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GFRA NEWS

The official newsletter of the Global FMD Research Alliance

IN THIS ISSUE

GFRA MEETING BANGKOK 2019: “living” for FMD discussion

by Alejandra Capozzo

One of the aims of the GFRA meetings is to discuss science behind FMD. The goals of our biennial meetings are getting together, share our research and discuss as much as possible, all the aspects around the new findings. This is something difficult to achieve during this kind of meetings, as time schedules are tight and sometimes there was no time left for questions. In many of our meetings before Thailand, we had remarkably interesting discussions sessions during closures, most of the leaded by Cyril Gay, one of the GFRA founders. These discussions were highly appreciated by the audience and gave us an idea of what upmost needed: space to share our comments.

In this regard, our last meeting in Bangkok was organized to maximize discussions. The scientific committee, led by Mariano Perez-Filgueira from Argentina, decided to leave time for discussions after each group of presentations. So, a “living room” was organized on stage and all the speakers took a seat there after the session and people form the audience lined up to ask their questions through the standing microphones situated in the aisles. It was amazing to see the dynamics of this activity and the way it flowed as it became more and more familiar to the audience. Each session started with a short introduction held by the chairs. This helped to put a framework for the session.

Each talk would go through, uninterrupted and at the end the speakers (principal investigators, graduate students, young scientists) and moderators, all sat together in the “discussion living-room”, answering and sharing ideas with the audience. It was a challenge for young students, particularly for those from non-English speaking countries, who struggled a little but valued the opportunity.

It was amazing to see the dynamics of this activity and the way it flowed as it became more and more familiar to the audience.

Anna Ludi, Cecilia Turco, Dachrit Nulibol, Yanmin Li, Michiel Harmsen and Pamela Opperman, discussing during one of the diagnostics sessions of the meeting

We knew from the attendee's feedback that this modality was well accepted and valued. We will keep this set-up for our next GFRA meeting that will be held in Buenos Aires in 2021. We will include of course, a dip of Argentinean hospitality, a good Malbec and some great beef as well. Looking forward to seeing you all again in Buenos Aires, to keep on "living FMD" research.

NEWS AND VIEWS

Good things happen when GFRA partners work together

by Wilna Vosloo

Researchers from the Australian Animal Health Laboratory and the Friedrich-Loeffler-Institut in Germany are jointly investigating measures to ensure safe transport of epithelial tissue samples from infected farms, without compromising sample integrity. These studies on simple but effective inactivation or preservation buffers will contribute greatly to attempts to contain the spread of FMD virus during the transport of samples from outbreaks in FMD-free countries like Germany and Australia. In addition, this will ensure that the state laboratories, where these samples might be tested, are not compromised and quarantined when they receive positive samples and can continue with their diagnostic work.

Nagendra Singanallur from AAHL looking happy in the laboratory at FLI

These studies on simple but effective inactivation or preservation buffers will contribute greatly to attempts to contain the spread of FMD virus during the transport of samples from outbreaks in FMD-free countries

The two GFRA partners are also investigating the survival of FMD viruses at different environmental conditions to assist with dispersal modelling. These data will help us to better trace potential sources of virus transmission during outbreaks, by taking into consideration weather conditions temperature and humidity) at specific time points.

Over the course of the past two years, AAHL researchers have visited and worked with experts at the FLI, for extended periods, and built up valuable relationships which fostered discussion of further collaborations beneficial to both Germany and Australia. This fruitful collaboration is an example of GFRA partners from different continents combining resources to reach a common and mutually beneficial goal to enhance FMD control measures.

The New European Union Reference Laboratory for Foot-And-Mouth Disease

by the EU Reference Laboratory for Foot-And-Mouth Disease

In 2019, the new European Union Reference Laboratory for Foot-and-mouth disease organized its first European Proficiency Test (EU-PT) on behalf of the European Commission, Health & Consumer Protection Directorate-General (DG-SANTE). This EU-PT was open to all National Reference Laboratories (NRLs) and Official Laboratories (OfLs) of the EU Member States, but also to the NRLs and OfLs of the EU-candidate members and neighboring countries. Thirty-seven laboratories participated to this PT. Participation of 7 laboratories from neighboring countries was covered by the special support of the EuFMD who funded the shipment of panels. The innovation of this PT lied on the use of LEILA, the online management and monitoring tool for Inter-laboratory Tests run by ANSES. This online platform is specifically dedicated to PT organization, from the registration process to the diffusion of individual results, allowing for an efficient communication between the participants and the EURL at all times (https://leila.anses.fr). The scenario of an FMD outbreak was presented to the participants, providing them with the possibility to ask for different panels of samples to process with all the techniques they deemed appropriate and wished to be tested on (viral isolation, molecular biology, serology). This EU-PT helped provide a better understanding of the strengths and needs for each EU laboratory, giving the EU-RL the tools to better assist them in their task as a NRL or an OfL of the EU.

The innovation of this first European Proficiency Test (PT) lied on the use of LEILA, an online management and monitoring tool for Inter-laboratory Tests run by ANSES and specifically dedicated to PT organization

Results of this EU-PT, as well as the results from the 2018 FMD EU-PT conducted by the Pirbright Institute were discussed during the annual Workshop (WS) held by the FMD EURL in early October. All NRLs and OfLs who participated to the 2019 EU-PT were invited to gather at the ANSES Campus in Maisons-Alfort for the event. This year, the event was a joint VSV-FMDV-SVDV Workshop, gathering 56 participants from all over Europe and the world for two days.

The program of the event included discussions on the results of both PT 2018 and 2019, the advances in analytical methods, advances in the theoretical knowledge of the diseases and the viral agents, updates on the epidemiology of the diseases, differential diagnosis and results of vaccine campaign surveys conducted in EU neighboring countries. Guests from EU neighboring countries as well as from the US provided the attendees with their expertise on their local epidemiological situation and feedback on the different initiatives present in each territory to fight FMD and VSV. The organization of the two-days workshop was supported by the European Commission, represented by Dr. Alf Füssel who attended and chaired this VSV-FMD-SVDV Workshop.

Group picture of the participants during the first European Proficiency Test (EU-PT)

A retirement gift signed by all participants was offered to Dr. Füssel as a recognition for his engagement alongside the European FMD laboratories over the years. A gift was also offered to Dr. Donald King and Dr. Anna Ludi on behalf of the European FMD laboratories to thank them for the many years of great work they achieved as EURL. The EURL for FMD would like to thank all speakers for their very interesting talks and addresses many thanks to all participants for the very nice discussions held during these two days and the EC for its support. These discussions provided an opportunity to settle on a common view of the challenges faced and set out the issues and expectations of the VSV-FMD-SVDV EU laboratories. As a result, it is hoped that the communication on those issues will help reinforce the European FMD surveillance network in the future.

Contributions of GFRA Scientific Meeting to our careers

by María Cruz Miraglia, Cecilia Turco and Guido Molina

The GFRA Scientific Meeting gave us the possibility to improve our capacities for making oral presentations in a foreign language as well as discussing and answering the questions of FMD experts and KOLs from all over the world. Although we have been investigating different aspects of the immune system, biotechnological tools, molecular biology, and general virology, we focused our work on FMDV during the last 2 years. The experience to interact face-to-face with experts of the FMD research community was one of the most important benefits of this conference and produced a big impact on our personal scientific career development. We also considered that the exchange of knowledge that came up from the experts´ opinions, questions, and comments led to a new analysis of the results with a different perspective.

To continue working in the development of tools for the prevention and the control of FMD, it was very enriching learn about the different approaches currently employed worldwide and the challenges to deal with the design and the use of vaccines in different regions.

One of the missions of the Global FMD Research Alliance is to establish and sustain global research partnerships to generate scientific knowledge and discover the tools to successfully prevent, control, and eradicate FMD. In this context, funding programs are essential and allow new researchers the possibility to interact and discuss with other professionals in this field. The financial support granted by the GFRA Organizing committee allowed Cecilia, María Cruz and Guido, post-doctoral fellows from Argentina, to attend the GFRA scientific meeting in Bangkok, Thailand. In this report, these three young researchers from the National Institute for Agricultural Technology (INTA) in Buenos Aires, share part of their works and tell us how this opportunity influenced them, both personally and professionally.

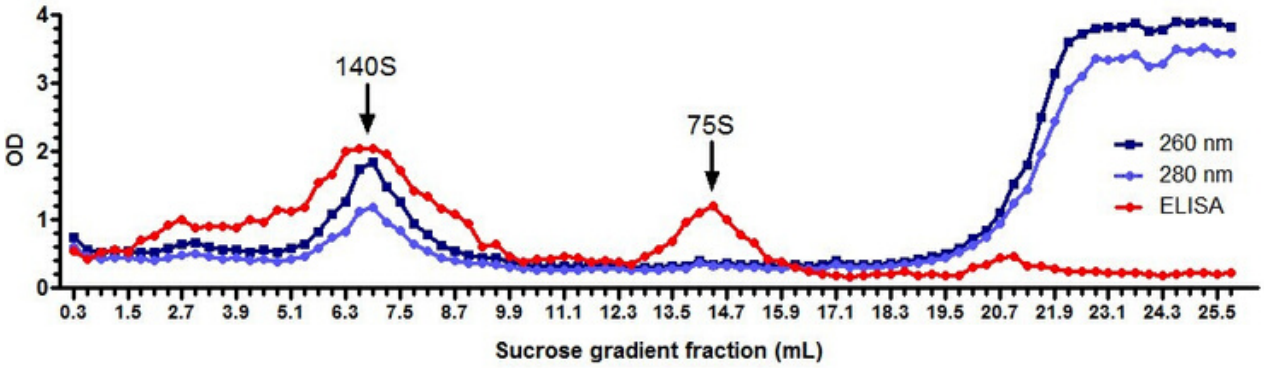
All this learning is paramount to decide the course of our projects. Moreover, some experts gave us very useful suggestions because they had already walked the same paths than us before, with different approaches but with similar goals. Specifically, the opinions about the mouse model or the complexity of the porcine immunology were the most interesting issues. Some biotechnological companies were also present in the GFRA Scientific meeting. We could talk with some representatives and we kept in contact with them after the meeting. The interaction with companies was beneficial for both sides and, in our case, learning requirements in terms of regulation and stability for an antiviral or a vaccine was especially useful for the course of our investigations.

Guido Molina, Marie Gismondi, Maria Cruz Miraglia, Guido König, Alejandra Capozzo, Mariano Perez-Filgueira and Cecilia Turco, the INTA's delegation at the GFRA Scientific Meeting in Bangkok, Thailand

It is important to note that as postdoc fellows, we do not have neither job stability nor certainty about our possibilities to keep on working in our institute. Furthermore, Argentina's economic crisis makes getting a research position in governmental institutions exceedingly difficult. In this scenario, the network we built during this GFRA Scientific meeting expanded our job possibilities to other countries. We could talk with several researchers of our areas of interest and we met them personally. For some of us, this meeting has been an exceptionally good first step in the search for future job positions. We also found that learning about the distinct problems, epidemiological situations and specific needs of each scenario is key for selecting control strategies, and how a biotechnological product which may not be fitted for some countries, can be, at same time, extremely relevant to others. We have discovered how the knowledge about the diversity of situations worldwide could enrich the projection of each work, the collaboration between countries and the conformation of a better global network.

Finally, a third impact was at the cultural level. Thailand, the location that was chosen for the GFRA Scientific meeting, is an amazing country with great cultural richness. This generated us a great expectation, as it was our first visit to Asia. We wanted to learn about their lifestyle, their traditions, and habits, and taste their typical tasty and spicy food. Bangkok captivated us with the contrast of the streets, where modern buildings “live together” with majestic temples, intricate streets, colorful markets and epic traffic. We enjoyed it a lot!

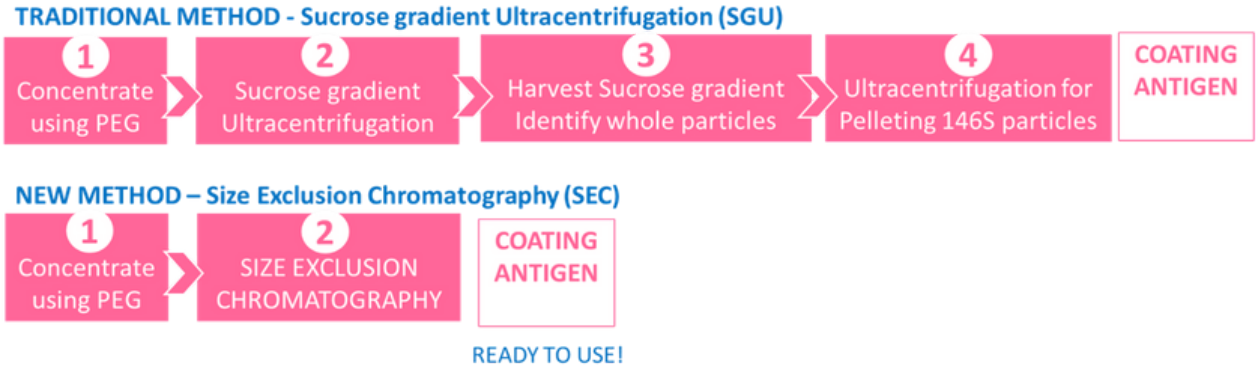
María Cruz Miraglia (a National Reseach Council, CONICET, post-doc fellow at INTA) tells us about her presentation in the frame of the pathogenesis and immunology session: "Our work is focused on identifying potential differences in the immune response generated by oil-adjuvanted vaccines formulated with FMDV A24/Cruzeiro natural purified empty capsids (75S) or the traditionally used whole-virus particles (140S)".



FMDV whole particles (140S) and natural empty capsids (75S) purified from the same antigenic preparation. The lower antigenic capacity of 75S-formulated vaccines is related to stability issues or to other structural features? More experiments to come!

Immunization experiments with both antigens in mice and cattle were shown, describing the time-course of both humoral and cellular immune responses. Results in both animal models indicated the existence of quantitative and qualitative differences in the adaptive immune response induced by FMD vaccines formulated with 146S or 75S antigens. The discussion at the end of the session focused on the stability of the empty particles compared to the whole viral particles and this discussion led her to reconsider and redesign some experiments for the immunological analysis.

Cecilia Turco (post-doc fellow at INTA) presented her work in the diagnostic session, a collaborative project between INTA (Alejandra Capozzo´s group) and the FMD World Reference Laboratory (Anna Ludi's group): "We aimed to adapt an alternative methodology to sucrose-gradient ultracentrifugation (SGU) to purify 146S viral particles to be used as a coating material in the indirect ELISAs. The size exclusion chromatography (SEC) is a simple 'low-tec' method to purify antigen particles from both vaccine and field virus grown in cell culture". According to this simple protocol, the virus suspension eluted from the column (SEC-FMDV), can be used directly as a coating material for ELISA plates.

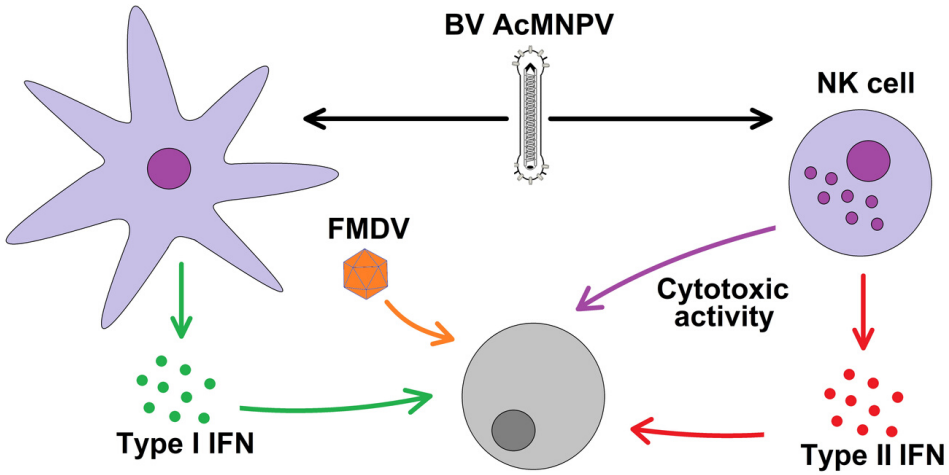


The purification process can be shortened from a week to a couple of hours of work using Size Exclusion Chromatography (SEC) instead of the traditional Sucrose Ultracentrifugation (SGU) protocols.

The integrity of SEC-purified FMDV was controlled by SGU and antigen ELISA confirming that the SEC-extracted fraction contains only 146S and eventually 75S particles. The comments from experts concerning this work caused a deeper inquiry about the yield of coating viral particles obtained in relation to SGU method. Furthermore, the GFRA Scientific meeting was important to gather with collaborators at the Pirbright Institute and strengthen collaborative bonds to continue this project.

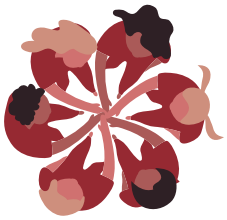
Guido Molina (a CONICET post-doc fellow at INTA) works in the development of antiviral strategies and new generation vaccines against FMDV. His presentation during the vaccine session described the use of the baculovirus *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), a virus that infect insects, in antiviral strategies against FMDV. Since the currently used FMDV vaccines induce a protective immune response between 4- and 7-days post-vaccination, antiviral strategies are needed to reduce the window of susceptibility in the case of an outbreak.

The intravenous injection of AcMNPV induced an antiviral status in mice providing protection against a lethal challenge with FMDV A/Arg/01 at 3 hours and 3 days post-inoculation. Type I IFNs induced by the AcMNPV and NK cells producing type II IFNs impeded the onset of the disease. AcMNPV also induced IFN-α-mediated antiviral activity in porcine peripheral blood mononuclear cells.



The proposed immune mechanisms behind the antiviral activity of the AcMNPV observed against the FMDV in pigs

Moreover, the intravenous inoculation of AcMNPV in pigs promoted the production of type I and II IFNs that were detected in sera during the first 9 hours after inoculation. The profile of antiviral mediators induced was able to protect porcine non-immune cells (LFBK) against an infection with FMDV, showing the potential of AcMNPV for the development of antiviral strategies against FMDV in pigs.



FMD Research in Argentina: the value of networking

by Mariano Perez-Filgueira

The FMD history demonstrates the risks and difficulties in controlling the incursions and outbreaks of the virus in disease-free regions, and Argentina has a long tradition in dealing with this old yet current threat. During the last decades, our country developed a solid network of public institutions intervening in different aspects of the problem, from the design and deployment of a robust national vaccination program and diagnostics network, to the support of basic research in different areas.

Together, SENASA (regional OIE FMD-reference laboratory), CEVAN (institution of the National Research Council, CONICET), and INTA have also held a strategic alliance with the private sector, implementing several collaborative projects with the main FMD local vaccine manufacturer, Biogenesis Bagó SA.

> Argentina will continue with this collaborative approach in the understanding that cooperation between countries synergizes individual capacities and represents the fastest and most efficient way to find innovative answers for the control of FMD

Soon after its foundation, the network also promoted international partnerships, starting in 2006 with a collaborative project with Sciensano (ex-CODA CERVA) from Belgium, followed by its participation in the FP7 FMD-DISCONVAC project between 2009 and 2014. In parallel, different INTA's research groups also held join initiatives with Spanish institutions such as INIA and the CBMSO.

The transference of newly developed assays from Argentina has also accounted for some of this exchange with several projects since 2014, including the ARC-OVR (South Africa), the Australian Animal Health Laboratory (AAHL-CSIRO), the Friedrich-Loeffler-Institut (FLI, Germany) and the Pirbright Institute (UK). With this aim, INTA was also one of the first institutions to join the GFRA as a full member in 2008, also accompanying the strategic alliance with the USDA through different collaborative projects with research groups in Plum Island Animal Disease Center (PIADC).

Following this approach, Argentinean research groups have participated in building fundamental and applied knowledge in a range of research topics such as immunology around the FMDV infection and vaccination in laboratory model and target species, development of new diagnostic techniques to evaluate immunity at individual and herd levels, experimental procedures to assess quality in FMD vaccines, molecular epidemiology of FMDV outbreaks, FMDV phylogeny and evolution, virulence factors associated to the RNA genome, vaccine matching and the immunological basis of the heterologous protection between FMDV strains, and development new FMD vaccines.

At present, several Argentinean laboratories are working in collaborative activities with scientists from other research and reference laboratories around the world.

INTA has held a series of consecutive projects with PIADC-USDA mostly focused on the immunology behind the infection and vaccination against FMDV in target species. In the frame of these projects, fundamental aspects of the local and systemic protective antibody responses were described, as well as the paramount role of the neutralizing antibodies in the control of FMDV systemic dissemination within the infected animals. In the current project, involving also SENASA and Biogenesis-Bago, the efforts are focused on understanding the immunological bases of the heterologous protection among FMDV strains in cattle.

SENASA, the OIE regional reference laboratory for FMD

As a regional reference laboratory, SENASA has also held twinning training projects ith its counterpart from Paraguay (SENACSA), sponsored by the OIE, and regularly participates and attends to the FAO/OIE Reference Laboratory Network Meetings. An ongoing project between INTA and The Biotechnology Research Institute (NRC-BRI) in Canada, also works on the development of FMDV virus-like particles, to be used as potential vaccine antigens which are produced using a transient expression system in mammalian cells developed at NRC-BRI.

INTA is also part of an international consortium which includes the ARC-OVR (South Africa) The Pirbright Institute (UK) and The University of Glasgow (Scotland), funded by the IVVN (International Veterinary Vaccinology Network). The project aims to study the antibody repertoire generated in buffalo and cattle immunized with different FMD vaccine protocols and identify potential cross-reactive antibodies. Also, the EuFMD has been funding a collaborative work between the World Reference Laboratory for FMD, the University of Glasgow and INTA to work on the validation of novel serological techniques to assess heterologous protection in natural hosts.

A molecular epidemiology project funded by the National Agency of Science and Technology (Argentina) also joins the expertise of the University of Minnesota and INTA to apply newly developed tools to measure transmission in different viruses through their evolution traces, using the 2001 FMDV outbreak in Argentina as a model.

From Argentina to the World. Current and recent activities on between Argentinean institutions and other laboratories working on FMD around the world

In the future, Argentinean institutions working on FMD will continue this approach in the understanding that cooperation between countries synergizes individual capacities and represents the fastest and most efficient way to find innovative answers in the fight against this long known adversary.

RESEARCH PAPERS

Follow the leader: FMDV Lpro enzymatic activities and their role in counteracting the host

by Gisselle N. Medina and Teresa de los Santos

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This article provides summarized data from a manuscript recently accepted in the Journal of Virology (2020) doi:10.1128/JVI.00341-20

Originally described as FMDV's second protease, Lpro cleaves itself from the nascent polypeptide chain at the L/VP4 junction (Strebel and Beck, 1986). In addition to its processing capability, Lpro antagonizes the host by targeting several cellular factors involved in innate immune responses (Fig. 1; (Medina et al., 2018)) granting its recognition as a 'security protein' (Agol and Gmyl, 2010).

The discovery of Lpro counter-defensive function was originally examined in a leader-deleted virus (leaderless, LLV) (Piccone et al., 1995) resulting in a highly attenuated virus in both cattle and swine (Mason et al., 1997; Chinsangaram et al., 1998; Eschbaumer et al., 2020). In vitro studies showed that attenuation of FMDV-LLV correlated with higher production of IFNα/β in supernatants from leaderless as compared to wild-type (WT) virus infected cells. Blockage of IFN expression during FMDV infection occurs fundamentally by the cleavage of the translation initiation factors eIF4GI and eIF4GII, preventing protein synthesis of capped host mRNAs (Devaney et al., 1988; Steinberger and Skern, 2014). Disruption of IFN induction has also been detected at the level of transcription. During infection, Lpro targets the transcription factors NF-κB and IRF3/7 for degradation resulting in the blockage of specific downstream signaling effectors of the antiviral innate immune response (de los Santos et al., 2007; Wang et al., 2010; Zhu et al., 2010). Although the precise mechanism for inducing the degradation of transcription factors by Lpro remains unclear, a putative SAP-DNA binding domain within Lpro was shown to mediate its nuclear localization during infection. Mutations of the SAP domain (I83A/L86A) abolished the abilities of Lpro to suppress type I IFN expression and to degrade signaling proteins during infection (de los Santos et al., 2009) while maintaining its proteolytic activity. Importantly, examination of Lpro SAP mutant resulted in severe attenuation in swine studies (Diaz-San Segundo et al., 2012). Lpro also targets the chromatin remodeling machinery by promoting its binding to the transcription factor ADNP (activity-dependent neuroprotective protein) and negatively regulate the activity of the IFN-α promoter (Medina et al., 2017). Paradoxically, in these studies, processing of ADNP was detected at later times of infection, suggesting that cleavage of ADNP generates products that may enhance transcription repressive activity. Future work to map Lpro-ADNP interaction domains may help elucidate the molecular mechanisms involved in this process.

Other Lpro cellular targets that antagonizes immune responses includes the cytosolic RNA sensors known as retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs). Recent studies have shown the interaction between the RLR LGP2 and FMDV Lpro followed by LGP2 degradation(Zhu et al., 2017; Rodríguez-Pulido et al., 2018). These results suggest that FMDV Lpro can target innate immune signaling pathways at many levels.

In addition to the proteolytic processing, FMDV Lpro has deubiquitinase (DUB) activity (Wang et al., 2011). In overexpression studies, Lpro displayed

DUB activity, catalyzing the removal of ubiquitin (Ub) from cellular substrates including RIG-I, TRAF3, TRAF6, and TBK. Modifications in the SAP domain of Lpro diminished DUB activity and its ability to block signaling to the IFN-β promoter (Wang et al 2011). Another Ub-like modifier is the IFN stimulated gene (ISG) 15 (ISG15) that is conjugated to target proteins in a process known as ISGylation by the consecutive action of three enzymes that make up the ISGylation machinery (E1-Ube1L, E2-UbcH8 and E3-HERC5).

However, unlike Ub, ISG15 and the ISGylation machinery are robustly induced by type I IFN and can be upregulated upon viral infection (dos Santos and Mansur, 2017). Recent studies conducted in BHK-21 cells have demonstrated that during FMDV infection cellular proteins undergo deISGylation (Swatek et al., 2018). Interestingly, the authors demonstrate that recombinant FMDV Lpro displays deISGylase activity on synthetic substrates and hydrophobic residues P99 and Leu102 are required for efficient deISGylase activity (Swatek et al 2018).

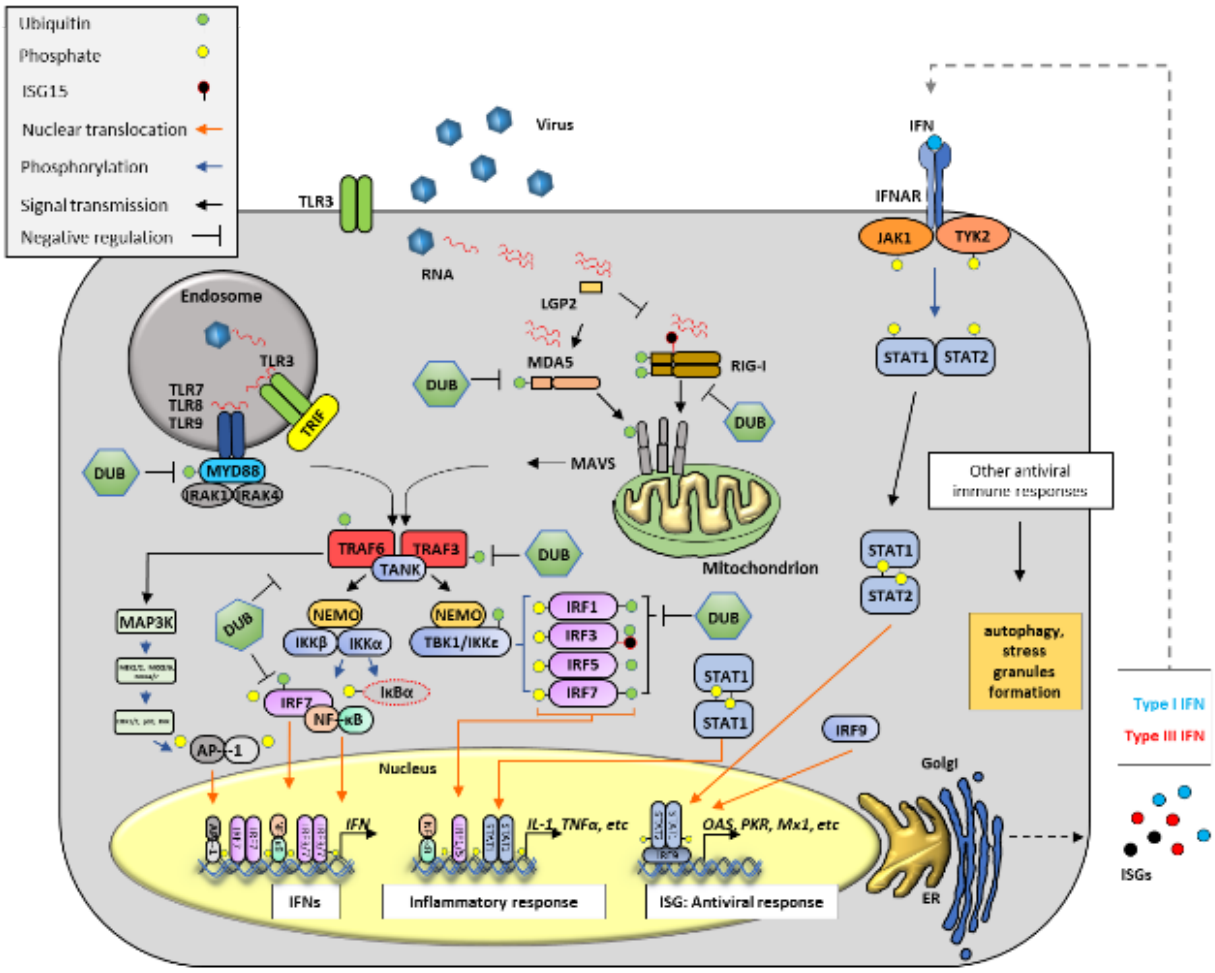

Figure 1: Innate antiviral immune responses druing viral infection

In our recent paper (Medina et al., 2020), we conducted structural analysis of FMDV Lpro followed by molecular modeling to reveal the presence of a conserved aromatic residue on Lpro, Trp,W105 (Fig.2A), that is required for optimal deISGylase activity. Importantly, engineering of an infectious clone carrying this mutation (LproW105A) rendered a viable FMDV with a perceptible level of attenuation and reduced deISGylation and DUB activity when compared to WT virus during infection of porcine cells (Fig. 2B-2D).

The observed reduction in Lpro DUB/deISGylation activity during FMDV infection suggested similar interactions between ISG15, Ub and Lpro. Intriguingly, infection with FMDV ILproW105A did not disrupt Lpro's ability to block IFN and ISGs expression (Medina et al 2020). Most importantly, in vivo inoculation with FMDV LproW105A using an FMD mouse model resulted in reduced lethality as compared to inoculation with WT virus (Fig.2E), suggesting that inability to remove ISG15 confers viral attenuation in vivo.

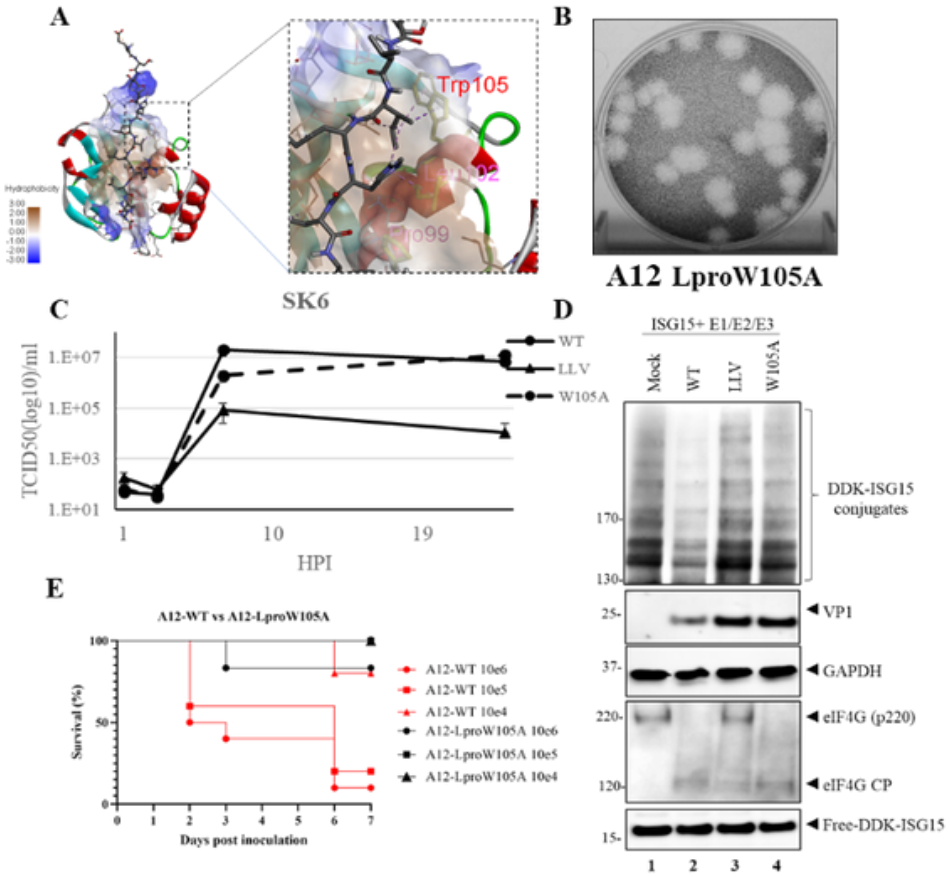

Figure 2: Hydrophobic aromatic residue in FMDV Lpro (W105) is requered for efficient deISGylase activity. (A) Close-up interactions between aromatic amino acid W 105 in red and ISG15 C-terminal peptide. (B) Plaque phenotype of viable FMDV carrying W105A mutation evaluated in BHK-21 cells. (D) LFPKaVβ6 transfected with plasmids encoding ISGylation machinery were infected with FMDVA12WT, LLV or LproW105A. At 6 hpi, cell lysates were prepared, and proteins were resolved in 3-8% SDS-PAGE followed by WB to detect DDK-tagged free or conjugated ISG15. FMDV VP1, eIF4G and GAPDH. (E) C57BL/6 mice were infected with A12 WT or A12-LproW105A and survival curves show data collected until day 7 after inoculation

Our studies reveal that educing/abolishing deISGylase activity in Lpro during infection renders the virus moderately attenuated independently of its ability to block the expression of type I IFN and other IFN-stimulated genes. Multifunctional enzymatic activities in the PLP Lpro indicate the evolvability of FMDV to counteract the immune response during infection. Our recent report demonstrates the separation of function between FMDV Lpro proteolytic activity -on canonical cellular substrates like eIF4G- from its DUB/deISGylase function.

Most importantly, we show for the first time the construction of a viable FMDV with impaired DUB/deISGylase activity. Although other Lpro residues mediating its interaction with ISG15 have been recently identified (Swatek et al 2018), we propose that Lpro W105 is important for modulating viral infection kinetics. Future studies to understand the specific contribution of Lpro DUB/deISGylase activity to counteract the immune response during virus infection opens new exciting avenues of research. This will ultimately lead to the development of effective live-attenuated vaccine candidates and other therapeutics to control FMD.

REFERENCES

-Agol, V. I., and Gmyl, A. P. (2010). Nat. Rev. Microbiol. 8, 867–878. doi:10.1038/nrmicro2452.

-Chinsangaram, J., Mason, P. W., and Grubman, M. J. (1998). Vaccine 16, 1516–1522. doi:10.1016/S0264-410X(98)00029

-de los Santos, T., Diaz-San Segundo, F., and Grubman, M. J. (2007). J. Virol. 81, 12803–12815. doi:10.1128/JVI.01467-07.

-de los Santos, T., Diaz-San Segundo, F., Zhu, J., Koster, M., Dias, C. C. a, and Grubman, M. J. (2009). J. Virol. 83, 1800–10. doi:10.1128/JVI.02112-08.

-Devaney, M. A., Vakharia, V. N., Lloyd, R., Ehrenfeld, E., and Grubman, M. J. (1988). J. Virol. 62, 4407–4409.

-Diaz-San Segundo, F., Weiss, M., Perez-Martin, E., Dias, C. C., Grubman, M. J., and de los Santos, T. (2012). J. Virol. 86, 1316–1327. doi:10.1128/JVI.05941-11.

-dos Santos, P. F., and Mansur, D. S. (2017). J. Interf. Cytokine Res. 37, 246–253. doi:10.1089/jir.2016.0103.

-Eschbaumer, M., Dill, V., Carlson, J. C., Arzt, J., Stenfeldt, C., Krug, P. W., et al. (2020). Pathogens. doi:10.3390/pathogens9020129.

-Guarne, A., Tormo, J., Kirchweger, R., Pfistermueller, D., Fita, I., and Skern, T. (1998). Embo Journal. Dec 15 1998; 17 7469.Mason, P. W.,

-Piccone, M. E., Mckenna, T. S., Chinsangaram, J., and Grubman, M. J. (1997). Virology 227, 96–102. doi:10.1006/viro.1996.8309.

-Medina, G. N., Azzinaro, P., Ramirez-Medina, E., Gutkoska, J., Fang, Y., Diaz-San Segundo, F., et al. (2020). J. Virol. doi:10.1128/JVI.00341-20.

-Medina, G. N., Knudsen, G. M., Greninger, A. L., Kloc, A., Díaz-San Segundo, F., Rieder, E., et al. (2017). Virology 505, 12–22. doi:10.1016/j.virol.2017.02.010.

-Medina, G. N., Segundo, F. D.-S., Stenfeldt, C., Arzt, J., and de los Santos, T. (2018). Front. Microbiol. doi:10.3389/fmicb.2018.02644.

-Piccone, M. E., Rieder, E., Mason, P. W., and Grubman, M. J. (1995). J. Virol. 69, 5376–5382.

-Rodríguez-Pulido, M., Sánchez-Aparicio, M. T., Martínez-Salas, E., García-Sastre, A., Sobrino, F., and Sáiz, M. (2018). PLOS Pathog. 14, 1–21.

-Steinberger, J., and Skern, T. (2014). Biological Chemistry doi:10.1515/hsz-2014-0156.

-Strebel, K., and Beck, E. (1986). J. Virol. 58, 893–9.

-Swatek, K. N., Aumayr, M., Pruneda, J. N., Visser, L. J., Berryman, S., Kueck, A. F., et al. (2018). Proc. Natl. Acad. Sci. 115, 2371–2376.
-Wang, D., Fang, L., Li, P., Sun, L., Fan, J., Zhang, Q., et al. (2011). J. Virol. 85, 3758–3766. doi:10.1128/JVI.02589-10.
-Wang, D., Fang, L., Luo, R., Ye, R., Fang, Y., Xie, L., et al. (2010). Biochem. Biophys. Res. Commun. 399, 72–78. doi:10.1016/j.bbrc.2010.07.044.
-Zhu, J., Weiss, M., Grubman, M. J., and de los Santos, T. (2010). Virology 404, 32–40. doi:S0042-6822(10)00271-0 [pii]\r10.1016/j.virol.2010.04.021.
-Zhu, Z., Li, C., Du, X., Wang, G., Cao, W., Yang, F., et al. (2017). Cell Death Dis. 8. doi:10.1038/cddis.2017.170

New fast FMDV subtyping tool

by Marco Cacciabue, Pablo Aguilera, María Inés Gismondi and Oscar Taboga

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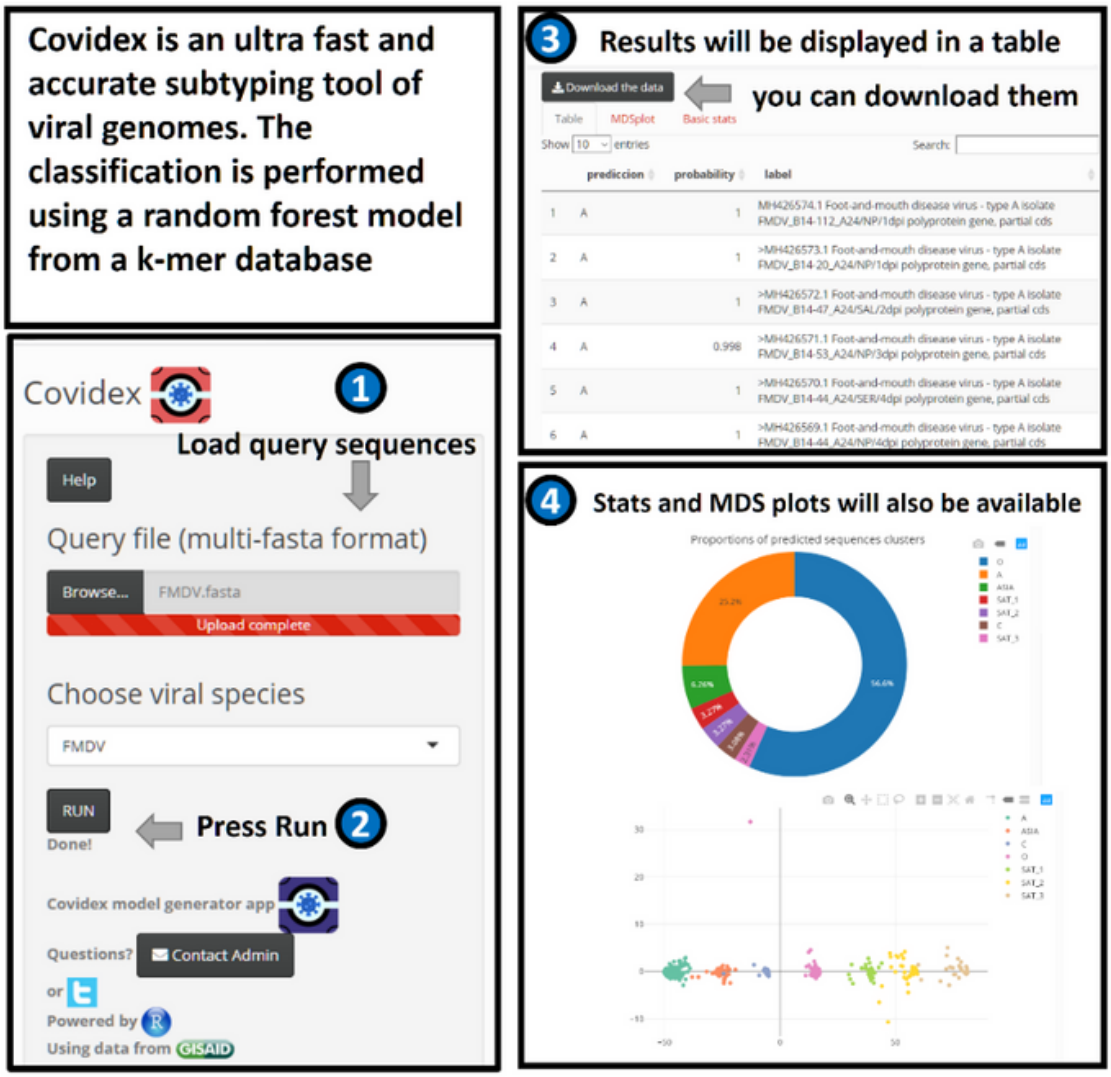
In the NGS era, a huge amount of sequence data is generated rapidly and continuously. In the case of FMDV, sequences may be used for virus classification for epidemiological purposes and for vaccine strain selection. FMDV subtyping is not trivial: current vaccines fail to cross protect FMDV serotypes and even between subtypes within the same serotype.
To facilitate FMDV sequence analysis, we present Covidex, a fast-open source alignment-free machine learning subtyping tool. It allows fast and accurate (Oob error rate < 1.5 %) classification of viral genomes in pre-defined clusters, all in a user-friendly interface (1). The program was originally developed for SARS-CoV-2 classification, and now it has been extended to FMDV.

Covidex is based on a fast implementation of random forest trained over a k-mer database composed of FMDV sequences deposited in GenBank (2, 3). Alternatively, user-uploaded models can be used. By training the classification algorithms over k-mer frequency vectors without the need of classic sequence alignment, Covidex substantially reduces computational and time requirements and can classify hundreds of FMDV full genomes in seconds. The software is available for download at

https://sourceforge.net/projects/covidex/

Users are encouraged to test it and feedback is very much appreciated!



Covidex app overview. The main steps for viral subtyping analysis are indicated in blue. The user is expected to load a sequence file and to select the model that will be applied for classification. Models may be selected from the default list or uploaded by the user. The program output (table and plots) is shown.

REFERENCES

-Chang W, Cheng J, Allaire J, Xie Y, McPherson J. 2020. shiny: Web Application Framework for R. Rpackage version 1.4.0.2. https://CRAN.R-project.org/package=shiny

-Wright MN, Ziegler A. (2017). Journal of Statistical Software 77

-Breiman L. (2001). Random Forests. Machine Learning 45:5-32

When does it end?!: Extinction dynamics of the foot-and-mouth disease virus carrier state

by Miranda R. Bertram, Shankar Yadav, Carolina Stenfeldt, George R. Smoliga, Ethan J. Hartwig, Ian H. Fish, Amy Delgado and Jonathan Arzt

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INTRODUCTION

Following the clinical phase of foot-and-mouth disease (FMD), a large proportion of ruminants remain persistently infected for extended periods. The existence of this carrier state in ruminants has practical implications in FMDV-endemic regions that are distinct from management practices in regions striving to regain FMD-free status after an outbreak (1). Appropriate practices for management of carriers have not been established in either context. Although extinction of the carrier state occurs continuously at the animal and population levels, studies vary widely in their estimates of the duration of persistent infection (2-4). The variability among distinct studies and analytical approaches impedes development of effective control measures to account for FMDV persistent infection. Most importantly there is a need for robust statistical models to capture the dynamics of persistent infection for the sake of guiding FMD control and trade policies. Longitudinal studies directly observe the dynamics of persistent infection in individuals over time. Unfortunately, longitudinal studies are labor-intensive, time-consuming, expensive, and logistically challenging. In contrast, cross-sectional studies have larger sample sizes and can be completed more rapidly and economically. However, it is unclear whether cross-sectional data is appropriate for modeling the dynamics of persistent infection. Alternatively, meta-analysis approaches combining data across several longitudinal studies could be used to mitigate small sample sizes while incorporating diverse field conditions (viral strain, host factors, husbandry, environmental factors) into a more holistic output (5).

The goals of the current study were to assess the utility of two distinct statistical models for predicting the probability of persistent FMDV infection post-outbreak at the level of individual carrier/ seropositive animals, and to compare different analytical methods to assess extinction of the carrier state. The current study incorporated data from three primary longitudinal studies of FMDV persistent infection in Vietnam and India using an individual participant data meta-analysis approach (5) to assess the dynamics of persistent infection across the three study populations. Generalized linear mixed models (GLMM) and accelerated failure time models (AFT) were developed to predict the probability of persistent infection in cattle and Asian buffalo (Bubalus bubalis) at various times following an FMD outbreak.

METHODS

The primary datasets incorporated in the analyses were derived within the scope of long-term, longitudinal projects on endemic FMD in India (n=2) and Vietnam (n=1) between 2010-2015 (2, 3, 6-8). For the current study, persistent infection was defined as the detection of FMDV RNA in oropharyngeal fluid (OPF). All animals were seropositive by anti-NSP antibody ELISA at the start of the study (2, 6, 7). Additionally, all animals in the two India studies were carriers at the start of the study, as determined by FMDV RNA detection in OPF (2, 7). For the purposes of this study, it was assumed that no reintroduction or subclinical circulation of the virus occurred on farms included in the study. The probability of persistent infection was investigated using accelerated failure time models (AFT) and generalized linear mixed models (GLMM).

	AFT Predictions				GLMM Predictions			
	Months Post-Outbreak				Months Post-Outbreak			
Species	**6**	**12**	**18**	**24**	**6**	**12**	**18**	**24**
India-1 Buffalo	99.5%	40.88%	2.47%	0.24%	91.05%	51.75%	10.16%	1.18%
India-1 Cattle	98.26%	16.49%	0.72%	0.07%	75.21%	24.23%	3.26%	0.35%
India-2 Cattle	99.75%	58.51%	4.9%	0.49%	77.56%	26.70%	3.70%	0.40%
Vietnam Buffalo	99.58%	45.61%	2.98%	0.29%	75.77%	24.79%	3.36%	0.36%
Vietnam Cattle	99.86%	71.08%	8.25%	0.85%	87.21%	41.82%	7.04%	0.79%
Overall	*99.23%*	*50.75%*	*5.76%*	*0.82%*	*80.38%*	*32.08%*	*5.17%*	*0.6%*

Table 1. Predicted probability of persistent infection following an FMD outbreak. Predictions were made from three longitudinal studies in Vietnam and India using an accelerated failure time model (AFT) and a generalized linear mixed model (GLMM)

To account for species differences and other study-site related variability, a combined study and species variable was created (study/species) and included in both models. Additionally, individual animal ID was included as a random variable in the GLMM to account for repeated measures on the same animals. The final model equations were used to predict the probability of FMDV RNA detection in OPF at 6, 12, 18, and 24 months post-outbreak. Estimates were generated for each study/species group.

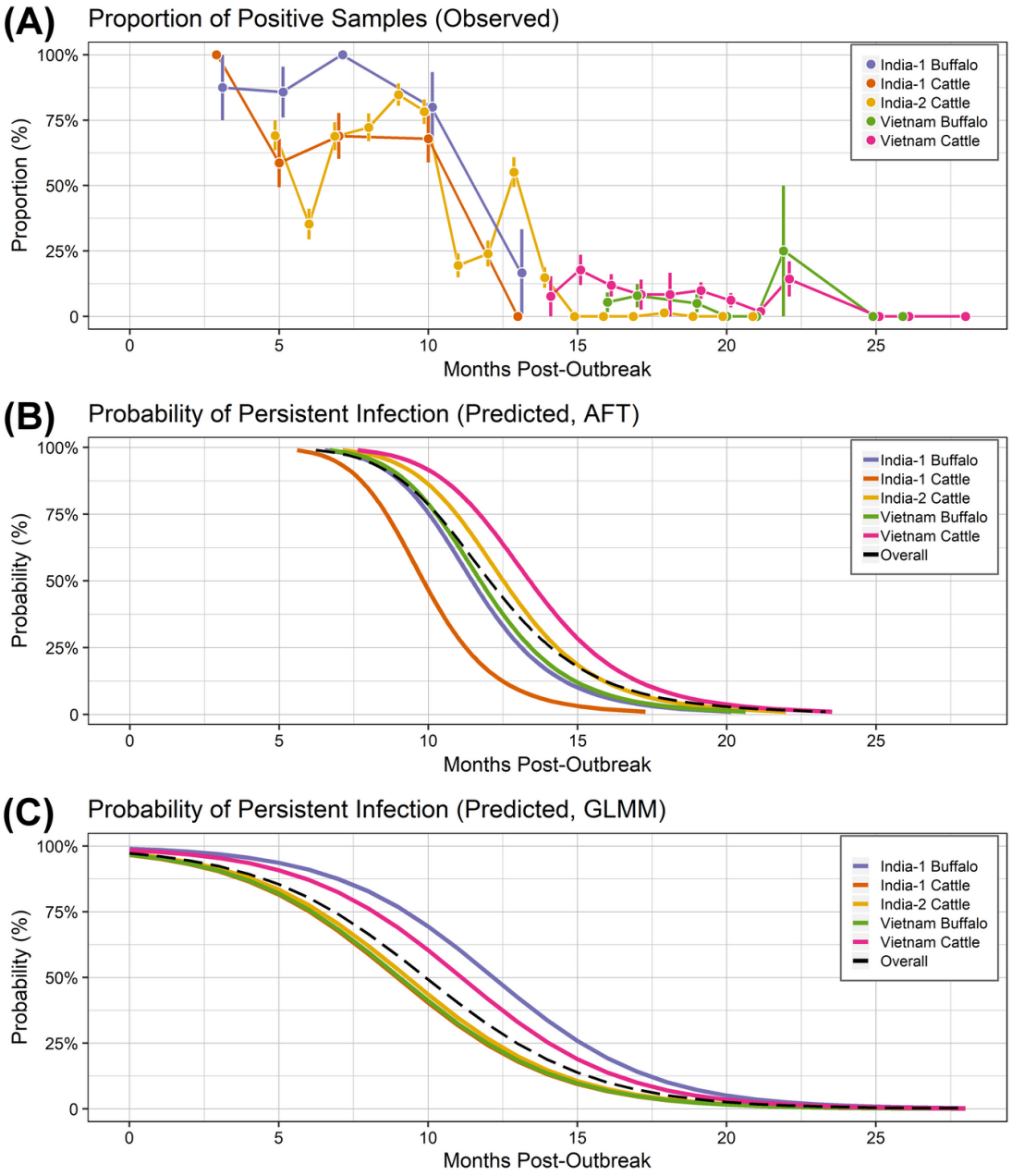

Figure 1. Probability of persistent infection, Vietnam and India data combined. (A) Observed proportion of OPF samples positive for FMDV RNA. Mean and standard error are shown. (B) Predicted probability of persistent infection, AFT model. (C) Predicted probability of persistent infection, GLMM model

RESULTS

The final dataset used to investigate the dynamics of extinction of persistent infection consisted of 2006 samples from 345 seropositive animals or identified carriers, across the 3 studies. FMDV RNA was detected in 90-100% of samples at 3 months post-outbreak, and the proportion of positive samples gradually decreased over time in all studies (Figure 1A). No FMDV RNA was detected after 15 months post-outbreak in the India-2 study, or after 25 months post-outbreak in Vietnam.

The two models estimated similar trends in the duration of persistent infection for the study/species groups included in the analyses, however the significance of the trends differed between the models. The overall probabilities of persistent infection were similar as predicted by the AFT and GLMM models: 6 months: 99% (AFT) /80% (GLMM), 12 months: 51% (AFT) /32% (GLMM), 18 months: 6% (AFT) /5% (GLMM), 24 months: 0.8% (AFT) /0.6% (GLMM) (Table 1, Figure 1).

DISCUSSION/CONCLUSIONS

These models utilizing diverse and robust data sets predict higher probabilities of persistence than previously published, suggesting greater endurance of carriers subsequent to an outbreak. Persistent infection with foot-and-mouth disease virus is a challenge for FMD control and eradication in endemic regions. Similarly, FMD-free regions must consider the existence of carriers when responding to incursions.

Trade and control policies have traditionally treated persistent infection as a binary state (present/absent) with a fixed duration. However, persistent infection is a dynamic process, and quantitative estimates of the probability of persistent infection at specified times post-outbreak may provide tools to more accurately assess the potential risks posed by persistently infected animals, which will help to guide control efforts.

Overall, the two models produced similar predictions in the meta-analyses, suggesting that either model may be satisfactory for describing the dynamics of FMDV carrier state extinction. Researchers should consider which model is more appropriate for a particular study based on the study design, data structure, and whether model assumptions are biologically appropriate. Additionally, results should be interpreted in consideration of the model used for analyses. Our results suggest that when only cross-sectional data are available, a GLMM approach may be suitable to model the probability of FMDV persistent infection in the study population. Alternatively, meta-analysis of several longitudinal studies can overcome some limitations of individual studies, such as small sample size and limited sampling frequency, while providing a more robust view of viral dynamics within animals over time. Furthermore, this approach can help researchers and disease control experts better understand how persistent infection varies across populations.

In the current study, the AFT and GLMM models predicted similar probabilities of persistent infection at 18 and 24 months post-outbreak, while the probability of detection of persistent infection was higher using the AFT model at 6 and 12 months post-outbreak. Because it may over-estimate probabilities at earlier timepoints, the AFT model is the more cautious approach for designing policies to reduce or eliminate the potential risk presented by persistently infected animals following an FMD outbreak. These analyses provide a more tailored approach to the development of control measures to minimize the risk posed by persistently infected animals. Additional studies with larger sample sizes and expanded meta-analyses, including more primary studies, are likely to provide more nuance and depth to our understanding of this dynamic process, leading to an improved understanding of FMDV persistent infection after outbreaks and how to predict and manage this disease state. These subjects are treated in greater detail in the article from Bertram et al in the Frontiers special issue "FMD Research: Bridging the Gaps with Novel Tools"

REFERENCES

1.Stenfeldt C, Arzt J. Pathogens (2020) 9(3):167. Epub 2020/03/04. doi: 10.3390/pathogens9030167.

2. Hayer SS, Ranjan R, Biswal JK, Subramaniam S, Mohapatra JK, Sharma GK, et al. Transboundary and Emerging Diseases (2017) 00:1-8. doi: 10.1111/tbed.12627.

3. Bertram MR, Vu LT, Pauszek SJ, Brito BP, Hartwig EJ, Smoliga GR, et al. Frontiers in Veterinary Science (2018) 5(174). doi: 10.3389/fvets.2018.00174.

4. Bronsvoort BM, Handel IG, Nfon CK, Sorensen KJ, Malirat V, Bergmann I, et al. Scientific Reports (2016) 6:29059. doi: 10.1038/srep29059. PubMed PMID: 27381947; PubMed Central PMCID: PMCPMC4933899.

5. Thompson S, Kaptoge S, White I, Wood A, Perry P, Danesh J. International journal of epidemiology (2010) 39(5):1345-59. Epub 2010/05/05. doi: 10.1093/ije/dyq063.

6. de Carvalho Ferreira HC, Pauszek SJ, Ludi A, Huston CL, Pacheco JM, Le VT, et al. Transboundary and Emerging Diseases (2017) 64(2):547-63. doi: 10.1111/tbed.12403.

7. Hayer SS, VanderWaal K, Ranjan R, Biswal JK, Subramaniam S, Mohapatra JK, et al. Transboundary and Emerging Diseases (2017) 00:1-12. doi: 10.1111/tbed.12774.

8. Brito B, Pauszek SJ, Eschbaumer M, Stenfeldt C, de Carvalho Ferreira HC, Vu LT, et al. Veterinary Research (2017) 48(1):24. doi: 10.1186/s13567-017-0424-7.

The role of viral particle integrity in serological assessments

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This article provides summarized data from a manuscript recently accepted in Plos One by Mansilla et al (2020) doi: 10.1371/journal.pone.0232782

The aim of this work was to assess humoral responses in FMDV vaccinated pigs, considering different aspects of the antibody response apart from neutralizing antibodies, such as total antibodies and isotypes induced. For this purpose, we developed indirect ELISA assays adapting those already used for bovine sera in our laboratory (1) and assessed the impact of virus particle integrity in serology.

A well-known fact among FMDV laboratories is that titers estimated by the currently used ELISAs, as Liquid Phase Blocking ELISA (LPBE) do not always correlate with those measured by the virus neutralization test (VNT). Others and we have proposed that the lack of correlation is a consequence of not measuring the correct protection marker (2, 3). In a recent study we published in PlosOne we demonstrated that, besides that, a simple technical issue like using disassembled viral particles may account, at least partially, to the low correlation sometimes observed (Figure 1), ; and that this phenomenon can be especially important for certain labile strains.

It is well known and demonstrated by our group and others, that type-O are less stable than A strains (4). In addition, different authors have demonstrated that disrupted viral particles (12S pentamers) are less immunogenic and elicit a diminished neutralizing response (5 ,6). Moreover, 12S particles may expose internal epitopes to B-cells, and antibodies against non-protective epitopes can be elicited. In order to analyze the immune response in these conditions we used an expired vaccine that contained only half the amount of the total O1 Campos-antigen as 146S particles. In contrast, due to the intrinsic stability features of A and O strains, most of the whole A24/Cruzeiro particles were preserved. Pigs were immunized with this vaccine and specific-total antibodies were measured with the standard liquid-phase blocking ELISA (LPBE). We also developed an indirect ELISA (IE) using sucrose gradient purified 146S particles as capture antigen to titrate total antibodies, IgM, IgG1 and IgG2.

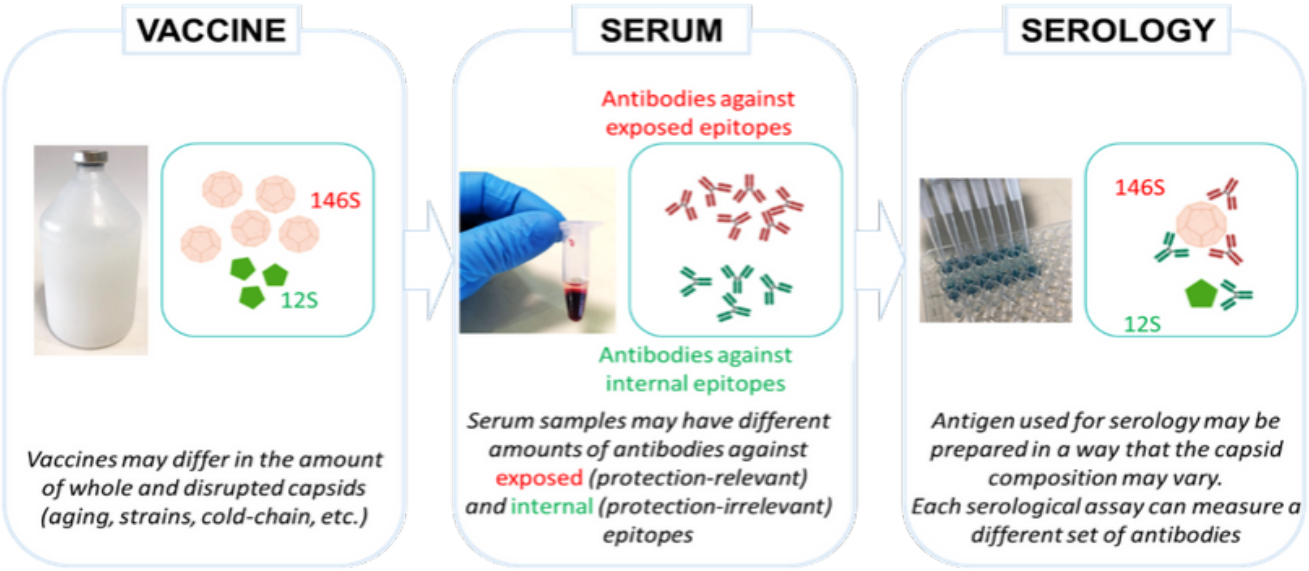

Figure 1. 12S pentamers derived from whole particles can be found in vaccines due to aging, the presence of additives, the intrinsic stability of each strain, etc. These pentamers can induce antibodies against epitopes that are not related to protection and may even be similar between different strains. If serology measures antibodies against exposed epitopes, then vaccine efficacy will be correctly estimated. However, if the serological assay does not ensure the absence of 12S particles, correlation with neutralizing titers will be low.

A good correlation was found between VNT titers and IgG-ELISAs for A24/Cruzeiro, with the lowest correlation coefficient estimated for IgG2 titers. For O1/Campos, however, the presence of antibodies against epitopes different from those of the whole capsid, elicited by the presence of 12S particles in the vaccine, hampered the correlation between LPBE and VNT, which was improved by using purified O1/Campos 146S-particles for the liquid-phase of the LPBE (Figure 2).

We also found that 146S particles but not 12S were efficiently bound to the ELISA plates, confirming the efficiency of the IE to detect antibodies against exposed epitopes.

Our results indicate that any serological test assessing total antibodies or IgG1 against epitopes exposed in intact 146S-particles correlate with the levels of serum neutralizing antibodies in vaccinated pigs, and might potentially replace the VNT, upon validation.

We recommend that antigen used for serological assays aimed to measure protective antibodies against FMDV should be controlled to ensure the preservation of 146S viral particles.

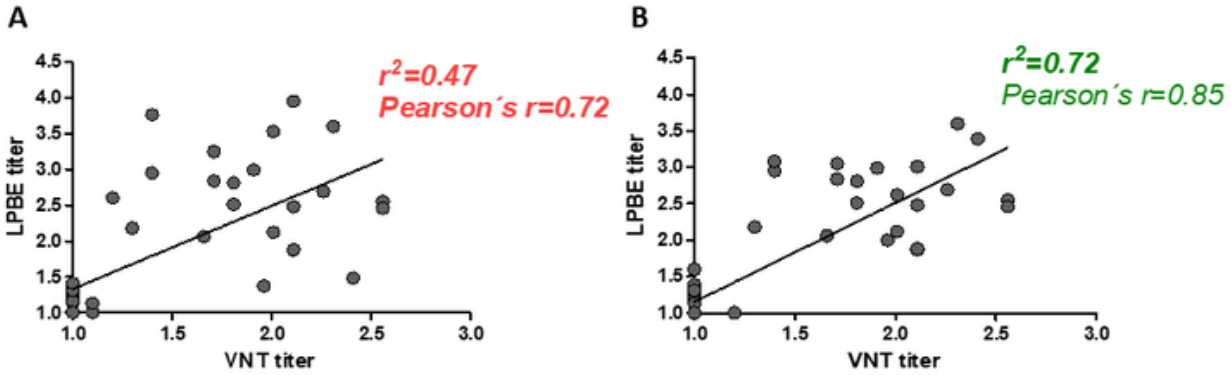

Figure 2. Effect of the presence of 12S particles in the correlation with VNT titers. Sera from animals vaccinated with 12S and 140S particles was used. LPBE was performed either with a virus suspension (A) or with whole-purified 146S particles (B). Titers were computed and plotted against their corresponding neutralizing titers. Each point depicts an individual sample. Linear correlation coefficients (r2) and Pearson´s r values are shown in each graph. In red: poor correlation between VNT and LPBE titers.

REFERENCES

(1) Lavoria M, Di-Giacomo S, Bucafusco D, Franco-Mahecha OL, Pérez-Filgueira DM, et al. (2012) Avidity and subtyping of specific antibodies applied to the indirect assessment of heterologous protection against Foot-and-Mouth Disease Virus in cattle. Vaccine 30: 6845-6850.

(2) Paton DJ, Reeve R, Capozzo AV, Ludi A (2019) Estimating the protection afforded by foot-and-mouth disease vaccines in the laboratory. Vaccine 37: 5515-5524.

(3) Brito BP, Perez AM, Capozzo AV (2014) Accuracy of traditional and novel serology tests for predicting cross-protection in foot-and-mouth disease vaccinated cattle. Vaccine 32: 433-436.

(4) Bucafusco D, Di Giacomo S, Pega J, Schammas JM, Cardoso N, et al. (2015) Foot-and-mouth disease vaccination induces cross-reactive IFN-γ responses in cattle that are dependent on the integrity of the 140S particles. Virology 476: 11-18.

(5) Doel TR, Chong WK (1982) Comparative immunogenicity of 146S, 75S and 12S particles of FMD virus. Arch Virol 73: 185-191.

(6) Rao MG, Butchaiah G, Sen AK (19. Doel TR, Chong WK (1982) Comparative immunogenicity of 146S, 75S and 12S particles of foot-and-mouth disease virus. Arch Virol 73: 185-191.94) Antibody response to 146S particle, 12S protein subunit and isolated VP1 polypeptide of FMDV type Asia-1. Vet Microbiol 39: 135-143.

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