GFRA NEWSLETTER

January 2024
Issue 14

Our Vision

A coordinated global alliance of scientists producing evidence and innovation that enables the progressive control and eradication of FMD.

Our Mission

To establish and sustain global research partnerships to generate scientific knowledge and discover the tools to successfully prevent, control and eradicate FMD.

INSIDE THIS ISSUE

GFRA 2023 SCIENTIFIC MEETING

***Forward* by: Alejandra Capozzo, GFRA CEO**

A Global Meeting of FMD Researchers in Africa by: Mariano Pérez-Filgueira, GFRA Science Director ('22-24)

GFRA Africa Chapter by: Frank Mwiine, GFRA President

Forward

The 2023 GFRA Scientific Meeting was held in the stunning Kampala, Uganda, from November 8 to 10. The Munyonyo Commonwealth Resort and Conference Centre, our chosen venue, seamlessly blended opulent conference rooms with a lush natural backdrop, providing an ideal environment for the gathering.

With nearly 100 participants from around the globe, a quarter of whom hailed from Africa, the event served as a crucial platform for delving into Foot-and-Mouth Disease (FMD) research in the region. Discussions encompassed the unique challenges associated with implementing FMD control measures.

Feedback from attendees echoed unanimous satisfaction, awarding the Scientific Meeting and its organization a stellar rating of 4.54 out of 5. The event successfully harmonized cutting-edge scientific presentations with social engagements, fostering a sense of camaraderie.

When asked about the highlights of the meeting, participants commended the superb scientific program and the exceptional quality of lectures that presented the latest findings in FMD research. Sessions on novel vaccine platforms captivated the FMD community's attention.

Maintaining a tradition of facilitating in-depth discussions, each session concluded with extensive deliberations, incorporating diverse perspectives from the global scientific community engaged in FMD research in both endemic and free countries.

The meeting cultivated a positive and collaborative atmosphere, mirroring the vision and mission of the GFRA. The intimate gathering of FMD-related professionals promoted effective communication and networking, with every session taking place in a unified space.

A noteworthy testimony to the impact of GFRA came from a first-time attendee, a young scientist, who shared:

"A year ago, I didn't even know GFRA existed, and now I am so glad I do! What I love most about GFRA is the emphasis on collaboration instead of competition! For the sake of science, it is much better to work together to move forward than each lab racing to make breakthroughs first. It's great to get perspectives from so many different sources!"

This sentiment aligns with the GFRA's vision and mission—collaborative, inclusive, and dedicated to advancing global FMD research.

Group Photo in Kampala, Uganda at the GFRA 2023 Scientific Meeting

GFRA 2023 Scientific Meeting: A Global Meeting of FMD Researchers in Africa

By: Mariano Pérez-Filgueira, GFRA Science Director ('22-24)

The GFRA 2023 Meeting in Uganda spanned three days, featuring a program organized by a Scientific Committee comprising more than 20 FMDV scientists from different countries. This committee oversaw abstract reviews and chaired oral presentation sessions within their areas of expertise. Authors worldwide, with a special emphasis on contributions from African countries, delivered a total of 36 oral and 35 poster presentations across nine thematically structured Sessions: Epidemiology, Diagnostics, Pathogenesis, Immunology, Vaccines, and Virology. Additionally, two sessions were exclusively dedicated to FMD research by African-based scientists.

Aiming to promote interactions among attendees, each session reserved its final minutes for engaging questions from the audience and open interactions with the presenting authors. In the same way, posters were displayed throughout the meeting, allowing delegates to visit these presentations at any time and giving them the chance to discuss with the authors during dedicated sessions over the first two days.

Following the introduction by the GFRA CEO Alejandra Capozzo, the opening session started with presentations from local government authorities (F. K. Tumwesabaze), the delegate of the World Organization of Animal Health for Eastern African countries (S. Wakhusama), the Food and Agriculture Organization of the UN (Fabrizio Rosso), and the GFRA (Cyril Gay). Following, the program advanced to the first session dedicated to FMD research in Africa. Susan Kerfua and Theo Night Jones chaired this session, addressing diverse topics such as the assessment of FMD vaccines in Uganda, heterologous protection against SAT1 strains in goats, the creation of FMDV SAT strains-specific peptide phage display libraries for epitope identification, and research on transmission dynamics and vaccine effectiveness in controlling endemic FMD in Ethiopia.

The afternoon session delved into the Diagnostics area, starting with a Keynote presentation by Donald King, who reviewed the global FMD situation based on the World Reference Lab activities and discussed the use of novel technologies for global FMDV surveillance. Vilna Vosloo and Donald King chaired subsequent oral presentations, covering diagnostic methods such as genome sequencing using nanopore technology, on-field FMDV detection through serological methods with lateral flow devices, alternative serological strategies for assessing cross-protection between FMDV strains, and evaluation and comparison of methods for reproducible FMDV challenges in swine.

The second day commenced with the Pathogenesis session, chaired by Jonathan Arzt and Toby Tuthill, featuring a Plenary presentation by Carolina Stenfeldt who discussed the distinct pathogenic features of FMDV infections according to the species of infected animals. Oral presentations included *in vitro* and *in vivo* studies on FMDV pathogenesis in cattle using recombinant leaderless FMDV infective particles, analysis of transmission during the FMDV incubation phase in bovines, and the association between FMDV persistent infection and AH receptor activation in cattle sera.

The subsequent session, chaired by Georgina Limon-Vega and Benedicto Byamukama, focused on the epidemiology of FMDV, including the study of genetic and antigenic variants of serotype O strains in Pakistan, characterization of FMDV in small ruminants in West Africa, a serological survey of serotypes A and O in wildlife interface areas, and a meta-analysis of emergence and control strategies for FMD in Uganda.

The traditional Gala dinner on the second evening of the meeting featured an interactive presentation from Brian Perry. He provided a historical overview of the evolution of FMD control and research in Africa, addressing the progress made over the years and highlighting remaining gaps and emerging opportunities. To conclude the presentation, a group of African scientists also joined the stage to share their experiences and perspectives.

In the afternoon of the second day, a Keynote presentation by Sandra Blaise-Boisseau discussed the interaction between FMDV and cellular type I IFN response in the frame of different experimental models. The subsequent oral presentation session, chaired by Giselle Medina and Michael Eschbaumer, covered topics such as the induction of ISG and its correlation with resistance to FMDV infection in mammals, the use of single-domain antibodies for mapping antigenic sites within FMDV particles, inter-serotypic recombination following superinfection in carrier cattle, and the development of FMDV minigenomes and their potential use in pathogenesis studies.

The final session of the second day was dedicated to research on FMDV immunology. Anna Ludi delivered a Plenary presentation on novel serology-based strategies to evaluate FMD vaccine suitability. Following, the session chaired by Eva Perez and James Zhu, comprised four oral presentations describing the heterologous cross-neutralization within serotype O strains provided by different FMD vaccine schemes in cattle, the improvement of the vaccination efficacy according to the prime-boost interval and the impact of maternally derived antibodies and pre-existing parasitic infections in adaptive immune responses in vaccinated bovines.

The last day kicked off with a session focused on FMD vaccines, chaired by Melanie Chitray and Mariano Perez-Filgueira. During this session, four presentations explored innovative vaccine approaches and discussed their future application. These included stabilized VLP-based vaccines targeting Eastern Africa strains, inactivated vaccines formulated with recombinant FMDV particles incorporating natural antigenic variations to enhance antigenic coverage, and two alternatives for live-attenuated FMDV vaccines generated through a genome recoding approach or codon deoptimization strategies.

The concluding session of FMD research in Africa was chaired by Alice Namatovu and Frank Mwiine. A Plenary presentation by Nina Hennings shared insights gained through the development of the AgResults initiative and discussed the project's criteria applied to enhance the quality of FMD vaccines used in Eastern African countries. The following oral presentations covered an array of results, including the genomic divergence of FMDV strains circulating in Egypt, the FMD situation in Nigeria and the economic impact of FMDV on the production of domestic small ruminants in Cameroon.

The Wrap-up Session, just before lunch on the last day, was conducted by Toby Tuthill and Mariano Perez-Filgueira, the incoming and outgoing GFRA Science Directors, respectively. The primary objective was to consolidate the most significant findings gathered from the chairs in each Session and share them with the audience for open discussions.

During the closing ceremony, Nagendra Singanallur, representing the GFRA Executive Committee, presented awards for poster presentations. The awards were conferred upon David Paton (1st Place Most Scientific Impact), Tamil Selvan Ramasamy Periyasamy (1st Place Best Visual Presentation & 2nd Place Most Scientific Impact) and Maruping Mangena (2nd Place Best Visual Presentation).

Following lunch, two satellite activities enriched the meeting's framework. The first, organized by the Star-IDAZ consortium, aimed to collect relevant information from attendees to construct an updated roadmap for FMD. This initiative served as a complement to the previous Gap Analysis Report conducted by the GFRA in December 2022 in Buenos Aires. Coordinated by Jeremy Salt from Star-IDAZ, the exercise brought together groups of scientists from different countries to identify and prioritize critical topics related to FMD diagnostics, vaccine development, epidemiology, and control strategies with a specific focus on Africa.

The second satellite activity, coordinated by Andrew Shaw, centered on the utilization of nanopore-based methods for sequencing FMDV isolates. This workshop-style activity featured two oral presentations, with Lina González detailing sampling strategies for MinION sequencing for FMDV, and Andrew Shaw providing a step-by-step guide for sequencing FMDV genomes using this innovative technology. As a concluding segment, a hands-on workshop allowed attendees the opportunity to load actual MinION flow cells and engage in the subsequent analysis pipeline.

The 2023 GFRA Scientific Meeting in Uganda successfully brought together scientists from around the world to discuss advancements in FMDV research, with a special emphasis on the current situation of the disease in Africa. The high level of attendance throughout all the sessions and activities offered during the meeting, the potential generation of an African-based GFRA Chapter, coupled with the very positive feedback received from the delegates, attests that the main goals of the meeting—such as promoting research on FMDV in Africa, help on interaction and collaboration initiatives and discussing the latest research advancements in different fields—have been fully accomplished.

Photo of the inaugural GFRA Africa Chapter Meeting Participants in Kampala, Uganda

GFRA AFRICA CHAPTER

By: Frank Mwiine, GFRA President

During the 2023 GFRA Scientific Meeting, delegates and scientists from Africa gathered for the inaugural meeting of the Africa Chapter of GFRA. The purpose of the gathering was to have an African Chapter, one body in Africa coming together to work on FMD research. There is a clear need for more representation from African countries at international meetings. This allows researchers the opportunity to collaborate, access resources, and reach research goals together. Ten countries in Africa were represented at the GFRA Scientific Meeting, leaving many countries missing from the conversation. Some countries do not have FMD research initiatives, and some are not connected to the global FMD community. The Africa Chapter of GFRA aims to interest scientists in these countries to engage in both FMD research and the larger GFRA community.

Future steps for the Africa Chapter include the organizational establishment of the committee and setting goals. The Chapter hopes to operate with a regional mindset – identifying a Representative from each region in Africa to coordinate activities and initiatives. Potential activities include joint grant writing, lobbying for funds, capacity building, mentorship, and promoting collaboration across regions and the Continent.

For more information regarding the Africa Chapter of GFRA, please contact GFRA President Frank Mwiine, fmwiine@gmail.com.

GFRA GAP-GRANT AWARDS

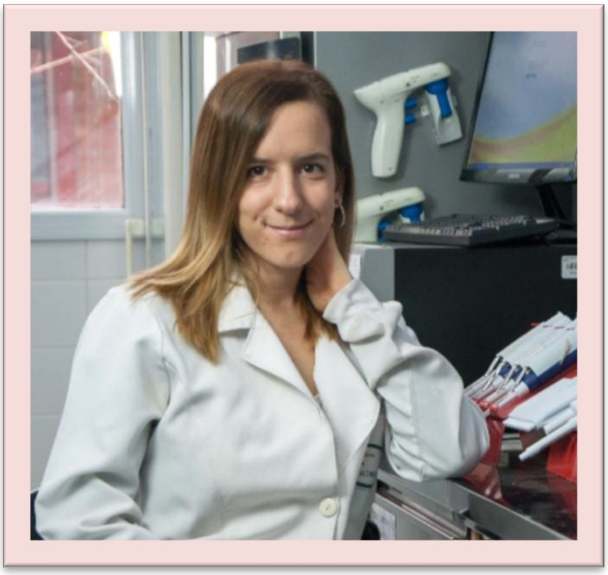
GFRA launched a call for proposals for grant awards in 2022, with winners announced in early 2023. The purpose of these grant awards was to establish and sustain global research partnerships that will generate scientific knowledge and tools to contribute to the successful prevention, control, and, where feasible, eradication of Foot and Mouth Disease (FMD). Grant opportunities were given to those who:

- Conduct strategic and multidisciplinary research to better understand FMD
- Determine social and economic drivers and impact of FMD
- Develop novel and improved tools to support the prevention and control of FMD
- Determine the impact of FMD prevention and control tools
- Serve as a communication and technology sharing gateway for the global FMD research community and stakeholders

Understanding how fascioliasis can influence Foot and Mouth Disease (FMD) vaccine-induced long-term immunity in cattle

By: Florencia Mansilla
Instituto de Virología e Innovaciones Tecnológicas, CICVyA, INTA Nicolás Repetto y Los Reseros s/n, Hurlingham, Buenos Aires, Argentina

Fasciola hepatica, a worldwide distributed helminth, has a robust immunoregulatory effect in the host, increasing the susceptibility to secondary. Despite the evidence of its immunoregulatory effects, the impact of fasciolosis on the immune response induced by Foot and mouth disease (FMD) vaccination in cattle has never been assessed. Our objective was to evaluate whether the infection by F. hepatica in cattle influences the long-term immunity elicited by the currently used commercial FMD-inactivated vaccines. These objectives have been achieved thanks to one of the research grants from the Global Foot-and-Mouth Disease Research Alliance.

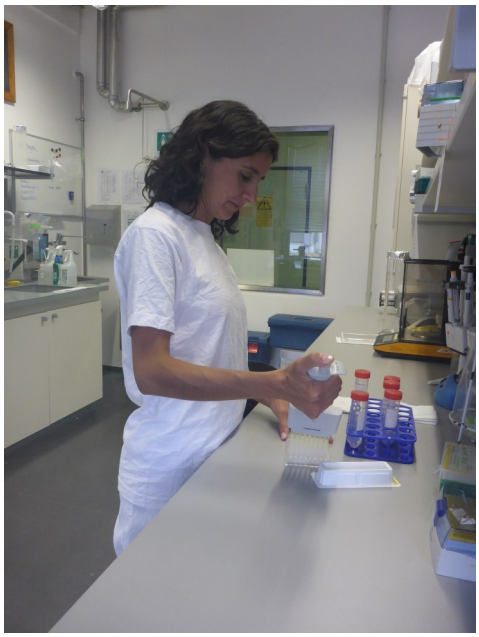


It was very beneficial for us, as all the proposed project objectives have been successfully achieved. The grant has allowed us to carry out this project and to divulgate its main findings. Our results show that F. hepatica infection modified anamnestic responses against FMDV, reducing serum IgG1 titers and avidity. This is the first report of immune-regulation of F. hepatica altering the immune response of FMD vaccines. These results were presented at the latest scientific GFRA meeting and are included in an article that has recently been accepted for publication in a high-impact journal.

Assessment of cross-protection using avidity and isotype ELISAs on FLI-Dr. Brehm´s 2008 samples

By: Nancy Cardoso
National Institute of Agricultural Research, INTA-CONICET, Buenos Aires, Argentina

The Gap-Grant allowed me to travel to the Friedrich Loeffler Institute, in Germany. The proposal aimed to characterize the humoral response induced in cattle by high potency monovalent vaccines followed by the heterologous experimental challenge. Neutralizing antibodies present in these sera have already been determined by VNT, in both homologous and heterologous challenges, by Brehm in 2008. Studies aiming to deepen the understanding of the cross-reactivity between different strains constitute a valuable tool to maximize the protective effect of multivalent vaccines, particularly against variants non-included in these formulations.



This project continued a collaboration, between INTA and FLI both GFRA members, started in 2018 by Dr Capozzo as part of a EuFMD FAR Research Grant. At that point, the collaboration was unfortunately interrupted because of a lack of financing and the recent pandemic situation. During the year 2023, the project could be resume, and we could analyze the samples by Avidity and Isotype ELISAs. The determinations tested allowed us to have interesting results. My stay at FLI was wonderful and provided me with a profitable experience and valuable knowledge for my career. The experience of interacting face-to-face with the FLI community was amazing and produced a huge impact on my personal scientific career development.

Dr. Eschbaumer and the people at the lab were always willing to collaborate and were very friendly and kind....they made me feel at home!

Comparison study of NGS and TGS technologies for FMDV sequencing

By: Lozano Calderón, Laura C[1]; Cacciabue, Marco[2]; Puebla, Andrea F[1]; König, Guido A[1]
[1]Instituto de Agrobiotecnología y Biología Molecular, INTA-CONICET, Argentina.
[2]Departamento de Cs. Básicas - Universidad Nacional de Lujan-CONICET, Argentina

*Name of GFRA GAP-GRANT Awardee underlined

The next-generation sequence (NGS) became available at the beginning of the 21st century with the ability to produce a large amount of data in a highly efficient, rapid, low-cost approach, and accurate DNA sequencing. Illumina is the leading technology in this segment. A new class of DNA sequencing, called third -generation sequencing (TGS), is currently under active development. This technology is capable of sequencing single DNA molecules without amplification and allows the production of reads much longer than NGS, being Nanopore sequencing one of the best examples of this technology.

For FMDV, access to whole genome sequences (WGS) allows multiple studies, from virus evolution to controlled experiments in cells, laboratory animals, and natural hosts. This approach has been used to characterize intra-host dynamics and diversity in viral populations.

As part of a bigger project, we analyzed 13 FMDV samples belonging to the same outbreak for WGS using both, NGS (Illumina) and TGS (Oxford Nanopore Technologies). Briefly, RNA extraction was performed using TRIzol®, followed by RT -PCR of seven overlapping fragments of 369, 702, 1563, 2014, 1884, 2044, and 1824 bp covering the whole FMDV genome. DNA libraries were prepared for the Illumina® and Nanopore® technologies. The NGS methodology was performed on a MiSeq instrument with a 2x250 paired-ended strategy while the Nanopore technique, on a MinION equipment. The generated sequences were filtered and trimmed by quality, length and presence of adapter contamination. The assembly was performed using a reference sequence to finally get a consensus sequence.

All samples have been already processed and sequenced by Illumina and Oxford Nanopore technologies. Preliminary analyses indicated no significant differences in the consensus sequences from both technologies, even though some discrepancies were found at the Single Nucleotide Variant (SNV) level.

This project is partially supported by the 2022 GFRA GAP -GRANT.

Mutational Spectra of Foot and Mouth Disease Viruses Isolated from Egypt, 2022-2023

By: Mohamed Fawzy
Department of Virology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

ABSTRACT

Background and Aim: Foot-and-mouth disease virus (FMDV) serotypes A, O and South African Territories (SAT2) are endemic in Egypt; each is presented by several partially related topotypes and lineages, depending on their geographical origin. Continuous mutations and the emergence of new topotypes that lead to occasional vaccination failures were frequently recorded, so this study aimed to Detect and genetically characterize of the circulating FMD viruses in Egypt at 2022-2023, focusing on amino acids variations in VP1 region.

Materials and Methods: A total of 32 oral tissue samples were collected from cattle and buffaloes in five farms, and 17 individual cases showed clinical signs suspected to be FMD in three Egyptian provinces (Ismailia, Suez, and Sharquia). The samples were tested by real-time (rt) RT-PCR to detect and quantify the virus. Furthermore, FMDV in collected samples was characterized by reverse transcription-polymerase chain reaction (RT-PCR) for amplification of full VP1 region, sequencing, and phylogenetic analysis.

Results: Out of 32 samples analyzed, 27 (84.37%) were positive by rt RT-PCR, but only 24 (75%) produced amplicons by conventional RT-PCR. Serotype O was predominant and detected in 16 samples (50%), serotype A was detected in 8 samples (25%), however serotype SAT2 wasn't identified during the study period. Sequencing and phylogenetic analysis of VP1 demonstrated clustering of serotype A and O in EURO-SA and EA-3 topotypes, respectively. Serotype A showed the highest pairwise identity at the amino acids level with the Venezuelan strains from South America. Serotype O showed the highest identity (92-99%) at the amino acids level with the recent circulating Egyptian strains. The three D structure of VP1 protein revealed amino acid substitutions of the studied isolates that impact the G-H loop. No amino acid substitutions have been detected in RGD motifs or C-Terminus.

Conclusion: The recent detection of the EURO-SA lineage samples may be explained due to imported animals from Colombia or Brazil which share geographical borders with Venezuela. Further studies and control measures of serotype A outbreaks are recommended to avoid further genetic mutation of FMDV.

PI Contact Information: Mohamed_ mohamed4@vet.suez.edu.eg; +002-01007995563

Validation of commercial kits for detection of FMD NSP antibodies in sheep – an example of GFRA collaboration

By: Nagendrakumar Singanallur Balasubramanian and Wilna Vosloo
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Geelong, Australia

Australia is free from foot-and-mouth disease (FMD) and to minimise the impact should an incursion occur, it is essential to implement control measures as soon as a disease is diagnosed. Highly contagious transboundary animal diseases like FMD pose grave and catastrophic economic consequences to the Australian agriculture sector.

Detection of antibodies using serological assays is essential for disease detection in the absence of overt clinical signs. These are vital tools for surveillance, post-vaccination monitoring and vaccine-matching studies. When we consider FMD, detecting antibodies to structural and non-structural FMD virus proteins can distinguish vaccinated from naturally infected animals, which will be crucial for proof of freedom testing when vaccination is used to control the disease. Serological tests (ELISAs) that detect antibodies to FMDV non-structural proteins (NSP) are indicative of FMD infection (both clinical and sub-clinical) and enable the identification of animals infected by any of the seven serotypes, irrespective of their vaccination status. Australia has approved two commercial kits for detecting FMD NSP antibodies. These kits have validation data for cattle but data for sheep and goats are limited.

We recently received funding from Agriculture Victoria (State of Victoria, Australia) to generate vital information on the diagnostic test characteristics of the commercial FMD NSP kits for use in sheep. Since we cannot handle live viruses in our high containment facility at the Australian Centre for Disease Preparedness, Geelong, due to our national policy, we lack samples suitable for our validation studies. One of the primary goals of the Global Foot-and-Mouth-Disease Research Alliance (GFRA) is to facilitate research collaborations and serve as a communication gateway for the global FMD research community. We therefore contacted European partners for samples and laboratory space. Two FMD national laboratories in Europe, the Wageningen BioVeterinary Research, Lelystad-The Netherlands (WVBR) and the Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna, Brescia-Italy (IZSLER) accommodated us with laboratory space and samples as this validation information is invaluable to the FMD research community. Adopting a collaborative approach, we managed to complete the validation exercise using 808 sheep and goat samples sourced from the field (Europe and Africa) by IZLER and another set of 941 sheep and goat samples sourced from the field (Netherlands) and during various experiments carried out at WVBR.

The results of this study will published soon to assist countries with small ruminants where these animals are included in surveillance activities to more accurately design their surveillance plans with known sensitivity and specificity data.

We express our gratitude to Agriculture Victoria for funding this project and to our collaborators, Dr Santina Grazioli, IZLER-Italy, Dr Phaedra Eblè and Dr Aldo Dekker, WVBR-Netherlands, for sharing their valuable samples and their hospitality.

We also take this opportunity to thank Dr Michael Eschbaumer and the Friedrich-Loeffler-Institut, Reims, Germany, another GFRA partner, for hosting our research team for the FMD Ready Project work on FMD virus inactivation kinetics studies.

Charting New Paths: introduction to our webinar coordinators and FMDV research speakers

By: Gisselle Medina
United States Department of Agriculture, Agricultural Research Service (USDA ARS)

Over the past six months, we have had the privilege of welcoming two speakers who provided invaluable insights into various facets of Foot-and-Mouth Disease Virus (FMDV). Our series commenced with an illuminating lecture from Dr. Lidia Lasecka-Dykes of the Pirbright Institute in the UK. Her presentation, entitled "*Usage of Replicons to Understand the Biological Significance of Variability in Foot-and-Mouth Disease Virus Genomes Observed in Nature*," showed the application of replicons as a method for investigating virulence factors in FMDV, allowing for acceleration of FMDV research by inclusion of laboratories which cannot handle the live virus.

Dr. Lasecka-Dykes's research notably emphasizes the study of pseudoknots—a complex secondary RNA structure whose function in FMDV replication remains understood. While the precise role of pseudoknots is yet to be fully elucidated, her findings suggest a potential involvement in the virus's packaging process. This research is a key part of the continuous work to better understand FMDV and greatly helps us learn how the virus operates and causes disease.

In addition, we also had the participation of Dr. Anna Jolles from the College of Veterinary Medicine in Oregon State University in the US. who provided an insightful exploration into "*The multiscale dynamics of FMDV in African Buffalo*" offering an engaging presentation on the disease's behavior in wildlife populations in Africa focusing on the SAT serotypes along with the variable antibody levels that may facilitate the virus's prolonged existence in these populations. Her findings are crucial for understanding and managing FMDV transmission between wildlife and livestock.

Together these webinars not only expanded our understanding but also invigorated our collecting quest for knowledge in the fight against FMDV.

It has been a distinct privilege to assume the role of coordinator for the GFRA webinar series in the latter half of 2023. As we step into the new year, I am excited about the prospect of collaborating with Dr. Melanie Chitray to curate a compelling roster of speakers and delve into even more fascinating topics related to FMDV.

A Call for More Research on the Economic impact of Foot and Mouth Disease in Africa

By: Dr. Simon Dickmu Jumbo and Dr. Ranyl Ngeuna
LANAVET, Cameroon

In Africa, more than 75% of livestock are raised under communal smallholder systems and they sustain the livelihoods of vulnerable groups such as women and children. The vulnerable groups are the most negatively impacted by trans-boundary animal diseases (TADs) which not only limits livestock productivity and well-being, but also the opportunities for regional trade of livestock and livestock products.

A free-range cattle herd in Cameroon

Most of the transboundary animal diseases affecting livestock in Africa have very high mortality and morbidity rate such as Contagious Bovine pleuropneumonia (CBPP) in cattle, Peste des Petits Ruminant (PPR) in sheep and goat, African Swine Fever (ASF) in pigs.

Foot and Mouth Disease (FMD) is another very important transboundary animal disease affecting cattle, pigs, sheep and goat. FMD has a high morbidity rate but with low mortality rate. The clinical signs of FMD include fever, lameness, and salivation (ptyalism) associated with the appearance of characteristic vesicular lesions in the oral cavity and in the interdigital clefts and coronary bands of the feet.

Africa is plagued with wars and other political crises. And as matters of health, Governments give priority to diseases affecting and killing humans such as malaria, HIV/AIDS, Tuberculosis and other vices with the limited resources available. Furthermore, Decision and policy makers in the livestock production and animal health sectors are more preoccupied with controlling zoonotic and Transboundary Animal Diseases (TADs) with high morbidity and mortality rates. They are less concerned with FMD which has high morbidity rate but low mortality rate where most animal affected by the disease do recover. They seem to neglect the economic loss incurred due to FMD. By the way, the real burden of FMD is underestimated.

Ruptured oral cavity vesicles that developed to wound in FMD affected herd in Cameroon

Source: Al Jazeera: Temporary Camps of internally Displaced people recent civil war in Sudan

In fact FMD constitutes a menace to the livestock sector in many countries of the world due to its extraordinary contagiousness and the threat it poses to international trade of livestock and livestock products It is one of the most important economic diseases of cattle, pigs, sheep and goat in the tropics limiting animal production and trade and contributes towards food insecurity in regions where there is a high demand for animal protein for an increasing human population . The lack of infrastructure, economic resources and vaccines tailored to particular condition renders many developing countries vulnerable to the spread and devastating effects of the disease.

The potential for high economic loss is exemplified by the devastating 2001 FMD epidemic in the United Kingdom that resulted in a total cost of over Five billion pound sterlings Another important and documented example is the Ugandan government's reported spending of 5.3million pound sterling and 7.5 million pounds sterling in the 2007/2008 and 2008/2009 budgetary year, respectively, to control FMD through short-term control measures (ring vaccinations, zoo-sanitary measures and quarantines to restrict the movement of livestock and livestock products to and from affected areas as well as ban on the slaughtering of animals in abattoirs within the affected zones) . A loss of 164,100 ± 18,436.8 FCFA was reported in cattle in the Northern Regions of Cameroon.

The ultimate aims of scientists all over the world and Africa in particular is to contribute in the eradication of FMD. For this to happen more research on economic impact of the disease should be carried out. Together with adequate advocacy, this will help decision and policy makers to actually see the enormous direct losses and indirect (incurred due to blockage to export of animal and animal products) caused by FMD. Furthermore, FMD will be given the right priority as far as disease control and eradication is concern. The control and the eradication of FMD will lead to increase in the revenue of vulnerable group (Women and children).

Housing in foot-and-mouth disease challenge tests

By: Aldo Dekker

Wageningen Bioveterinary Research, PO box 65, 8200AB Lelystad, the Netherlands

ABSTRACT

Foot-and-mouth disease is an economic important disease of even-toed animals. For the control foot-and-mouth disease vaccine is often the best option, but only if the quality is good. To test foot-and-mouth disease vaccine quality a potency test is performed (PD_{50} test or protection against podal generalisation). To make sure that the protection data of animals are independent the design of the experiment should be considered. The probability of transmission between animals determines how animals have to be separated after challenge, this is different for pigs, cattle and sheep. Pigs should be separated after challenge, housing cattle in a tie-stall is the best option, for sheep and goats group housing is probably not a big concern. Without independent observations the outcome of a potency test is not valid.

INTRODUCTION

Foot-and-mouth disease (FMD) is a contagious disease which is present in Africa, Asia and one country (Venezuela) in South America. The economic costs of infection in dairy cattle are high. The Netherlands was in 1953 the first country that implemented prophylactic vaccination in all cattle, which was later followed by other European countries and countries in South America (Barteling and Vreeswijk 1991). The World Organisation for Animal Health (previously called OIE; Office des Epizooties) is responsible for setting standards for vaccine potency testing. The method using different dilutions has become the standard for potency testing in Europe, however in South America a different approach was chosen, called PGP test (Protección a la Generalización Podal) in which 16 - 18 cattle are vaccinated with a full dose (Servicio Nacional de Sanidad y Calidad Agroalimentaria 2006; Goris et al. 2008; Anonymous 2017). For animal experiments the 3R principle is very important. The 3R's stand for Replacement of animal studies, reduction of number of animals in experiments and refinement of the experiment. When animal experiments are performed it is essential that they are designed and analysed to ensure robust and reproducible findings (National Centre for the Replacement Refinement & Reduction of Animals in Research 2022). This discussion paper will address the housing used FMD challenge tests with the objective to have robust and reproducible outcomes. As it is a discussion paper (we are planning to write a position paper) any comments can be sent to Aldo.Dekker@wur.nl.

FMDV POTENCY TEST DESIGN

As described above in both, the PD_{50} test as well as the PGP test, the individual results of individual animals are evaluated. From a methodological point of view these observations should be independent. So, infection in one animal should not influence the outcome in the other animals. FMD transmission has been studied extensively. One of the first transmission studies that analysed the time at which transmission of FMDV can occur in cattle was using pair-wise transmission set-up; The transmission from an infected donor steer to a non-vaccinated contact steer was studied for each day after infection. The cattle were kept in contact for approximately 1 day and housed together, free roaming. FMDV transmission occurred on day 1 - 8 after inoculation of the donor steer (Graves et al. 1971). The use of a pair-wise design allows the quantification of infectivity and susceptibility (Hagenaars et al. 2011). After the 2001 outbreak in the Netherlands limited transmission was observed in young cattle and this limited transmission was then confirmed in experimental studies that were setup to quantify the transmission rate (Bouma et al. 2004). The most striking observation in this study, using young calves, was that no transmissions of FMDV occurred when contact between calves was limited, even though in the experiment direct mouth contact between infected and contact calf was possible (see photo).

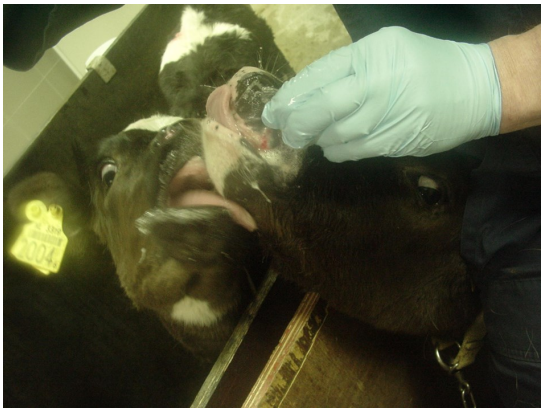


Figure 1: Contact between calves housed in boxes. An FMD lesion is seen in the calf in the front, still no transmission occurred.

Transmission was also studied in calves of the same age that were housed in free roaming groups of 4 calves (2 infected calves and 2 contact calves). In the free roaming calves, the estimated reproduction number was 3; significantly above 1. This shows that the reduction of contact reduces the transmission in calves significantly.

In previous studies it was shown that vaccinated infected cattle can transmit FMDV to non-vaccinated contact cattle housed in a tie-stall (see Figure 2) (Terpstra et al. 1990).

Figure 2: Cattle housed in a tie-stall during a PD_{50} test. There is a drinker for at least every second cow.

That transmission is limited in a tie-stall is also seen in a study where we tested 3 vaccines using 5 cattle per vaccine, based on the antibody titres we decided to challenge with a type A strain, therefore the 5 type O vaccinated cattle were not vaccinated. But as all cattle were housed in one tie-stall the 5 type O vaccinated cattle remained in the same room till the end of the experiment 8 days after challenge. At the end of the experiment none of the type O vaccinated cattle showed FMD specific lesions, not even the cow that was housed next to one of the non-vaccinated control cattle using the same drinking bowl (Dekker, personal observation). These experiments show that in a tie-stall transmission is also limited like the results in the experiments with young calves in crates.

In pigs, potency tests have been performed like the cattle PD_{50} test, there is however no consensus on the challenge method. Transmission studies in pigs have shown that separation of pigs reduces the transmission of FMDV (Eblé et al. 2006; van Roermund et al. 2010; Dekker et al. 2020), and transmission between vaccinated pigs housed in a group can be observed (Orsel et al. 2007). Transmission between pigs is not caused by airborne infection (Alexandersen and Donaldson 2002), but most likely by intradermal infection when fighting for food. Therefore, separation of pigs is such an efficient method to reduce transmission. In animal challenge studies on should make sure that all challenged pigs are exposed to the same challenge dose and route, therefore the pigs must be separated (see figure 3).

Figure 3: pigs separated after challenge in individual pens with individual drinking nipples.

For FMDV challenge tests in sheep inoculation in the coronary band is often used (Burrows 1968), but this has the disadvantage of losing one foot for evaluation. We therefore use tongue inoculation as has been described for cattle using the same dose as used in cattle and using cattle passaged FMDV. With most cattle adapted FMDV strains we get clinical disease in sheep (Hamers et al. 2016; Hamers et al. 2016). Studies on the transmission rate of sheep show that transmission between non-vaccinated free roaming sheep is limited and no transmission was observed in free roaming vaccinated sheep (Orsel et al. 2007; Bravo de Rueda et al. 2014; Bravo De Rueda et al. 2015; Eble et al. 2015). There is therefore limited need to separate sheep after challenge, and they can be kept in groups.

ANIMAL WELFARE LEGISLATION

In many countries there is legislation for the use of animals in animal experiments. In Europe this is directive 2010/63/EU on the protection of animals used for scientific purposes. Preambles 13 of the directive states: "The choice of methods and the species to be used have a direct impact on both the numbers of animals used and their welfare. The choice of methods should therefore ensure the selection of the method that is able to provide the most satisfactory results and is likely to cause the minimum pain, suffering or distress. The methods selected should use the minimum number of animals that would provide reliable results and require the use of species with the lowest capacity to experience pain, suffering, distress, or lasting harm that are optimal for extrapolation into target species". This preamble clearly states 3 important issues:

Good scientific method (see also article 4.1)

Lowest number of animals (see also article 4.2)

Optimal extrapolation to the target species (not covered by the directive)

GOOD SCIENTIFIC METHOD.

The challenge of animals in FMDV potency should be standardised, using a standardised route that ensures that the same dose and route is applied to all animals, and the failure of vaccine induced protection should not influence the outcome in other animals. As discussed above transmission between pigs occurs more easily and that will influence the outcome of a vaccination challenge study. In vaccine challenge studies using pigs it is therefore essential to separate pigs as shown in figure 3. Transmission in cattle is sufficiently reduced by keeping cows in a tie-stall, therefore there is no need to separate the cattle completely. In non-vaccinated sheep the transmission rate is low (the reproduction number is close to 1), therefore we see no evidence to separate sheep in FMDV vaccine potency tests.

In directive 2010/63/EU Annex III §3.3a it is stated that animals that do not live solitary should be housed in stable groups, unless there are scientific reasons to house them individual (article 33.3). The duration of the single housing should be limited to a minimum period and visual, auditory, olfactory and/or tactile contact should be maintained. In pig vaccination challenge studies, pigs can be housed in groups up to the time of challenge, after challenge the pigs should be separated, but the separations should have windows to maintain visual contact (see figure 3, NB this is a refinement), the open top should allow auditory and olfactory contact. To limit infection between cattle after challenge a tie-stall should be used, more restrictions are not necessary, in this type of housing cattle have visual, auditory, olfactory and tactile contact, but most contact is limited to the neighbouring cow (thereby limiting FMDV transmission to maximum 2 other cattle and the limitation in contact limits also the probability of transmission).

LOWEST NUMBER OF ANIMALS.

The numbers of cattle used in a PD_{50} test and PGP test are prescribed in the WOAH terrestrial manual (WOAH 2023); 3 groups of 5 vaccinated cattle and 2 controls in a PD_{50} test, 16 vaccinated cattle and 2 controls in a PGP test. The precision of one experiment is low, in a study testing 10 times the same vaccine the overall potency (using 3 x 50 vaccinated cattle) calculated using logistic regression was 15 PD_{50}/dose with a 95% CI of 8 to 25 PD_{50}/dose (based on the delta method). Using logistic regression, the observed potency per experiment ranged from 5 - 90 PD_{50}/dose. Because the numbers are prescribed lowering the number is not an option (also not because of the low reproducibility). Increasing the experiment with a lower dose, for 4-fold dilutions that would be a 1/64 dose has advantages. With good vaccines it more likely to find vaccinated animals with low antibody responses that are not protected, which then allows for validation of serological tests and gives the opportunity to replace challenge tests.

OPTIMAL EXTRAPOLATION TO THE TARGET SPECIES.

In human medicine many research questions are studied in laboratory animals, as doing the same studies in humans would not be possible. EU directive 2010/63 all live vertebrates (including foetal forms in the last third of their development) as well as live cephalopods are included. Apart from non-human primates, all species are considered the same value. For this reason, studying FMD vaccination responses in mice or guinea pigs should not be allowed; for the optimal extrapolation to the target species, a study in the target species is more appropriate. The EU directive does not consider suffering of small laboratory animals less because they are smaller. In fact, lesions in small laboratory animals are often more severe.

DISCUSSION AND CONCLUSION

The main question addressed in this paper is the housing of animals in FMD challenge studies. When the outcome in individual animals is important in the analysis, infection in one animal should not influence the outcome in another animal. In pig and cattle studies (where the R_0 is high in unvaccinated animals) separation of animals after challenge is essential. Group housing should not be used in cattle and pig studies, whereas the in sheep (and probably in goats) the R_0 is close to 1 in unvaccinated animals, group housing will not influence the outcome very much.

REFERENCES

Alexandersen, S., Donaldson, A. I. 2002. Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. Epidemiol. Infect. 128(2); 313-323.

Anonymous (2017). Foot-and-mouth disease (ruminants) vaccine (inactivated) (monograph 0063). European Pharmacopoeia 9.5. EDQM**:** 1115-1116.

Barteling, S. J., Vreeswijk, J. 1991. Developments in foot-and-mouth disease vaccines. Vaccine 9; 75-88.

Bouma, A., Dekker, A., de Jong, M. C. M. 2004. No foot-and-mouth disease virus transmission between individually housed calves. Vet. Microbiol. 98(1); 29-36.

Bravo de Rueda, C., de Jong, M. C., Eble, P. L., Dekker, A. 2014. Estimation of the transmission of foot-and-mouth disease virus from infected sheep to cattle. Vet. Res. 45; 58.

Bravo De Rueda, C., Dekker, A., Eblé, P. L., De Jong, M. C. M. 2015. Vaccination of cattle only is sufficient to stop FMDV transmission in mixed populations of sheep and cattle. 143(11); 2279-2286.

Burrows, R. 1968. The persistence of foot-and mouth disease virus in sheep. J. Hyg. (Lond). 66(4); 633-640.

Dekker, A., van Roermund, H. J. W., Hagenaars, T. J., Eblé, P. L., de Jong, M. C. M. 2020. Mathematical Quantification of Transmission in Experiments: FMDV Transmission in Pigs Can Be Blocked by Vaccination and Separation. Front. Vet. Sci. 7(955).

Eblé, P., de Koeijer, A., Bouma, A., Stegeman, A., Dekker, A. 2006. Quantification of within- and between-pen transmission of Foot-and-Mouth disease virus in pigs. Vet. Res. 37(5); 647-54.

Eble, P. L., Orsel, K., van Hemert-Kluitenberg, F., Dekker, A. 2015. Transmission characteristics and optimal diagnostic samples to detect an FMDV infection in vaccinated and non-vaccinated sheep. Vet. Microbiol. 177(1-2); 69-77.

Goris, N., Maradei, E., R, D. A., Fondevila, N., Mattion, N., Perez, A., Smitsaart, E., Nauwynck, H. J., La Torre, J., Palma, E., De Clercq, K. 2008. Foot-and-mouth disease vaccine potency testing in cattle using homologous and heterologous challenge strains: Precision of the "Protection against Podal Generalisation" test. Vaccine 26(27-28); 3432-3437.

Graves, J. H., McVicar, J. W., Sutmoller, P., Trautman, R. 1971. Contact Transmission of Foot-and-Mouth Disease from Infected to Susceptible Cattle. J. Infect. Dis. 123(4); 386-391.

Hagenaars, T. J., Dekker, A., de Jong, M. C., Eble, P. L. 2011. Estimation of foot and mouth disease transmission parameters, using outbreak data and transmission experiments. Rev. Sci. Tech. 30(2); 467-77.

Hamers, C., Dekker, A., Mouton, L., Besset, M., Bleijenberg, M., Schaijk, B. v., Coco-Martin, J., Curet, M., Goutebroze, S. Efficacy of an FMD inactivated vaccine (Aftovaxpur DOE), administered at a 1 ml dose. Open Session of the EuFMD Standing Technical Committee, Cascais, Portugal 26 - 28 October 2016.

Hamers, C., Mouton, L., Besset, M., Dekker, A., Bleijenberg, M., Schaijk, B. v., Coco-Martin, J., Curet, M., Goutebroze, S. Development of a virulent FMD challenge model in sheep. Open Session of the EuFMD Standing Technical Committee, Cascais, Portugal 26 - 28 October 2016.

National Centre for the Replacement Refinement & Reduction of Animals in Research. (2022). "Definitions of the 3Rs." Retrieved 27 December, 2022, from https://nc3rs.org.uk.

Orsel, K., de Jong, M. C. M., Bouma, A., Stegeman, J. A., Dekker, A. 2007. Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. Vaccine 25(34); 6381-6391.

Orsel, K., Dekker, A., Bouma, A., Stegeman, J. A., de Jong, M. C. M. 2007. Quantification of foot and mouth disease virus excretion and transmission within groups of lambs with and without vaccination. Vaccine 25(14); 2673-2679.

Servicio Nacional de Sanidad y Calidad Agroalimentaria. (2006). "Resolución 351/2006 Actualízase la reglamentación que permite el control de las vacunas destinadas a la prevención de la Fiebre Aftosa." Retrieved 28 December, 2022, from http://servicios.infoleg.gob.ar/infolegInternet/anexos/115000-119999/117636/norma.htm.

Terpstra, C., van Maanen, C., van Bekkum, J. G. 1990. Endurance of immunity against foot-and-mouth disease in cattle after three consecutive annual vaccinations. Res. Vet. Sci. 49(2); 236-42.

van Roermund, H. J. W., Eblé, P. L., de Jong, M. C. M., Dekker, A. 2010. No between-pen transmission of foot-and-mouth disease virus in vaccinated pigs. Vaccine 28(28); 4452-4461.

WOAH (2023). Foot-and-mouth disease (infection with foot-and-mouth disease virus). Manual of diagnostic tests and vaccines for terrestrial animals, World organisation for animal health**:** 1-34.

GFRA Executive Committee 2024-2026

Chief Executive Officer	**Alejandra Capozzo** National Institute for Agricultural Technology, Argentina capozzo.alejandra@inta.gob.ar
President	**Frank Mwiine** Makerere University, Uganda fmwiine@gmail.com
Science Director	**Toby Tuthill** Pirbright Institute, UK toby.tuthill@pirbright.ac.uk
Finance Director	**Michael Eschbaumer** Friedrich-Loeffler-Institut, Germany michael.eschbaumer@fli.de
Secretariat	**Anna Ludi** Pirbright Institute, UK anna.ludi@pirbright.ac.uk
Members	**Melanie Chitray** ARC: Onderstepoort Veterinary Research Vaccine and Diagnostic Development, South Africa chitraym@arc.agric.za
	Phaedra Eblé Wageningen Bioveterinary Research, The Netherlands phaedra.eble@wur.nl
	Gisselle Medina United States Department of Agriculture, Agricultural Research Service, USA gisselle.medina@usda.gov
	Mariano Pérez-Filgueira National Institute for Agricultural Technology, Argentina perez.mariano@inta.gob.ar
	Nagendra Singanallur Australian Centre for Disease Preparedness, CSIRO, Geelong, Australia nagendra.singanallur@csiro.au

STAY IN TOUCH WITH GFRA

* Visit the GFRA website: www.ars.usda.gov/gfra/
* Follow GFRA on Twitter @gfra_official
* To join the GFRA mailing list to stay up to date on GFRA news, events, and opportunities, please email GFRA secretariat tcoughtreydavenport@crdfglobal.org