chemical inactivation of foot-and-mouth disease virus in bovine tongue epithelium for safe transport and downstream processing.

9.2 Subproject 2

Appendix 4.1: Understanding the vulnerability of beef producers in Australia to an FMD outbreak using a Bayesian Network predictive model
Appendix 4.2: Using a Bayesian Network predictive model to understand vulnerability of Australian sheep producers to a foot and mouth disease outbreak
Appendix 4.3: The goat industry in Australia: using Bayesian network analysis to understand vulnerability to a foot and mouth disease outbreak
Appendix 4.4: Durong beef IP: Chain of Response
Appendix 4.5: Esparance Sheep IP: L.I.V.E. – preparedness against disease outbreaks
Appendix 4.6: Biosecurity Fact Sheet: My pig is sick – what should I do?
Appendix 4.7: Maffra Dairy IP: NLIS promotion flyer
Appendix 4.8: Goat Diseases – The Farmers' Guide

9.3 Subproject 3

Appendix 5.1: A simulation study of the use of vaccination to control foot-and-mouth disease outbreaks across Australia.
Appendix 5.2: Comparing surveillance approaches to support regaining free status after a foot-and-mouth disease outbreak.
Appendix 5.3: Economic benefits of implementing trading zones for Australian livestock disease outbreaks of limited duration.
Appendix 5.4: The economic benefits of targeted response strategies against foot-and-mouth disease in Australia.

9.4 General

Appendix 1.1: List of the researchers who participated in the project
Appendix 8.1: FMD Ready Project: Communications, Engagement and Extension Report
Appendix 8.2: FMD ready Project: Monitoring and Evaluation Report
Appendix 8.3: List of all the project publications, conference and meeting presentations and other project outputs