Issue 8
June 2018

GFRA

NEWSLETTER

Fighting Foot-and-Mouth Disease together

Global Foot-and-Mouth Disease Research Alliance

EuFMD OS 2018
Puglia, Italy 29–31 October 2018

Dedication to Dr. Leslie Lobel

MD, PhD, Virologist and Infectious Disease Physician

(1956-2018)

Leslie Lobel, MD, PhD, was an American-born Israeli virologist and physician working on infectious diseases at Ben-Gurion University of The Negev (BGU), Israel, where he was a lead researcher developing diagnostics, vaccines and therapeutics against infectious diseases, of both humans and animals, primarily Ebola and Foot-and-Mouth disease.[1] He was the Chair of Department of Virology and Developmental Genetics, and Vice Chair of the Department of Microbiology, Immunology and Genetics at BGU. [2] Leslie was also a member of the Global Foot and Mouth Disease Research Alliance (GFRA). Partnering with both the U.S. military and the Uganda Virus Research Institute, he was developing human monoclonal antibodies as a therapeutic which could be manufactured and administered to people to provide rapid immunity against a number of infectious diseases. He hoped to create a "passive" vaccine which would offer protection immediately after being given.[3]

Early years: Born in Queens, New York, Lobel received his B.A., Summa Cum Laude, in chemistry, from Columbia College of Columbia University, followed by a Ph.D. in virology. He received an M.D. from Columbia University College of Physicians and Surgeons in 1988. [2] He also did post-graduate work at M.I.T. in Boston. He stated that he went into virology since the field was small and less studied. Later, his interest expended also to oncology and the study of human immune response to cancer.

Late years: After his transition to BGU, Leslie focused his work on isolating monoclonal antibodies for various viral diseases including West Nile Fever, Hepatitis C, foot-and-mouth disease and Ebola.
He was one of the world's experts [4] and the only academic researcher in Israel working on Ebola.[5] “Ebola is the most lethal virus," he stated. "It can kill 90% of those afflicted. And there is no therapy to counter it. There is a vaccine thus far tested in animals, but it takes a

relatively long time to build immunity."[6] As monoclonal antibodies are proteins that attack specific targets, including viruses, bacteria or other foreign bodies, "the antibodies will neutralize the virus, and avoid the dangerous side effects of the existing vaccine," he said. He anticipated that antibodies therapy will also be useful for treating other diseases like smallpox in the event of an outbreak, and would give protection immediately instead of weeks or the month required by typical vaccines.[6]

During his Ebola research, he used to travel constantly to Uganda, with the aid of U.S. and other foreign grants, where he studied a variety of viral hemorrhagic fever (VHF) viruses. The research focus on understanding the residual memory immune response was done by obtaining and analyzing blood samples from survivors of Ebola and other VHF diseases. Currently, there are over 100 Ugandan survivors of Ebola and other VHF infection that he studied during his visits, checking their humoral and cellular immunity levels over time, trying to discover how their immune system helped them survive. Such personal surveys would be less possible, he said, had not Israel invested time and effort in partnering with African medical organizations over many decades and gained their trust.[7]

Lobel added that besides Ebola, there are other "bad" viruses out there, which the developed world prefers to ignore so long as they aren't affected.

Dr. Lobel strongly believed that working together to control infectious disease through engagement partnerships we will not only have a positive influence on economic development but will also facilitate mutual understanding between different cultures, and in the end this will win hearts and minds. An additional example of this approach, was seen in one of his latest studies, where he collaborated with the United States Department of Agriculture (USDA), Agricultural Research Service (USDA-ARS) and the Cooperative Biological Engagement Program (CBEP) of the Defense Threat Reduction Agency (DTRA) in a project aimed facilitate biological research and disease management by designing novel countermeasures for the progressive control of FMD in Uganda and the development of novel improved diagnostics.

As Leslie once said, "Although integration of cultural differences between societies remains a challenge for globalization in the 21st century, the one thread that binds us all is infectious disease. … It is a common bond of mankind that can unite us all".

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FMD Exposed - Camillids To the Rescue: Introducing Camelid Nanobodies for FMDV DIVA Diagnostics in Economically Constrained Environments

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Foot-and-mouth disease (FMD) is an extremely contagious viral disease of cloven-hoofed animals, most notably cattle, pigs and sheep [1]. Despite recent successes in controlling the disease in Europe and some parts of South America, FMD remains one of the most important infectious disease of farm animals due to the possible impact of an FMD outbreak on trade in animals and animal products [2]. The FMDV exists in the form of seven serologically and genetically distinguishable serotypes named A, O, C, Asia I, and South African Territories (SAT1, SAT 2, and SAT3), with multiple subtypes within each serotype [3]. Epidemiological studies on outbreaks incidences showed that six of the seven serotypes of FMD (O, A, C, SAT1, SAT2, and SAT3) have occurred in Africa [4].

Today, global FMD control strategy includes reliable and effective surveillance and is greatly supported by competent laboratory diagnostic services [3, 5]. Currently, most detection assays for FMD DIVA relies on antibodies to NSPs targets mainly 3ABC [6-9], and several commercial tests (kits) are available [10]. Although used worldwide these tests vary in sensitivity and specificity and are expensive for developing countries [10, 11]. As such, development of a simple, rapid, sensitive and broadly reactive DIVA diagnostic that is extremely cost effective for economically constrained regions of the world is still much in need [5]. With the introduction of new technological platforms implanted into the field of diagnostic, camelid-derived single-domain antibodies fragments so-called Nanobodies, have demonstrated great efficacy for the development and improvement of serological diagnostics [12]. Their high solubility and stability coupled with strong antigen affinity have made them a great new generation of detection component for various diagnostic applications [13].

To address the necessity for effective inexpensive and economically sustainable FMD diagnostic kits to meet the needs of developing countries and the world, a novel Nanobody-based FMD ELISA, for the serological detection of antibodies against FMD Non-Structural Proteins (NSP) was developed and validated. The work lead by researchers from Ben Gurion University in Israel, the United States Department of Agriculture, the Uganda Virus Research Institute in Uganda, and the Uganda Ministry of Agriculture was part of a new research project in designing novel countermeasures for the progressive control of FMD in Uganda and the

development of novel improved diagnostics. Such capacities' in return will result in a positive impact on the country economy, facilitate capacity building and enhance educational programs and positively impact human- and regional security in Uganda. The pilot study had focused its efforts on meeting FMD challenges in Uganda, a perfect environment, given its complex and diverse FMDV ecosystem. As part of this new research project, a set of nanomolar affinity anti-FMD NSP nanobodies were isolated, purified and characterized for their capacity to be use as diagnostic components. These nanobodies were tested in several ELISA formats with cattle sera collected in Uganda and Israel, which represented various FMD status. Using a specific high affinity Nanobody, as a competitive component, an in-house competitive ELISA for the detection of FMD NSP antibodies in cattle serum was eventually design and validated. The new developed assay demonstrated high diagnostic sensitivity and specificity of above 95%, with the capability to detect NSP-specific antibodies against multiple FMD serotype infections.

Since every assay development has its own sets of merits and demerits various parameters that extensively differ between non-endemic and endemic surroundings, such as those seen in Uganda, were taken under consideration during the assay design to fit the country needs. Further studies with large sample cohorts and across different animal species must still be carried out to completely validate the assay performance, before regulatory authorities can adopt it for routine use. However, this novel, sensitive and specific NSP ELISA, clearly demonstrate a strong potential to be used as an alternative/ supplemental way for simple, low-cost and effective countermeasures for routine disease diagnosis, and the improvement of FMD management and control in endemically complex environments, such as those found in Africa.

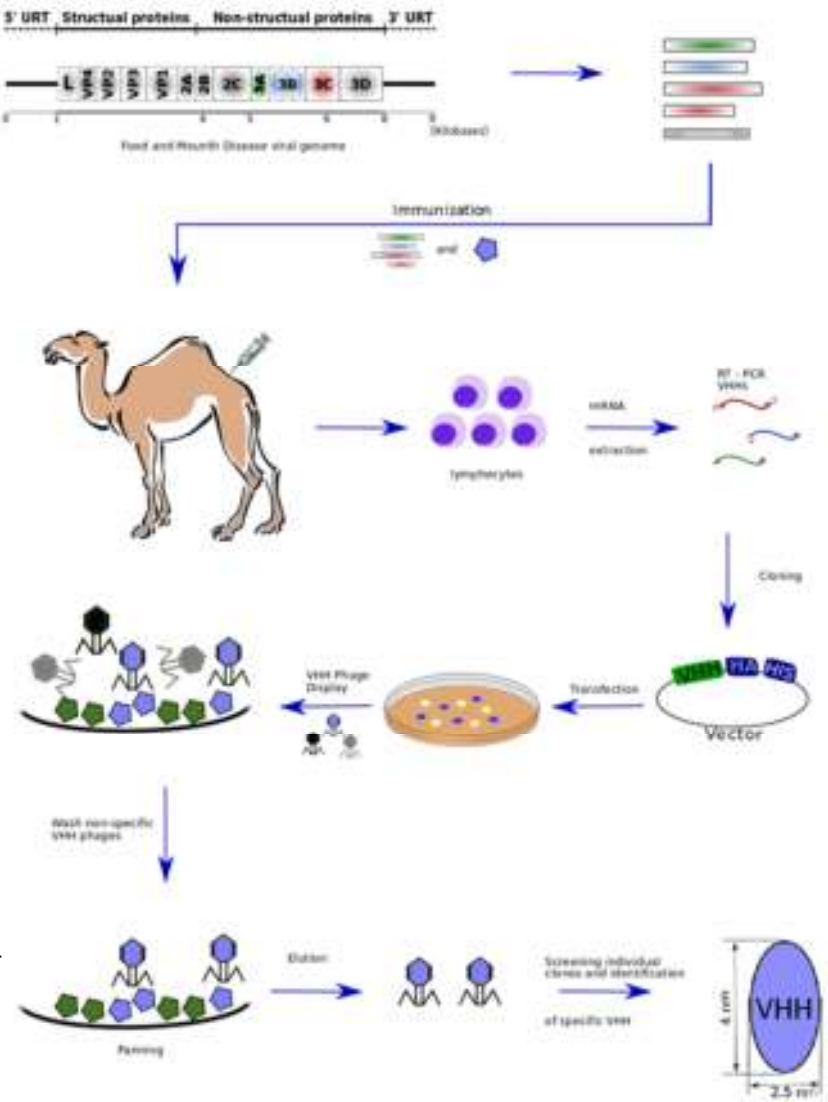

Figure 1. Schematic over-view of anti-FMD NSP Nanobodies generation .

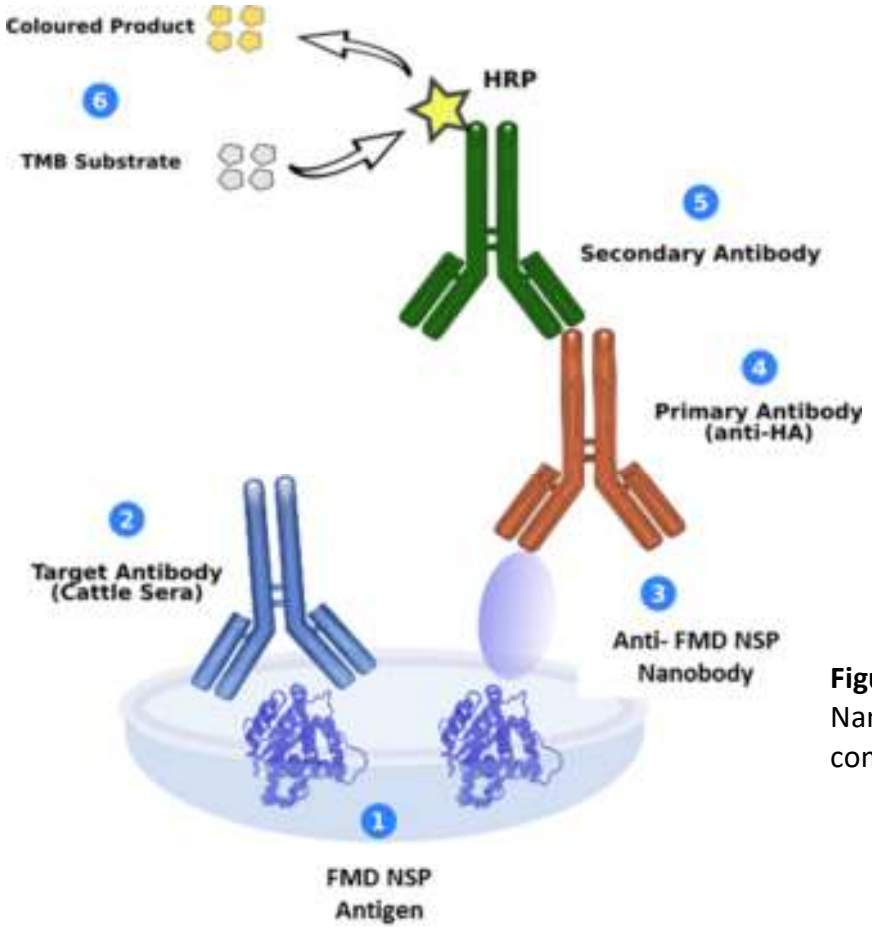

Figure 2. Schematic overview of the Nanobody based in-house FMD NSP competitive ELISA.

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GFRA scientific meeting – Incheon, South Korea 25-27 October 2017

The sixth GFRA Scientific Meeting was held on the 25th–27th October 2017 in Incheon, South Korea, attended by more than 150 people from at least 20 different countries.

The meeting was dedicated to the memory Dr. Ngo Thanh Long, who was GFRA president from 2014–2015. Dr. Long was a very productive FMD researcher who collaborated with many GFRA scientists from different countries and his untimely death leaves a gap within the GFRA family.

The organising committee consisted of the executive committee of GFRA, members of the Animal and Plant Quarantine Agency in Korea as well as Prof Young Lyoo from the Konkuk University, Korea. A local company, InnoN, provided the logistical support while CRDF Global provide invaluable secretarial support.

The GFRA currently has 52 GFRA partners, collaborators and stake holders from over 26 different countries. We aim to foster research cooperation and technical exchange to complement and enhance each partner's specific expertise. As a result of the GFRA framework, there are dozens of collaborative research projects among GFRA partners, collaborators and stakeholders throughout the world.

To support this already strong alliance, the overall theme for the biennial meeting was Science and Innovation for FMD Control and Response. Every day addressed a theme with supporting sessions. With 53 talks and 86 posters, the programme was jam-packed with exciting research.

Theme: FMD in global perspective	
Session	Global and regional disease status reports
	FMD in Korea, remarks from APQA
	FMD Ecology and Epidemiology: Differences in Africa and Asia
	Socio-Economics of FMD: Endemic and Non-endemic Settings
Theme: Understanding and combatting FMD	
Session	FMD vaccines in the 21st century
	FMD in swine: pathogenesis and immunology
	Vaccine efficacy/potency testing
	Vaccine delivery routes and adjuvants
	Research on diagnostics, including sample collection and management and disinfection
	Intervention strategies and control methods
Theme: Research gaps for the control of FMD	
Session	FMD modelling: connecting theory, experiments and reality
	Persistent FMD: old problem – new knowledge
Workshop: Research gaps identified	

The workshop during the last morning utilised the experience and knowledge of those at the meeting and was an introduction to a further gap analysis that GFRA will perform in 2018 to identify the most pressing areas that need attention to allow us to better control FMD. The outputs will be used to direct research focus areas.
Fortunately, there was time for fun and interaction, and thanks to our generous sponsors, the functions were entertaining, not to mention gastronomical delights.

The Defense Threat Reduction Agency, the Global Challenges Research Fund and the Pirbright Institute sponsored participation of a great number of delegates from developing countries. In addition, there were a number of commercial sponsors that contributed to a very successful meeting.

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SEPPIC,
Intercare,
Life Technologies,
MSD Animal Health.

Meeting the people who worked so hard to make it all happen

Development and application of a European foot-and-mouth disease spread model – EuFMDiS

Graeme Garner, Mark Hovari, Keith Sumption
European Commission for the Control of Foot-and-Mouth disease (EuFMD)

Transboundary animal diseases like foot-and-mouth disease (FMD) have the potential to spread rapidly and cause severe economic impacts. Disease managers face many challenges when developing plans and policies to deal with potential outbreaks, such as choice of control strategy (including if, when and how to use vaccination), managing potentially scarce resources and effective post-outbreak management strategies to regain FMD-free status as quickly as possible. Disease models are increasingly being used to support disease planning and preparedness in many countries. However, where countries share land borders, modelling outbreaks at a single country level fails to take into account the complexities of transmission risks and outbreak management. This is particularly the case where there is normally (before an outbreak is confirmed) relatively free movement across borders as occurs between the FMD free countries of Europe which are part of the EU or accepted into the common system for trade in livestock and livestock products. Improving readiness to manage an FMD outbreak is a priority of the EuFMD program and improving the use of decision-support tools has been a focus of EuFMD training. Two EuFMD-funded disease modelling training workshops were held in 2014 and 2016. They used the Australian Department of Agriculture and Water Resources' foot-and-mouth disease (FMD) model, the Australian Animal Disease Spread Model (AADIS) (Bradhurst et al., 2015) as a training tool. These workshops were viewed very successfully by participants and the potential to more widely use disease models to support FMD preparedness in Europe recognized.

Following a regional disease modelling initiative proposed by Austria in 2016, a proposal for a European multi-country modelling project was submitted for funding under EUFMD's Funds for Applied Research (FAR) and approved in 2017. The project was approved in October 2017. Under this pilot project, EuFMD is working with seven central European countries (Austria, Bulgaria, Croatia, Italy, Hungary, Romania and Slovenia) to develop a multi-country FMD disease modelling capability to support FMD planning, training and exercises. The project objective is to develop a modelling tool (the European FMD Spread – EuFMDiS – model) that can be used to simulate spread and control of FMD both within and between participating countries.

A workshop of the participating countries was held in Vienna, Austria in December 2017. The aim of this initial workshop was to bring together the countries and other interested parties with EuFMD experts to discuss the scope of the project. The main output of the workshop was to agree on the country-specific data required to set up and parameterize the EuFMDiS model. Another key outcome was a set of agreed milestones, with the aim to have a working model prototype by 30 June 2018.
In addition to considering scope and functionality of a European multi-country FMD spread model, the Vienna workshop also discussed the data required for setting up livestock populations for disease modelling studies and information needed to simulate disease transmission and control measures. An important step was to identify regions within countries to capture the major environmental aspects and livestock production and marketing characteristics, and agree on common definitions for classifying herds based on size, production type and FMD risk. The country herd data files used are provided in a fully anonymized format. Approaches and data required for representing FMD transmission within and between countries was discussed. For between country spread, countries would use

their access to the Trade Control and Expert System (TRACES) that records commodity movements between EU Member States. Relevant and de-identified data on livestock movements was extracted with the help of instructions and an "R" script. Another key feature of the development has been to ensure that nomenclature and control measures for FMD control are consistent with the European Union legislation. Finally, data requirements for modelling disease control, including resources, and approaches to assessing the economic impact of outbreaks and costs of control measures were also discussed.

EuFMDiS is based on the conceptual hybrid modelling approach developed for the AADIS model (Bradhurst et al. 2015, 2016). FMD transmission within herds is simulated using equation-based modelling (EBM) and transmission between herds is simulated using agent based modelling (ABM). The ABM allows for multiple pathways of transmission including local spread, spread associated with animal movements or indirect contacts (contaminated products, people, vehicles, etc), longer distance wind-borne spread, and spread associated with livestock assembly centres. Disease control is based on the measures described in the European FMD directive (European Union 2003). Effectiveness of control depends on availability of resources to implement the prescribed measures.

The project is progressing well. Countries have defined livestock production regions, and herd datasets and between country spread information has already been provided by most of the countries. Figure 1 shows the participating countries and livestock production regions.

The work to date has focused on software development, supporting the participating countries in data analysis and preparing instructions and data reporting templates that will assist new countries to join the project in the future. In order to ensure close cooperation and mutual support of the participants, regular on-line meetings hosted by EuFMD, with the help Adobe Connect software are held to discuss the current steps and share experiences. The drive of this small, but dedicated community conveys a strong sense of ownership and is able to deliver despite the ambitious time frame. The staff of EuFMD are ensuring that question and requests made by the countries involved in the pilot are met in a timely and proficient manner. The project is on track to deliver a functional model by June 2018.

The current project will end with a second workshop, planned in Budapest, Hungary in July 2018. The aim of the second workshops is to install the EUFMDiS software and train local within-country experts on the use and application of the model. All of the countries participating in the pilot project will receive within this package a "user manual" that will support subsequent use of the model.

Future developments could include adding additional countries, adaption of the model to other diseases and incorporation of a wildlife component.

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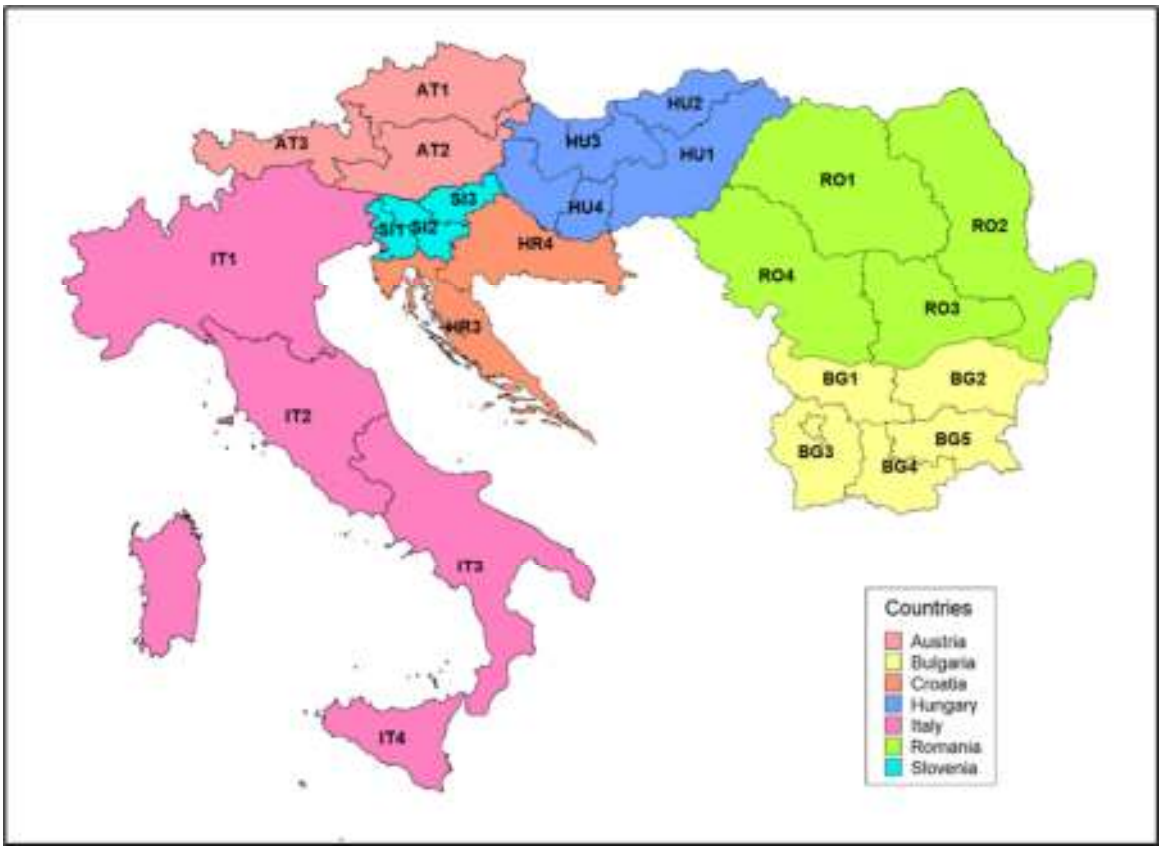

Figure 1: Participating countries (n=7) and livestock production regions (n=25) for the EuFMDiS Project.

Foot-and-mouth disease virus vaccine quality

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Introduction

FMDV vaccination is one of the best tools to control FMDV transmission (Orsel, 2008). To assess the quality of FMDV vaccine, standards have been set by the World Organisation for Animal Health (OIE) and the European Pharmacopoeia. The current paper will discuss which standards should be applied dependent on the objective of the study. The same standards can be used for quantifying heterologous protection. Sera from standardised vaccine trials can be used for defining serological criteria for vaccine release as well as post-vaccination monitoring.

FMDV vaccine control

The OIE has defined clear standards for FMDV vaccine control; the vaccine should contain at least 3 PD50/dose, where the PD50 is the dose that protects 50% of the animals, or protect at least 75% of the primo vaccinated cattle against homologous challenge (the PGP test is mostly used in South-America). In both approaches, cattle are vaccinated and 3 - 4 weeks after vaccination challenged by injection of FMDV into the dermis of the tongue. Cattle are deemed protected when they do not develop foot lesions (OIE, 2017). To replace, reduce and refine the use of animals in experiments, these challenge tests should only be performed for registration of new vaccine strains and correlates between the immune response and protection should be established to enable the release of future batches using only serology, or even better, by assessing the antigen concentration and adjuvant quality of the vaccine.

Table 1: Percentage of outcomes that pass the criterion 3 PD_{50}/dose or 75% protection			
	True potency of the vaccine (PD_{50} per dose)		
	3	6	15.7
Spearman Kärber ≥ 3 PD_{50}/dose	55 %	89 %	99.8 %
12 or more out of 16 cattle protected	56 %	90 %	99.8 %

There has always been a discussion between the use of the PGP test where 16 cattle are vaccinated with a full dose and 75% should be protected, and the PD50 studies, in which cattle are vaccinated with 3 different doses of the vaccine (often 1, ¼ and 1/16 dose) and the volume that protects 50% of the cattle is determined. If the cut-off is set at at least 75% protection, or at least 3 PD50/dose, then both tests perform similarly (Reeve et al., 2011). Based on the dose response relation (Jamal et al., 2008) the probability of each possible outcome in a potency test can be calculated. Based on that probability the percentage of vaccines passing the test, given a defined potency, can be calculated (table 1).

The advantage of the PD50 experiment is, however, that in addition to determining if the vaccine passes the criterion, it also allows calculation of quantitative information on the potency of the vaccine. This quantitative potency is essential to determine whether or not a vaccine will protect against heterologous challenge (see below).

As stated before, challenge experiments should be limited to those in which the correlation between the immune response and protection is determined. To determine this correlation, approximately 50% of the vaccinated cattle should become infected. So for good quality vaccines a fourth dose should be added to the standard PD50 experiment (full, ¼, 1/16 and 1/64 dose) (Dekker et al., 2016). There have been many studies on correlation between antibody response and protection. In both cattle and pigs, antibody responses correlate very well with protection (Eblé et al., 2009; Goris et al., 2008; Mackowiak et al., 1962; Maradei et al., 2008; Pay and Hingley, 1986; Terpstra et al., 1976; van Bekkum, 1969; van Maanen and Terpstra, 1989). The correlation between cellular immune responses and protection has also been evaluated (Parida et al., 2006), but it is statistically less strong than that between antibody response and protection. Usually, for a VNT or an ELISA it is easy to repeat a failed test, but tests for quantifying cellular immunity are difficult to repeat or to standardise.

Antibody titres can be determined using the virus neutralisation test (VNT) as well as ELISAs (Dekker, 2008). Recently isotype specific ELISAs as well as an avidity ELISA were evaluated (Brito et al., 2014). The ROC curves of both ELISAs and VNT had a similar area under the curve, so from a statistical point of view there is no advantage in adding these isotype specific and avidity tests unless it is not possible to do the VNT. Standardisation of tests is, however, an important international issue, not only in diagnostic tests (Mackay et al., 1996) but also in vaccine evaluation (Dekker, 2008). It was shown that inclusion of a standard serum (e.g. a serum equal to 50% protection in cattle) will help the standardisation between laboratories, and can be useful for post-vaccination monitoring (see below).

Heterologous protection

The current international standard to predict heterologous protection is the r1-value, which is the quotient of the arrhythmic heterologous titre and the arrhythmic homologous titre of a post-vaccination/infection serum raised against the vaccine strain. Although a valuable parameter, it is only one part of the equation. Heterologous protection is best determined using a potency test with heterologous challenge. In that case one could assume that the outcome of the heterologous challenge and the outcome of the homologous challenge would be:

Heterologous potency = vaccine match x homologous potency

So heterologous protection depends not only on the vaccine match, but also on the homologous potency. In the equation above the "vaccine match" is the quotient between heterologous and homologous potency. In some studies this "vaccine match" corresponded very well with the r1-value (Fishbourne et al., 2017; Nagendrakumar et al., 2011), but there is currently insufficient data to evaluate this fully. Using the equation above, one can estimate the minimal vaccine match if the homologous potency is known. E.g. if the homologous potency is 6 PD50/dose and it is assumed one needs at least 3 PD50/dose against the heterologous strain, then the "vaccine match" should not be below 0.5. In the study reported by Fishbourne et al. (2017) the estimated homologous potency was 17 PD50/dose. So an r1-value of 0.2 was sufficient to yield a heterologous potency larger than 3 PD50/dose.

A well-established relation between antibody response and protection is only available for a limited number of vaccine strains, although vaccine producers have the data, these are not publically available. More studies should be performed in which it is possible to establish this relationship, as well as the development of standard sera (e.g. equal to 50% protection) to standardise tests between laboratories.

Post-vaccination monitoring

Determining the antibody response in a vaccinated population was the standard when Europe still was using prophylactic vaccination, but data were only published in a few cases (Fish et al., 1969; van Bekkum et al., 1969). Nevertheless, most European countries considered evaluation of vaccine responses after vaccination essential to determine both the quality of the vaccine and the vaccination campaign.. Quality of the vaccine can best be determined by vaccinating a small number of cattle close to the point of entry of the vaccine. Efficiency of the vaccination campaign should be a risk based study in which cattle in various parts of the country are sampled. It is then essential that the vaccination sampling interval is approximately the same in each study, and can be correlated with protection in potency tests (see above), e.g. by inclusion of a standard serum which relates to 50% protection. Both VNT as well as (commercial) ELISAs can be used for this post-vaccination monitoring (Smitsaart et al., 1998).

Conclusion

Although very valuable, cross-protection studies have shown that good quality vaccines can cross-protect (Brehm et al., 2008). However, many studies lack quantitative estimates of the homologous potency. The fact that many cross-protection studies show protection even when r1 -values are low, has resulted in questioning the validity of r1-values. But in most discussions the quality of the vaccine is not mentioned, in addition to knowledge on the homologous potency which is essential, as is shown in equation above. There is currently an increased interest in quantifying heterologous and homologous protection; the outcomes of those studies will be used to evaluate the validity of the r1-values.

The current serological techniques for quality control of vaccine batches can also be used to standardise post-vaccination monitoring. However, it is essential that standard sera (e.g. equal to 50% protection) are generated for all possible vaccine strains.

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Laboratory report from Sciensano, Brussels, Belgium

David Lefebvre
Sciensano, Brussels, Belgium

Name change from CODA-CERVA to Sciensano
On April 1st 2018 the former Veterinary and Agrochemical Research Center CODA-CERVA has merged with the former Institute for Public Health WIV-ISP to form a new One Health Institute called Sciensano (www.sciensano.be) The FMD laboratory is found in the Service for Exotic Viruses and particular diseases in the Directorate for Infectious Diseases in Animals.

New EU-Reference Laboratories for FMD
The European Commission has appointed the FMD laboratories of ANSES (Maisons-Alfort, France) and Sciensano as the new EU-RL for FMD from January 1st 2019. This is a consequence of Brexit as the current EU-RL for FMD is the Pirbright Institute (UK). In this new EU-RL ANSES takes the lead and Sciensano is a full partner.

Results from international collaborations
Sciensano, an OIE Collaborating Center and an FAO Reference Centre, has a bilateral collaboration with the Botswana Vaccine Institute (BVI), an OIE Reference Center, with particular emphasis on hands-on training of BVI staff on genome sequencing and phylogenetic analysis. Similar to 2016, Sciensano participated in 2017 in an Inter Laboratory Comparison diagnostic trial organized by BVI. Field samples from several African countries were characterized by means of viral isolation, antigen ELISA and real time PCR. Sequencing and phylogenetic analysis of the positive samples are ongoing.

Sciensano is 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. Sciensano provides laboratory training to scientists and technicians from the NVRI. Previously, our joint effort identified a new SAT1 topotype X in Nigeria as well as FMD virus strains from topotypes O/West Africa and O/East Africa-3, SAT2 topotype VII and A/Africa/G-IV (Vandenbussche et al., 2018). A close relative of the latter strain has caused outbreaks of FMD in Algeria and Tunisia in 2017, demonstrating for the first time an epidemiological link of FMD between West and North Africa. In 2017, staff from NVRI sampled animals with clinical signs of FMD in 5 different Nigerian states. After characterization by real time PCR, virus isolation and antigen ELISA the positive samples were send to Sciensano for sequencing and phylogenetic analysis, which is ongoing. In 2018, 3 scientists from Sciensano have provided hands-on laboratory training at NVRI with particular emphasis on real time PCR and virus isolation and different types of ELISA.
The results of the OIE Laboratory Twinning Program between Sciensano and NVRI help to fill the knowledge gap of FMDV dynamics in the West African region to support local and regional development of vaccination-based control plans and international risk assessment.

Sciensano has a bilateral collaboration with the National Veterinary Laboratory (LNV) from Bujumbura, Burundi. Nearly 200 serum and 200 tissue samples were taken in 6 different Burundese provinces from animals with clinical signs of FMD. These samples were analyzed and characterized at Sciensano and the results will be presented at the EuFMD Open Session 2018 in Italy.

Research updates
Sciensano is involved in the development of next generation vaccines for FMD through the Anihwa -funded project Transcriptovac, involving different partners from France, Germany and Sweden. A first paper on this collaboration was published in 2018 (De Vleeschauwer et al.)

Science reports
Scientific publications from Sciensano 2018

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Alejandra Capozzo, across the Pacific and Down Under, as a Frederick McMaster Fellow in Australia

Nagendra Singanallur, Jacquelyn Horsington, Leanne McNabb, Michelle Cummins and Wilna Vosloo

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Leanne, Alejandra and Michelle loving what they're doing!

Sir Frederick McMaster (1873–1954) was a prominent grazier who was a generous benefactor of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia. Based on his bequest, CSIRO created the Sir Frederick McMaster Bequest Trust Fund. This fund supports distinguished overseas scientists to visit CSIRO and perform research in veterinary science or agriculture. Fellows are also required to undertake a program of visits within CSIRO, seminars to scientific societies, and addresses to industry groups.

The FMD research team at the Australian Animal Health Lab (AAHL) aims to have tools to mitigate the potential devastating effects of FMD, and measures to counter and manage an incursion of this high priority disease into Australia. Due to Australia's 146 years of FMD freedom, we are limited to working with only inactivated reagents. Vaccination could be an option during the course of an outbreak and we need in vitro methods to assist with our decision making processes, especially estimating vaccine efficacy.

As one of the founding members of GFRA, AAHL has always been engaged with the wider FMD research community. The South American experiences with control of FMD have been inspiring and the role played by the laboratories like INTA, SENASA and CONICET are noteworthy. Dr Alejandra Capozzo has been a leader in developing alternative methodologies for evaluating FMD vaccines. Development or adaptation of new methodologies for infectious diseases management has been a focus at AAHL. Recognising the scientific merits of the new methods for vaccine evaluation developed by Alejandra and her group, we successfully applied for the Frederick McMaster Fellowship, which allowed Alejandra to travel to AAHL and transfer the techniques for performing the avidity assay and IgG isotyping assay. Thanks to her training, AAHL scientists can now perform the novel, high-throughput avidity ELISA to predict vaccine efficacy. The sera collected during the animal trials performed with many of our GFRA collaborators over the years will be used to further evaluate the assays.

Alejandra shared her South American experience in controlling FMD using vaccination, in the form of invited talks at the Department of Agriculture and Water Resources-Office of the Chief Veterinary Officer forum, Canberra, and a seminar at AAHL. The FMD research group in conjunction with the diagnostic group at AAHL will continue to work with Alejandra and her team to evaluate the avidity and IgG isotyping ELISAs for deployment in South East Asian countries, where improved assays for measuring vaccine efficacy are needed. We trust this visit will be the basis for a longstanding future collaboration with Alejandra and her group in Argentina. Alejandra's visit Down Under with the McMaster Fellowship is an example of GFRA's vision of building a global alliance of scientists producing evidence and innovation that enables the progressive control or eradication of FMD, and its mission of establishing and sustaining global research partnerships to generate scientific knowledge. Amidst of all the exciting work in the laboratory, Alejandra also found time to enjoy the sandy beaches of Geelong and some challenging walks in the bush with Wilna, as well as the different cuisines on offer in Victoria with Jacq, Michelle and Leanne. Muchas gracias to Alejandra for accepting the fellowship and visiting AAHL. We also acknowledge CSIRO for conferring the Sir Fredrick McMaster Fellowships to Alejandra to make her visit possible.

Alejandra searching for the elusive platypus in Lake Elizabeth.

Planned closure of Lindholm

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DTU Vet, Lindholm.

It is planned that, at the end of 2019, the laboratory on the island of Lindholm, in Denmark, will cease to operate as a high containment facility for the study of exotic virus infections, including FMD. The laboratory part of the veterinary contingency activities is funded by the Danish Veterinary and Food Administration. These activities will move from being the responsibility of the National Veterinary Institute, part of the Technical University of Denmark (DTU), to Copenhagen University (that has the Vet School), in partnership with the State Serum Institute (SSI) that is also located in Copenhagen.

The island of Lindholm is only about 500m long (see picture) and it is located about 100km south of Copenhagen. The staff reach the island each day by ferry from Kalvehave. Work on FMD at Lindholm began in about 1925 and has included a variety of different activities including the production of FMD vaccine, until the early 1990's. Currently, it maintains the diagnostic capabilities for FMD and other important viral diseases of animals including Classical swine fever, African swine fever and rabies. It also has research activities associated with these diseases. In addition, Lindholm has undertaken diagnostic and research activities associated with various emerging diseases, for example caused by bluetongue and Schmallenberg viruses, when these have become a threat to Denmark and after their introduction into the country.

The SSI is currently responsible for the diagnosis of human infections and thus the relocation of the veterinary contingency to SSI will bring together the diagnostic activities for human and animal health under a single "One health" umbrella.

A new high containment building is being planned for SSI. This should have facilities for work with FMDV, CSFV, ASFV etc. but will not have facilities for experiments in large animals. However, this building is not expected to be functional by the beginning of 2020 and thus temporary measures will be introduced to cover the required diagnostic functions.

It is anticipated that staff will relocate from Lindholm to SSI/Copenhagen University.

Aerial view of the laboratory on the island of Lindholm, Denmark.

Effect of stabilizers on immune-reactivity of Foot-and-Mouth Disease Virus serotype 'O' virus like particle (VLP) on lyophilisation

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Foot-and-mouth disease (FMD) virus (FMDV), an Aphthovirus within the family Picornaviridae, is highly infectious and one of the most economically important diseases of cloven-hoofed livestock and other artiodactyl species. In endemic countries like India, the reliable maintenance of an adequate cold-chain for FMD vaccines from the manufacturer to the field is a challenge. Therefore, more stable FMD vaccine is necessary which could improve the quality of vaccine thereby improve the duration of immune response in animals and additionally be less reliant on the cold-chain.

In recent years virus-like particles (VLPs) have received much attention as next-generation vaccine platforms. VLPs are poly-protein structures that mimic the organization and conformation of native viruses but lack the viral genome. Since VLPs do not contain the genetic material of the native virus but have immunogenic properties similar to the native virus, they require no inactivation process and are recognized to be safer. Traditional virus vaccines use the inactivated virus as vaccine antigen. However, the inactivation process can lead to a reduction in immunogenicity because of structural alterations (Fan et al., 2015). Likewise the structural and immunogenic properties of VLPs are influenced by factors such as ionic strength, temperature, pH and choice of host cell (Kim, 2017). Therefore, identifying the unique properties of a VLP preparation is critical for controlling its quality as a vaccine.

Little attention has been paid to investigate the influence of freeze-drying on FMDV antigens since virus is highly fragile losing its antigenicity at extreme temperature. Recovering such multi protein complexes after freeze-drying without loss of vaccine potency remains a major challenge (Hansen et al., 2015). However, freeze-drying can provide the best way to increase the stability of protein-based biopharmaceuticals and eliminate the need for cold chains. In the present study, the stability of FMDV VLPs in the liquid and freeze-dried state over a four-week period was investigated, and the immune-reactivity of freeze-dried preparations was examined for the first time.

Expression and harvesting of FMDV serotype 'O' capsid proteins in Tn5 cells

Trichoplusia ni (Tn5) insect cells $20x10^6$ in $175cm^2$ culture flasks were infected with FMDV "O" capsid recombinant baculovirus at multiplicity of infection 7.5 in SF-900 II SFM serum free media and incubated at 27°C. Infected cells were harvested 3 days post infection by pelleting at 4500rpm for 10min. The cells were lysed in freshly prepared 3.0 ml of PBSV (consisting of 0.2 M sodium phosphate, 0.1 M NaCl, pH 6.0) and sonicated (amplitude of 27% for 1 minute with pulse of 10 seconds and pause of 20 seconds). Lysed cells were frozen at overnight 70°C and thawed at room temperature. The cell lysate was centrifuged at 9000 rpm for 30 min and the supernatant was collected and stored at 4°C.

Preparation of FMDV serotype 'O' lysate containing VLP for Lyophilisation

FMDV serotype 'O' capsid protein lysate was re-suspended in 500µl of PBSV containing 15% (w/v) trehalose or 10 % (w/v) sorbitol as stabilizers separately. Non-stabilizer conditions refer to the use of storage buffer PBSV alone. Samples were frozen at −80°C and lyophilized in a freeze-dryer overnight. Multiple set of samples were prepared and kept at 4°C. For recovery, the lyophilized samples were re-suspended in 500 µl distilled

water. Capsid proteins 1) lyophilized 2) stored 4^{o}c in aqueous state, were evaluated by sandwich ELISA using guinea pig and rabbit 146S hyperimmune serum. Our study indicated that VLPs stored in the aqueous state in sugar stabilizers at 4^{o}C helped in maintaining its immune reactivity in sELISA. Also VLPs stored in lyophilised state in the presence of stabiliser maintained their immune reactivity.

Discussion

VLPs are empty capsids without viral RNA , whereas in native viral capsids the space is filled with genetic material which interact with the adjacent capsid proteins. The empty space could lead to unstable VLPs due to nucleophilic attack in aqueous state, and the rate of dissociation of empty capsid proteins is dependent on the ionic strength of storage buffer and temperature (Hansen et al., 2015). Hence FMDV VLPs may require the use of a stabilizer for freeze-drying or for long-term storage. Sugars have been used as stabilizer to protect proteins against loss of activity due to chemical or thermal denaturation in solution. Sugars are thought to increase the surface tension of water, which increases the energy required to create the cavities responsible for protein denaturation. Sorbitol and Trehalose are widely used stabilizers that appear to increase the thermal denaturation temperature of proteins. In agreement with this, we found that Sorbitol (10%) or Trehalose (15%) stabilized the immune-reactivity of FMDV VLPs in the aqueous and lyophilised state at 4°C (Fig1 &2). Whereas, VLPs stored in PBS-V show decrease in optical density of 0.45 without lyophilisation. Therefore, there is a danger that the recovered capsid protein will not maintain the VLPs in the correct conformation. Water has a critical effect on protein structure and interactions. The study showed that FMDV VLPs in the presence of stabilizers can be recovered in a good condition after lyophilisation indicating stabilizer effect on FMDV VLPs during freeze-drying without loss of immune reaction. The findings are encouraging to FMD vaccine research as the availability of lyophilized formulations is required for FMD diagnostics and vaccines, as cold chains are totally unaffordable especially in developing countries. Increased familiarity with VLP lyophilisation promises to extend their usefulness in vaccine formulations.

Acknowledgement

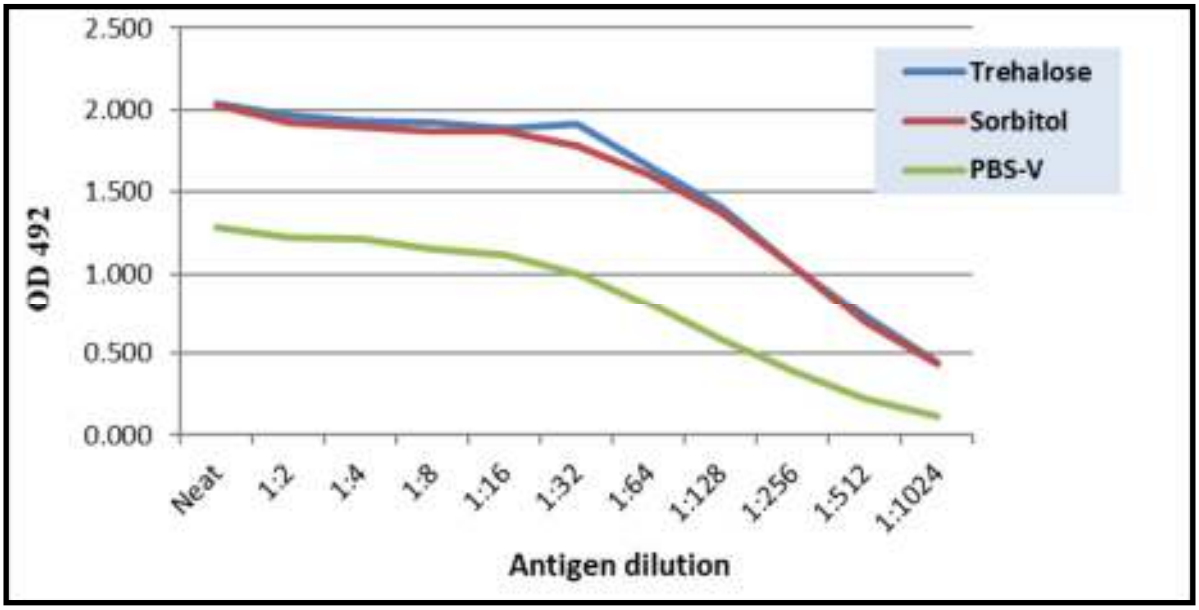

Figure 1: Immuno-reactivity of FMDV'O' VLP without lyophilisation in Sandwich ELISA (s-ELISA) [Samples stored for 4wk].

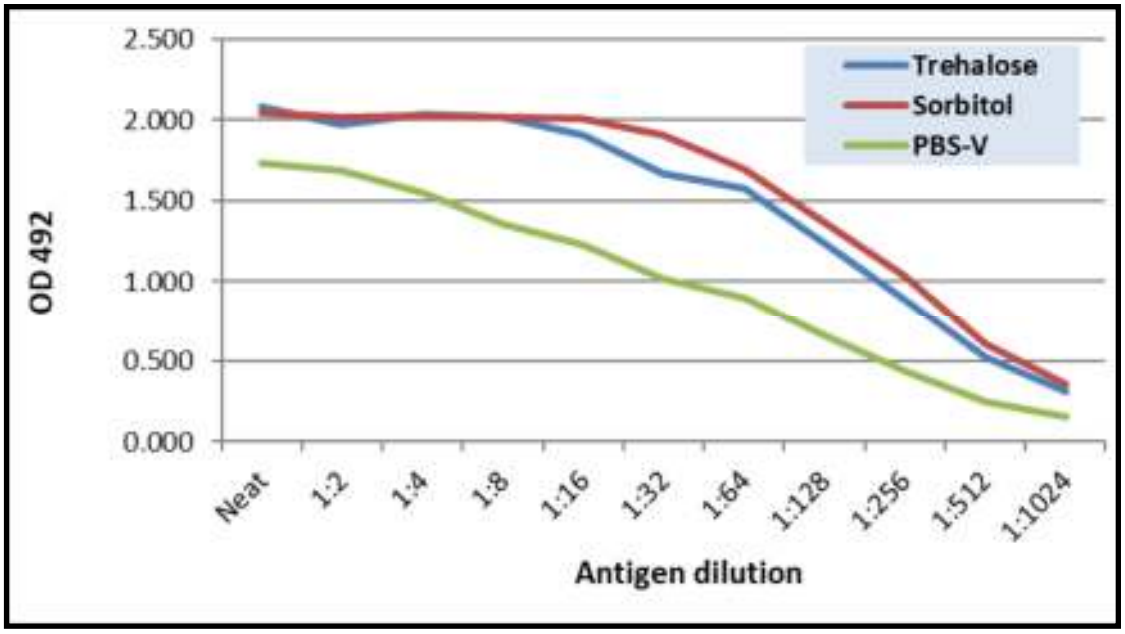

Figure 2: Immuno-reactivity of FMDV'O' VLP after lyophilisation in Sandwich ELISA (s-ELISA) [Samples stored for 4wk].

Acknowledgement
Authors acknowledge the Director, IVRI and Joint Director, IVRI Bangalore for providing the facilities to carry out this study.

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FMD-LL3B3D Vaccine Platform: Safe, Highly Potent, Fully DIVA Compatible, Inactivated FMD Virus Vaccines

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Foot and Mouth Disease Virus (FMDV) is the most infectious disease known and affects swine, cattle, sheep, goats, buffalos, and other cloven-hoofed animals. The disease causes severe production losses and disrupts a wide range of agricultural, industrial, and social activities. The FMD status of a country represents the single largest barrier to trade in the agricultural sector. A single outbreak in a FMD-free country can result in an economic impact in the range of $3 billion to greater than $150 billion US dollars. In the United States, FMDV is considered a Tier One Select Agent and is also restricted by legislation. The resultant regulations and restrictions complicate the discovery, development, and manufacture of FMD vaccines that use virus pathogens that could cause FMD.

Genetic and antigenic diversity of FMDVs among the seven 'global pools' forms the basis of the 7 serotypes and more than 60 recognized subtypes, and drives the difficulty in vaccine matching for effective FMD control in smallholder livestock (e.g., cattle, goats, sheep). Licensed FMD vaccines (monovalent and multivalent) are comprised of virulent, wild -type viruses chemically inactivated and formulated with adjuvants. These vaccines confer acceptable protection against FMDVs closely related to the vaccine strain. However, four major drawbacks of traditional FMD vaccines are the fact that (1) large quantities of infectious, virulent FMD virus are necessary to produce vaccine antigen, with the associated risk of virus escape from manufacturing facilities or incomplete inactivation during the vaccine formulation process; (2) the vaccine strain must match the wildtype FMDV responsible for the outbreak as vaccines provide little or no cross-protection; (3) traditional vaccines produced from wild-type FMDV are not fully DIVA compatible, since small amounts of nonstructural proteins may still be present; and (4) they do not fully protect animals from persistent infection.

To address the necessity for effective and safer vaccines a novel, antigenically marked, attenuated FMDV-LL3B3D vaccine platform was developed by Zoetis, Inc. and United States Department of Agriculture - Agricultural Research Service. The vaccine platform consists of an attenuated FMD A24 Cruzeiro virus that has been modified in three ways; (1) The leader gene has been completely deleted, (2) restriction sites have been inserted to flank the capsid coding region, and (3) negative antigenic markers have been engineered in the non-structural proteins 3B and 3Dpol. This vaccine platform allows for a rapid response capability by virtue of the easy exchange of capsid coding sequences using the unique restriction sites flanking the capsid coding region (Figure 1). In contrast to wild-type FMD vaccine viruses, the recombinant FMDV-LL3B3D platform vaccine viruses are fully attenuated as they induce no clinical signs of FMD and no shedding of virus in cattle or pigs when inoculated as a live virus.

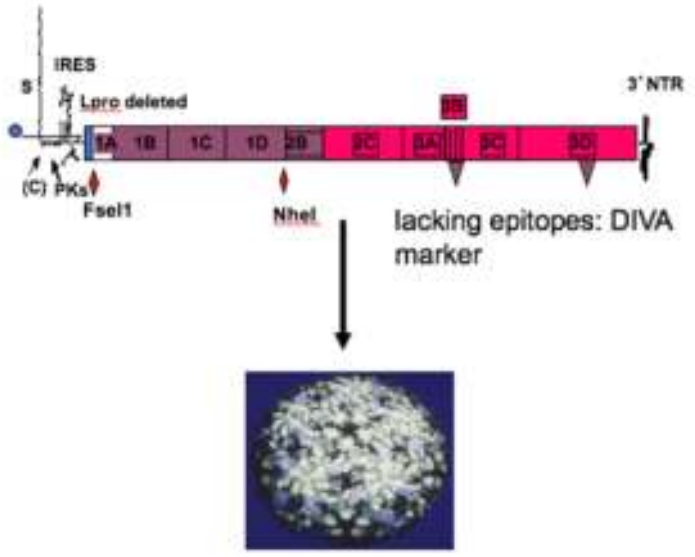

Figure 1. Schematic overview of FMD-LL3B3D Vaccine platform .

This vaccine platform may use existing FMD vaccine manufacturing technology and significantly lowers biosafety risks associated with FMD vaccine production. The finished vaccine is formulated with a proprietary adjuvant system that significantly boosts the humoral and cellular immune responses. Upon exclusion from the United States Select Agent Program authorized in April 2018, the FMDV-LL3B3D vaccine platform will be developed to accommodate production of high potency, fully DIVA compatible FMD vaccines in the United States. Cattle immunized with a variety of chemically inactivated FMD-LL3B3D vaccine constructs were protected from challenge with parental virus. Two commercially available DIVA companion kits (PrioCheck and VMRD FMDV Antibody test kit, cELISA (Chung et al 2018 In Press) are compatible with the negative markers built into the FMD-LL3B3D vaccine platform and facilitate the full DIVA capability (Figure 2).

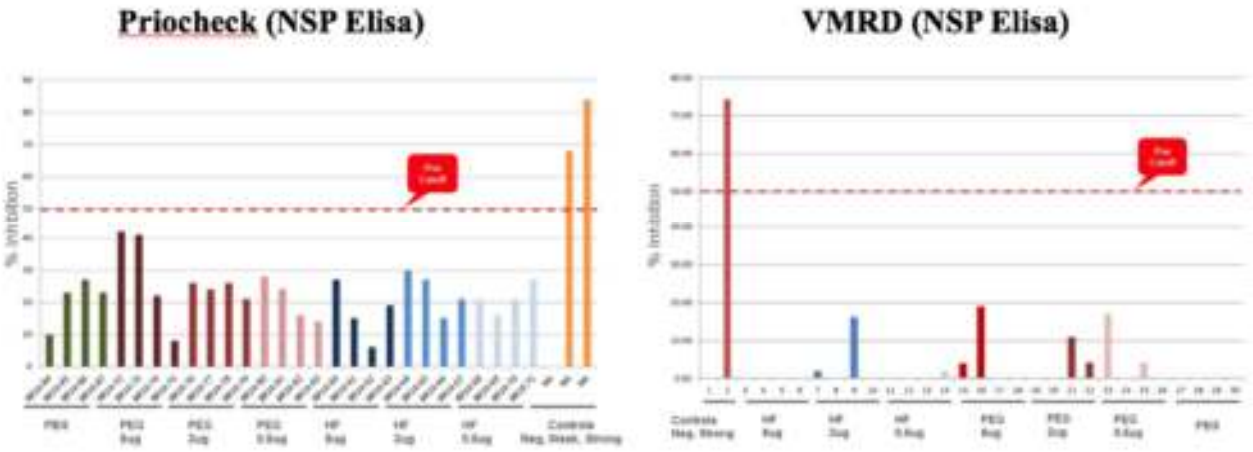

Figure 2. Serological Immune Responses to A24 FMD-LL3B3D Vaccine in cattle.
DIVA compatibility of the FMD-LL3B3D A24 Cruzeiro vaccine with the Prionics and VMRD FMD ELISA assays using serum from cattle vaccinated with varying volumes of FMD 24 Cruzeiro vaccine (prepared with antigen produced by either PEG precipitation or hollow fiber filtration and adjuvanted with a proprietary Zoetis adjuvant system.

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Inyathi antibodies in FMD diagnostics

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Foot-and-mouth disease (FMD) ranks as one of the most economically important and socially devastating livestock diseases throughout the world where several parts of Asia, most of Africa and the Middle East remain endemic. Southern Africa is endowed with an abundance of wildlife, which has been well protected within national parks and game reserves. In communities neighbouring these parks, the livestock/wildlife interface presents unique challenges to livestock disease control (Thomson et al., 2003; de Garine-Wichatitsky et al., 2013). A consequence of this is that the threat to livestock agriculture and the effective management of FMD in southern Africa differs radically from elsewhere. Additionally, the epidemiology of FMD in southern Africa differs significantly from other parts of the world such as South America, the Middle East and Asia. 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), which maintains the three SAT serotypes (SAT1, SAT2, SAT3) of the FMD virus (FMDV), and an independent cycle maintained within domestic animals (Condy et al., 1985; Thomson et al., 2003).

To ensure proper control and vaccination programs are carried out in an event of an FMD outbreak, it becomes important that the laboratory diagnosis of FMD is rapid and precise. In endemic, resource poor regions, such as sub-Saharan Africa, the timeline can be long from the period a suspected FMD case is reported to the time samples are analysed. Additionally, samples received at the laboratory can be of poor quality due to an ineffective cold chain and long transport periods, making diagnosis impossible. It is evident that sub-Saharan Africa requires diagnostic tools to eliminate this situation, which will subsequently allow for rapid diagnosis and the appropriate measures taken for control. The current recommended World Animal Health (OIE) diagnostic assay for routine diagnostics or screening of field samples for FMDV during surveillance projects is the liquid phase blocking ELISA (LPBE). Although LPBEs for detecting antibodies to SAT1, SAT2 and SAT3 viruses are well established; given the prevalence and genetic diversity of the SAT-type viruses (Van Rensburg and Nel, 1999; Knowles and Samuel, 2003; Maree et al., 2011), this assay is plagued with inadequacies due to a lack of specificity most likely due to the use of polyclonal capture and detection antibodies. This inadequacy thus highlights the need for the improvement of the sensitivity and specificity of the SAT1, SAT2 and SAT3 LPBEs.

Although hybridoma technology has contributed to major scientific advances, this technology has several limitations, amongst others, its high cost, it requires considerable time and expertise and the technology relies on the use of laboratory animals (Willats, 2002). Antibody phage display libraries (Hoogenboom et al., 1998; Van Wyngaardt et al., 2004), which enables the selection of specific recombinant antibodies, are considered to represent the most successful in

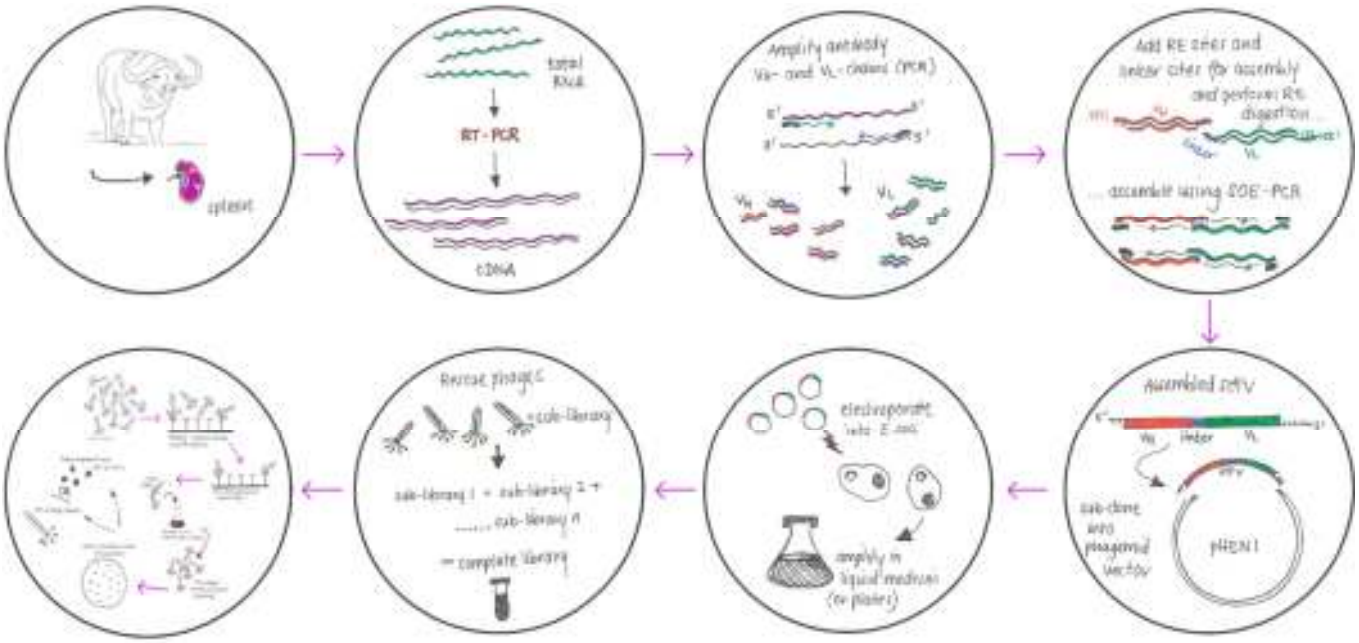

Figure 1: Diagram representing the construction of the immune FMD *Inyathi* (buffalo) library. Total RNA was extracted from spleens of three African buffalos infected with a FMD serotype SAT1, SAT2 and SAT3 virus, followed by cDNA synthesis and the amplification of the coding sequences for the immunoglobulin variable heavy (V_H) and variable light (V_L) chains by PCR. Linker and restriction enzyme sites were incorporated to enable assembly and restriction enzyme digestion respectively. A splicing with overlap extension PCR (SOE-PCR) was performed to assemble the VH and VL chains. The assembled scFv was cloned into the pHEN vector, transformed into electrocompetent E.coli cells and the library was amplified. Following the amplification of the Inyathi library, phages were rescued and the complete library obtained was 3.9 x 107 phages. To obtain FMD-specific scFvs, the Inyathi library is being panned against one SAT1 virus, two SAT2 viruses and one SAT3 virus. For the biopanning process, the FMD antigen is coated onto immunotubes and the phage library is added to allow relevant/specific phages to bind to the target antigen. Non-binding phages are washed off and phages that bound to the target antigen are eluted and re-infected into E.coli cells to amplify the pool of binders. Phages are then rescued and the biopanning round repeated at least 3 to 4 times. Thereafter, individual clones are selected and the phage and scFv formats of the FMD-specific binders tested further. Figure courtesy of Susan Wemmer (ARC-OVR).

vitro antibody selection technology and has allowed for the selection of recombinant antibodies against a wide variety of different targets (Carmen and Jermutus, 2002). There are several advantages of phage-displayed scFv (single chain fragment variable) over monoclonal antibodies (MAbs). Firstly, phages are more stable and can be stored up to several years at 4°C (Burritt et al., 1996). Secondly, they can be produced rapidly and inexpensively by infecting E.coli (Wang et al., 1995). Thirdly, their genes can be easily manipulated and lastly they can be produced by circumventing hybridomas and immunization, which requires animal ethics approvals (Marks et al., 1991). Two different types of antibody phage display libraries exist i.e. an immune library that is derived from animals immunized with a target antigen and a naïve library that is derived from non-immunized animals (Willats, 2002).

Tailor-made recombinant scFv antibodies have become potential alternatives to "conventional" immunodiagnostic reagents (Chowdhury and Wu, 2005). The functionality of scFvs as immunological reagents has been discovered in several different assay formats (Emanuel et al., 2000). The immunology division at the ARC-OVR has developed a large semi-synthetic single-chain variable fragment phage display library based on chicken immunoglobulin genes (van Wyngaardt et al., 2004), which has been panned against viruses such as: Bluetongue virus (Rakabe, 2009), FMDV SAT2 (Opperman, 2012), African horsesickness virus (van Wyngaardt et al., 2013) and Enteroviruses (Nukarinen, 2016) amongst others. The FMD SAT-specific scFvs have been used to map antigenic footprints as well as in ELISA formats as capturing and detecting reagents.

To further enhance this technology and to obtain more specific scFvs for FMDV diagnostic capture and detection reagents in the development of serotype specific assays, we constructed a FMD immune Inyathi (buffalo) library. Immune libraries are derived from animals immunized with a target antigen (in this case, FMDV) and are therefore pre -biased towards antibody fragments with desirable affinities and specificities. For the Inyathi library construction, RNA was extracted from spleen samples of buffalo infected with a SAT1, SAT2 and SAT3 FMD virus (Maree et al., 2016). The extracted RNA was converted into cDNA by RT-PCR and the coding sequences for the immunoglobulin light and heavy chains amplified. The heavy and light regions were joined with a linker sequence and cloned into a phagemid vector. See below the schematic diagram (Figure 1) for a brief explanation of this process. The pHEN phage vector harbouring the buffalo scFv gene was transfected into electrocompetent cells and the library amplified. The Inyathi library consists of 3.9 x 107 different clones and deemed large enough for an immune library. Currently we are in the process of panning the immune buffalo library against one SAT1, two SAT2 and one SAT3 FMD virus to extract SAT-specific scFvs. These virus specific scFvs will then be tested in an ELISA assay to determine if they can be used as capturing and detecting reagents with the goal of improving the specificity of the current FMD diagnostic assays used at the ARC-OVR.

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