VACCINATION AGAINST FMD – PRINCIPLES AND PRACTISE

Second GFRA/EUFMD virtual symposium co-hosted by OIE and FAO

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Introduction

A joint virtual GFRA/EuFMD/OIE/FAO regional workshop for Asia and South East Asia was held on Thursday, 25 March 2021.

Our vision was to share the principles and practical experiences of different parts of the world in topics of vaccination that included vaccine matching strategies, post vaccination monitoring, *in vivo* testing of vaccines, monitoring vaccine quality and novel vaccines.

The workshop brought together those researchers, policy makers and implementers interested and involved in vaccination as one of the means for FMD control to share knowledge, experience and ideas. Following the scene setting that involved invited speakers, we had a round table discussion where we invited participants to seek clarification and share their experience and viewpoints from their countries/regions. An online chat function, running concurrently with the talks, provided an additional vibrant forum for raising and answering questions from peers.

There were 368 registrations from 63 countries.

The recording of the meeting can be found at: https://www.youtube.com/watch?v=CikzDBeVcIU

Report

David Paton set the scene with a presentation entitled: 'Serological monitoring of FMD vaccination - Principles and Practise'.

Population immunity serosurveys can address two related but different objectives. Firstly, they can help to determine how well vaccination has been carried out and secondly, how well the target animals are likely to be protected from disease. Ideally, the first objective requires knowledge of how animals respond to correctly administered vaccine under the conditions of a particular target species, background immunity, vaccine batch, vaccination regime, post-vaccination sampling interval and test methodology. The second objective requires knowledge of how the antibodies that are measured corelate with protection against the threat posed by the specific viruses circulating in the field. In practise, precise knowledge of different outcome determinants and their influences is often missing, and so assumptions and simplifications must be made. For example, there is often information about antibody responses and sometimes about protection against vaccine homologous viruses, 3-4 weeks after a single dose of vaccine has been given to a naïve steer. However, the expected level of antibody or protection is less clear at other time periods, against other field viruses, and in animals with different vaccination and infection histories. David's talk discussed the pros and cons of different compromises with a view to making the best use of the options that are available (Appendix A).

Anna Ludi presented on: 'A new model for independent FMDV vaccine QA/QC as an aid to vaccine selection'.

Relationship coefficients (r_1-values) are often used to understand the antigenic relationship between a foot-and-mouth disease (FMDV) field strain and a vaccine. Values greater than 0.3 are suggestive of a close antigenic relationship between the field isolate and the vaccine strain; potent vaccine containing the vaccine strain is likely to confer protection. While r_1-values are often useful and highlight antigenic trends (such as the antigenic mismatch of the A/ASIA/G-VII field isolates with current vaccines) they have their limitations. To carry out r_1-value work one needs the vaccine virus from the vaccine company. In addition, the test is based on monovalent bovine serum collected 21 days after a single vaccination. However, in FMDV endemic countries, vaccines often contain multiple strains, and a booster vaccine is required to achieve appropriate antibody levels against circulating field strains. To overcome some of these limitations we are proposing a new model for FMDV vaccine QA/QC with the aim to roll this out at AU-PANVAC in Africa. This model does not require the vaccine virus or monovalent bovine serum. Instead, a reference panel of viruses is identified and tested against sera that have been obtained from animals vaccinated with commercial, often multi-strain, vaccine used in the field. Multiple vaccines are tested against the reference panel and by assessing the neutralisation titres the most suitable vaccine and testing regime can be determined. Anna's talk considered the benefits and limitations of these different serological approaches to vaccine selection and how this new model could be implemented in other regions (Appendix B).

Wilna Vosloo asked the question: '*In vivo* testing of vaccines in South East Asia – how well do antigen matching correlate with protection?'

Antigenic matching data are generated *in vitro,* for a quick analysis of the emerging viruses against available epidemiologically relevant FMD vaccine strains. This method has advantages such as speed of analysis, cost, and most importantly, circumventing animal ethics considerations. It provides a relatively easy and rapid way to test the suitability of available vaccine strains against a large number of field isolates and a statistical estimate to predict protection (r_1-value). However, interpretation of data can be challenging, especially in heterologous systems, where the r_1-value is not always a good indicator of vaccine efficacy. In such cases, testing of vaccines *in vivo* in target hosts and challenge with a representative heterologous field virus (r_1-value <0.3 by VNT) can provide valuable information on vaccine efficacy. Such studies also provide insights into the pathogenicity and infectiousness of novel variants of FMDV. Due to the costs incurred and ethical implications, many of these live virus challenge studies were/are performed as collaborations between laboratories and data shared to inform on decisions on vaccine strains to be included in vaccine banks or for routine use in the field.

Over the last 10 years a number of laboratories have investigated vaccine efficacy using the major commercially available vaccines in cattle, pigs and sheep against endemic and variant viruses from the A/Asia/SEA-97 and O/ME-SA/Mya98 lineages that have been circulating in South East Asia (Pool 1). In addition, *in vivo* testing was also performed against the newly introduced O/ME-SA/Ind-2001 lineage. Although the A/ASIA/G-VII lineage has not been found in SEA so far, the eastward spread of the O/ME-SA/Ind-2001 lineage has given concern that such novel lineages may also be introduced from neighbouring geographical pools.

In most cases, emergency vaccines that contain high levels of antigen (>6PD_{50}/dose) provide protection against clinical disease, decrease the clinical signs, and lower the amount of virus excreted as well as duration of excretion into the environment. This is despite low r_1-values in some cases. Therefore, even if animals are not fully protected, vaccination can be a useful additional control measure to minimise spread and economic damage. This presentation gave an overview of some of the *in vivo* vaccine efficacy trials and discussed the results (Appendix C).

Elizabeth Rieder gave a presentation on: ‘Novel FMD vaccines and their future use in developing countries’.

Chemically inactivated, oil adjuvanted FMD vaccines are critical to FMD control in endemic countries and have been successfully used in eradication programs in the past. Although these vaccines are effective in inducing protective immunity in livestock species, the response is short-lived with limited cross-protection and are unable to eliminate virus from persistently infected animals, prevent viremia or shedding. Vaccine manufacturing requires growing large volume of live virus in expensive high containment biosecurity level 3 facilities for production. To address the shortcomings of inactivated vaccines, current efforts are devoted to develop novel recombinant vaccines including marker inactivated vaccines, empty capsids, recombinant protein vaccines, and synthetic peptide vaccines. Elizabeth reviewed the progress on experimental and advanced development stages of these vaccine platforms and how they could support global FMD control and eradication. These next generation FMD vaccines offer improved production technologies to rapidly respond to new emerging strains. In addition, have the potential to reduce costs by eliminating the need for high level containment for production and downstream processes since they are fully DIVA compatible (Appendix D).

The final presentation was given by David Mackay on: 'Principles and best practice for official batch control of FMD vaccines'.

Official control authority batch release ("official control") is the process by which regulatory authorities confirm that vaccines released onto their national markets comply with the specifications that are set during the process of approval for licensing (also termed registration or marketing authorisation). Official control generally relies on a combination of review of the batch records provided by the manufacturer and re-testing. The emphasis placed on these two aspects varies between regions and between regulatory authorities. As it is not usually possible to replicate the full range of in-process and final products tests that are performed by the manufacturer, where re-testing is carried out, the aim is to independently verify the key parameters of the vaccine that determine its quality, safety and efficacy. In the case of FMD vaccines the tests to be performed are described in Section 4 of Chapter 3.1.8 of the OIE Manual of Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (the OIE Manual). For FMD vaccines the primary concerns relate to confirmation of innocuity to demonstrate freedom from live FMD virus and potency. Independent confirmation of potency represents a particular challenge in the case of FMD vaccines. The definitive test is the *in vivo* challenge test in cattle as described in the OIE Manual. *In vitro* alternatives involving testing of sera from vaccinated animals may be used, provided that the cut-off in the test has been validated with respect to the minimum acceptable potency defined in the authorisation. The ability of regulatory authorities to independently evaluate potency is greatly facilitated by cooperation with manufacturers to gain access to the protocols, vaccines strains and reference sera used to validate and perform the manufacturer's batch potency test (Appendix E).

Discussion

The presentations were followed by a round table discussion where the moderators and rapporteurs grouped and simplified some of the most relevant questions and discussed them with the panellists.

The main topics were:

How widely is post vaccination monitoring (PVM) used in parts of the world and for different vaccination systems? And if it is not widely used, why not?

- It varies a lot. For example, in South America where there have been large scale vaccination campaigns, it is used on a regular basis. In other parts of the world where vaccination is done intermittently, there is almost no PVM done at all, or when it is done it is done in a haphazard way. More effort needs to be put into PVM. In FMD free countries where vaccination is used as emergency measure, there is pressure to use PVM when trying to recover free status.

How should the various thresholds (PD50; titres and protection) be used by policy makers or those planning and designing campaigns?

- The neutralisation and ELISA assays are not currently harmonised between different laboratories. Different laboratories use their own cut-off values, as determined in those laboratories. The VNT cut-off for protection is still under investigation, particularly at WRL. Regardless of cut-off, one can still get an idea of vaccines that are giving no response to your reference panel, versus those that do give a response. It is difficult to have high precision about predicting protection. The purpose of your investigation is important to determine what to look for and what to infer from titres and thresholds. For example, you should know what titres to expect at which time points post vaccination, and check that this is what you are getting in the field. The level of protection you get as a result of that is a secondary consideration. As part of the authorisation procedure, a vaccine manufacturer may use a serological test to measure potency but they have to validate the relationship between titre and protection (as measured by PD50) in relation to the vaccine for which approval is sought.

Is harmonisation of VNT assays even possible, when there are so many variables (cell types, cell passage history, virus passage history) between labs?

- An equivalence study is being conducted by WRL. Reference sera and calibrator sera are needed so that different labs can adjust their neutralisation titres.

Should a prime, or prime-boost be used when testing vaccines?

- The performance of a vaccine in a lab study is often measured at 21 days, while in the field animals get infected at a whole range of different days post vaccination. There is an argument to move towards understanding the response in vaccinated animals across the range of days post vaccination, including the waning of immunity over time between the points at which animals are vaccinated. There is very little published literature on this, and this is a huge gap. There is a need to perform studies in the field where animals are vaccinated and serum samples collected at different time points. The WRL would be interested to contribute to any such studies planned by colleagues worldwide. It is important to distinguish vaccine potency and efficacy – two terms that are often confused. Potency has to do with quality of the vaccine, what is the immunogenic potential of that vaccine, which is what is measured in the potency testing. Efficacy is closely

related to potency, but also involves the vaccination schedule, and other factors in the field. Potency and efficacy need to be considered together.

Variability of potency testing is also an issue – is it really possible to distinguish between 3 and 6 PD50?

- Potency values are very hard to calibrate and they are very variable between testing. There is an argument to move away from using 3 or 6 PD50 and rather say that vaccines should have a certain standard amount of antigen that yields good potency and efficacy. However, the potency of the final formulation of vaccine depends not only on the amount of antigen but also on the interaction between the antigen and the other components of the vaccine, particularly the adjuvant. Tests that have been used for decades, like the cattle challenge test, become the gold standard, even though when you investigate deeper you realise that they are not as accurate or predictable as you would like. It would probably not pass if it were to be added as a new test now, but it has stood the test of time. There also hasn't been much of a cry to change the pharmacopeial standard other than to maybe define what dose would be necessary for a single dose regime. There are different applications of FMD vaccines (emergency vaccine vs. ring vaccination vs. routine vaccination), and different vaccines to fit those different applications, and economics/affordability also plays a role in PD50 levels required.

What will be the cost of new generation FMD vaccines compared to existing vaccines on the market? Will these new generation vaccines be affordable by developing countries where FMD is endemic? When is it expected that new generation vaccines will be ready for widespread use? Is there a new vaccine on the horizon that is better or cheaper to make?

- At present it is difficult to predict the cost of novel vaccines as it will depend on the technology. Virus like particles could be cheaper but RNA or other technologies are likely to be much more expensive. There are some new vaccines that have advanced to the level where adoption could be achieved in the near future. One of the advantages of the new candidate vaccines is that the high level of biocontainment for current vaccines are not required, thereby potentially lowering the cost. These next generation vaccine platforms are fully DIVA compatible and no downstream processes are necessary to eliminate non-structural viral proteins, with a cost reduction potential. Stockpiling of DNA to make the vaccines is also easier.

Timelines for new FMD vaccines?

- The development part is quick (couple of weeks) for derivation of new strains of interest, but regulatory process is time consuming. You can get accelerated timetables of 150 days, in general 210 days. But that doesn't include time it takes for manufacturers to answer questions. COVID has shown how regulators are able to speed up the process when under pressure. Platforms can be licensed (in the USA), which will speed up eventual vaccine licensing. When using existing technologies, realistically it will take a minimum of around 1 year to get a new vaccine licensed, but it could be sped up during emergency outbreak situations.

What has been learned from the COVID19 pandemic and accelerated vaccine approval, how could this be taken advantage of for FMD? Can some of the platforms for COVID19 be used for FMD? Which new FMD development is the most promising?

- The molecular aspects of the new vaccines are relatively straightforward, but the regulatory side will still take long to clear. Speeding up the regulatory processes should be worked on and the lessons learnt from COVID should be applicable to the veterinary domain. In terms of COVID

vaccine platforms, DNA and the adenovirus vector vaccines are being investigated, mRNA vaccine candidates are available.

There is no AU PANVAC in Asia, how should the SEA region proceed? Is the viral intelligence in East Africa good enough to select the relevant field strains, or will something be missed?

- It needs to be a joint effort and should be discussed at meetings like the SEACFMD meetings. The networks need to decide how they go about it and do it as a combined effort. There should be agreement between countries in the region to define reference antigens that are representative of circulating strains, and WRL can supply those. There had been effort in the last 5 years to increase surveillance in endemic regions, to improve viral intelligence. Ongoing surveillance to identify new strains is still necessary, despite having a reference panel of antigens.

Questions posted during the workshop

Some questions were answered in the chat/Q&A section by various other participants, these are summarised below

General:

- Question to Ronello Abila (OIE SEACFMD): In the OIE SEACFMD Roadmap is it possible to harmonize regulatory requirements in the region with the help of authorities, to have quick review and registration of FMD vaccines?
    - Answered by Ronello Abila: The issue will be discussed with the SEACFMD members.

Combination of vaccines:

- Is there a possibility of using FMD vaccine simultaneously with lumpy skin disease vaccine in cattle?
    - Mixing of rabies and FMD vaccines has been done before which worked well and saved money since veterinarians only needed to visit the animals once. But mixing of vaccines is something that needs to be tested in animal trials by including groups vaccinated with mixed and non-mixed vaccines, while also evaluating vaccine reactions which could be more severe when mixing. For LSD specifically it should be possible but research in the field is lacking. It would depend on how the LSD vaccine virus is produced and inactivated, and formulations need to be tested mixed and non-mixed. Currently FMD vaccines are inactivated while LSD vaccines are live attenuated, thus mixing might not be possible at this time. Vaccination schedules also differ, one may need a 6 monthly application and the other once per year, and this will also affect how practical it is to combine vaccines. Licensing the mixing of vaccines that are already licensed is expensive and laborious as it essentially creates a new product. Licensing combined products from the outset is generally more straightforward. Licensing the simultaneous administration of two vaccines from the same manufacturer (at different sites) is more straightforward but still requires considerable investment for which there is only sometimes a commercial return.

- It would be great if you could share your experience on results of combined vaccine of a virus i.e. FMDV and a bacteria i.e. Haemorrhagic septicaemia. As a pilot experiment this has been used in India in one of the State and results are quiet encouraging.
    - There are many publications from India and products are also available. FMD, FMD+HS, FMD+HS+BQ vaccines are available. Some of the states are already using them.

DIVA:

- Are serological tests able to differentiate between animals that were naturally infected and those that were vaccinated? Or is this only possible when a DIVA vaccine is used?
    - In animals that received a single vaccination, and at the relevant age category to monitor the infection status in the population, the current NSP tests are very specific. Even when using non-purified vaccines, most animals do not develop an NSP response after one dose of vaccine. However, specificity is reduced in animals that were vaccinated multiple times, so these animals should not be included in monitoring.
- What is the detectable duration and interpretation of NSP antibodies in ELISA, incl. issue of thresholds in commercial and in-house test systems?
    - Specificity and sensitivity vary between NSP tests and should be demonstrated and published as part of commercialisation or use. For a manufacturer to claim that their vaccine has DIVA properties they should follow the requirements in the OIE Terrestrial Manual with respect to the method of manufacture and demonstrating that vaccination does not induce NSP antibodies. Only vaccines for which these requirements have been met should include DIVA claims on their label.

Booster vaccinations:

- Question/comment directed at Don King: Don stated that booster vaccination is often applied, but OIE standards require good protection after one shot. Economically using a good potency vaccine is better than boosting with a poor potency vaccine. So, boosting should not be the standard, but used for overcoming the immunity gap or to control an outbreak in a vaccinated population
    - Response by Don: the point I was trying to make is that measurement/assessment of vaccine performance is rarely done at time points outside of the rather rigid protocols (and time points) defined in the OIE Manual. In the field this raises an important gap because we do not have well -validated metrics to define what might expected for a serological cut-off to define a "protected" animal in field studies.
    - But duration of immunity is usually determined after a two-dose primary course and it is difficult to sustain heterologous protection for 6 months without such a regime for naïve animals. If reactive vaccination is used in response to an incursion, then a long duration of immunity may not be needed if the outbreaks are controlled quickly.
    - From a regulatory perspective it is important to differentiate potency from efficacy, even though the two factors are closely related. In order to have consistency and comparability, potency testing is performed 21 days after a single vaccination and can be measured with the vaccine strain as challenge (i.e. homologous strain). Efficacy is demonstrated in line with the vaccination protocol described on the label and may involve one or two vaccinations depending on the claim made by the manufacturer.

When the vaccine strain is used for challenge in the potency test described in the OIE Manual, this represents the highest possible level of protection that can be afforded by the vaccine following a single dose. Efficacy under field conditions will be different depending on a wide number of factors including the vaccination protocol applied (number of doses and age at vaccination) and the antigenic relationship between field and vaccine strains.

- Also related to booster vaccination - is 21 days booster vaccination required even if the animals develop protective titres after a single vaccination?
    - Countries may decide to buy vaccines that induce sufficient antibody titres after one vaccination, which may not be available. The vaccination campaign is usually the most expensive part of control and paying double for a vaccine that is effective after a single dose can save money.
    - But PVM needs to be performed to determine, confirm and monitor the required vaccine schedule. There may be a trade off on the number of doses available and the potency and number of strains included in the vaccine, relating to production capacity and/or cost. Also the repeated rounds of vaccination allow young animals not eligible at the last round of vaccination to be vaccinated, rather than wait for the next round of vaccination in six months.
- Should a booster regimen be part of vaccine potency test?
    - No, the OIE guideline is clear. Vaccine should protect 75% of the cattle (or 3 PD50/dose) after a single vaccination. A booster dose increases homologous titres that will subsequently increase the heterologous titres as well. Potency is to pass a batch, but efficacy depends on the antibody response and challenge in the field. So, it is always recommended to administer a booster in primo-vaccinates in the field.

Population immunity and surveys:

- How many samples need to be collected to conduct a population immunity survey? What is the recommended age range of cattle and small ruminants in post-vaccination surveys to assess herd level immunity? In West Eurasia, often 4-18 months (or 12 for small ruminants) are used, to limit the influence of maternal immunity and on the other hand the possible build-up of NSP antibodies with higher age, and produce results with reasonable informational value about the population.
    - The number of samples needed depends upon the design, the population size and heterogeneity and the precision required. Examples can be found in the OIE/FAO PVM Guidelines at (http://www.fao.org/3/i5975e/i5975e.pdf). Two key considerations for PVM survey design are to i) decide if you want to measure individual animal or herd immunity, and ii) stratify the population into young animals that will have had few vaccinations and older ones that will have had many. In endemic vaccinated populations 7-18 months would be a reasonable age-range, but if you have enough animals to choose from minimising the range e.g. focussing on 12 month old would allow more precise interpretation.
- As per the OIE guidelines, post vaccination serological monitoring can be done using solid phase competitive ELISA. In India, Directorate on FMD has developed an in house very well validated SPCE which is being used for testing the samples throughout the country. Your

views, please. If the liquid phase blocking ELISA (LPBE) is not available, can the solid phase competitive ELISA be used instead for population immunity surveys?

- Most of the serological tests correlate with protection, but the VNT predicts protection better. Most likely as it only detects neutralising antibodies against the intact virus. There is evidence that serological tests using purified 146S are equally effective (Mansilla, F. C., C. S. Turco, M. C. Miraglia, F. A. Bessone, R. Franco, M. Perez-Filgueira, J. M. Sala and A. V. Capozzo (2020). "The role of viral particle integrity in the serological assessment of foot-and-mouth disease virus vaccine-induced immunity in swine." PLoS One 15(5): e0232782.).
- Currently it is not possible to determine a titre directly using SPCE. It only gives you a dichotomous positive or negative result. You need to titrate the sera and validate it against a VNT or LP-ELISA. For this reason, standardised sera are required and are available (standardised to protection in potency tests.

- Why is only 75% population herd immunity required, and not 80% or more? How is a correlation made between PD50 and probability of protection (i.e. 75% = 6 PD50).
  - The level of herd immunity required is not fixed, but depends on the transmission dynamic (reproduction ratio, R) of the infection to be controlled (not only influenced by viral factors, but also by animal density and contacts, other control measures, etc) and on the effectiveness of vaccination. In Europe vaccine efficacy has been standardised in the 1960s due to a lot of discussion between European countries on quality of FMDV control and export-import. It was agreed that 3 PD50/dose would be sufficient and that became the standard for vaccine producers in Europe and is now also the standard in the OIE Terrestrial Manual. This potency correlates approximately with 75% protection. In South America a different batch release test was implemented, but also using 75% protection as cut-off. These standards have been successful to combat endemic FMDV. In hindsight if you estimate the R_0in a population it is not very high for FMD; often less than 4 but mostly less than 2. For an $R_0$of 4, vaccination of 75% of the animals would reduce the R-naught below 1 and then eventually (after a long time) the epidemic will stop. Luckily the R_0 of FMDV is often lower and 75% protection will reduce an outbreak much quicker. In a case where the outbreak virus is different from the vaccine strain, a higher potency vaccine is needed. The r1-value is probably a good predictor of the heterologous potency. So, to have at least 3 PD50/dose for heterologous protection with an outbreak strain that has an r1-value of 0.5 you need at least 6 PD50/dose against homologous challenge. But even with low r1-values e.g. 0.1, a vaccine that has a homologous potency of 30 PD50/dose will probably still protect against the outbreak strain. If it is difficult to obtain high potency vaccines, it is possible to use boosting with the normal vaccine around outbreaks thereby improving the potency. The need for tailored vaccines is limited, but a broad panel of good producing vaccine strains is needed.
  - NB – That said R_0 is highly variable and dependent on the setting and production system, e.g.R_0 >50 for transmission within an intensive dairy herd, and the level of coverage needs to be factored in. These issues also get confusing when looking at R_0 for between herd transmission (often used for FMD) but considering vaccine effectiveness for individual animals.

- Protective titres are specifically mentioned as $\log_{10}$ 1.7; why not $\log_{10}$ 1.8 or more? In another presentation it was stated as $\log_{10}$ 1.5.
    - A universal cut-off is not possible due to many situation-specific influences (virus serotype and strain, nature and timing of challenge, test characteristics, host variability, etc). Differences obtained with similar tests between laboratories can be as high as 1 $\log_{10}$ so standard sera are essential to standardise serology. Vaccine manufacturers and reference laboratories may be able to advise on test selection and cut-offs and there is guidance at: http://www.fao.org/3/i5975e/i5975e.pdf
- A $\log_{10}$ 1.7 VNT titre (i.e. 1:50) is mentioned as 75% protection for O/A/Asia1. Will this be the same for polyvalent vaccine with O/A/Asia1?
    - Cut-offs that are determined in one laboratory with one test cannot be used in other laboratories. Each laboratory has to validate their own tests using sera from potency tests. There are very limited studies on polyvalent vaccines and protection. There is no evidence that the correlation between antibody response and protection is different in multivalent vaccines, but that could be due to the limited amount of data. The OIE Terrestrial Manual (and European Pharmacopoeia) requires a serological threshold corresponding with protection to be established for each strain in trials with monovalent vaccine. Once established, the same threshold can be used when this strain is used in combination with any other. The onus is on the vaccine producer to carry out these analyses and submit as a part of their dossiers. Countries can request or insist on such results before ordering the vaccines, if needed. Both passed and failed batches should be included in such analyses.
- Different manufacturers use different adjuvant formulations for vaccine preparation. Doesn't this affect population immunity dynamics? Sometimes there is a choice of vaccines based on different adjuvants, e.g. aqueous or oil based, from the same manufacturer and with the same virus. Which will be the optimal choice in terms of its effect on potency and post vaccination titre?
    - Formulation (146S content and adjuvant) both influence potency of a vaccine. FMD adjuvants are often categorised as oil or aqueous, but there are also other ingredient differences as well as in the way that the emulsions are formed. Adjuvant is probably at least as important than 146S content. But many adjuvants work well, so it is not easy to say which adjuvant is better. In Europe, FMDV was controlled mainly with Aluminium Hydroxide adjuvanted vaccines. In South America Oil emulsion vaccines are used. Both were successful. Only oil adjuvant vaccines are suitable for pigs.
- Is there a protective titre difference between antibody induced by vaccination versus field virus infection?
    - Yes, but a wide range of antibody titres can be elicited depending upon the extent of virus replication and disease in infected animals and on the dose and number of boosters given in case of vaccinations. But, in general for FMD, infection will elicit a stronger and more long-lasting immunity than vaccination.
- How can a cut-off value for protection be determined for each serotype?
    - The cut-off is different for each strain, not only for each serotype. This can be determined by testing sera from potency tests. So the OIE standard potency tests should be performed 2 or 3 times for every new vaccine strain and sera (sufficient volumes of the 21 days post vaccination sera) should be stored for later evaluation

and sharing with consumers that want to validate their serological tests used in their country.

- Does animal breed influence the protective titre?
- This has not been widely studied for FMD and relevant livestock species. However, in outbred animal populations, genetics is expected to influence the immune response. There could be association with production stress. For more information, please read the open access papers: https://link.springer.com/article/10.1007/s40003-013-0063-9 and https://pubmed.ncbi.nlm.nih.gov/23895140/.
- The mere presence of antibodies against a strain in a serum sample is not enough to be happy about a field vaccination. Low amounts of certain quality of antibodies are related to protection. In some countries PVM is performed by using commercial kits, some of them blocking ELISAs that actually measure antibodies against just one epitope… that may detect "some level" of antibodies, but says nothing about the vaccine capacity to potentially protect against infection. Any comments? Why do we always dance around VNT… this is a complicated assay to harmonize. There are other immune parameters to use, for potency testing and also to have an idea of expected protection of a vaccine in the field. We need new serological assays.
    - Statistical analysis in South America has shown that the prediction of protection is better with VNT than LPBE, still they choose the LPBE because of simplicity. We have evaluated O Manisa potency test in the Priocheck type O ELISA, if I remember well a 1/40 dilution correlated with 50% protection (so 8 times higher dilution than the standard 1/5 dilution).
    - The advantages of VNT are i) that it can be adapted readily to incorporate different virus strains according to their relevance in the field, and ii) it corelates better than many other tests with protection. Efforts continue to develop ELISAs that can be as good indicators but that will be simpler to use.
- In endemic regions, a large proportion of animals may have pre-existing immunity against FMDV, due to the past infection history. Would this lead to a different serological response pattern after (boost) vaccination?
    - Yes, this should improve immunity in general and after vaccination.
    - Booster is for primo vaccinates. But repeat vaccination in adult animals has to be timed. Booster vaccination in primo vaccinates is mainly to cover the immunity gap. When dams are vaccinated well, first vaccination in young stock should not be given early (not before ca. 10 weeks in pigs or ca. 4 months in cattle). Then often one vaccination with a good quality vaccine is sufficient. But when outbreaks in young stock are seen the quality of the vaccine should be checked. And if the quality is sufficient early vaccination and boosting could be considered.
- Comment related to principles and practices for serological monitoring of FMD vaccination: The PD50 challenge model is inherently variable. In practice you would need to repeat the challenge studies multiple times to narrow down the real value, which is not feasible considering the multiplicity of strains and the availability of animal facilities.
- Related to principles and practices for serological monitoring of FMD vaccination, the speaker (David Paton) indicated that one can choose to monitor at the peak of antibody response after vaccination, or at the time of re-vaccination. Would it be important to have a fixed time interval to be able to evaluate the response?

    - A systematic approach will make it easier to build up knowledge of what to expect and to compare results from one campaign to another. A practical approach is to mainly measure peak immunity (in the population at large) and to interpret this information in light of complementary smaller studies that estimate the duration of immunity.
- Can antibody response to homologous vaccine and multivalent and heterologous vaccine be similar, if the vaccine strains are the same for homologous and heterologous?
- The virus used in a test to measure the antibody response to vaccination depends on the purpose of the test. If the test is to measure the 'quality' of the vaccine (i.e. does the vaccine match the specification on the label/authorisation in terms of potency), then testing against the vaccine (homologous) strain, or a strain shown to be antigenically similar/identical, is appropriate. If the intention is to estimate likely protection against field strains in the area where the vaccine is used (i.e. fitness-for-purpose) then testing against field (heterologous) strains is appropriate. In such cases the relationship between homologous vs. heterologous titres should be established first in order to be able to differentiate a failure of vaccine quality from a lack of vaccine relevance. Question to David Paton and Anna Ludi: What do you think of using only heterologous virus for Post Vaccination Monitoring Program since it is not possible for us (Malaysia) to test our vaccinated animal sera with the homologous virus.
    - This should be fine if you use heterologous viruses that represent likely field strain threats. A small longitudinal follow-up to vaccination done under controlled conditions will help to calibrate the responses that can be expected for the vaccine and vaccination regime selected.
- How often are lab specific cut-off values for titres of protective immunity changed?
    - Probably less often than they should be, but as we have discussed, this requires data that may not be available without investing in appropriate studies.
- Are there criteria requirements for private labs for testing?
    - This may be a matter for local regulation, but the key considerations are the quality and safety indicators.

Vaccine matching, r_1-values, antigen payload and potency:

- What is the ideal antigen payload recommended in standard potency and high potency vaccine for O, A and Asia1?
    - This is serotype dependent and also will be influenced by antigen stability and adjuvant. Antigen payload is only one consideration. Quality and stability of the antigen is also important. Vaccine producers should perform potency tests and determine a serological release criterion and show that the vaccines are stable during shelf-life.
- Is the r_1 value >0.3 equally applicable to countries with complex ecology of viruses, larger geography and larger animal populations like India? Would a vaccine with a higher PD50 per dose be more efficacious?
    - r_1 value cut-off of 0.3 is based on the assumption that the vaccine has more or less a standard potency. But it has been shown that high potency vaccines >30 PD50/dose can protect against strains that have an r_1-value of 0.1 or lower. The r_1-value is a good estimator of which homologous potency you need in your vaccine (or you have to

boost). A standard of 6 PD50/dose for emergency vaccines is an arbitrary cut-off. One should consider r_1-value and homologous potency in the evaluation. As a rule of thumb for an outbreak with a virus with an r_1-value of 0.1 you need a 30 PD50/dose homologous potency to get 3 PD50/dose against the outbreak virus.

- Does r_1-values equal to or above 0.3 indicate a closer antigenic relationship between the vaccine strain and the field strain?
    - Yes, r_1 measures antigenic relationships, but other factors influence protection. The r_1-value cut-off of 0.3 is not cut in stone. It depends on the homologous potency. The r_1-value is a good predictor which homologous potency you need.
- Is variation in antibody titer among vaccinated cattle a critical consideration in determining vaccine potency? What is the maximum acceptable variation?
    - Vaccine potency should be determined in cattle that are free of FMDV antibodies at the time of vaccination. The variation in the antibody response after vaccination is normal, it is representative for the population where the same variation is observed. With 7 cattle vaccinated and evaluating the antibody response in well validated serological test the estimation of the potency can be as precise as in a potency test using challenge in 15 vaccinated and 2 control cattle. But potency tests have to be performed to validate the serological test.
- What is the best virus inactivation agent for FMD killed vaccine?
    - Any agent that produces linear inactivation will be good. Formaldehyde does not produce linear inactivation curves. BEI is often used, as it produces linear inactivation. In the EuPharm the inactivation should be efficient. In 2/3 of the inactivation time less than 1 particle per 10 000 liter should be achieved. This can be tested in an inactivation curve.
    - Bromoethylene imine (BEI) that is cyclicised using NaoH on Bromoehtylene amine is the best so far (economical as well). Other one is Beta propiionolactone (but very expensive and used in human vaccines). You need to have data on the inactivation kinetics for each batch of BEI you use. We call them chemicals with first order kinetics. Time needed for inactivation depends on dose and temperature. The inactivation kinetics should be tested and controlled. BPL is more effective against enveloped virus than nonenveloped. Possible human carcinogen too. Formaldehyde and BEI are also human carcinogen so they should be handled with care.
- Comment: If we move from PD50 to antigen payload based quality assessment of vaccines, then we should have a reliable assay to do antigen estimation in the formulated vaccine.
- Question to Wilna: From your presentation, combination of different strains can broaden immunity. Is it always true for any strains of each serotype? Can there be heterologous interference?
    - We don't fully understand the role combinations of strains can play in broadening immunity. Evidence from the field is that there is not interference between strains and addition leads to better protection. The added amount of antigen could be the reason we see better protection, but how different strains react together is largely unknown.
- When different manufacturers are using different adjuvants in the vaccine, should serology-based potency testing be optimized for each manufacturer?

- Yes. Each manufacturer should validate the serological test they use as a correlate of *in vivo* vaccine performance and establish a cut-off corresponding with protection for each strain included in a vaccine and for each vaccine. 'Bridging studies' may be sufficient to show that the serological titre in one strain/vaccine can also be applied to other strains/vaccines.

- Which serology test can be used for *in vitro* potency testing of a vaccine? How many samples and intervals do we need to collect serum samples?
    - Manufacturers should validate the protocol that they use for *in vitro* potency testing. Example protocols are provided in the OIE Terrestrial Manual, but the manufacturer would still be expected to validate that the test performs as expected, and the that the thresholds still apply, when the test is performed in their hands.

Novel vaccines

- In VLP construct (new generation FMD vaccines) what is the need of 3B protein. Does this carry any function?
    - FMDV 3B (also known as VPg) is present in three similar but nonidentical copies (3B1, 3B2, and 3B3) (20), and there are no reports of naturally occurring FMDV strains with fewer than three copies of 3B. Although not all of the three copies are essential for FMDV replication, the copy number of 3B is critically associated with the host range and the virulence of FMDV. Recently, 3B was identified as a new antagonistic factor of host innate immune response (https://doi.org/10.4049/jimmunol.1901333).

General information and Agenda

Organising committee	
GFRA	Wilna Vosloo (CSIRO), Nagendra Singanallur (CSIRO), Alejandra Capozzo (INTA)
EUFMD	Nadia Rumich, Fabrizio Rosso, Alessandra Alviti, Ludovica Nela, Costanza DeLaurentiis
Moderators	Kees van Maanen (GD Animal Health, FAO/EuFMD consultant for FMD), Theo Knight-Jones (International Livestock Research Institute)
Rapporteurs	Nagendra Singanallur (CSIRO), Petrus Jansen van Vuren (CSIRO), Wilna Vosloo

Programme	
Opening session (Alejandra Capozzo (CEO GFRA); Fabrizio Rosso (EUFMD); Ronello Abila (OIE SEACFMD)	
Serological monitoring of FMD vaccination - Principles and Practise ***David, Paton**, Anna Ludi, Donald King* The Pirbright Institute, Ash Road, GU24 0NF, Woking, Surrey, UK	Appendix A
A new model for independent FMDV vaccine QA/QC as an aid to vaccine selection ***Anna Ludi**, Ginette Wilsden, Madeeha Afzal, Amin Asfor, Alison Burman, David Paton, Don King* The Pirbright Institute, Ash Road, GU24 0NF, Woking, Surrey, UK	Appendix B
In vivo testing of vaccines in South East Asia – how well do antigen matching correlate with protection? ***Wilna Vosloo[1]**, Nagendra Singanallur[1], Petrus Jansen van Vuren[1], Jacquelyn Horsington[1], Jong-Hyeon Park[2], Charles Nfon[5], Don King[3], Anna Ludi[3], Phaedra Eblé[4], Aldo Dekker[4]* [1]CSIRO-Australian Centre for Disease Preparedness, Geelong, Australia [2]Animal and Plant Quarantine Agency, Gimcheon, Gyeonsangbuk-do, 39660, Republic of Korea [3]The Pirbright Institute, Ash Road, GU24 0NF, Woking, Surrey, UK [4]Wageningen Bioveterinary Research, Lelystad, Netherlands [5]National Centre for Foreign Animal Disease, Winnipeg, Canada	Appendix C
Novel FMD vaccines and their future use in developing countries *Elizabeth Rieder* USDA Plum Island Animal Disease Center, ARS, USDA, Greenport NY, US	Appendix D
Principles and best practice for official batch control of FMD vaccines *David Mackay* Advisor on Veterinary Medicines, United Kingdom	Appendix E
Round table discussion and question time (*moderators: Kees van Maanen and Theo Knight-Jones*)	