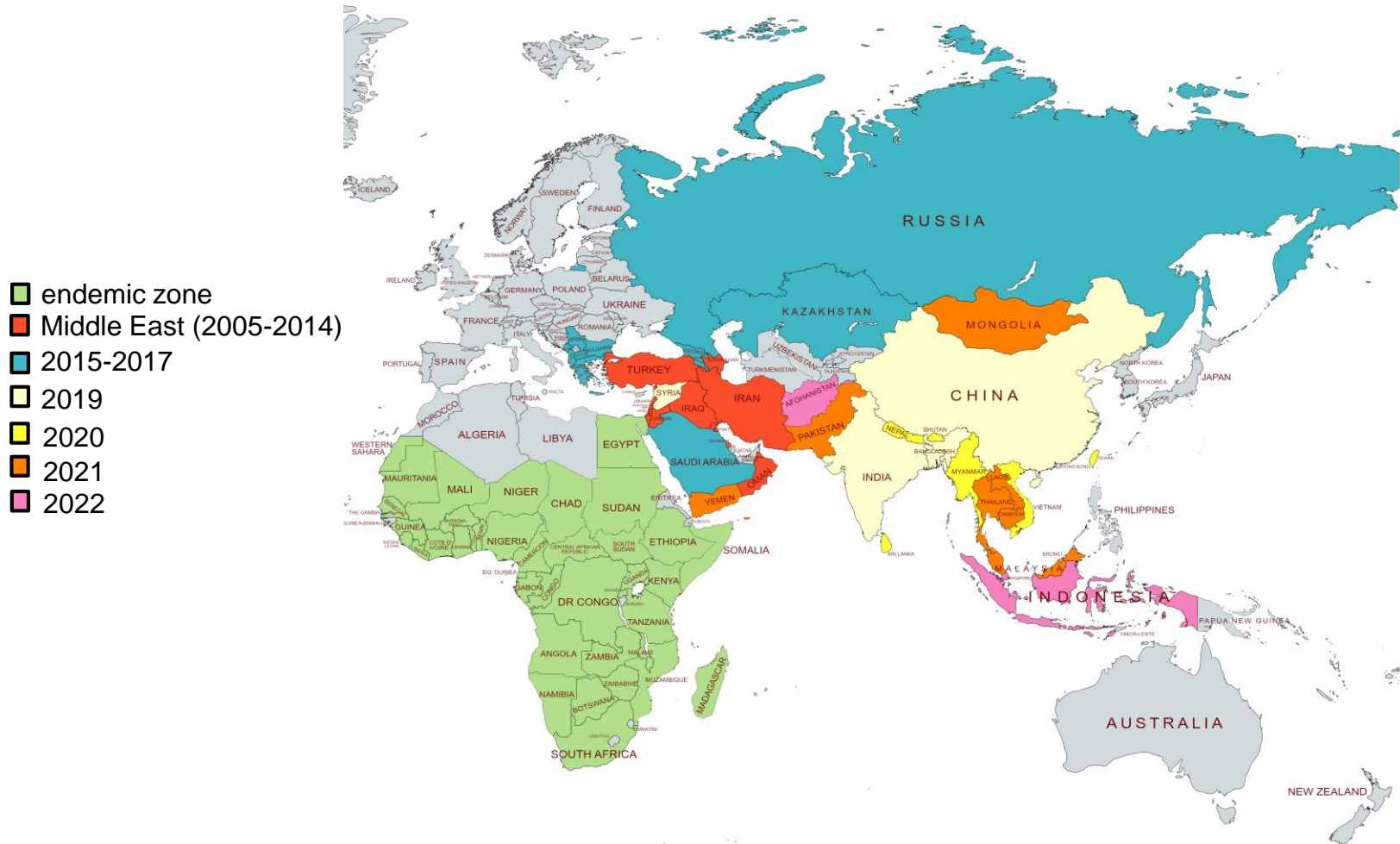




Global LSDV situation with focus on the recombinant LSDV strains predominating the LSDV epidemic in South-East Asia

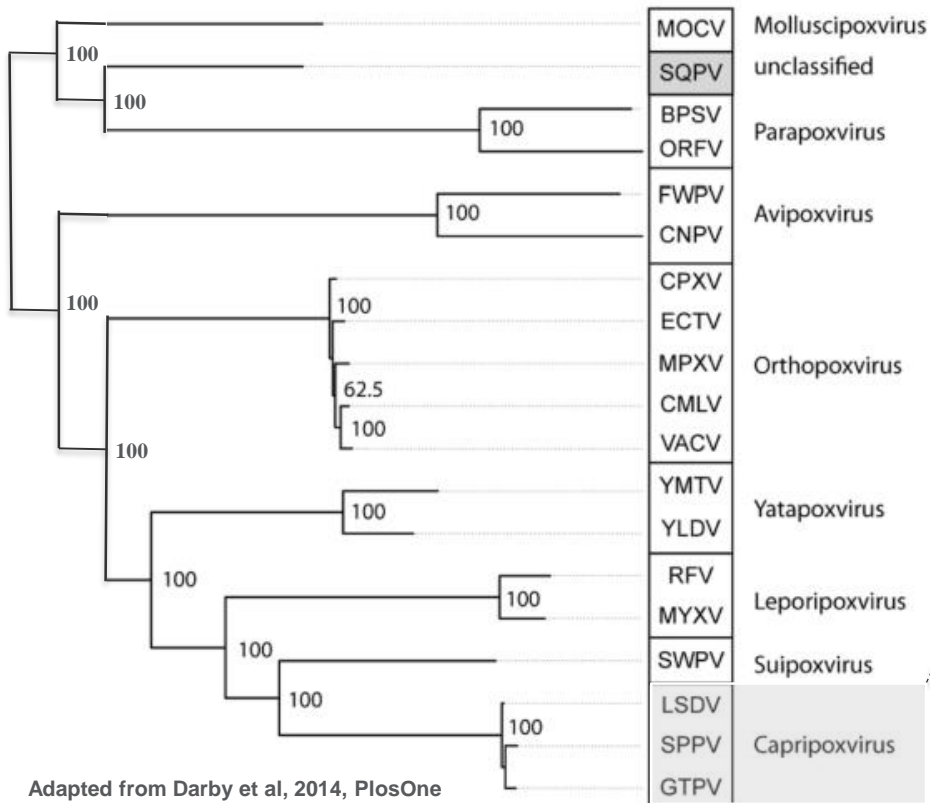
NICK DE REGGE

LSDV epidemiology

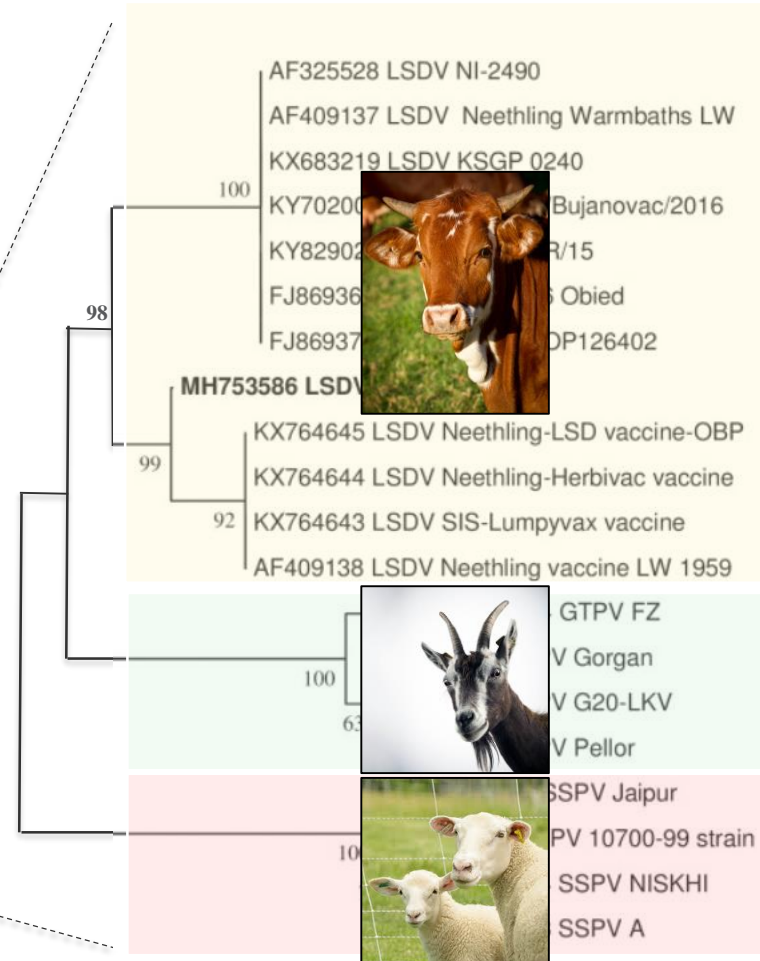


Poxviridae

Chordopoxvirinae

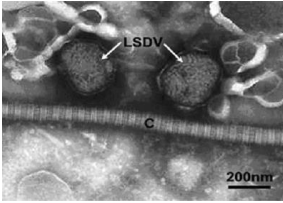


Adapted from Darby et al, 2014, PlosOne



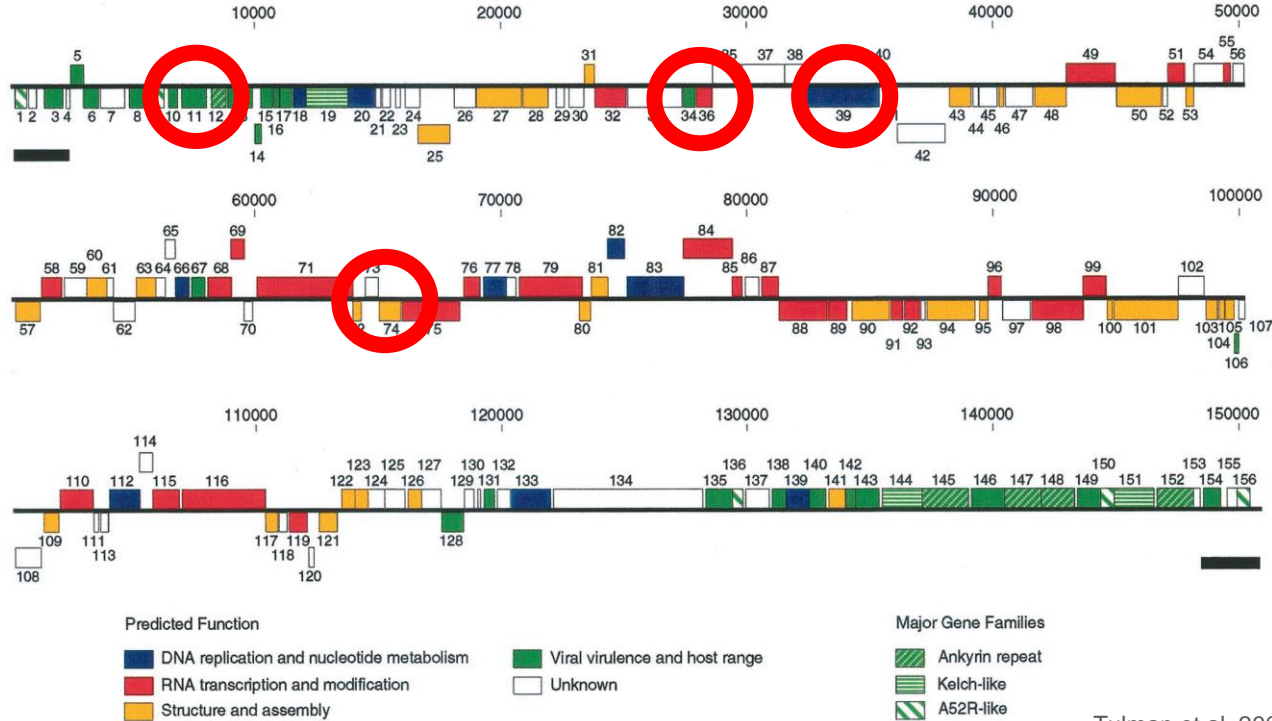
Adapted from Sprygin et al, 2018, PlosOne

LSDV genome



Tageldin et al 2014

- Not segmented, dsDNA
- Size: 150 to 160 kbp → +/- 150 putative genes
- High nucleotide identity between SPPV, GTPV, LSDV: +/- 96%

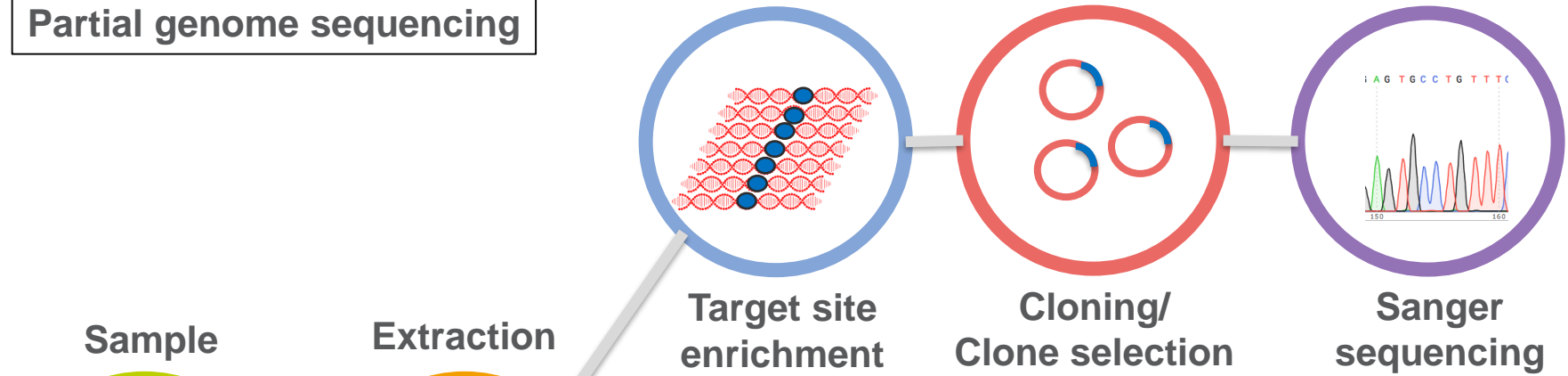


Tulman et al. 2001

FIG. 1. Linear map of the LSDV genome. ORFs are numbered from left to right based on the position of the methionin ORFs transcribed to the right are located above the horizontal line; ORFs transcribed to the left are below. Genes with sim members of gene families are colored according to the figure key. ITRs are represented as black bars below the ORF map.

LSDV genome sequencing

Partial genome sequencing



Sample

Extraction

Target site enrichment

Cloning/
Clone selection

Sanger sequencing


host + viral DNA

LSDV enrichment

Library prep

NGS 

Contents lists available at [ScienceDirect](#)

 **Journal of Virological Methods**

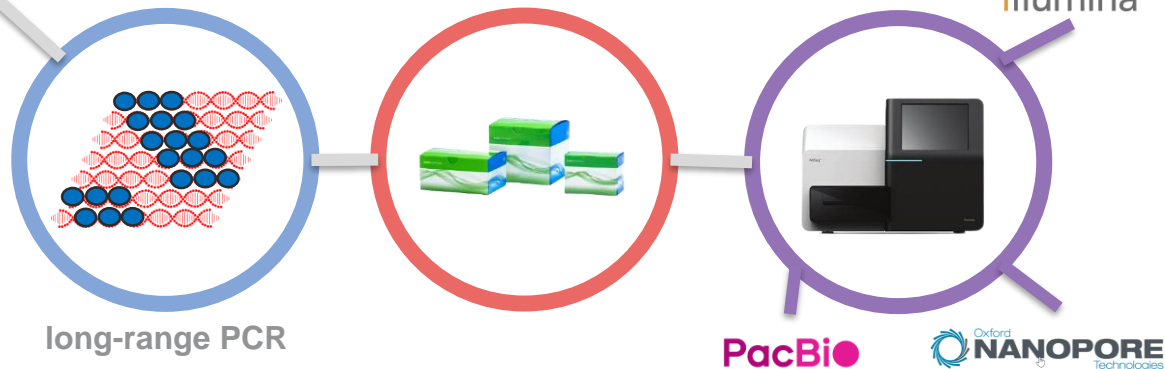
journal homepage: www.elsevier.com/locate/jvromet

Protocols

A robust, cost-effective and widely applicable whole-genome sequencing protocol for capripoxviruses

Elisabeth Mathijs*, Andy Haegeman, Kris De Clercq, Steven Van Born, Frank Vandenbussche

Full genome sequencing

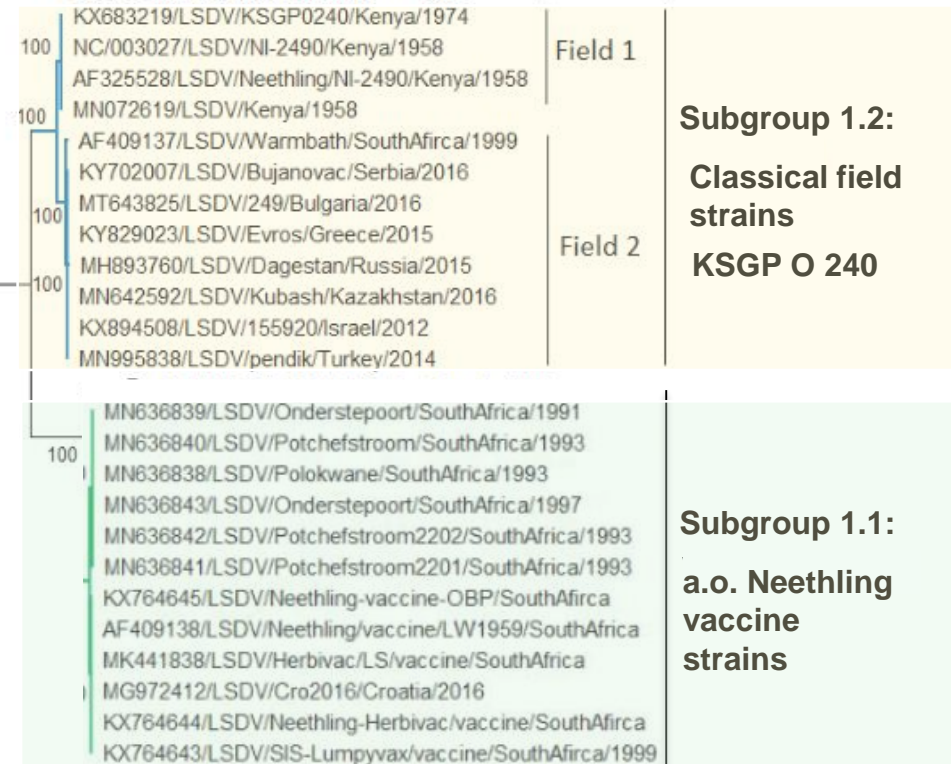


long-range PCR





LSDV epidemiology till 2017

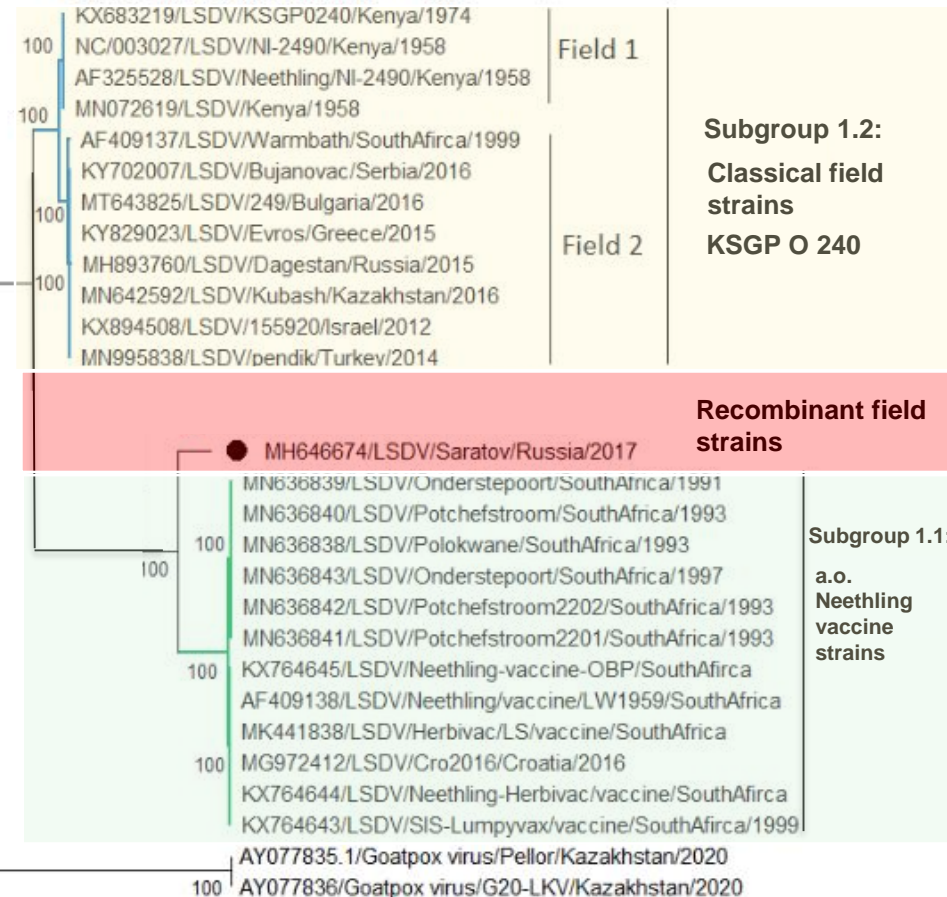


AY077835.1/Goatpox virus/Pellor/Kazakhstan/2020
100 AY077836/Goatpox virus/G20-LKV/Kazakhstan/2020

Poxviruses have low mutation rate



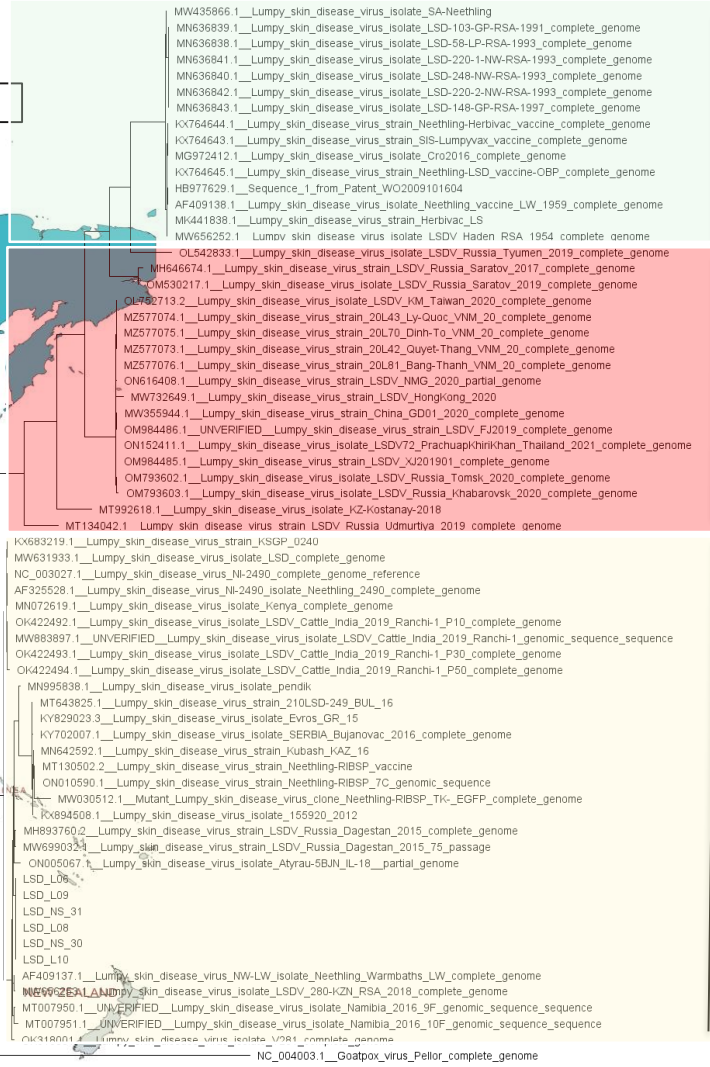
LSDV epidemiology till 2017



Recombinant strains cluster more closely to vaccine strains, but behave as wild type field strains

Adapted from Ma et al, 2022 – based on full genome sequences

LSDV epidemiology 2019-2022



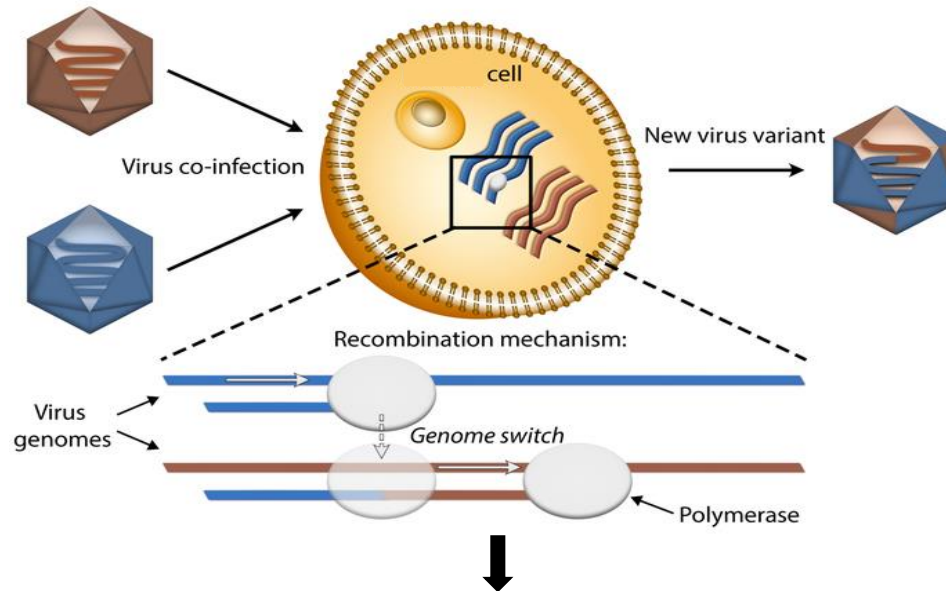
Subgroup 1.1:
a.o. Neethling vaccine strains

Recombinant field strains

Subgroup 1.2:
Classical field strains
KSGP O 240

NC_004002.1_Sheeppox_virus_17077-99_complete_genome

Recombinant LSDV strains



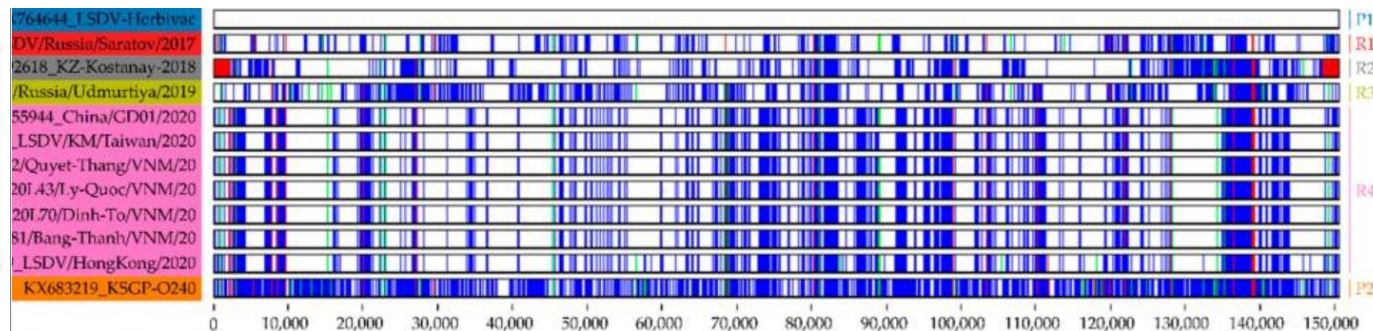
Single recombination

LSDV recombinants

Neethling vaccine

Recombinant strains

KSGP-O-240



> 120 recombination events

Recombinant LSDV strains - origin

- Natural recombination in the field? Safety of LAV LSDV vaccines?
- Posterior vaccine control of LSDV vaccine used in Kazakhstan before the emergence of recombinant strains

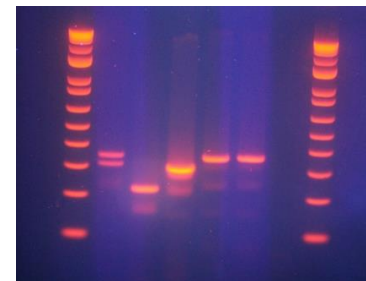
1. Confirming vaccine virus Titer ✓

2. Check for contaminants using real-time PCR ✓

- Bluetongue virus - BTV
- Foot-and-mouth disease virus – FMD
- Epizootic hemorrhagic disease virus – EHDV
- Rift Valley fever virus – RVFV
- Parapox – PaPx

3. Confirming capripox, LSDV, vaccine status using (real-time) PCR !

- Pan Capripox
- DIVA 1: Vac: Pos - Field type: Pos !!
- DIVA 2: Vac: Pos - Field type: Pos !!
- DIVA 3: LSDV – Field type SPPV/GTPV

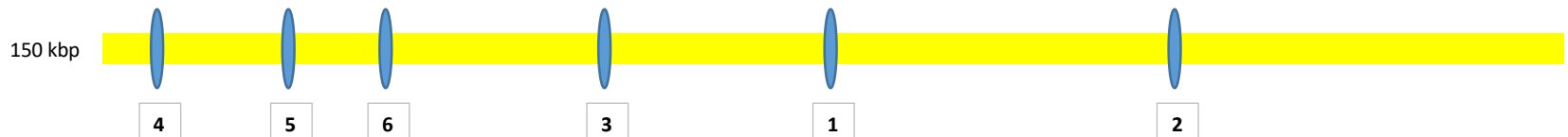


1kb ladder
1. Lumpivax vaccine
2. SPPV RM65 vaccine
3. Field type SPPV
4. Field type LSDV
5. LSDV vaccine (Neehtling)
6 Neg
1kb ladder

Recombinant LSDV strains - origin

4. Partial genome sequencing

- Region 1, 2, 3 → 1 clone sequenced → **Field type GTPV**
- Region 6 → 1 clone sequenced → **LSDV**
- Region 4 → 24 clone sequenced → **Field type GTPV + Field type LSDV + Neethling vaccine**
- Region 5 → 22 clone sequenced → **Field type LSDV + Neethling vaccine**



- Neethling vaccine LSDV
- Field type LSDV
- Field type GTPV
- Suggestive for presence of recombinants



Article

The Importance of Quality Control of LSDV Live Attenuated Vaccines for Its Safe Application in the Field

Andy Haegeman ^{1,*}, Ilse De Leeuw ¹, Meruyert Saduakassova ², Willem Van Campe ³, Laetitia Aerts ⁴, Wannes Philips ⁴, Akhmetzhan Sultanov ², Laurent Mostin ³ and Kris De Clercq ¹

Recombinant LSDV strains - origin

5. Full length genome sequencing of two vaccine batches using Illumina
 - De novo assembly failed
 - Mapping reads using perfect mode
 - Presence of neethling strain
 - Presence of KSGP-O-240
 - Presence of GTPV

 - Clear differences in constitution between both vaccine batches
 - Iterative assembly strategy
 - Presence of neethling strain
 - Presence of KSGP-O-240
 - Presence of GTPV
 - Presence of recombinant strains

Recombinant LSDV strains - origin

5. Full length genome sequencing

- Conclusion



- Neethling like LSDV vaccine strain
- KSGP-like LSDV vaccine strain
- Sudan-like GTPV strain
- Multiple recombinant strains (almost) identical to recently described recombinant vaccine-like strains
- Most likely source of recombinant strains in the field
- Highlights importance of an independent vaccine quality control

One specific badly produced and insufficiently controlled LSDV vaccine was responsible for the release of recombinant LSDV strains in the field

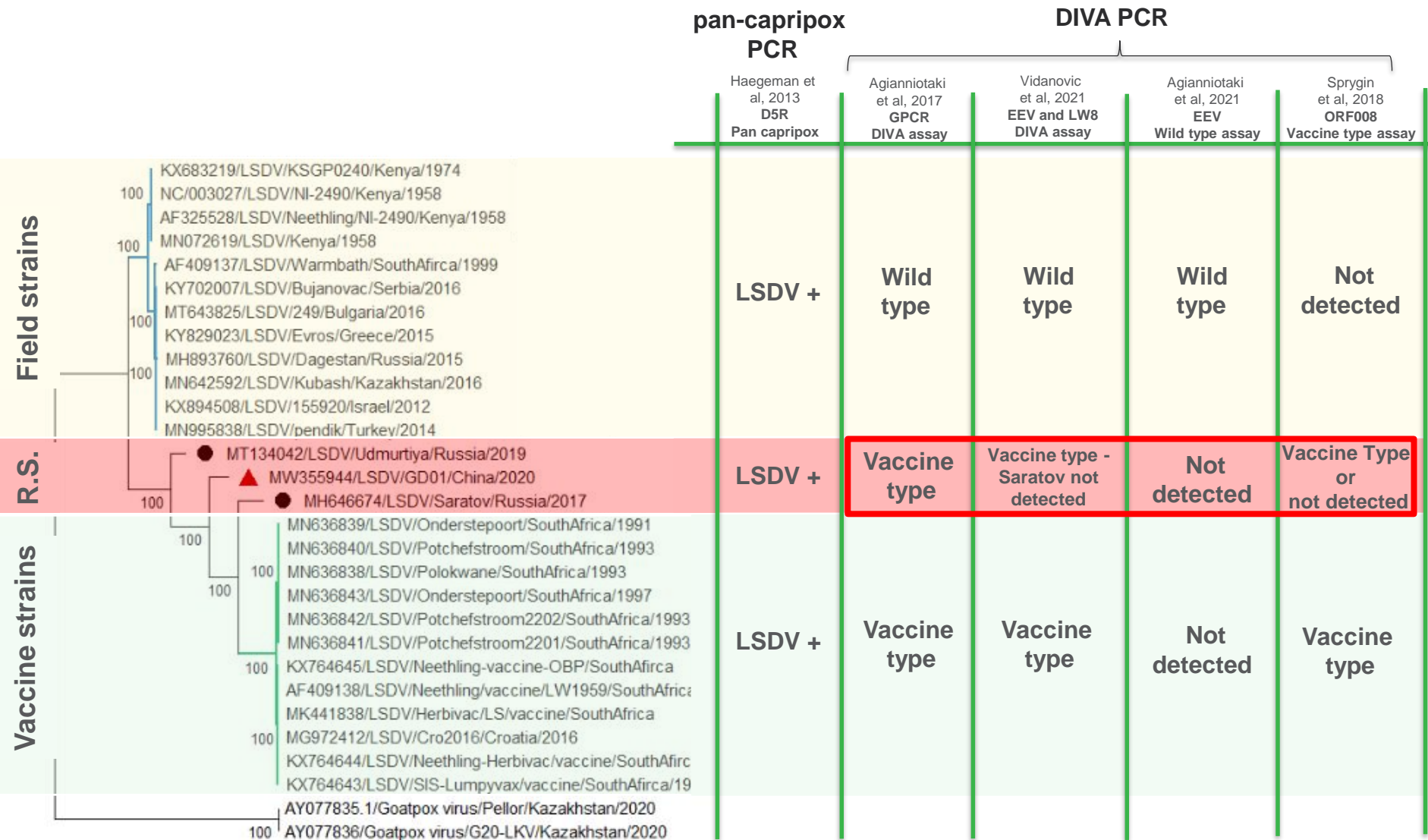


Article

Recombinant LSDV Strains in Asia: Vaccine Spillover or Natural Emergence?

Frank Vandenbussche ^{1,*}, Elisabeth Mathijs ^{1,*}, Wannes Philips ¹, Meruyert Saduakassova ², Ilse De Leeuw ³, Akhmetzhan Sultanov ², Andy Haegeman ³ and Kris De Clercq ^{3,*}

Recombinant LSDV strains - diagnostics



Recombinant LSDV strains - diagnostics

Haegeman et al, 2013
D5R
Pan capripox

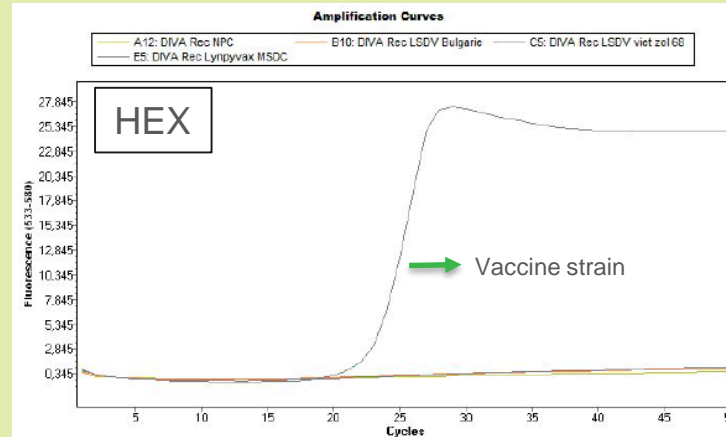
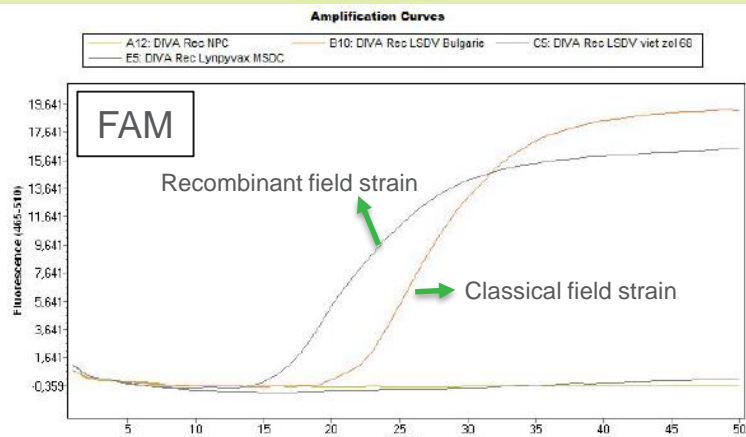
Agianniotaki et al, 2017
GPCR
DIVA assay

Vidanovic et al, 2021
EEV and LW8
DIVA assay

Agianniotaki et al, 2021
EEV
Wild type assay

Under development
DIVA assay

- New DIVA test under development at EURL:
 - 'recombinant' and 'classical' field strains identified as wild type strains
 - Neethling based vaccine strains identified as vaccine strains
 - in silico: ok!
 - validation parameters: ok!



- Combination with existing DIVA test will allow to downstream differentiate 'recombinant' from 'classical' field LSDV strains

Wild type

Wild type

Vaccine type

Field strains

R.S.

Vaccine strains

Recombinant LSDV strains – pathogenesis, transmission, vaccine efficacy

➤ Impact pathogenesis-virulence?

➤ [Transbound Emerg Dis. 2021 May;68\(3\):1377-1383. doi: 10.1111/tbed.13798. Epub 2020 Sep 20.](#)

A lumpy skin disease virus which underwent a recombination event demonstrates more aggressive growth in primary cells and cattle than the classical field isolate

Svetlana Kononova¹, Aleksandr Kononov¹, Irina Shumilova¹, Olga Byadovskaya¹, Alexander Nesterov¹, Pavel Prutnikov¹, Shawn Babiuk², Alexander Sprygin¹

➤ Impact transmission?

➤ [Front Vet Sci. 2022 Oct 20;9:1001426. doi: 10.3389/fvets.2022.1001426. eCollection 2022.](#)


Experimentally controlled study indicates that the naturally occurring recombinant vaccine-like lumpy skin disease strain Udmurtiya/2019, detected during freezing winter in northern latitudes, is transmitted *via* indirect contact

Alexander Nesterov¹, Ali Mazloun¹, Olga Byadovskaya¹, Irina Shumilova¹, Antoinette Van Schalkwyk^{2,3}, Alena Krotova¹, Vladimir Kirpichenko⁴, Irina Donnik⁵, Ilya Chvala¹, Alexander Sprygin¹

SHORT COMMUNICATION

Transboundary and Emerging Diseases WILEY

Overwintering of recombinant lumpy skin disease virus in northern latitudes, Russia

Irina Shumilova¹ | Alena Krotova¹ | Alexander Nesterov¹ | Olga Byadovskaya¹ | Antoinette van Schalkwyk² | Alexander Sprygin¹ 

 pathogens



Article

A Recombinant Vaccine-like Strain of Lumpy Skin Disease Virus Causes Low-Level Infection of Cattle through Virus-Inoculated Feed

Irina Shumilova¹, Alexander Nesterov¹, Olga Byadovskaya¹, Pavel Prutnikov¹, David B. Wallace^{2,3}, Maria Mokeeva¹, Valeriy Pronin¹, Aleksandr Kononov¹, Ilya Chvala¹ and Alexander Sprygin¹ 

➤ Impact vaccine efficacy?

Thanks to the EC for the support

EU Reference Laboratory for Capripox viruses



Funded by the
European Union

Contact: nick.deregge@sciensano.be