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EuFMD Cascais, Portugal

Foot-and-mouth disease transmission

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Europe is free of foot-and-mouth disease (FMD) without vaccination. However, since 1992, when the EU's non-prophylactic vaccination policy for FMD was implemented, seven minor or major FMD outbreaks were documented within the EU. In recent years, other FMD-free countries, like Korea and Japan, have also experienced outbreaks. This shows that every officially free country is still at risk. For this reason all FMD-free countries have contingency plans that focus on early detection of infected herds and the prevention of further spread.

Experimentally, the within-pen reproduction number is high in pigs and cattle and relatively low in sheep (Eblé and others 2006b; Eblé and others 2008; Orsel and others 2007a, b; Orsel and others 2005). The reproduction number represents the average number of new infections caused by an average infectious individual. It was also shown that separations between pens could limit transmission in pigs (Eblé and others 2006a; van Roermund and others 2010). This supports the notion that transmission between farms depends on the contact structure between the farms. Experimental data can not be extrapolated

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directly to a field situation. Therefore field data are needed to optimise control measures as laid down in contingency plans.

Analysis has shown that there is clear relation between distance and probability of infection (Boender and others 2010; Keeling and others 2001). It should be realised, however, that the underlying mechanisms for this relation are not clear. The spatial analysis shows that some transmissions occur over a considerable distance, this is exemplified in figure 1. In graph A the relationship between distance to an infected herd and the probability of infection is depicted in a situation when there is a stand-still of movement of animals implemented. This graph shows that the farms near to an infected herd have the highest probability of infection. Graph B shows the number of farms in relation to distance, and in this example is based on a uniform distribution of 1 farm per km². Graph C is the result of the product of the first two graphs and shows the average number of new infections caused by one infectious farm in relation to distance. This graph shows that most infections travel a considerable distance (peak around 2 km from the source), and

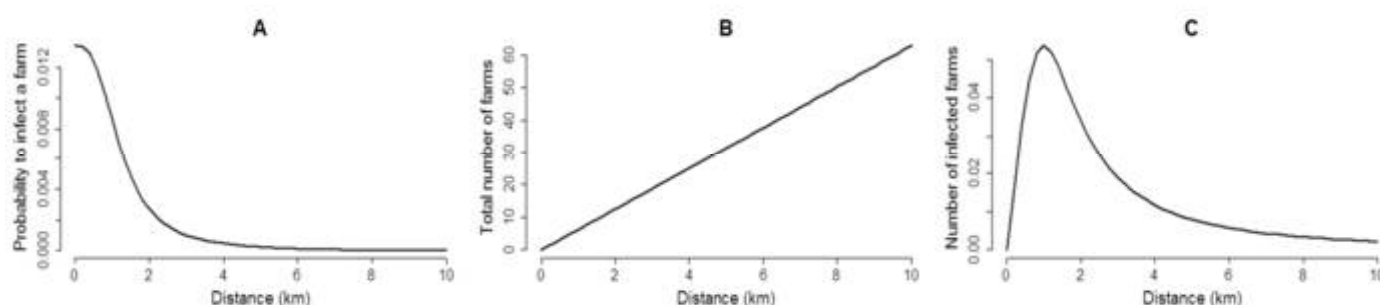


Figure 1: Relation between the probability of transmission and distance (graph A) (Boender and others 2010). Graph B shows the number of herds in relation to distance based on a uniform distribution of farms with a density of 1 farm per km². Graph C shows the consequential average number of infections caused by an infectious farm in relation to distance, which is the product of the graph A and B.

it is therefore unlikely that when transport of animals is not possible, new infections are caused by direct contact between animals on the farms.

So the most likely transmission route is through the gate and not over the fence. Airborne transmission has been described in FMD infection, but when airborne transmission is modelled with mean numbers of virus excretion and susceptibility, and not with the maximum ever found, the probability of airborne transmission over distances more than 1 km is very low. Therefore the observed transmissions over more than 1 km are most likely caused by people.

There is also an indication of increased indirect transmission by humans after implementation of control measures. In the 2001 UK outbreak the estimate of the between farm reproduction number was 3.3 (Woolhouse and others 2001) before control measures were implemented and 1.1 after control measures were implemented. It has been shown in the past that approximately 77% of the known transmission routes were animal transport related (Dijkstra 1955). If we extrapolate this number to the FMD outbreak in the UK in 2001, the ban on animal transport would have resulted in a between farm reproduction number of 0.8 (100 - 77% times 3.3). The fact it was still 1.1 suggests the development new of transmission routes after the implementation of control measures (Although these numbers should be interpreted with care). Illegal movement of animals cannot fully be excluded, but the increase could be also caused by an increased number of people, involved in disease control, moving between farms.

These two examples show that, although we quantified parts of the transmission process, it is still essential to identify specific risk factors for FMD transmission. A case-control study on risk factors for FMD virus transmission was carried out during the 2007 UK outbreak showing that the only significant risk factor was a biosecurity risk score (Ellis-Iversen and others 2011). This biosecurity risk score was composed of various factors such as presence/use of gates/barriers to livestock areas, signs prohibiting entry, boot dips and farm-specific clothing. This study shows the importance of biosecurity and sets an example which is hopefully copied by others.

This text has been modified from a text published before as invited editorial in the Veterinary record (2011; 168: 126-127).

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EuFMD Cascais, Portugal 2016

A prime boost vaccination strategy to prevent serotype O FMDV infection in cattle

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Foot-and-mouth disease remains one of the most economically important infectious diseases of production animals globally. Vaccination can help to control this disease, however, current FMD vaccines are imperfect. They are based on chemically inactivated FMDV that has to be grown on a large scale in cell culture. Virus escapes from vaccine production plants have been a significant cause of FMD outbreaks. The inactivated virus vaccine is also sensitive to heat and confers relatively short-term immunity, especially after only 1 or 2 vaccinations. Thus, there is a need for improved, safe and effective vaccines to control FMD.

The FMDV particles (as present within the current vaccines) comprise a protein shell (the capsid) consisting of 60 copies of 4 different proteins surrounding the RNA genome. Immunity against FMDV infection is generated following vaccination and protection is correlated with the level of anti-FMDV capsid protein antibodies that are present within the sera of animals. There are seven known serotypes of the virus and there is no cross protection between them, thus infection or vaccination against one serotype does not confer protection against virus of another serotype. Serotype O is the most abundant globally followed by serotype A. Within FMDV-infected cells, the FMDV proteins are all produced from a single “polyprotein” that has to be cut by virus-encoded proteases to produce the mature products (plus multiple precursors). If the FMDV capsid protein precursor (P1-2A) is expressed along with the FMDV 3C protease (3Cpro) then production of the

processed capsid proteins can be achieved and these are capable of self-assembling into “empty capsid” particles. These empty capsids are non-infectious but have similar antigenicity as the whole virus. There have been many studies that have achieved the production of FMDV empty capsids within cell culture systems, however these products can suffer from many of the same drawbacks as the inactivated vaccines (although they can be produced outside of high containment).

We have now achieved the expression of FMDV empty capsids within cells using a virus vector system that can infect cells within host animals. This may allow presentation of the FMDV proteins to the immune system in a manner that is closely matched to that achieved during a FMDV infection. We have used a “single cycle” alphavirus expression system that employs a packaged, self-replicating RNA based on Semliki Forest virus (SFV) to express the FMDV capsid precursor P1-2A together with the 3Cpro. It has been shown that the expected FMDV capsid proteins are expressed by these recombinant virus vectors (rSFV-FMDVs) within infected cells, furthermore the processed FMDV proteins were shown to assemble into empty capsids. When cattle were vaccinated once with these rSFV-FMDV vectors then an anti-FMDV antibody response was observed but this was insufficient to confer protection against FMDV challenge. However, it was noted that there was a much higher anti-FMDV antibody response in the vaccinated animals, post challenge, than in the naïve animals, i.e. the animals had been primed to respond to FMDV by the rSFV-FMDV vector. Thus a prime-boost vaccination strategy was designed.

Cattle were vaccinated with the rSFV-FMDV vectors and then boosted (14 days later) with purified empty capsid particles (prepared using a recombinant vaccinia virus expression system). Following challenge with serotype O FMDV the cattle were found to be protected against disease.



These animals had no virus in their blood. High levels of anti-FMDV antibodies were achieved prior to virus challenge. In contrast, unvaccinated animals, or animals that received the same vaccine components in the opposite order, displayed clinical signs of disease following virus challenge and had high levels of virus in their blood. Thus the expression of the FMDV capsid proteins, from the rSFV-FMDV vector within the host animal cells, is clearly important for the priming step of this vaccination strategy. This prime-boost vaccination system, using reagents that can be generated outside of high-containment facilities, offers significant advantages to achieve control of FMD by

vaccination. Further studies are required to determine the duration of immunity achieved by this system.

The results of this study were presented at the EuFMD Open Session meeting in Cascais, Portugal and have recently been published as: Gullberg, M, Lohse, L, Bøtner, A, McInerney, GM, Burman, A, Jackson, T, Polacek, C & Belsham GJ. (2016) A prime-boost vaccination strategy in cattle to prevent foot-and-mouth disease using a “single-cycle” alphavirus vector and empty capsid particles. PLoS ONE 11(6):e0157435. Doi:10.1371/journal.pone.0157435

Ensuring wildlife and livestock coexistence vital for international trade and environmental protection: Southern Africa moves to capitalise on benefits of coexistence of livestock and wildlife


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VICTORIA FALLS, ZIMBABWE (November 15, 2016) — Animal health and wildlife conservation experts from the five-nation Kavango Zambezi Transfrontier Conservation Area (KAZA TFCA) have just concluded a breakthrough meeting on additional, environmentally-friendly ways to manage trade-sensitive animal diseases like foot and mouth (FMD), with an aim towards easing tensions at the livestock-wildlife interface. The more than 500,000 square kilometer KAZA landscape, on the verge of becoming the largest land mass dedicated to wildlife conservation in Africa if not the world, is located in the Okavango and Zambezi river basins and includes, for example, the Chobe National Park, the Okavango Delta (the largest Ramsar site in the world and a World Heritage Site) and the Victoria Falls World Heritage Site. KAZA is also home to spectacular wildlife, including approximately 250,000 elephants, likely more than half of all of the elephants left in Africa. Addressing disease issues of importance to the international trade of beef is critical in order for wildlife and livestock to finally be able to peacefully coexist. The importance of the KAZA transfrontier conservation area to the

region was reaffirmed in August 2011 when the Presidents of Angola, Botswana, Namibia, Zambia and Zimbabwe signed a binding Implementation Treaty legally establishing the transboundary area focused on economic opportunity and peace among nations, all as grounded in the conservation of the region’s extraordinary biodiversity.

Given the importance of both the livestock and wildlife sectors to many countries across the region, southern Africa has been reevaluating how best to manage risks from diseases like FMD, as well as the costs and environmental impacts of various disease management options, including veterinary fences. The primary goal is to help Africa's pastoralists and farmers, while also protecting free-ranging wildlife. Ensuring that beef-importing countries have full confidence that any products they are buying pose minimal threats to their own agricultural sector of course remains of paramount importance.

At a just completed conference in the heart of KAZA organized by the Food and Agriculture Organization of the United Nations (FAO), the KAZA Secretariat, and the Cornell University College of Veterinary Medicine, “**Towards**



Implementation of Commodity-Based Trade of Beef in the KAZA Transfrontier Conservation Area: Opportunities for Integrating Livestock Agriculture & Wildlife Conservation,” southern African and international experts agreed to pilot new approaches to the safe trade of beef and beef products based on the meat production process itself (aka- “commodity-based trade”), rather than solely on livestock’s geographic origin as delineated by fencing. By applying systems already widely used to ensure that food is safe for human consumption – and by focusing on straightforward livestock management principles, meat hygiene, and quality processing – beef and related products free of animal diseases of concern can be produced.


Moving this new approach forward would mean that the poorest livestock farmers living closest to wildlife are no longer excluded from global beef markets, and veterinary cordon fencing, often devastating to migratory wildlife, is no longer the only option for managing foot and mouth disease in southern Africa. The KAZA Transfrontier Conservation Area is thus approaching a critical turning point in regards to resolving the more than half century-old conflict between (a) international beef trade policy based on foot and mouth disease control fencing in the southern African context and (b) the migratory needs of free-ranging wildlife in the region and beyond.

“With colleagues from KAZA countries adopting commodity-based trade as an additional regional standard as per new 2015 guidelines from the OIE [Office International des Epizooties – the World Organization for Animal Health], the door is open to a truly win-win opportunity both for livestock farmers and for tourism and related industries involved with transfrontier conservation areas or ‘peace parks,’” noted Professor Steve Osofsky of Cornell University’s College of Veterinary Medicine and the AHEAD (Animal & Human Health for the Environment And Development) Program. “If livestock agriculture is no longer solely dependent on veterinary fencing to deal with foot and mouth disease, then KAZA’s vision for the restoration of major movement corridors for the region’s spectacular wildlife, including the world’s largest remaining population of

elephants, indeed has a chance of being realized. In addition, with commodity-based beef trade and the local value-added processing it encourages, livestock farmers previously excluded from accessing markets may for the first time be able to find traction in the wider regional economy, and beyond,” Professor Osofsky added.

“There is still a lot of groundwork to be laid to optimize regional land-uses so that transfrontier conservation and livestock agriculture can literally find common ground in the interest of regional economic development underpinned by earnest environmental stewardship” noted Dr. Patrick Otto, the UN Food and Agriculture (FAO) Head of Livestock Development for Southern Africa. “Over time, as the region gets more experience with commodity-based trade, we hope southern Africa will be able to seize upon the socioeconomic as well as conservation opportunities offered by SADC’s collective vision for transfrontier conservation areas as enabled by strategic realignment of selected veterinary cordon fences. At the same time, commodity-based trade should facilitate expansion of livestock farmers’ access to regional and global markets based on additional, practical disease control policy options,” added Dr. Otto. Meeting co-organizer Dr. Morris Mtsambiwa, Executive Director of the KAZA TFCA Secretariat, noted “We are so pleased to have the FAO, Cornell University, and the AHEAD Program join the KAZA Secretariat to support this week’s milestone planning meeting, as we all share in the belief that sustainable development and environmental conservation are in fact inextricably linked, especially when it comes to improving the lives and livelihoods of communities across KAZA.”

The Animal & Human Health for the Environment And Development (AHEAD) Program of Cornell University’s College of Veterinary Medicine is a convening, facilitative mechanism, working to create enabling environments that allow different and often competing sectors to literally come to the same table and find collaborative ways forward to address challenges at the interface of wildlife health, livestock health, and human health and livelihoods. AHEAD convenes stakeholders; helps delineate conceptual



frameworks to underpin planning, management and research; and provides technical support and resources for projects stakeholders identify as priorities. *AHEAD*, launched in 2003 as one of the world's first applied One Health programs, recognizes the need to look at health and disease not in isolation but within a given region's socioeconomic and environmental context.

Achieving food security for all is at the heart of FAO's efforts – to make sure people have regular access to enough high-quality food to lead active, healthy lives. Our three main goals are: the eradication of hunger, food insecurity and malnutrition; the elimination of poverty and the driving forward of economic and social progress for all; and, the sustainable management and

utilisation of natural resources, including land, water, air, climate and genetic resources for the benefit of present and future generations. The Kavango Zambezi Transfrontier Conservation Area, or KAZA TFCA, is potentially the world's largest conservation area, spanning five southern African countries; Angola, Botswana, Namibia, Zambia and Zimbabwe, centered around the Caprivi-Chobe-Victoria Falls area. The goal of the KAZA TFCA is "To sustainably manage the Kavango Zambezi ecosystem, its heritage and cultural resources based on best conservation and tourism models for the socio-economic wellbeing of the communities and other stakeholders in and around the eco-region through harmonization of policies, strategies and practices."

Ongoing studies of FMDV persistence in cattle at Plum Island Animal Disease Center

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Recent and ongoing research projects at the Foreign Animal Disease Research unit at Plum Island Animal Disease center (USDA-ARS) have focused on investigating the FMDV carrier state using a multifaceted approach. Preliminary work included validation of a novel challenge system (1) and characterisation of early infection (2). Subsequent studies were aimed at investigating anatomic sites of virus persistence, characterising patterns of virus shedding throughout different phases of infection, as well as determining how these parameters were influenced by vaccination (3). Continued work has focused on characterising how distinct aspects of the host immune response are associated with clearance versus persistence of FMDV (3-5) as well as investigating potential associations between specific alterations of the FMDV genome and the FMDV carrier state.

Background

The carrier state of FMD continues to be a determinant of aggressive control policies in

response to FMD outbreaks that occur in countries that are previously free of the disease. Despite having been a focus of substantial research efforts spanning multiple decades, the mechanisms involved in establishment and maintenance of persistent subclinical FMDV infection in ruminants have not been fully elucidated. Similarly, there is still less than complete clarity regarding the potential roles of the FMDV carrier state in FMD endemicity.

The FMDV carrier state was initially described by J.G. van Bakkum et al in 1959 (6). Continued investigations by P. Suttmoller and co-workers led to the currently accepted definition of FMDV persistence characterised by FMDV detection in oropharyngeal fluid (probang samples) beyond 28 days post infection (7, 8). The first experimental investigation of the pathogenesis of FMDV persistence in bovine tissues was carried out by R. Burrows, who described localisation of persistent FMDV to the mucosa of the dorsal soft palate and dorsal nasopharynx (9). Interestingly, although science and scientific methodology have substantially progressed since these works were published during the 1960s, much of what we know about the FMDV carrier state in cattle is still derived from these distinguished publications.

Methods and results

The overarching objective of the current projects was to investigate the pathogenesis of FMDV persistence in vaccinated and non-vaccinated cattle, with specific emphasise on elucidating factors associated with the divergence between animals that develop persistent infection (“carriers”) and those that clear the infection (“terminators”). Animals included in the investigations were Holstein cattle of approximately 200kg that were either naïve or vaccinated using a recombinant adenovirus-vectored vaccine. All vaccinated cattle (n=25) were completely protected against clinical FMD, whereas non-vaccinated cattle (n=21) developed moderate to severe FMD after intra-nasopharyngeal inoculation (1) with FMDV A24 Cruzeiro.

Analysis of an extensive set of tissue samples from diverse anatomic sites during early phases of infection confirmed that the nasopharyngeal mucosa constitutes the site of primary FMDV infection in both vaccinated and naïve cattle (2) (Figure 1).

Further investigations using multi-channel immunofluorescence (MIF) and laser-capture microdissection (LCM) localised this early infection to follicle-associated epithelium of the dorsal nasopharynx and dorsal soft palate. Local, subclinical, infection of the upper respiratory tract was confirmed in the majority of vaccinated animals despite absence of clinical signs of infection. Primary infection of vaccinated cattle was associated with early activation of the local innate immune response characterised by increased expression of interferon (IFN) mRNA and marked influx of antigen-presenting cells (MHC II+/CD11c+). Contrastingly, in naïve cattle, these aspects of the host response were not detected until the occurrence of viremia and generalisation of infection (2). Viremia was associated with a strong systemic IFN response in non-vaccinated cattle, whereas neither viremia nor systemic IFN induction were detected in vaccinated cattle (4). Virus challenge of non-vaccinated cattle induced a decrease in circulating lymphocytes (relative lymphopenia).

Contrastingly, a transient increase in monocyte

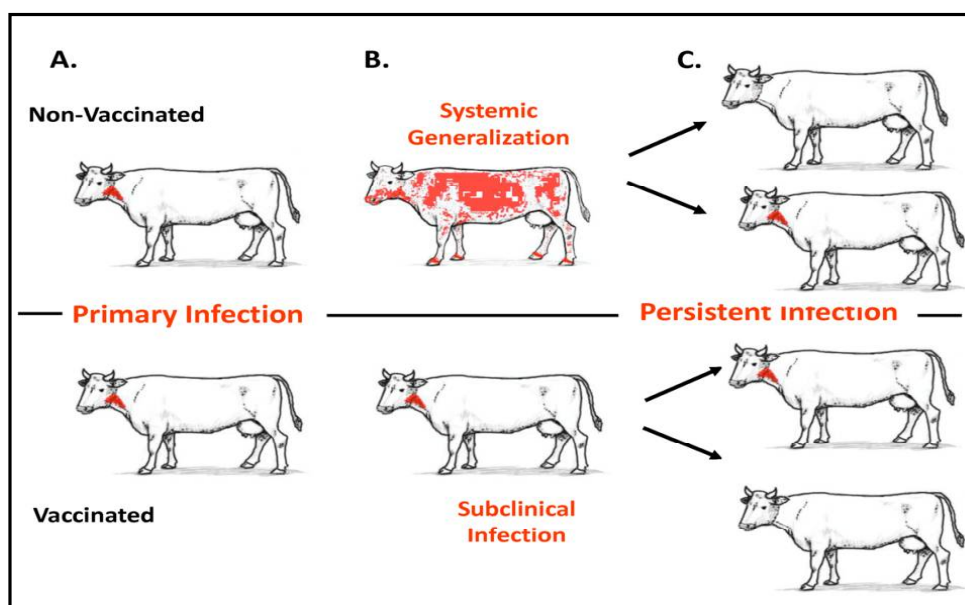


Figure 1: Divergence between FMDV carriers and terminators

Temporo-anatomic progression of FMDV infection in non-vaccinated (top row) and vaccinated (bottom row) cattle. **A)** The primary infection is localised to distinct segments of epithelium of the nasopharyngeal mucosa. The distribution and quantity of virus during primary infection is similar in naïve and vaccinated cattle. **B)** In non-vaccinated cattle, there is a systemic generalisation of FMDV. The clinical phase of infection is characterised by high-titre viraemia and vesicular lesions at sites of secondary viral replication. In vaccinated cattle that are protected against clinical FMD, the infection remains restricted to the nasopharyngeal mucosa. **C)** After resolution of the clinical phase of disease, there is a similar divergence (both vaccinated and non-vaccinated cattle) between animals that clear infection and those that maintain persistent FMDV infection. In carrier cattle, the anatomic distribution of infectious FMDV is again restricted to the nasopharyngeal mucosa.

and lymphocyte counts occurred concurrent with virus challenge (4).

The prevalence of FMDV persistence was similar in both groups of animals (62% vaccinated; 67% non-vaccinated) despite vaccinated cattle having been protected from clinical disease. Vaccinated cattle shed lower quantities of FMDV in oral and nasal secretions during the early phase of infection. However, there was no difference between the groups in quantities of FMDV detected in probang samples during persistent infection (3). Continuous monitoring of detection of FMDV in probang samples suggested that the divergence between cattle that cleared infection and those that developed persistent infection occurred earlier than previously recognised. Specifically, in vaccinated cattle, all animals that cleared infection were probang-negative by 10 days post challenge (dpc) whereas FMDV detection in probang samples from persistently infected cattle was consistent through 35 dpc. In non-vaccinated cattle, this divergence had occurred by 21 dpc (3).

Similar to the early phase of infection, persistent FMDV was localised to follicle-associated epithelium of the nasopharyngeal mucosa (Figure 1). Both structural and non-structural FMDV proteins were detected within segments of this highly specialised epithelium that directly overlies follicles of nasopharyngeal-associated lymphoid tissue (NPALT; Figure 2)(3). FMDV genome was detected within follicle associated epithelium as well as in the subjacent lymphoid follicles, but there was no detection of non-structural FMDV protein within lymphoid tissue. Interestingly, in non-vaccinated terminators, there was an increased expression of IFN- λ mRNA in follicle-associated epithelium, and reduced expression of IL10 in submucosal lymphoid follicles during what was defined as the transitional phase of infection (10-21 dpc; corresponding to the established time frame of virus clearance within this cohort). In contrast to this, there was a negative association between FMDV genome quantities and mRNA levels of anti-viral cytokines (IRF-7, CXCL10, IFN- γ , IFN- λ) in LCM-processed samples of nasopharyngeal mucosa during FMDV persistence.

To further investigate the role of the host immune response in development of persistent FMDV infection, a comprehensive investigation of potential modulation of the local host response to infection was carried out using a bovine whole-transcriptome microarray (5). Samples of nasopharyngeal mucosa from cattle defined as either FMDV carriers or non-carriers were analysed for the purpose of identifying modulation of the local immune response associated with FMDV persistence versus clearance. It was concluded that genes associated with induction of regulatory T cells and production of prostaglandin E2 were overexpressed in tissues from FMDV carriers. In contrast, tissues from non-carrier animals expressed higher levels of complement regulators and pro-apoptotic genes that could promote virus clearance (5).

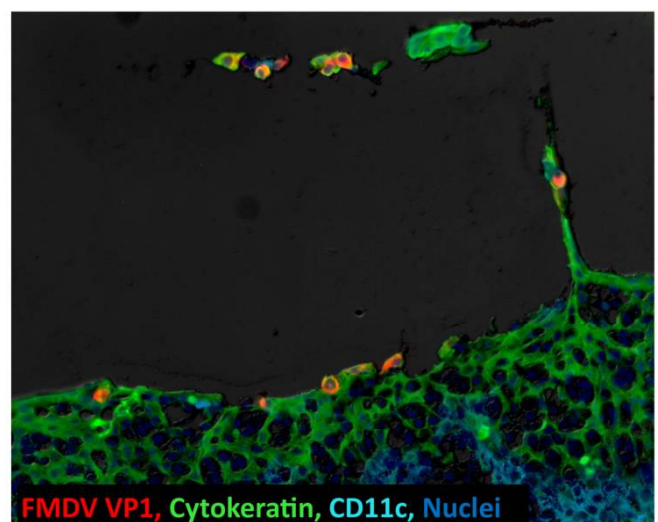


Figure 2: FMDV shedding within infected nasopharyngeal epithelial cells

Persistent FMDV infection in lymphoid-associated epithelium of the bovine nasopharynx. FMDV antigen (red) localised to cytokeratin⁺ (green) epithelial cells of the dorsal soft palate at 35 days post challenge of a steer infected with FMDV A24 Cruzeiro. CD11c⁺ (aqua) antigen-presenting cells are found subjacent and interspersed within epithelium. Some FMDV-infected epithelial cells have sloughed from the mucosal surface (released into secretions). Multi-channel immunofluorescence microscopy. 20x magnification. Mouse monoclonal antibodies: Anti FMDV-VP1 (red) and anti-bovine CD11c (aqua). Rabbit polyclonal anti-pan cytokeratin (green). Nuclear counterstain (dark blue). *This image is reproduced from an open access article distributed under the terms of the creative commons attribution 4.0 international license: Copyright (C) 2016 Stenfeldt et al Journal of Virology 90 (14) pp6344-6364. DOI: 10.1128/JVI.00388-16.*

Discussion

A novel, standardised experimental model was applied to a series of in-vivo experiments for the overarching objective of establishing a comprehensive and detailed overview of FMDV pathogenesis in cattle spanning from early to persistent phases of infection.

Initial investigations were consistent with previous works (10-12) in confirming the bovine nasopharynx as the site of primary and persistent FMDV infection in cattle. Additionally, it was demonstrated for the first time that primary (subclinical) infection of vaccinated cattle was highly similar to primary infection of non-vaccinated animals. While non-vaccinated cattle developed clinical disease with viremia and dissemination of virus in tissues, infection in vaccinated cattle remained restricted to the nasopharyngeal mucosa (Figure 1). Beyond resolution of clinical FMD in the non-vaccinated cohort, anatomic distribution of virus was again similar in the two cohorts. Throughout the investigations, it was concluded that differences in the host response to infection during early infection were dictated by the animal's vaccination status, whereas differences detected during late infection were instead associated with the divergence of FMDV persistence versus clearance.

Our finding of persistent FMDV localised to distinct segments of epithelium within the bovine nasopharynx differs slightly from previous works that have reported localization of persistent FMDV to lymphoid tissue (13, 14). However, these distinct findings may not be mutually exclusive and continued investigations are warranted to further clarify relative contributions of lymphoid tissue and associated epithelium in establishment and maintenance of persistent FMDV infection.

FMDV detection in probang samples was consistent in cattle that developed persistent infection, regardless of vaccination status. In contrast to this, clearance of virus in the non-carrier cohort occurred earlier than previously defined (10 dpc in vaccinates and 21 dpc in non-vaccinates). This finding is critical for continued research aiming to identify factors associated with

successful clearance of FMDV from the bovine upper respiratory tract. We hypothesize that the specific virus-host interactions which define persistence occur within this (transitional) temporal window in which divergence occurs. Further investigations of gene expression in nasopharyngeal tissues using whole-transcriptome microarray suggested that FMDV persistence is associated with an impaired cellular anti-viral response in infected tissues.

Ongoing efforts include in-depth investigations of host factors associated with clearance versus persistence of FMDV during the period defined as the transitional phase of infection. Additionally, continued work to elucidate the interplay between nasopharyngeal epithelial cells and associated lymphoid tissue is under way.

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Immunological and vaccine formulation parameters affecting heterologous protection for FMD vaccines in cattle

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Background

The wide antigenic diversity of the FMDV may affect the efficacy of vaccine-based strategies during incursions of the virus, due to the poor cross-protection among different strains, even within the same serotype. For this reason, it is essential to understand the mechanisms and variables underlying the generation of protective responses by FMD vaccines against virus strains not included in the formulation. In this work, we assessed the effect of antigen payload, revaccination or inclusion of multiple strains for FMD oil vaccines applied to cattle further challenged with a heterologous strain. The experimental design was based on the A24/Cruzeiro, a commonly used FMD vaccine strain, and the A/Arg/2001 strain, one of the strains responsible for the FMD outbreaks in Argentina during 2000 and 2001. The poor antigenic relatedness between these two strains was previously described *in vivo* (56% to 25% of protection in PPG tests for cattle vaccinated with A24/Cruzeiro and challenged with A/Arg/2001) as well as *in vitro*, with r_1 values <0.2 for VNT and <0.3 for LP-ELISA.

Experimental Design

Three experimental vaccines were formulated as water-in-oil emulsions, containing 10 µg of inactivated A24/Cruzeiro (low payload monovalent), 40 µg of inactivated A24/Cruzeiro (high payload monovalent), or 40 µg of inactivated FMDV, combining 10 µg of A24/Cruzeiro, 10 µg of C3/Indaial and 20 µg O1/

Campos (trivalent). Groups of steers (n=5 each) were immunized with (1) one dose of the low payload monovalent vaccine; (2) one dose of the high payload monovalent vaccine; (3) one dose of the trivalent vaccine, or (4) two doses of the low payload monovalent (at 0 and 15 days post-vaccination, dpv). Two unvaccinated steers were used as negative controls. Vaccines were applied intramuscularly and serum and whole blood samples were obtained on weekly bases until challenge at 30 dpv. Serum samples were assayed for total antibodies (liquid-phase blocking ELISA, LPBE), neutralising antibodies (virus neutralisation test, VNT), avidity index (AI, avidity ELISA) and IgG1/IgG2 titres (isotype indirect ELISA) specific for the A/Arg/2001 strain. Whole blood samples were assessed for IFN-γ production specifically induced against the homologous (A24/Cruzeiro) or heterologous (A/Arg/2001) strains.

Experimental challenge was performed using the A/Arg/2001 strain (10^4 TCID₅₀) applied by the intradermolingual route. After challenge, clinical FMD symptoms were daily followed for a week; serum and whole blood samples were also obtained every 24 h. Clinical scores were established for each individual based on the detection of hyperthermia or blistering /lesions in feet.

Results

The evaluation of different immunological responses against the heterologous strain just prior to the experimental challenge, revealed few significant differences among groups. Total antibody titres against A/Arg/2001 measured by LPBE only showed statistical differences between group 4 (revaccinated) and group 1 (low payload monovalent vaccine; group 4>group 1, $p<0.05$). Neutralising antibody titres against the A/Arg/2001 strain showed additional differences: group 4 was higher ($p<0.001$) than group 1 and group 3 (trivalent vaccine), and group 2 (high payload monovalent vaccine) was higher than groups 1 ($p<0.01$) and 3 ($p<0.001$). No statistical

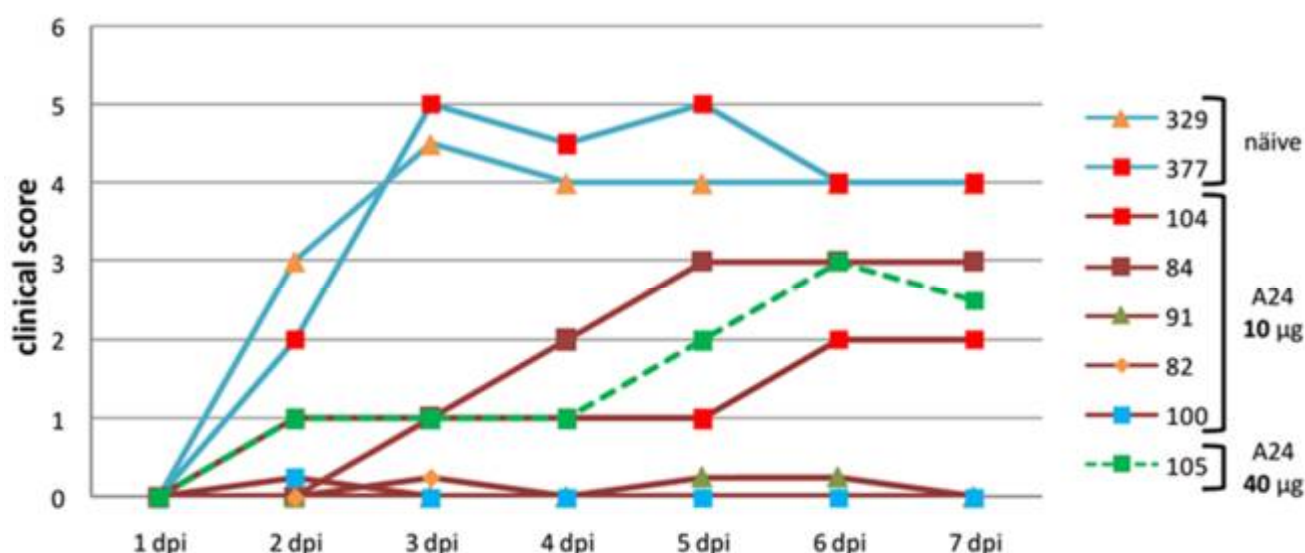


Figure 1: Clinical scores from steers after heterologous challenge. Animals without any FMD-compatible symptoms were excluded.

differences were found among groups for A/Arg/2001-specific IFN- γ production, IgG1/IgG2 titres, or AI.

Starting two days after challenge, FMD clinical signs were detected in animals from groups 1 (2 out of 5) and 2 (1 out of 5). Both groups received a single dose of the monovalent formulations. These 3 non-protected animals showed less severe and more delayed clinical signs than naïve controls (fig.1). The rest of the animals from the A24 10 μ g vaccination group showed at least 1 one day with a slight hyperthermia during the week following the challenge.

Challenge results are summarised in table 1. Our observations are in line with previous reports for vaccine matching assays with the same strains (10 μ g A24/Cruzeiro oil vaccine, single dose), which showed between 56% and 25% of protection after challenge with the A/Arg/2001 strain at 30 dpv. Analyses of VNT titres, LPBE titres and AI against the A/Arg/2001 strain for individual samples

Table 1: Protection observed in all experimental groups after challenge with A/Arg/2001 strain.

Experimental group	# protected animal /total
A24 10 μ g	3 / 5 (60%)
A24 40 μ g	4 / 5 (80%)
A24 10 μ g x2	5 / 5 (100%)
A24/C3I/O1C	5 / 5 (100%)
PBS	0 / 2 (0%)

revealed some differences among these three assays. According to VNT or LPBE assays, all three non-protected animals showed titres above those established for 75% of expected protection (EPP75) for these assays. Consequently, VNT or LPBE mean titres were not statistically different between non-protected and protected animals. On the contrary, the AI from the three non-protected steers were the lowest observed, and the mean AI from these animals was significantly lower than that of the protected individuals ($p < 0.01$, fig.3).

Conclusions

Only experimental groups receiving two doses of the low payload monovalent vaccine or a single dose of the trivalent vaccine were fully protected against FMD-generalisation clinical signs after the heterologous challenge. This may indicate that revaccination and antigenic diversity could favour the response against heterologous FMDV strains. On the contrary, both monovalent formulations, either with low or high antigenic payloads, failed to protect at least one animal of the group, in line with previously reported results.

Although no statistical differences were found among groups for many of the immunological parameters analysed, significant differences found for A/Arg/2001-specific VNT titres indicated that

revaccination could effectively boost ($p < 0.001$) mean neutralizing antibody titres in this group compared to those immunized only once with vaccines containing 10 µg of A24/Cruzeiro (low payload monovalent and trivalent formulations). However, neither VNT nor LP-ELISA titres could show a clear correlation with the protection against the heterologous infection. While all non-protected animals were above EPP75% cut-off values for these assays, five animals, which showed VNT or LPBE titres below the EPP75% threshold, resulted protected against the experimental heterologous challenge. AI analyses, though, indicate that non-protected animals

showed the lowest values of all. Hence, determination of the avidity of sera against the heterologous strain (A2001) was the only assay capable of differentiating protected from non-protected animals. While these studies should be extended to other serotypes and strains, our results highlight the value of incorporating new assays when analysing the protection in a heterologous setup, as well as experimenting with new formulations and vaccination schedules which may broaden the antigenic spectrum of the immunity induced.

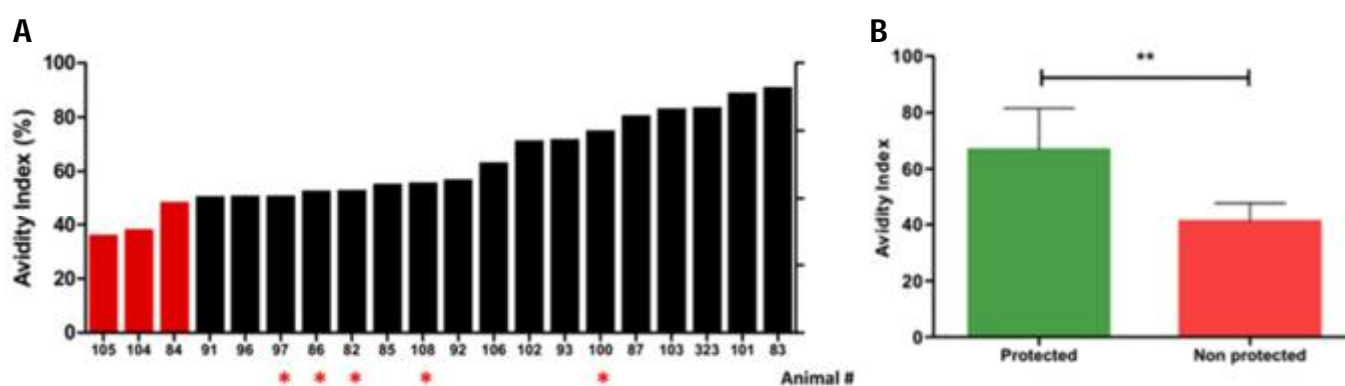


Figure 3: (a) Individual animals ordered according to AI values. Red bars correspond to those steers not protected against the A/Arg/2001 challenge. Red asterisks indicate individuals which showed VNT or LPBE titres below the EPP75% threshold for these assays. (b) Statistical differences in AI values between protected and non-protected cattle ($p < 0.01$).

Genetic characterisation of foot-and-mouth disease viruses circulating in Balochistan, Pakistan

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Foot-and-mouth disease is endemic in Pakistan and three serotypes (and multiple sub-types within each serotype) of FMDV are involved in outbreaks. Continuous surveillance of the disease is essential for determination of subtypes and selection of appropriate vaccine strains for control of the disease. Pakistan is currently in stage 2 of the FAO/EuFMD/OIE progressive control pathway for FMD (FMD-PCP). Characterisation of FMDVs circulating in

Balochistan Province of Pakistan, the largest province in the country sharing borders with Iran and Afghanistan, was presented by Syed M. Jamal as oral presentation in the Open Session 2016 of the Scientific and Technical Committee of the European Commission for the control of foot-and-mouth disease (EuFMD), held in Cascais, Lisbon, Portugal during November 25-28, 2016. Three different sublineages within A-Iran05 strain, including two previously described and one new sublineage and Sindh-08 (Group-VII) strain of FMDVs were found circulating in the region.

Participation of Syed M. Jamal was supported by GFRA.

In vitro antiviral efficacy of Ribavirin on foot-and-mouth disease virus replication

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Introduction

Foot-and-mouth disease is endemic in India and it is caused by serotype O, A and Asia1 viruses. In enzootic countries like India, mass vaccination of susceptible livestock is the method of choice to build up herd immunity and for ultimate control and eradication of FMD. However, in the absence of movement control of animals between territories, and other difficulties associated with complete vaccination coverage, the disease still causes high morbidity and mortality not only in domestic animals but also in captive wild animals and in natural settings. In these situations, emergency ring vaccination is practiced to control further spread of virus. However, these vaccines are only able to induce complete clinical protection 4–7 days post vaccination. This results in a window of susceptibility in unexposed animals and animals exposed to infection may succumb to the disease. The other possible alternatives would be the use of antiviral agents that inhibit FMDV replication. Therefore in this present study, we aimed to explore the *in vitro* antiviral efficacy of ribavirin by the way of their cytopathic effect (CPE) inhibition in a BHK21 cell culture system at different time periods against FMD virus serotypes O, A and Asia1.

Methodology

Maximum Non Toxic Concentration (MNTC) of Ribavirin in BHK-21 cells

50 µL of 2-fold serially diluted ribavirin from 500–0.97 µg/50µL, was added in duplicate wells of 96 well TC plates containing confluent layers of BHK-21 Cells. After 24 hours and 48 hours of incubation, the cytotoxicity was assessed by visual

observation under light microscope and 0.4% trypan blue dye exclusion method.

CPE inhibition assay for Ribavirin

Three different concentrations (5, 10, 15µg /50µL) of ribavirin below MNTC, were tested against three serotypes of FMDV in 96-well TC microtitre plates containing confluent monolayers of BHK-21 cells. The supernatants were sampled at 6, 12, 24 and 48 hrs (For FMDV O) and 24 and 48 hrs (For FMDV-A and FMDV-Asia 1). Three controls including the drug ribavirin, virus and cell were also included in the assay. The titre of each serotype of FMDV was calculated at different time intervals against each concentration of ribavirin. The titre was compared with the virus control and subsequently reduction in titre was estimated. Percentage of inhibition of ribavirin against all three serotypes of FMDV was calculated using the formula: Percent Inhibition (PI) = [1- (Antilog test titre/Antilog Control titre)] × 100.

Detection of FMDV O viral antigen/nucleic acid in ribavirin treated timed samples

The samples that were scored negative in CPE inhibition assay were further tested for the presence of viral antigen with serotype-specific sandwich ELISA. The samples which gave OD more than the blank cut-off OD were scored as positive and percentage inhibition was calculated. The 6, 12, 24 and 48 hr samples which were negative in sandwich ELISA, were further tested by PCR using serotype specific primers (Giridharan *et al* 2005). The 24 and 48 hr samples that were negative in gel-based PCR, were further tested by SYBR green based real-time PCR.

Results and Discussion

Maximum Nontoxic concentration (MNTC) of Ribavirin

Wells treated with ribavirin at a concentration of

15.62 µg/50µL showed 66.47% and 52.59 % cell viability at 24 hr and 48 hr post drug treatment, respectively, compared to the cell control. The cell viability increased as the concentration of the drug was decreased.

At 2 and 4 hpi, little CPE could be observed in ribavirin treated cells with all three drug concentrations tested. It was difficult to detect CPE as early 4 hpi, as a single replication cycle of FMDV takes approximately 5–6 hrs (Grubman and Baxt, 2004). However, at 6 hpi the CPE was evident in the virus control wells, but the titre was less compared to the titre of virus with ribavirin. The absence of virus induced CPE in ribavirin treated cells indicate that the ribavirin can reduce virus replication during the initial stages, and continued availability of the drug may result in reduced virus secretion in infected animals and thereby reduce the transmission events. The inhibitory effect of ribavirin on FMDV was proved authentically at 12, 24 and 48 hrs post treatment as shown by the high percent inhibition of CPE estimated by cell bio-assay, sandwich ELISA, RT-

PCR and real-time PCR. Ribavirin at a concentration of 15 µg/50 µL was found to inhibit FMDV serotype O, A and Asia 1 to an extent of 99% at 48 hr post treatment. The results shown validate the earlier reports of ribavirin on the FMDV replication *in vitro* and *in vivo* (De la Torre *et al.*, 1987; Goris *et al.*, 2007). The inhibitory concentrations of ribavirin against other viruses are also shown to be in the range of concentrations tested against FMDV.

In summary, we successfully applied a BHK21 cell model for evaluating the anti-FMDV activity of ribavirin. The concentration of ribavirin tested in this study was effective in inhibiting the *in vitro* replication of FMDV O, A and Asia 1. However, it is required to be tested in *in vivo* models such as suckling mice, guinea pigs, pigs and cattle. Therapeutic application of the drug would be useful in treating FMDV infected animals and also prevent the spread of the infection across the herd.

Table 1: Antiviral activity of ribavirin against FMDV O (virus titres in terms of TCID₅₀/ml) at different times. Samples assessed using cell bio-assay, sandwich ELISA, PCR and SYBR Green based real-time PCR

Time of Harvest (hrs post treatment)	Ribavirin concentration (µg/50µL)				Ribavirin concentration (µg/50µL)			
	5	10	15	Virus Control	5	10	15	Virus control
	Log ₁₀ TCID ₅₀ / mL (Cell Culture)				Log ₁₀ TCID ₅₀ / mL (Sandwich ELISA)			
6	0	0	0	3.80	4.80	4.80	4.55	4.80
12	3.80	3.80	2.80	5.80	ND	ND	ND	ND
24	6.05	4.80	3.80	6.80	7.55	6.55	4.80	7.80
48	5.80	4.80	4.05	7.80	6.80	5.80	5.80	7.80

Time of Harvest (hrs post treatment)	Ribavirin concentration (µg/50µL)				Ribavirin concentration (µg/50µL)			
	5	10	15	Virus Control	5	10	15	Virus Control
	Log ₁₀ TCID ₅₀ / mL (PCR)				Log ₁₀ TCID ₅₀ / mL (Real Time PCR)			
6	5.80	5.63	5.55	5.80	ND	ND	ND	ND
12	7.80	6.93	5.80	7.80	ND	ND	ND	ND
24	7.55	7.55	6.80	7.80	7.80	7.05	6.80	7.80
48	7.93	7.55	7.55	7.80	7.80	7.80	6.80	7.80

ND - Not done

ANSES, Animal Health Laboratory – Maisons-Alfort France

Labib Bakkali-Kassimi and Stéphan Zientara

Inauguration of new FMD Laboratory “ICube”

13th of October 2016: the new laboratory facility “ICube” of the Anses Animal Health Laboratory of Maisons-Alfort (France) has been inaugurated by M Stéphane Le Foll, French Minister of Agriculture, Valérie Pécresse, President of the Regional Council of Île-de-France and Dr Monique Eloit, Director General of OIE.

This facility is divided into two BSL3 laboratories: one dedicated to FMDV manipulation (about 300 m²), according to EuFMD minimum standard, and another one (300 m² also) dedicated to handling zoonotic viruses (West Nile, Japanese encephalitis, Hepatitis E, etc.) and includes an insectarium.

This new facility will offer the Anses Animal Health Laboratory of Maisons-Alfort (which is an OIE reference lab for FMD) a new modern tool to work and to develop research projects on FMDV, meeting the international standards on biosafety and biosecurity.



92nd Executive Committee Meeting of the EuFMD



92nd EuFMD Executive Committee meeting

26th and 27th of September, 2016: the Anses Animal Health Laboratory of Maisons-Alfort hosted the 92nd Executive Committee of the EuFMD.

Other information:

From the 30th of November to the 2nd of Dec 2016, the Anses Animal Health Laboratory of Maisons-Alfort will organise the annual OIE/FAO FMD Reference Laboratory Network meeting.

The OIE twinning project on FMD and other transboundary animal diseases between Mongolia and Japan

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The National Institute of Animal Health (NIAH) is the only institution providing definitive diagnosis of FMD in Japan, authorised by the Ministry of Agriculture, Forestry and Fisheries of Japan, and has been designated as a collaborating centre on diagnosis and control of animal diseases and related veterinary product assessment in Asia by the World Organization for Animal Health (OIE). The NIAH also serves as reference laboratories of the OIE for several transboundary animal diseases (TADs) and contributes significantly to animal health in Asian countries.

For enhancement of control of TADs in the East Asian region, in July 2015 the NIAH proposed a twinning project to the OIE in order to improve diagnostic techniques for TADs, including FMD, in the State Central Veterinary Laboratory (SCVL) of Mongolia. The proposition was accepted by the OIE Biological Standards Commission in September, 2015. A contract for the twinning project was completed between the SCVL and NIAH in December, 2015. The SCVL can currently diagnose FMD by molecular and serological methods; however, virus isolation using cell culture and serotyping of a causal FMD virus in an outbreak, which is necessary for effective control of FMD using a vaccine, are not performed. Nucleotide sequencing and analysis of viral genomes for molecular epidemiological analysis of viruses and pen-side diagnostic methods are not also applied sufficiently in the SCVL. Furthermore, establishment of an appropriate transportation system of clinical samples from an outbreak site to a laboratory, and application of anatomical and pathological diagnoses of TADs which may occur in Mongolia in the future are strongly required. In the twinning project, the

NIAH will receive 4 staff of the SCVL as trainees between January, 2016 and December, 2018, and give them training on the abovementioned techniques in Japan. In addition, the NIAH will dispatch 10 staff of the NIAH to Mongolia and aim to establish the techniques in Mongolia by running workshops.

A meeting on control of FMD was held in Mongolia in 2010, and Mongolian and Japanese experts actively discussed control measures of FMD in both of the countries. A staff member of the SCVL received training between May and November, 2012, at the NIAH in order to acquire skills in basic diagnostic methods of FMD under the OIE/JTF project for FMD control in Asia. Several meetings on control of FMD were also held in Mongolia under the same project in 2013. Many researchers and government officers in East Asian countries participated in the meetings and discussed the control of FMD in the East Asian region. The past activities in both countries are important bases for implementation of the twinning project.

With the twinning project it will be possible to improve diagnostic systems and to perform appropriate control measures to TADs including FMD, and as a result, reduce economic losses from outbreaks of such diseases in Mongolia. In addition, the twinning project will lead to the reduction of outbreaks of TADs around the East Asian region. Furthermore, in the future it is desirable that the SCVL will be able to facilitate diagnosis and control of TADs, including FMD, in the East Asian region.



Group photo from pre-training of the OIE twinning project held in the SCVL in April, 2016

CODA-CERVA, Belgium

EuFMD Open Session – Cascais, Portugal (October 25th–28th 2016)

At the EuFMD Open Session in Portugal, scientists from the CODA-CERVA were involved in 3 oral presentations and 3 poster presentations, which are summarised below.

ENCOURAGING THE USE OF VACCINATION-TO-LIVE AS A CONTROL STRATEGY FOR FMD OUTBREAKS: PERSPECTIVES AND ISSUES (Ryan E. et al.)

Vaccination-to-live as a control strategy in countries previously free of FMD without vaccination is complicated by uncertainties surrounding the impact of this policy to regain official OIE FMD-free status and the implications for access to markets. Across Europe, public tolerance for culling has decreased. Countries using sustainability as a part of their marketing strategy are less likely to use vaccination-to-kill as a control strategy. The likelihood of a successful vaccination campaign is increased if vaccination is started promptly; constraints to the use of vaccination-to-live should be identified in advance and addressed. It is important to convince large food retailers that there is no impediment to the marketing and consumption of meat/milk from vaccinated animals. Bringing stakeholders into the discussion builds awareness of the implications of vaccinating or not vaccinating, increasing the likelihood of consensus in the face of an outbreak. Specific guidelines for data interrogation for NSP surveys are needed, especially in relation to spatial clustering and the extent of positivity. A six month waiting period following vaccination-to-live does not necessarily provide more confidence in disease freedom compared with a three month period; the quality of the vaccination strategy and post-outbreak serosurveillance, including post-vaccination monitoring, have more effect on the probability of (detecting) virus circulation than an additional three months wait; criteria by which these elements can be evaluated are needed. Allowing countries a choice to provide either a comprehensive package of quality assurance data and an epidemiological analysis demonstrating that it had reached a high level of confidence in

disease freedom after three months, or to wait an additional three months until the current six month period is reached, would address one major constraint to the adoption of vaccination-to-live.

DETECTION AND MOLECULAR CHARACTERISATION OF FMD VIRUSES FROM OUTBREAKS IN NORTHERN NIGERIA 2013-2015 (Ehizibolo D. et al.)

Blood and epithelial samples were collected from cattle (C) and sheep (S) during FMD outbreaks in four Northern Nigerian States in 2013-2015. Seventy-three % of cattle (131/178) and 64% (47/73) of sheep sera were antibody-positive against FMDV NSPs. Using solid phase competition ELISA on NSP-antibody positive sera, antibodies were predominantly detected against FMDV serotypes O (C: 101/127, S: 13/26), A (C: 43/127, S: 8/26) and SAT2 (C: 66/127, S: 14/26). Using rRT-PCR, FMDV genome was detected in 97.3% (73/75) of the epithelial tissues and from 29/75 samples FMDV could be isolated. Isolates belonged to serotypes O, A, SAT1 and SAT2. Phylogenetic analysis of the virus isolates revealed that two serotype O topotypes, East Africa-3 (EA-3) and West Africa (WA), were circulating as well as FMDV strains belonging to the Africa genotype (G-IV) of serotype A and FMDV SAT2 topotype VII strains. The isolated SAT1 strain was genetically distinct from SAT1 strains previously isolated in the region. In conclusion, this study provides evidence of co-occurrence of different FMDV serotypes and topotypes in Nigeria. These data may help to fill the knowledge gap of FMDV dynamics in Nigeria and West Africa and support local and regional control plans.

COMPLETE GENOME SEQUENCES OF THREE AFRICAN FMD VIRUSES FROM CLINICAL SAMPLES ISOLATED IN 2009 AND 2010 (Van Borm S. et al.)

To evaluate the feasibility of complete genome sequencing from clinical samples using unbiased RNA sequencing methods, three strongly positive epithelial samples from symptomatic cattle from Zambia and Namibia were homogenized in PBS, pretreated by filtration and nuclease, and RNA

was extracted. cDNA was synthesized using random hexamer primers and sequencing libraries were prepared and checked for quality. Sequencing was performed with the Illumina MiSeq technology. The obtained sequences were trimmed and assembled. The complete genome sequences of SAT2/ZAM18/2009 and O/ZAM14/2010 were obtained, with average coverages of 2,151X and 732X, respectively. These genomes contain a single open reading frame (ORF) of 7,008 and 6,999 nucleotides (nt), respectively, encoding a polypeptide precursor protein. The contig representing SAT1/NAM01/2010 contains a single 7,020-nt ORF. As only a limited number of reads were available for this sample, two gaps were closed using PCR amplification and Sanger sequencing, while the average coverage was <10X. These data demonstrate the feasibility of direct sequencing of complete FMDV coding sequences from samples from symptomatic animals (rRT-PCR CT range of 14.63 to 16.18) using an unbiased cDNA sequencing approach. However, targeted approaches using FMDV-specific cDNA synthesis primers or PCR amplification may result in a better sensitivity for whole-genome sequencing.

IMMUNE RESPONSES TO FOOT-AND-MOUTH DISEASE VIRUS IN GUINEA PIGS AFTER VACCINATION WITH CANINE ADENOVIRUS VECTOR (Lacour S. et al.)

A recombinant canine adenovirus-based FMD vaccine, Cav-P1/3C, expressing the P1 precursor along with the non-structural protein 3C protease of the FMDV strain O/FRA/1/2001 was evaluated in guinea pigs as a novel vaccine against FMD. Guinea pigs were vaccinated intramuscularly twice with a three week interval. Three weeks after the last vaccination, all guinea pigs were challenged intradermally with the guinea pig-adapted O1 Manisa/Turkey/1969 strain. A humoral immune response was elicited in guinea pigs following vaccination with Cav-P1/3C. The Cav-P1/3C recombinant vaccine protected guinea pigs from FMD to a similar extent as did a high potency double-oil-emulsion O1 Manisa vaccine. These results demonstrate that the Cav-based vector can express immunogenic FMDV antigens, offer protection against FMD in guinea pigs and suggest that Cav-P1/3C can be considered as a

potential marker vaccine against FMD.

TAILED PRIMERS ENHANCE REAL-TIME RT-PCR DETECTION OF FMD VIRUS (Vandenbussche F. et al.)

In this presentation it was shown that the incorporation of short A/T rich “tails” at the 5'-end of the primers has a positive effect on the detection of FMD viral RNA by real-time RT-PCR. In the 5'-UTR assay (Reid et al., 2002) fluorescence accumulated faster and to higher levels in reactions with tailed primers. This effect was more pronounced for SAT serotype strains and at lower target concentrations. Tailed primers significantly delayed the formation of PCR artefacts that are known to reduce amplification efficiency and restored the sigmoidal shape of the curves. Further, tailed primers altered the utilization patterns of the degenerate primers and increased the number of primer variants that participate in the reaction. In the 3D assay (Callahan et al., 2002) the effect of tailed primers was less pronounced but for 5 out of 50 FMDV strains and from 4 different serotypes the C_q values were markedly lower (3.43 ± 0.11) with tailed primers. Sequence analysis revealed several mutations in the inter-primer region that extend an existing hairpin structure immediately downstream of the forward primer binding site. Stabilization of the forward primer with a tail sequence restored the sensitivity of the assay, suggesting that the enhancing effect is due to a more efficient extension of the forward primer. In conclusion, primer tailing can alter amplification through various mechanisms that are determined by both the assay and target region. The enhancing effect also depends on the viral isolate and the target RNA concentration. These data are now available as an Open Access paper in PLOS ONE.

GENETIC AND ANTIGENIC CHARACTERISTICS OF FOOT AND MOUTH DISEASE VIRUS STRAINS ISOLATED IN 2011 AND 2015 IN NORTHERN BOTSWANA (Seoke L. et al.)

In the present study the genetic and antigenic characteristics of SAT2 viruses from two outbreaks in 2011 and 2015 in Botswana were analyzed. Sequencing of the VP1 gene showed 100% amino acid similarity between the two

outbreaks. Comparison with the recent SAT2 vaccine strain SAT2035 showed 5% amino acid variation and comparison with the vaccine strain SAT251 showed 12% variation. The r_1 values as measured by two-dimensional virus neutralization tests ranged between 0.51 – 0.87 for SAT2035 and 0.36 – 0.55 for SAT251, evidencing that these vaccines are relevant to confer protection against the circulating field strains. It was concluded that the vaccine strains are genetically and antigenically related to the circulating field virus strains and that minimal mutation occurred in the field strains intra- and inter-outbreaks.

Soon-to-be published work

Scientists from the CODA-CERVA contributed to a **book chapter** on “**Antiviral therapies for foot-and-mouth disease**” (Chapter 15) in the book “Foot-and-mouth disease virus: current research and emerging trends” that is edited by Francisco Sobrino and Esteban Domingo (Caister Academic Press). This book will be available for sale in 2017.

International collaborations

The **CODA-CERVA**, an OIE Collaborating Center, has a **bilateral collaboration** with the **Botswana Vaccine Institute (BVI)**, an OIE Reference Center. Within this framework, scientists from BVI visited the CODA-CERVA for a hands-on training with a particular emphasis on genome sequencing and analysis. Several FMD virus strains from Southern-Africa were isolated and characterized by full genome sequencing and phylogenetic analysis. The results were recently published in the journal *Genome Announcements*.

The **CODA-CERVA** is also involved as a parent collaborating center in an **OIE Laboratory Twinning Program** for capacity building via a technical and scientific collaboration with the **National Veterinary Research Institute (NVRI) from Vom, Plateau State, Nigeria**. The main aims are 1) to identify key-gaps in the laboratory practices with recommendations to improve current practices, 2) to strengthen and enhance safe and secure diagnostic laboratory practice and

skills and 3) to improve laboratory surveillance and disease reporting in Nigeria.

The CODA-CERVA provides laboratory training to scientists and technicians from the NVRI. From a scientific perspective, particular attention is given to extensive molecular characterization of Nigerian FMDV isolates, and includes sequencing, sequence analysis and phylogeny. Publication of results is foreseen in 2017.

Science reports

Scientific publications on foot-and-mouth disease involving CODA-CERVA in 2016

De Vleeschauwer AR, Lefebvre DJ, Willems T, Paul G, Billiet A, Murao LE, Neyts J, Goris N, De Clercq K. A Refined Guinea Pig Model of Foot-and-Mouth Disease Virus Infection for Assessing the Efficacy of Antiviral Compounds. *Transbound Emerg Dis*. 2016 Apr;63(2):e205-12. doi: 10.1111/tbed.12255.

Van Borm S, Rosseel T, Haegeman A, Fana ME, Seoke L, Hyera J, Matlho G, Vandenbussche F, De Clercq K. Complete Genome Sequences of Three African Foot-and-Mouth Disease Viruses from Clinical Samples Isolated in 2009 and 2010. *Genome Announc*. 2016 May 5;4(3). pii: e00326-16. doi: 10.1128/genomeA.00326-16.

Vandenbussche F, Mathijs E, Lefebvre D, De Clercq K, Van Borm S. A Tale of Tails: Dissecting the Enhancing Effect of Tailed Primers in Real-Time PCR. *PLoS One*. 2016 Oct 10;11(10):e0164463. doi: 10.1371/journal.pone.0164463.



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The New Zealand National Biocontainment Laboratory Project – Innovative approaches to meet testing requirements in the event of an FMD outbreak

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(as presented at the EuFMD Open Session 2016 in Cascais, Portugal)

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Introduction

In October 2015 the Ministry for Primary Industries (MPI) in New Zealand began construction on the National Biocontainment Laboratory (NBL), a state of the art, 3400 square metre high containment laboratory facility to replace its current enhanced physical containment level 3 laboratory facility. Although New Zealand is free from FMD and many high priority animal diseases this facility is an important contingency to support diagnostic testing for such diseases.

Design considerations to meet FMD testing requirements

A number of critical factors had to be addressed when designing the NBL and considering FMD testing in New Zealand:

1. The NBL will be the only enhanced physical containment level 3 facility in New Zealand and as such should meet a variety of needs, be resilient and provide continual service.



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2. The NBL should be seismically resilient to minimise any potential impacts of a major earthquake on the facility and its integrity.
3. The NBL should offer a highly flexible work environment.
4. The NBL should provide the ability to meet surge testing capacity.
5. The NBL should provide sufficient space without being too big or expensive to operate.
6. The NBL should be able meet current and future international standards for work with FMD including meeting the requirements of the EU FMD guidelines.

Discussion

A range of innovative design approaches were employed to address the above considerations including:

Potential outbreaks of FMD in New Zealand were modelled and business process analysis undertaken to understand testing capacity requirements based on different outbreak scenarios. This enabled MPI to make an informed decision on how big the laboratory should be.

Surge capacity to support diagnostic testing of a significant exotic animal disease was addressed by having the ability to convert the PC2 laboratory space to enhanced PC3.

Flexibility in laboratory space was attained through use of reconfigurable casework. Seismic resilience was ensured through a range of design strategies including base isolation, use of anchor piles, use of a highly rigid steel super structure, use of an integrated seismic bracing system and use of flexible service connections.

In summary, use of a range of different innovative approaches has enabled the design of an adaptable and efficient high containment laboratory that will significantly enhance New Zealand’s testing capability for FMD in the unlikely event of an incursion.

Field diagnostic testing to aid rapid differential diagnosis of foot and mouth disease and endemic causes of vesicular disease in New Zealand – new project

Rudolfo Bueno, Richard Spence

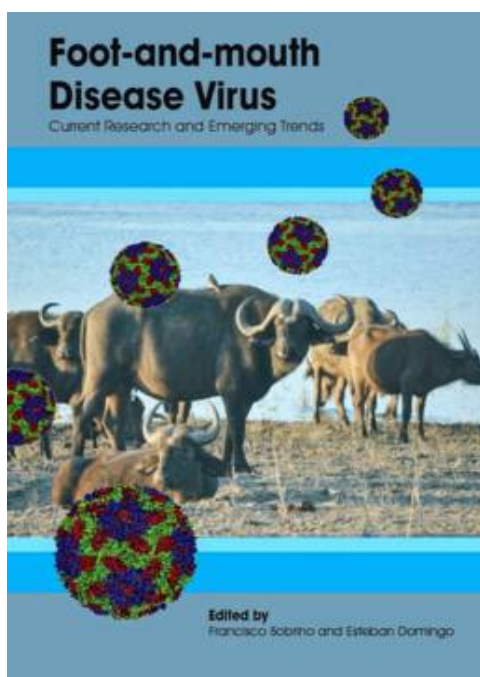
A new operational research project, funded by the Ministry for Primary Industries (MPI) Operational Research Programme, will explore the usefulness of field based tests to further enhance the efficiency of MPI's animal disease investigation, diagnosis and early disease outbreak response. MPI's Animal Health Laboratory (AHL) is the only laboratory capable of testing for suspected exotic infectious diseases in New Zealand. A challenge for AHL can be the transit of samples from a source farm, especially from remote locations, to the laboratory. Any lag time in transit may cause a delay in performing disease diagnostic testing. Examples of important exotic animal diseases that require rapid diagnosis and immediate control include foot and mouth disease (FMD), highly pathogenic avian influenza, Newcastle's disease and classical swine fever.

Field deployable diagnostic tests offer potential as first response tests to detect bio-threat organisms; as a rapid and reliable diagnostic tool in low resource areas; to support animal, plant and aquatic disease investigations; and in

conducting research testing in isolated field environments. Field based tests, if fully developed and validated, can be employed by MPI to enable prompt diagnosis of animal diseases on-farm to support clinical findings. This permits a more rapid presumptive diagnosis and allows implementation of immediate interventions and disease control while awaiting confirmatory diagnosis from AHL.

This three years undertaking, which started last September 2016, is a collaboration between MPI, Massey University and the Australian Animal Health Laboratory (AAHL). The project will focus on developing a "model" multiplex field deployable test based on selected exotic and endemic differential diagnosis of vesicular lesions in cattle. Primary emphasis of the work will include the selection of a "fit for purpose" field deployable diagnostic platform; development of a rapid, field ready sample processing method and multiplex assays for detecting the selected disease agents; and field validation of the test around New Zealand and in a country where the disease is endemic.

NEW PUBLICATION



Several GFRA partners and members contributed to a new book that will be available early 2017, entitled:

Foot-and-mouth Disease Virus Current Research and Emerging Trends

Caister Academic Press, edited by Francisco Sobrino and Esteban Domingo.

<http://www.caister.com/fmdv>

GFRA Scientific Meeting

Seoul, South Korea

25-27 October 2017



- FMD in Swine: Pathogenesis/Immunity
- FMD Modelling: More Data, Better Models?
- Persistent FMD: New Knowledge - Old Problem
- FMD Ecology: Africa vs. Asia
- FMD Vaccines in the 21st Century
- Socioeconomics of FMD



Want to know more?

The Global Foot-and-Mouth Disease Research Alliance (GFRA)

A worldwide association of animal health research organisations to assist the global control and eventual eradication of foot-and-mouth disease.

www.ars.usda.gov/gfra



The GFRA Executive Committee

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Secretarial Assistance: The GFRA ExCo has engaged the services of CRDF Global to provide secretarial assistance with a kind financial contribution from the USDA. CRDF is an independent nonprofit organization that promotes international scientific and technical collaboration through grants, technical resources, training, and services.

See more at: <http://www.crdfglobal.org/who-we-are/our-story#sthash.OgxqDdZF.dpuf>

This arrangement is for a trial period of one year and if successful, will need additional funding to continue engaging their services.

Cover and internal photos from the EuFMD Open Session 2016, Cascais, Portugal

Newsletter compiled by Jacquelyn Horsington, FMD Risk Management Project, CSIRO-AAHL

**Please note the contents of this newsletter are not peer reviewed.*