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The Immune Response to FMD Vaccines

Tim Doel

I am sure that if a poll was conducted on the subject of what FMD scientists and users think about FMD vaccines, a favourite topic would be the perceived inadequacy of the immune response with particular reference to duration of immunity. Here I want to explore whether or not this perception is justified, drawing on the FMD literature and, particularly, our extensive knowledge of the immune response to other vaccines.

It is necessary to emphasise that the quality of commercial FMD vaccines will vary with the competency of the manufacture and I am only addressing what good quality FMD vaccines have achieved or could be expected to achieve.

The Primary Immune Response.

When young animals are first vaccinated against FMD, it is often recommended to do so when maternal antibodies have declined to very low levels, otherwise the response to the vaccine will be impaired. Maternal antibodies probably inhibit the primary B cell response of vaccinates by sequestering antigen, thus limiting B cell activation, proliferation and differentiation. While some authorities wait up to six months before starting the primary vaccinations, it is also common practice to vaccinate at several months of age when maternal antibodies will often be present in significant titres. There is no hard-and-fast rule here. The timing of the first vaccination(s) is very much driven by the local disease threat. Therefore, in an endemic situation, vaccinating at two months of age would usually be considered the best approach. In this way, animals with significant maternal antibody titres would, at least, be protected by their maternal antibodies although they would not respond well to vaccination; whereas animals in which maternal antibodies had declined significantly would be expected to develop a protective antibody response to the vaccine.

The good news from work with other antigens is that the presence of maternal antibodies still allows priming of memory B cells, and T cell responses appear to be largely unaffected. In addition, it has been shown with measles and hepatitis A that it is possible to overcome the inhibitory effect of maternal antibody by using higher payload vaccines. This suggests the possibility of FMD vaccines formulated with a higher than normal payload for exclusive use as the first or second priming doses[1]. It needs to be stressed, however, that the FMD vaccine immune response-payload relationship appears to be sigmoidal and there is no evidence of any benefit in using very high payloads, with particular respect to booster immunisations.

Conventionally, young animals are vaccinated twice with an interval of three to four weeks between the FMD vaccine doses. While the second dose is often referred to as a booster, the consequence is largely the induction of more of the same antibody specificity and affinity stimulated by the first dose. Boosting, strictly, refers to much later vaccinations when immune memory is stimulated, leading eventually to the production of high affinity antibodies.

The plasma B cells and antibodies induced by priming vaccination(s) with non-live vaccines are relatively short-lived. However, a small proportion of the plasma cells acquire the capacity to survive, notably within the bone marrow, and can produce specific antibodies for extended periods. (These cells are distinguished from memory B cells which do not produce antibody).

Although short-lived, the priming response antibody population is capable of protecting cattle against a severe FMD challenge. Notably, protection at 4 dpv using aerosol challenge by infected pigs. In fact, cattle protection can be quite remarkable with good quality vaccines (6 PD_{50} or greater). With intradermolingual challenge of some cattle, we did not observe any evidence of virus replication around the needle tracks used to inject the virus. Unfortunately, pigs seem much more difficult to protect and we and others have observed how the immune system of a vaccinated pig can be overwhelmed by the levels of virus excreted by other pigs in a cohort. Clearly, much more needs to be done to develop improved vaccines for use in pigs.

Results with other non-live vaccines indicate that the interval between the first and the second priming doses of vaccine is important in terms of developing sufficient antibody and should be at least three weeks. When we conducted experiments in cattle, there was a strong increase in antibody titre with a two month interval and no increase at all when the interval was only two weeks. The four week interval group gave an intermediate antibody increase (unpublished). However, it must be said that delaying the second 'priming' dose may pose a risk in environments where FMD is particularly prevalent and/or where the first priming dose is not very effective. It would also be necessary to determine whether a delayed second priming dose would have negative consequence for memory development.

[1]In this article, I suggest that vaccines could be formulated for specific purposes (e.g. high payload priming vaccines, low payload boosting vaccines) and different scenarios (e.g. endemic or non-endemic, low or high density animal populations). Regrettably, the reader should not conclude that low payload vaccines would be much cheaper. Much of the cost involved in manufacturing a GMP product are fixed – e.g. bottles, adjuvant, packaging, QC testing.

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One explanation for the success of live vaccines (including lifelong immunity) is that they replicate in the host and establish numerous germinal centres. In contrast, a killed vaccine will remain largely at the site of inoculation and rely on an encounter with patrolling immature dendritic cells (DCs) and eventual transport to secondary lymph nodes where the induction of specific T and B cells occurs. Thus, immune system activation by a killed vaccine is much more limited than that due to a live vaccine[2]. This prompts the question of whether the sites and/or routes of vaccination used for FMD vaccines could be improved to increase the frequency of encounters with DCs. The routes used are subcutaneous for aluminium hydroxide/saponin vaccines and subcutaneous or intramuscular for oil vaccines, depending on the formulation of the latter. The precise sites of inoculation are selected to avoid vaccine depots and damage in the more valuable parts of a carcass. Thus, the neck region may be used for cattle or behind the ear of pigs. Other sites include the dewlap of cattle and even the caudal fold.

DCs are numerous in well vascularized muscle but are far less prevalent in adipose tissue. Interestingly, the dermis contains high numbers of DCs and work at the Plum Island Animal Diseases Centre with needleless dermal injection demonstrated complete protection of cattle 31 dpv using 1/16 of a normal vaccine antigen payload. Given particularly the difficulty of protecting pigs in situations where virus is circulating freely and at high levels, it would seem appropriate to investigate alternative vaccine formulations for this species with particular reference to routes and sites of vaccination.

Duration of Immunity

Despite the reputation, there is good evidence that the duration of immunity conferred by FMD vaccines can be quite long. Van Bekkum and colleagues showed many years ago that Dutch cattle given two or more vaccine doses had a strong duration of antibody up to the end of one study (107 weeks post last vaccination). They also reported duration up to 4 years in another study. These results are consistent with several European reports of antibody titres in cattle sera, taken six or seven years after vaccination was prohibited in the European Union.

This prompts the question of how FMD vaccines inherited the 'poor duration of immunity' label. A clue lies in the different vaccination protocols used worldwide which are not necessarily equivalent to those used in Europe before 1990. During the period of Van Bekkum's studies, vaccination of young cattle consisted usually of an early priming dose or doses (i.e. 0 and 21 days) followed by a booster dose at 6 months and, thereafter, yearly. Nowadays, it is not uncommon to see much more frequent vaccinations of cattle and even pigs.

A critical characteristic for many killed vaccines is the establishment of immune memory. This normally develops over several months following the initial vaccine priming and requires a minimum interval of 4–6 months between the priming and boosting (6 month) doses in order to stimulate high levels of immune memory, including very high affinity B memory cells. Antigen introduced (i.e. vaccination) in the period between priming and boosting has a negative impact on the memory and affinity maturation process and should be avoided. In the case of FMD, this aspect is clearly most relevant to long-lived livestock. However, a long delay between the priming and boosting doses could present difficulties if FMD is rampant and there is need to quickly raise and sustain antibody titres. Thus, frequent boosting with FMD vaccine would probably solve the immediate threat, resembling essentially a succession of primary immune responses but accompanied by poor or non-existent development of immune memory. Such an approach clearly contrasts with the vaccination protocols used in Europe before 1990.

While not necessarily practical as far as all routine applications of FMD vaccines are concerned, lower priming doses of non-live antigens are thought to drive the subsequent memory response more strongly than higher priming doses.

Perhaps more interesting in the context of FMD booster vaccination is the fact that quite low doses of antigen will boost the immune response. Black et al (1984) showed that vaccine doses as low as 7 ng of 146S boosted virus neutralising antibody titres to protective levels in cattle vaccinated 127 days previously with a 5 µg dose vaccine. Although boosting with higher payloads gave higher titres, all of the antibody kinetics converged over the following months to more or less the same titre as the 7 ng booster vaccine. This work suggests that, in stable situations with very low-level endemicity, booster vaccines could be formulated with lower payloads sufficient to ensure antibody titres were maintained at protective levels.

A particular feature of a 'typical' memory response is the development of very high affinity antibody driven by somatic hypermutation in the immunoglobulin genes. In the case of FMD, relatively little is known about the importance of affinity in singly or multiply vaccinated animals. Some years ago we showed that, with paired sera having similar virus neutralising antibody titres, the high affinity sera were protective whereas the lower affinity sera were not. In the author's view, any study to examine novel ways of synthesising, formulating or presenting FMD vaccines to animals should be accompanied not only by simple antibody titre assays but should include affinity immunoassays.

Regrettably, in this short review it has not been possible to develop the subjects of mucosal immunity, persistence and T cell responses and memory. Their omission does not reflect their importance and they are subjects which demand more attention.

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[2]A replicating recombinant FMD vaccine would at least offer the possibility of more extensive immune stimulation.

Isolation and Characterisation of a SAT 3 FMDV from a Long-horned Ankole Calf within Uganda.

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Foot-and-mouth disease (FMD) is endemic in much of Africa and indeed 6 of the 7 known serotypes of FMD virus (FMDV) have been found to circulate there (the only exception being Asia 1). However serotype C has not been detected anywhere in the world since 2005 and SAT3 was last identified in samples collected in South Africa in 2006 but is believed to continue to circulate there within buffalo populations. In contrast, SAT1 and SAT2 FMDVs are reported quite frequently within sub-Saharan Africa and indeed a major incursion of SAT2 virus occurred into the Middle East in 2012.

As part of a DANIDA (Danish Development Agency) funded project (DTU-Vet in conjunction with Copenhagen University, Makerere University (Kampala, Uganda), Ministry of Agriculture Animal Industry and Fisheries (Entebbe, Uganda) and the Foot-and-Mouth Disease Laboratory (Embakasi, Kenya)) we have undertaken an analysis of the FMDVs circulating in Uganda and Kenya both in domestic livestock and in wildlife (especially the African buffalo, Syncerus caffer). The buffalo are found within National Parks in these countries and the domestic livestock around the parks frequently come into close contact with the buffalo while grazing and at sources of water. In South Africa it is believed that buffalo within National Parks are a source of FMD outbreaks within domestic animals. These Parks are fenced and buffer zones, in which vaccination is practiced, are used to try to reduce the spread of the disease.

Figure 1: *Ankole cattle heading for water and grazing within QENP, Uganda*

As part of the analysis of the importance of interaction between domestic animals and wildlife in Uganda, 20 long-horn Ankole cattle (ca. 6 months old) were introduced as sentinel animals into a farm in Nyakatonzi (Kasese district), in close proximity to the Queen Elizabeth National Park (QENP) which has more than 6000 buffalo. These cattle moved regularly into the QENP for pasture and water and freely mixed and grazed with buffalo (observed within a few metres of each other) and other local cattle (see Figure 1). At the time of their introduction to this region, these sentinel cattle, originating from another area within Uganda with no reported FMD outbreaks for about 10 years, had no detectable antibodies against FMDV non-structural proteins (NSPs). The calves were closely monitored; both blood and probang (comprising oropharyngeal scrapings and fluid) samples were obtained from individual animals, at 2 week intervals, after their entry to the farm. Within 1 month of their arrival in this region, two of the sentinel calves were found to have seroconverted against FMDV as detected using ELISAs specific for the NSPs and also serotype specific tests. In one calf the serology suggested an infection with SAT1 virus while in a second calf (#34) reactivity against both SAT1 and SAT3 was observed (although it is commonly found that there can be some cross-reactivity in these assays during the first stages of the immune response).

No clinical signs of FMD were apparent in these calves but high levels of FMDV RNA could be detected in a probang sample collected from calf #34 some 14 days post arrival of the animals into the farm. An aliquot of the probang sample from this calf was inoculated onto primary bovine thyroid cells for virus isolation. Cytopathic effect was observed within 48 hrs and the virus harvest was assayed using serotype specific antigen ELISAs; a strong signal indicative of SAT3 FMDV was observed with no significant signal for other serotypes. The presence of high levels of FMDV RNA in the cell harvest was demonstrated using real time RT-PCR assays indicative of isolation and growth of FMDV. Using conventional RT-PCR, the VP1 coding region within the FMDV genome was amplified and the amplicon (ca. 820 bp) was sequenced. Comparison of the VP1 coding sequence with other FMDV sequences from East Africa clearly identified the isolate as being SAT3 (Figure 2), consistent with the antigen ELISA result. The new isolate was named SAT3 UGA/1/13. Within the VP1 coding region, the new virus was most closely related (ca. 80% identity) to earlier SAT3 viruses isolated from buffalo in Uganda and differed by about 36% from SAT3 viruses from South Africa. The complete genome sequence of the new SAT3 virus was then determined. There are only 3 other full genome sequences for SAT3 FMDVs in the database and they are each from isolates of virus from southern Africa that are about 50 years old. These differ in sequence by about 20% across the whole genome compared to the UGA/1/13 virus.

Conclusions

These results indicate that SAT3 FMDV is still in circulation within Uganda and that it can spread into domestic animals albeit that this may not result in clinical disease (within the indigenous Ankole cattle at least). A fuller description of

fuller description of this virus has been published by Dhikusooka et al., (2015).

It is noteworthy that other serotypes of FMDV are also still circulating in Uganda and Kenya. Recent work, from the same project, reported by Namatovu et al (2015) has shown that serotypes O, A, SAT1 and SAT2 have each been responsible for outbreaks of disease during 2012-2013 within Uganda. Similarly the same 4 serotypes have been identified within Kenya as well (Wekesa et al., 2015). To date, it seems that the virus populations that cause disease in domestic animals are distinct from those present in buffalo (albeit only a small number of buffalo isolates have been obtained) and thus it is not established that spillover of FMDV between wildlife and domestic animals is common in East Africa.

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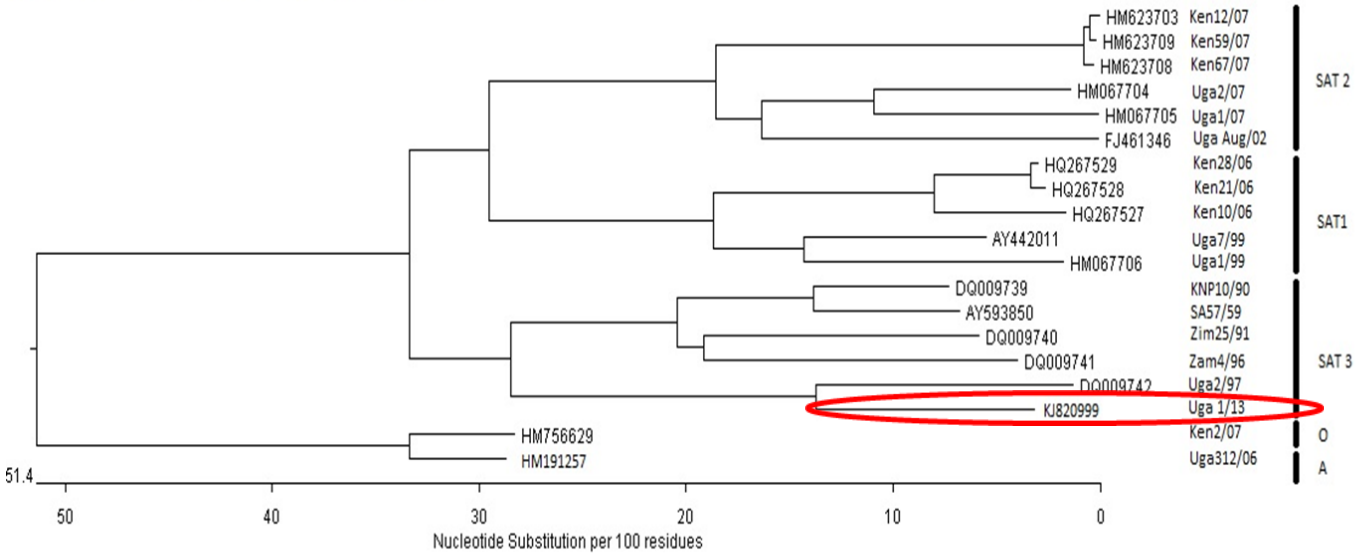

Figure 2: *Phylogenetic comparison of complete FMDV VP1 coding sequences from East Africa. The UGA/1/13 sequence is marked with the red oval. The accession number for the complete genome sequence is KJ820999.*

Quantitative Risk Assessment Methodology to Measure FMD Risk Posed by Chilled Deboned Beef Produced in a Non-FMD Free Location

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Recently an international standard (Article 8.7.25 of the OIE's Terrestrial Animal Health Code) has been provided to facilitate trade in deboned beef where the locality of production is not in a FMD-free country or zone. However, this standard presents practical difficulties for locations in southern Africa where extensive wildlife conservation is a major consideration (Thomson et al., 2013). The primary difficulty with that standard is a requirement for certification that FMD, whether clinical or sub-clinical and in any susceptible species, has not occurred within 10 km of the 'establishment' from which the cattle that provided the beef were derived in the last 30 days prior to slaughter. For most production systems in southern Africa such certification would lack credibility.

With this as background, we concluded that under present circumstances, to achieve better access to beef markets for most locations in southern Africa would necessitate proving that FMD risk management of the value chain concerned attains at least the same level of protection as could be achieved through application of Article 8.7.25 i.e. demonstration of 'equivalence'.

This approach for the beef value chain in the Zambezi Region (ZR) of Namibia was therefore investigated. That involved assessing the level of risk reduction achieved currently (labelled 'current') by the value chain and comparing that with two alternative risk release scenarios, *viz.* theoretical application of Article 8.7.25 (labelled 'OIE') and a modification of the current risk management strategy focusing risk mitigation on critical control points along a modified value chain (labelled 'VC' – Fig. 1). The

assessments were conducted independently for different cuts of matured (pH <6.0) beef from which bones and lymph nodes had been removed. Potential exposure assessment was based on swine consuming waste beef in a theoretical importing country.

A separate part of this study confirmed that SAT viruses could not be identified in striated muscle of carcasses of cattle experimentally infected with three separate SAT virus isolates or from body fat or bone marrow immediately after slaughter and exsanguination. The detection method used was real-time PCR. Conversely, high levels of infectivity were detectable in lymph nodes of the same carcasses.

The mean (range) number of FMD virus contaminated carcasses that could potentially pass through all safeguards of each scenario on an annual basis was estimated to be 0.48 (0, 8), 0.18 (0, 4), and 0.02 (0, 2), for the 'current', 'OIE' and 'VC' scenarios respectively. Estimated probabilities of infection of swine were consistently highest for 'current' and lowest for 'VC' scenarios. Boxed fillet was associated with the lowest risk of all evaluated beef cuts. The probability (range) that a box of fillets would cause FMD virus infection in exposed swine in an importing country was $4.3x10^{-8}$ (0, $1.4x10^{-4}$), $1.6x10^{-8}$ (0, $8.7x10^{-5}$), and $2.1x10^{-9}$ (0, $5.3x10^{-5}$) for 'current', 'OIE' and 'VC' scenarios respectively. This showed that risk reduction achieved by the value chain approach was more effective than either of the other two scenarios, thereby demonstrating equivalence with the international standard.

Novel features of the risk analysis conducted were that it formally compared the risk of three different scenarios that incorporated probabilistic and quantitative aspects. In contrast, most quantitative risk assessments are based on either simple probability pathways or a quantitative value that is reduced by various risk mitigation steps.

This approach has the potential to benefit many thousands of resource poor cattle farmers, regional trade in beef and wildlife conservation in many regions of southern Africa where FMD is endemic. Until such time as the current standard is modified to render it more applicable to circumstances in southern Africa, risk assessment will be needed to demonstrate equivalence with the existing standards. The approach outlined here (to be published) offers a way forward in the interim.

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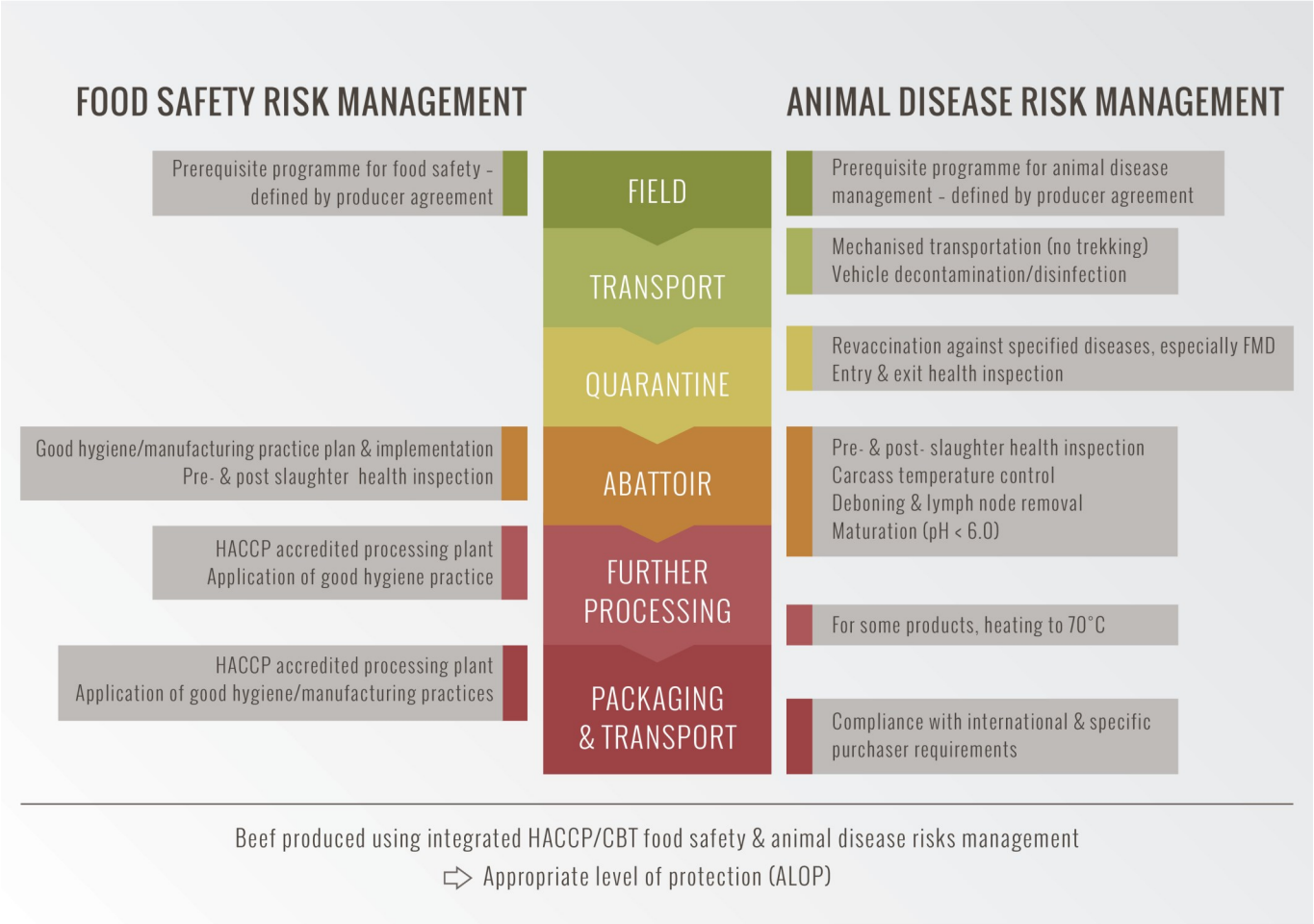

Figure 1: *The integrated (food safety & FMD) risk management system for deboned beef produced in the Zambezi Region of Namibia*

Improving Quality Assurance along the FMD Vaccine Production and Supply Chain

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The effectiveness of FMD vaccines is influenced by multiple factors such as route of vaccination, dose, similarity to circulating virus and adjuvant. Antigen integrity is also imperative for vaccine efficacy; FMD vaccines that are comprised of dissociated viral particles do not elicit adequate levels of protective neutralising antibodies. This problem is exacerbated by the fact FMDV capsids (146S particles) readily dissociate at mild acidic conditions and at room temperature into their constituent subunits (12S particles). In addition, chemical inactivation further decreases the stability of the FMDV capsid (Doel and Baccarini, 1981).

A research team headed by Bryan Charleston at The Pirbright Institute in the UK has recently focused on (i) the development of alternative FMDV vaccines based on virus-like particles (VLPs), and (ii) the generation and characterization of more stable infectious virions and VLPs. To quantify the antigen content and integrity of these new vaccine candidates two different assays have been developed. The first, termed the thermofluor assay, is a qPCR-based technique that monitors viral genome release as an indicator of capsid disassembly using a dye sensitive to the presence of nucleic acid during a slow increase in temperature. The second assay is ELISA-based and uses llama single-domain antibodies (VHHs) that are specific for intact viral capsids. Michiel Harmsen and Aldo Dekker at the Central Veterinary Institute Wageningen UR, The Netherlands (CVI), have developed the selection of such 146s specific VHHs using phage display libraries derived from llamas immunized against FMDV. Both assays are user -friendly and can be quickly performed with minimal training using standard laboratory equipment. In combination, the techniques complement each other and have the potential to be applied for quality control of FMDV vaccines, both during and after the production process, as well as for the characterisation of optimal vaccine storage conditions.

At the 2014 EuFMD meeting in Croatia, the use of both techniques as diagnostic tools for FMDV capsid stability was presented, generating a lot of interest amongst the FMDV community. This has led to a successful application for EuFMD-FAR funding. The collaborative project combines the technical expertise of Julian Seago and Eva Perez at TPI in performing the assays with established methodologies at CVI in the production of VHHs. The project will develop and assess the assays on FMD vaccine strains currently being produced in East Africa by vaccine companies, with the intention of transferring the technologies for their future application; this will be mediated through Nick Lyons at TPI and Vish Nene at the International Livestock Research Institute (ILRI) in Kenya.

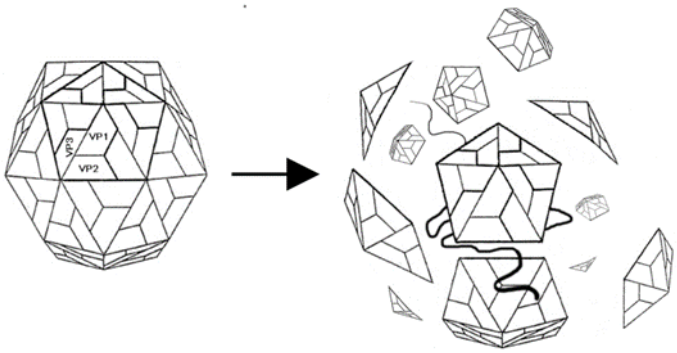

Figure 1: *Schematic representation of a FMDV virion (146S) dissociating into its constituent pentameric subunits (12S) and RNA genome. Positions of the three structural proteins (VP1-3) on the external surface of the capsid are*

Foot-and-mouth Disease Vaccine Antigen Engineering to Promote Cell Culture Adaptation, Capsid Stability and Antigen Immunofocussing to Innovate Vaccine Candidates

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FMD is not simply a contagious disease of cloven-hooved livestock, but can be considered as a complex of diseases caused by numerous genetic and antigenic variants with different geographic distributions and epidemiologies, belonging to the seven serotypes, A, O, C, Asia-1, Southern African Territories (SAT) type 1, SAT2 and SAT3 . In Africa, the epidemiology of FMD is influenced by two different patterns, i.e. a cycle involving wildlife, in particular the African buffalo (Syncerus caffer), and an independent cycle maintained within livestock. Another unique feature of FMD epidemiology in Africa is the presence of the three SAT serotypes, which are maintained within the African buffalo populations. Therefore, the presence of large numbers of African buffalo provides a potential source of sporadic infection to livestock and other wildlife species.

Although the precise mechanism of transmission of FMD from buffalo to cattle is not well understood, it is facilitated by direct contact between these two species. Once cattle are infected they may maintain SAT infections without the further involvement of buffalo (Thomson et al., 2003). Southern African Development Community (SADC) is endowed with an abundance of wildlife, which has been preserved within national parks and game reserves. However, in communities neighboring these parks, the livestock/wildlife interface presents unique challenges to livestock disease control. In addition, the creation and expansion of transfrontier conservation areas (TFCAs) in SADC presents a particular challenge to the management of FMD (Jori et al., 2009; Miguel et al., 2013).

For these reasons SADC countries rely largely on the implementation of strategies such as separation of wildlife and livestock, repeated vaccination of cattle herds at risk, control of animal movements and careful assessment of the risk of introduction of FMDV into disease-free areas (Ferguson et al., 2013). However, the lack of veterinary infrastructure, human resources, movement controls, and appropriate vaccines render smallholder farmers and many developing countries particularly vulnerable to the spread and poor control of the disease. Unless ways of profiting from the large livestock populations of the arid and semi-arid regions of sub-Saharan Africa can be found, the human populations of those regions will be consigned to continuing poverty. To manage FMD in the face of increasing integration of land-use in future will necessitate improved tailored vaccines, and novel control measures that relates to the conditions in Africa. Furthermore, the vaccination and disease control strategies need to enable fit-for-purpose approaches to FMD control in Africa (reviewed in Maree et al., 2014).

Although the current inactivated vaccines have proven effective in reducing clinical disease in FMD-endemic areas and have been critical to the success of FMD control programs in South America and Europe, these vaccines are not ideal. In Africa, the diversity of circulating field strains makes the selection of sufficiently cross-protective FMD vaccines a challenge. Some viruses are very difficult to adapt to cell culture, slowing the introduction of new vaccine strains, reducing vaccine yield, and potentiating through prolonged passage the selection of undesirable antigenic changes. In addition, the hot climate in many African regions calls for vaccines with improved stability and which are less reliant on a cold-chain. However, FMDV is notoriously unstable, especially viruses of serotypes SAT1, SAT2 and O. It is believed that unstable vaccines are less immunogenic due to degradation of antigen before and after inoculation.

In the last 15 years researchers of the Agricultural Research Services (ARS) of the USA Department of Agriculture (USDA) and the Agricultural Research Council (ARC) of South Africa have conducted collaborative studies using a reverse genetics technology to address the current limitations of vaccines for the control of FMD in South Africa and the larger SADC region. Genome-length viral RNA (vRNA) derived from complementary DNA (cDNA) clones of FMDV is infectious when transfected into suitable mammalian cells (van Rensburg et al., 2002). These cDNA clones are readily amenable to genetic engineering to introduce changes to the virus genome that allow for the replacement of the external capsid-coding region or structural, surface-exposed antigenic loops with the corresponding regions of an emerging virus.

Toward this end we have designed intra- and inter-serotype chimera-derived FMD vaccines by replacing the surface-exposed, capsid-coding region (1B-1D/2A) of an infectious copy genome-length clone (Fig. 1) and tested these vaccines in cattle. The chimeric viruses exhibited comparable infection kinetics, virion stability and antigenic profiles to the parental viruses, thus indicating that the functions

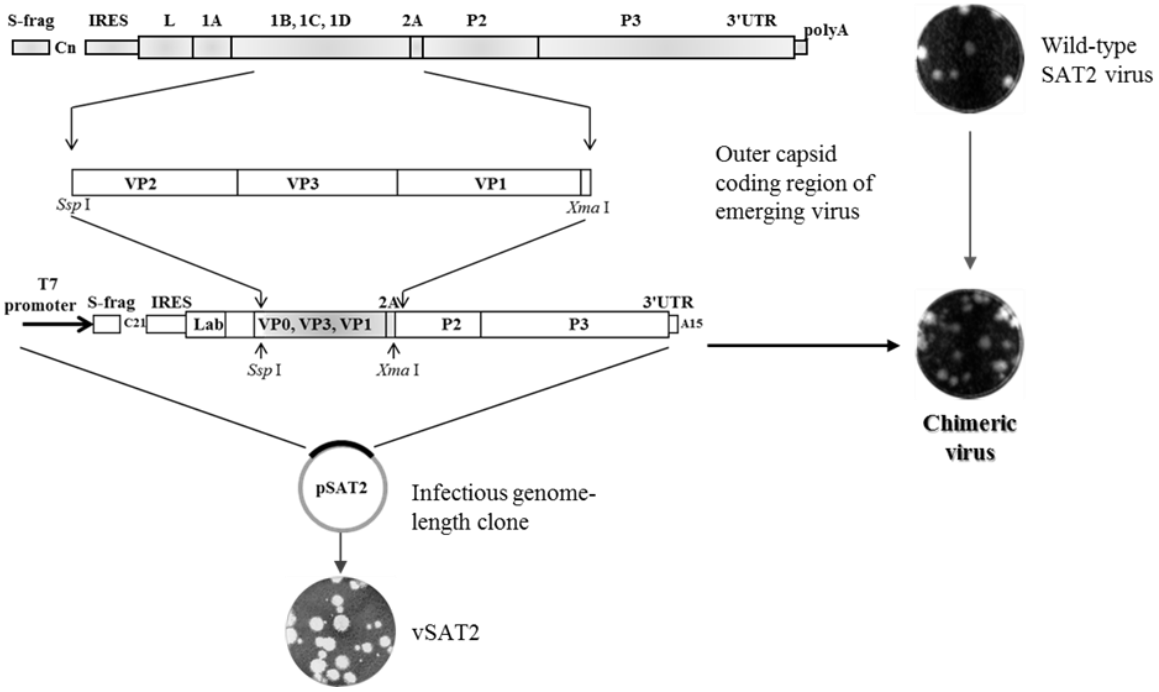

Figure 1: *Schematic representation of the cloning strategy generate chimeric viruses.*

provided by the capsid can be readily exchanged within and between serotypes (Fig. 1). We showed that chimeric vaccines successfully elicit protective immune responses in FMDV host species, accompanied by neutralizing antibody responses. This approach therefore has the potential to make a valuable contribution to the 16-year framework for the progressive control of FMD in SADC countries (Lubroth et al., 2007). However, capsid swapping may transfer other undesirable traits such as capsid instability and poor cell culture adaptation, which are limitations that can be overcome by site-directed mutagenesis of the amino acid(s) associated with improved performance as vaccine candidates. We provide three examples where the desired traits were successfully designed into the infectious clones.

The development of new vaccine FMDV strains relies strongly on virus growth and high antigen yields of the new strain in the production cell line. We have engineered SAT1 and SAT2 viruses with symmetrically arranged positive charged amino acids around the five-fold axis of the virion, linked with enhanced glycosaminoglycan (GAG) receptor binding and more efficient cell entry. The mutations resulted in the chimeric viruses having a predisposed characteristic to grow to high tires on BHK-21 monolayer and suspension cells when compared to the respective wild-type virus. These viruses are available for use in large-scale production fermenters.

The stability of vaccines is of crucial importance in Africa, where the logistical process to get the vaccine from the manufacturer to the animal may take months and in many remote regions is in the absence of a cold-chain. The residues at the capsid inter-pentamer interfaces, and their interactions, are important for the infectivity and stability of the virion. However, experimental studies on the relative importance of residues and molecular interactions in capsid stability are still very limited. The Wellcome Trust Translational award research groups (The Pirbright Institute, Oxford University, ARC-OVI, USDA & Intervet) have predicted residue substitutions that could increase stability at inter-pentameric interfaces. We could demonstrate using biochemical and reverse genetic approaches, the effect of the stabilizing mutations in the capsid on virus infectivity and particle stability. The stability of the mutated SAT2 capsid was found to be 10% more stable than the wild-type, confirming the predictions, and vaccinated animals generated improved neutralising antibody responses (over a 6 month period and after storage) to stabilised particles compared to the parental viruses or wild-type capsids. These changes have been incorporated in prototype FMDVs for vaccine development.

Next, we used infectious genome-length clones to rationally engineer antigenic relevant sites with an increased avidity to antibodies and a broader antigenic spectrum within a serotype of FMDV. We have targeted specific residues identified as antigenically significant, as well as variable residues identified as epitopes in other serotypes to design broadly protective vaccine strains. Interestingly, we not only found the VP1 G-H loop as antigenically important but were also able to map a unique epitope to residues 71-72 of the VP2 protein for SAT2 viruses (Opperman et al., 2013). Using this immunofocussing technology we believe we can design vaccines that will provide improved coverage against the various antigenic types within a serotype. However, further structural and functional studies are necessary to better understand the structural basis of antigenic variation and the interaction of the FMDV epitopes in antibody binding.

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L-R: Lia Rotherham, Theresa de los Santos, Katherine Scott, Elizabeth Rieder, Melanie Chitray, Pamela Opperman, Francois Maree.

Novel Countermeasures to Support the Surveillance and Control of Foot-and-mouth Disease Virus in Uganda

Frank Mwiine[1], Julius Lutwama[2], Leslie Lobel[3], Francois F. Maree[4], Andres Perez[5], Zaheer Ahmed[6], Luis Rodriguez[6], and Elizabeth Rieder[6]

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[4] *Onderstepoort Veterinary Institute, Agricultural Research Council (ARC-OVI), South Africa*
[5] *University of Minnesota, USA*
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Despite recent successes in controlling the disease in Europe and some parts of South America, FMD remains one of the most important infectious diseases of farm animals due to the potential impact of an outbreak on trade in animals and animal products. The World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO) of the United Nations have called for the global control and eradication of trans-boundary diseases, with FMD identified as one of the priority infectious diseases of livestock. FMD impacts millions of people dependent on livestock for food and farm work. Usually associated with developing countries that lack the resources to control it, FMD impacts productivity and commerce, and adds food insecurity to regions of the world facing high population increases that will demand secure meat supplies as a main source of protein.

Livestock is an important element for the livelihoods of millions of Ugandans and considerable efforts at economic development by the government of Uganda have focused on the livestock sector. Livestock, being one of the main users of natural resources, are an important economic resource for some 80% of Ugandans, providing power for cultivation, nutrients for farmland, investment opportunities and animal protein. The livestock sub-sector contributes approximately 7.5% of GDP and 17% of agricultural GDP. It is an integral part of the agricultural system of many parts of the country. Mixed farming small holders and pastoralists own over 90% of the cattle herd and 100% of the small ruminants and non-ruminant stock. Cattle are the most important of all the livestock. Livestock production has continued to grow, at a rate of over 4% per annum, in response to increasing demand for milk and meat in the local market. At least five of the seven serotypes, i.e. A, O, SAT1, SAT2 and SAT3 are known to occur in Uganda, causing disease in wildlife and domestic animals. FMD is highly contagious and the high morbidity caused by the disease has serious impact on growth and production and the resulting disruption of trade and decrease in market value of livestock products. Most importantly there is a need to improve surveillance, detection of FMD, to characterize viral strains and perform effective vaccine matching in order to select the appropriate vaccines.

Researchers from the United States Department of Agriculture (USDA), Agricultural Research Council (ARC-OVI) of South Africa, Makarere University (MU) and Uganda Virus Research Institute (UVRI) in Uganda, Ben Gurion University in Israel, the University of Minnesota and the Uganda Ministry of Agriculture initiated a research project in 2014 to design novel countermeasures for the progressive control of FMD in Uganda. Uganda has had more than 34 districts out of 112 reporting FMD outbreaks since July 2014. The main goal of the project is to implement the necessary tools for disease surveillance and diagnosis of FMD in Uganda. It is envisaged that the epidemiological knowledge and the selection of vaccines that properly match FMDV strains circulating in Uganda will enable the progressive control and increase the opportunities of the eventual eradication of FMD in Uganda.

Surveillance is the first necessary component of a vaccine research program leading to an effective control strategy. The information derived from the surveillance and vaccine matching studies will provide detailed understanding of the FMDV strains circulating in Uganda, for which little information is currently available. Scientists at UVRI, Ben Gurion University and the USDA-ARS have joined efforts to develop strain-specific, affordable diagnostic kits, for locally circulating FMDV strains. At the end of the project it is expected there will be a better understanding of the FMDV serotypes and subtypes circulating in Uganda, and the capacity to develop locally affordable FMD diagnostic tools, as well as providing relevant training and educational workshops.

In this regard, activities conducted in Uganda in 2014 consisted of gathering equipment and material to prepare the laboratories at Makarere University and UVRI to initiate FMD surveillance and diagnostics. Two training courses were provided to staff scientists from UVRI, Makarere University and MAIFF scientists during the first year of this project. The USDA-ARS team, with OVI scientists and epidemiologist from the University of Minnesota, conducted training on molecular and serological diagnostics, sample collection including probang and blood, as well as epidemiology and surveillance of FMDV. A training manual was developed for this workshop and distributed to all the participants in the training. During a field visit for attending the FMDV outbreak, all the team members were taken to District Mbale. They were trained to take probang, tissues and blood samples from FMDV suspected animals and then properly store them for shipment while maintaining the cold chain (see attached pictures). The same samples were then taken to the lab at UVRI, where lab scientists received training on sample processing, RNA extraction and rRT-PCR and serological analysis using ELISA for diagnosing FMDV.

A second hands-on workshop was held at UVRI, Uganda, also in 2014. This workshop was conducted by scientists from Ben-Gurion University (Israel), in collaboration with UVRI and MU (Uganda) project staff. The training focused on FMDV recombinant protein expression, purification and analysis.

Serum and probang samples have been collected from 22 districts of Uganda, from all the 4 regions of Uganda including Central, Eastern, Northern and Western regions during the first months of execution of this project. The map of Uganda below (Fig 1), shows all the districts selected for sampling from all 4 regions. The blue, red, green and yellow

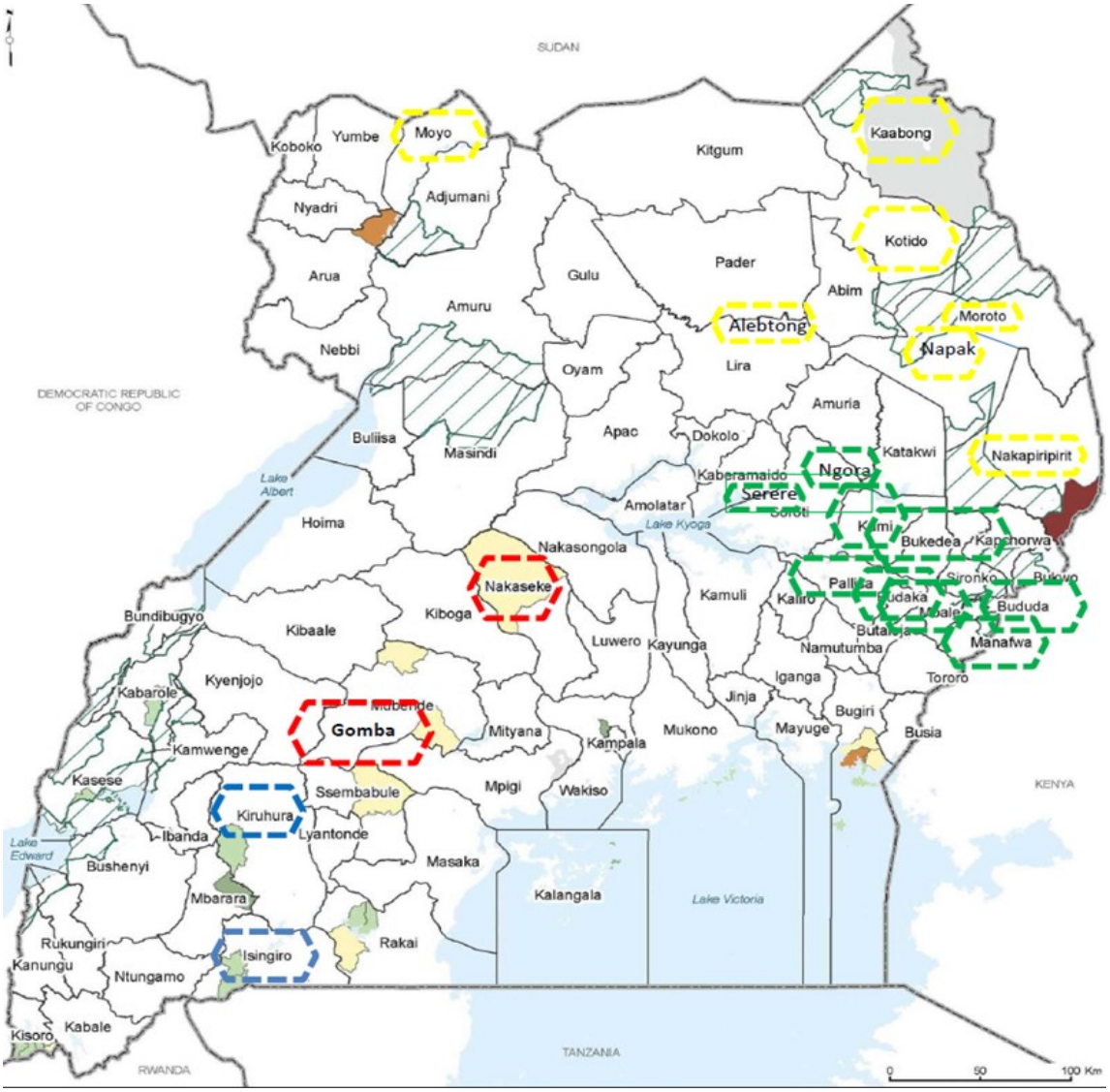

Figure 1: *Map of Uganda showing details of districts selected for FMDV surveillance*

colours indicate western, central, eastern and northern regions, respectively.

Scientists from MU and UVRI laboratories of Uganda have collected a total of 808 serum, 4317 probangs and 3 tissue samples from 22 districts from all 4 regions of the country. Accompanying questionnaires and GPS coordinates for each of the sample units and sampled animals have also been collected. Diagnostics of the samples obtained is being conducted at UVRI, MU and USDA-ARS, PIADC laboratories. A summary of samples tested at PIADC are provided in Table 1.

Samples received at PIADC were processed for virus isolation, rRT-PCR and P1 sequencing. Probang samples collected from 12 districts was processed for virus isolation, rRT-PCR and sequencing. Approximately 47% of the probang samples, tested so far, were positive for FMDV by rRT-PCR while a total of 34% of these positive samples showed CPE on cell culture. The P1 sequencing of 15 viruses isolated from the Eastern region of Uganda confirmed FMDV serotype O, the phylogeny of which indicates that this serotype O belongs to topotype EA-2.

In addition to the above activities, reference serum samples have been produced in cattle in Uganda for antigen matching studies that will be performed at the USDA-ARS and ARC-OVI laboratories, according to the serotypes identified (serotypes O, SAT 1 and SAT 2) in Uganda.

Table 1: *District-wise details of samples collected from 22 districts of Uganda.*

DETAILS OF SAMPLES RECEIVED FROM UGANDA SHIPMENT 1 OCTOBER 23, 2014					
Sr #	District	Code	Probang received	Serum received	Tissue Received
1	ALEBTONG	ALB	26	172	0
2	BUDUDA	BUD	10	18	0
3	MANAFWA	MAN	20	32	0
4	NAKAPIRIPIRIT	NKP	39	276	0
5	MBALE	MBE	6	11	3
6	MOYO	MOY	0	155	0
7	KOTIDO	KOT	0	179	0
8	BUGIRI	BUG	70	282	0
9	BUDAKA	BDK	53	208	0
10	BUKEDEA	BKD	22	33	0
11	KUMI	KUM	25	90	0
12	KIRUHURA	KIR	16	107	0
13	PALISA	PAL	77	413	0
14	GOMBA	GMBS	14	23	0
15	NAKASEKE	NSKF	15	30	0
16	ISINGIRO	ISG	4	6	0
SUB TOTAL			**397**	**2035**	**3**
DETAILS OF SAMPLES RECEIVED FROM UGANDA SHIPMENT 2 DECEMBER 5, 2014					
17	Ngora	NGO	75	403	0
18	Serere	SER	69	412	0
19	Kaabong	KAB	69	401	0
20	Kotido	KOT	57	268	0
21	Napak	NAP	80	406	0
22	Moroto	MOR	61	392	0
SUB TOTAL			**411**	**2282**	
GRAND TOTAL			**808**	**4317**	**3**

Future Activities

I. Continuous surveillance and monitoring of FMDV strains present in different regions of Uganda.
II. Continue development of affordable diagnostic tools tailored to FDMV serotypes prevalent in Uganda.
III. Conduct antigenic matching for serum collected during surveillance in Uganda.
IV. Provide additional training to Ugandan scientists at MU and UVRI working on FMDV.

Glimpses of field & lab trainings conducted in Uganda in 2014

Guidelines for Implementation of a Value Chain Approach to Management of Foot and Mouth Disease Risk for Beef Exporting Enterprises in Southern Africa

G. Thomson [1,2] & M-L. Penrith[1,2]

[1]*University of Pretoria, South Africa*
[2]*ARC-OVI, Pretoria, South Africa*

New AHEAD Publication Offers Potential Breakthrough for Pastoralist Market Access and for Transfrontier Conservation

In southern Africa the vast majority of cattle are located in areas not free of foot-and-mouth disease (FMD), leaving owners of these cattle with limited access to regional and international beef markets. This situation constrains investment in cattle production, thereby limiting rural development and helping to entrench rural poverty in one of the least developed regions of the world.

For decades this situation has been accepted as irredeemable because the type of FMD prevalent in the region is maintained by wildlife from which it is technically very difficult or impossible to eliminate. Moreover, until recently, international trade rules and conventions were founded on the need for the locality of beef production to be free of FMD. Fortunately, this situation is changing and options include, among others, management of risk of FMD along a particular value chain. These guidelines are provided to inform management of enterprises based on beef production of the nature of these changes and specifically how, step-by-step, the value chain approach can now be assessed and potentially exploited to broaden market access and thereby profitability.

This presents a new vista for beef production in many parts of southern Africa, and potentially beyond. To download the **Guidelines for Implementation of a Value Chain Approach to Management of Foot and Mouth Disease Risk for Beef Exporting Enterprises in Southern Africa**, click on the icon in the upper right hand corner at http://www.wcs-ahead.org/workinggrps_kaza.html. And let us know what you think!

LAB UPDATES

The Animal Health Laboratory (AHL), Ministry for Primary Industries (MPI) New Zealand

Richard Spence, Reinhold Kittelberger, Rick Clough, Rudolfo Bueno

Completed collaborative project

In the 2014 GFRA Newsletter, we reported in detail on our collaborative project with the National Centre for Foreign Animal Diseases (NCFAD) in Canada on the FMD in deer project titled "Establishing critical diagnostic capability for foot-and-mouth disease in deer." The study included experimental infection of ten red deer with a serotype O FMD virus, sampling of 1200 uninfected red deer and evaluation of 14 FMD test methods. The project is now successfully completed. A manuscript with the findings, entitled "Foot-and-Mouth Disease in Red Deer – Experimental Infection and Test Methods Performance" has been accepted for publication in the journal "Transboundary and Emerging Diseases" and will be in print in due course. This was a highly successful collaboration between MPI's AHL and the NCFAD that met all milestones and objectives on-time and on-budget.

New Projects

The laboratory has recently applied for internal funding for two new projects relating to FMD virus diagnostic testing. The first is a follow up to the work described above and will seek to understand the performance of FMDV test methods in deer experimentally infected with serotype A and Asia 1 FMD viruses. As before, it is hoped this will be a collaborative project with NCFAD in Canada. The second is a project to investigate the utility and performance of field deployable tests for FMDV in New Zealand. We would be very interested to speak to other laboratories who have investigated or are planning to investigate field deployable tests for FMDV.

At this stage both projects are under consideration but we will hear the outcome of our funding applications by June 2015. There has been strong industry support for both these proposed projects and we are hoping that if either (or both) of these projects are approved industry will work with MPI to realise them.

International Collaborations

In May 2014 another member of AHL staff attended the excellent FMD diagnostic training course run by the FMD World Reference Laboratory in Pirbright. As always this provided an invaluable opportunity to gain first-hand experience of working with live FMDV (New Zealand is FMD free) and to network with colleagues from around the world involved in FMD diagnostic testing. AHL have sent several members of staff on this training in recent years as part of its ongoing FMD preparedness work and it is greatly valued by AHL and MPI.

CODA-CERVA, Belgium

David Lefebvre[1], Annebel De Vleeschauwer[1], Andy Haegeman[1], Ilse De Leeuw[1], David Ehizibolo[2], Jean-Baptiste Hanon[3], Steven Van Borm[4], Elliot M. Fana[5], George O. Mathlo[5], Joseph M.K. Hyera[5], Kris De Clercq[1]

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[2]National Veterinary Research Institute, Vom, Nigeria
[3]CDD-ERA, OD Interaction and Surveillance, CODA-CERVA, Belgium
[4]Molecular Platform, OD Virology, CODA-CERVA, Belgium
[5]Botswana Vaccine Institute, Gaborone, Botswana

New projects

The CODA-CERVA is involved in the ERA-Net ANIHWA (Animal Health and Welfare) research project Transcriptovac that started in March 2015 with a duration of 36 months. The full project title is 'Host response gene signatures associated with FMDV infection, vaccination and persistence'. This collaborative project brings together five public research groups from four EU countries and one private international company. The project coordinator is Stéphan Zientara from the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). The aims of the project are 1) to identify innate immune gene signatures that are associated with long-term antibody

Workshop at NVRI, Vom, Nigeria

Field sampling team

responses in sheep to define molecular targets for the development of new adjuvants and vaccines, 2) to identify factors within pathogen and host gene signatures associated with FMDV infection and persistence that can be targeted to prevent persistent infection with FMDV and 3) to develop novel viral vectored vaccines that could enhance the adaptive and local immune response against FMDV. The CODA-CERVA is particularly involved in the evaluation of long-term antibody responses in sheep in WP1 and in the preliminary evaluation of novel viral vectored vaccines in guinea pigs in WP3.

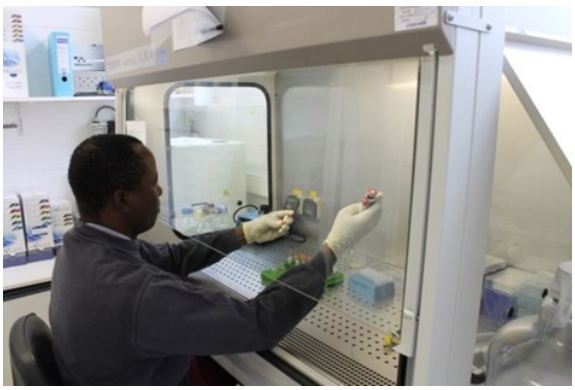
International collaborations

Since June 2014, the CODA-CERVA has been 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. From November 4th to November 13th 2014 Annebel De Vleeschauwer and Jean-Baptiste Hanon from CODA-CERVA organized a first workshop in Vom that was attended by 12 executives, scientists and technicians from NVRI. The workshop topics included epidemiology, laboratory techniques and biosafety, field sampling procedures and sample shipment. Several pastoralist herds were visited and samples were taken in duplicate for analysis in NVRI and CODA-CERVA, respectively. From November 17th till December 12th 2014, David Ehizibolo visited the Unit for Vesicular and Exotic diseases from the CODA-CERVA. David received training in biosafety procedures in a BSL3 laboratory and routine serological and virological diagnosis of FMDV. Particular attention was given to viral RNA extraction from different sample matrices, real-time RT-qPCR and molecular characterization of field samples from Nigeria including sequencing of the FMDV VP1 gene, sequence analysis and phylogeny. David also represented the NVRI at the annual meeting of the OIE/FAO Reference Laboratories Network at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) in Brescia, Italy on November 26–27th 2014 where he presented an update of the regional FMDV situation in Nigeria and West Africa. From April 14th to July 7th 2015 David Ehizibolo will receive extensive 3-month training at the CODA-CERVA with particular emphasis on full characterization of field samples from Nigeria. In addition, two-week technical training courses are scheduled for employees of NVRI at CODA-CERVA.

Field sampling of pastoralist herds

Preparation of samples for shipment

David Ehizibolo at CODA-CERVA



Analysis of samples at CODA-CERVA

The CODA-CERVA, as an OIE Collaborating Center, has a **bilateral collaboration** with the **Botswana Vaccine Institute (BVI)**, an OIE Reference Center. On June 4th 2014, the CODA-CERVA was visited by an official delegation from Botswana including eminent members of BVI, the Ministry of Agriculture, the Department of Veterinary Services and the Embassy of Botswana. The delegation attended a general presentation of the CODA-CERVA and a presentation of the Unit for Vesicular and Exotic diseases as an NRL and research center for FMDV. The visit was completed with a guided tour of the outer box of a BSL3 laboratory, with visual access to the inner part of the BSL3 laboratory. From July 28th to August 8th 2014,

LaToya Seoke from BVI received two-weeks training at CODA-CERVA with particular emphasis on viral RNA extraction from different sample matrices, real-time RT-qPCR and molecular characterization of field samples from Botswana including sequencing of the FMDV VP1 gene, sequence analysis and phylogeny.

Funding opportunities

The CODA-CERVA is involved in the **ERA-Net ANIHWA** research proposal **DiagnosTech** that was submitted in February 2015. The full title is 'Realising the potential of new diagnostic technologies to control livestock diseases'. This project proposal brings together eight public research groups from eight EU countries and is coordinated by Donald King from The Pirbright Institute, UK. The aims of the project proposal are 1) to expand the scope of technologies for rapid, simple and decentralised diagnostics (novel laboratory and field based assays and those suitable for low-resource laboratories), 2) to increase confidence in their use, 3) to maximize potential uses and 4) to develop a robust business case for these new technologies. The CODA-CERVA is particularly involved in the field validation of FMDV penside diagnostics tests through existing collaborations in Nigeria and Botswana, including training of end users and confirmatory testing and in the molecular characterization of viral nucleic acids recovered from lateral flow devices.

National Centres for Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba and Lethbridge, Alberta, Canada

Soren Alexandersen

New Projects

We are currently working on the development of multiplex bead-based assays for simultaneous diagnosis of vesicular diseases (foot-and-mouth disease, swine vesicular disease and vesicular stomatitis) and serotyping of FMD in livestock. With a multiplex bead-based assay, FMD, SVD and VS can be assayed at the same time in one assay format, reducing both the time and amount of serum sample that is required for separate assays. Furthermore, the FMD serotyping assay will combine NSP and VP1-based multiplex Luminex immunoassays to enable the detection of infection and identification of FMDV serotype at the same time.

The NCAD is part of a consortium of Canadian, US and UK partners working on the use of oral fluid samples to monitor virus shedding and antibody responses in pigs experimentally infected with high consequence swine viruses (foot-and-mouth disease, African swine fever, swine vesicular disease and classical swine fever viruses). Oral fluid (OF) samples are easy to collect and use in the assessment of group/herd health status and this is becoming a more attractive sampling option for most swine production systems. Some commercial kits for endemic diseases have been adapted for use with this sample type. Similarly, work has been done on the use of OF in FMD, CSF and ASF diagnosis. However, these assays based on OF haven't been developed and/or fully validated for routine surveillance of these and other exotic swine diseases. Development and/or validation of OF-based assays for foreign animal diseases (FADs) are therefore critical to ensure potential use of this sample type for FAD diagnostic assays.

International Collaborations

CSIRO-Australian Animal Health Laboratory (AAHL), Geelong, Australia
Iowa State University, Ames, Iowa, USA
The Roslin Institute, University of Edinburgh
RapidAssays ApS, Copenhagen S, Denmark

Science Report

A multiplex lateral flow immunochromatographic strip test (multiplex-LFI) was developed for rapid detection and serotyping of FMD viruses. The FMDV serotype specific monoclonal antibodies (mAbs) were used as capture antibodies and a colloidal gold-conjugated serotype independent mAb was used as the detection mAb. The strips used in this study contained one control line and three test lines, which correspond to one of the three serotypes, O, A or Asia 1.

The newly developed multiplex-LFI strip test was able to specifically identify serotype O (n=46), A (n=45) and Asia 1 (n=17) in all tested field isolates. The sensitivity of this strip test was comparable to the double antibody sandwich ELISA for serotypes O and A, but lower than ELISA for serotype Asia 1. All tissue suspensions collected from animals experimentally inoculated with serotype O, A or Asia 1 were identified as positive by the multiplex-LFI strip test. The capability of the multiplex-LFI strip tests to produce rapid results for multiple serotypes of FMDV make it a valuable tool to detect FMDV at outbreak sites.

A competitive enzyme-linked immunosorbent assay (cELISA) for FMDV/A antibody detection was developed using two mAbs (F66A22-14 and F67A22-18) that demonstrated high ability to compete with the polyclonal anti-FMDV/A serum. The cELISA demonstrated positive antibody response at 4-5 days post inoculation for bovine, porcine and ovine experimentally inoculated with FMD A24 Cruzeiro or A22 Iraq 24/64. To investigate antibody responses in FMDV/A vaccinated animals, sheep were vaccinated with the FMDV A22 Iraq vaccine and challenged with A/Vietnam/13 (in collaboration with AAHL, Australia). The A/cELISA detected positive antibody responses at 9 dpv/5 dpi for all 6 animals and remained positive until the end of experiment (35 dpi). This result was in accordance with that of the VNT. Meanwhile the cELISA was negative for antibody responses in serum samples from sheep vaccinated and challenged with FMDV serotype O indicating that the cELISA is serotype specific. This is a simple, reliable test to detect antibodies against FMDV serotype A in infected and/or vaccinated animals.
A new isothermal assay for detection of FMD on a portable user-friendly instrument is being validated. The user-friendly

assay successfully detected viral RNA from a large and diverse panel of 61 FMDV strains representing all seven serotypes and showed no cross reactivity with VSV, SVDV and VESV. The assay also detected FMDV RNA in extracted clinical samples, as well as unprocessed vesicular fluid from experimentally infected animals.
A previously developed multiplex assay for detection of FMDV and other high consequence livestock viruses is being integrated into a fully automated assay that does not require user-handling after sample introduction. To date, virus-specific capture probes have shown good reactivity with target pathogens, and no cross reactivity with a large panel of bacteria and viruses associated with livestock.

CSIRO-Australian Animal Health Laboratory, Geelong, Australia

Wilna Vosloo, Jacquelyn Horsington, Jacqui Morris, Tim Bowden, Nagendra Singanallur

Introduction

The FMD Risk Management Project (FMD-RMP) was originally identified as being of benefit to Australia following wide-spread consultation with all relevant livestock industries and government departments. The project is in its 5th year and it is satisfying to reflect on the lessons learned and to celebrate our successes.

Due to the restrictions on importing live FMDV into Australia, we engaged several international animal facilities as collaborators for this work, to enable us to work with a variety of animal species and perform laboratory assays involving the live virus. Without the support within the GFRA community, this would not have been possible.

The project to date has focused largely on assessing the effectiveness of the vaccines held in the Australian Vaccine Bank against viruses currently circulating in South East Asia (SEA). A number of trials have been performed involving cattle, sheep and pigs to ascertain whether the vaccines will protect these animals against clinical disease and measure the extent of the immune response. In addition we have studied the course of disease through viraemia (blood) and the amount of virus excreted in secretions such as saliva, nasal fluids, and oro-pharyngeal scrapings (probangs), information that will assist vaccination strategy decisions by Government. The project is also addressing other objectives including capacity building and networking with laboratories in SEA and further abroad to improve our diagnostic capability and surveillance.

The first four years of the project contributed significantly to Australia's FMD preparedness in the areas of:

- vaccine efficacy and matching
- disease pathogenesis
- diagnostic capability
- virus evolution and trends
- capacity building in Australia and SEA

This information will be valuable to government to help model and predict disease dispersion in Australia and will assist with the design of FMD control strategies for the nation.

Summary of Results

The vaccine efficacy studies have shown that for most viruses and vaccines tested to date, the antigenic relatedness (r1-values) between the outbreak and vaccine strains are not an accurate indicator of protection with high potency vaccines. Even when *in vitro* predictions suggested that the vaccine would not protect, the trials *in vivo* showed that high potency vaccines, such as those Australia will receive in an outbreak, protected against clinical disease. And in most cases, the animals were challenged using methods that are more severe than would be expected during an outbreak (needle infection versus aerosol), further indicating that the vaccines are efficacious. However, even in the absence of clinical disease, the vaccines did not always prevent virus replication, a well-known fact about the inactivated FMD vaccines. In these cases, vaccination decreased the amount of virus found in bodily excretions and will therefore decrease the amount of environmental contamination, assisting with containing disease. It was also evident from the results that some virus strains are more virulent and that vaccination may not be as efficacious against those viruses in certain species. These findings will help refine strategies by species to optimise the benefits of vaccination during an outbreak. The results also indicated that the time between diagnosing an outbreak and the decision on the most suitable vaccine strain, could most likely be made based on nucleotide sequence data that could be available within less than 24 hours, compared to the r-values, that could take up to 4 days to perform, therefore decreasing the time prior to ordering the vaccine.

Collaborative *in vitro* surveillance work with the OIE Regional Reference Laboratory (RRL) based in Pakchong, Thailand indicated that for serotype A there is a constant evolution of virus strains, with new genetic lineages arising over time with variable antigen matching results with the Australian vaccine strains. More work is needed to follow these changes over time with other serotypes as well, and also to use these results to guide the *in vivo* challenge studies. Despite the findings so far, we need to continue with live animal challenges to ensure no new variants emerge that could completely escape vaccine protection.

Future Work

During 2015, the following trials are planned:

a. During 2014–15, a different lineage of serotype O (O/IND/2001), previously confined to Asia and the Middle East, spread into northern Africa and caused severe outbreaks. The *in vitro* assays indicated that the current serotype O vaccine strains may not be fully protective against this newly emerged virus and we plan to test the efficacy of the serotype O vaccine strains in the Australian bank for protection at 7 and 21 days post vaccination (dpv) in cattle in collaboration with the Central Veterinary Institute, Lelystad, The Netherlands, and Merial, Lyon, France.

b. In addition, in 2008, a new lineage of Asia-1 was identified (Asia1/Sind-08) that had very poor antigenic matching to the current vaccine strain. Some evidence has emerged that high potency vaccines will be efficacious against this virus, but no experimental data are available for sheep. The intra-nasopharyngeal route of inoculation will be used to assess the pathogenesis and determine the efficacy of the vaccine at 7, 14 and 21 dpv in collaboration with the National Centre for Foreign Animal Diseases (NCFAD), Winnipeg, Canada.

c. Finally, we will test the efficacy of A MAY 97 and A IRQ 22 in pigs at 7 and 14 dpv against challenge with the 2014 SEA variant of serotype A in collaboration with NCFAD.

UPCOMING EVENTS

Understanding Disease Ecology, Transmission and Vaccine Matching Toward FMD Control

* Role of Asian buffalo in FMD ecology
* Vaccines: quality control, post-vaccinal monitoring, improvement in vaccines, vaccine matching, challenges
* Development and application of diagnostic tools
* Socio-economic impact of FMD
* Disease modelling

Venue: Pullman Hotel, Hanoi

Important Dates

Deadline for early bird registration and abstract submission	Monday 27 July 2015
Deadline for sponsorship applications:	Monday 29 June 2015

Website: www.gfra2015.org

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

CSIRO
Australian Animal Health Laboratory

GFRA
Global Foot-and-Mouth Disease Research Alliance

The GFRA Executive Committee

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