Recommendations for International Standard Guidelines for ASF First Generation Live Attenuated Virus Vaccines

2023 GARA Gap Analysis

December 05-07, 2023

Manila, Philippines

Cyril Gay and David Brake

USDA-ARS/GARA/WOAH SPONSORED, CONTRACTED (CRDF GLOBAL)
2022-2023 PROJECT TO BIOQUEST ASSOCIATES, LLC.

Global African Swine Fever Research Alliance

Concerns with ASF live vaccines

Global African Swine Fever Research Alliance

NEWS & REPORTS

Warning over substandard ASF vaccines

By Arabella Gray

THE World Organisation for Animal Health (WOAH) has warned veterinary authorities and the pig industry about the risk of using 'substandard' African swine fever (ASF) vaccines.

Against the backdrop of the ongoing ASF outbreak, which has seen large swathes of Europe affected by the disease, WOAH has reiterated the importance of using only 'high-quality ASF vaccines with proven efficacy and safety', and ones that have been subject to 'regulatory evaluation and approval' by WOAH international standards.

The use of non-compliant and poor-quality vaccines may not only fail to confer protection against ASF, but also risks spreading vaccine viruses that could result in acute or chronic disease, WOAH explained in a recent press release.

'Additionally, these vaccine viruses could also recombine with field strains to generate novel strains that could evade detection and result in acute
infect

The organisation also noted that, regardless of vaccine efficacy, vaccination programmes should be implemented as part of a 'comprehensive prevention and control strategy', which should also include biosecurity measures, movement controls and import measures.

Postvaccination monitoring and surveillance, as well as an exit strategy for the cessation of vaccination, should also always be included in such programmes, it said.

WOAH is currently monitoring the progress of several ASF vaccine candidates at various stages of development. Some countries have already approved modified live vaccine candidates, while others are currently conducting field trials.

In recent months ASF has been detected in wild boar in Sweden for the first time. It is not yet known how the disease was introduced into the country, or the genotype of the virus; the Swedish National Veterinary Institute has said it believed the most likely route of entry to be

ASF outbreaks in domestic pigs and wild boar across the rest of Europe have decreased considerably since 2022, although there have been increased cases in the Balkan states in pig farms recently.

Nonetheless, EU pork production remains 'at its lowest level in almost a decade', according to the latest Defra outbreak assessment, due to a combination of factors including ASF limiting export opportunities, input
id cost-of-living
mers.

Only high-quality ASF vaccines with proven efficacy and safety should be used

Vet. Rec. 2023 Nov 18;193(10):390

- THE World Organisation for Animal Health (WOAH) has warned veterinary authorities and the pig industry about the risk of using 'substandard' African swine fever (ASF) vaccines.
- WOAH has reiterated the importance of using only 'high-quality ASF vaccines with proven efficacy and safety', and ones that have been subject to 'regulatory evaluation and approval' by WOAH international standards.

PROJECT BACKGROUND

Global African Swine Fever Research Alliance

- Goal. Develop recommendations for ASF MLV first generation vaccine international guidelines for standards and submission to the WOAH Biological Standards Commission.
- Process. Recommended guidelines were developed using:
  1. Vaccine production and quality control, purity, potency and stability principles and standards described in the relevant chapters of the WOAH "Terrestrial Manual".
  2. Current guidelines for veterinary LAV/MLV vaccines on purity, potency, safety and efficacy published by: EMA CVMP, USDA CVB, VICH, and WOAH – *with a strong emphasis on the SAFETY component*
  3. Results from *PEER-REVIEWED published studies* on ASF MLV vaccine lead candidates from leading ASF vaccine research/discovery laboratories in the U.S., Europe, Africa, and Asia
  4. Consensus and dissention outcomes from (5) x ~3 hr. virtual meeting workshops (SMEs, KOLs, Regulatory Experts)
  5. Supplemental information obtained from other sources (GARA Workshops [2018, 2022]; lab surveys, 1-on-1 information exchanges w/ SMEs)

PROJECT BACKGROUND (cont'd)

Global African Swine Fever Research Alliance

- Primary focus on standards associated with first generation, ASF modified-live virus vaccine candidates targeting genotype II pandemic strain
- Draft recommendations for ASF MLV first generation vaccine international guidelines delivered APR23 to the WOAH Biological Standard Commission
- Key outcomes circulated in WOAH Biological Standards Commission SEP23 meeting report (Annex 16, Item 5.1- pgs. 196-213; Appendix 1, pgs. 214-219)
- Vaccine manufacturers should consider these draft standards when developing and evaluating ASF MLV vaccine candidates for regulatory approval

Report of the Meeting of the WOAH Biological Standards Commission

Original: English (EN)

4 to 8 September 2023

Paris

Introduction and Member contribution

A meeting of the WOAH Biological Standards Commission (hereafter called 'the Commission') was held from 4 to 8 September 2023 at the WOAH Headquarters in Paris, France. During the meeting, 15 chapters from the WOAH *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) were approved for circulation for first-round Member comment, six Reference Centre applications and eight nominations for replacement experts were also evaluated.

Draft Standards for African Swine Fever (ASF) Modified Live Virus (MLV) Vaccines for Domestic and Wild Pigs

I. Background

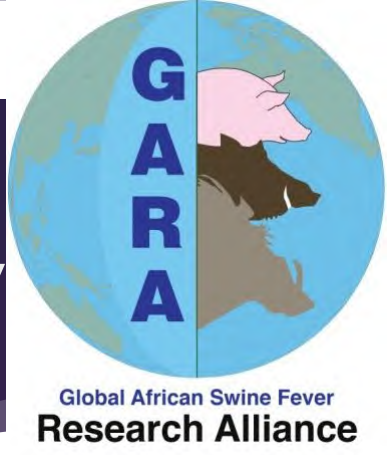
Under a project funded by a Collaborative Agreement between the WOAH and USDA-ARS, and in collaboration with CRDF Global, a consultant, Dr David Brake of BioQuest Associates, LLC, was engaged to develop guidelines on the development and manufacture of safe and efficacious ASF vaccines.

Summary of Key Discussion Areas - Safety

Global African Swine Fever Research Alliance

- Breed and Gender
- Age/Weight Range
- Group Size and Housing
- Route of Immunisation
- Dose Studies
- Clinical observations: Frequency, Duration, Rectal Temp, Disease/Clinical Scoring
- Analytical readouts: viremia shedding
- Short vs. long term
- Post-mortem readouts: pathology, tissue persistence
- Transmission studies
- Reversion to Virulence
- Recombination
- Pregnant animals
- Wild boars
- **Definitions – minimum standards for fever, clinical signs, residual virulence, viremia, shedding**

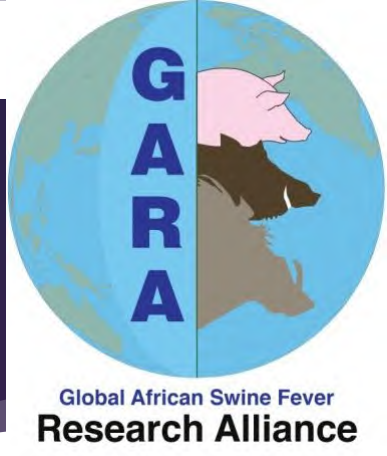
Summary of Key Discussion Areas - Efficacy



- Breed and Gender
- Age/Weight Range
- Group Size and Housing
- Dose
- Challenge route
- Challenge strain and dose
- Challenge timepoint
- Clinical observations: Frequency, Duration, Rectal Temp, Survival, Clinical Scoring

- Analytical readouts: Viremia, shedding, challenge virus transmission
- Protective dose (MID vs. PD)
- Duration of immunity
- Cross (heterologous) protection
- DIVA
- Wild boars
- **Definitions – minimum standards for fever, clinical signs, "prevents" vs. "reduces"**

Consensus Minimum Standards Laboratory Safety



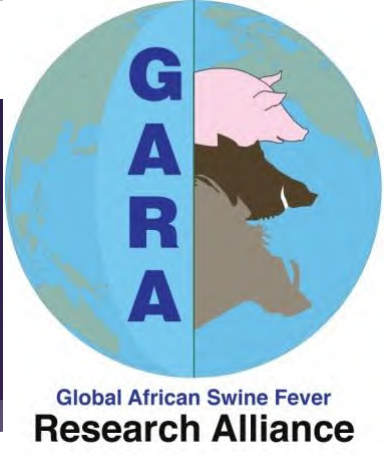
1. MLV transmission more important than shedding; vaccine safety should include measurement of MLV transmission to naïve pigs, *particularly in regions where several wild type or unauthorised MLV vaccine strains/genotypes may be co-circulating.*

2. General lack of correlation between: viremia and residual virulence, viremia and ability to shed, viremia and ability to transmit; thus, viremia may not be a highly informative parameter to evaluate vaccine safety

3. Measure both virus isolation and RT-PCR (blood and swabs); however hard to set safety quantitative thresholds.

4. In reversion to virulence studies, not essential to conduct next generation/deep sequencing on ASF MLV virus full genome obtained after the last in-vivo passage, however, consideration may be given to limiting sequence analysis to genome regions containing gene deletion(s).

MLV Vaccine Safety - Consensus Must Demonstrate



1. Absence of fever (*draft definition - average body temperature increase for all vaccinated piglets (group mean) for the observation period does not exceed 1.5°C above baseline; and no individual piglet shows a temperature rise above baseline greater than 2.5°C for a period exceeding 3 days*).

2. Minimum horizontal transmission (defined as no naive, contact piglet shows notable signs of disease by ASF related clinical signs, gross pathology and a low percentage of contact piglets testing both RT-PCR positive and seropositive).

3. Absence of an increase in virulence (genetic and phenotypic stability) (defined as complies with the reversion to virulence test).

TOP LINE CONCLUSIONS

Global African Swine Fever Research Alliance

- There was sufficient consensus on Chapter 3.9.1 African Swine Fever – draft Section C - for consideration by the WOAH Biological Standards Commission at their SEP2023 meeting
- There are (4) major areas – *technical gaps in draft standards* - which could benefit from future input:

1. ASFV challenge dose (range);

2. Establish numerical cut-off values for fever definition for both vaccine safety and vaccine efficacy;

3. Define 2–3 most important clinical sign observations to measure and make decision on whether or not to use a standard numerical scoring for each clinical sign; and

4. Yes/No on the use of prescriptive timepoints and sample types for subsequent passages for regulatory reversion to virulence study

GARA - Recommended Next Steps
International Standard Guidelines for ASF
First Generation Live Attenuated Virus Vaccines

Global African Swine Fever Research Alliance

1. Conduct semi-annual review to identify any **new peer-reviewed publications** and new technical information on existing and any new ASF MLV candidates
2. Conduct a comprehensive review of **peer-reviewed literature** on **current ASF MLV licensed (Vietnam) vaccines and top vaccine candidates (~12)** and generate vaccine safety and efficacy comparative summary tables *(methods used and key results)*
3. Provide **feedback to the WOAH Biological Standards Commission** on the *current published draft (*Terrestrial Manual Chapter 3.9.1 African Swine Fever – draft Section C (available on-line)

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 4–8 September 2023

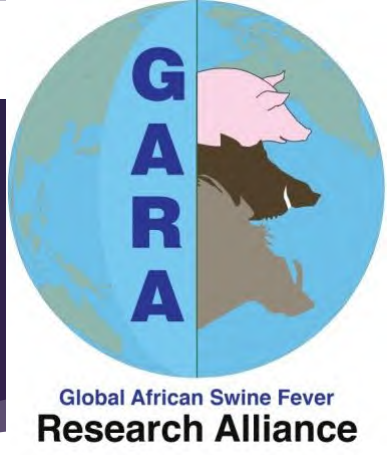
SECTION 3.9.

SUIDAE

CHAPTER 3.9.1.

AFRICAN SWINE FEVER
(INFECTION WITH AFRICAN SWINE FEVER VIRUS)

Requirements for ASF Vaccines Introduction



- Vaccines should be prepared in accordance with Chapter 1.1.8 *Principles of veterinary vaccine production*.
- ASF modified live virus (MLVs) vaccines are based on the live virus that have been naturally attenuated or attenuated by targeted genetic recombination
- ASF MLV first generation vaccines – defined as those for which peer-reviewed publications are in the public domain
- ASF MLV first generation vaccines allowing the differentiation of infected animals from vaccinated animals (DIVA) by suitable methods (e.g. serology-based tests) are preferred.
- Demonstration of MLV safety and efficacy in breeding-age boars, gilts and pregnant sows, and onset and duration of protective immunity, are also preferred but are not required to meet the minimum standard.

Requirements for ASF Vaccines Background

Global African Swine Fever Research Alliance

- The ASF p72 genotype II strain (ASFV Georgia 2007/1 lineage) (NCBI, 2020) is recognised to be the current highest global threat for domestic pig production worldwide (Penrith *et al.,* 2022).
- The ASF MLV vaccine production facility should meet the requirements for containment outlined in Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*.

Requirements for ASF Vaccines Background

Global African Swine Fever Research Alliance

- An optimal ASF MLV first generation vaccine for the target host should have the following general characteristics (minimum standards):
  - ✓ Safe: demonstrate absence of fever and clinical signs of acute or chronic ASF in vaccinated and in-contact animals, minimal and ideally no vaccine virus transmission, and absence of an increase in virulence (genetic and phenotypic stability);
  - ✓ Efficacious: protects against mortality, reduces acute disease and reduces vertical and horizontal disease transmission;
  - ✓ Quality – purity: free from wild-type ASFV and extraneous microorganisms that could adversely affect the safety, potency or efficacy of the product;
  - ✓ Quality – potent: the log10 virus titre maintained throughout the vaccine shelf life that guarantees the efficacy demonstrated by the established minimum immunising (protective) dose.
  - ✓ Identity: based on the capacity to protect against the ASFV B646L (p72) genotype II pandemic strain or other p72 genotypes of recognised epidemiologic importance.

Requirements for ASF Vaccines Background

Global African Swine Fever Research Alliance

- Ideally, ASF MLV first generation vaccines that meet the minimum standards should also fulfil the following additional general characteristics**: i) prevents acute and persistent (carrier state) disease; ii) prevents horizontal and vertical disease transmission; iii) induces rapid protective immunity (e.g. < 2 weeks); and iv) confers stable, life-long immunity.**
- Furthermore, ASF MLV second and future generation vaccines should meet the minimum safety and efficacy standards as ASF MLV first generation vaccines, and ideally provide additional product profile benefits, including but not limited to**: i) contain a negative marker allowing the differentiation of infected from vaccinated animals (DIVA) by reliable discriminatory tests such as serology-based tests; and ii) confer broad range of protection against other p72 genotype field strains of varying virulence (low, moderate, and high).**

Requirements for ASF Vaccines
2.3.2. Safety requirements

Global African Swine Fever Research Alliance

i) Safety in young animals

Carry out the test by each recommended route of administration using, in each case, piglets a minimum of 6-weeks old and not older than 10-weeks old.

The test is conducted using no fewer than eight healthy piglets, and preferably no fewer than ten healthy piglets.

Use vaccine virus at the least attenuated passage level that will be present in a batch of the vaccine.

Administer to each piglet a quantity of the vaccine virus equivalent to not less than ten times the maximum virus titre (e.g. 50% haemadsorption dose [HAD_{50}], 50% tissue culture infective dose [$TCID_{50}$], quantitative PCR, etc.) (maximum release dose) likely to be contained in one dose of the vaccine. To obtain individual and group mean baseline temperatures, the body temperature of each vaccinated piglet is measured on at least the 3 consecutive days preceding administration of the vaccine.

Requirements for ASF Vaccines
2.3.2. Safety requirements

Global African Swine Fever Research Alliance

ii) Safety test in pregnant sows and test for transplacental transmission

There is currently an absence of published information on ASFV pathogenesis in breeding-age gilts and in pregnant sows associated with ASFV transplacental infection and fetus abortion/stillbirth. If a label claim is pursued for use in breeding age gilts and sows, then a safety study in line with VICH GL44 (*Guidelines on Target Animal Safety for Veterinary Live and Inactivated Vaccines, Section 2.2. Reproductive Safety Test*, 2009[46]) should be completed.

Requirements for ASF Vaccines
2.3.2. Safety requirements

Global African Swine Fever Research Alliance

iii) Horizontal transmission

The test is conducted using no fewer than 12 healthy piglets, a minimum of 6-weeks old and not older than 10-weeks old and of the same origin, that do not have antibodies against ASFV, and blood samples are negative on real-time PCR. All piglets are housed together from day 0 and the number of vaccinated animals is the same as the number of naïve, contact animals. Co-mingle equal numbers of vaccinated and naïve, contact piglets in the same pen or room.

Use vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine. Administer by each recommended route of administration to no fewer than six piglets a quantity of the vaccine virus equivalent to not less than the maximum virus titre (maximum release dose) likely to be contained in 1 dose of the vaccine.

Requirements for ASF Vaccines
2.3.2. Safety requirements

Global African Swine Fever Research Alliance

The vaccine complies with the test if:

- No vaccinated or naïve contact piglet shows abnormal (local or systemic) reactions, reaches the predetermined humane endpoint defined in the clinical scoring system or dies from causes attributable to the vaccine;
- The average body temperature increase for all naïve, contact piglets (group mean) for the observation period does not exceed 1.5°C: above baseline; and no individual piglet shows a temperature rise above baseline greater than 2.5°C for a period exceeding 3 days;
- No naïve, contact piglet shows notable signs of disease by gross pathology and no virus is detected in their blood or tissue samples
- No naïve contact pigs test positive for antibodies to the vaccine virus.

Requirements for ASF Vaccines
2.3.2. Safety requirements

Global African Swine Fever Research Alliance

iv) Post-vaccination kinetics of viral replication (MLV blood and tissue dissemination) study

Prior to the reversion to virulence study (Section C2.3.2.v. below), a minimum of one study should be performed to determine the post-vaccination kinetics of virus replication in the blood (viremia), tissues and viral shedding.

The test consists of the administration of the vaccine virus from the master seed lot to no fewer than eight healthy piglets, and preferably ten healthy piglets, a minimum of 6-weeks old and not older than 10-weeks old and of the same origin, that do not have antibodies against ASFV, and blood samples are negative on real-time PCR.

Requirements for ASF Vaccines
2.3.2. Safety requirements

v) Reversion to virulence

The test should be carried out consistent with VICH GL41 (Examination of live veterinary vaccines in target animals for absence of reversion to virulence, 2008[47]).

The test for increase in virulence consists of the administration of the vaccine master seed virus to healthy piglets of an age (e.g. between 6-weeks and 10-weeks old) suitable for recovery of the strain and of the same origin, that do not have antibodies against ASFV, and blood samples that are negative on real-time PCR. This protocol is typically repeated five times.

Requirements for ASF Vaccines
2.3.2. Safety requirements

Global African Swine Fever Research Alliance

At a minimum, a safe MLV vaccine shall demonstrate ALL the following features (minimal standards):

- Absence of fever (defined as average body temperature increase for all vaccinated piglets (group mean) for the observation period does not exceed 1.5°C above baseline; and no individual piglet shows a temperature rise above baseline greater than 2.5°C for a period exceeding 3 days);
- Absence of chronic and acute clinical signs and gross pathology over the entire test period or minimal chronic clinical signs (defined as mild swollen joints with a low clinical score that resolve within 1 week).
- Minimal (defined as no naïve, contact piglet shows notable signs of disease by clinical signs and gross pathology and no or a low percentage of contact piglets test both real-time PCR positive and seropositive) or no vaccine virus transmission (defined as no naïve, contact piglet shows notable signs of disease by clinical signs and gross pathology and no contact piglets test both real-time PCR positive and seropositive) over the entire test period;
- Absence of an increase in virulence (genetic and phenotypic stability) (complies with the reversion to virulence test).

In addition, the vaccines in their commercial presentation before being authorised for general use should be tested for safety in the field (see chapter 1.1.8 Section 7.2.3). Additional field safety evaluation studies may include but are not limited to: environmental persistence (e.g. determination of virus recovery from bedding or other surfaces), assessment of immunosuppression, and negative impacts on performance.

Requirements for ASF Vaccines
2.3.3. Efficacy requirements

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i) Protective dose

Vaccine efficacy is estimated in immunised animals directly, by evaluating their resistance to live virus challenge. The test consists of a vaccination/challenge trial in piglets a minimum of 6-weeks old and not more than 10-weeks old, free of antibodies to ASFV, and negative blood samples by real-time PCR. The test is conducted using no fewer than 15 and preferably no fewer than 24 vaccinated pigs, and no fewer than five non-vaccinated control piglets.

The test is conducted to determine the minimal immunising dose (MID) (also referred to as the minimal protective dose [MPD] or protective fraction); using at least three groups of no fewer than five and preferably not fewer than eight vaccinated piglets per group, and one additional group of no fewer than five non-vaccinated piglets of the same age and origin as controls. Use vaccine containing virus at the highest passage level that will be present in a batch of vaccine.

Requirements for ASF Vaccines
2.3.3. Efficacy requirements

Global African Swine Fever Research Alliance

ii) Assessment for horizontal transmission (challenge virus shed and spread study)

The ASF basic reproduction number, R0, can be defined as the average number of secondary ASF disease cases caused by a single ASFV infectious pig during its entire infectious period in a fully susceptible population (Hayes *et al.*, 2021). In general, if the ASFV effective reproduction number Re=R0 × (S/N) (S= susceptible pigs; N= total number of pigs in a given population) is greater than 1.0, disease is predicted to spread. Ideally, ASF vaccination should reduce Re to less than 1.0 by reducing the number of susceptible, naïve, contact pigs exposed to vaccinated, infected pigs.

To evaluate ASF vaccine impact on ASF disease transmission, the test consists of a vaccination/challenge trial in piglets a minimum of 6-weeks old and not older than 10-weeks old, free of antibodies to ASFV, and negative blood samples by real-time PCR.

The test is conducted using no fewer than 15 healthy piglets at a ratio comprising twice the number of vaccinated piglets to naïve piglets (e.g. ten vaccinated and five naïve). Use vaccine containing virus at the highest passage level that will be present in a batch of the vaccine.

ASF Vaccine Master Seed Purity: Absence of Parental ASFV

Global African Swine Fever Research Alliance

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Check for updates

Confirming the absence of parental African swine fever virus as a potential contaminant of recombinant live attenuated ASF vaccines

Lauro Velazquez-Salinas [a,b,*], Elizabeth Ramirez-Medina [a], Ayushi Rai [a,c], Sarah Pruitt [a], Elizabeth A. Vuono [a,d], Nallely Espinoza [a], Cyril G. Gay [e], Steve Witte [f], Douglas P. Gladue [a,**], Manuel V. Borca [a,***]

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[f] Biologics Development Module, National Bio and Agrodefense Facility, Agricultural Research Service, USDA, Manhattan, KS, USA

Peer-reviewed Publications
ASF Reversion to virulence Studies

Global African Swine Fever Research Alliance

viruses

MDPI

Article

Evaluation of the Safety Profile of the ASFV Vaccine Candidate ASFV-G-ΔI177L

Xuan Hanh Tran [1,*], Le Thi Thu Phuong [1], Nguyen Quang Huy [1], Do Thanh Thuy [1], Nguyen Van Dung [1], Pham Hào Quang [1], Quách Võ Ngôn [1], Ayushi Rai [2], Cyril G. Gay [3], Douglas Paul Gladue [2,*] and Manuel Victor Borca [2,*]

npj | vaccines

www.nature.com/npjvaccines

ARTICLE OPEN

Check for updates

Assessment of African swine fever vaccine candidate ASFV-G-ΔMGF in a reversion to virulence study

Paul Deutschmann[1], Jan-Hendrik Forth[1], Julia Sehl-Ewert[1], Tessa Carrau[1], Elisenda Viaplana[2], Jose Carlos Mancera[3], Alicia Urniza[2], Martin Beer[1] and Sandra Blome[1]✉

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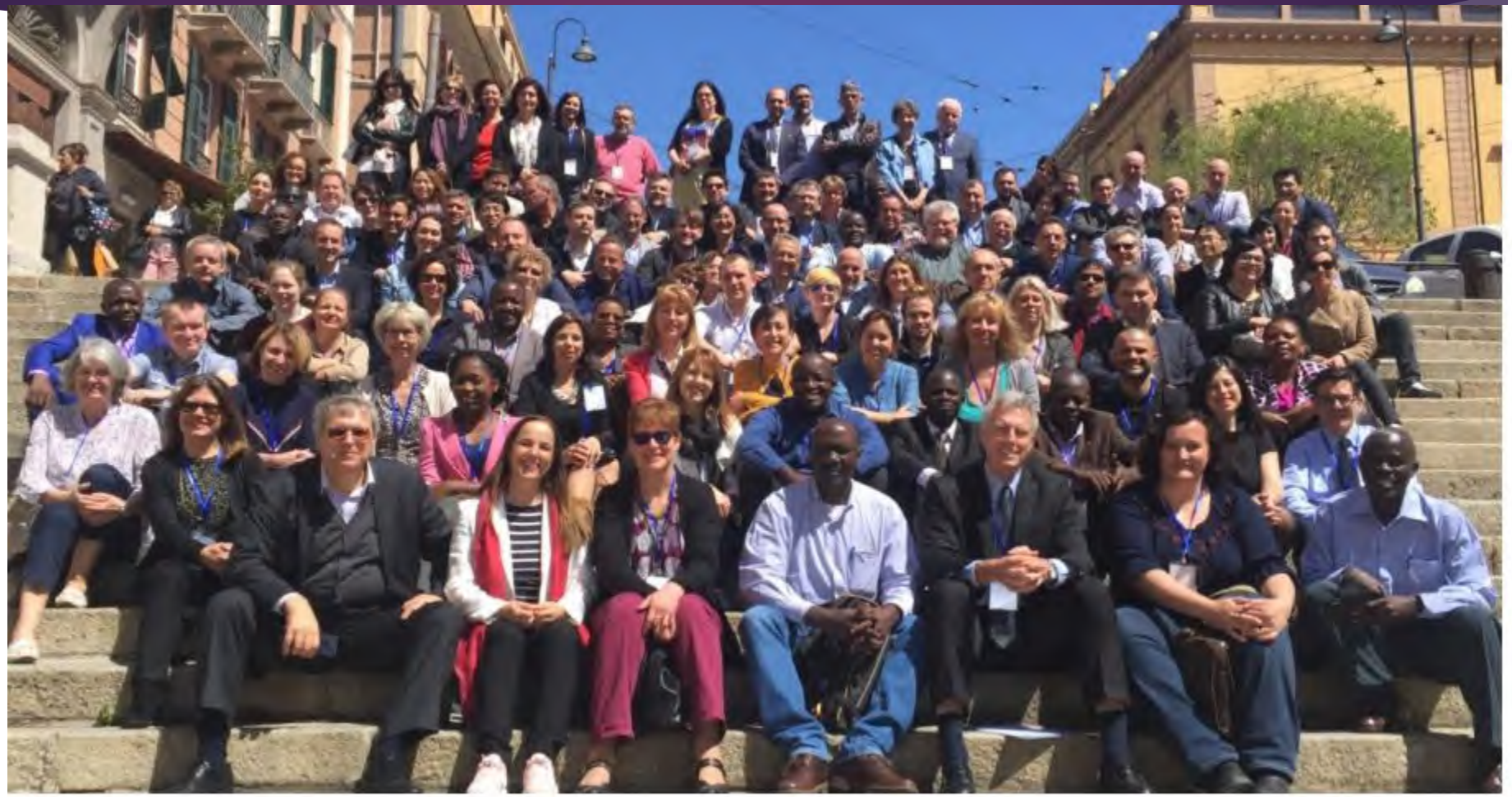
- GARA ASF Vaccine Researchers

Thank you!



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Research Alliance