Vaccine development for the control of African swine fever

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Founder: Genomics and Vaccines, LLC
Director: Global Alliance for African swine fever Genomics Center

Disclaimer: None of the Vaccine opinions are from my previous employer at the USDA or my current employer Seek Labs, Inc. The opinions are solely my own based on published and collaborative studies.

USDA –NAVETCO Partnership 2020

ORIGINAL ARTICLE

African swine fever virus vaccine candidate ASFV-G-ΔI177L efficiently protects European and native pig breeds against circulating Vietnamese field strain

Manuel V. Borca and Douglas P. Gladue contributed equally to this study.

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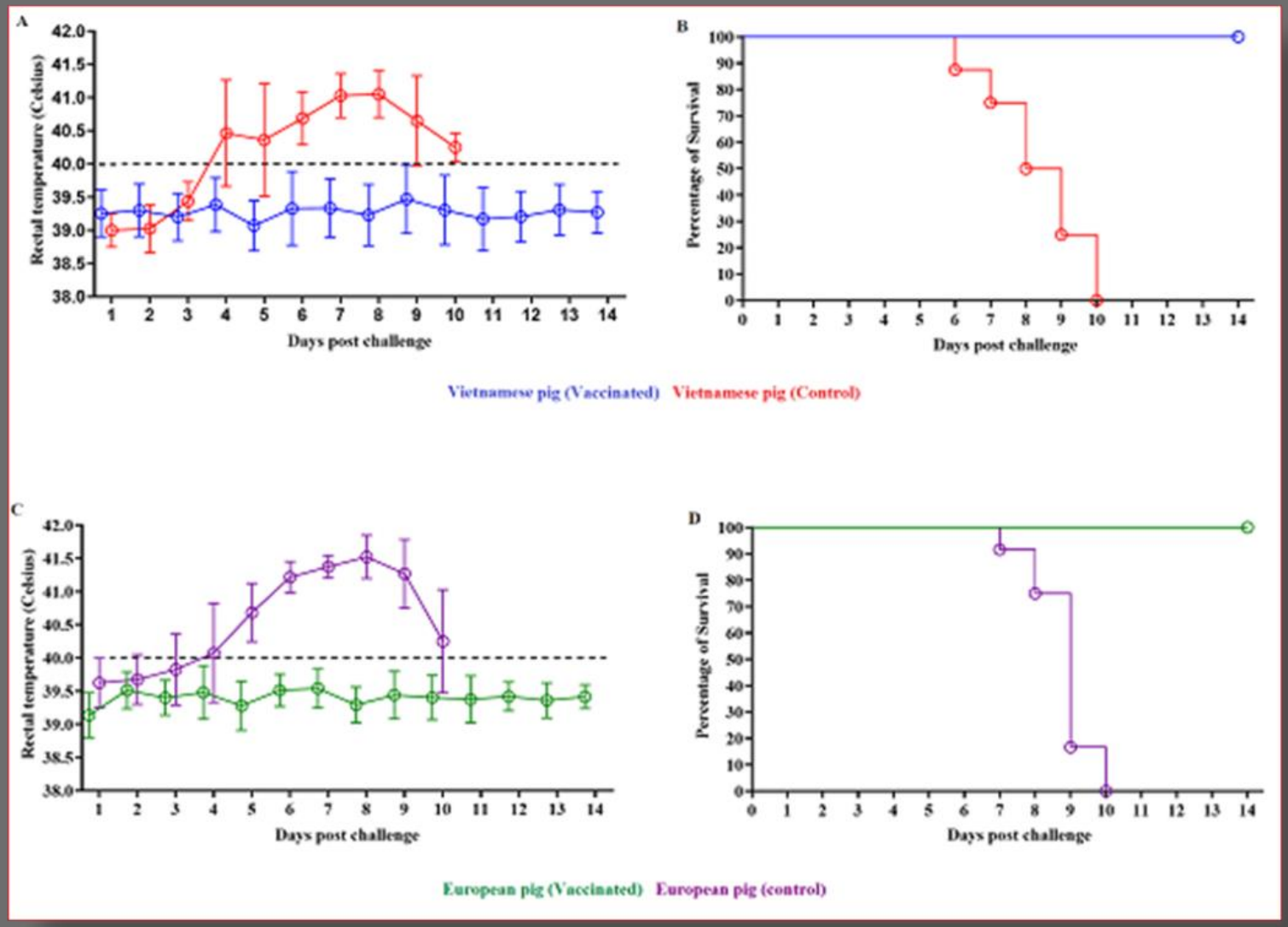
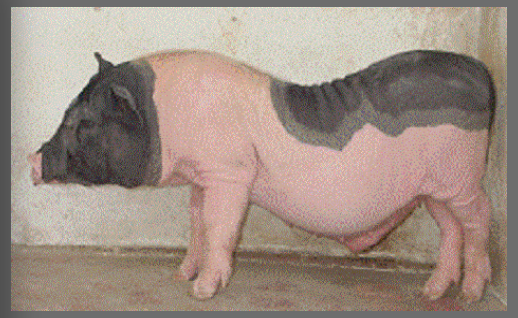
Transboundary and Emerging Diseases

WILEY





ASFV-G-ΔI177L is an effective vaccine in both Local breeds and European pigs

USDA, DAH, NCVI, NCVD- Additional I177L studies

No transmission in experimental conditions
Limited transmission in field conditions (R0<1)

Overdose (10x dose) does not induce local or systemic problems

Field trials in North and South Vietnam confirm experimental safety data and 100% efficacy in representative group of animals challenged under experimental conditions

Reversion to virulence studies successfully completed: vaccine is phenotypically and genetically stable

Long term study (6 months) showed absence of residual virulence

ASFV Vaccine Development Status

Discovery Projects	Licensing Partnerships	Phase I Vaccine Safety and efficacy	Phase II Expanded trials	Phase III Field Trials	Registration/ Regulatory Approval	Launch

ARS patent → CRADA.MTRA → Involvement of PIADC scientists

1. Vaccine for global market
2. Vaccine for US-stockpile

ASFV-G-ΔI177L – Navetco →

Licensing

ASFV-G-Δ9GLΔUK (Undisclosed Partner) →

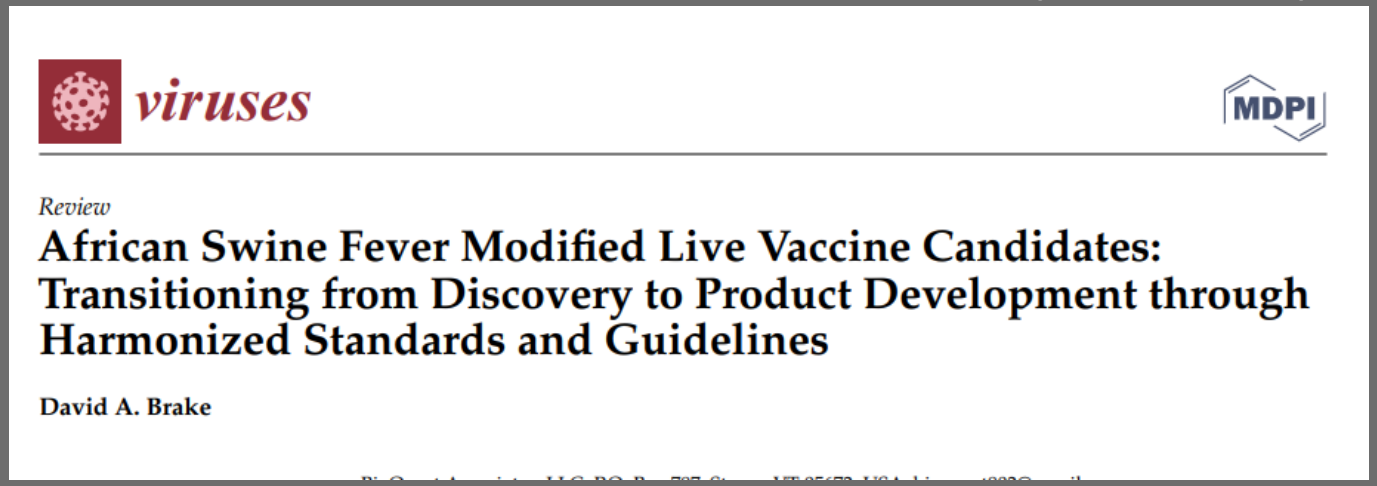
ASFV-G-ΔMGF Aptimmune/AVAC →

ASFV-G-ΔI177L ΔLVR – Komipharma →

ASFV-G- ΔI177L ΔLVR-Dabaco →

LAV ASFV Vaccines for widespread use

- Both Vaccines have been publicly under development for years
- LAV ASFV vaccines were first reported in 1996
- WOAH guidelines for safety studies for LAV vaccines are Still not available
  - The primary cause of delayed deployment of current vaccines
  - The cause for Random Reversion to Virulence studies with often misleading information, some pharmaceutical companies report on only on candidates they are not pursuing – destroy competition
  - David Brake as hired to interview ASFV experts and publish findings.



viruses

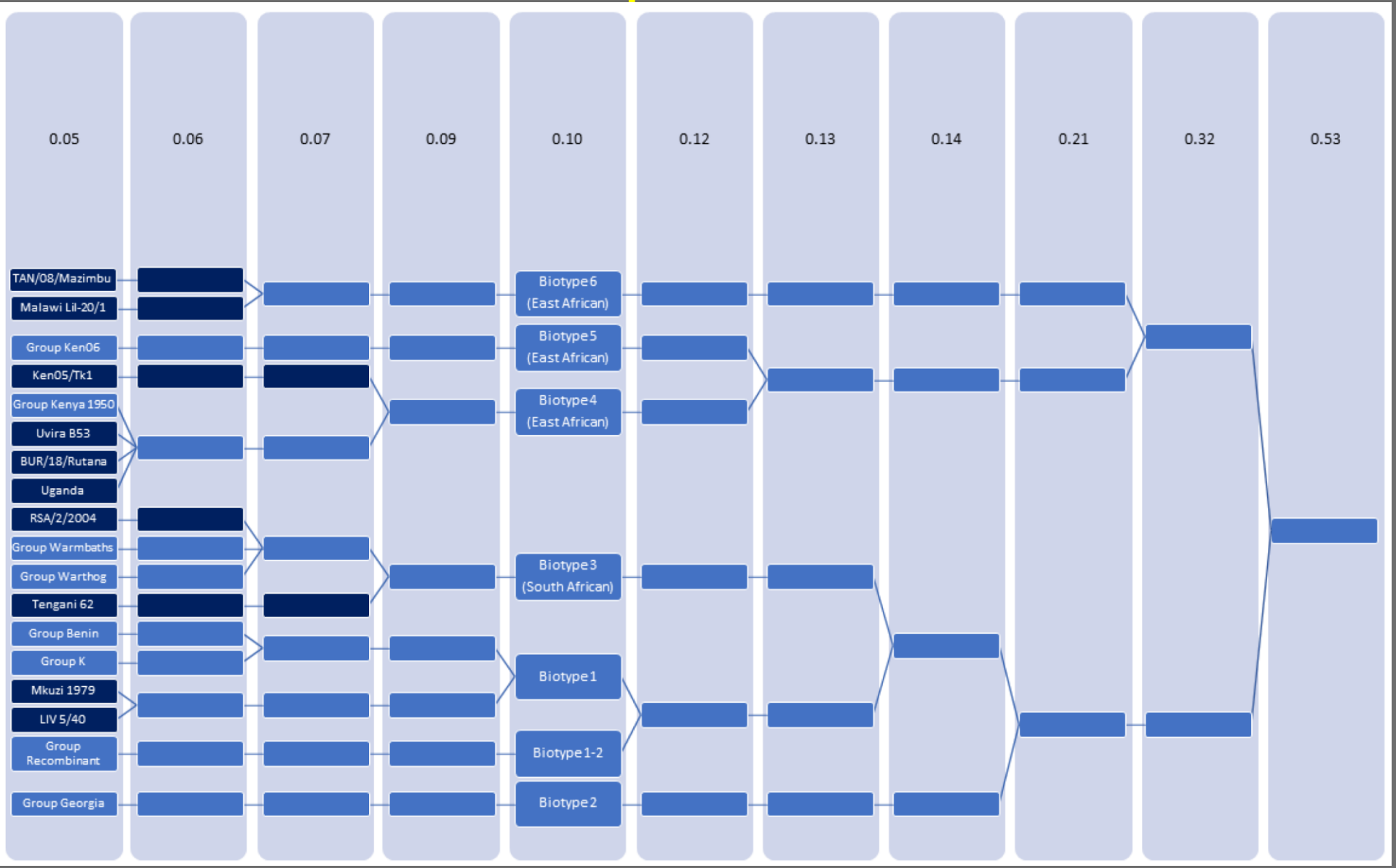
MDPI

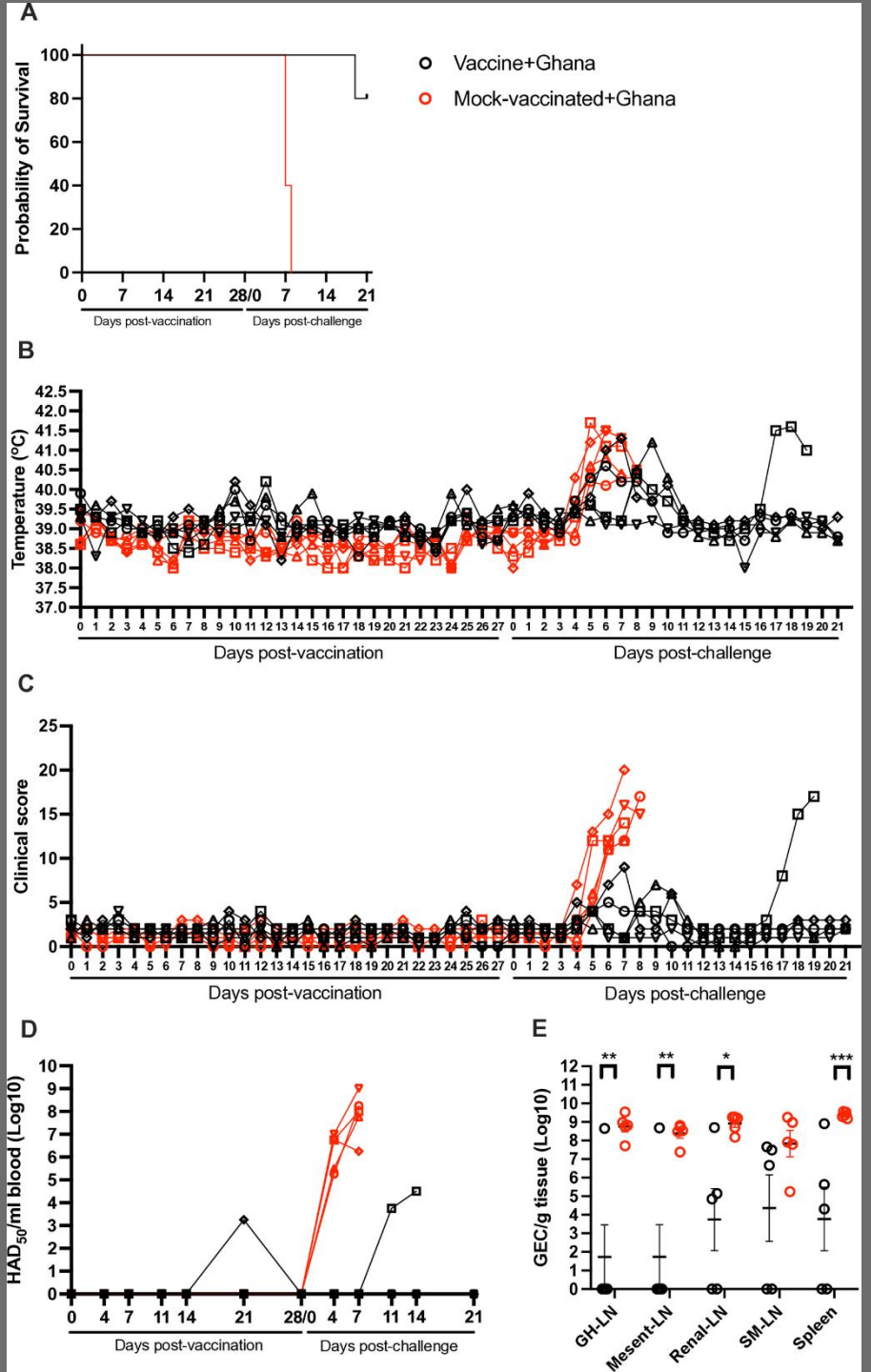
Review

African Swine Fever Modified Live Vaccine Candidates: Transitioning from Discovery to Product Development through Harmonized Standards and Guidelines

David A. Brake

Cross-Protection of NAVETCO I177L using biotype as a predictive model

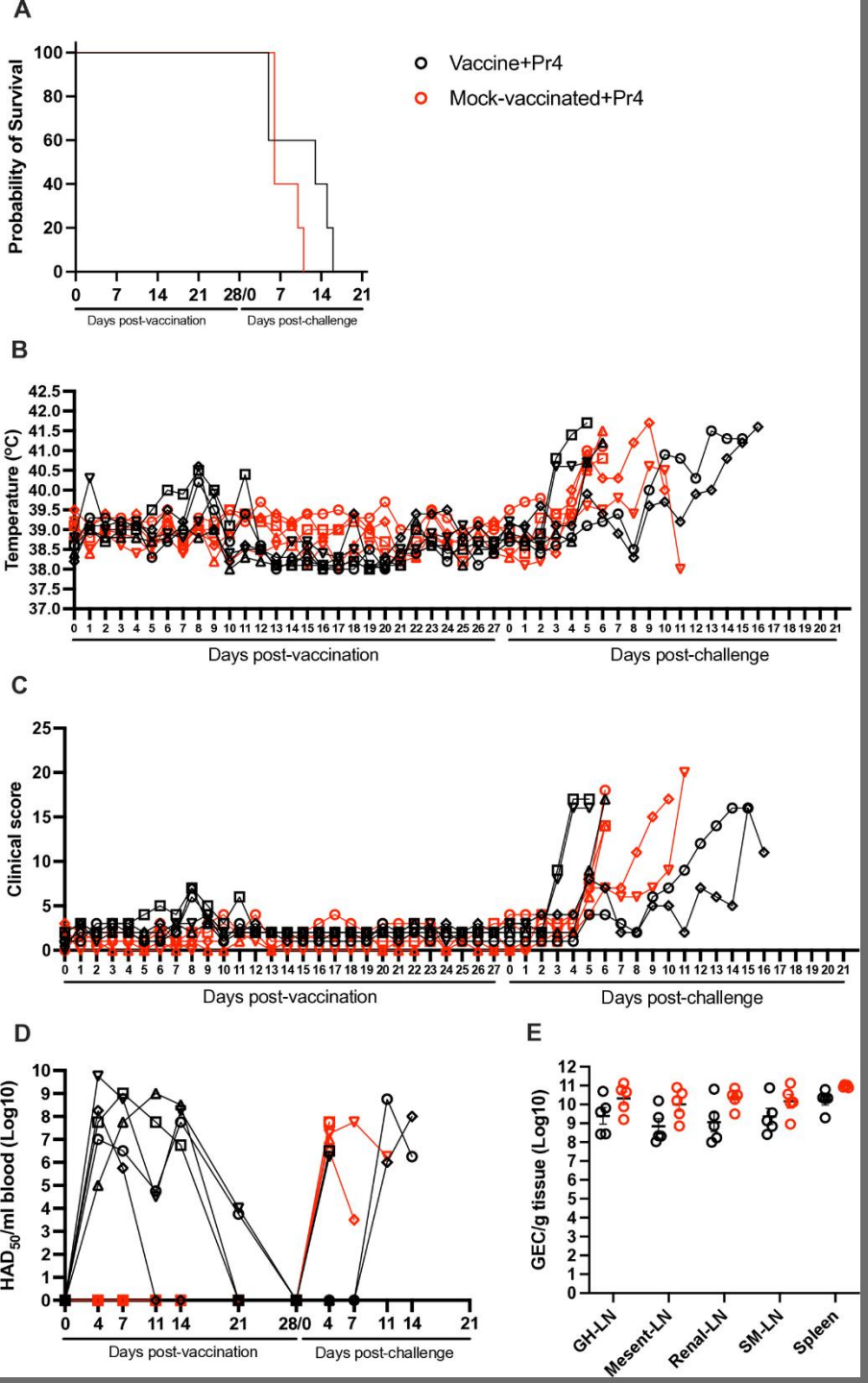




ASFV-G-ΔI177L Vaccinated

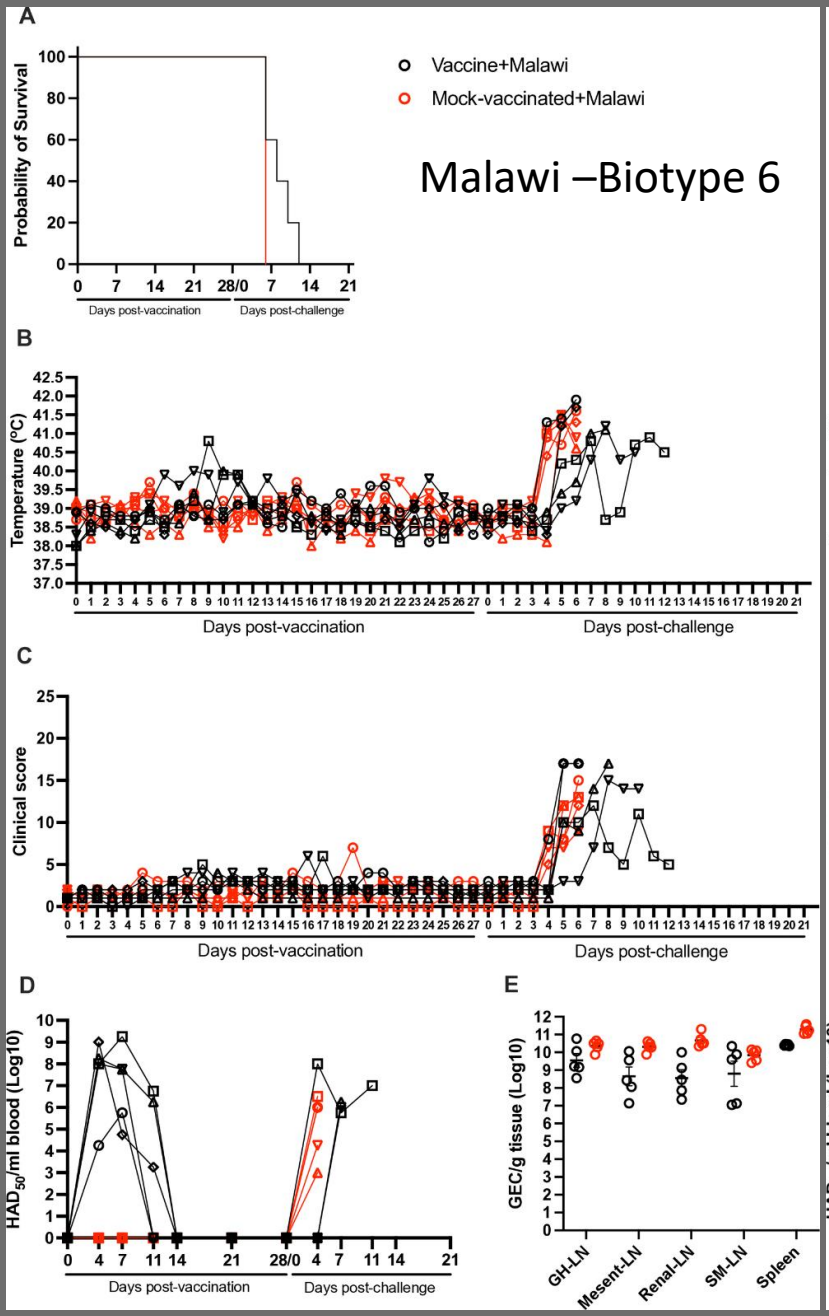
Ghana2021- Biotype I

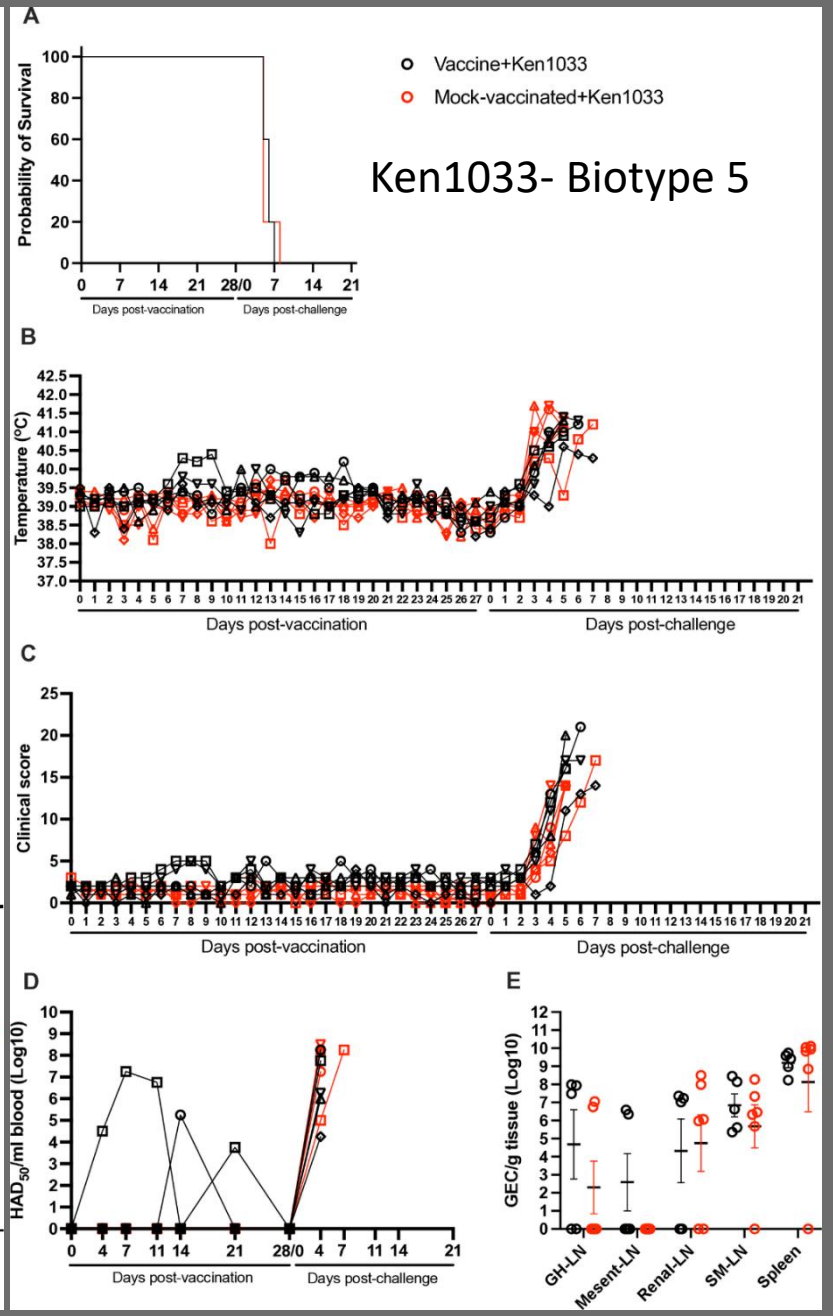
80% survival

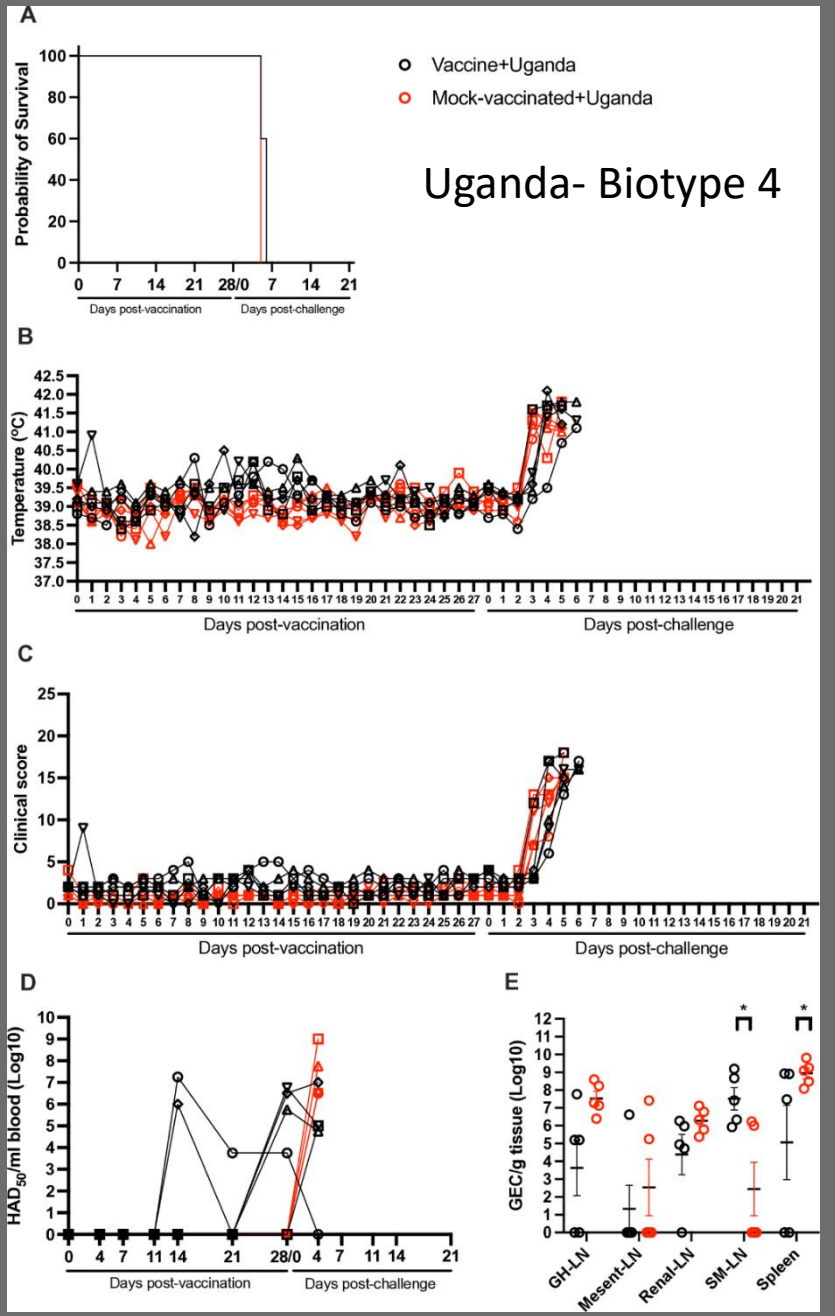


Pretoria Biotype 3
Identical P72 to ASFV-Georgia

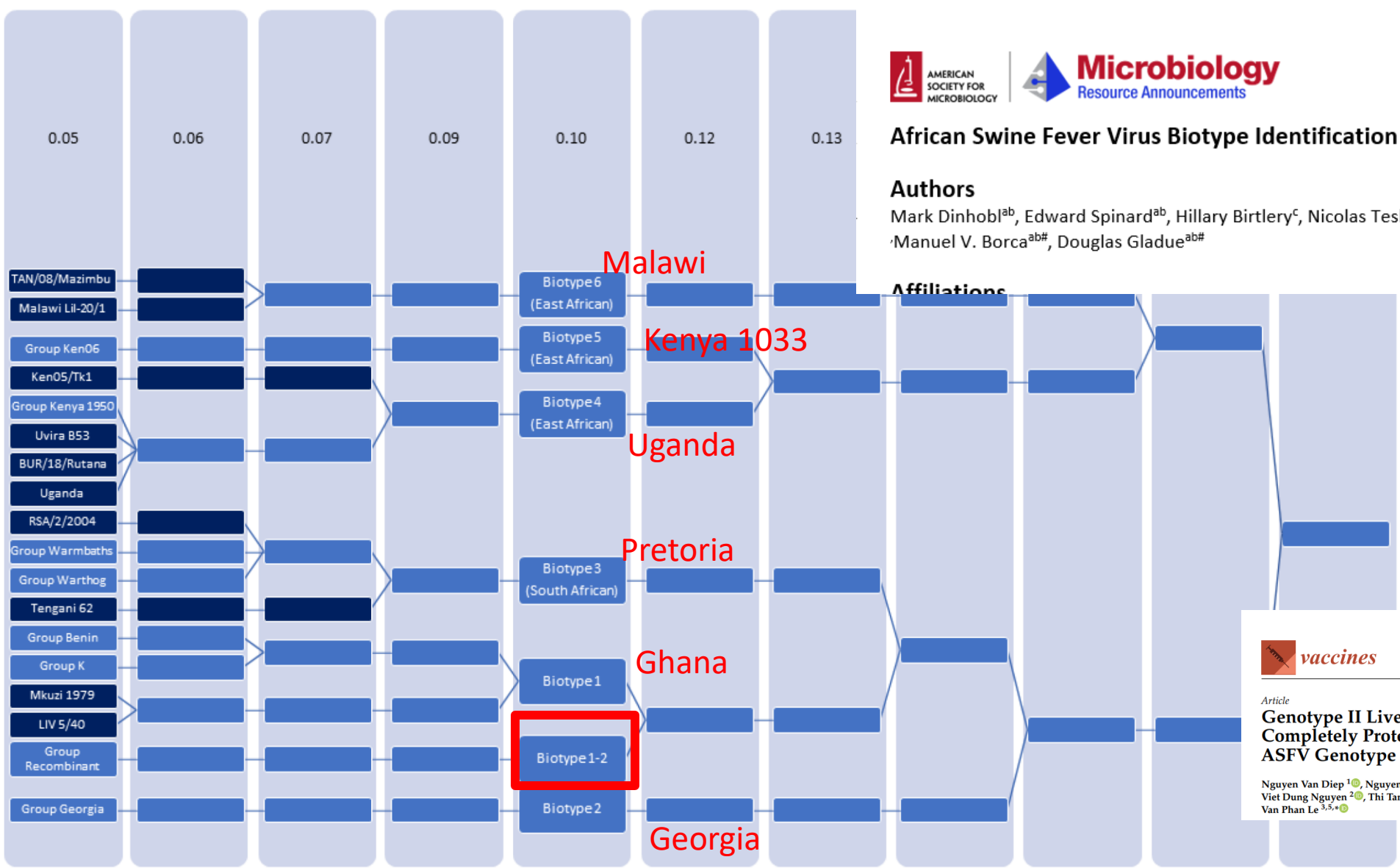
Some extension of survival


A
Probability of Survival
Vaccine+Malawi
Mock-vaccinated+Malawi
Malawi –Biotype 6
Days post-vaccination
Days post-challenge
B
Temperature (ºC)
C
Clinical score
D
HAD50/ml blood (Log10)
E
GEC/g tissue (Log10)
GH-LN
Mesent-LN
Renal-LN
SM-LN
Spleen


A
Probability of Survival
Vaccine+Ken1033
Mock-vaccinated+Ken1033
Ken1033- Biotype 5
Days post-vaccination
Days post-challenge
B
Temperature (ºC)
C
Clinical score
D
HAD50/ml blood (Log10)
E
GEC/g tissue (Log10)
GH-LN
Mesent-LN
Renal-LN
SM-LN
Spleen


A
Probability of Survival
Vaccine+Uganda
Mock-vaccinated+Uganda
Uganda- Biotype 4
Days post-vaccination
Days post-challenge
B
Temperature (ºC)
C
Clinical score
D
HAD50/ml blood (Log10)
E
GEC/g tissue (Log10)
GH-LN
Mesent-LN
Renal-LN
SM-LN
Spleen

Cross-Protection of NAVETCO I177L using biotype as a predictive model



AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

African Swine Fever Virus Biotype Identification Tool

Authors

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Affiliations

vaccines MDPI

Article

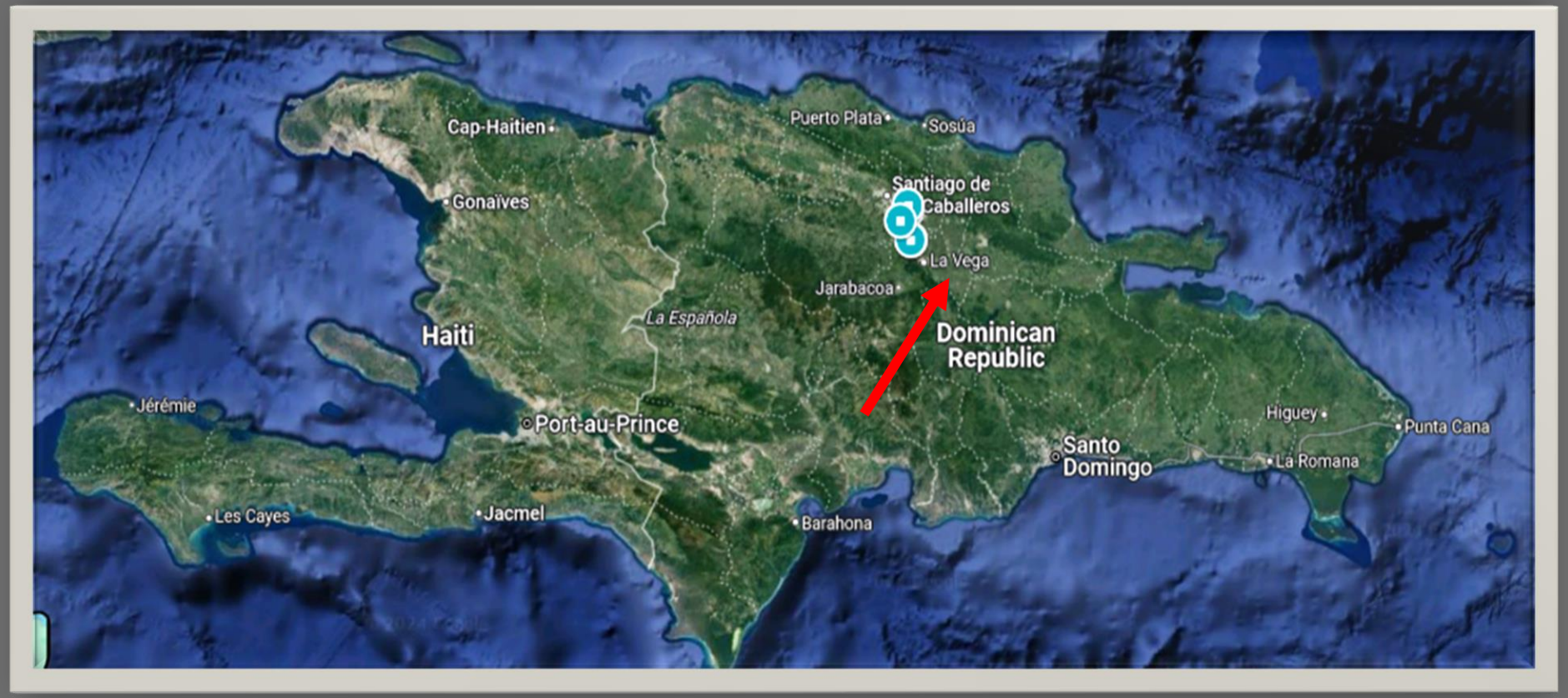
Genotype II Live-Attenuated ASFV Vaccine Strains Unable to Completely Protect Pigs against the Emerging Recombinant ASFV Genotype I/II Strain in Vietnam

Nguyen Van Diep [1], Nguyen Van Duc [1], Nguyen Thi Ngoc [1], Vu Xuan Dang [1], Tran Ngoc Tiep [1], Viet Dung Nguyen [2], Thi Tam Than [3], Dustin Maydaniuk [4], Kalhari Goonewardene [4], Aruna Ambagala [4,*] and Van Phan Le [3,5,*]

Genetic Differences to Predict Cross Protection?

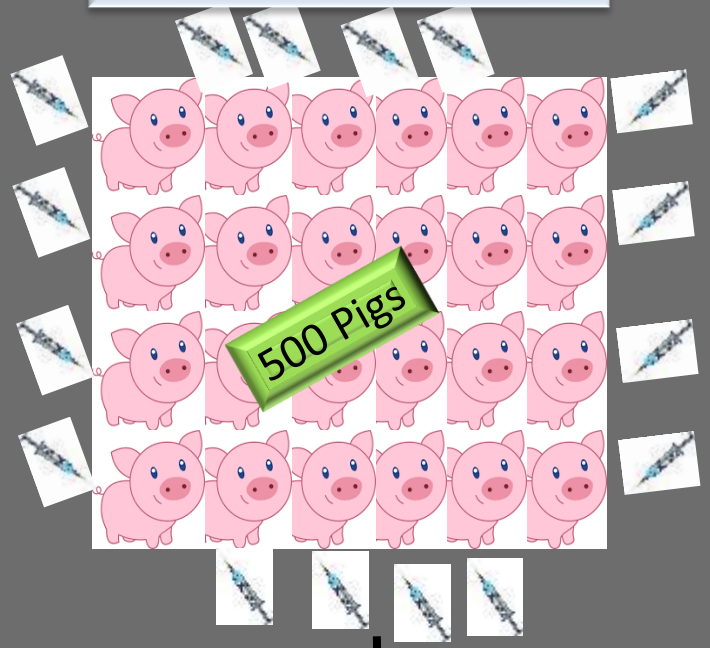
- Biotypes are diverse too many changes to accurately predict which proteins could be involved for cross protection or lack of cross protection by Biotype Alone
- Why was there cross protection between Biotype II vaccines and Biotype I but not with Biotype II vaccines and Biotype I/II
- There were 74 proteins with genetic changes between Biotype I/II and Biotype II vaccine. 73 of these changes were also in Biotype I. Interestingly, the changes in Biotype I were additive to I/II.

Study Site: La Vega Province, DR



Prospective Cohort Study (Study Design)

Cohort 1
Definition = Vaccinated

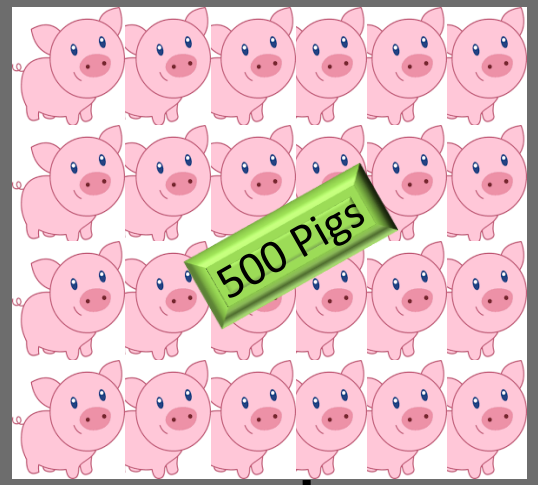


Monitoring

Time = 30d

- Live pigs
- Dead pigs
- Mild reactions
- Weight gain
- Diag (Sero + PCR)

Cohort 2
Definition = Not Vaccinated



Monitoring

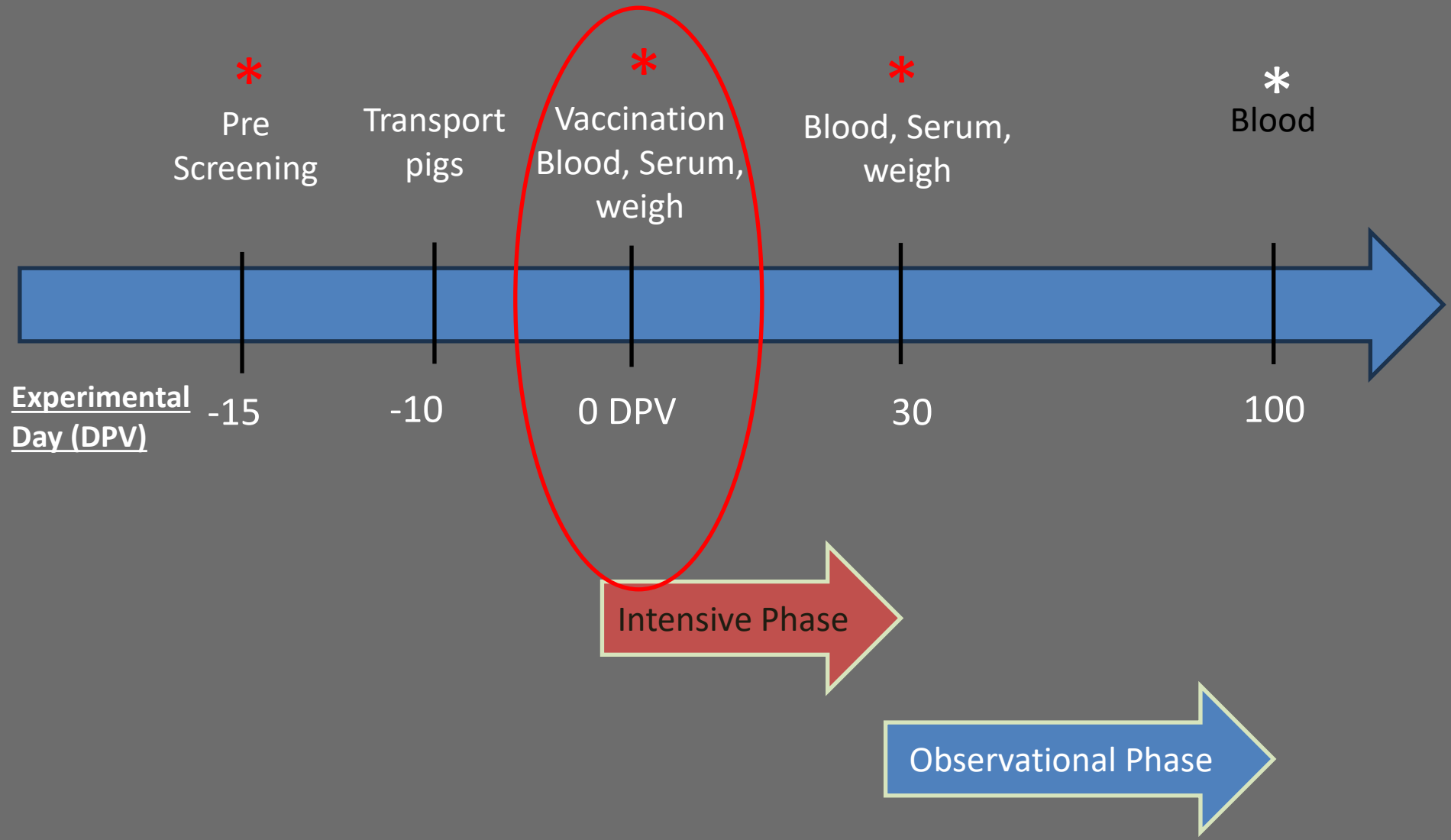
Time = 30d

OBSERVATIONS

- Live pigs
- Dead pigs
- Mild reactions
- Weight gain
- Diag (Sero + PCR)

Vaccinated Cohort
Control Cohort

Simplified study timeline (4 sampling points (*)



Results 3: Antemortem Diagnostics 0-30-90d

Purpose of samples

- Serum for anti-ASFV Ab (hint of efficacy)
- Blood for ASFV vacc DNA

Table 3. Serology for ASFV antibodies and detection of vaccine DNA in whole blood

	Day 0	
	ASFV DNA	ASF sero-positive
Vaccinated	0.0	0.0
Control	0.0	0.0

*At day 100 blood was sampled for ASFV (vaccine) DNA, but not serology

Important points:

- Good seroconversion
- No transmission of vaccine
- Low prevalence and quantity of DNA detection in vaccinates

Results 4: fatalities

	Total Fatality per Week (Vacc)	Total Fatality per Week (Control)	Total Fatality per Week
Week Number			
Pre-Vacc	6	8	14
1	3	3	6
2	5	5	10
3	4	4	8
4	4	0	4
5-8	11	7	18
9-12	30	25	55
13-16	15	12	27
Total Fatalities	78	64	142
Total Since Vaccination	72	56	128
	14.4%	**11.2%**	

Results 4b: Fatalities

Cause of Death/Euthanasia in Vaccinated and Control Pigs

	Traumatic Lameness		Hemorrhage (Mycotoxin) Syndrome		Neurologic Syndrome		Other Cause or Undetermined	
	Vacc	Control	Vacc	Control	Vacc	Control	Vacc	Control
Week Number								
(-1) Pre-Vacc	2	2	0	0	1	4	3	2
1	2	0	1	3	0	0	0	0
2	4	0	1	2	0	0	0	3
3	4	2	0	1	0	0	0	1
4	2	0	0	0	1	0	1	0
(5-8) Post-Experimental Observation Period		1	7	2	0	1	4	3
(9-12) Post-Experimental Observation Period	0	0	26	15	0	0	4	10
(13-16) Post-Experimental Observation Period	0	0	13	5	0	0	2	7
Total Since Vaccination	12	3	48	28	1	1	11	24

Shaded cell represents period in which category "Other" included cases consistent with PRRS

Conclusions (within this study):

- NAVET-ASFVAC did not cause differences in:
  - Temperatures of pigs
  - Weight gain
  - Fatalities

- Induced high prevalence of anti-ASFV antibodies
- Low prevalence and quantity of vaccine DNA at 90 DPV
- Vaccine was not transmitted between sheds when adequate biosecurity was implemented

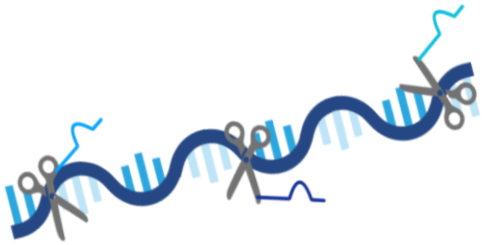
What is the solution to ensure food safety with ASF spreading to secure the food supply?

- Subunit or Vectored vaccines? Likely not in the near future, likely require multiple antigens and are likely not cost effective.
- Live attenuated vaccines Safe when matched to outbreaks, and with biosecurity / government oversight
- Can we all agree that ASFV should be controlled?
- If no vaccines to control ASFV a universal therapeutic is urgently needed for food security.
    - Small molecules likely to produce disease resistant strains (as seen in HPAI, etc) when used widely on farms

A solution exists for ASFV and future emerging viruses without the need for RtV studies

Programmable Target Ablation Platform

Seek Labs is transforming how diseases are treated by engineering CRISPR-based therapeutics using only sequencing information for rapid discovery, development, and deployment of precision therapeutics.



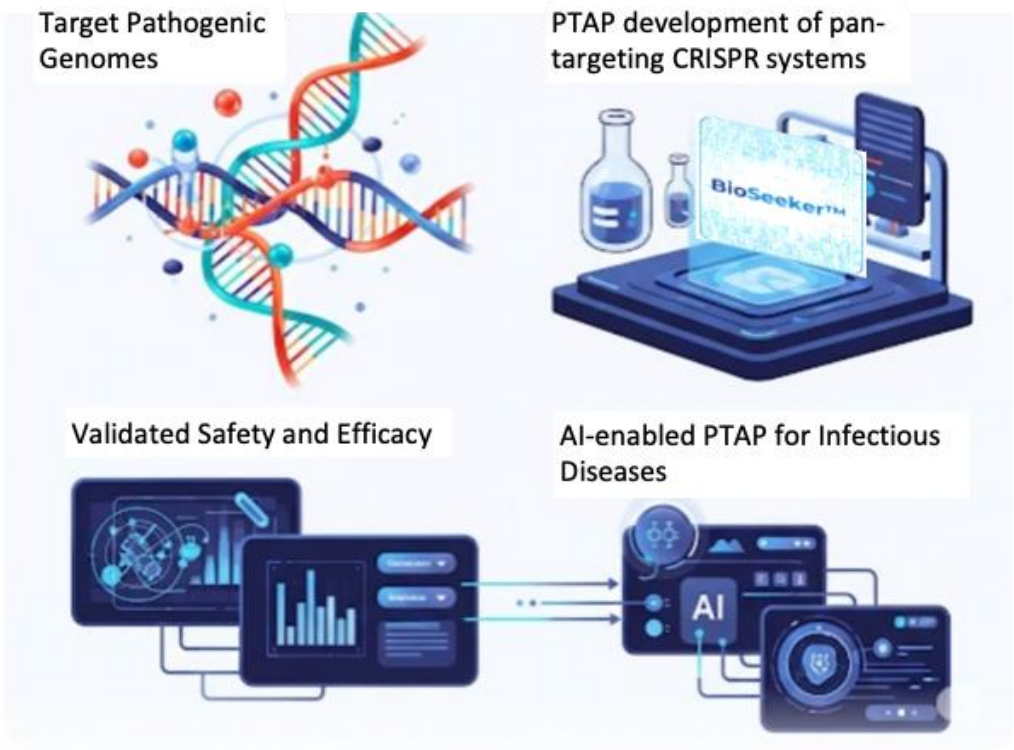
A CRISPR guide molecule is programmed to precisely target ands cleave pathogen's genome.

The invading pathogen is no longer able to replicate, limiting disease burden and spread to facilitate recovery.

Feature	RRET	Monoclonal Antibodies	Vaccines	Small Molecule Antivirals
Adaptability to Unknowns	Programmable per sample	Predefined antigens	Predefined viral genomes	Target-based discovery
Sample-to-Formulation	≤12 hours	3–6 months	6–18 months	Months to years
Per-Dose Latency	On-demand, ≤12 hrs	Batch-scale, weeks	Mass-manufactured	Dependent on production cycle
Manufacturing Requirement	In-system synthesis	Centralized, scaled	Biologic scale-up	Centralized synthesis
Delivery Infrastructure Required	Modular, point-of-care ready	Cold chain + infusion	Cold chain + global rollout	Oral or IV (centralized)

BioSeeker: AI-programmable Antiviral TX

BioSeeker automates and accelerates discovery and design of new therapeutics for Infectious Diseases

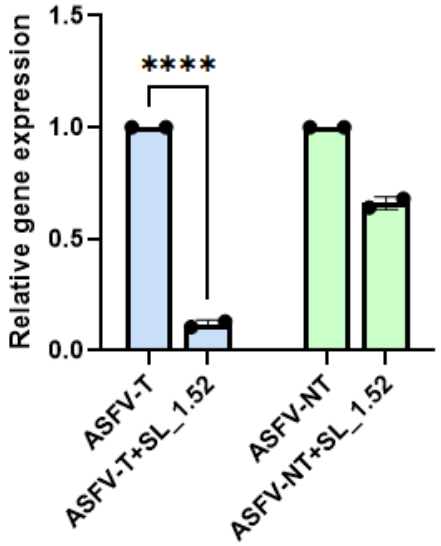


- Computational approach for Pan-targeting of Viral Diseases
- Disease dependent cleavage across all genomes
- Minimal number of gRNAs for efficient viral ablation
- gRNA design accounting for tolerated mismatches
- Structural tolerance of gRNAs for efficient Cas binding
- Processing features based on disease dependent positioning of multiplexed gRNAs
- Driven by constant updates on sequence and experimental data
- Proprietary machine learning algorithms for AI-enabled design
- Delivery systems dependent on site or viral target
- Pre-screens gRNAs to ensure no off-targeting in host species

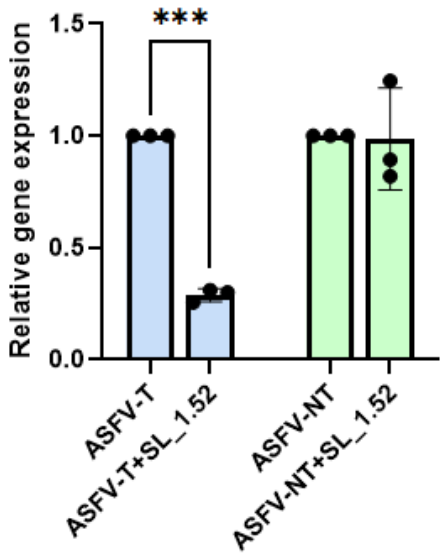
In Vitro Validation of ASFV Gene Knockdown

SL_1.52 Programmed CRISPR construct targets and cleaves a targeted ASFV gene (ASFV-T) in mammalian cells

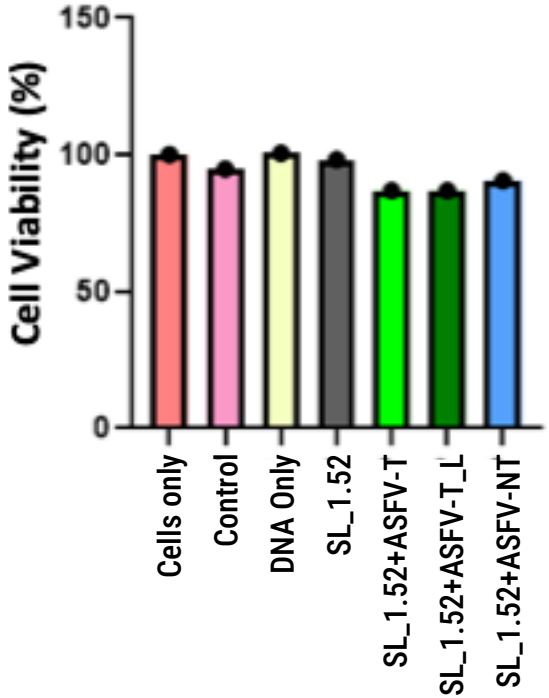
ASFV-T gene DNA reduced by 85%



Linked RFP reporter reduced by 75%



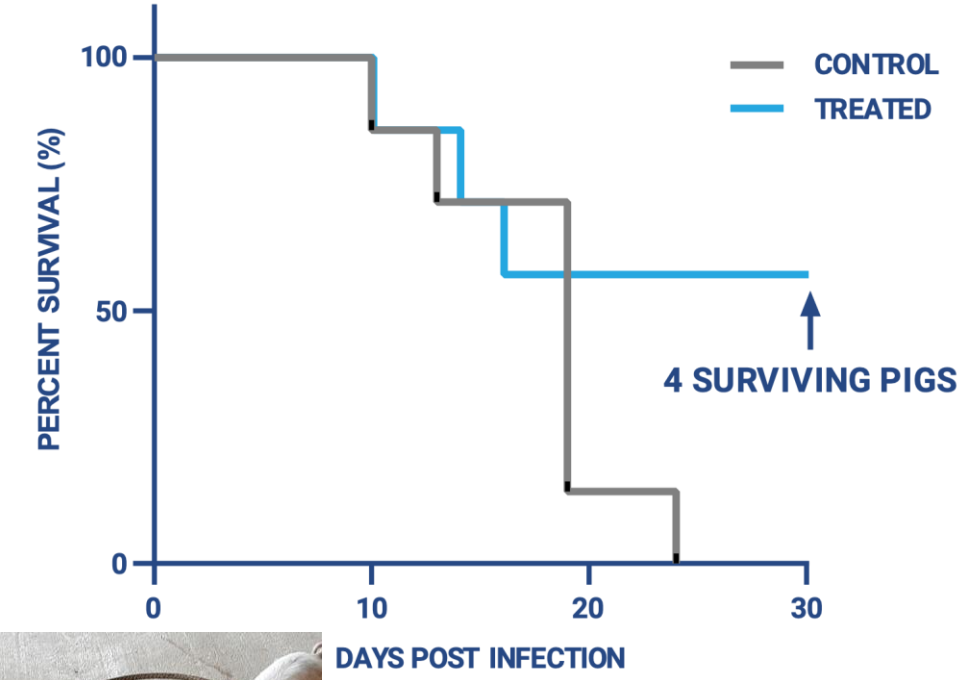
Minimal impact on cell viability



- SL_1.52 robustly and reproducibly knocks-down a targeted ASFV gene and
- SL_1.52 also decreased levels of a linked RFP-reporter gene in HEK293T
- Cell viability was not impacted by CRISPR activity

SL_1.52 In Vivo Efficacy: 4/7 pigs recovered from ASF

In one treatment cohort, 4 of 7 SL_1.52 treated ASFV-Infected pigs survived the trial (57% pigs recovered)

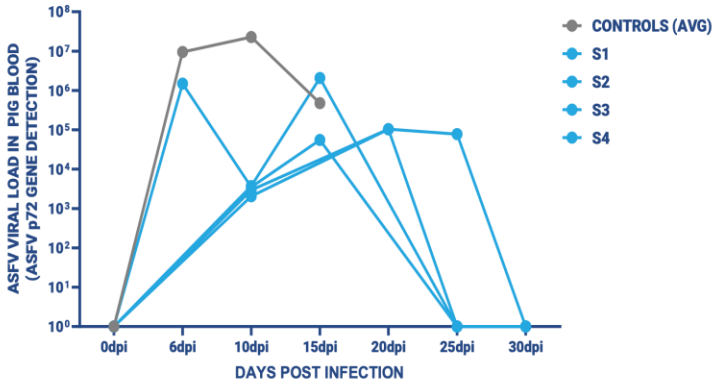


- Intramuscular lethal infection
- Treated on predicted days of clinical symptoms
- In ASFV-infected pigs, CRISPR-treatment enabled 4 of 7 pigs to fully recover from infection while all untreated controls succumbed to infection.
- Surviving pigs quickly recovered from initial clinical symptoms
- Natural challenge infection would be seen on infected farms, a less robust challenge test

SL_1.52 Reduced Viremia and Increased Antibodies

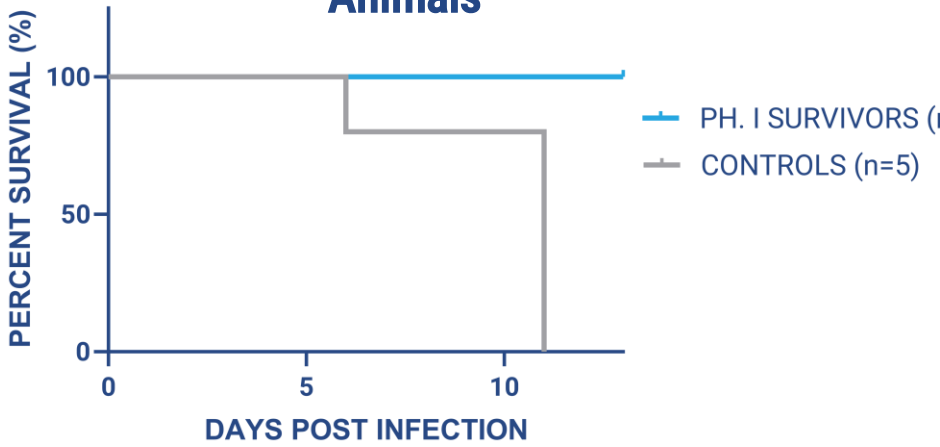
Surviving pigs had lower ASFV viral load, robust antibodies and demonstrated clearance of virus in blood

Treatment Muted Viral Replication in Survivors



Increased Pathogen-Specific Antibody Response in Survivors

Outcomes of Re-challenged Phase 1 Surviving Animals vs. Naive Control Animals



- **ASFV-CRISPR is an effective strategy to control the spread of ASFV**
  - *Has the potential for a universal treatment of ASFV*
- **First control approach that does not require strain-specific matching**
  - *Our CRISPR system targets conserved regions across multiple strains*
- **Our ASFV-CRISPR systems provide an inexpensive and accessible alternative to culling infected animals in outbreak or endemic areas.**
  - *In many endemic areas, governments do not have sufficient funds to reimburse farmers for loss resulting in quick sales of infected/exposed animals not showing clinical symptoms leading to further spreading of outbreaks to other farmers*

Cross protection studies USDA-ILRI collaboration

Plum Island/NBAF
Elizabeth Ramirez
Alyssa Valladares
Leanna Burton
Ediane Silva

Previous PIADC Involved in Presented vaccine work:

Douglas Gladue
Vivian O'Donnell
Manuel Borca
Devendra Rai
Alexia Alfonso
Michael Singer

Edward Spinard
Keith Berggren
Jolene Carlson
Paul Azzinaro
Dr. Lu
Lauro Velazquez-Salinas

Amanda Meyers
Ryan Baker-Branstetter
Ignacio Fernandez-Sainz
Lauren Holinka-Patterson
Betty Bishop
Hillary Birtley

Mark Dihbol
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Navetco, Vietnam 2023

Thank you!

Questions?

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