New developments in vaccines against African swine fever

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International Veterinary Vaccinology Network Webinar
30 November 2021

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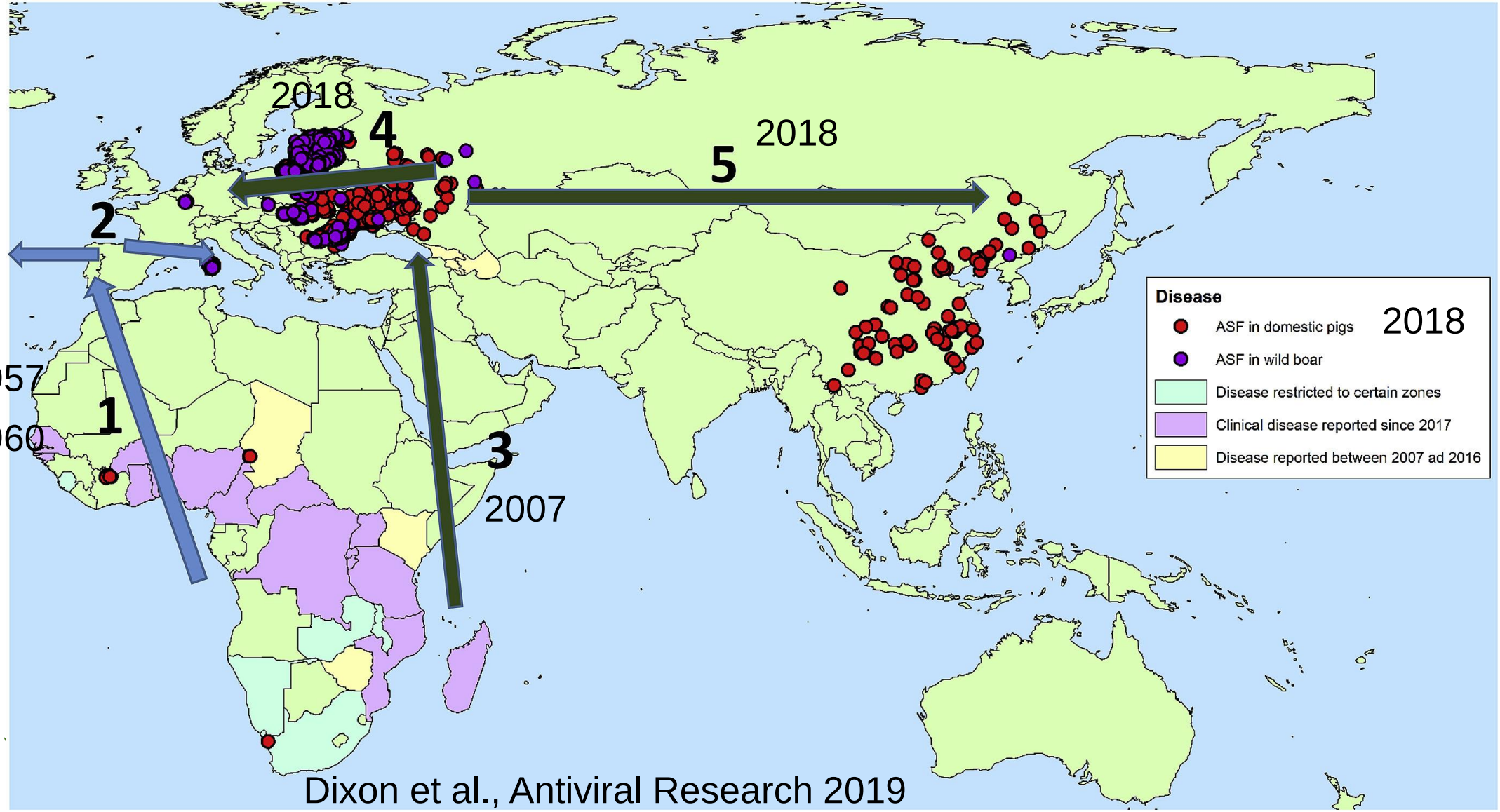
Spread of African Swine Fever Virus



Dixon et al., Antiviral Research 2019

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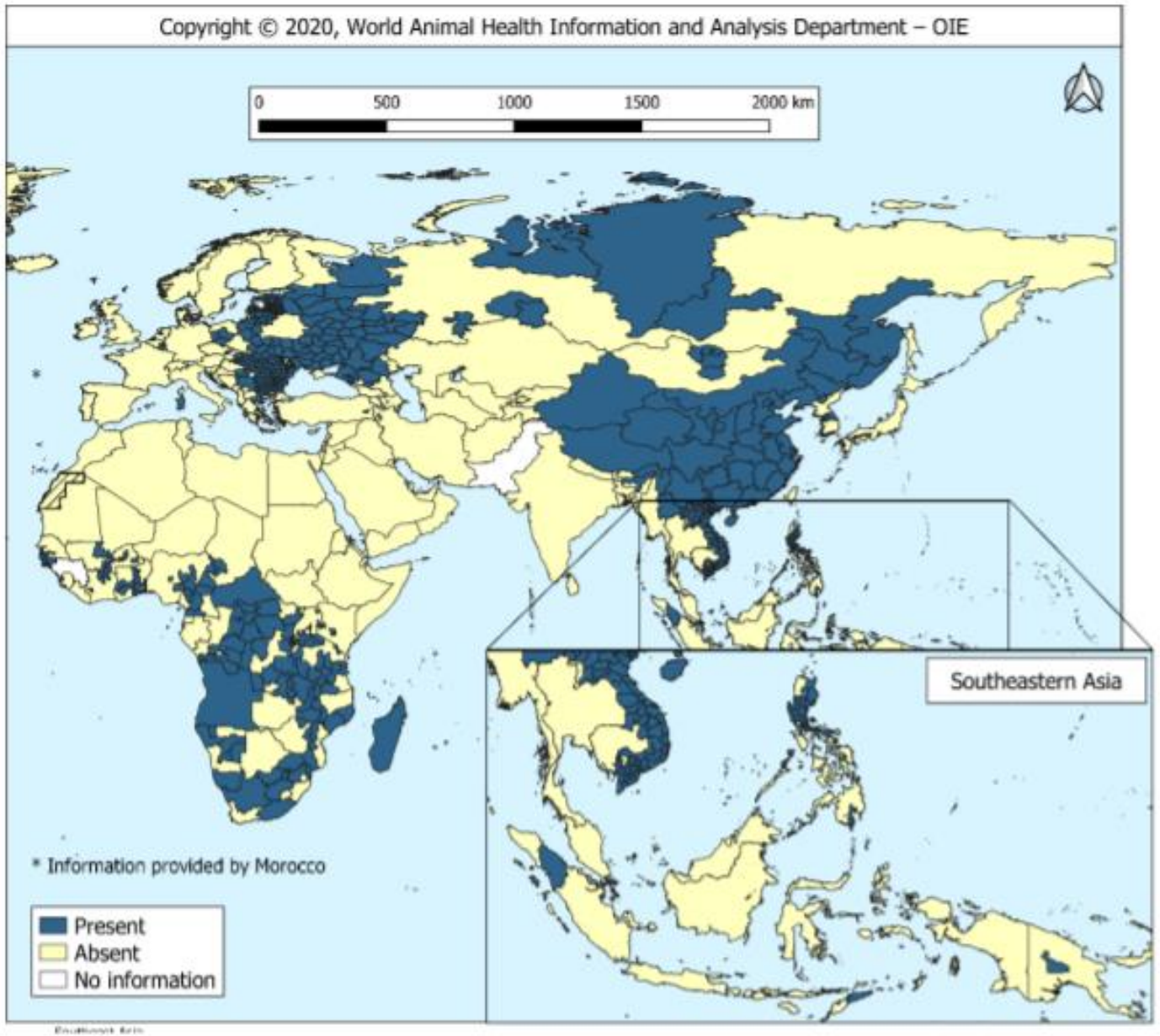
African Swine Fever Status (2016-2020)



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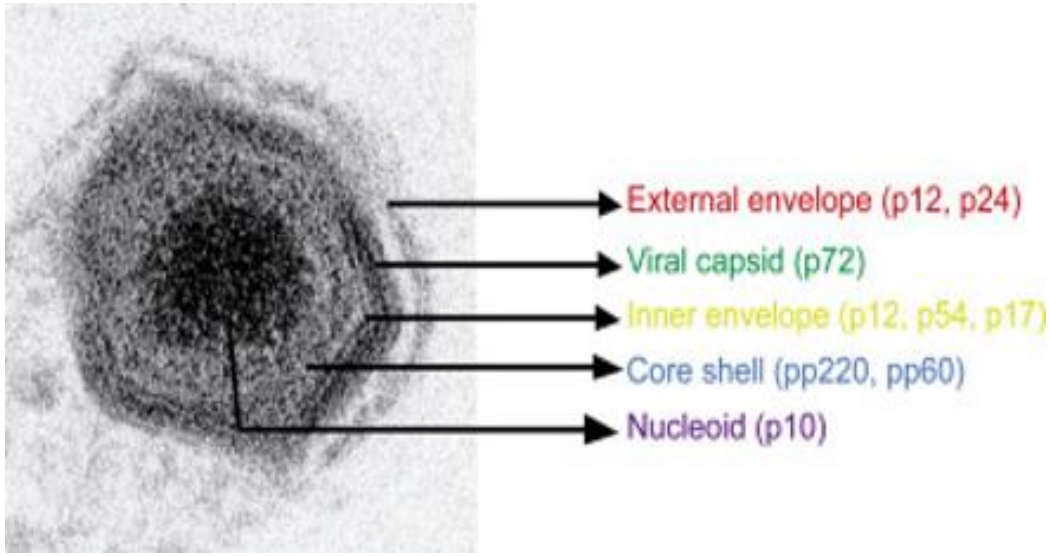
African Swine Fever in Africa

- Large DNA virus, Asfaviridae family
- Approximately 160 genes, number depending on isolate.
- 2 genotypes present in China, one in Europe.
- ASFV present in about 26 African countries.
- All 24 genotypes are present in Africa.
- There is a wildlife reservoir: warthogs and bush pigs.
- Wild boars are susceptible.
- Soft ticks of the genus Ornithodores are involved in transmission of ASFV.



From “Encyclopedia of Virology” Chap.: African swine fever” by L.Dixon and D. Chapman. 2008

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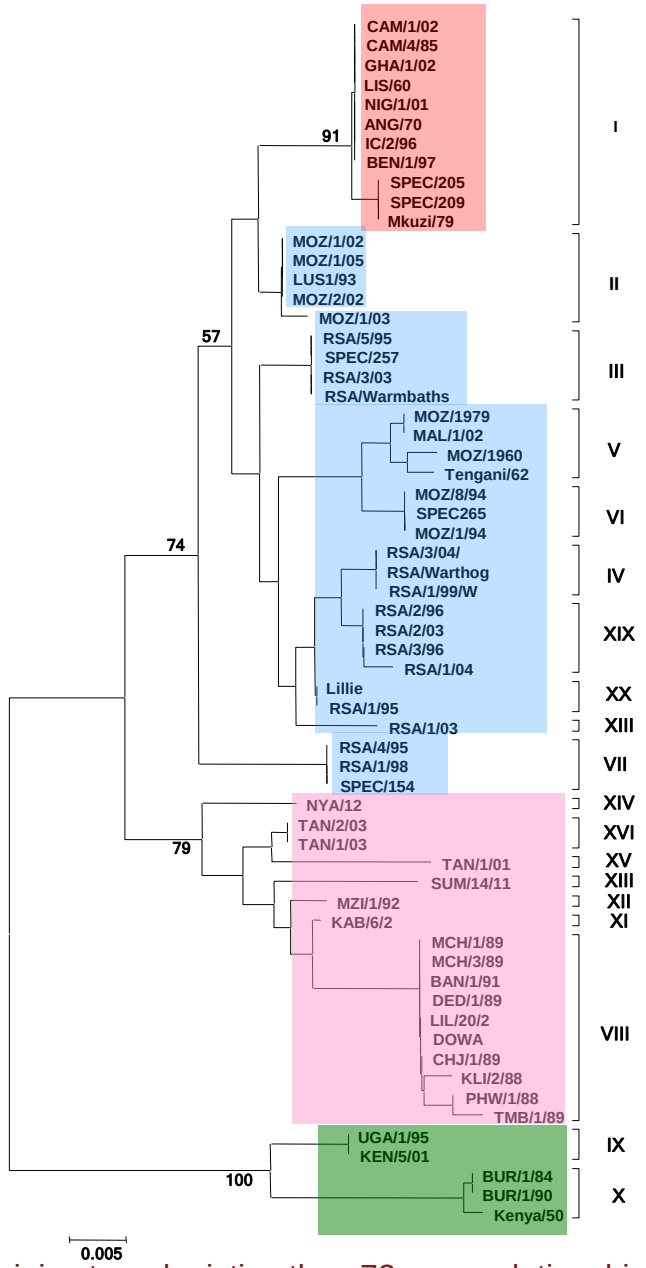
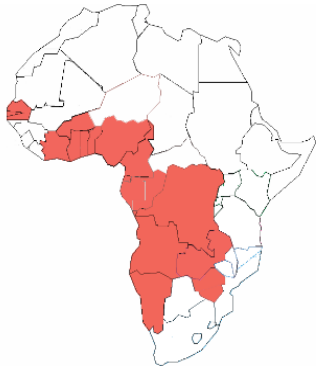
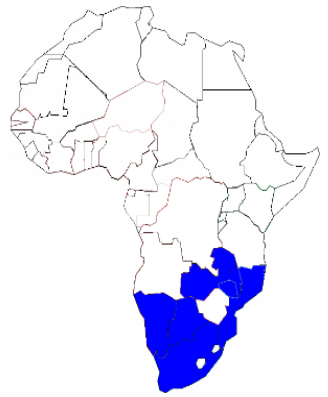
p72 gene



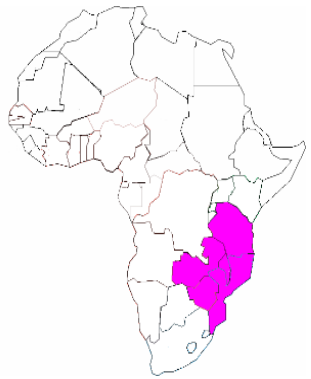
Neighbor-Joining tree depicting the p72 gene relationships and geographical distribution of the major ASFV genotypes



Genotype I



Genotype II,III, IV, V, VI, VII, XIX, XX, XIII



Genotype VIII, XI, XII, XIII, XV, XVI, XIV

Genotype IX, X

Contribution: Livio Heath (ARC-OVI)

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TOWARDS A LIVE ATTENUATED VACCINE FOR AFRICAN SWINE FEVER

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Vaccine Candidates

Development of a Highly Effective African Swine Fever Virus Vaccine by Deletion of the I177L Gene Results in Sterile Immunity against the Current Epidemic Eurasia Strain

Manuel V. Borca[a], Elizabeth Ramirez-Medina[a,b], Ediane Silva[a,c], Elizabeth Vuono[a,d], Ayushi Rai[a,e], Sarah Pruitt[a,e], Lauren G. Holinka[a], Lauro Velazquez-Salinas[a,c], James Zhu[a], Douglas P. Gladue[a]

Deletion of the African Swine Fever Virus Gene DP148R Does Not Reduce Virus Replication in Culture but Reduces Virus Virulence in Pigs and Induces High Levels of Protection against Challenge

Ana L. Reis, Lynnette C. Goatley, Tamara Jabbar, Pedro J. Sanchez-Cordon,* Christopher L. Netherton, David A. G. Chapman,* Linda K. Dixon
The Pirbright Institute, Pirbright, Woking, Surrey, United Kingdom

African Swine Fever Virus Bearing an I226R Gene Deletion Elicits Robust Immunity in Pigs to African Swine Fever

Yanyan Zhang,[a] Junnan Ke,[a,b] Jingyuan Zhang,[a] Jinjin Yang,[a] Huixian Yue,[a] Xintao Zhou,[a] Yu Qi,[a] Rongnian Zhu,[a] Faming Miao,[a] Qian Li,[a] Fei Zhang,[a] Ying Wang,[a] Xun Han,[a] Lijuan Mi,[a] Jinmei Yang,[a] Shoufeng Zhang,[a] Teng Chen,[a] Rongliang Hu[a]

First Oral Vaccination of Eurasian Wild Boar Against African Swine Fever Virus Genotype II

Jose A Barasona [1], Carmina Gallardo [2], Estefanía Cadenas-Fernández [1], Cristina Jurado [1], Belén Rivera [1], Antonio Rodríguez-Bertos [1 3], Marisa Arias [2], Jose M Sánchez-Vizcaíno [1]

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Vaccine Candidates

- **Efficacy**
  - High level of protection, 100 % in many cases in various doses
  - Under optimal timing, 4 weeks post immunization
  - Duration of immunity ?

- **Safety**
  - Different dose studies for some vaccine candidates.
  - Very different clinical readout system, some use clinical score systems with many parameters (King 2011 and Galindo-Cardiel 2013), others use single parameters, e.g., fever.

Absence of Long-Term Protection in Domestic Pigs Immunized with Attenuated African Swine Fever Virus Isolate OURT88/3 or BeninΔMGF Correlates with Increased Levels of Regulatory T Cells and Interleukin-10

Pedro J Sánchez-Cordón [1], Tamara Jabbar [2], Dave Chapman [2], Linda K Dixon [# 2], María Montoya [# 2 3]

- **Route of immunization**
  - Initially: intramuscularly
  - Orally route became interesting because of wild boar
  - Less viremia using orally route

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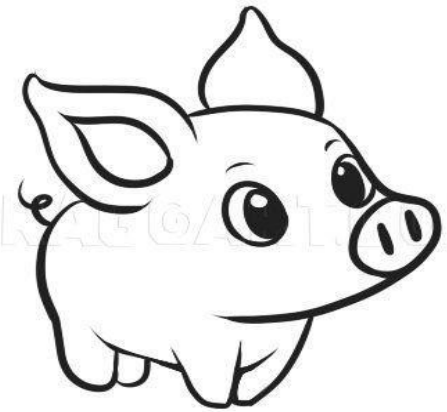
ILRI ASFV Vaccine Activities

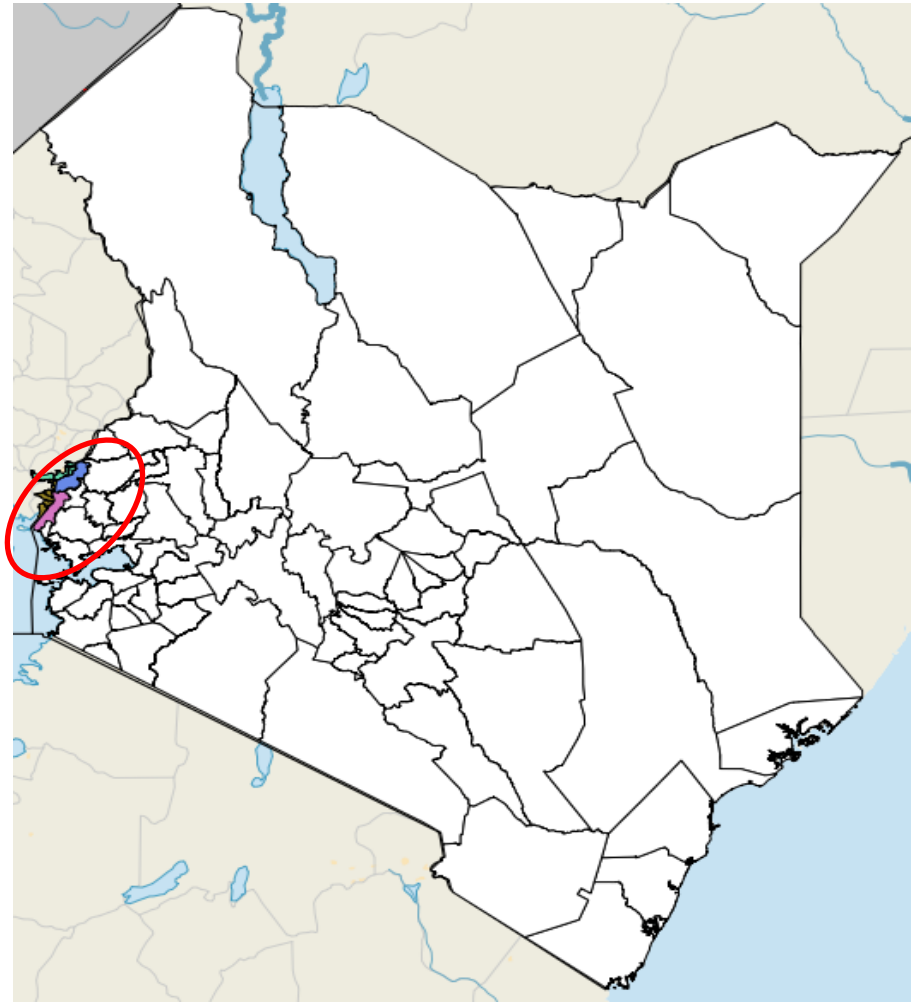
- Live vaccine (CRISPR/Cas9 deletions) and synthetic approach
  - Deletion of genes for attenuation
  - Testing in established animal model



- Subunit vaccine – activities
  - Screening of antigens
  - Viral vectors as delivery

Isolated Virus

- Kenya 1033 (genotype IX) isolated by ILRI and DVS Kenya.
- Genotype IX and X are especially circulation in Eastern Africa.
- Isolated from a zone with outbreaks.
- Used as the challenging virus in the animal model
- Used as backbone for deletion of genes to generate attenuated viruses.



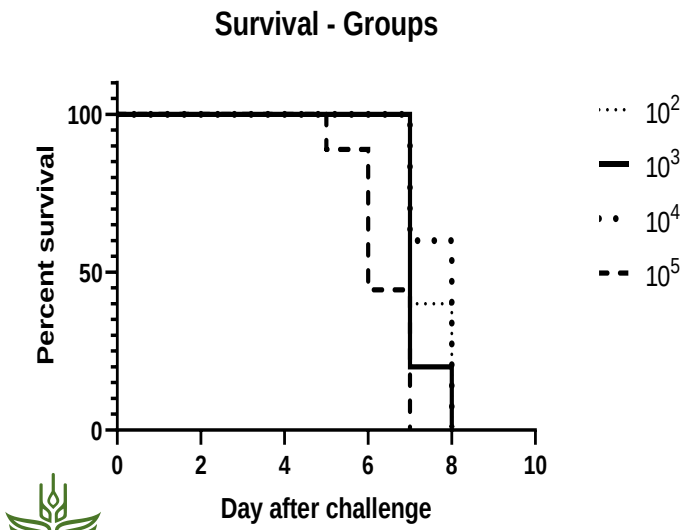
Gallardo C et al. A.J. Biotech 2011

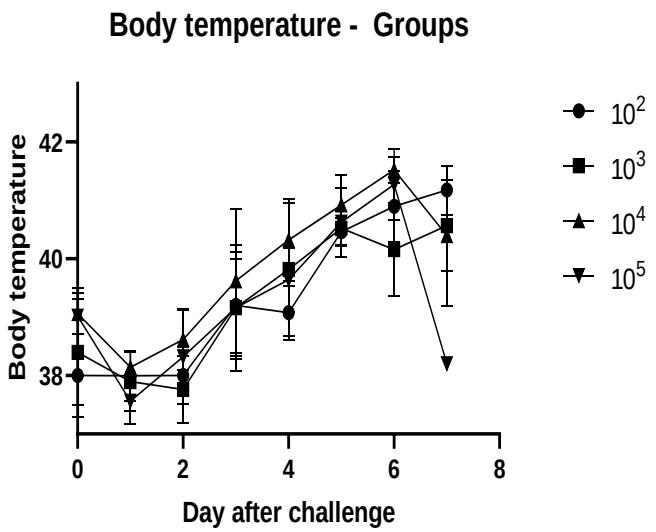
Onzere C. et al. Virus Genes 2018

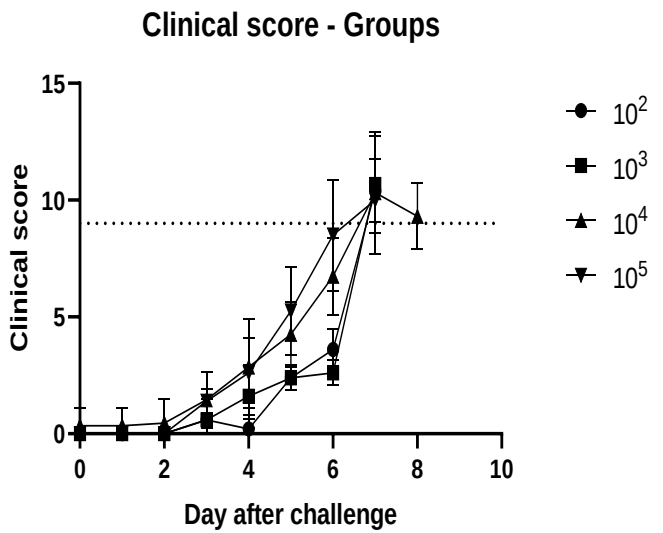
ASFV Kenya 1033 – Virus Batch for Challenge

- This virus is very similar to the other genotype IX and X viruses.
- Animal model was set up. Different doses were tested.

5 animal per group, intramuscular injection.







Scoring system: Galindo-Cardiel 2013

Genomic Stability and Production Cell Lines

Problems with instability of genomes in cell lines

JVI
Journals.ASM.org

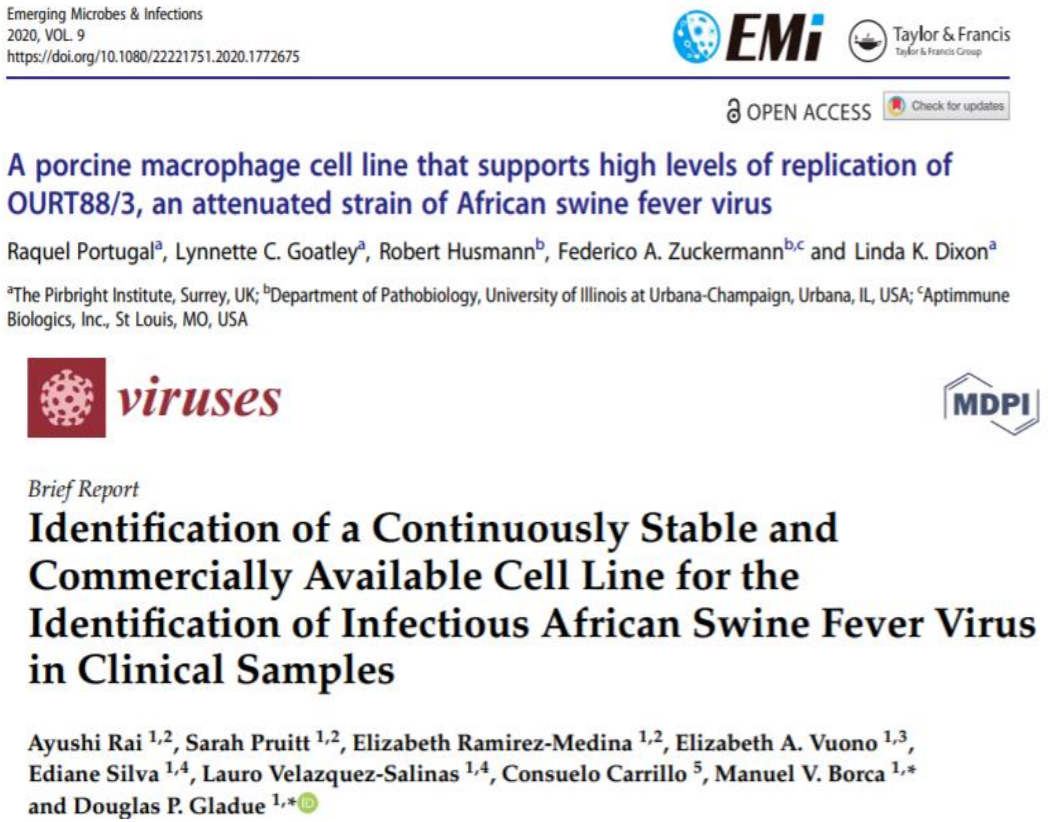
The Progressive Adaptation of a Georgian Isolate of African Swine Fever Virus to Vero Cells Leads to a Gradual Attenuation of Virulence in Swine Corresponding to Major Modifications of the Viral Genome

Peter W. Krug,[a] Lauren G. Holinka,[a] Vivian O'Donnell,[a,b] Bo Reese,[c] Brenton Sanford,[a] Ignacio Fernandez-Sainz,[a,b] Douglas P. Gladue,[a,b] Jonathan Arzt,[a] Luis Rodriguez,[a] Guillermo R. Risatti,[b] Manuel V. Borca[a]

ZMAC – pig macrophage cell line

MA-104 cell line (Green monkey kidney epithelial cell line)

Progress on production cell lines



Emerging Microbes & Infections
2020, VOL. 9
https://doi.org/10.1080/22221751.2020.1772675

EMi Taylor & Francis

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A porcine macrophage cell line that supports high levels of replication of OURT88/3, an attenuated strain of African swine fever virus

Raquel Portugal[a], Lynnette C. Goatley[a], Robert Husmann[b], Federico A. Zuckermann[b,c] and Linda K. Dixon[a]

[a]The Pirbright Institute, Surrey, UK; [b]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA; [c]Aptimmune Biologics, Inc., St Louis, MO, USA

viruses

MDPI

Brief Report

Identification of a Continuously Stable and Commercially Available Cell Line for the Identification of Infectious African Swine Fever Virus in Clinical Samples

Ayushi Rai [1,2], Sarah Pruitt [1,2], Elizabeth Ramirez-Medina [1,2], Elizabeth A. Vuono [1,3], Ediane Silva [1,4], Lauro Velazquez-Salinas [1,4], Consuelo Carrillo [5], Manuel V. Borca [1,*] and Douglas P. Gladue [1,*]

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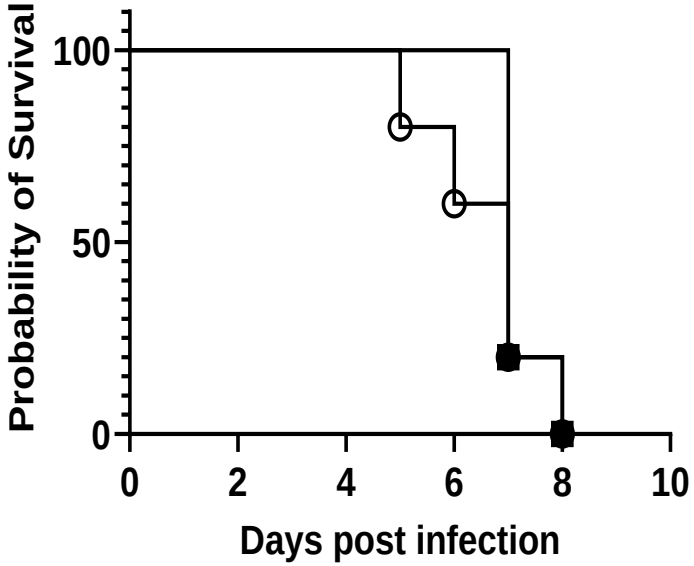
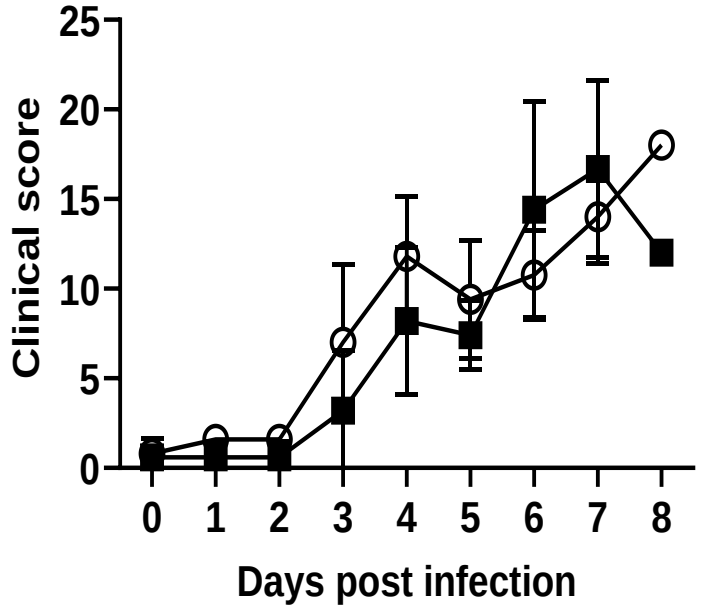
Virulence of WSL Adapted WT-Virus

- WSL (from FLI) is a fetal wild boar lung cell line, not immortalized.
- ASFV Kenya 1033 was adapted to WSL (20+ passages)
- 10^2 $TCID_{50}$ was chosen to test if the virus grown in WSL cells was still lethal



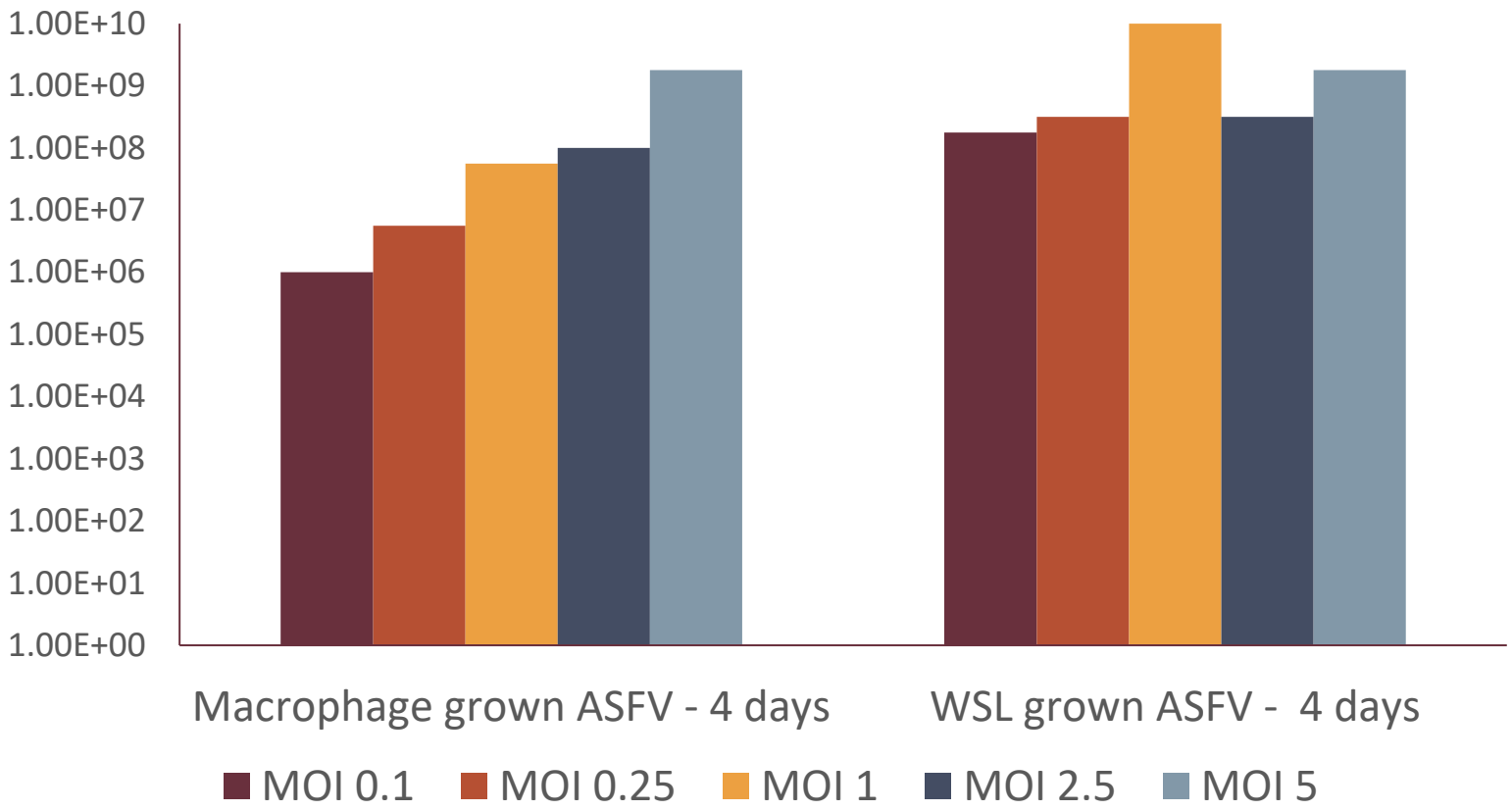


Challenge with wild type virus.

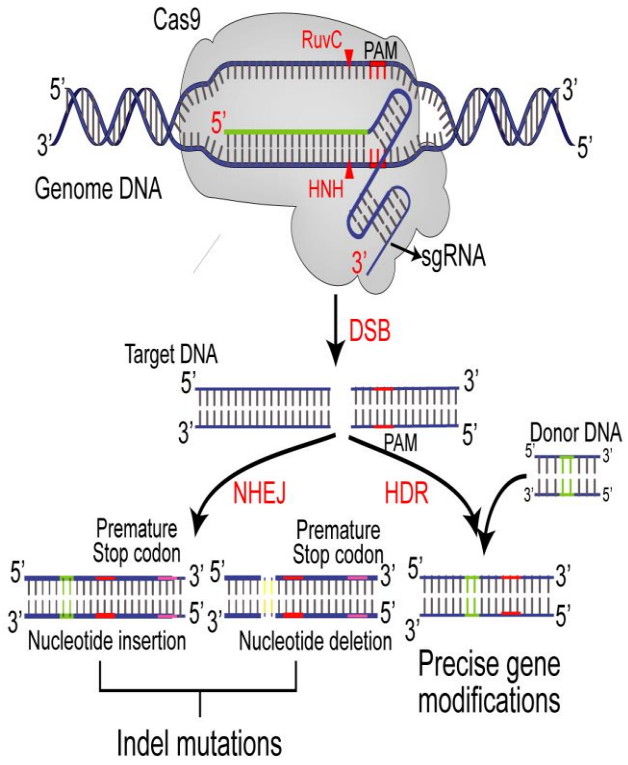
Open circles: WSL cell line grown virus , Solid squares: Macrophage grown virus

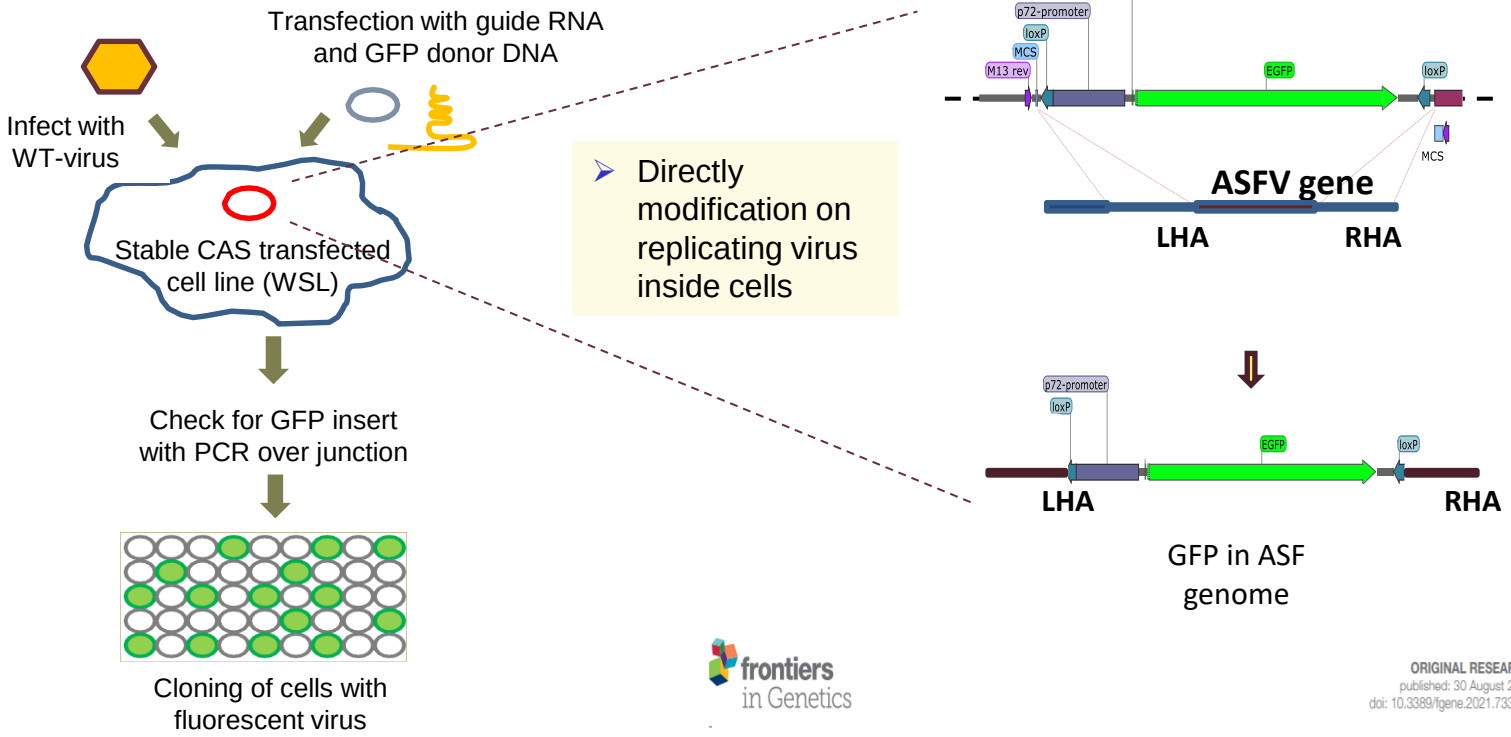
Scoring system: King et al. 2011

Titers of ASFV Ken-1033 in WSL



CRISPR-Cas Editing of African Swine Fever Virus





Constructed 7-10 different viruses

frontiers in Genetics

ORIGINAL RESEARCH
published: 30 August 2021
doi: 10.3389/fgene.2021.733674

Rapid CRISPR/Cas9 Editing of Genotype IX African Swine Fever Virus Circulating in Eastern and Central Africa

Hussein M. Abkallo[1], Nicholas Svitek[1], Bernard Oduor[1], Elias Awino[1], Sonal P. Henson[1], Samuel O. Oyola[1], Stephen Mwalimu[1], Nacrya Assad-Garcia[2], Walter Fuchs[3], Sanjay Vashee[2] and Lucilla Steinaa[1]*

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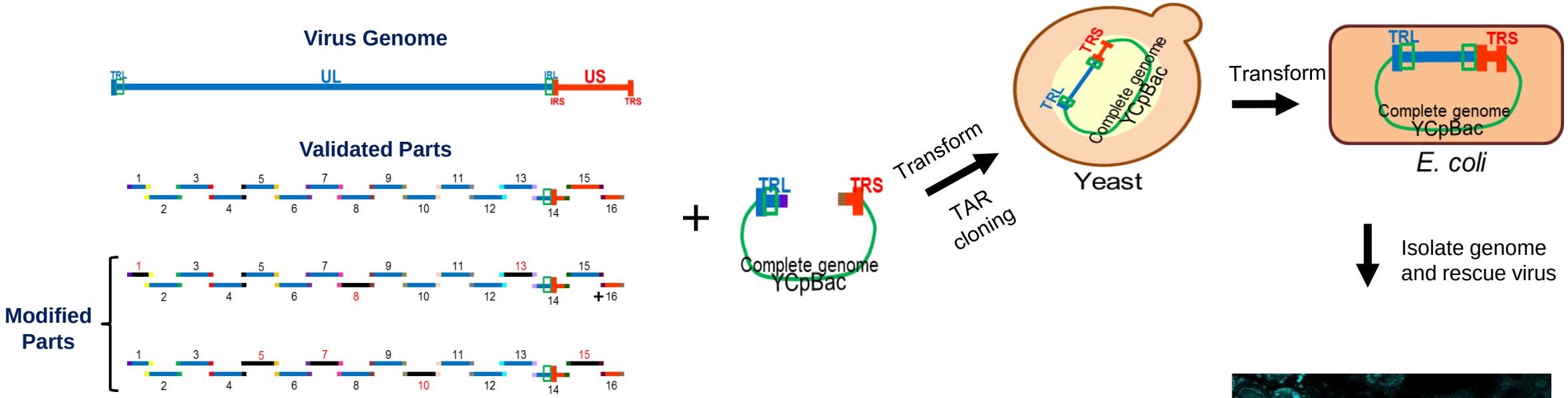
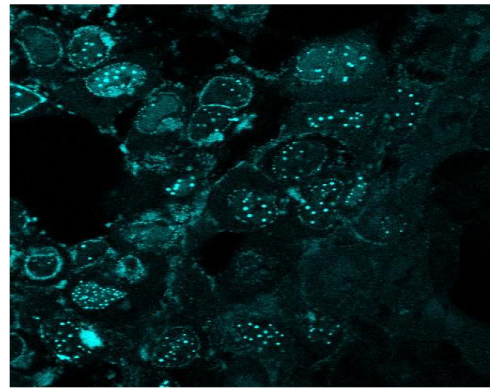
Synthetic Construction of African Swine Fever Virus





- Capacity to efficiently perform genome-wide changes in the virus genome in a combinatorial manner to understand virus biology.
- Capacity to produce clinically-relevant viruses without extensive passaging in tissue culture.
- Streamlines process to generate various designer vaccine candidates and oncolytic viruses.

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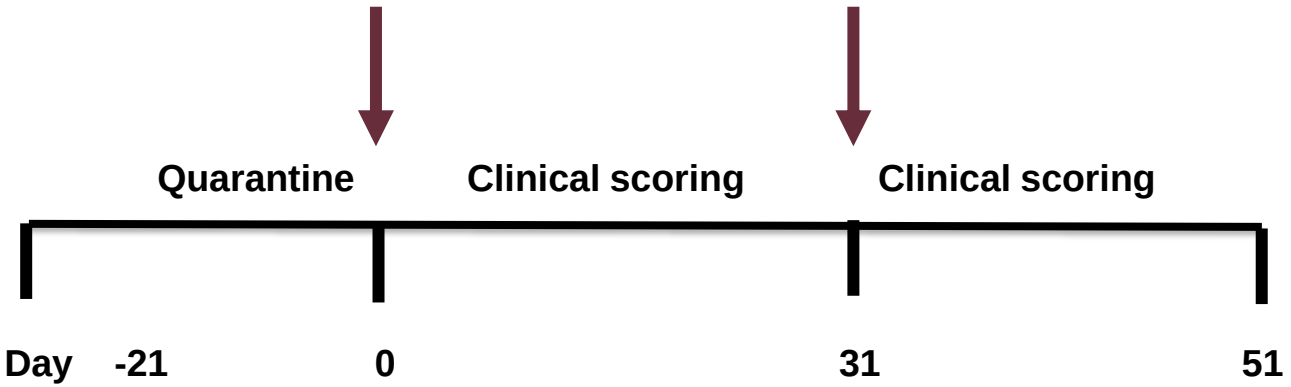
First Viruses: Experimental Setup

Immunisation (1 injection)		Challenge
10^4 ASF1033_ΔCD2v	(9x)	10^2 ASF1033 (8x)
10^4 ASF1033_ΔCD2vΔA238L	(9x)	10^2 ASF1033 (8x)
PBS	(9x)	10^2 ASF1033 (8x)



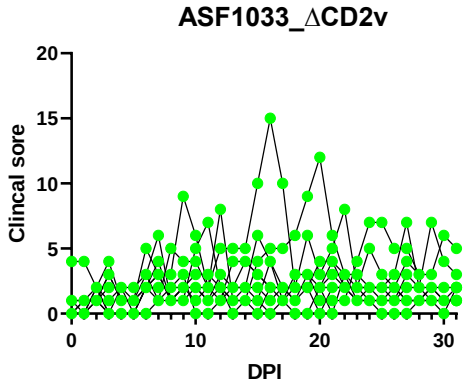
CD2v

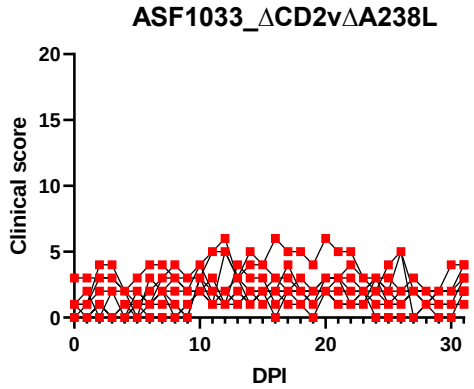
Immunomodulatory molecule promoting apoptosis of lymphocytes.

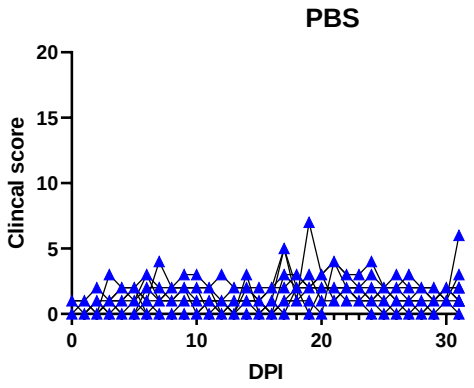
A238L

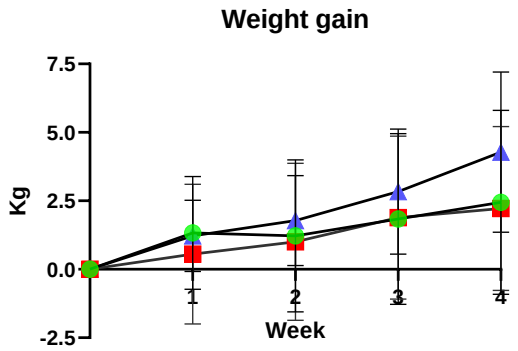
Mimic NFκB subunit, inhibits NFκB activity, which is crucial in the pro-inflammatory response.

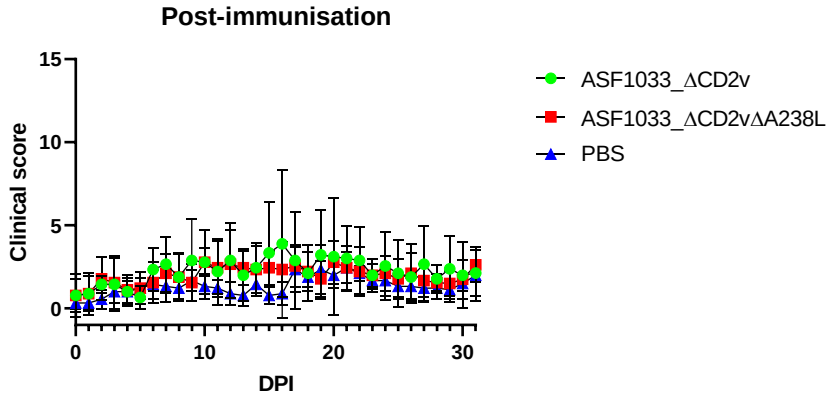
Clinical Scores After Immunization











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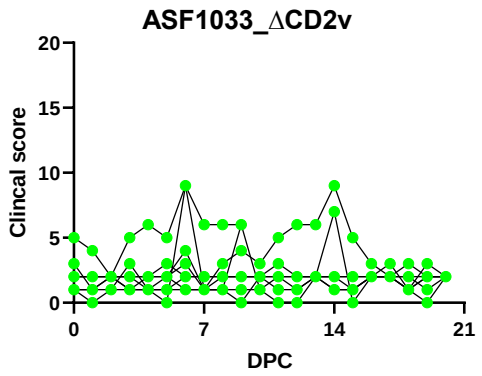
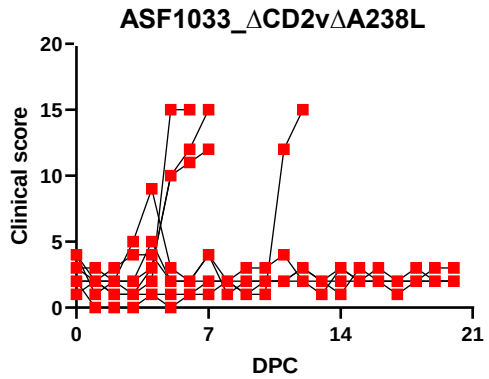
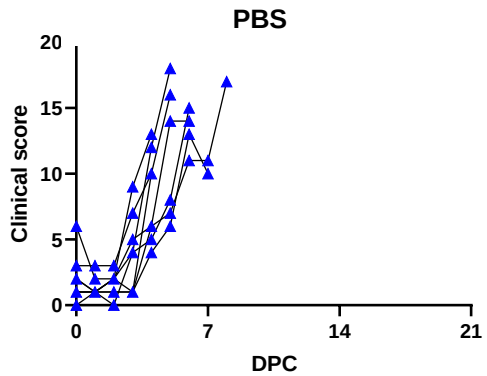
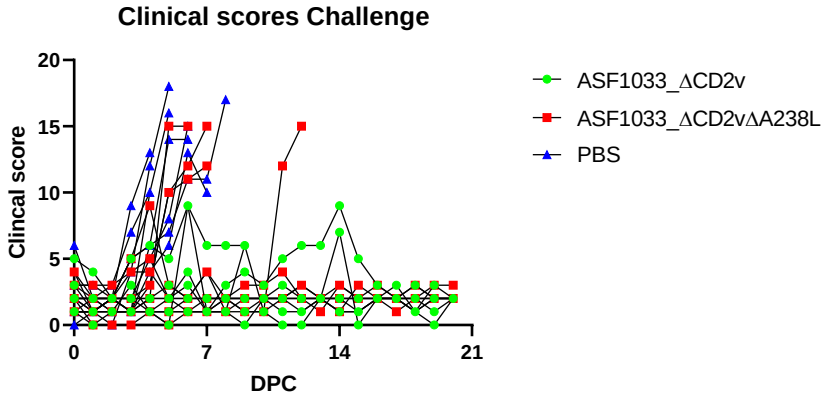
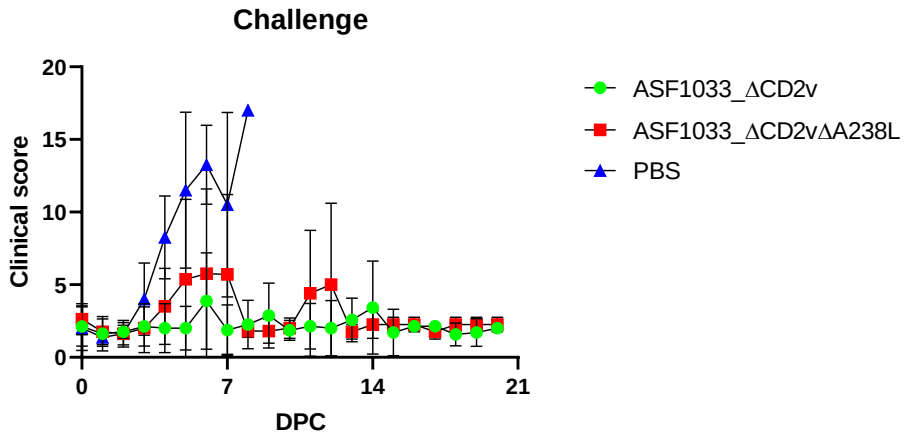
Clinical Scores After Challenge











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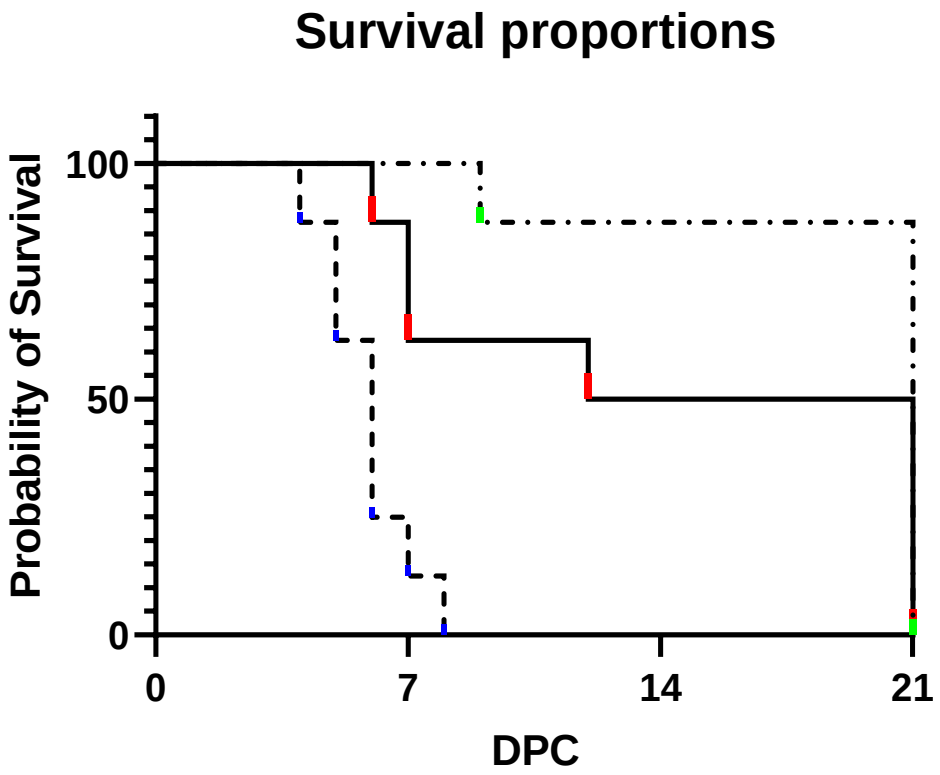
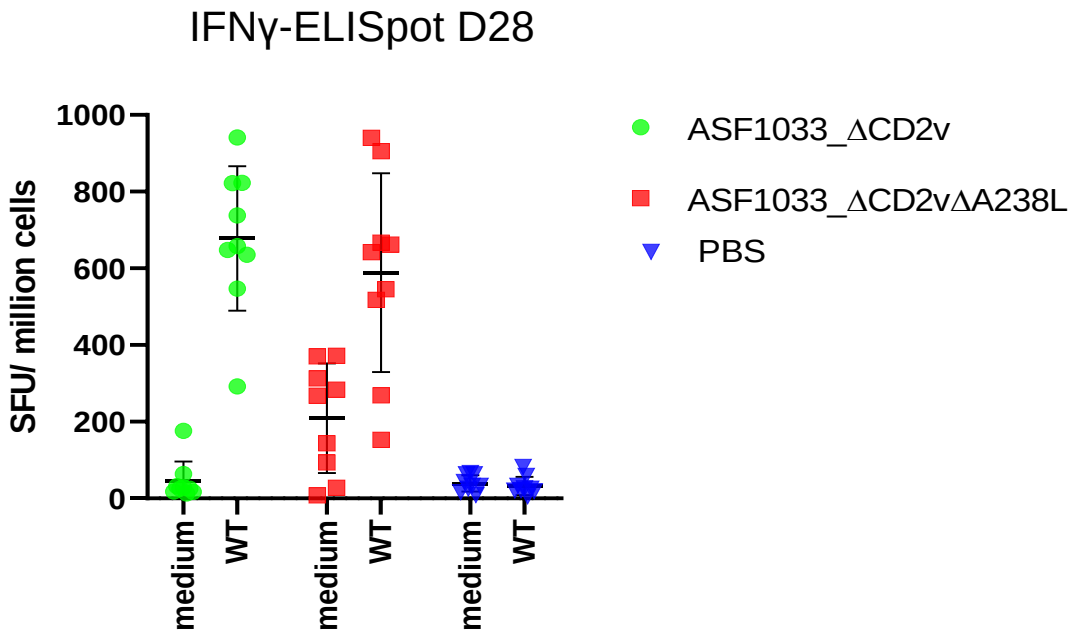
Survival Plot



ASF1033_ΔCD2v

ASF1033_ΔCD2vΔA238L

PBS



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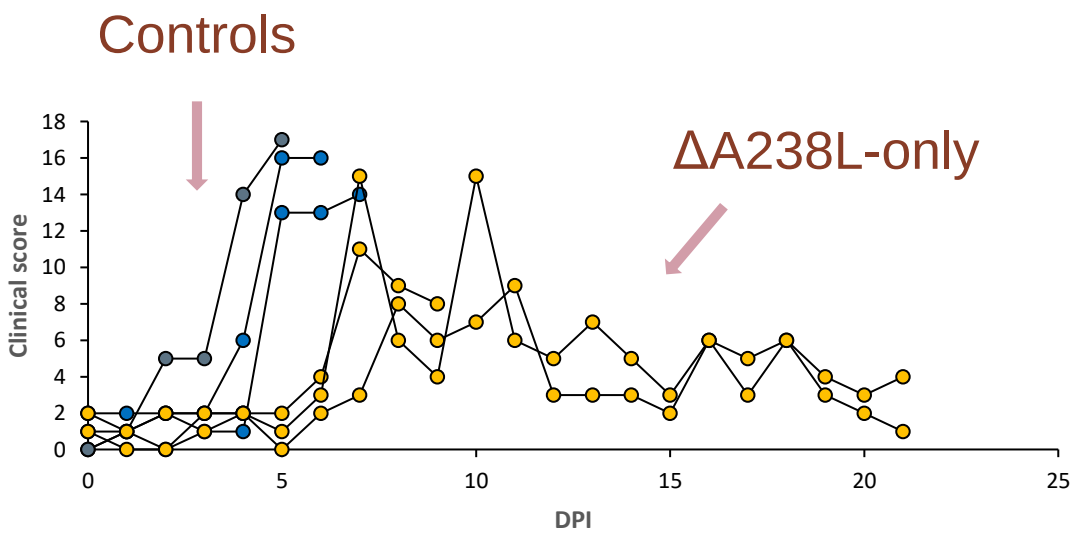
Conclusion

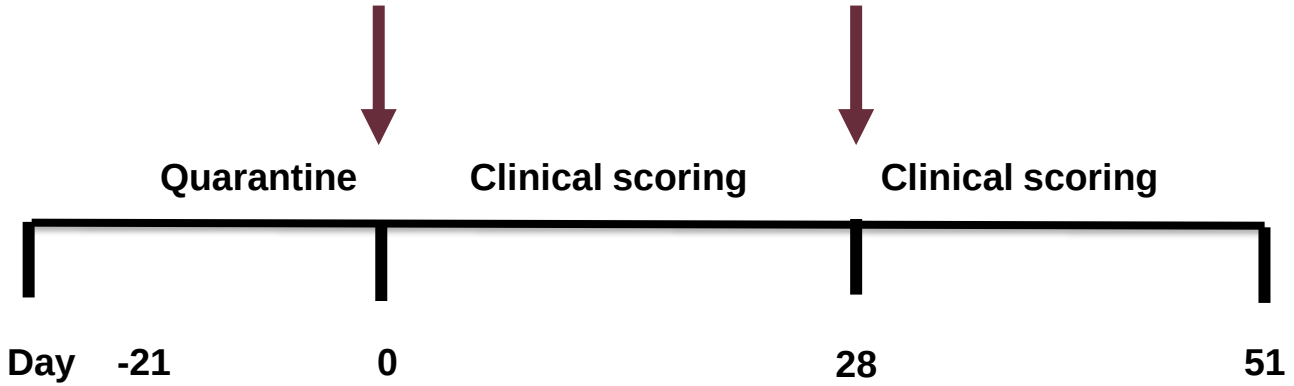
- ΔCD2v is more efficient than the double knockout but less attenuated. 87.5% protection versus 50%.

- ΔA238L seems to add to the attenuation, but with a loss in ability to protect.

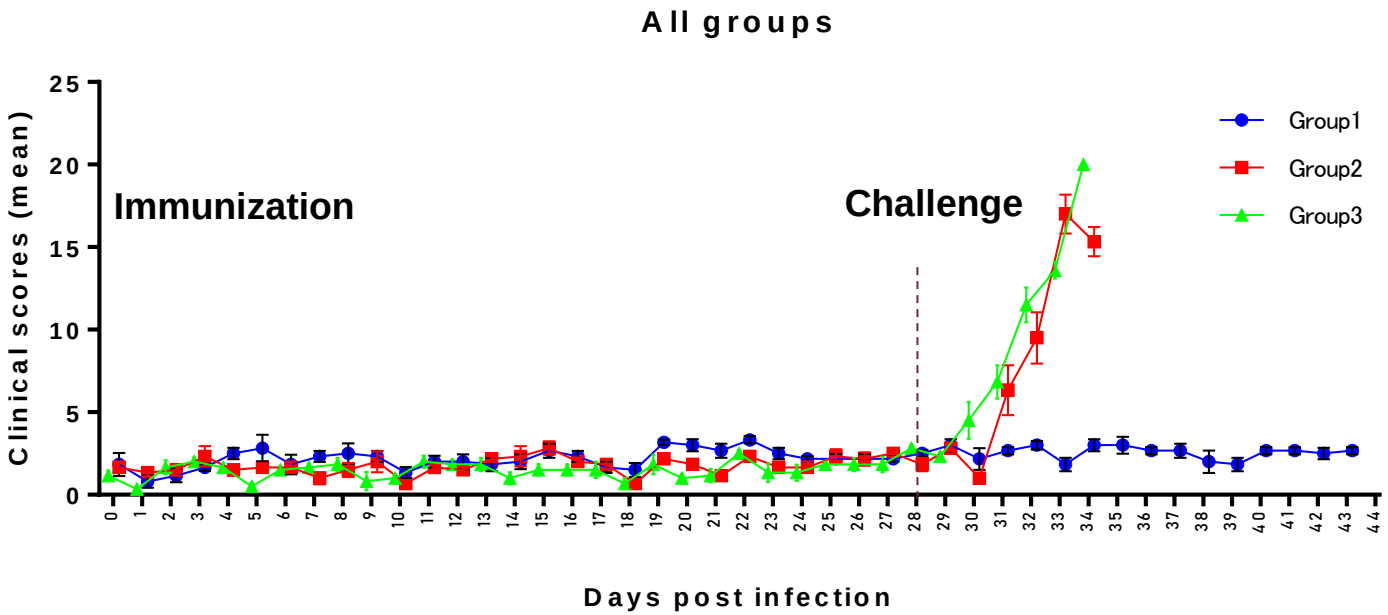


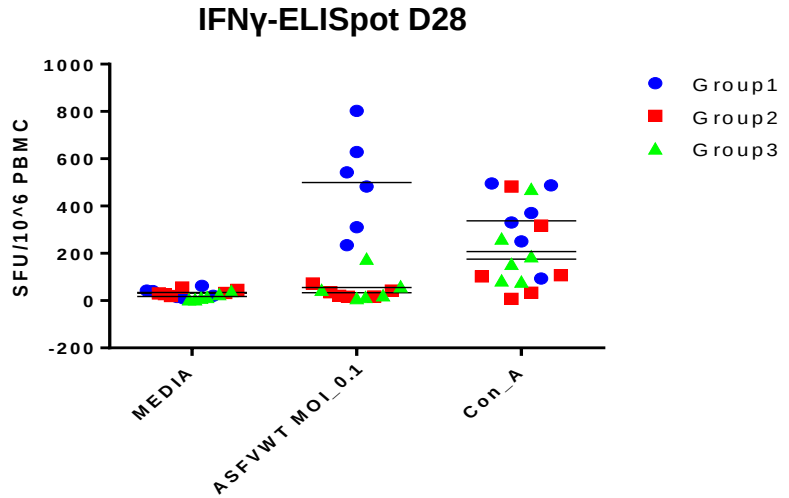
Second viruses: Experimental Setup

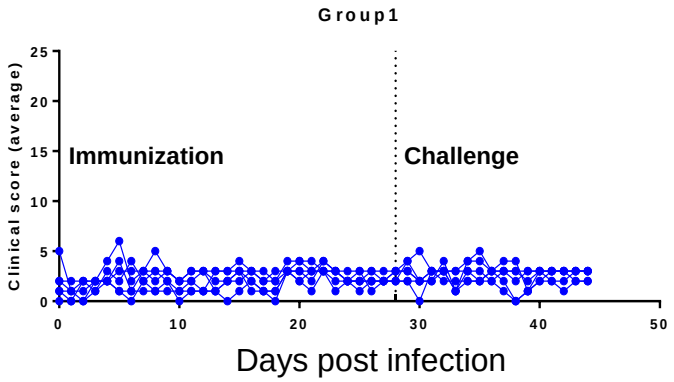
Immunisation (1 injection)		Challenge
10^3 ASF1033_ΔX	(7x)	10^2 ASF1033 _ΔX (6x)
10^3 ASF1033_ΔY	(7x)	10^2 ASF1033 _ΔY (6x)
PBS	(7x)	10^2 ASF1033 (6x)

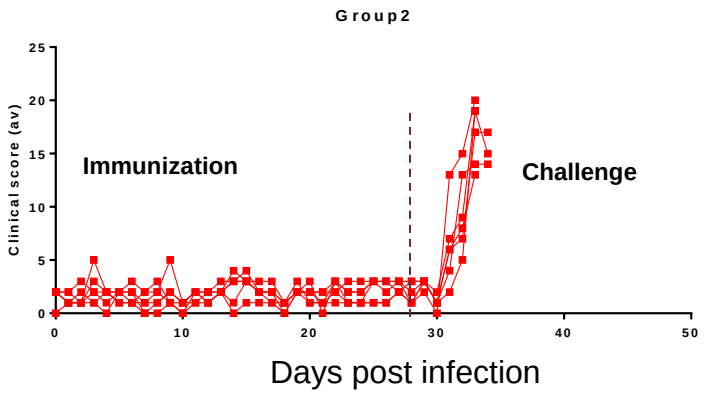


New Gene-Deleted Viruses









Remaining data:

Viremia data

Antibody titers

PM data

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Acknowledgements

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Bernard Odour
Emanuel Khazalwa
Lucilla Steinaa

ΔCD2v virus / WT-virus

Friedrich Loeffler Institute
Gunther Keil
Raquel Portugal
Sandra Blome

ILRI
Richard Bishop, now WSU
Edward Okoth

Collaborators

Sanjay Vashee,
J. Craig Venter Institute

Walter Fuchs,
Friedrich Loeffler Institute

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The International Livestock Research Institute (ILRI) is a non-profit institution helping people in low- and middle-income countries to improve their lives, livelihoods and lands through the animals that remain the backbone of small-scale agriculture and enterprise across the developing world. ILRI belongs to CGIAR, a global research-for-development partnership working for a food-secure future. ILRI's funders, through the CGIAR Trust Fund, and its many partners make ILRI's work possible and its mission a reality. Australian animal scientist and Nobel Laureate Peter Doherty serves as ILRI's patron.

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