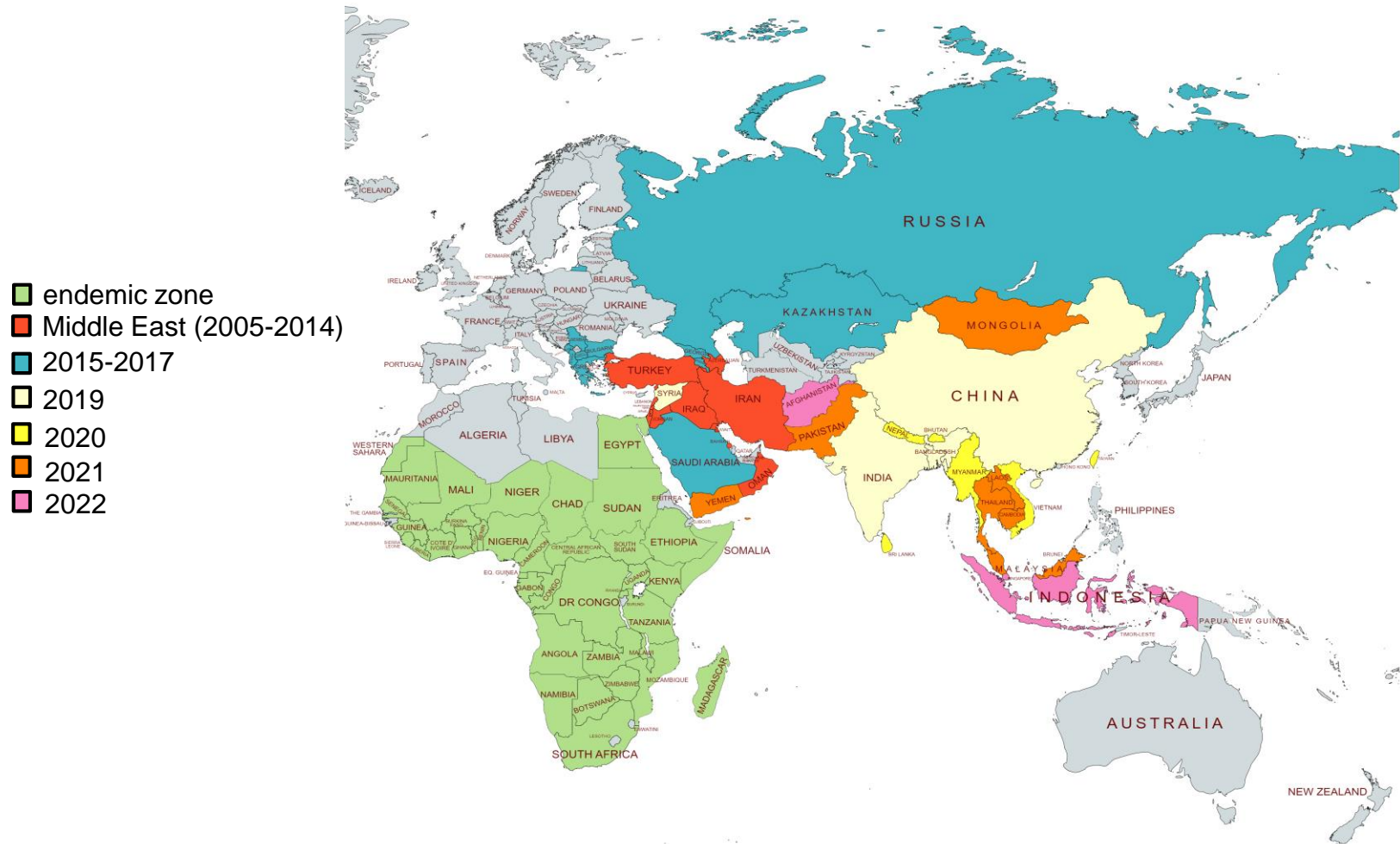




# EURL ACTIVITIES IN SUPPORT OF LSDV DIAGNOSIS AND CONTROL

-  
NICK DE REGGE

# LSDV epidemiology



# LSDV epidemiology till 2017

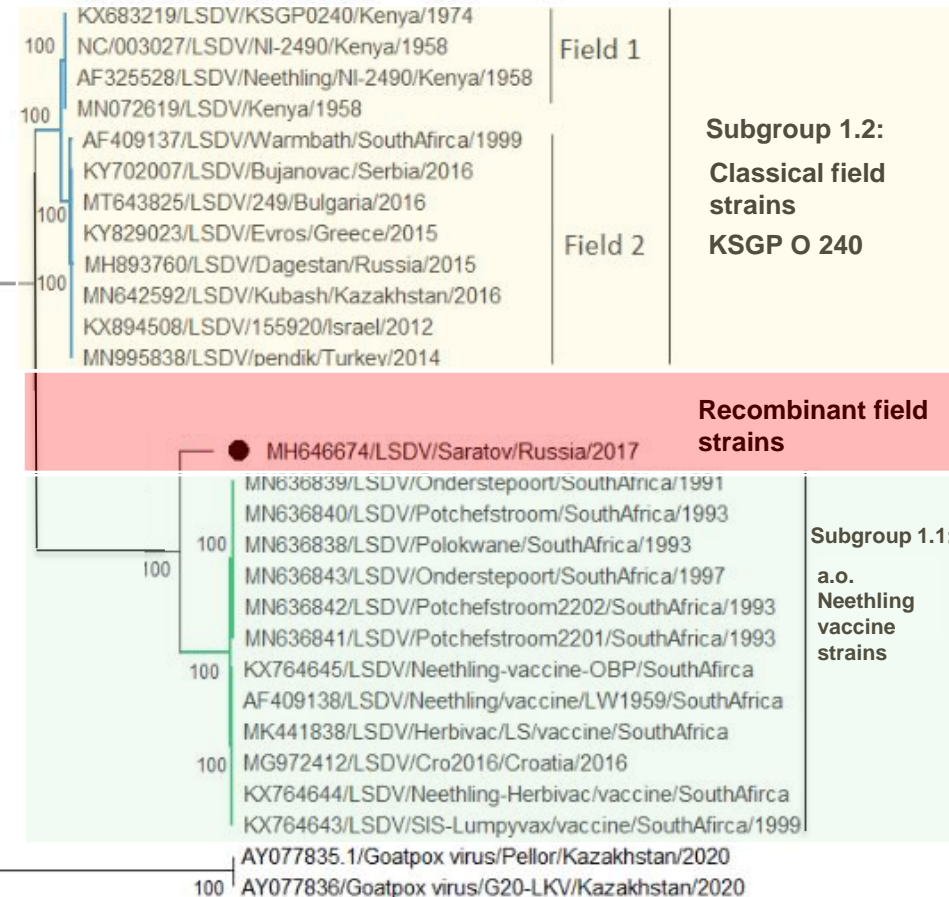


100	KX683219/LSDV/KSGP0240/Kenya/1974	Field 1	<b>Subgroup 1.2:</b> <b>Classical field strains</b> <b>KSGP O 240</b>
100	NC/003027/LSDV/NI-2490/Kenya/1958		
100	AF325528/LSDV/Neethling/NI-2490/Kenya/1958		
100	MN072619/LSDV/Kenya/1958	Field 2	
100	AF409137/LSDV/Warmbath/SouthAfrica/1999		
100	KY702007/LSDV/Bujanovac/Serbia/2016		
100	MT643825/LSDV/249/Bulgaria/2016		
100	KY829023/LSDV/Evros/Greece/2015		
100	MH893760/LSDV/Dagestan/Russia/2015		
100	MN642592/LSDV/Kubash/Kazakhstan/2016		
100	KX894508/LSDV/155920/Israel/2012		
100	MN995838/LSDV/pendik/Turkey/2014		
100	MN636839/LSDV/Onderstepoort/SouthAfrica/1991	<b>Subgroup 1.1:</b> <b>a.o. Neethling vaccine strains</b>	
100	MN636840/LSDV/Potchefstroom/SouthAfrica/1993		
100	MN636838/LSDV/Polokwane/SouthAfrica/1993		
100	MN636843/LSDV/Onderstepoort/SouthAfrica/1997		
100	MN636842/LSDV/Potchefstroom2202/SouthAfrica/1993		
100	MN636841/LSDV/Potchefstroom2201/SouthAfrica/1993		
100	KX764645/LSDV/Neethling-vaccine-OBP/SouthAfrica		
100	AF409138/LSDV/Neethling/vaccine/LW1959/SouthAfrica		
100	MK441838/LSDV/Herbivac/LS/vaccine/SouthAfrica		
100	MG972412/LSDV/Cro2016/Croatia/2016		
100	KX764644/LSDV/Neethling-Herbivac/vaccine/SouthAfrica		
100	KX764643/LSDV/SIS-Lumpyvax/vaccine/SouthAfrica/1999		
100	AY077835.1/Goatpox virus/Pellor/Kazakhstan/2020		
100	AY077836/Goatpox virus/G20-LKV/Kazakhstan/2020		

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



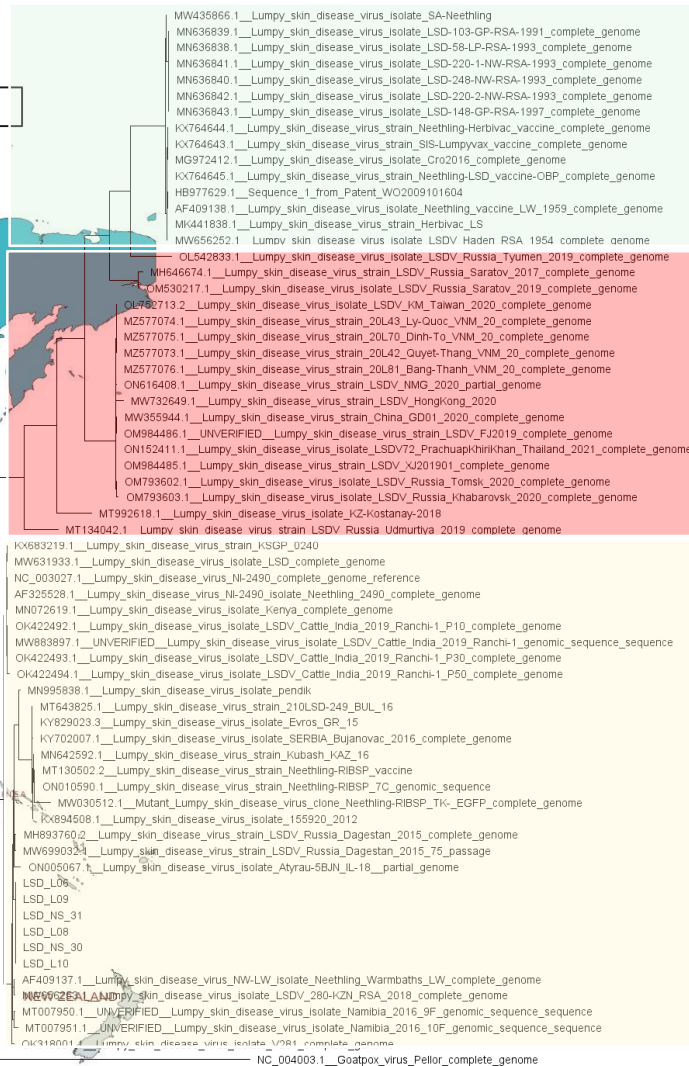
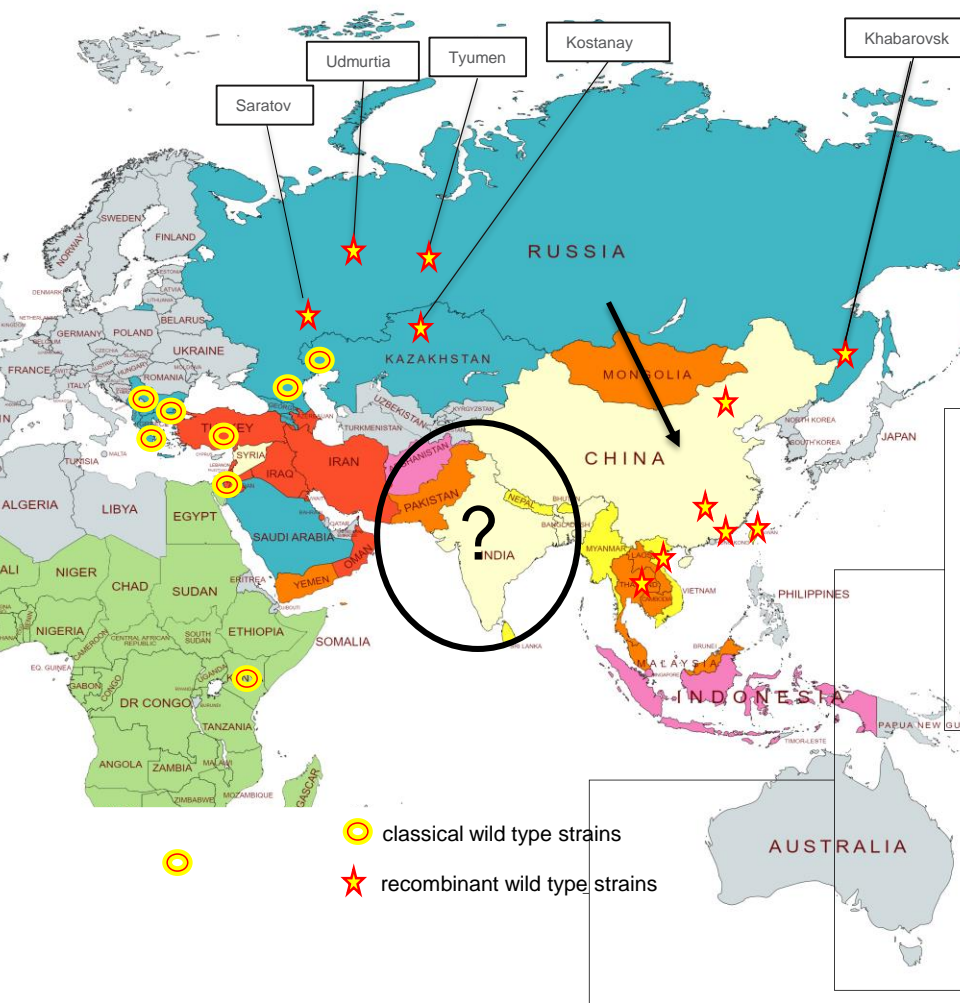
# LSDV epidemiology till 2017



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

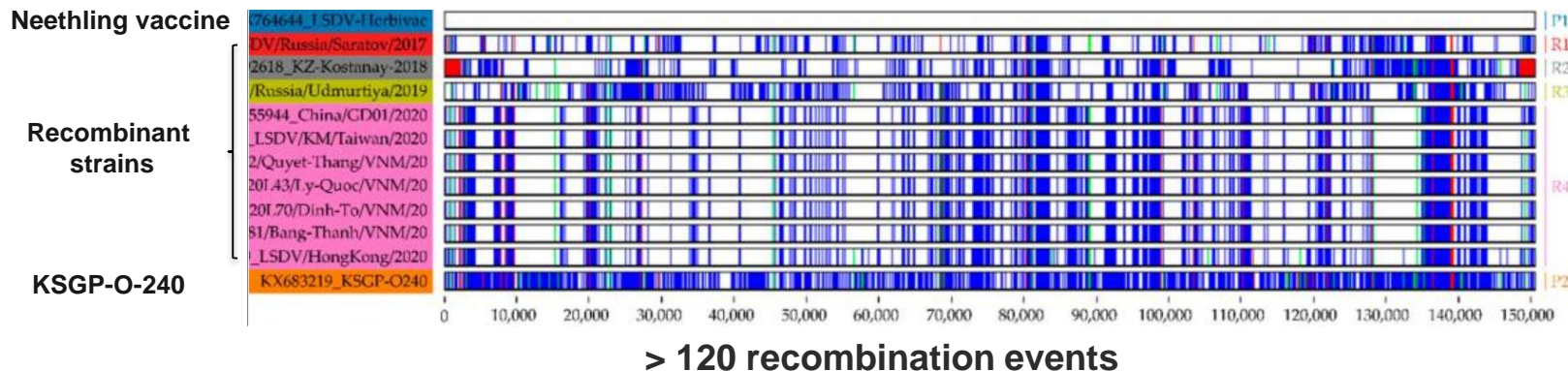
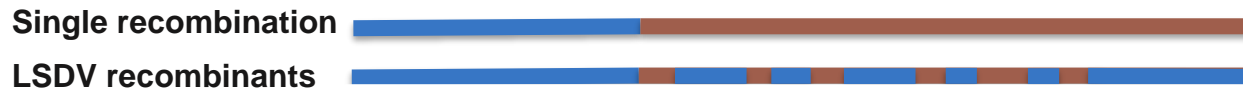
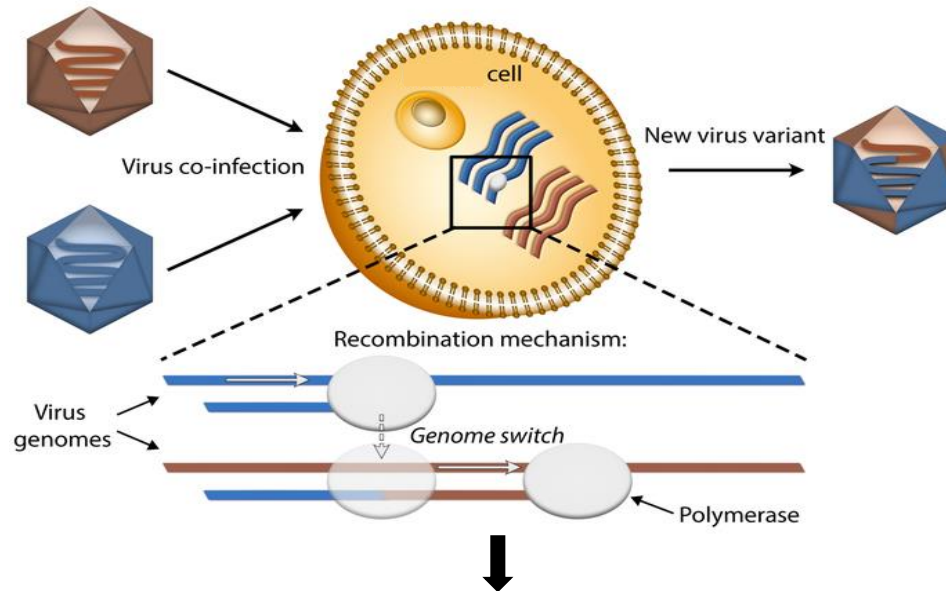
Adapted from Ma et al, 2022 – based on full genome sequences

# LSDV epidemiology 2019-2022





# Recombinant LSDV strains



# 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

Vaccine quality control: PCRs, partial sequencing, full length genome sequencing



- 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



Article

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

Andy Haegeman <sup>1,\*</sup>, Ilse De Leeuw <sup>1</sup>, Meruyert Saduakassova <sup>2</sup>, Willem Van Campe <sup>3</sup>, Laetitia Aerts <sup>4</sup>, Wannes Philips <sup>4</sup>, Akhmetzhan Sultanov <sup>2</sup>, Laurent Mostin <sup>3</sup> and Kris De Clercq <sup>1</sup>



Article

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

Frank Vandenbussche <sup>1,†</sup>, Elisabeth Mathijs <sup>1,†</sup>, Wannes Philips <sup>1</sup>, Meruyert Saduakassova <sup>2</sup>, Ilse De Leeuw <sup>3</sup>, Akhmetzhan Sultanov <sup>2</sup>, Andy Haegeman <sup>3,\*</sup> and Kris De Clercq <sup>3,\*</sup>

# 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

Vaccine quality control: PCRs, partial sequencing, full length genome sequencing



- 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

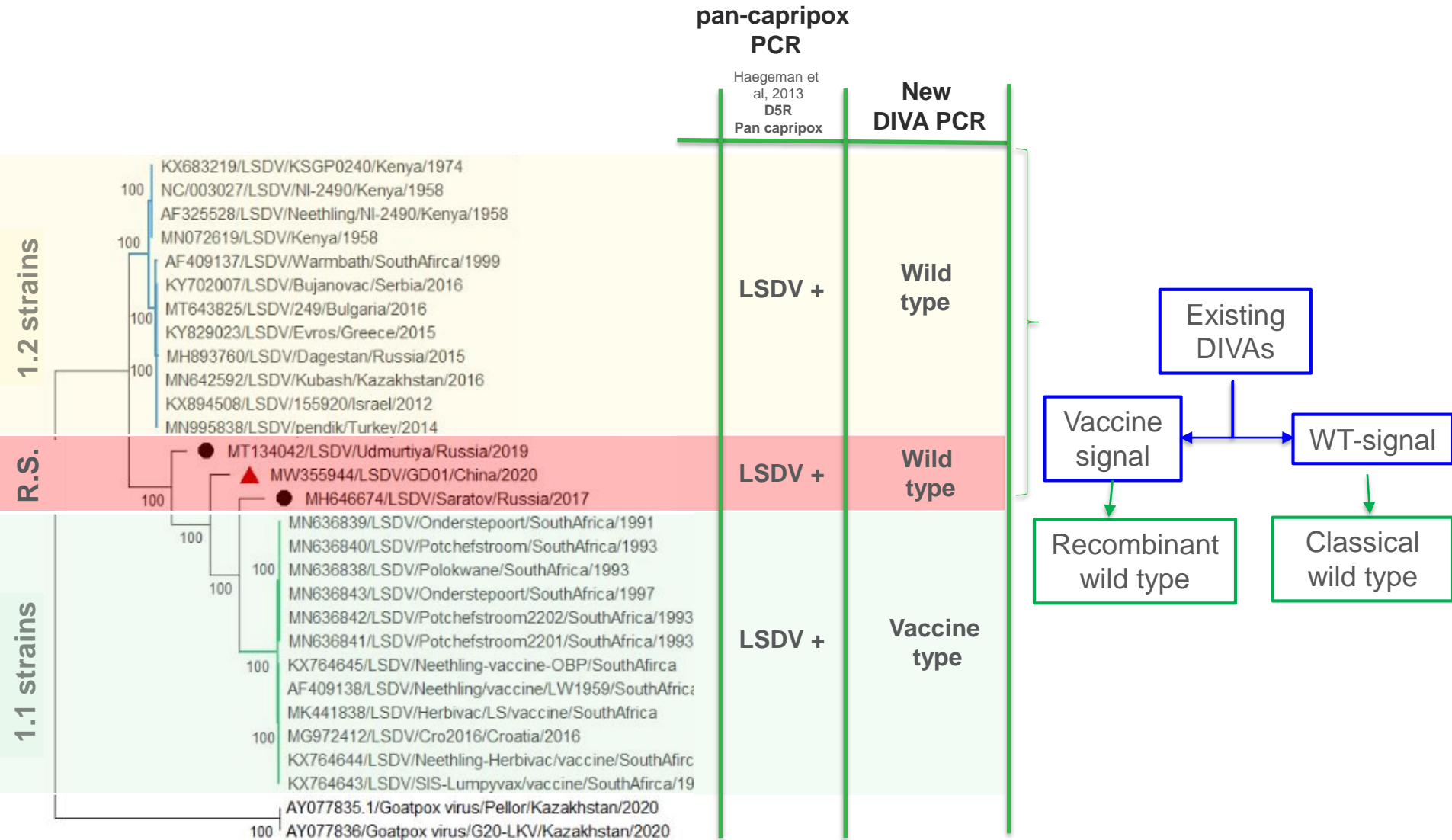
↳ Highlights that efforts need to be done to stimulate a thorough vaccine batch quality control



# Recombinant LSDV strains - diagnostics

		pan-capripox PCR		DIVA PCR		
		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	Sprygin et al, 2018 ORF008 Vaccine type assay
1.2 strains	<p>KX683219/LSDV/KSGP0240/Kenya/1974 NC/003027/LSDV/NI-2490/Kenya/1958 AF325528/LSDV/Neethling/NI-2490/Kenya/1958 MN072619/LSDV/Kenya/1958 AF409137/LSDV/Warmbath/SouthAfrica/1999 KY702007/LSDV/Bujanovac/Serbia/2016 MT643825/LSDV/249/Bulgaria/2016 KY829023/LSDV/Evros/Greece/2015 MH893760/LSDV/Dagestan/Russia/2015 MN642592/LSDV/Kubash/Kazakhstan/2016 KX894508/LSDV/155920/Israel/2012 MN995838/LSDV/pendik/Turkey/2014</p>	LSDV +	Wild type	Wild type	Wild type	Not detected
R.S.	<p>● MT134042/LSDV/Udmurtiya/Russia/2019 ▲ MW355944/LSDV/GD01/China/2020 ● MH646674/LSDV/Saratov/Russia/2017</p>	LSDV +	Vaccine type	Vaccine type - Saratov not detected	Not detected	Vaccine Type or not detected
1.1 strains	<p>MN636839/LSDV/Onderstepoort/SouthAfrica/1991 MN636840/LSDV/Potchefstroom/SouthAfrica/1993 MN636838/LSDV/Polokwane/SouthAfrica/1993 MN636843/LSDV/Onderstepoort/SouthAfrica/1997 MN636842/LSDV/Potchefstroom2202/SouthAfrica/1993 MN636841/LSDV/Potchefstroom2201/SouthAfrica/1993 KX764645/LSDV/Neethling-vaccine-OBP/SouthAfrica AF409138/LSDV/Neethling/vaccine/LW1959/SouthAfrica MK441838/LSDV/Herbivac/LS/vaccine/SouthAfrica MG972412/LSDV/Cro2016/Croatia/2016 KX764644/LSDV/Neethling-Herbivac/vaccine/SouthAfrica KX764643/LSDV/SIS-Lumpyvax/vaccine/SouthAfrica/19 AY077835.1/Goatpox virus/Pellor/Kazakhstan/2020 AY077836/Goatpox virus/G20-LKV/Kazakhstan/2020</p>	LSDV +	Vaccine type	Vaccine type	Not detected	Vaccine type

# Recombinant LSDV strains - diagnostics





# Recombinant LSDV strains - diagnostics

- Wild type reaction

WT reaction	GGAACTCTGTGCAGAAATAAAGTACGA	-----	CATAAAATAGAGGGATTTGATGAG	---	CAGTGC GTTCCCTTCGG
LSDV WT Kubash2016 MN642592	.....	TGGCGAA	TTT	.....	TTT
LSDV WT 155920 2012 KX894508	.....	TGGCGAA	TTT	.....	TTT
LSDV WT Dagestan2015 MH893760	.....	TGGCGAA	TTT	.....	TTT
LSDV WT Evros GR2015 KY829023	.....	TGGCGAA	TTT	.....	TTT
LSDV WT SERBIA Bujanovac2016 KY702007	.....	TGGCGAA	TTT	.....	TTT
LSDV WT V281 OK318001	.....	TGGCGAA	TTT	.....	TTT
LSDV WT 280KZN RSA2018 MW656253	.....	TGGCGAA	TTT	.....	TTT
LSSV WT BUL16 MT643825	.....	TGGCGAA	TTT	.....	TTT
LSDV WT LW AF409137	.....	TGGCGAA	TTT	.....	TTT
LSDV Rec Saratov2017 MH646674	.....	CGGTGAA	TTT	.....	TTT
LSDV Rec Saratov2019 OM530217	.....	CGGTGAA	TTT	.....	TTT
LSDV Rec Khabarovsk2020 OM793603	.....	TGGCGAA	TTT	.....	TTT
LSDV Rec Tomsk2020 OM793602	.....	TGGCGAA	TTT	.....	TTT
LSDV KM Taiwan2020 OL752713	.....	TGGCGAA	TTT	.....	TTT
LSDV Thailand2021 ON152411	.....	TGGCGAA	TTT	.....	TTT
LSDV India Ranchi1 2019 OK422493	.....	TGGCGAA	TTT	.....	TTT
LSDV Rec 20L81 Bang Thanh 2020 MZ577076	.....	TGGCGAA	TTT	.....	TTT
LSDV Rec 20L43 Ly-Quoc 2020 MZ577074	.....	TGGCGAA	TTT	.....	TTT
LSDV Rec HongKong2020 MW732649	.....	TGGCGAA	TTT	.....	TTT
LSDV Rec China GD01 2020 MW355944	.....	TGGCGAA	TTT	.....	TTT
LSDV Rec Udmurtiya2019 MT134042	.....	TGGCGAA	TTT	.....	TTT
GTPV Turkey MN072622	.....	TGGCGAA	TTT	.....	TTT
GTPV Vietnam MN072621	.....	TGGCGAA	TTT	.....	TTT
GTPV India MN072620	.....	TGGCGAA	TTT	.....	TTT
GTPV Gorgan KX576657	.....	TGGCGAA	TTT	.....	TTT
GTPV V103 MW020570	.....	TGGCGAA	TTT	.....	TTT
GTPV Yemen MN072625	.....	TGGTGAA	TTT	.....	TTT
GTPV Sudan MN072624	.....	TGGTGAA	TTT	.....	TTT
SPPV Saudi Arabia MN072630	.....	TGGTGAA	TTT	.....	TTT
SPPV Pendik MN072629	.....	TGGTGAA	TTT	.....	TTT
SPPV Nigeria MN072628	.....	TGGTGAA	TTT	.....	TTT
SPPV V293 MW167071	.....	TGGTGAA	TTT	.....	TTT
SPPV NISKHI AY077834	.....	TGGTGAA	TTT	.....	TTT
SPPV A AY077833	.....	TGGTGAA	TTT	.....	TTT
LSDV Vac Herbivac LS MK441838	.....	CGGTGAA	TTT	.....	TTT
LSDV Vac Cro2016 MG972412	.....	CGGTGAA	TTT	.....	TTT
LSDV Vac OBP KX764645	.....	CGGTGAA	TTT	.....	TTT
LSDV Vac SIS Lumpyvax KX764643	.....	CGGTGAA	TTT	.....	TTT
LSDV_Vac_LW1959_AF409138	.....	CGGTGAA	TTT	.....	TTT



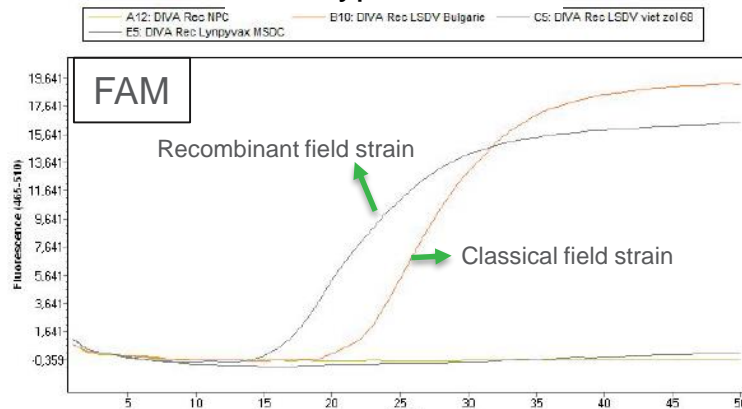
# Recombinant LSDV strains - diagnostics

- Vaccine type reaction

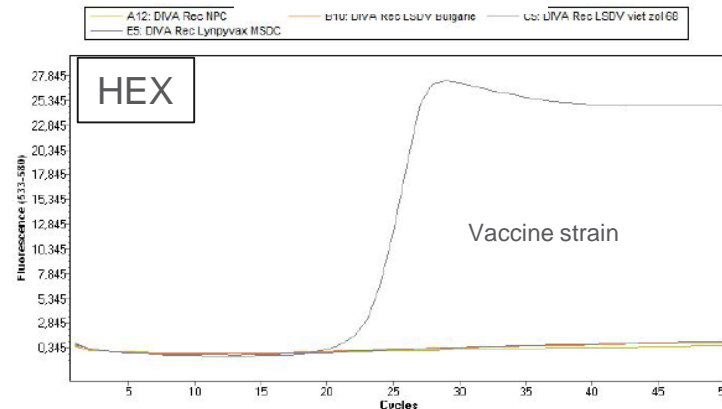
Neethling-like vaccine reaction	GGATTTATTTATATTGTGGGTGGAATT	CTCTCGGAATAGGCTATGAAGG	GAACCCATTACGACATACAAAAA
LSDV Vac Herbivac LS MK441838	.....	.....	.....
LSDV Vac Cro2016 MG972412	.....	.....	.....
LSDV Vac OBP KX764645	.....	.....	.....
LSDV Vac SIS Lumpyvax KX764643	.....	.....	.....
LSDV Vac LW AF409138	.....	.....	.....
LSDV WT Kubash2016 MN642592	.....A..A..G..T..	.....	.....
LSDV WT 155920 2012 KX894508	.....A..A..G..T..	.....	.....
LSDV WT Dagestan2015 MH893760	.....A..A..G..T..	.....	.....
LSDV WT Evros GR2015 KY829023	.....A..A..G..T..	.....	.....
LSDV WT SERBIA Bujanovac2016 KY702007	.....A..A..G..T..	.....	.....
LSDV WT V281 OK318001	.....A..A..G..T..	.....	.....
LSDV WT 280KZN RSA2018 MW656253	.....A..A..G..T..	.....	.....
LSDV WT BULL16 MT643825	.....A..A..G..T..	.....	.....
LSDV WT LW AF409137	.....A..A..G..T..	.....	.....
LSDV Rec Saratov2017 MH646674	.....A..A..G..T..	.....	.....
LSDV Rec Saratov2019 OM530217	.....A..A..G..T..	.....	.....
LSDV Rec Khabarovsk2020 OM793603	.....A..A..G..T..	.....	.....
LSDV Rec Tomsk2020 OM793602	.....A..A..G..T..	.....	.....
LSDV KM Taiwan2020 OL752713	.....A..A..G..T..	.....	.....
LSDV Thailand2021 ON152411	.....A..A..G..T..	.....	.....
LSDV India Ranchi1 2019 OK422493	.....A..A..G..T..	.....	.....
LSDV Rec 20L81 Bang Thanh 2020 MZ577076	.....A..A..G..T..	.....	.....
LSDV Rec 20L43 Ly-Quoc 2020 MZ577074	.....A..A..G..T..	.....	.....
LSDV Rec HongKong2020 MW732649	.....A..A..G..T..	.....	.....
LSDV Rec China GD01 2020 MW355944	.....A..A..G..T..	.....	.....
LSDV Rec Udmurtiya2019 MT134042	.....A..A..G..T..	.....	.....
GTPV Turkey MN072622	.....C..A..A..G..T..	.....	.....
GTPV Vietnam MN072621	.....C..A..A..G..T..	.....	.....
GTPV India MN072620	.....C..A..A..G..T..	.....	.....
GTPV V103 MW020570	.....C..A..A..G..T..	.....	.....
GTPV Yemen MN072625	.....C..A..A..G..T..	.....	.....
GTPV Sudan MN072624	.....C..A..A..G..T..	.....	.....
SPPV Saudi Arabia MN072630	.....A..A..G..T..	.....	.....
SPPV Nigeria MN072628	.....A..A..G..T..	.....	.....
SPPV V293 MW167071	.....A..A..G..T..	.....	.....
SPPV NISKHI AY077834	.....A..A..G..T..	.....	.....
SPPV_A_AY077833	.....A..A..G..T..	.....	.....

# Recombinant LSDV strains - diagnostics

Wild type reaction



Vaccine reaction



- Diagnostic sensitivity and specificity
- Analytical sensitivity
- No cross reaction with other viruses
- Limited cross reactions with SPPV and GTPV in pure cell cultures
- Co-detection of wild type and vaccine strains
- Good intra and inter run variability

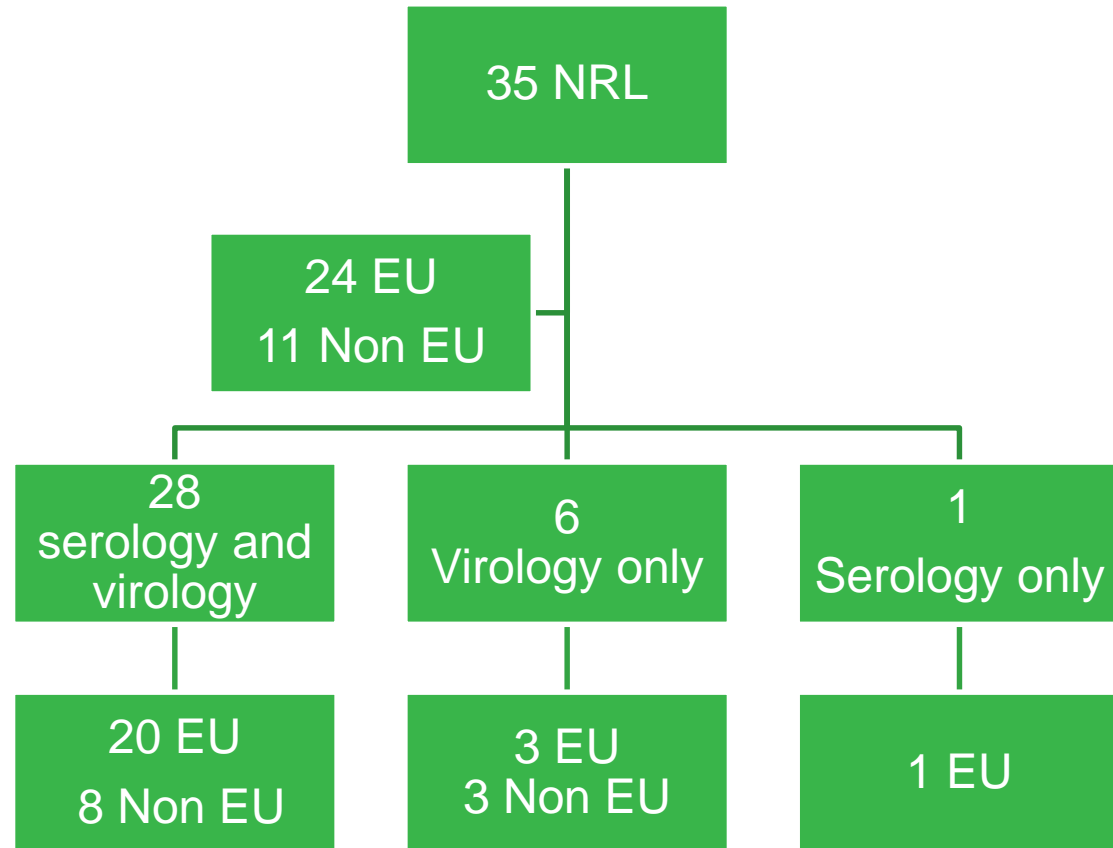
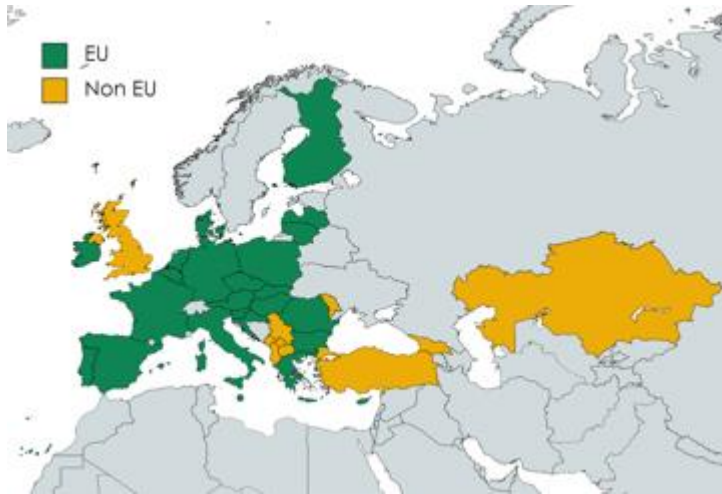


[Funding Article](#)

Development and validation of a new DIVA real-time PCR allowing to differentiate wild type lumpy skin disease virus strains, including the Asian recombinant strains, from Neethling-based vaccine strains.

Andy Haegeman <sup>1</sup>, Ilse De Leeuw <sup>1</sup>, Wannes Philips <sup>2</sup>, Nick De Regge <sup>1</sup>

# Proficiency test capripox viruses 2022



- Serology
- Virology: - capripox virus DNA detection
  - species differentiation
  - wild type vs vaccine type



# Proficiency test capripox viruses 2022

For the detection of specific antibodies to capripox virus in bovine and ovine sera, 28 out of 29 participating laboratories achieved a satisfactory performance on all tests

For the detection of capripox virus nucleic acid in the PT samples, 33 out of 34 participating laboratories achieved a satisfactory performance on all of their PCR tests. 1 out of 34 participating laboratories achieved a satisfactory on one of their PCR tests and an unsatisfactory performance on the two other PCR tests.

For the differentiation of capripox virus species, 17 out of 22 participating laboratories achieved a satisfactory performance, 2 out of 22 participating laboratories achieved an unsatisfactory performance, misclassifying 1 (no complete dataset) and 5 samples, and 3 out of 22 participating laboratories did not submit a complete dataset and could therefore be not awarded a satisfactory performance

# Proficiency test capripox viruses 2022

For the differentiation between capripox virus vaccine and field strains, 10 out of 22 participating laboratories achieved a satisfactory performance, 11 out of 22 participating laboratories achieved did not submit a complete dataset and could therefore be not awarded a satisfactory performance, 1 out of 22 participating laboratories achieved an unsatisfactory performance, misclassifying 6 samples

Sample ID	REP	Expected results	# of GTPV	# of LSDV Wild	# of LSDV Vaccine	# of SPPV Wild	# of SPPV Vaccine	# of NEG	# of ND	%
TP1	1	GTPV	6	0	0	0	0	10	6	27
TP2	1	LSDV Wild	0	21	0	0	0	1	0	96
TP3	1	SPPV Wild	0	0	0	10	0	7	5	45
TP4	2	LSDV Wild	0	42	0	0	0	2	0	96
VP1	1	LSDV	0	2	11	0	0	3	6	59
VP2	1	LSDV Vaccine	0	0	21	0	0	0	1	96
BP1	1	SPPV Vaccine	0	0	0	0	10	7	5	45
BP2	1	LSDV Wild	0	21	0	0	0	1	0	96
BN1	1	NEG	0	0	0	0	0	19	3	86



# Recombinant LSDV strains – vaccine efficacy

➤ Impact on vaccine efficacy and LSDV control?

In vivo experiment planned to look at efficacy of homologous LAV against a challenge with a recombinant strain.



# Duration of immunity induced by LSDV vaccines

## ❖ Vaccination

### ➤ Most commonly used: Live attenuated vaccines (LAV)

- Homologous vaccines (based up LSDV)



vaccines



Article

#### Comparative Evaluation of Lumpy Skin Disease Virus-Based Live Attenuated Vaccines

Andy Haegeman <sup>1,\*</sup>, Ilse De Leeuw <sup>1</sup>, Laurent Mostin <sup>2</sup>, Willem Van Campe <sup>2</sup>, Laetitia Aerts <sup>3</sup>, Estelle Venter <sup>4,5</sup>, Eeva Tuppurainen <sup>6</sup>, Claude Saegerman <sup>7</sup> and Kris De Clercq <sup>1</sup>

- Heterologous vaccines (based up SPPV, GTPV)

### ➤ Recent: inactivated vaccines

- Homologous vaccines (based up LSDV)



vaccines



Article

#### High Efficiency of Low Dose Preparations of an Inactivated Lumpy Skin Disease Virus Vaccine Candidate

Janika Wolff <sup>†</sup>, Martin Beer and Bernd Hoffmann <sup>\*✉</sup>

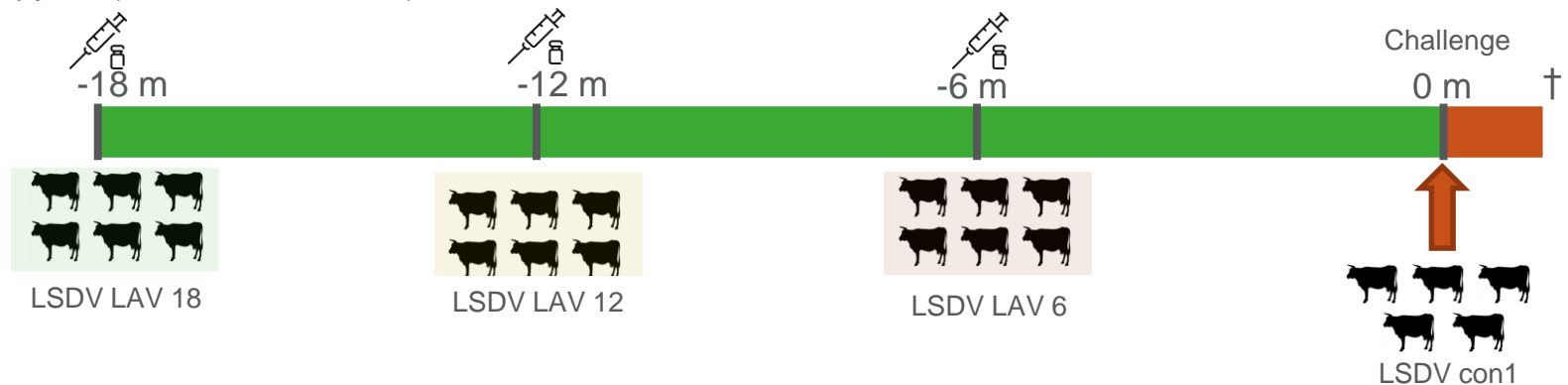
Information available on safety and efficacy



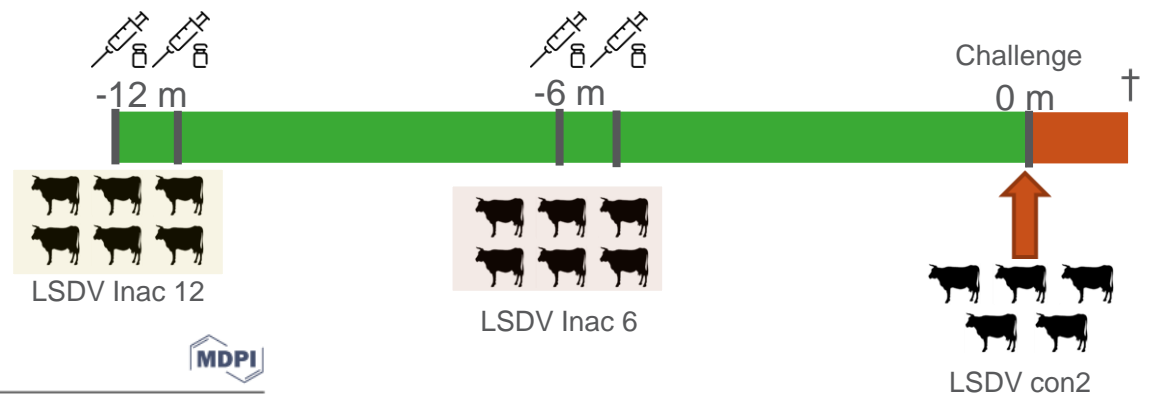
Duration of protective immunity

# Duration of immunity induced by LSDV vaccines

## ❖ LAV: Lumpyvax (MSD Animal Health)



## ❖ Inac: (MCI Santé Animale)



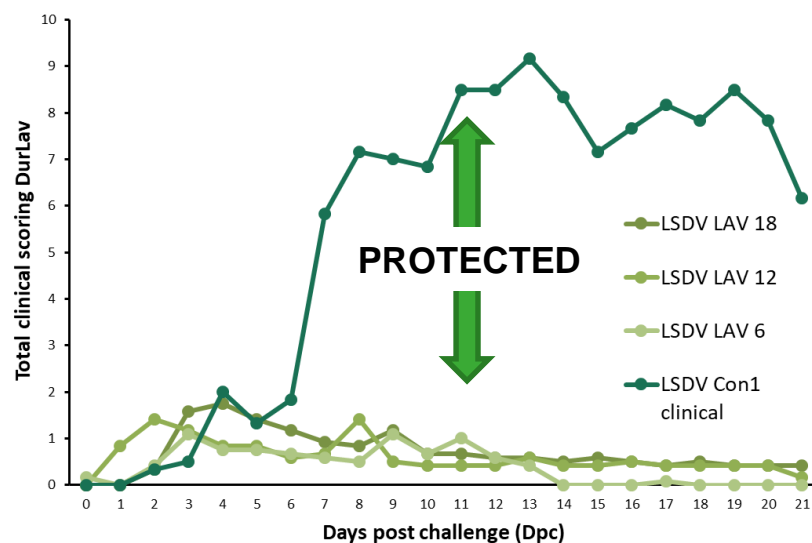
Challenge: LSD/OA3-Ts.MORAN;  
titer 6.5 TCID<sub>50</sub>/ml

# Duration of immunity induced by LSDV vaccines

## Live attenuated vaccine

Total clinical scoring

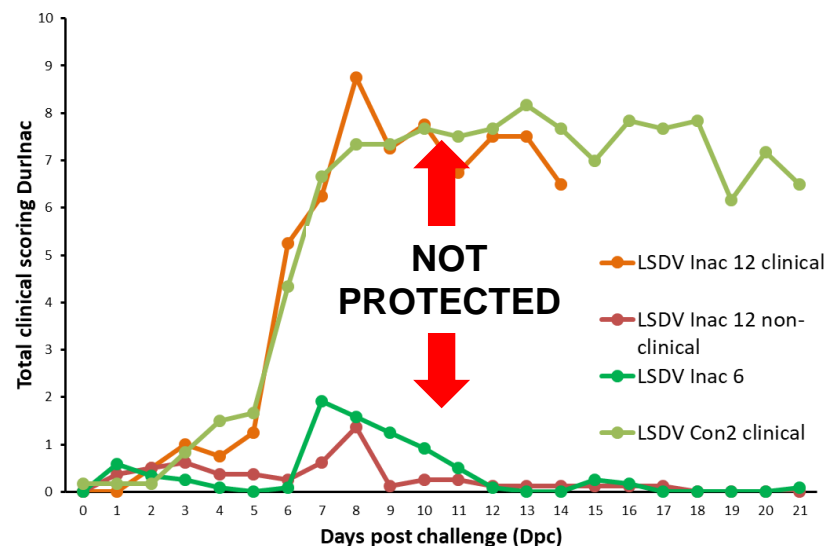
- No nodule formation
- Almost no clinical scoring



- Only 1 vaccination necessary
- Limited side effects upon vaccination
- Complete protection for at least 1,5 years

## Inactivated vaccine

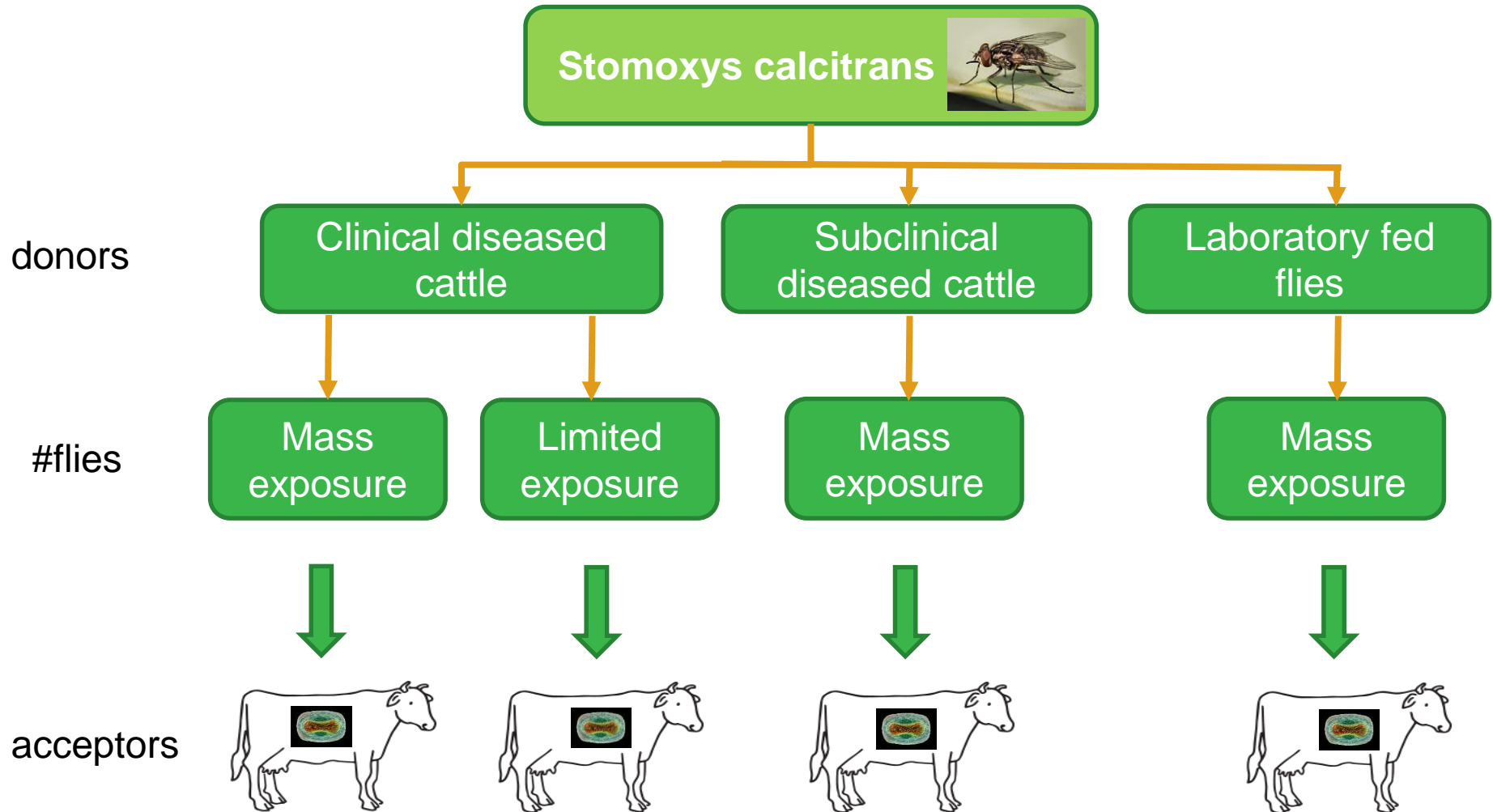
- Nodule formation in LSDV Inac 12 (2/6)
- Clinical scoring in 2 animals LSDV Inac 12



- Prime/boost vaccination necessary
- Almost no side effects upon vaccination
- Complete protection up to six month, but not after one year



# LSDV transmission by *Stomoxys calcitrans*



# Sheeppox virus



- Confirmation diagnostics
- Whole genome sequencing SPPV strain
- Spanish SPPV isolate obtained
- EUVET mission
- Vaccine safety study SPPV vaccine
- Vaccine safety/efficacy study alternative SHPP vaccines
- Development of a SPPV infection model using intranasal inoculation
- SPPV inactivation in milk

# Thanks to the EC for the support

## EU Reference Laboratory for Capripox viruses



Funded by the  
European Union

**Contact: [nick.deregge@sciensano.be](mailto:nick.deregge@sciensano.be)**