JULY 2021 ISSUE 11

GFRA NEWS

The official newsletter of the Global FMD Research Alliance

Save the date: 1-3 November 2021

GFRA Scientific Meeting – now virtual!

Click here: Register today!

It is our very great pleasure to announce the **VI Global Foot-and-Mouth Disease Research Alliance (GFRA) Scientific Meeting** to be held **virtually** due to the COVID-19 pandemic, from 1-3 November 2021. This time the ongoing COVID-19 pandemic banned the chance of meeting in person in Buenos Aires as we have planned and announced during our last meeting in Thailand 2019.

The **GFRA 2021** meeting will bring together experts from across the globe to share their research on basic virology, immunological insights around the vaccination and infection in different species, epidemiology, diagnostics, and novel treatments to control FMD.

The virtual event, as well as all our previous meetings, will prioritize long fruitful discussions. Special attention will be brought to the delegate's contributions, and poster presentations will be the landmark of this virtual meeting. We will take advantage of the virtual environment to make it the perfect place for each of you to share your research with the global community and start or keep on "networking."

Registration is now opened https://www.gfra2021.com/registrations/ - join us! All your contributions will be visualized and interaction will be encouraged through the virtual platform. We look forward to welcoming you all to the first virtual **GFRA 2021** meeting!

Dr. Alejandra Capozzo
GFRA Chief Executive Officer

IN THIS ISSUE:

NEWS AND VIEWS

First EuFMD-GFRA Virtual Regional Meetings

***Dr. Alejandra Capozzo**, GFRA-CEO, CONICET-INTA, Buenos Aires, Argentina*

***Dr. Wilna Vosloo**, GFRA ExCo, CSIRO-Australian Centre for Disease Preparedness*

EuFMD and GFRA are the most important organizations dealing with foot-and-mouth disease (FMD) worldwide. While EuFMD is more focused on policies and disease control strategies, GFRA leads research and addresses research gaps that are identified during ad hoc meetings.

In this effort of getting together most of the FMD actors around the globe, we organized two virtual meetings. The first one called "FMDV in the Americas· (March 10th, 2021) focused on regional Vaccine Banks and Epidemiology. The second one was based in Asia (March 25th, 2021) and explored vaccination principles and practice.

First GFRA/EuFMD virtual symposium: FMDV in the Americas

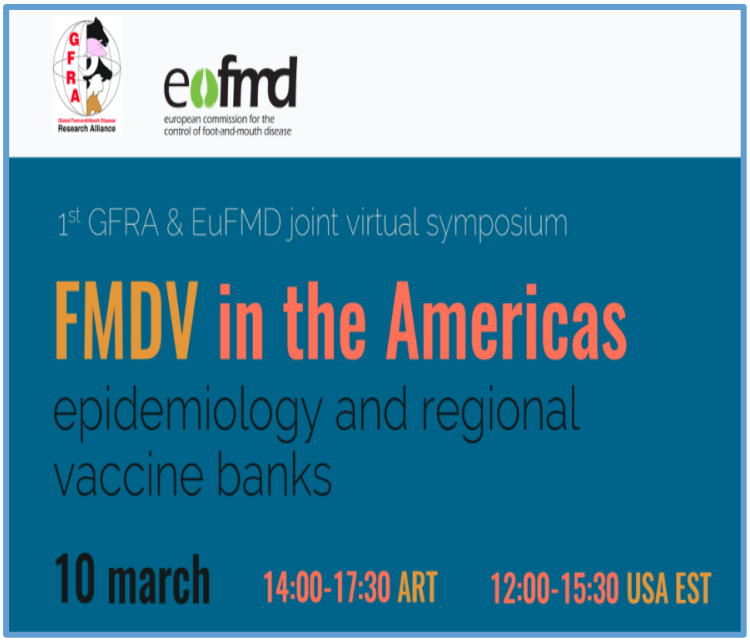
This was the first time EuFMD and the GFRA worked together and promoted a bilingual meeting that enabled the involvement of Latin-American professionals including scientists, experts, veterinarians, and graduate students who are sometimes excluded from these discussions due to language. We fulfilled our commitment of "giving a voice" to all the FMD actors in our vast continent.

The meeting focused on Vaccine Banks and Epidemiology, two highly relevant, current issues in the Americas. Our continent has different epidemiological situations although it is mostly free of FMD with or without vaccination, while outbreaks have been sporadic and controlled by vaccination. Many countries are pursuing the cessation of vaccination and move to a vaccine-free status, supported by a vaccine bank to respond to an emergency. Countries that pursue continuing vaccination also rely on vaccine banks to respond to introductions of new strains.

In this scenario, more information on the available regional vaccine bank was needed, together with the novel tools and epidemiological data that can be applied to have knowledge of the situation and organize an accurate contingency plan in the case of an emergency. Representatives from the different banks in the continent were gathered and as well as experts on the epidemiology in the region. Participants received and discussed novel information provided by the experts and guided by the moderators. See the complete report on our webpage:

Symposium Report

Workshop Recording

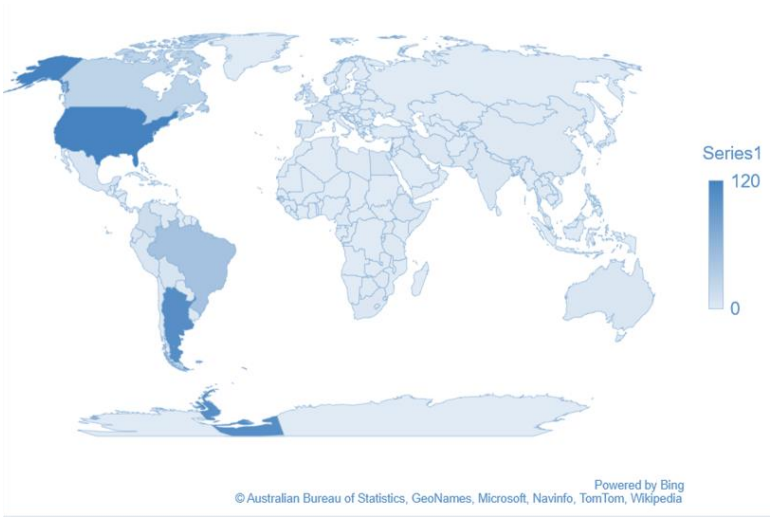


Dr. Alejandro M. Rivera from PANAFTOSA (Brazil) presented the Regional FMD Antigens and Vaccines Bank BANVACO. Dr. Jamie Barnabei (USDA) introduced the North American FMD vaccine bank (NAFMDVB), and the National Animal Vaccine and Veterinary Countermeasures Bank (NAVVCB), while Dr. Ana Taffarel (SENASA, Argentina) overviewed the experience of the Argentinian Vaccina Bank.

The session provided strong evidence of functional vaccine banks in the region, although the actual collaboration between them is not as active as it could be, and this can be understood by defence issues, among others. However, the existence and interchange of a global community of scientists, regulatory agencies and field actors with open communication can work towards a common aim: fill the gaps in our knowledge of FMD-surveillance, vaccine matching, diagnostic tools, viral reservoirs, among other issues. FMD is recognized as a transboundary global disease that should be tackled through strong regional collaboration to improve science, particularly that of the low-middle income countries. It is worth noting that sporadically endemic areas and those that still perform vaccination programs are in Central and South America and constitute most of the countries in this continent.

The epidemiology session held two lectures by Dr Andres Perez (UMN, USA) who overviewed surveillance approaches for FMD and Dr Manuel Sanchez (PANAFTOSA, Brazil) who presented examples of targeted surveillance approaches for FMD in South America.

The discussion moved around barriers and challenges to surveillance in the context of The Americas, how to approach the analysis of hazard and risks and how to harmonize surveillance systems in the region, among other themes. The experts agreed on the need to explore the use of targeted (risk-based) or enhanced passive surveillance approaches for the early detection of the disease as the most efficient strategy to mitigate the impact of a hypothetical FMD incursion into a free country or region. Regardless of the approach, they highlighted that high quality data are critical to ensure effective surveillance in areas, countries, and regions.



We had more than 400 registrations from different countries, and most of the attendees were from Argentina and USA, followed by Brazil and Canada. Almost all Latin American countries were represented. The workshop also captured the attention of colleagues from Europe, Africa, Asia, and Australia.

Second GFRA/EuFMD virtual regional workshop for Asia and Southeast Asia

This workshop brought together 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.

David Paton set the scene with a presentation entitled 'Serological monitoring of FMD vaccination—Principles and Practice.' Anna Ludi presented on 'A new model for independent FMDV vaccine QA/QC as an aid to vaccine selection.'

Wilna Vosloo asked the question: 'In vivo testing of vaccines in Southeast Asia – how well do antigen matching correlate with protection?' Elizabeth Rieder gave a presentation on 'Novel FMD vaccines and their future use in developing countries.' David Mackay gave the final presentation on 'Principles and best practice for official batch control of FMD vaccines.'

There were 368 registrations from 63 countries, demonstrating the interest of the regional FMD community in the practice of vaccination. The participants were interested in aspects such as combination of vaccines with vaccines to other diseases, booster FMD vaccinations, population immunity and surveys, vaccine matching, r1-values, antigen payload and potency.

These questions were addressed in the report available at the link below. The recording of this meeting is available on YouTube.

This Workshop shared 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.

Regional Workshop Report ***Workshop Recording***

Wageningen Bioveterinary Research: New FAO Designation

We proudly announce that Wageningen Bioveterinary Research (WBVR), The Netherlands, has been designated as FAO Reference Centre for foot-and-mouth disease. WBVR has focused its FMD research on quantification of (cross-)protection. Historically, WBVR has been producing FMD vaccine and currently it is regularly performing potency test for commercial companies. WBVR has an inventory of 3-week post-vaccination sera from such potency tests which can be used for standardization of diagnostic tools (e.g. standardization of the relation antibody response and protection). Our unique serum collection is especially valuable for vaccine matching studies. Another unique feature of our lab is that we have a collection of llama single domain antibodies (VHHs) available that have been developed for in-vitro evaluation of FMDV antigens (llama single domain antibodies; see below). The FAO reference center is led by A. (Aldo) Dekker and P.L. (Phaedra) Eblé.

RESEARCH PAPERS

Characterization of llama single domain antibodies

WBVR has a broad panel of llama single-domain antibodies (VHHs) against a diverse panel of FMDV strains. We especially have selected capsid specific VHHs that thus do not bind 12S particles. In a collaborative research with Lanzhou Veterinary Research Institute and The Pirbright Institute we recently discovered that some of these VHHs bind well to natural (75S) empty capsids and virus-like particles (VLPs) while others only bind 146S particles (Table 1). The VHHs were tested with FMDV serotype A, O, C, Asia1 and SAT2 vaccine strains. We aim to make these reagents available to the FMDV research community.

Table 1. Specificity in ELSA of FMDV binding VHHs available at WBVR.

VHH	Serotype Specificity	Strains recognized (SAT1 / SAT 3 unknown)	Particles bound in ELISA (Yes/No/Not Determined) 12S	146S	75S	VLPs
12S specific						
M3ggsVI4q6e	O, A, C, Asia 1	All strains tested except SAT2 SAU/2/2000	Y	N	N	N
M3F	O, A, C, Asia 1	All strains tested except SAT2 SAU/2/2000	Y	N	N	N
Capsid specific						
M170F	O	O1 Manisa, O1 BFS	N	Y	Y	Y
M210F	O	O1 Manisa, O1 BFS	N	Y	ND	Y/N
M377F	SAT2	SAT2 SAU/2/2000	N	Y	Y	Y
M691F	A	Most serotype A strains	N	Y	N	N
M702F	A	About 50% of serotype A strains	N	Y	Y	Y
M332F	Asia 1	Asia 1 Shamir	N	Y	Y	Y
M8F	O, A, C, Asia 1	All strains tested	Y	Y/N	Y	Y
12S and capsid binding						
M98F	Asia 1	Asia 1 Shamir, Asia 1 Bahrain	Y	Y	ND	Y
M220F	O, A, C, Asia 1	All strains tested	Y	Y	ND	Y
M311F	SAT2	SAT2 SAU/2/2000	Y	Y	ND	Y

Novel research on VHHs at WBVR now focusses on epitope mapping of especially the capsid specific VHHs. This research is done in close collaboration with Lanzhou Veterinary Research Institute. That group recently published the Cryo-EM structure of complexes of VHHs M8 and M170 with FMDV (Dong et al., 2021; https://doi.org/10.1007/s13238-021-00828-9). M8 recognizes a site on the VP1 GH loop. M170 was found to bind a site on VP3 that has an altered conformation in 12S particles. This shows that the FMDV capsid is not a rigid structure but flexible.

These very specific VHHs can be used as tools for the characterization of FMDV antigens, even after formulation with adjuvant. Furthermore, we are currently in the process to isolate VHHs against the more conserved VP4 protein to develop an 146S specific ELISA suitable for all serotypes. This approach is inspired by unpublished work from Amin Asfor and Toby Tuthill from The Pirbright Institute. For further information and requests for specific VHHs please contact M.M. (Michiel) Harmsen (Michiel.Harmsen@wur.nl).

MGPKαVβ6, a porcine cell line free from BVDV and other adventitious agents, offers optimal growth of all FMDV serotypes

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This article provides summarized data from a manuscript in press in the journal Biologicals (available online, June 04, 2021).

The porcine LFBKαVβ6 cell line features great capability for detection, isolation diagnostics, and propagation of all FMDV serotypes1. However, their initial characterization revealed the presence of an adventitious agent in the form of the non-cytopathic (NCP) bovine viral diarrhea virus (BVDV). This type of contamination becomes problematic when use of this cell line is directed for vaccine production, an aspect that has not been examined before. In this study, we developed a three-prong methodology to completely remove BVDV from these cells without affecting the viral growth of the different FMDV serotypes. We have named the clean cell line MGPK αVβ6-C5. Additionally, isolation of FMDV from field oro-pharyngeal samples (OPF), was successful at the same sensitivity as in the BVDV-contaminated LFBKαVβ6 cell line. Our results identified a direct method to efficiently eliminate BVDV from porcine cells without altering FMDV permissiveness, diagnostic value and potential use for vaccine manufacturing.

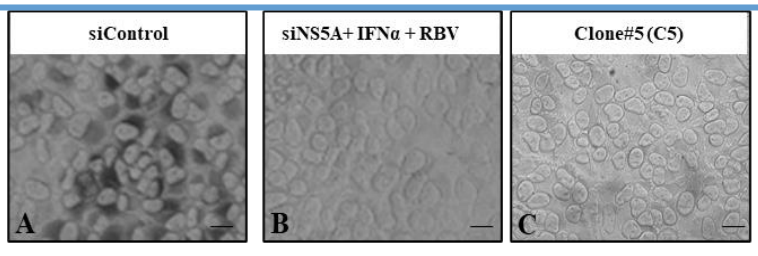

Figure 1. Combination treatment with siNS5A, Ribavirin and IFNα effectively eliminates BVDV from LFPKαVβ6. Cells were transfected with siNS5A (40nM) in combination with Ribavirin (10nM) and IFNα (100 U/ml), or siRNA control. Forty-eight hours post transfection cells were fixed and stained to evaluate for the presence of BVDV as described in Figure 1. A: cells untreated with the antivirals. B: pool of cells treated with siNS5A, Ribavirin and IFN-α. C: single cell derived clones from treated cells. Scale bar 30μm.

In order to eliminate BVDV from LFBKαVβ6, we use siRNA against regions of the BVDV genome (siNS5A) previously described to inhibit BVDV replication 2. Stable depletion (~99%) of BVDV was achieved 48h post siRNA transfection with no cytotoxicity detected. Under these conditions, additional treatments with antivirals known to inhibit the replication of pestivirus (IFNα and Rivabirin) 3–5 was used in order to ensure complete removal of the adventitious agent (Figure 1).

Combinatorial treatment of porIFN-α and Ribavirin in previously siNS5A-treated LFBKαVβ6 cells completely eliminated BVDV positive signal as determined by immunohistochemistry (Figure 1B) when compared to siControl treated cells (Figure 1A). Next, we derived single clones of BVDV- free LFBKαVβ6 cells (Figure 1C) and determine their stability after repeated passage, and the maintenance of their susceptibility to FMDV infection.

Clean cells (MGPKαVβ6-C5) were infected with animal-derived FMDV of different serotypes and their titers were measured by plaque assays (Figure 2). Bovine or porcine derived FMDV O1 Taiwan, A24, ASIA, SAT-1, SAT-2, SAT-3 or C3 Resende were detected at similar titers and plaque morphology in LFBKαVβ6 or MGPKαVβ6-C5 cells (Figure 2A-B). Together these results indicated that MGPKαVβ6-C5 cell free of BVDV maintain their susceptibility to FMDV infection.

In addition to BVDV, MGPKαVβ6-C5 cells were evaluated by NGS analysis for the presence of other extraneous agents, including Reovirus, Rabies Virus, Blue Tongue Virus, Bovine Adeno Virus, Bovine Parvo Virus, Bovine Respiratory Syncytial Virus, Porcine Adenovirus, Porcine Parvovirus, Transmissible Gastroenteritis Virus and Porcine HA Encephalitis virus. Genome sequences obtained from the total RNA isolated from MGPKαVβ6-C5 showed no contig formation for any of the extraneous agents mentioned above during blast analysis.

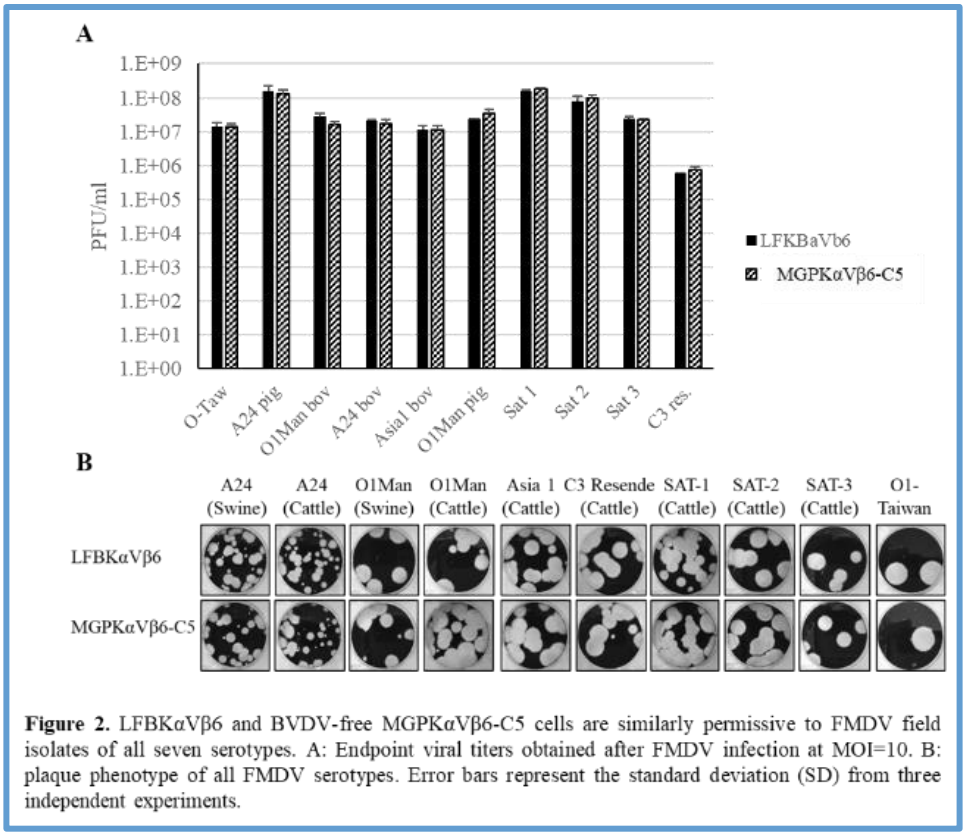

Figure 2. LFBKαVβ6 and BVDV-free MGPKαVβ6-C5 cells are similarly permissive to FMDV field isolates of all seven serotypes. A: Endpoint viral titers obtained after FMDV infection at MOI=10. B: plaque phenotype of all FMDV serotypes. Error bars represent the standard deviation (SD) from three independent experiments.

Our results demonstrated, for the first time, that the combinatorial use of siRNA, porIFN-α and ribavirin can completely remove NCP BVDV from infected porcine cells.

The methodology described in this work6 could potentially be utilized to eliminate the presence of other adventitious agents not only in porcine but other mammalian cell lines of interest. Furthermore, these cells may provide a useful platform for diagnostics and propagation to high titers of other viruses of interest for the animal health community.

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