GFRA Forum at the EuFMD Open Session 2024: Integrating Science, Regulation, and Producers for Effective FMD Control

The third day of the EuFMD Open Session 24 was hosted by GFRA and was dedicated towards discussion on three major topics: (1) why should we control FMD, (2) FMD control using improved diagnostics and (3) control of FMD using novel vaccines. Each session had introductory talks by key experts to provoke conversation, followed by a substantial discussion involving the speakers, panellists, and audience.

Session 1: Why should we control FMD?

Melissa McLaws kicked off the meeting with a talk on the costs and benefits of FMD control. The impact on livestock disease could be seen as branching into direct (immediate impact such as loss of milk production) and indirect (human interventions like vaccines and social impact). The measurements for impacts can be difficult for certain parameters such as the impact of diagnostic testing and the use of disinfectant. Indirect costs also affect FMD-free countries which pay a premium to import from countries free from FMD and other transboundary diseases. In endemic countries, there are knowledge gaps in net impact of FMD, specifically due to the difficulties in measuring indirect impacts. Livestock owners, especially dairy and pig producers, often experience the worst consequences, which are more apparent than smallholders, However, there might be a delay in recognizing these impacts, as they primarily affect finishing costs. The question of who will bear the cost is also often discussed and will likely change along the control pathway where initially the government may take on the burden (PCP 1-2) with down the line costs being transferred to private industry (PCP 3-4). In conclusion, key impacts depend on perspective: individuals (production gains, livelihoods), countries (market access) or global (poverty reduction). It may be necessary to consider updating cost-benefit analysis to reflect these diverse impacts more accurately.

The conversation then continued with the viewpoints from FMD-endemic countries (Africa and South-East Asia), FMD-free countries (Europe) and countries transitioning to free without vaccination (South America).

Simon Dickmu started this session with his experience on the cost/benefit analysis of FMD in endemic settings in Africa. At a global level, around 2% of the world's cattle population has FMD on an annual basis with annual impact of between USD 6.5 and 21 billion (Knight-Jones, 2013). However, this publication has no global estimates on welfare, environment, and livelihoods. The Uganda government spent between £5.3 million and £7.5 million between 2007-2009 on FMD control. Why is more research needed? Seventy-five percent of communal smallholders sustain livelihoods of vulnerable groups (woman and children). While transboundary animal diseases (TADs) have high mortality and morbidity, governments often prioritize human disease and political crises, over animal health issues. Therefore, more research on the economic impact of FMD is needed to highlight the importance of the disease to the governments and partners. Due to the porous borders between countries, regional vaccination and control programs should be implemented. Ultimately the control and eradication of FMD will lead to an increase in revenue for vulnerable groups, particularly women and children.

Mariano Perez-Filgueira next presented the current situation in South America which is transitioning from free with vaccination to free without vaccination. The key factors to freedom include mandatory, systematic, and controlled vaccination plans with the use of high-quality purified vaccines (DIVA-compatible), improved diagnostics, epidemiological monitoring, early detection, and disease alerts. Vaccination campaigns are being suspended in most of Brazil and Bolivia, which over time will result in fewer vaccinated animals. What's next? Can the success of South America including the local production of good quality vaccines be replicated in other regions? What is the main risk of reintroductions of exotic strains to South America? How is the region prepared for a potential reemergence of FMD? What is the availability of vaccine banks and local vaccine manufacturers? What is the impact of Venezuela? Should the WOAH code be updated to favour free with vaccination?

Aldo Dekker then presented the situation in Europe (specifically The Netherlands) which is an FMD-free country. In Europe most people don't think about FMD. FMD was the first virus discovered because it caused a reduction in milk production, now it is mostly talked in the context of its effects on blocking trade. The advantages of being free include: (1) free trade as exports for The Netherlands account for 60-80% of agricultural products and (2) no trade restrictions (including whole carcasses that can be sold abroad as animal byproducts). There are different perspectives in society: Veterinary officials think it is important to control FMD, farmers are interested in being free, but they don't agree with strict control measures, agricultural industry wants to be free and the public does not understand the need to kill animals. For The Netherlands, a good contingency plan is key to ensure that everyone is onboard and prepared in case of an outbreak.

Wilna Vosloo discussed the current situation in South-East Asia which is endemic for FMD. The situation in the region is complex with WOAH SEACFMD (12 countries) being involved in the region since 1994. WOAH SEACFMD assists with designing roadmaps which are agreed with the countries and assist with FMD control. The concern is that outbreaks are not being serotyped; in 2023 this accounted for 89.11%. Cattle are the main species being detected but this is misleading as not all outbreaks are investigated. There are attempts to use vaccination but that is not always well coordinated. Recently bilateral agreements between countries have been established – these rely on quarantine stations. It will be interesting to see how this works and whether they help to control the disease. To summarise, there is a lack of political support and funding, and FMD is considered a low priority disease compared to other endemic diseases. However, there is growing demand for high-quality protein, which impacts trading patterns. There is always hope as there are motivated people (veterinary services, field workers, scientists) in this region that want to help with controlling the diseases, which opens the door to involve the private sector.

The speakers as well as the panellists (Tsviatko Alexandrov and Georgina Limon-Vega) were then asked to come to the front. The discussion was started with a comment from Georgina Limon-Vega who stated that un-intended costs of control measures can have more of an effect than the disease itself. We should think globally about how free zones impact other countries from ever having access to the free market. Also, the overall impact of subsistence farmers needs to be better understood and taken into consideration. The discussion was then opened to the floor.

Harsh control measures aimed at becoming FMD-free will have a huge impact not only on tourism but also on the mental health of farmers. This is why a robust contingency plan is important so these things can be considered and modelled. An example was given for Uganda where three quarters of the country were under quarantine which had a huge social impact. Weddings were delayed because there were no dowries, burials couldn't take place as the custom of slaughtering animals couldn't happen and the stigma of having sick animals affected mental health. An additional comment was made that this also affected neighbouring countries (Congo) that depend on the trade from the country having outbreaks. An example was given as to the use of disease-free zones which promote local, regional markets in Kenya. A question was also raised as to whether more research needs to be done on breeding animals which are less susceptible to FMD.

Keith Sumption raised a challenge to the FMD community highlighting the significant impact of FMD on huge numbers of animals. He questioned whether efforts to combat FMD could be linked to funding to combat climate change (by improved vaccine control of FMD) or antimicrobial resistance (these are often used to treat secondary infections of FMD lesions)? Requests for funding need to be in the language of economists. Aldo Dekker then mentioned that we need to ensure that FMD is perceived as still relevant. In 1936, The Netherlands reported 100,000 outbreaks; endemic countries do not report anywhere close to this number. If we want to highlight that FMD is important and we need funding, then we need to record

outbreaks to highlight to governments how huge the issue is. Even if FMD is linked to global warming, no one will care as the number of reported outbreaks doesn't highlight the impact it has. There are a lot of barriers to reporting and there needs to be further investigation as to how to motivate people to report outbreaks.

Discussion then moved to the South American experience and the effect of stopping vaccinations. The stopping of vaccination means that there is less direct contact of veterinary services with animals, not just for FMD surveillance but also for monitoring other diseases. Mariano Perez-Filgueira stated that, based on the South American experience, it is impossible to move ahead without regional input. Even if there are disagreements there needs to be discussion as there will be points that are in common, especially as some countries will buy vaccine from other countries. Of course, a strong industry and contribution from farmers in buying and applying the vaccines have been essential in the region and in many countries in South America this was strongly guided by the government. The situation in South-East Asia is different as each country has a different language, religion, and culture, therefor it is difficult to find common ground for discussion. Min Park gave a brief update on the WOAH code FMD chapter, which has recently been updated. The newly adopted chapter allows vaccinated animals to enter countries free from FMD without vaccination with provisions. A question was then raised as to why vaccination needs to be stopped if it is working?

A comment was made not to equate the situation in Europe and South America to that in different parts of Asia. They are culturally, economically, and politically different regions. Efforts must be made to understand the other disease priorities and see if FMD vaccines can be delivered along with vaccines for other endemic diseases of importance to the different regions in Asia.

A comment was made that maybe there needs to be a push towards plant protein, raising the question whether we can link animal husbandry with plant protein production, as manure will be critical to produce plant protein,

The discussion ended with comments on whether control programs could include other TADs to make them more effective. Even if countries are free from FMD they may not be able to trade because of other endemic diseases.

Session 2: FMD control using improved diagnostics

The second session was dedicated to diagnostics and what new advancements have been made to control the disease. Labib Bakkali Kassimi started with giving an overview of the current diagnostic tools and the current approaches for improved control. The iceberg example for the reported FMD cases was given; a huge number of outbreaks underneath the visible tip of the iceberg are not reported. FMD may not be a priority in certain countries, there may also be difficulties accessing certain areas, a lack of access to laboratories and difficulty in shipping samples. Current virological diagnostic tools include the detection of the virus (virus isolation which needs to be confirmed to be FMDV), identification of viral proteins (Ag-ELISA, both mAb and polyclonal based) and viral RNA (non-serotype specific real-time RT-PCR and conventional RT-PCR). Conventional RT-PCR can be followed by sequencing and phylogenetic analysis. Serological methods include anti-SP (SPCE, LPBE and VNT) and anti-NSP detection (detecting all serotypes). However, there is an overall lack of commercial kits available, much fewer than there are for other diseases.

What are the new approaches? Papers have been published on RT-loop mediated isothermal amplification (RT-LAMP), portable rtRT-PCR as well as lateral flow immunochromatographic tests (for detection and typing). The main issue is what can be done after an outbreak has been characterised to control the disease. There is also a difficulty in shipping samples to reference laboratories; packaging and dry ice are difficult to procure in certain regions. There are three ways to inactivate samples and make them safer for

shipment, with the additional benefit of being able to be shipped at room temperature. FTA cards (not yet fully validated and may not fully inactivate the sample), acid-treated LFDs and chemical inactivation of liquid samples. Many papers show that even after inactivation you can carry out genome detection by rtRT-PCR, serotype by RT-PCR and VP1 sequencing, and can transfect to recover live virus which could be used for vaccine matching (although depends on RNA integrity).

There are other new approaches for surveillance such as the use of drones to monitor large areas that are difficult to access, for outbreak management including providing real-time data on the spread of the disease, and to assist in the delivery vaccines or other medical supplies. But the cost might be prohibitive.

In conclusion, lab diagnostics play an important role in the control of FMD. Although several advances have been made there is still under-reporting and there needs to be a move towards developing simple and less costly methods. Capacity building and technology transfer are also essential to improve FMD control and must continue.

The next presentation by Nagendra Singanallur started with his observation of samples moving from the field to the laboratory. His thoughts are that one should think beyond traditional roles to maximise the information and samples being taken by field team members (i.e., do not use veterinarians and other para-veterinary staff for vaccination only; they can also act as first responders). Good quality clinical samples are often hard to come by as farmers want to treat the lesions on their animals – making them unsuitable for sample collection. Therefore, the primary veterinarian should be the one collecting the sample before treatment. With COVID, people were trusted to carry out the testing why is this not true for other diseases?

The presentation continued with the use of point-of-care devices such as LFDs. Validation requirements for LFDs are needed and the WOAH manual could be used to approve these devices especially if they will be used in the field. In an endemic situation, the collection of tissue samples may also be important alongside LFDs as tissue samples allow for further characterisation. However, consideration must include how these samples will be shipped including packaging and cold chain if necessary.

Lastly, Michael Eschbaumer gave the German perspective on the regulatory aspects of pen-side diagnostic tests. Their Animal Health Act states that in-vitro diagnostics for notifiable diseases may only be placed on the market after an official marketing authorization by the FLI has been granted. An FMD LFD did receive marketing authorization in 2015 although the sensitivity was much less than real-time RT-PCR and it could not detect all SAT 2 strains. The limited sensitivity had to be noted on the packaging insert and the test was authorized for vesicular fluid and epithelium samples only. One batch of LFDs was released; anyone could buy these tests, and the concern was that negative results would not be followed up correctly (i.e., by laboratory investigation) even though it was clearly stated in the package insert that this is required. It is unclear whether this test was ever used, but the released batch has expired, and the company no longer produces them. The marketing authorization has now been withdrawn. There is good laboratory infrastructure including a PTS carried out by FLI and over 20 regional/state veterinary diagnostic laboratories can rule out FMD with RT-PCRs (accredited to ISO17025). For an outbreak, most of the time is lost between when an animal is infected and when it is realised that there is a suspect case. Once the sample is in the lab, it will only take hours/days for the testing. LFDs may be useful as a secondary test when, if the test is positive, one can make hard decisions on control measures. But with the limited sensitivity and the need to have epithelial samples, you will only get positives with clearly visible clinical signs – in that case, do you still need a positive LFD to confirm? Maybe it is dangerous to put these tests in the field, as the problem with false negatives can jeopardise timely identification of an introduction.

The talks finished and the panellists (Melanie Chitray and Santina Grazioli) were asked to join the speakers. The discussion started with Melanie Chitray sharing her experience in South Africa and the outbreaks they are dealing with. Currently they are only using conventional methods as they know these work and they

have been fully validated. In her opinion LFDs are unlikely to work in to the field as they could be mis-used leading to potential further spread of the disease. Keith Sumption questioned where more research needs to be placed, for example environmental and milk samples.

Discussion was also had on the use of ELISAs and VNTs for vaccine quality. This is difficult to address as VNTs require high containment and can be highly variable. On the other hand, ELISAs are easy to perform but there does not appear to be a clear correlation to protection. It is also difficult to change out the antigen ELISA to make it suitable for different situations as this requires re-validation and access to a panel of diverse sera is in short supply.

A question was raised as to what the gaps in sampling were and how these can be closed. Don King mentioned a study where the findings stated that to measure the burden of the disease 120 samples per pool per year are needed. This does occur in some pools, but it is inherently very biased. It was also mentioned that maybe a more holistic approach should be taken with samples also being taken for other diseases.

The inclusion of farmers to control the disease was considered vital to eradicating the disease in South America. When meetings were held, the CVO sat next to the farmers and all contributed. This discussion continued with the audience agreeing that the private sector must also be included along with farmers being part of the outbreak and hearing the outcome.

Loic Comtet from ID.VET gave his point of view from industry. There needs to be a clear overview of whether LFDs are needed. There is a question of market size and whether this is big enough to warrant the development of these. It is also difficult for them to access strains, etc. that may be needed as well as the funds. One way to support the industry is to have more collaborative research projects with laboratories that could help to develop and validate the tests; however, most funding calls are not open to industry. It was also raised that maybe LFDs would have more of a benefit if they could identify the serotype?

Each diagnostic test also requires a separate registration (sometimes by batch) with each country and these all require different things. It would be beneficial if there was mutual recognition of registrations between countries – for instance within the EU. There is currently no provision for diagnostics if an outbreak occurs – there was discussion of a bank, but this did not materialise on a transnational level.

The conversation then went back to LFDs and their benefit. It was felt that one benefit is that the samples can be tested before they are shipped to ensure that they are positive and thus useful for further studies. In addition, they can be shipped at room temperature which is much simpler; in certain countries they will still be considered dangerous goods as they contain FMDV genome. Maybe a pilot study needs to be carried out to look at the economic impact.

Session 3: Control of FMD using novel vaccines

The last session focused on the control of FMD using novel vaccines. Danny Goovaerts delivered the first presentation, discussing conventional vaccines and why new technology is needed. Since 1976, there has been no major change in vaccine production. No manufacturers are located in the USA and Australia. While conventional vaccines are great in controlling outbreaks, such as those in South Korea in 2010 and 2011, the question remains: why do we need new technologies? Most people underestimate the challenges involved in producing high-quality FMD vaccines. The limitations stem from three key areas: (1) virus-related aspects, (2) vaccine-related aspects and (3) manufacturing challenges. Antigen production requires a BSL3+ facility or higher, which means a high initial investment (50-200 million USD, roughly 1 USD per dose to be produced annually). In addition, FMD vaccine plants have limited versatility in their operations. In Danny Goovaerts' opinion, small-scale manufacturing plants (<50 million annual doses of monovalent vaccine) lack the economy of scale and cannot be competitive. A detailed analysis of the

economic costs associated with vaccine production was then presented. Manufacturing yields are relatively low while inactivated vaccines require large amounts of antigen per dose to achieve good potency. For a high-quality vaccine, an average of 7 ug of 146S per serotype is needed per dose, resulting in a total of around 21 ug for one dose of trivalent vaccine, equivalent to 10 mL of BHK virus culture. This is very high! As a comparison, the production of 1 million doses of inactivated FMD vaccine needs 500-1000 L of serum while only 0.5-1 L is needed for the same number of doses of a live-attenuated PPR vaccine.

Gisselle Medina gave a brief overview of the current approaches and advancements in alternative vaccines. The new vaccine technologies include virus-like particles (VLPs) (produced in vitro and purified or delivered using a viral vector), peptides, DNA/mRNA and modified-live attenuated vaccines. However, no single approach meets all the requirements that are needed to reach an ideal FMD vaccine. Gisselle Medina then highlighted the positives and negatives of each technology. Some positives are that the leaderless vaccine platform allows for easy swap of FMDV strains and the chimeric/mosaic FMD vaccines may provide broader coverage. Purified VLPs, which were discussed on the first day of the conference, are safe to produce outside of BSL3+; however, they still require a booster vaccination and are difficult to make. There are various different expression systems that are used for the expression of VLPs; however, most of these have not moved beyond the mouse model. An overview of the different recombinant viral vectors used was also given (e.g., adenovirus, MVA, fowlpox virus, attenuated pseudorabies virus and Semliki Forest virus which can encode the P1 and 3C coding sequences of FMDV). However, these can be expensive and do not prevent persistence. There are also peptide vaccines, which include FMDV epitopes for T-cells and B-cells. These appear to have low immunogenicity and require prime/boost vaccination strategies. The DNA and RNA vaccines have low immunogenicity and need multiple applications. They also require transcription and/or translation processes. Of course, one also needs to think about modified live-attenuated vaccines (LAVs); studies have looked at these and there is a small chance they may revert when only a few amino acids are mutated, particularly in the Lpro region, to achieve attenuation. There are promising advancements in codon deoptimization technologies, which may mitigate the reversion issue by introducing many synonymous mutations in the coding regions of the FMDV genome without altering the amino acid sequence. She shared encouraging results from cattle trials using a codon-deoptimized vaccine candidate. In conclusion, while novel vaccine platforms try to address limitations on current inactivated vaccines, further research and validation (particularly in livestock) are needed.

The panellists (Jacquelyn Horsington, Elizabeth Rieder and David Mackay) were then asked to join all the speakers upfront. Clarification came from David Mackay as to the registration of live-attenuated vaccines. It is not a tick-the-box exercise as to whether the product is safe or not; it is all done through risk assessment. The risk should include the environment and must be quantitative – this takes a thorough study. Recombination may be accepted if the end product is less virulent than what is in the field.

Further discussion then talked about live-attenuated vaccines and the fact that you may go too far and have no antibody response, or you may not go far enough and have reversion to virulence and/or recombination. However, would live-attenuated vaccines have a longer duration of immunity? Maybe look at polio vaccine, as why would FMD be different? It was then mentioned that the difference between FMD and polio is that the FMD vaccine needs to contain multiple strains and it's not clear how that would work. Also, what happens with animals that have previously been exposed? The O/CATHAY strain in Pool 1 is probably from a live-attenuated vaccine and this will be a criticism if these types of vaccines are in the field again.

The perspective from industry was then given. Pascal Hudelet shared their experience of looking at an alternative vaccine. One such technology looked very promising in cattle and cost about the same; however, when it was tried in pigs it didn't work and it also didn't work with multiple strains, so it went nowhere. Registration is different in each country and can require additional studies – which makes it very expensive. The price of vaccines is very high (£2/dose) – and drastic action needs to be taken to reduce this

especially with twice a year vaccination. Studies should also be carried out to look at the difficulties and costs of re-vaccinating animals.

Chriche du Plessis stated that the development of new vaccines takes time as a process needs to be followed and this cannot be shortened. The efficacy studies that are needed are a regulatory hurdle to register vaccines. Between cattle, sheep and goats, a vaccine registration can easily require 40+ trials. A question was raised as to whether the vaccine manufacturers could work together and carry out one global registration.

It is important to remember the Nagoya Protocol and how it limits the strains that can be used for vaccine production. Maybe this is something that should be addressed in the WOAH manual?

New technologies may be cheaper as they are produced in BSL2 and involve simpler processing. This conversation then led to the duration of immunity and how important that is. Adjuvants help to increase the duration of immunity. Romina Sierra from CDV (FMD vaccine producer in South America) is currently looking at different adjuvants.

A question was also raised about long-term immunity (including memory cells) and whether animals would still be protected if these are around. Do we know what the duration of immunity is after natural infection? There was no consensus on this issue, indicating a need for further investigation. Experimental studies on vaccine efficacy typically do not address the duration of immunity, particularly for modified live attenuated vaccine (LAV) candidates that are expected to induce long-term protection. Several audience members expressed their willingness to collaborate in these efforts.

There seemed to be agreement that it is difficult to distinguish between a good and a bad vaccine in an endemic setting when the vaccination program is not optimal. Also, there are currently no commercial tools available to check vaccine quality. PVM studies shouldn't be undertaken if the quality of the vaccine is not known (i.e. no homologous potency testing was done).

Summary of Key Points

1. Why Should We Control FMD?

- **Cost-Benefit Analysis of FMD Control**:
  Previous studies on the cost-benefit of controlling FMD have faced significant challenges due to the complexity of factors involved, such as the impact of movement restrictions on local cultures. There is a need for closer collaboration with economists to develop effective strategies.
- **Vaccination Campaigns in South America**:
  With efforts to phase out vaccination in South America, the necessity of stopping vaccination campaigns is questioned. Further studies are needed to evaluate the costs of vaccination in relation to the risks of potential FMD reintroduction.
- **Maintaining FMD Relevance**:
  Discussions highlighted the importance of keeping FMD a priority for countries, motivating them to analyze samples and stay vigilant. Broader funding approaches, such as leveraging climate change or antibiotic resistance initiatives, may help ensure the continued relevance of FMD control.

2. FMD Control Using Improved Diagnostics

- **Simplifying Diagnostics**:
  Despite advances in diagnostics, underreporting remains a significant issue. There is a push for developing simpler and more cost-effective methods, such as lateral flow devices (LFDs). However, questions remain about their adoption, timing, and industry investment for commercialization.
- **Understanding Country-Specific Challenges**:
  A bottom-up approach was proposed, starting with the specific needs of endemic or free countries and working backwards to identify diagnostic gaps and develop tailored solutions.
- **Innovative Technologies**:
  More innovation is needed in diagnostic tools, including air sampling technologies with rapid analysis capabilities to enhance FMD surveillance and response.

3. Control of FMD Using Novel Vaccines

- **Defining Immune Response Goals**:
  There is a lack of clarity on what constitutes a robust immune response and how long protection lasts after initial infection. Without this understanding, setting benchmarks for vaccine development remains difficult.
- **Reducing Industry Risk**:
  The high cost and risk associated with developing and testing vaccines using new technologies deter industry investments. Collaborative efforts to share resources and expertise are needed to mitigate these risks.
- **Challenges with Live-Attenuated Vaccines**:
  While the registration of live-attenuated vaccines is possible, the extensive safety evidence and testing required may discourage industry efforts to advance these technologies, yet these challenges also present an opportunity for innovation and collaboration to enhance vaccine development.

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