JULY 2022, ISSUE 12

GFRA NEWS

The official newsletter of the Global FMD Research Alliance

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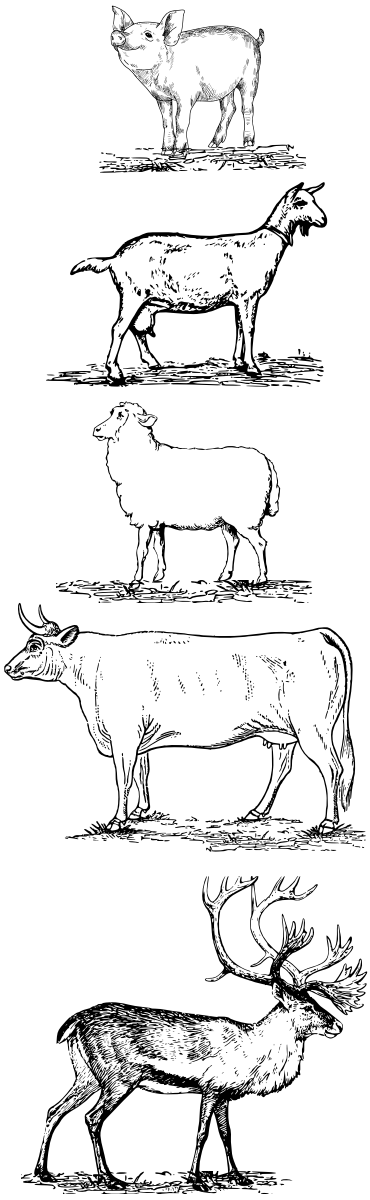


123RD EUFMD-GFRA VIRTUAL REGIONAL MEETINGS

3rd GFRA/EuFMD Regional Virtual Workshop: FMD in Africa
February 2022

This workshop ran over two days and focused on challenges that are unique to Africa to work towards the control of foot-and-mouth disease.

PAST EVENTS

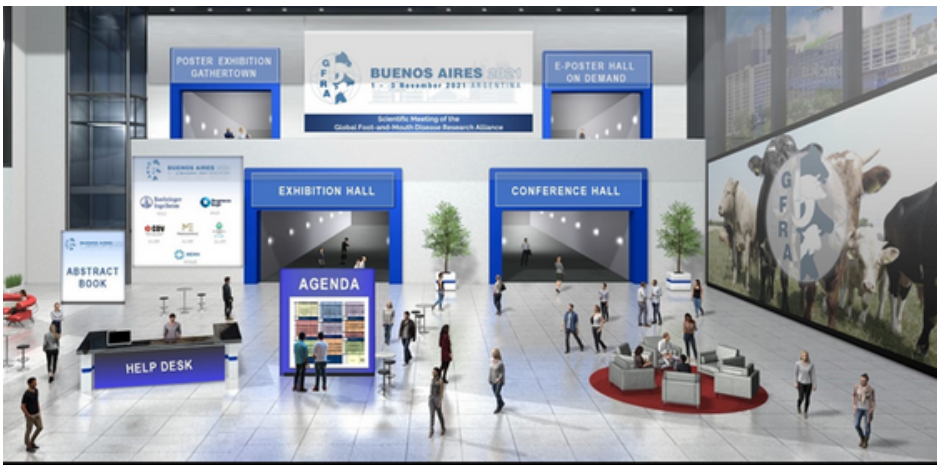
2nd GFRA/EuFMD Virtual Symposium: Vaccination Against FMD-Principles and Practise
March 2021

GFRA/EuFMD Regional Meeting: FMDV in America
March 2020

GFRA 2021 VIRTUAL SCIENTIFIC MEETING

Alejandra Capozzo

The 2021 GFRA meeting, expected to be held in Buenos Aires, Argentina, moved to the virtual format due to the COVID-19 pandemic. This format is definitely not the same as in-person meetings but the technical committee mostly conformed of young Argentinean researchers, worked really hard to produce a less distant experience through our computers. They built “gather town,” a virtual space where each participant can have an avatar, walk around, have a look at posters, meet with other participants and even give and receive a virtual hug!



VISION

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

MISSION

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

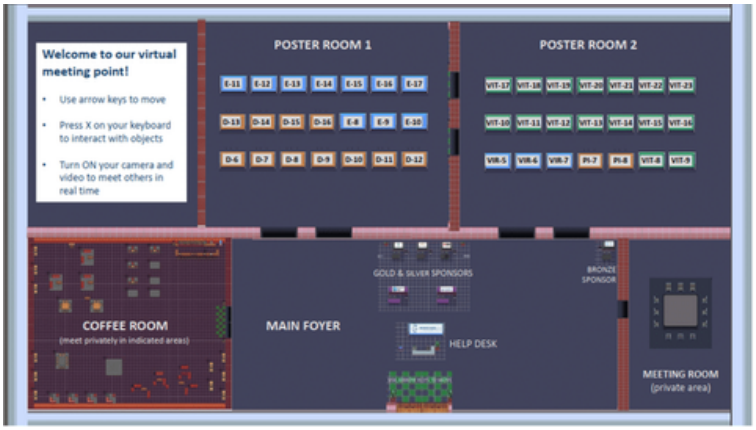
GFRA 2021 VIRTUAL SCIENTIFIC MEETING

continued

Alejandra Capozzo

The meeting was structured as usual, covering our usual topics as epidemiology, vaccines, immunopathology, virology, and diagnostics. We had keynote speakers for each thematic block and invited speakers, all of them selected by the scientific committee.

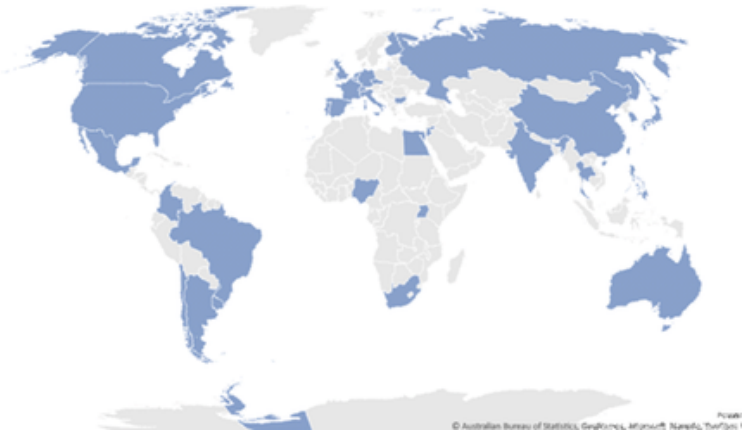
The scientific committee deserves a special mention. It was an interdisciplinary, gender-balanced group with worldwide representation, which worked through virtual contacts and committed to the meeting. Apart from the usual activities expected for this committee they also helped to the last-minute selection of best presentations.



Assistance and contributions

The meeting had 275 registered participants, with live 140 – 180 participants along the three days, even though the time-zones made it complicated for colleagues from Australia, Asia, and western north America.

We received 71 abstracts, 29 oral presentations and 42 posters from all over the world.



The Highlights

The most interesting highlights of the meeting were examples on how basic research is continuously translated into applied development. One example of translational research was the unveiling the immune-suppressive activity of structural proteins and how this information can be used to improve immunogenicity of vaccines. The knowledge on capsid structure produced over the years was also paramount for the development of new-generation VLP vaccines.

The need of using new assays for post-vaccination monitoring was also discussed. Avidity of specific antibodies upraise as a feasible alternative to the virus neutralization tests. This was approached by the use of different platforms.

Sequencing tools were also discussed, together with new approaches for molecular diagnostics.

Opening Session

The opening session included an overview of the FMD worldwide situation with a focus on America. Dr. Manuel J Sanchez Vazquez, Coordinator of the Epidemiology Area & Interim Coordinator of the Foot-and-Mouth Disease Area at PANAFTOSA-PAHO/WHO, provided an overview of the recent evolution of FMDV South America emphasizing the region has had steady progress in eliminating the risk of FMD and progressively adding new FMD free zones recognized by the OIE. The PHEFA 2021-2025 plan is being implemented with the general purpose of completing FMD eradication and mitigating the north of Andean region risks.

This talk was followed by two regulatory laboratories in the region, one from an FMD- free country without vaccination by Dr Roberto Navarro López from SENASICA Mexico and the other from Argentina that has areas free with vaccination, by Sabrina Galdo Novo (SENASA). Both speakers updated the audience on how their countries are organized to tackle a possible incursion of the virus by installing "epidemiological surveillance contact points" in key areas, in the case of Mexico; while in Argentina is aligned with the PHEFA 2021-2025 plan, updating the vaccine bank and vaccine control methods.

INVITED SPEAKERS AND KEYNOTE LECTURES

Anna Munsey
Carolina Stenfeldt
Caroline Wright
Catalina Esther Avendaño Valenzuela
Cecilia Caldevilla
Charles Nfon
Cindy Bernelin-Cottet
Cristian Malnero
David Mackay
Emma Brown
Fayna Díaz-San Segundo
Gisselle Medina
Ian Fish
James Zhu
Jeffrey Hammond
Joaquin Bozzo
Julian Seago
Keith Sumption
Laura Camila Lozano Calderon
Lidia Dykes
Livio Heath
Lucy Gordon
Manuel Sánchez Vázquez
Margarita Sáiz Zalabardo
María Victoria Iriarte Barbosa
Mariano Pérez Filgueira
Micaela Ziraldo
Michael Eschbaumer
Michael Puckette
Natasha Edwards
Nick Knowles
Nicolas Cardenas
Petrus Jansen van Vuren
Roberto Navarro López
Ruben Marrero Diaz de Villegas
Sabrina Galdo Novo
Samia Metwally
Soumendu Chakravarti
Syed Jamal
Umanga Gunasekara
Zhidong Zhang

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WINNERS

Best oral presentations

Lidia Dykes
The Pirbright Institute, UK
Mutagenesis Mapping of RNA Structures within the Foot-and-Mouth Disease Virus Genome Reveals Functional Elements Localized in the Polymerase (3Dpol)-Encoding Region

Ian Fish and **Carolina Stenfeldt**
U.S. Department of Agriculture, United States
Both oral presentations are part of the same research line) Frequent inter-serotypic recombination of foot-and-mouth disease virus during subclinical coinfection with serotype o and a viruses / simultaneous and staggered foot-and-mouth disease coinfection of cattle

Micaela Ziraldo
Centro de Virología Animal, Argentina
Characterisation and efficacy assessment of an adenovirus-based vaccine candidate which delivers the expression of FMDV O1/Campos/Brazil/58 virus-like particles.

Best poster

Juan Manuel Sala
INTA, Argentina
Avidity ELISA as an alternative to the virus neutralization test for measuring antibodies in foot-and-mouth disease vaccinated buffaloes (Bubalus bubalis)

SPONSORS

A special mention deserves the commercial companies who sponsored the meeting: Boerihnger Ingelheim and Biogénesis Bagó (Gold Sponsors), Medical Ethics, Indian Immunologicals Limited and CDV Diagnóstico y Vacunas (Silver Sponsors), and Median Diagnostics (Bronze Sponsor).

AN UPDATE ON USDA'S NATIONAL BIO AND AGRO-DEFENSE FACILITY

Dr. Alfonso Clavijo, NBAF Director

The National Bio and Agro-Defense Facility (NBAF) in Manhattan, KS will be the premier center of scientific excellence for the study of transboundary, emerging, and zoonotic animal diseases that threaten the U.S. food supply, agricultural economy, and public health. This state-of-the-art facility will replace the Plum Island Animal Disease Center (PIADC) where USDA scientists and their global partners have researched Foot-and-Mouth Disease (FMD) for the last 65 years. Importantly, NBAF will continue to expand on the legacy set by PIADC and represent a national asset to help protect our Nation's agriculture and its citizens against the threat and potential impact of serious animal diseases.

USDA's Agricultural Research Service (ARS scientists working on FMD research at PIADC) are actively working and collaborating with members of the Global FMD Research Alliance (GFRA). At NBAF, ARS scientists will continue to support the strategic mission of GFRA, and with access to a next-generation research facility to continue the successful legacy they established at PIADC.

The U.S. Department of Homeland Security Science and Technology Directorate (DHS S&T) has been building NBAF to standards that fulfill the mission needs of the USDA, which will own, manage, and operate NBAF once construction and commissioning activities are complete.

NBAF is currently undergoing a phased transition of operational responsibility from DHS to USDA. In 2020, to minimize delays caused by COVID-19, the DHS Science & Technology Directorate and USDA, started working to realign the transition schedule. NBAF construction is currently estimated to be completed by late spring of 2022 and commissioning is expected by the summer of 2022.

The NBAF campus is about 48 acres with more than 700,000 square feet of facility space where about 574,000 square feet are dedicated to biocontainment. Once fully operational, the facility will run 24/7..

Currently, ARS and the Animal and Plant Health Inspection Service (APHIS) conduct research, diagnostics, and training at the PIADC and will transfer their respective missions to NBAF where they will operate the facility jointly.
In addition to having biosafety level-2 and -3 containment laboratories, USDA will expand its scientific work at NBAF and be the first in the U.S. to provide maximum biocontainment (biosafety level-4 or BSL-4) laboratories capable of housing large livestock to study especially dangerous zoonotic diseases.

NBAF's BSL-4 facilities will help scientists continue and expand on Plum Island's mission by including zoonotic diseases in its mission to protect U.S. agriculture and food systems against terrorist attacks, major disasters, and other emergencies.

AN UPDATE ON USDA'S NATIONAL BIO AND AGRO-DEFENSE FACILITY

Dr. Alfonso Clavijo, NBAF Director

Continued...

With the first BSL-4 containment laboratories capable of holding large animals, NBAF is already unique. But another first in the animal health arena is NBAF's Biologics Development Module (BDM). It is a proof-of-concept facility that will take NBAF's basic research and turn it into applied science, such as vaccines and other disease countermeasures. The BDM will help form industry partnerships that can take research on disease-fighting products to prevent animal disease outbreaks around the world. From advising researchers and small-scale production to testing and scaled-up production processes, the BDM team will work to provide industry partners with step-by-step instructions to fight foreign and emerging animal diseases in large livestock.

Establishing diverse collaborations among researchers will strengthen the Nation's response to animal diseases. Though the BDM represents a small part of NBAF's capabilities, it will help speed up the time it takes for a product to go from research to the hands of people who are on the frontlines of the fight against animal diseases. The major program components of the BDM have been designed to support the development and license of products and reagents discovered and developed at NBAF. The BDM has also been designed to support translational studies by producing study materials and documenting manufacturing processes for potential therapeutic and preventive materials, products, and testing results in research and clinical applications.

USDA's NBAF team has well established relationships with community leaders and organizations. In addition to participating in various outreach activities, NBAF leaders meet quarterly with a group of local and regional leaders (known as NBAF's Community Liaison Group, or CLG) who represent a broad range of community interests.

NBAF's website (www.usda.gov/nbaf) has a broad array of news, facility history, design and construction information, and frequently asked questions. Our communications team strives to keep the website updated with the most recent news so the public can have access to the wide variety of information. .

Anyone with questions about NBAF is encouraged to submit them to the communications team at: nbaf@usda.gov

Someone will be there to answer your inquiries!

NBAF
NATIONAL BIO AND AGRO-DEFENSE FACILITY

PEGYLATED INTERFERON VS STANDARD INTERFERON: A PROMISING BIOTHERAPEUTIC PLATFORM TO GET STRONG AND LONG-LASTING ANTIVIRAL ACTIVITY AGAINST FMDV

Dr. Fayna Diaz-San Segundo, Dr. Gisselle N. Medina, and Dr. Teresa de los Santos

Plum Island Animal Disease Center (PIADC), ARS, USDA, Greenport, NY, 11944, USA. 2National Bio and Agro-Defense Facility (NBAF), ARS, USDA, Manhattan, KS 66502, USA.

The use of interferons (IFNs) to block rapid replication and spread of foot-and-mouth disease virus (FMDV) has been under investigation for few decades now. Since vaccines fail to provide protection until approximately seven days post-vaccination, use of anti-viral molecules that could suppress the spread of FMDV prior to development of adaptive immune response is appealing and needed.

Delivery of porcine IFNα/β using a replication deficient human adenovirus 5 vector (Ad5-poIFNα), has shown effective to protect swine challenged with FMDV A24 Cruzeiro as early as one day post-inoculation (Chinsangaram et al., 2003). In some instances, protection could last for 3 to 4 days post-Ad5-poIFNα-inoculation (Moraes et al., 2003) and was effective against multiple FMDV serotypes (Dias et al., 2011). Moreover, the effect of type II IFN has also been tested in swine using the Ad5 platform for delivery of poIFNγ, demonstrating enhanced potency against FMD when poIFNα and poIFNγ were co-administered, either formulated as the combination of Ad5-poIFNα and Ad5-poIFNγ (Moraes et al., 2007) or using an Ad5- vector that expressed poIFNα and poIFNγ bicistronically (Kim et al., 2014). Moreover, in proof-of-concept studies Moraes et al. (2003) demonstrated that a combination of Ad5-poIFNα with an Ad5 vaccine that delivers FMDV A24Cru empty capsids (Ad5-FMD-A24) could induce complete protection of swine challenged with FMDV at 1-3 days post-infection (dpi), while a strong adaptive immune response was mounted. Although the use of type I IFN using the Ad5 platform has proven very successful in swine, preventive therapy only had limited efficacy in cattle (Wu et al., 2003). On the contrary, boIFN-λ3, a type of IFN that belongs to the type III IFN family, plays a critical role in the innate immune response of cattle against FMDV (Diaz-San Segundo et al., 2011); certainly, FMD could be controlled in bovines by treatment with Ad5-boIFNλ3 (Perez-Martin et al., 2012). Similarly to what had been described for porcine, combination of Ad5-boIFNλ3 and Adt-FMD-O1Manisa vaccine resulted in complete protection against disease when cattle were challenged with FMDV O1Manisa 3 days after vaccination (Diaz-San Segundo et al., 2016).

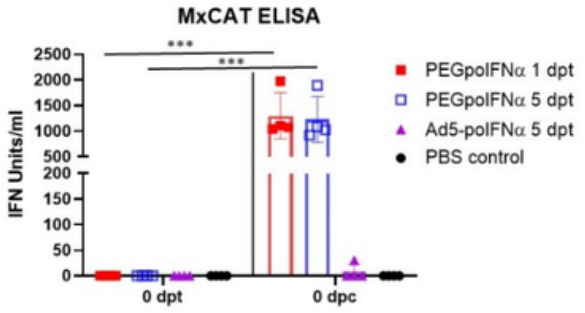
Although the delivery of IFNs using the Ad5 vector has proven effective in vivo, this platform might be expensive to be further developed in the veterinary field. Furthermore, strong protection using this platform is only achievable for 2 or 3 days after treatment.

A cost-effective alternative to the use of IFN delivered by an Ad5 vector, is the use of recombinant proteins expressed in bacteria or other eukaryotic systems such as yeast or insect cells. Over the last 30 years many such studies have been performed using IFNs to control human pathogens such as Hepatitis C, Hepatitis B, Venezuelan Equine Encephalitis and others (Friedman, 2008; Hoofnagle and Seeff, 2006). However, the use of IFNs requires extensive testing in the species of interest in order to evaluate the metabolic rate and potential adverse systemic effects of individual preparations. In this regard, many approaches to change the pharmacokinetic profiles of IFNs have been examined. These include the covalent modification of IFN with polyethyleneglycol (PEG) molecules (PEGylation) (Dozier et al.,2015) or the expression of recombinant IFN fused to other proteins such as immunoglobulins Fc fragments, albumin or others (Czajkowsky et al., 2012). These modified-IFNs have been tested for the treatment of multiple human diseases such as hepatitis, multiple sclerosis and cancer (Fried et al., 2002; Lazear et al., 2019; Ortiz et al., 2018; Woo et al., 2017). Use of these new IFN-modified platforms should improve its biotherapeutic function in the animal setting as well.

Dr. Fayna Diaz-San Segundo, Dr. Gisselle N. Medina, and Dr. Teresa de los Santos

In our recent paper (Diaz-San Segundo et al., 2021), we evaluated the antiviral activity of pegylated poIFNα (PEGpoIFNα) against FMDV in swine. Preliminary pharmacokinetics studies demonstrated that there is a sustained systemic detection of IFN, lasting for over 96 h after a single inoculation of PEGpoIFNα. Based on these results with PEGpoIFNα and our previous data from animals inoculated with Ad5-poIFNα (Dias et al., 2011), we decided to evaluate the efficacy of PEGpoIFNα using the IM inoculation route at a dose of 200ug/kg. For comparison, we also inoculated a group of animals with 1010 pfu/animal of Ad5-poIFNα. Animals were challenged at 1- or 5- days post treatment (dpt). As seen in Figure 1, all animals inoculated with PEGpoIFNα showed a statistically significant increase on the levels of systemic antiviral activity at 1 or 5 dpt which was when animals were challenged with infectious FMDV (0 dpc), as measured by an MxCAT ELISA method. (Fig. 1A; Table 1).

A.



B.

	PEGpoIFNα 1dpt	PEGpoIFNα 5dpt	Ad5-poIFNα 5dpt	Control
CCL2	2.97 ± 1.93	8.32 ± 6.11	11.01 ± 16.83	0.09 ± 0.07
CCL20	5.38 ± 6.62	786.77 ± 1519.3	73.84 ± 125.54	2.62 ± 4 .36
CCR2	1.01 ± 1.57	35.04 ± 43.67	1.71 ±2.11	0.06 ± 0.09
CXCL10	40.41 ± 23.65	37.65 ± 42.47	3.81 ± 2.1	0.92 ± 1.46
IFNα	0.14 ± 0.24	30.37 ± 52.85	1.22 ±1.34	0.02 ± 0.03
IFNβ	0.99 ± 0.88	729.48 ± 1039.5	584.57 ± 1006.68	1.12 ± 1.9
IRF-3	0.28 ± 0.18	1.09 ± 2	0.06 ± 0.09	0.05 ± 0.05
IRF-7	1.54 ± 0.44	6.17 ± 12.08	0.25 ± 0.37	0.13 ± 0.07
ISG15	72.07 ± 126.19	36.89 ± 48.04	0.46 ± 0.72	19.33 ± 30.38
mda-5	14.11 ± 12.36	32.7 ± 31.95	0.51 ± 0.44	0.55 ± 0.66
Mx1	35.27 ± 58.46	61.89 ± 46.12	0.69 ± 0.85	0.17 ± 0.17
OAS1	24.31 ± 29.06	27.98 ± 45.88	0.48 ± 0.58	0.42 ± 0.56
PKR	1.61 ± 2.14	2812.99 ± 3645.2	257.78 ± 428.21	0.45 ± 0.77
rig-I	19.66 ± 17.92	36.64 ± 49.68	0.39 ± 0.63	2.6 ± 3.98
USP18	8.62 ± 6.22	304.32 ± 570.67	2.88 ± 3.68	1.4 ± 1.5

<2
2 to 5
5 to 10
10 to 50
50 to 100
100 to 1000
>1000

Figure 1: Systemic antiviral response induced by treatment. Bioactivity against FMDV assayed by MxCAT ELISA (A.) was determined in sera of treated animals with PEGpoIFNα or with Ad5-poIFNα after 1- or 5-days post-treatment (dpt) right before the animals were challenged (0 dpc). A group of PBS-treated animals was included as a negative control. For all groups, samples taken before any treatment (0 dpt) were used as baseline. Graph represents group average (bar) and individual values of each animal in the group at indicated time points. ***, P ≤ 0.001. (B.) Gene expression in PBMCs of treated animals with PEGpoIFNα or with Ad5-poIFNα after 1 or 5 dpt [right before the animals were challenged (0 dpc)] was measured by qRT-PCR. A group of PBS-treated animals was included as negative control.

The panel of analyzed genes included IFNα and IFNβ, and a small list of well characterized ISGs described in INTERFEROME [a database dedicated to chronicling all genes significantly regulated by IFN (Rusinova et al., 2013)] including INF-Induced GTP-Binding Protein Mx1, double-stranded RNA-activated protein kinase (PKR), 2',5'-Oligoisoadenylate Synthetase-Dependent (OAS1), IFN regulatory factor (IRF) -3 and-7, C-C Motif Chemokine Ligand 2 (CCL2), C-C Motif Chemokine Ligand 20 (CCL20), C-X-C Motif Chemokine Ligand 10 (CXCL10), C-C Motif Chemokine Receptor 2 (CCR2), IFN-Stimulated Protein, 15 KDa (ISG15), Ubiquitin Specific Peptidase 18 (UPS18), and also pattern recognition receptors retinoic acid-inducible gene I (rig-I) and melanoma differentiation-associated protein 5 (mda-5). Results are expressed as relative fold induction values with respect to 0 dpt (ΔΔCT). GAPDH expression was used as normalizer

Interestingly, the levels of antiviral activity in the PEGpoIFNα treated animals were sustained for 5 days, while no antiviral activity could be detected at that time in the animals treated with Ad5-poIFNα (Fig. 1A; Table 1). Parallel to these results, real-time RT-qPCR analysis of a panel of IFN stimulated genes (ISGs), previously used in our lab to demonstrate the systemic effect of Ad5-poIFNα on PBMCs (Diaz-San Segundo et al., 2010), showed upregulation in 9 out of 15 analyzed genes at one day after PEGpoIFNα inoculation, and all genes except for IFN regulatory factor-3 (IRF-3) were upregulated at 5 days after PEGpoIFNα treatment. (Fig. 1B). On the contrary, only 6 out of 15 genes were upregulated in animals treated with Ad5-poIFNα by 5dpt, and levels of upregulation were low for the most part.

Clinical and serological disease outcome after challenge with FMDV A24Cru were also evaluated (Table 1). As expected, animals in the PBS control group developed clinical signs of FMD at 2 dpc, with all animals reaching high clinical scores (10 to 15). Similarly, animals treated with Ad5-poIFNα and challenged at 5 dpt, showed vesicular disease comparable to the PBS control group, with no statistically significant difference between the two groups (data not shown). Remarkably, none of the animals treated with PEGpoIFNα either 1 or 5 days prior to challenge, developed vesicular disease at any time post-FMDV challenge.

Parallel to the development of disease, all animals in the PBS control group and animals treated with Ad5-poIFNα, that were challenged at 5 dpt, showed detectable viral RNA in serum and nasal swabs. Additionally, infectious virus was isolated from both, serum and nasal swabs, except for one animal in the PBS control group. On the other hand, infectious virus could not be isolated from any of the animals treated with PEGpoIFNα, and viral RNA was only detected in 1 out of 8, and 2 out of 8, sera and nasal swabs samples, respectively. Furthermore, only the groups of animals that showed clinical FMD developed significant levels of neutralizing antibodies against FMDV.

Dr. Fayna Diaz-San Segundo, Dr. Gisselle N. Medina, and Dr. Teresa de los Santos

Group (n=4)	Treatment	Challenge (dpt)[a]	Antiviral at 0dpc[b]	Clinical Score[c]	Viremia TCID50[d]	Viremia GCN[e]	Shedding TCID50[f]	Shedding GCN[g]	SNT[h]
1	PEGpoIFNα (200ug/kg)	1 dpt	1975.5	0	0	$3/1.2x10^2/1$	0	0	0
			1043.4	0	0	0	0	0	0
			1107.3	0	0	0	0	$3/1.4x10^3/1$	0
			1073.2	0	0	0	0	0	1.2
2	PEGpoIFNα (200ug/kg)	5 dpt	1893.1	0	0	0	0	0	0
			1086.4	0	0	0	0	0	0.9
			920.4	0	0	0	0	$4/9.7x10^2/3$	0
			1023.6	0	0	0	0	0	0
3	Ad5-poIFNα (10^{10}pfu/pig)	5 dpt	0	4/12	$3/1.3x10^4/1$	$1/1.7x10^7/5$	$7/5.0x10^1/1$	$3/1.3x10^5/5$	2.7
			30	5/9	$5/1.3x10^3/1$	$3/2.7x10^5/3$	$3/7.9x10^1/2$	$3/9.5x10^5/5$	2.4
			0	4/13	$4/7.9x10^3/1$	$2/3.1x10^8/6$	$2/5.0x10^1/3$	$2/2.1x10^5/6$	2.1
			0	2/16	$2/3.2x10^6/1$	$2/5.3x10^8/6$	$2/5.0x10^2/3$	$2/1.2x10^5/6$	2.1
4	Control (PBS)	1 dpt	0	2/14	0	$3/6.4x10^6/5$	0	$3/2.1x10^5/5$	3.3
			0	3/14	$3/3.2x10^7/1$	$1/8.5x10^9/7$	$3/1.3x10^2/1$	$3/1.6x10^6/5$	3
			0	4/15	$2/7.9x10^7/1$	$2/2.3x10^{10}/6$	$2/7.9x10^3/3$	$2/1.1.x10^7/7$	3
			0	4/10	$4/2x10^8/1$	$2/2.6x10^8/6$	$2/7.9x10^1/3$	$2/1.5x10^5/7$	2.7

Table 1. Serological and Clinical Response of Swine Pretreated with pegylated poIFNα (PEGpoIFNα) or Adenovirus Type 5 Vector Containing a poIFNα Gene and Intradermal Challenged with Foot-and-Mouth Disease Virus A24Cruzeiro.

a - Days after treatment.
b - Antiviral activity (U/mL) measured by MxCAT ELISA at the moment of challenge (0 days post-challenge [dpc]).
c - Dpc at which first signs of lesions/highest lesion score.
d - First dpc that viremia was detected by virus isolation, maximum amount of viremia in TCID50/mL detected in sera samples, and the duration (days) of viremia.
e - First dpc that viremia was detected by real-time RT-qPCR (rRT-qPCR), maximum amount of viremia in genome copy number (GCN)/mL detected in sera samples, and the duration (days) of viremia.
f - First dpc that shedding virus was detected by virus isolation, maximum amount of shedding virus in TCID50/mL detected in nasal swab samples, and the duration (days) of shedding.
g - First dpc that shedding virus was detected by real-time RT-qPCR (rRT-qPCR), maximum amount of shedding virus in genome copy number (GCN)/mL detected in nasal swab samples, and the duration (days) of shedding.
h - Serum neutralizing antibody response reported as serum dilution yielding a 70% reduction in the number of plaques (PRN70) at 14 dpc.

In sum, our results demonstrate that treatment with PEGpoIFNα induces a sustained strong and systemic antiviral state capable of protecting animals against challenge with FMDV for at least 5 days post-administration, being the first time a pegylated protein has been tested against an important veterinary pathogen. Use of a recombinant purified pegylated protein represents a cost-effective alternative as compared to protein-expression platforms delivered by live vectors such as Ad5. Ultimately, a combination of PEGpoIFNα with a FMD vaccine should provide early and long-lasting protection against a viral disease that spreads so rapidly during an outbreak currently causing devasting consequences in vast areas of the World. In this regard, IFNα would not only be effective at blocking initial FMDV replication, as demonstrated in this note, but would also be an effective adjuvant for the FMD vaccine, as it has been previously described (de Avila Botton et al., 2006; Duan et al., 2020; Su et al., 2013). Further studies using the PEGpoIFNα in combination with FMD vaccines to confirm this adjuvant effect are warranted. Moreover, it will be worthy to explore the efficacy of this type of molecules in other FMDV hosts, such as cattle, and hopefully design experiments to better understand the mechanisms of protection in each susceptible species to keep walking in a path that could lead towards FMD eradication.

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RECODING THE GENOME OF FMDV: A PATH FOR THE DEVELOPMENT OF LIVE ATTENUATED VACCINE (LAV) CANDIDATES AGAINST FMD

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Recoding of viral RNA genomes using different approaches to alter the use of specific set of codons without affecting their amino acid composition can result in the production of viruses with significant reduction in viral fitness. Use of codon deoptimization (CD) or codon-pair deoptimization(CPD) can serve as a platform for the development of live attenuated vaccine (LAV) candidates. Since recoded viruses can still infect and replicate in the host, immune responses developed (innate and adaptive) should provide a level of protection to control disease. In fact, this platform has influenced multiple studies targeted to develop novel LAV candidates against different diseases including those that affect livestock animals (Burns et al., 2006; Mueller et al., 2006; Wang et al., 2015; Velazquez-Salinas et al., 2016; Park et al., 2020). Specifically, in the last few years we have seen an increase in the number of publications related to the use of CD or CPD in viruses when compared to a decade ago (NCBI, 2022). This article highlights the recent progress made in the development of LAVs against FMDV using codon deoptimization and the need to understand the mechanisms of attenuation of the mutant viral strains to improve vaccine safety and efficacy.

In proof-of-concept studies, we have demonstrated that CPD is tolerated by FMDV (Diaz-San Segundo et al., 2016) and results in a distinct phenotype (i.e., plaque size, growth kinetics, viral protein expression, etc.) when compared to WT strain. Importantly, this approach did not affect the viral genome stability after multiple passages in cell culture. Recoding of the P1 structural region resulted in a FMDV strain (A12-P1Deopt) that was highly attenuated in mice and in swine at a dose ~100 fold higher than the dose of homologous WT virus. Interestingly, high levels of neutralizing antibodies were detected in sera suggesting that swine inoculated with the mutant virus could be protected against FMD.

Other studies involved the CD of FMDV P2 and P3 regions encoding non-structural proteins. Using this platform, viruses were engineered to carry convenient restriction endonuclease cleavage sites that allow for effective capsid swapping within A24Cruzeiro infectious clone while bearing a DIVA marker in the P3 region (Diaz-San Segundo et al., 2021). Interestingly, such a broad approach led to viable viral progeny that exhibited different degrees of attenuation in cell culture, mice and swine. Highest level of attenuation was observed in viral strains encoding

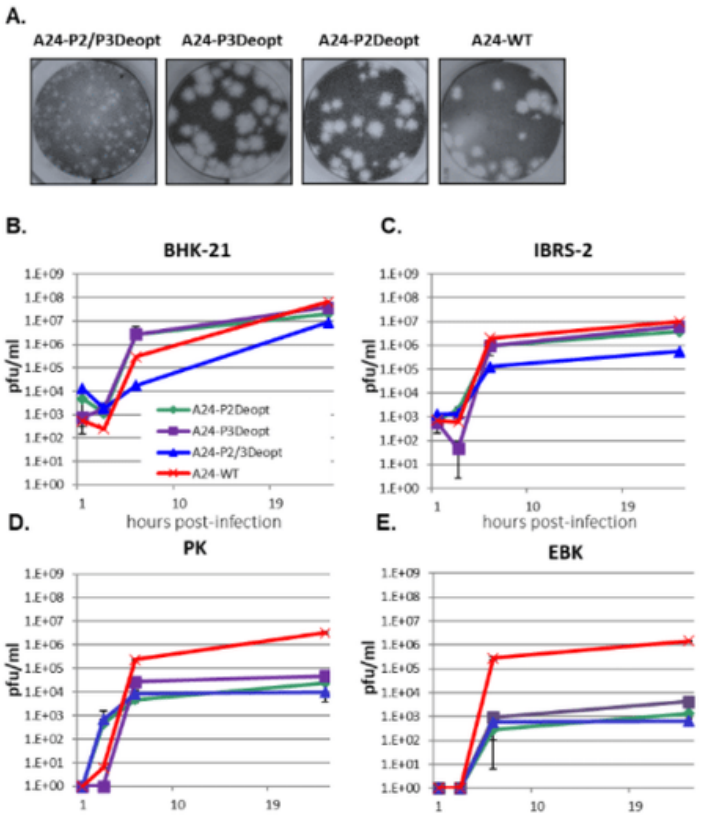

Figure 1. Plaque morphology and kinetics of growth in cell culture. (A) Plaque morphology of WT and deoptimized viruses. Kinetics of growth in multiple cell lines: (B) BHK-21 and (C) IBRS-2 cell lines or (D) PK and (E) EBK cells were infected with FMDV A_{24}Cru wild type (A24-WT) or deoptimized variants, A24-P2Depot, A24-P3Depot, and A24-P2/3Depot.

combined recoding of the P2 and P3 regions (Figure 1), supporting previous evidence that links the extent of recoding and the level of attenuation (Mueller et al., 2006). Clinical evaluation of swine inoculated with lower doses of these deoptimized FMDV strains showed no evidence of FMD, and viral RNAs were not detected in serum or nasal swabs. Under these conditions, neutralizing antibody titers were undetectable in inoculated animals. Not surprisingly, challenge with WT virus caused disease in all the swine previously inoculated with the deoptimized strains.

Although there is not a consistent correlation between the induction of neutralizing antibodies and protection against FMD, we think that induction of a strong level of neutralizing antibodies is critical for the efficacy of this vaccine platform, at least in pigs. Lack of detectable viable virus in serum could have contributed to the overall lack of antibody induction. This may indicate that viral fitness of these FMDV P2/P3 deoptimized strains is substantially impaired to efficiently replicate and/or translate in levels of viral protein in the host animal that could elicit a detectable and hopefully protective immune response. Identification of the specific deoptimization parameters for FMDV should help avoid the generation of "over-attenuated" viral strains. In this sense, successful vaccine candidates derived from recoded strategies will need to have an ideal balance between attenuation and immunogenicity to provide effective protection.

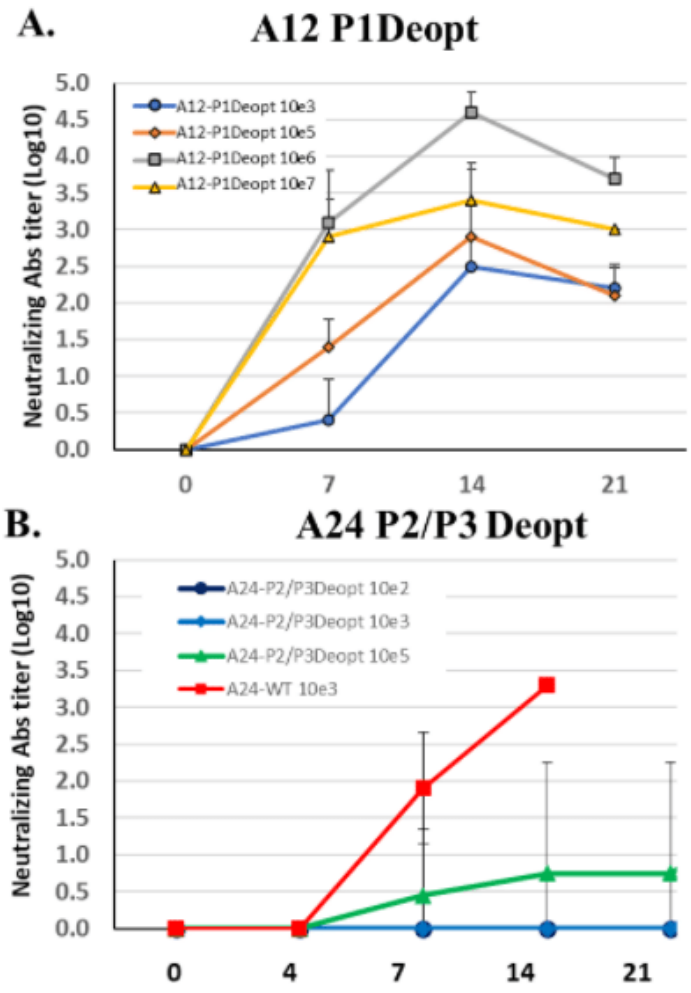

Figure 2. Determination of FMDV neutralizing antibodies in the serum of swine inoculated with different doses of (A) A12-P1Depot or (B) A24-P2/P3Deport.

The biological mechanisms underlying the attenuation of codon deoptimized viruses is not well understood. Previous investigations have demonstrated that recoding of viral genomes results in the unintentional enrichment of dinucleotides CpG and UpA which can act as immunological molecules inducing antiviral responses (Tulloch et al., 2014; Simmonds et al., 2015; Gonçalves-Carneiro and Bieniasz, 2021). Although we have also detected an increased in CpG and UpA in many FMDV deoptimized strains, correlation with the level of attenuation was not consistent across our multiple studies. Others have proposed that attenuation of these modified viruses was the result of decreased stability of the genome, loss of needed RNA secondary structures, a decrease in the rate of translation, co-translational misfolding, and a decreased rate of replication (Gonçalves-Carneiro and Bieniasz, 2021). Further investigation is warranted to dissect the dynamics of attenuation induced by this recoding strategy for FMDV and hopefully increase the safety and efficacy profiles of this provocative vaccine platform.

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REINVENTING THE FMD RESEARCH TEAM – ACTIVITIES DURING COVID-19 LOCKDOWNS

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FMD research with infectious FMDV is not permitted in Australia. Therefore, we are heavily reliant on our Regional and GFRA research partners globally to perform any research that involves life virus. Similar to some other veterinary research laboratories, the FMD team at CSIRO-Australian Centre for Disease Preparedness (ACDP) was impacted by the COVID pandemic and particularly since we were unable to travel and work with our research partners. In fact, the team had to leave the Friedrich Loeffler Institute in Germany, in haste, in March 2020 before Australia closed its borders to returning citizens. Our team expresses a special word of thanks to Michael Eschbaumer and the FLI team for completing some of the work in our absence; this is genuine GFRA collaboration.

True to our spirit and the resilience of our research team, we offered our expertise when an opportunity was presented to participate in the evaluation of SARS-CoV-2 vaccine candidates. CSIRO-ACDP was entrusted with the responsibility of carrying out preclinical studies for two front-runner candidate vaccines using the ferret infection model early in 2020. Our team proposed the use of a Systems Immunology based approach to evaluate the performance of the vaccines using our expertise gained during evaluation of FMD vaccines. This resulted in a comprehensive report to the Coalition for Epidemic Preparedness Innovations (CEPI) who had sponsored the study and was subsequently submitted to the regulatory authorities for emergency approval of two vaccine for use in humans. This initial success led to more collaborative work on COVID-19 for is. We were part of a team that was successful in obtaining funding from the Australian Medical Research Future Fund (MRFF) to initiate a new area of research using stem cell models. We propose to use the systems biology-augmented, stem cell-derived, multi-tissue panel for rapid screening of approved drugs as potential COVID-19 treatment. We were also part of a team successful in obtaining a United States Food and Drug Administration (USFDA) grant for strengthening COVID-19 animal models and regulatory science using a systems biology approach.

We are proud that our team could demonstrate the true spirit of a One Health Approach and being able to demonstrate that expertise in animal infections has broader applications into other areas of animal and human health.

THE NEOTERIC LOOPHOLE WITHIN THE CARRIER CONUNDRUM

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Field studies from areas of endemic FMD have suggested that animals can be simultaneously infected by more than one distinct variant of FMDV [1], potentially resulting in emergence of novel viral strains through recombination [2]. However, there has been limited investigation of the mechanisms of in vivo FMDV coinfections under controlled experimental conditions.

In a recent study conducted at the Plum Island Animal Disease Center (PIADC), distinct cohorts of cattle were experimentally exposed to two FMDV strains of serotypes O and A. One cohort was simultaneously infected with both viruses, while additional cohorts were initially infected with FMDV A24 and subsequently superinfected with FMDV O1 Manisa, after 21 or 35 days. Coinfections were confirmed during acute infection, with both viruses concurrently detected in blood, lesions, and secretions. Staggered exposures resulted in overlapping infections as convalescent animals with persistent subclinical FMDV infection were superinfected with the heterologous virus. Staggering virus exposure by 21 days conferred partial clinical protection as six of eight cattle did not develop FMD following the second exposure. All animals were, however, subclinically infected following the heterologous virus exposure. By contrast, the cattle that were superinfected at 35 days post initial infection developed fulminant FMD. All animals that were simultaneously coinfected, or superinfected at 21 days post initial infection, maintained persistent infection with just one of the two viruses, while clearing the other. By contrast, the cohort that was superinfected at 35 days after initial infection efficiently cleared both viruses.

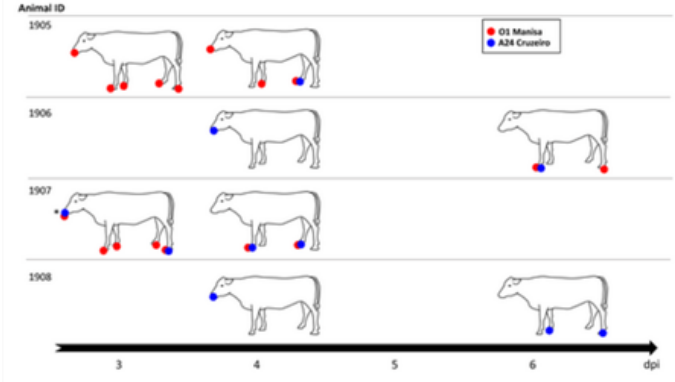

Figure 1. Strain-specific detection of FMDV A24 and O1M in vesicular lesions of acutely co-infected cattle. Virus identity in vesicular lesions and sampled during acute FMDV co-infection. Colored circles represent vesicular lesions caused by FMDV O1M (red) or A24 (blue) on the feet or in the oral cavity. Overlapping circles of both colors indicate that both viruses were detected in one sample. Each animal was sampled twice during the clinical phase of infection. Sampled vesicles do not represent all vesicles observed in the animals *= Two distinct vesicles ere harvested from the the dental pad and tongue on day 3 from animal #1907. Both vesicles contained genomes of both viruses.

FMDV (full length) sequence analysis confirmed the simultaneous presence of both infecting viruses in lesions, blood and secretions in acutely coinfected animals. However, these acute coinfections did not give rise to any dominant recombinant FMDVs. By contrast, recombinant viruses with O1M-derived capsid coding regions and A24-derived non-structural coding regions were detected as the dominant (consensus level) genomes in serial samples of oropharyngeal fluid (OPF) from 5 superinfected cattle. The recombinant viruses were detected within 10 days of superinfection, and were out-competed in all but one animal by a non-recombinant parental virus by the end of the study at 28 or 35 days post superinfection. The dominant recombinant viruses were detected in superinfected FMDV carriers regardless of the clinical status of the animals. Thus, a subset of animals were subclinically superinfected and shedding FMDV in oronasal secretions without any clinical signs of FMDV.

We hypothesize that the generation of recombinant FMDVs in the superinfected FMDV carriers may be facilitated by the lack of immunity to the superinfecting virus. This theory was corroborated by the finding that all of the recombinant genomes had capsid-coding regions derived from the most recent virus exposure, to which the animals did not yet have neutralizing antibodies. Additional studies in which the order of exposure to the two viruses are reversed will further elucidate this concept.

It is thoroughly established that during the carrier phase, FMDV may be detected by probang sampling of OPF, but is not shed in typical oronasal secretions. Neoteric (early) subclinical infection is different from the carrier phase in that FMDV may be detected in oronasal secretions, indicating shedding which may lead to transmission [3].

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The superinfected animals in these studies were simultaneously carriers of FMDV-A and neoterically infected with FMDV-O; and, they were shedding FMDV and generating O/A recombinants. Consequently, viral recombination occurring during the early stages of superinfection may promote emergence and transmission of recombinant viruses. Thus, the findings from the current study suggest a previously unidentified role of persistently infected FMDV carriers as mixing vessels in which novel strains may rapidly emerge through superinfection and recombination. This may be viewed as a loophole in the conventional wisdom that carriers do not transmit FMDV.

Ongoing work at PIADC is aimed at further characterizing obtained recombinant FMDVs in relation to the parental virus isolates. Additional forthcoming work will seek to investigate transmission from superinfected FMDV carriers to naïve in-contact animals.

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SLAUGHTERHOUSE SURVEILLANCE FOR SUBCLINICAL FOOT-AND-MOUTH DISEASE VIRUS IN VIETNAM

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The objective of this study was to evaluate sampling of clinically healthy livestock at slaughterhouses as a strategy for genomic FMDV surveillance. In two slaughterhouses in southern Vietnam, 1200 serum and OPF samples were collected from asymptomatic cattle and buffalo (2017 to 2019) as a pilot study on the use of slaughterhouses as sentinel points of surveillance. FMDV VP1 sequences were analyzed using discriminant principal component analysis (DAPC) and time-scaled phylodynamic trees. Delineation of different clusters enabled tabulation of when and where distinct FMDV variants were detected. Viruses isolated from slaughterhouses clustered together with viruses recovered from farms during the same period, indicating that slaughterhouses are representative of FMDV circulation at the farm level. Six of seven serotype O and A clusters circulating in southern Vietnam from 2017-19 were detected at least once in slaughterhouses, sometimes pre-dating outbreak sequences associated with the same cluster by 4-6 months.

Within the scope of this study, circulating viruses in Vietnam were associated with the serotype A SEA/97 lineage and the serotype O Cathay, Pan Asia and Mya-98 lineages, with Pan Asia being the most common. This finding is consistent with other recent molecular epidemiology studies in Vietnam. Some (17/56, 30.1%) sequences in one of the serotype A-cluster were isolated and identified as recombinant sequences within a different study analyzing full-length sequences (https://journals.asm.org/doi/10.1128/MRA.01263-20). Recombinants were identified in both clinical and subclinical samples. The P1 portion of these viruses aligned with A/Sea-97, whereas P2-P3 were from O/ME-SA/Pan-Asia.

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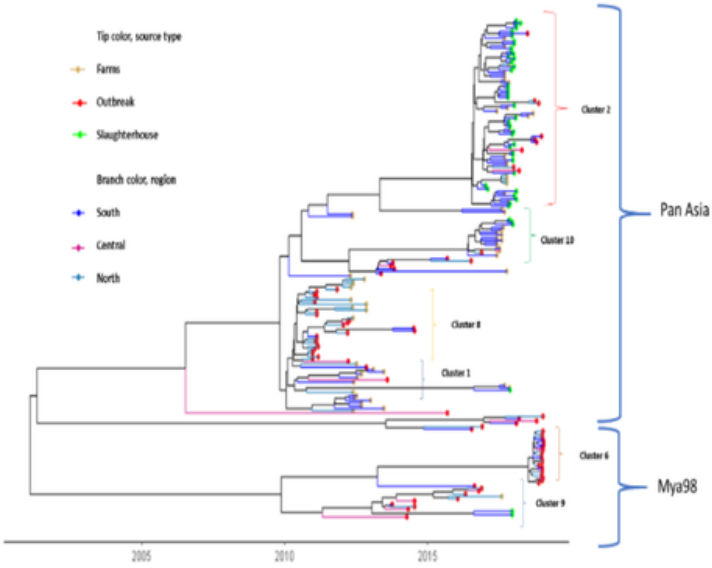

Figure 1. Time-scaled phylogeny for serotype O sequences isolated in Vietnam. Tip color indicates the source type of data (slaughterhouse, farm and outbreak). Different branch colors show the region of the country where sequences were isolated. Small brackets identify the clusters, and the large brackets identify the lineages each cluster belongs to.

Our results suggest that slaughterhouse-based surveillance can provide more timely information on circulating or emerging FMDV variants as compared to passive detection through outbreaks. Specifically, some variants were detected at slaughterhouses four to six months prior to their association with reported outbreaks. These results demonstrate the potential utility of systematic genomic surveillance across a network of slaughterhouses in endemic countries.

We also identified FMD high risk areas in Vietnam using phylogeographic data accounting for the regional connectivity where active surveillance could be prioritized. We explored whether the distribution of reported clinical FMD outbreaks across space and time is better explained by models that incorporate population connectivity based upon FMDV movement (inferred by discrete phylogeographic analysis) as opposed to spatial adjacency. These alternative connectivity matrices were incorporated into a Bayesian space-time regressions to assess which measure of population connectivity best explained the pattern of reported FMD outbreaks across the country. Both the spatial adjacency and phylogeographic risk models were used to identify high-risk areas and risk factors for FMD in Vietnam. We conclude that accounting for virus movement through phylogeographic analysis serves as a useful proxy for population connectivity in spatial-temporal risk models when movement data are not available. This approach may contribute the design of surveillance and control activities in countries in which movement data are lacking or insufficient.

Looking forward, an ongoing study (unpublished) is evaluating within-host evolution of FMDV in carrier animals in Vietnam over the same time period as the slaughterhouse work. In this study, animals were separated into carriers and serially infected animals. P1 sequences were analyzed from within an individual to determine where nonsynonymous amino acid changes occurred. This was done by comparing amino acid sequences in a pairwise, chronological process. A dN/dS analysis was run on the full set of serotype A and O samples separately. P1 sequences from carrier and serially infected animals were compared in a pairwise, chronological process to examine the mutation rate of FMDV within carrier animals. Mutation rates between VP1 and P1 were compared to determine differences in mutation rate across these regions of the genome within carriers. Results of these analyses have been intriguing, and will soon be published in a super-fabulous, high-impact journal!

These findings are all derived from the long-standing collaboration between the two GFRA members ARS/USDA and DAH Vietnam, which in recent years have benefited from further collaboration with scientists at the University of Minnesota. Over the years, several studies have been performed and many papers published, many of which have emphasized molecular epidemiology and subclinical infection. This work is ongoing and intended to contribute to understanding viral evolution and circulation between clinical FMD, neoteric subclinical infection, and the FMDV carrier state.

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PHOTO DESCRIPTIONS

Provided by Jonathan Arzt

Veterinarians from GFRA members Department of Animal Health (DAH), Vietnam and Agricultural Research Service (ARS), USDA conducting field work collecting oropharyngeal fluid samples (probangs) from FMDV-carrier goats in southern Vietnam.

GFRA members Department of Animal Health (DAH), Vietnam and Agricultural Research Service (ARS), USDA investigating outbreaks of African Swine Fever in Northern Vietnam.

Veterinarians from GFRA members Department of Animal Health (DAH), Vietnam and Agricultural Research Service (ARS), USDA conducting training sessions on oropharyngeal fluid sample (probang) collection during field outbreaks near Hanoi, Vietnam.

Veterinarians from GFRA members Department of Animal Health (DAH), Vietnam and Agricultural Research Service (ARS), USDA conducting training sessions on oropharyngeal fluid sample (probang) collection during field outbreaks near Hanoi, Vietnam.

Scientists from GFRA members ICAR-Directorate of Foot and Mouth Disease (DFMD) and Agricultural Research Service (ARS), USDA conducting training sessions on oropharyngeal fluid sample (probang) collection during field outbreaks near Hanoi, Vietnam.

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