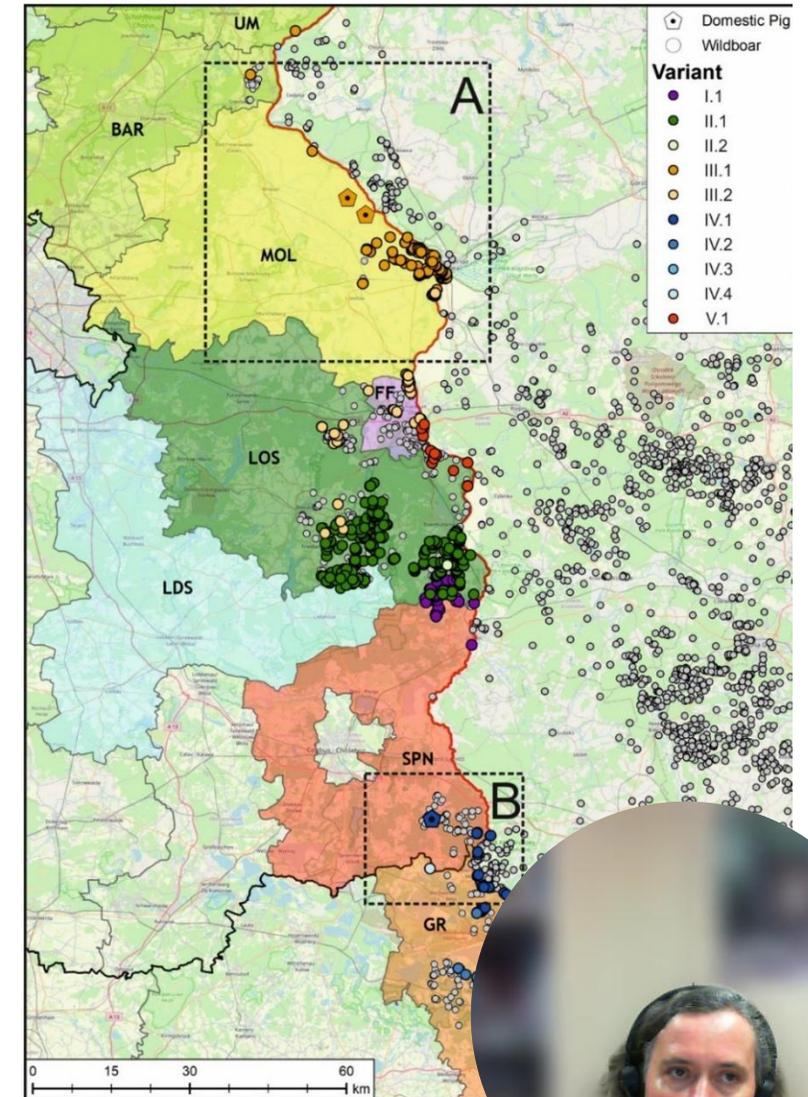
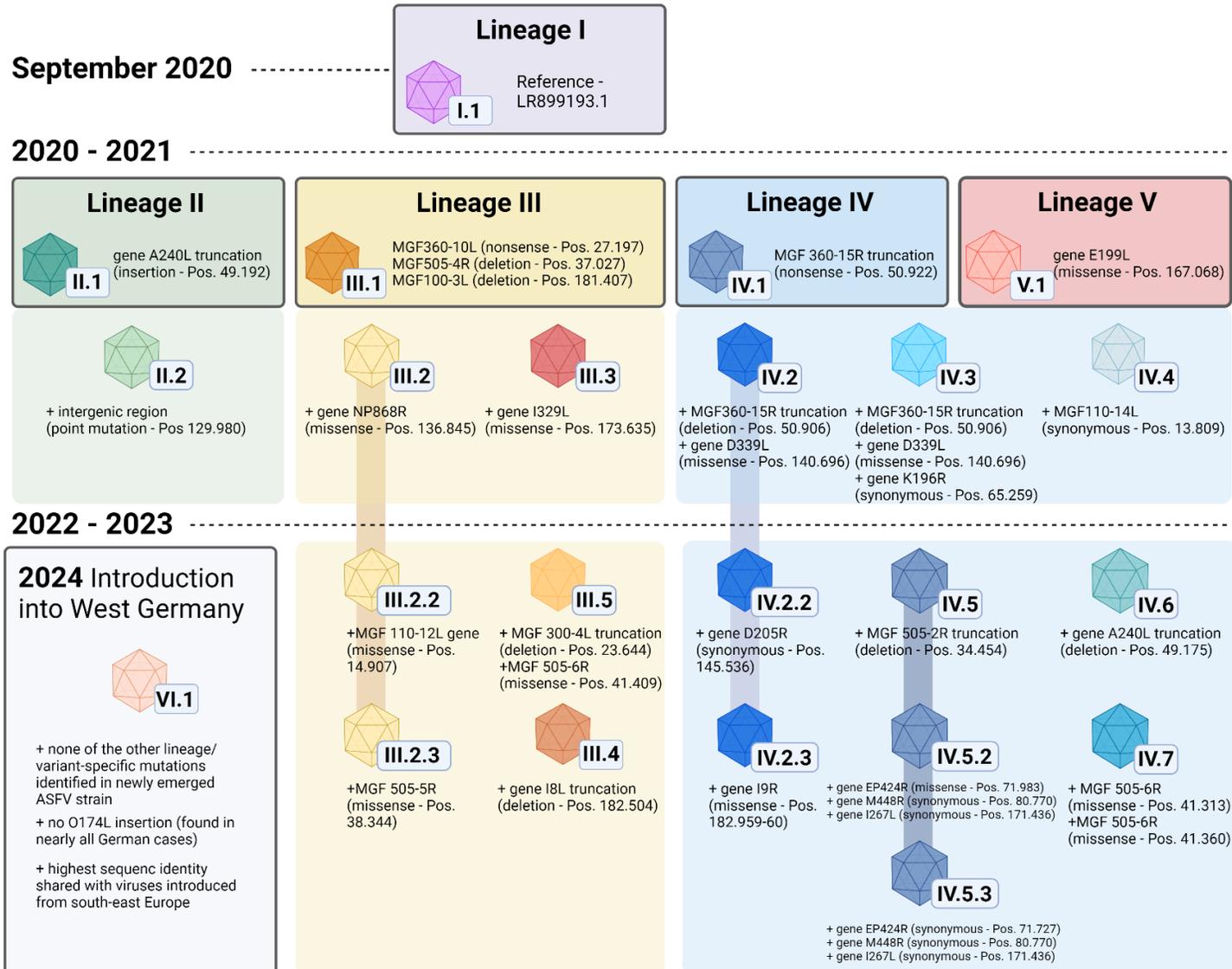
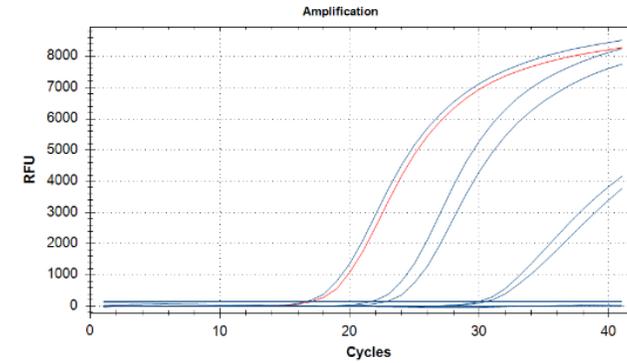


Variants on the rise - evolution of viruses



NRL Impressions OR: Poor sample is a bad excuse...

ID	County	Matrix	Age and sex	Sample origin	Stage of decomposition	LLBBIDEXX ASFV	NRL ASFKing PCR	NRL ASFvirotype ASFV	NRL ASFtignon PCR
1	Lk Spree bite	Bone marrow	Adult, female	☞	III	24	23	21	25
2	Lk Oder-Spree	Swab suspension	Subadult, female	♣	I	30	29	27	29
3	Lk Oder-Spree	Blood clot suspension	Piglet	☞	I	20	21	21	21
4	Lk Oder-Spree	Swab suspension	Subadult, female	☞	II	30	25	29	25
5	Lk Oder-Spree	Swab suspension	Adult, female	☞	II	25	25	24	25
6	Lk Oder-Spree	Swab suspension	Piglet	☞	II	25	25	24	32
7	Lk Oder-Spree	Swab suspension	Subadult, female	☞	II	24	26	23	NT
8	Lk Oder-Spree	Swab suspension	Piglet	☞	II	28	28	25	29
9	Lk Oder-Spree	Swab suspension	Piglet	♣	I	26	25	24	27
10	Lk Spree bite	Bone marrow	Subadult, male	☞	III	27	30	27	31
11	Lk Spree bite	Bone marrow	Piglet	☞	III	33	35	29	37
12	Lk Spree bite	Bone marrow	Subadult, male	☞	III	29	No cq	33	38
13	Lk Spree bite	Bone marrow	Piglet	☞	III	31	36	30	37
14	Lk Oder-Spree	Swab suspension	Subadult, female	☞	I	23	21	NT	NT
15	Lk Oder-Spree	Bone marrow	Adult, female	☞	IV	30	33	31	34
16	Lk Oder-Spree	Swab suspension	Piglet	☞	II	26	25	23	25
17	Lk Spree bite	Bone marrow	Piglet	☞	IV	37	35	No cq	34
18	Lk Spree bite	Bone marrow	Piglet	☞	IV	19	20	17	20
19	Lk Spree bite	Bone marrow	Piglet	☞	NK	21	18	17	19
20	Lk Spree bite	Bone marrow	Adult, female	☞	IV	23	23	22	24
21	Lk Oder-Spree	Swab suspension	Piglet	☞	II	22	21	18	22
22	Lk Oder-Spree	Swab suspension	Piglet	☞	II	26	25	24	27
23	Lk Oder-Spree	Bone marrow	Female, no age differentiation	☞	IV	35	36	29	35
24	Lk Oder-Spree	Bone marrow	Only bones, probably female	☞	IV	32	31	29	32
25	Lk Oder-Spree	Bone marrow	Piglet	☞	IV	20	19	18	21
26	Lk Oder-Spree	Bone marrow	Only bones, probably male	☞	IV	26	27	23	28
27	Lk Oder-Spree	Bone marrow	Severely decomposed, no age and sex	☞	IV	28	27	26	29
28	Lk Oder-Spree	Bone marrow	Adult, female	☞	III	26	25	24	27
29	Lk Oder-Spree	Bone marrow	Severely decomposed, no age and sex	☞	IV	25	22	21	24
30	Lk Oder-Spree	Bone marrow	Female, no age differentiation	☞	IV	20	24	21	NT
31	Lk Oder-Spree	Bone marrow	Piglet	☞	II	19	19	18	NT
32	Lk Oder-Spree	Swab suspension	Adult, male	☞	I	25	27	23	NT



No problems with ASF detection via PCR



ASF diagnosis in wild boar

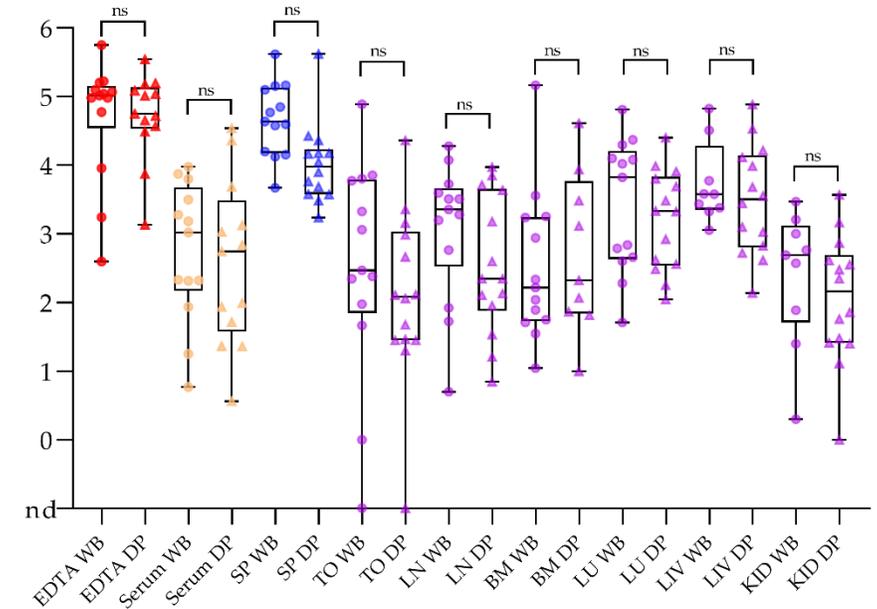


No difference with routine samples...

BUT:



Source: Food and Veterinary Service of Latvia; scheme drawing: J. Sehl, FLI

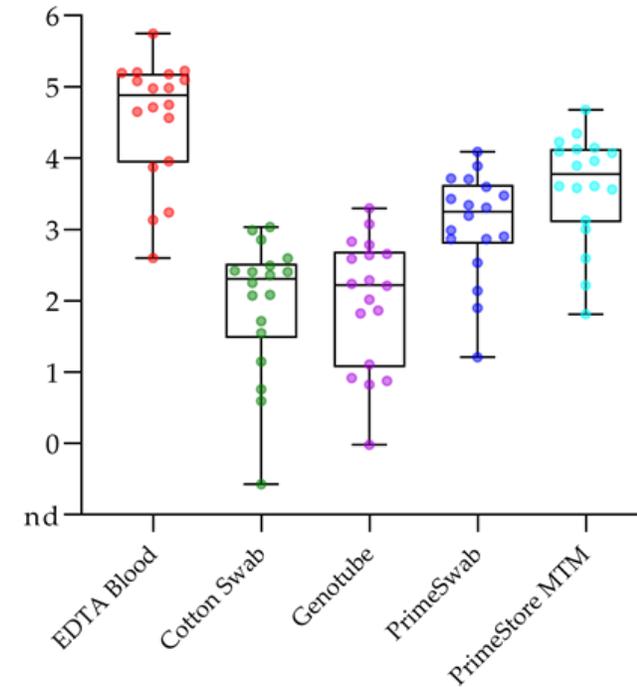
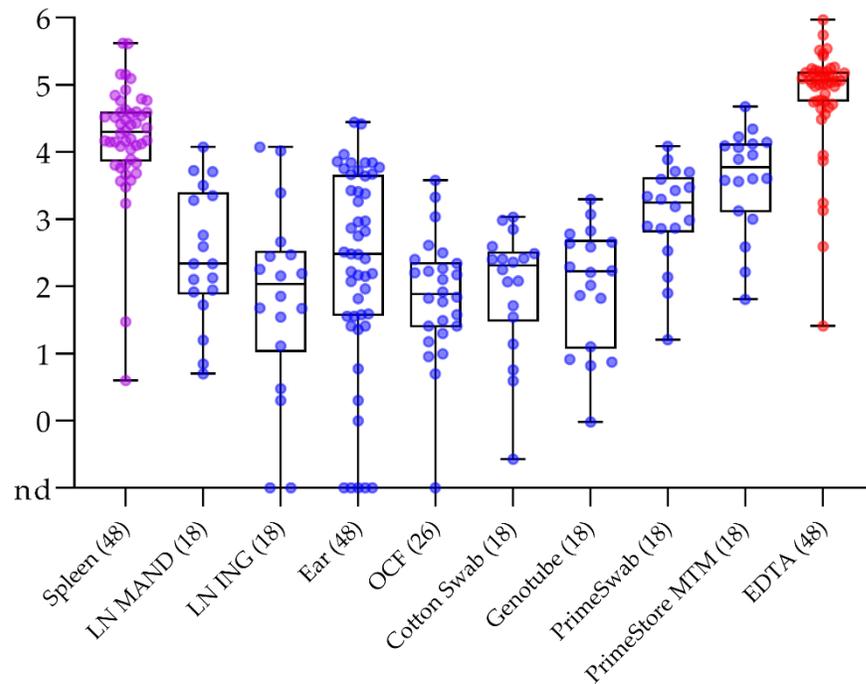


- Sampling is not easy
- Rotten carcasses are smelly and disgusting...
- Some organs are not available anymore
- Blood (decent fluid) is
- Some carcasses are ju



Alternative sampling

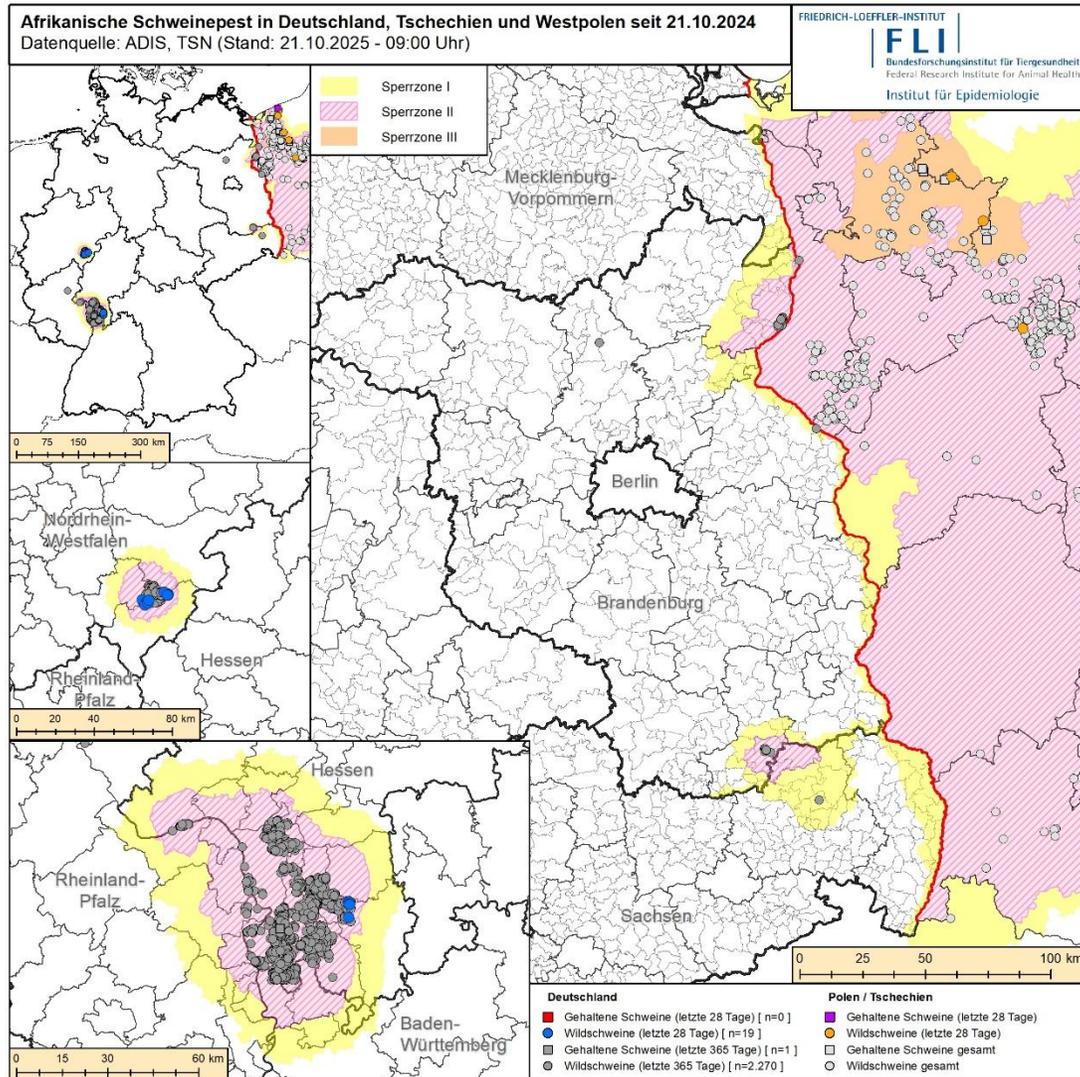
- Different stages of infection
- Different genotypes
- Wild boar and domestic pigs



Pikalo et al., 2021



Current situation in Germany

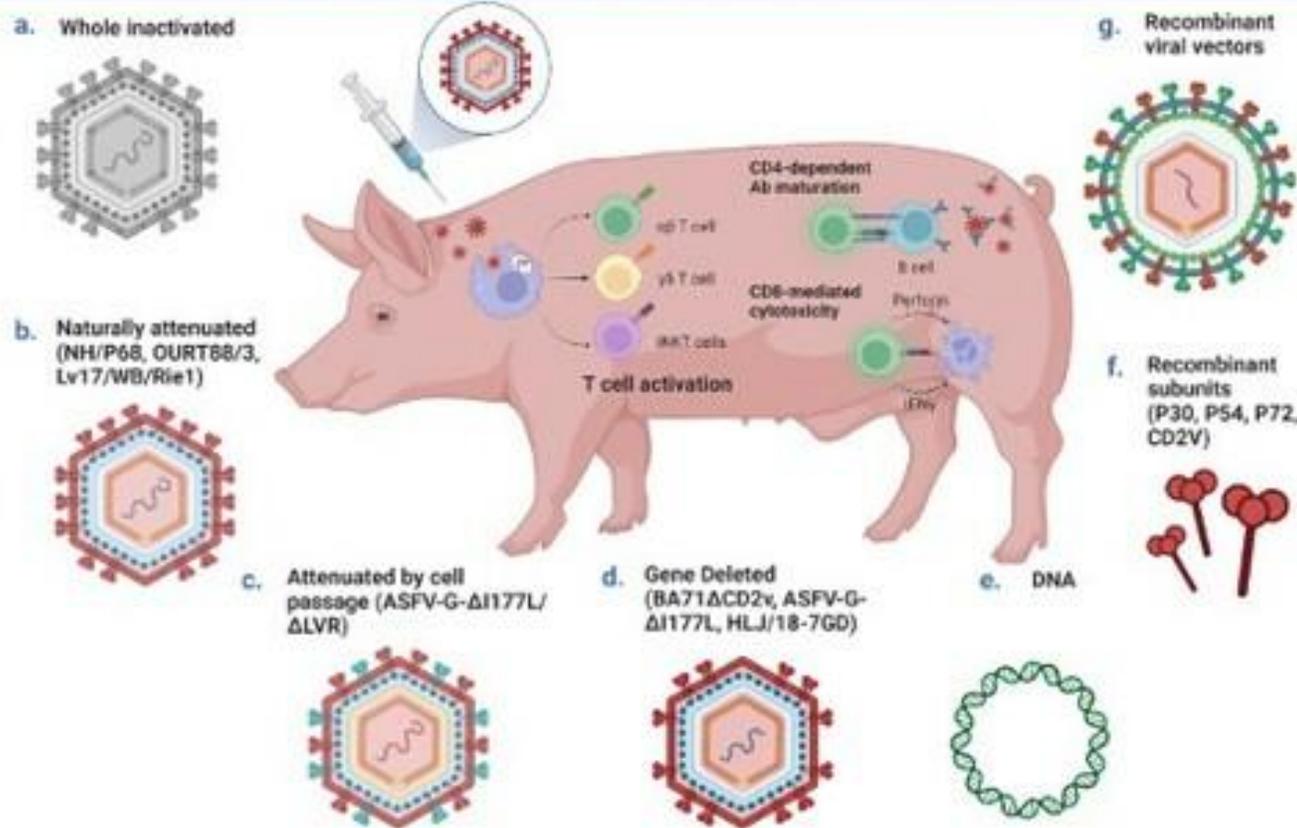


- All core areas in Brandenburg lifted
- ASF was eradicated in the first affected areas
- The last case occurred there in October 2021
- Positive results also in MVP (restriction zones were lifted) and SN (lifted)
- Problem areas (e.g. open-case mining, border areas)
- Still some infection pressure on the border with Poland, with cases in the high-risk corridor
- Independent events in Hesse, RLP and BW
- Very recent introduction into NRW



Vaccines against ASF

Approaches to ASFV Vaccine Development



Urbano and Ferreira 2022, doi: 10.1080/22221751.2022.2108342

Inactivated vaccines

- good safety profile
- have never induced robust immunity

Naturally attenuated strains and live vaccines obtained by passage

- Induction of humoral and cellular responses
- Oral administration possible
- Partly negative side effects, chronic infections
- Different results with different candidates

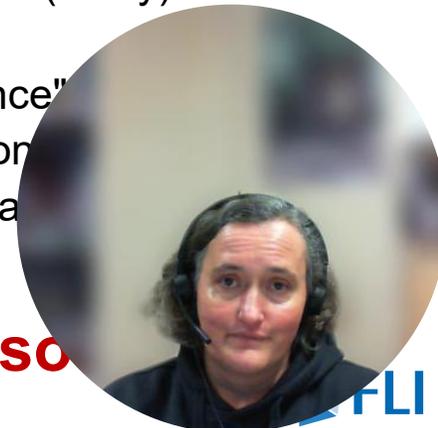
Vector vaccines, subunit, DNA vaccines

- Easy to scale up, good safety profile
- Depending on the system, both humoral and cellular reactions, high DIVA potential
- So far only partial protection (if any)

Gene-deleted live vaccines

- Deletions targeting "virulence"
- Cellular and humoral responses
- All advantages and disadvantages of live vaccines

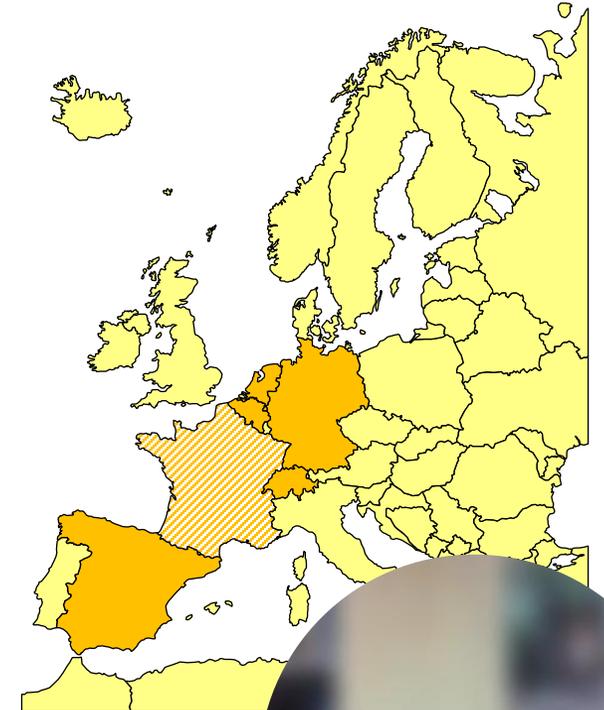
→ Only live vaccines showed real promise so far



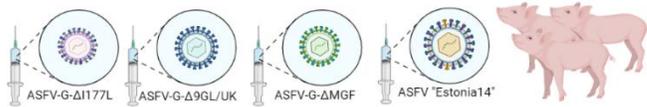
EU-Projekt (HORIZON): ASFaVIP



FLI
 Wageningen
 Sciensano
 UFZ Leipzig
 IABS-EU
 UCLM
 Büro WildVet
 IVI
 Zoetis
 USDA
 Widening: BIOR (LV)

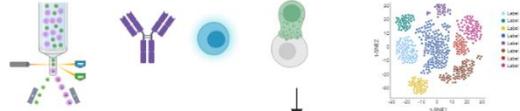


Theme 1: Establish standards and define correlates and mechanisms of protection



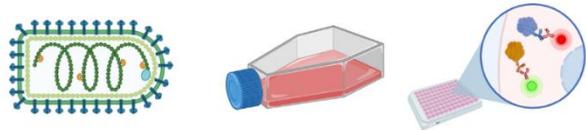
Multi-center studies using established vaccine candidates and comparators

Harmonization of immunization and challenge, laboratory procedures and read out



Investigate correlates and mechanism of protection and immunopathology

Theme 2: Explore possibilities to generate innovative next generation vaccine approaches



Use knowledge generated in Theme 1 to express new antigens in established systems



Further development of effective candidates - strengthening of the DIVA potential

Theme 3: Move ASFV-G-Δ1177L towards central licensing at the European Medicines Agency



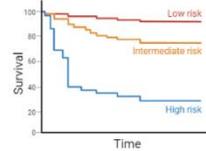
Safety and efficacy tests with oral and intramuscular vaccination

Bait uptake studies and proof of concept in European wild boar



Establish contact with the European Medicines Agency and other regulators towards licensing

Theme 4: Designing and validating vaccination concepts for domestic pigs and wild boar



Modelling vaccination using safety and efficacy data generated in the project



Optimize oral vaccination of wild boar taking into account wild boar ecology



Involve regulators, hunters, farmers, veterinary authorities and other stakeholders

Understanding performance characteristics of live attenuated vaccines for the prevention and control of African swine fever in wild boar and domestic pigs

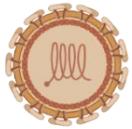
ASF vaccines - Turning dream into reality?



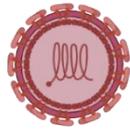
	Safety Studies	Efficacy Studies	Oral or ON studies	Field experience
ASFV-G-ΔI177L	✓ IM	✓ IM	✓	✓ IM
ASFV-G-ΔMGF	✓ IM	✓ IM	✓	(✓) IM
ASFV-G-Δ9GL/UK	✓ IM	✓ IM	✗	✗

Why's it gotta be so complicated?

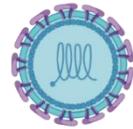
CSF



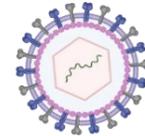
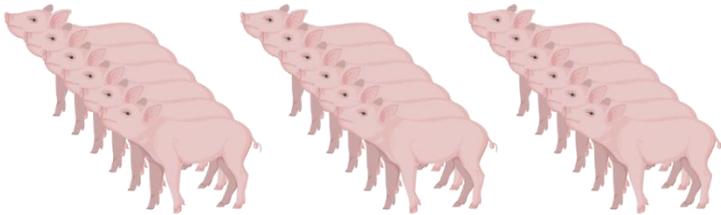
CP7_E2alf



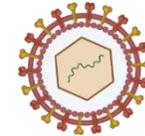
flc11



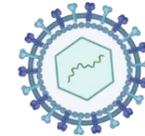
C-Strain



ASFV-G-ΔI177L

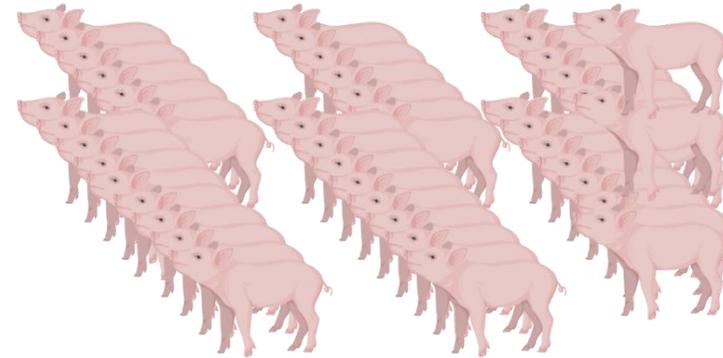


ASFV-G-Δ9GL/UK



ASFV-G-ΔMGF

ASF



Response rate:

- CP7_E2alf: 100%
- flc 11: 100%
- C-Strain: 100%

Challenge - efficacy in responders

- CP7_E2alf: 100%
- flc 11: 100%
- C-Strain: 100%

Response rate:

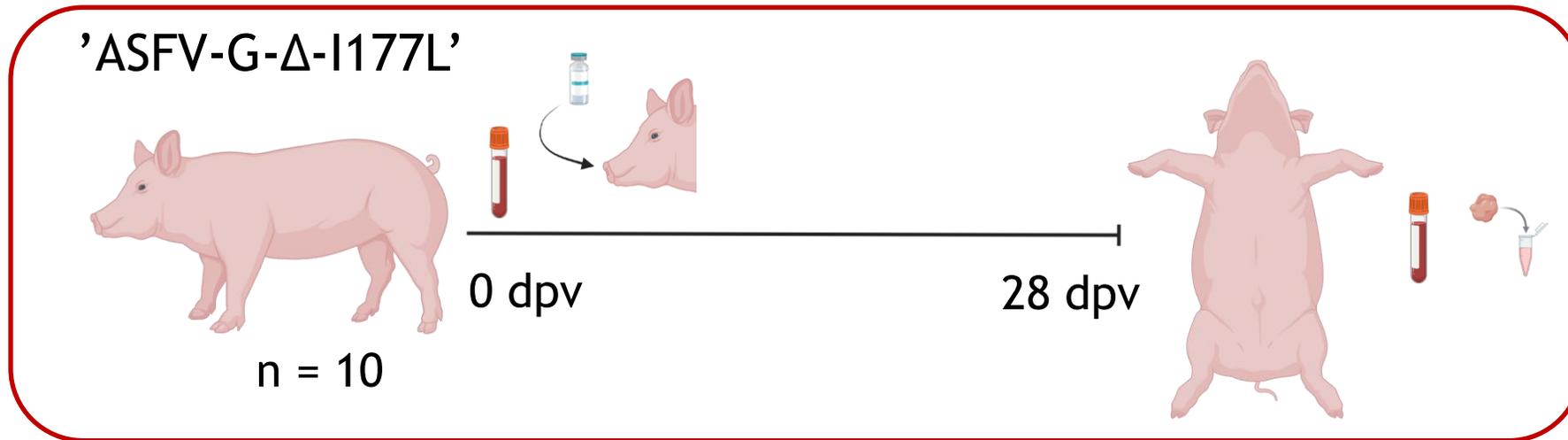
- ASFV-G-ΔI177L: 20%
- ASFV-G-Δ9GL/UK: 0%
- ASFV-G-ΔMGF: 0%

Challenge - efficacy in responders

- ASFV-G-ΔI177L: 100%
- ASFV-G-Δ9GL/UK: no responders, no protection
- ASFV-G-ΔMGF: no responders, no protection



Optimization of oral bait vaccination



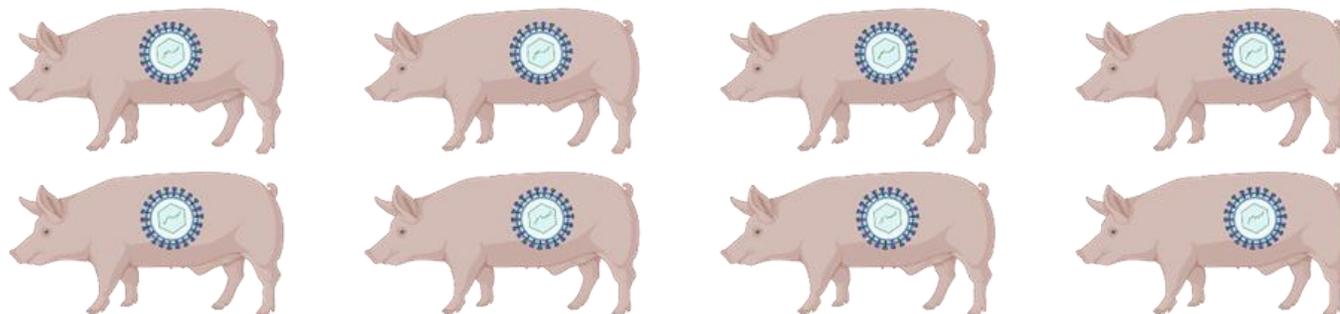
1. Without bait and increased titer
2. Montanide GR
3. Inactivated Mycobacteria
4. Walnut shreds
5. 5 x bait
6. Montanide Gel



Safety in adult breeding boars

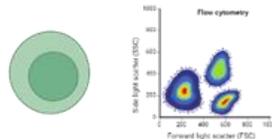


Intramuskuläre Inokulation
ASFV-G-ΔI177L 10^{4.5} HAU



Probennahme: Spermia, Nativblut und EDTA-Blut an den Tagen -4, 0, 4, 7, 10, 14, 21 und 28 nach der Inokulation.
Nicht-invasive Kastrickbeprobung in den Stalleinheiten.
Pathologisch-anatomische und histopathologische Untersuchung

Durchflusszytometrie
und Restimulation



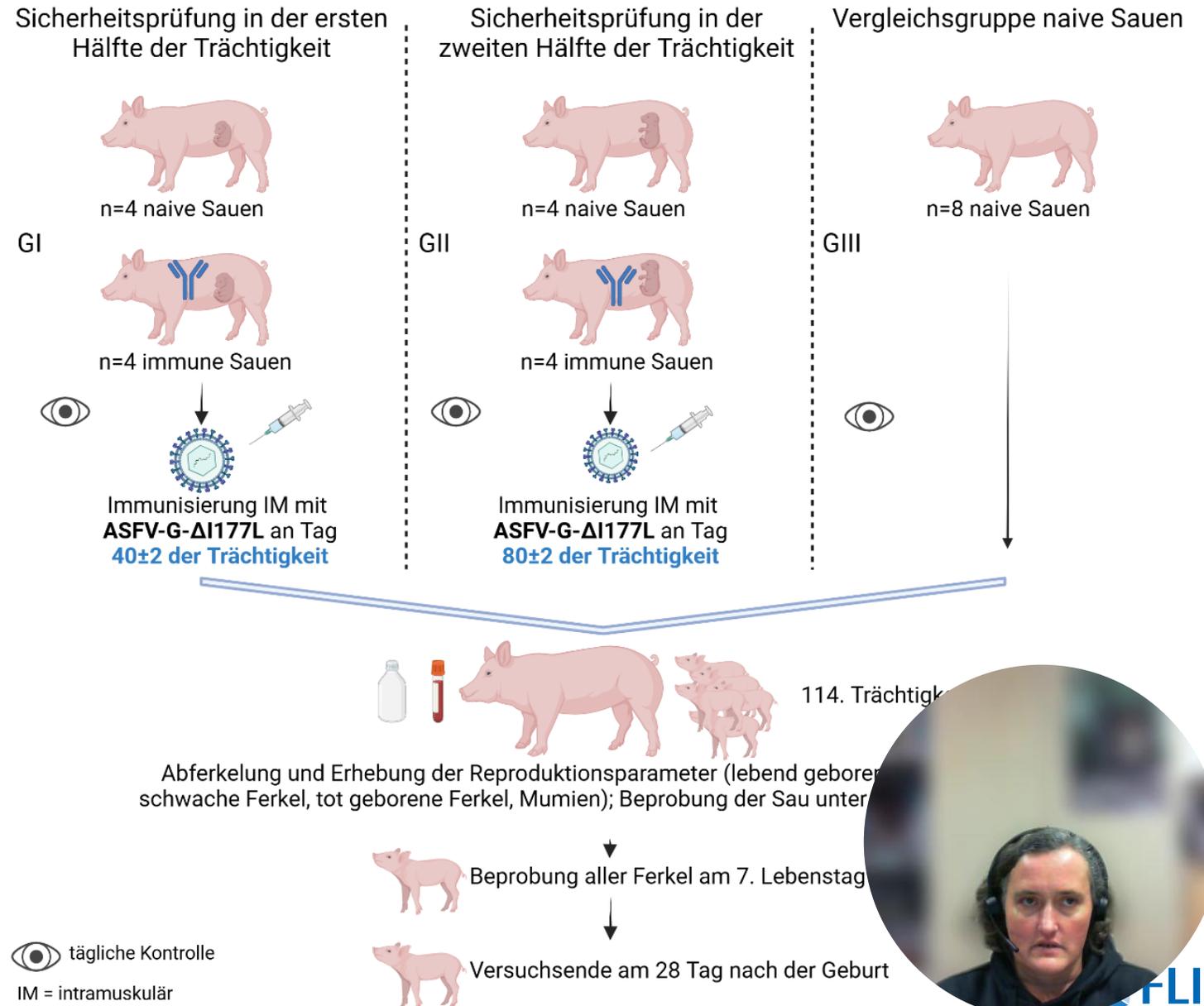
qPCR aller Proben; ggf. Virusisolierung für
positive Matrizes, Serologie (ELISA und IIPT)



In a nutshell: the vaccine is NOT safe for adult boars...

- High fever (even over 41 °C) in almost all boars
- High viral (genomic) loads in blood and organs
- Detection of viral genome and virus in semen
- 4 out of 8 boars reached the moderate humane endpoint

Ongoing and future studies



Between the devil and the deep blue sea

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN An African swine fever vaccine-like variant with multiple gene deletions caused reproductive failure in a Vietnamese breeding herd

Thanh Che Nguyen^{1,2,4,11}, Nga To Thi Bui^{3,11}, Lua Thi Nguyen³,
Tram Ngoc Thi Ngo⁴, Cuong Van Nguyen⁴, Luan Minh Nguyen⁴, Janin Nouhin⁵,
Erik Karlsson⁵, Pawin Padungtod⁶, Nakarin Pamornchainavakul⁷,
Sawang Kesdangakonwut^{2,8,9}, Roongroje Thanawongnuwech^{2,10} & Duy Tien Do⁴



- Introduction of a vaccine like ASFV variant into a breeding farm without prior outbreak or vaccination history
- Farm with 2.400 sows (Land race/Yorkshire); very high replacement rate from external sources
- Poor biosecurity, active ASF circulation in the surrounding area
- No clinical signs in gilts, but reproductive disorders in pregnant animals
- Ulcerative lesions on the udders of lactating sows, necrotizing vasculitis, suspected immune system involvement
- Virus with characteristics of ASFV-G- Δ MGF, but also additional mutations (including EP402R)



Like that wasn't enough...

nature communications

Explore content ▾ About the journal ▾ Publish with us ▾

[nature](#) > [nature communications](#) > [articles](#) > [article](#)

Article | [Open access](#) | Published: 29 May 2023

Highly lethal genotype I and II recombinant African swine fever viruses detected in pigs

[Dongming Zhao](#), [Encheng Sun](#), [Lianyu Huang](#), [Leilei Ding](#), [Yuanmao Zhu](#), [Jiwen Zhang](#), [Dongdong Shen](#), [Xianfeng Zhang](#), [Zhenjiang Zhang](#), [Tao Ren](#), [Wan Wang](#), [Fang Li](#), [Xijun He](#) & [Zhigao Bu](#) 

[Nature Communications](#) **14**, Article number: 3096 (2023) | [Cite this article](#)

15k Accesses | 87 Citations | 20 Altmetric | [Metrics](#)

- Recombinant of genotypes I and II
- Our vaccines would NOT protect against this chimera
- Reported in China, Vietnam and the Russian Federation
- New vaccine deletion mutants are under development...

