



EURL activities of importance to the LSD diagnosis and vaccine control

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Content

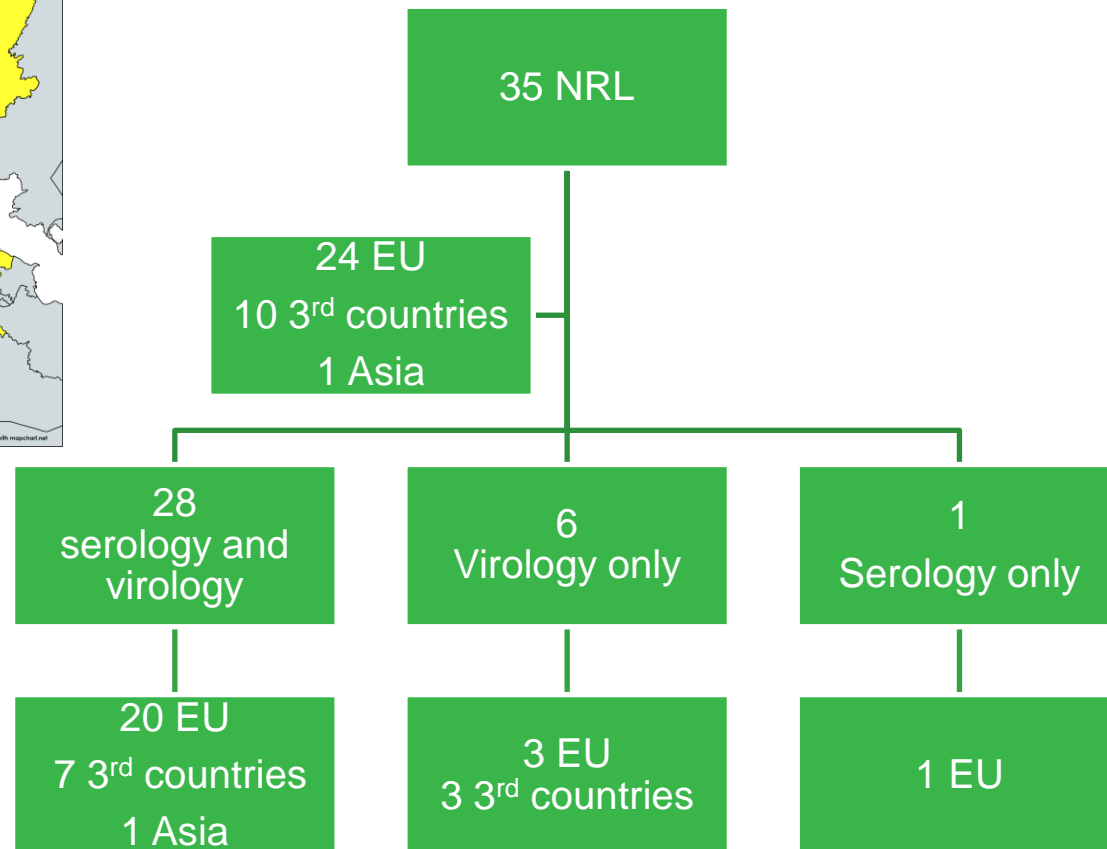
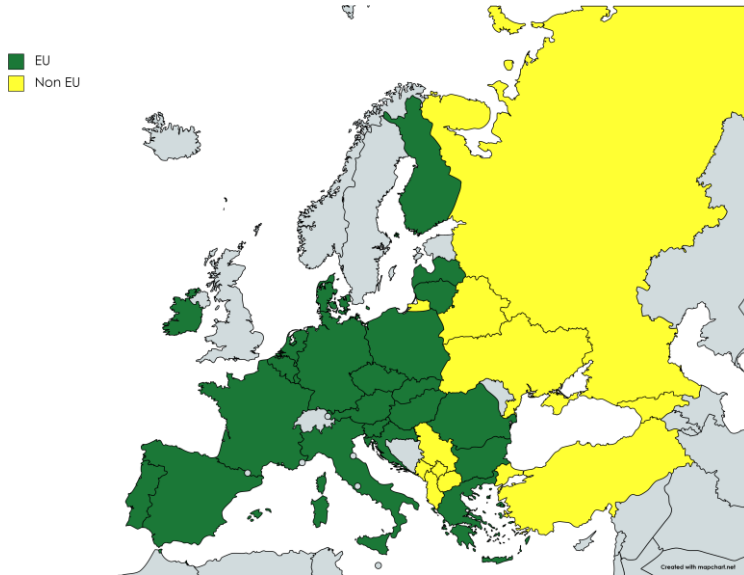


Part I: Tasks related to South-East European epidemic

Part II: Preparedness for Central Asian epidemic

Part I: Proficiency test

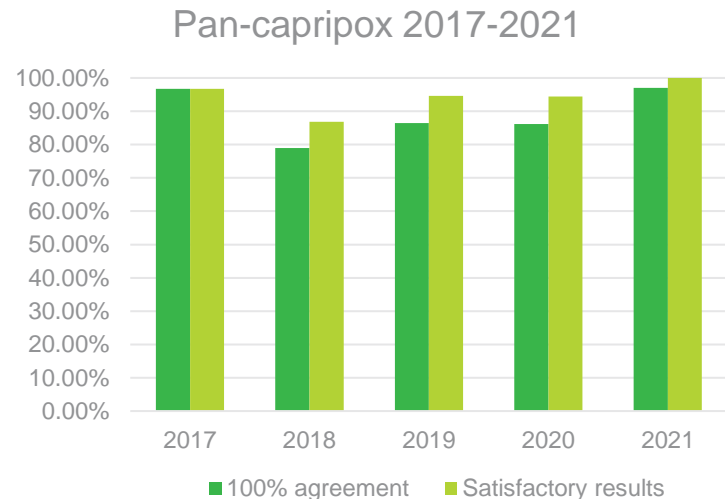
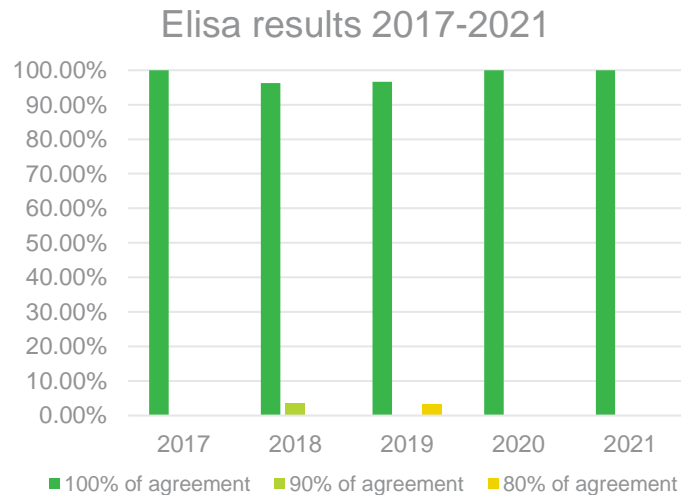
Objective: to assess the ability of NRLs to perform
Capripox virus serological and molecular virology diagnosis



Part I: Proficiency test

For the detection of specific antibodies to capripox virus in bovine and ovine sera, **all** participating laboratories achieved a satisfactory performance

For the detection of capripox virus nucleic acid in the PT samples, **all** participating laboratories achieved a satisfactory performance



Part I: Training

- Annual meeting: update of the most recent epidemiological situation and diagnostic tools
- Training for Pendik Institute (Turkey): LSDV diagnostics, vaccines and vaccine quality control

Part I: In vitro studies to improve diagnostics

- LSDV DIVA PCRs: are important to differentiate adverse reactions after vaccination from clinical disease induced by wild type strains

> [Mol Cell Probes](#). 2021 Dec;60:101778. doi: 10.1016/j.mcp.2021.101778. Epub 2021 Nov 11.

A TaqMan probe-based multiplex real-time PCR method for the specific detection of wild type lumpy skin disease virus with beta-actin as internal amplification control

Eirini I Agianniotaki ¹, Serafeim C Chaintoutis ², Andy Haegeman ³, Kris De Clercq ³, Eleni Chondrokouki ⁴, Chrysostomos I Dovas ⁵



microorganisms



Article

Validation of TaqMan-Based Assays for Specific Detection and Differentiation of Wild-Type and Neethling Vaccine Strains of LSDV

Dejan Vidanović ^{1,*}, Bojana Tešović ¹, Milanko Šekler ¹, Zoran Debeljak ¹, Nikola Vasković ¹, Kazimir Matović ¹, Andrey Koltsov ², Kiril Krstevski ³, Tamaš Petrović ⁴, Ilse De Leeuw ⁵ and Andy Haegeman ⁵

Part I: In vivo studies in support of policy decisions

- Evaluation of safety/efficacy of inactivated and live-attenuated vaccines



Article

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

Andy Haegeman ^{1,*}, Ilse De Leeuw ¹, Laurent Mostin ², Willem Van Campe ², Laetitia Aerts ³, Estelle Venter ^{4,5}, Eeva Tuppurainen ⁶, Claude Saegerman ⁷ and Kris De Clercq ¹

- LSDV transmission by *Stomoxys calcitrans* flies and *Haematopota* horseflies from LSDV infected cattle with clinical signs

**SCIENTIFIC
REPORTS**
nature research

Experimental evidence of mechanical lumpy skin disease virus transmission by *Stomoxys calcitrans* biting flies and *Haematopota* spp. horseflies

C. Sohler^{1,3}, A. Haegeman^{1,3}, L. Mostin¹, I. De Leeuw¹, W. Van Campe¹, A. De Vleeschauwer¹, E. S. M. Tuppurainen², T. van den Berg¹, N. De Regge¹ & K. De Clercq¹



Part I: In vivo studies in support of policy decisions

- Potential role of subclinical LSDV infected cattle in transmission



microorganisms



Article

Detection of Clinical and Subclinical Lumpy Skin Disease Using Ear Notch Testing and Skin Biopsies

Laetitia Aerts ^{1,†}, Andy Haegeman ^{2,*}, Ilse De Leeuw ², Wannas Philips ¹, Willem Van Campe ³, Isabelle Behaeghel ⁴, Laurent Mostin ³ and Kris De Clercq ^{2,*}

- Evaluation ongoing of LSDV transmission by *Stomoxys calcitrans* flies from subclinical LSDV infected cattle
- Evaluation ongoing of transmission of vaccine strains (LSD vaccine (OBP) and Lumpivax (Kevevapi)) by *Stomoxys calcitrans* flies

Part I: Vaccine control

- In vitro vaccine batch control
 - European LSDV vaccine bank
 - Turkish batch control for LSDV and SHPX vaccines
- In vitro and in vivo quality control of Lumpivax LSDV vaccine (Kevevapi)



vaccines



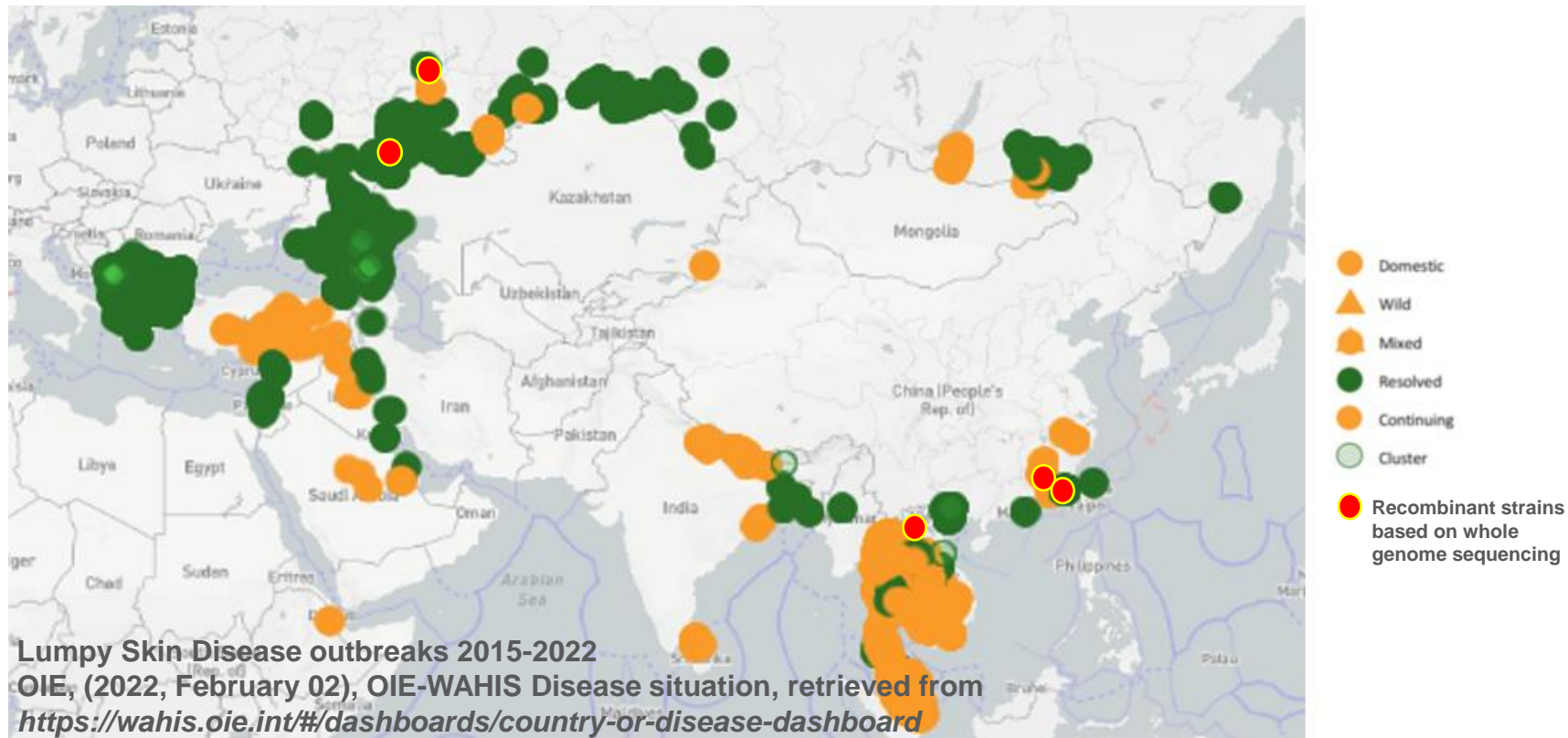
Article

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

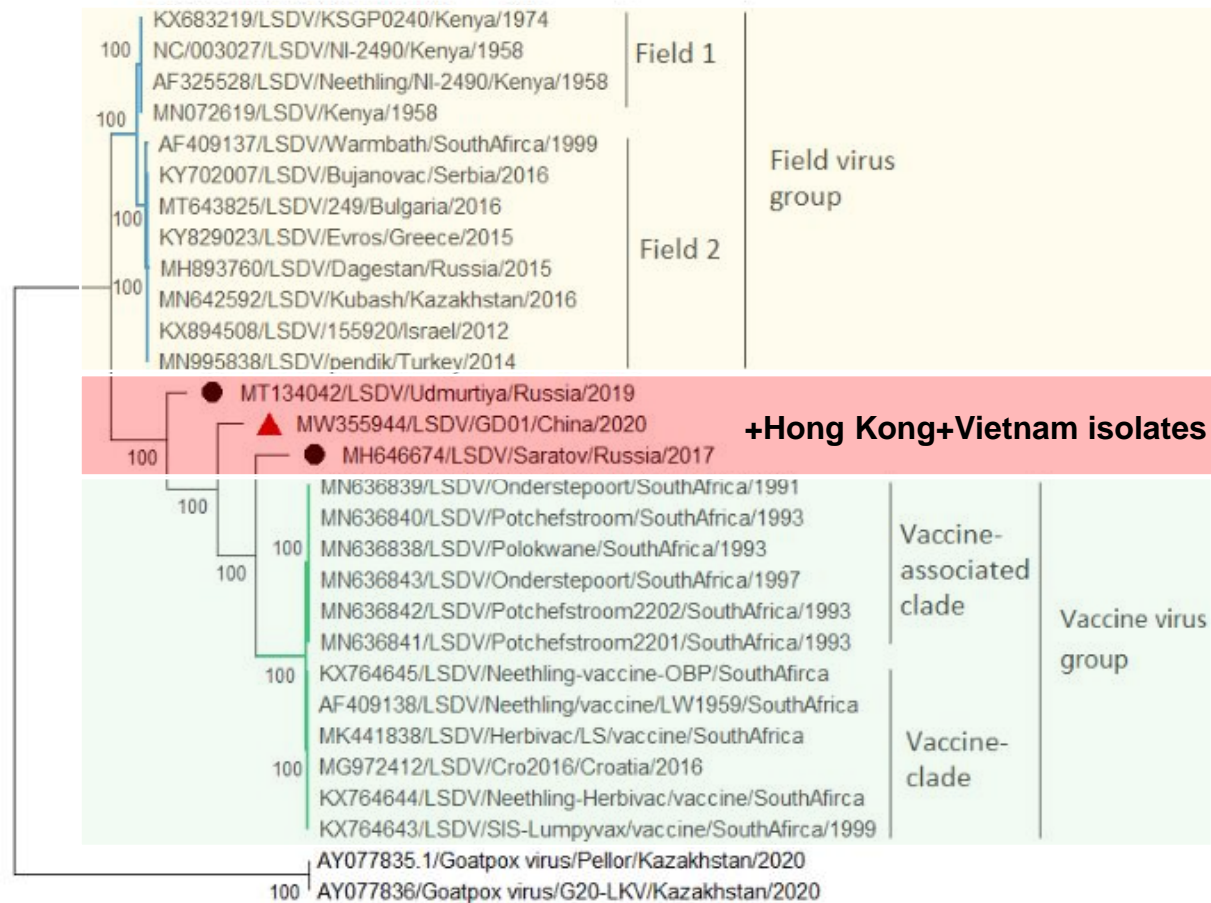
- ✓ Contained genomic sequences of LSDV vaccine and field type strains and goatpox virus
- ✓ Indications of recombinant strains in vaccine – result of a poorly controlled production process
 - follow-up studies via NGS ongoing
- ✓ Provided protection against challenge infection

Part II: Central Asia – recombinant strains



- Recombinant LSDV strains seem to have a Neethling-like vaccine strain backbone combined with sequences of field type LSDV strain(s), resulting in a wild type phenotype

Part II: recombinant strains - phylogeny



Ma et al, 2022 – based on full genome sequences

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

Part II: recombinant strains – full genome sequencing

- Important to collect and share information on circulating strains during an epidemic – important for preparedness
- Full genome sequencing is advised for a correct classification

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



Protocols

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

Elisabeth Mathijs*, Andy Haegeman, Kris De Clercq, Steven Van Borm, Frank Vandebussche

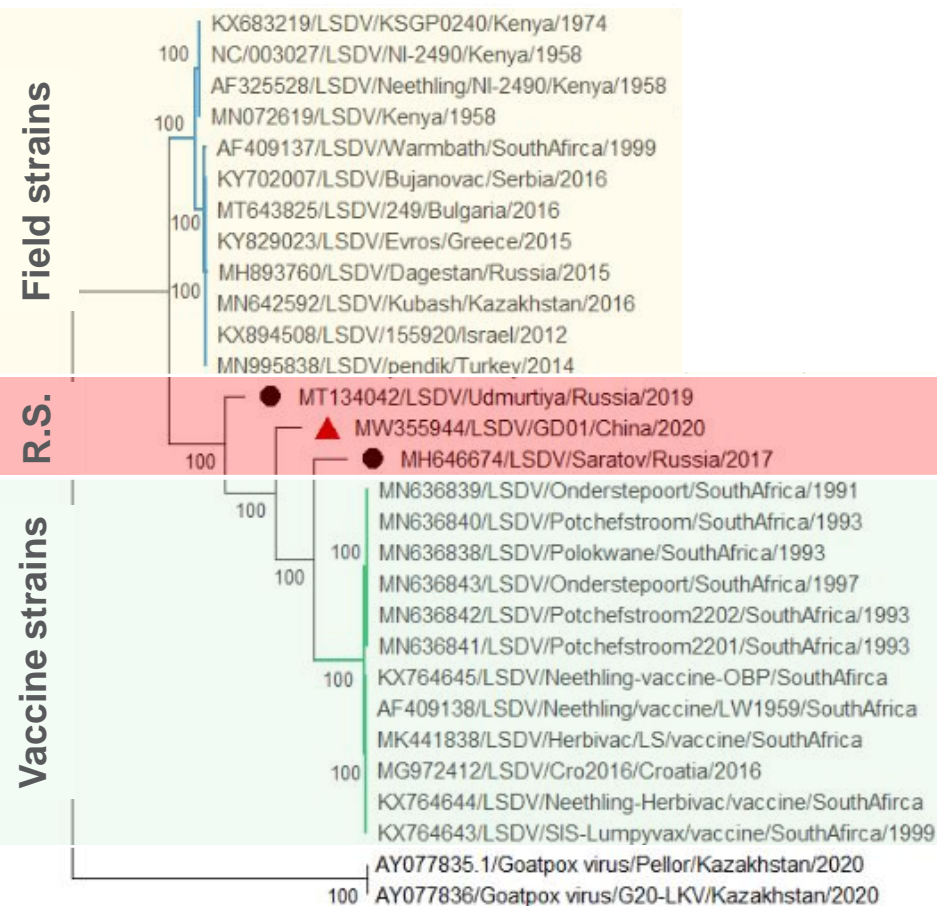
Infectious Diseases in Animals, Sciensano, Rue Juliette Wytsmanstraat 14, 1050, Brussels, Belgium



- EURL is ready to provide help to countries for strain characterization

Part II: recombinant strains – DIVA PCRs

- DIVA PCRs are important to differentiate adverse reactions after vaccination from clinical disease induced by wild type strains



► [Transbound Emerg Dis. 2021 Nov;68\(6\):3020-3024. doi: 10.1111/tbed.13942. Epub 2021 May 13.](#)

Performance of the currently available DIVA real-time PCR assays in classical and recombinant lumpy skin disease viruses

Olga Byadovskaya¹, Yana Pestova¹, Aleksandr Kononov¹, Irina Shumilova¹, Svetlana Kononova¹, Alexander Nesterov¹, Shawn Babiuk², Alexander Sprygin¹

- IDVet (commercial)
- Biosellal (commercial)
- Agianniotaki et al, 2017
- Sprygin et al, 2018
- Kononov et al, 2019

➤ Recombinant field strains classified as vaccine strains in several DIVA PCRs

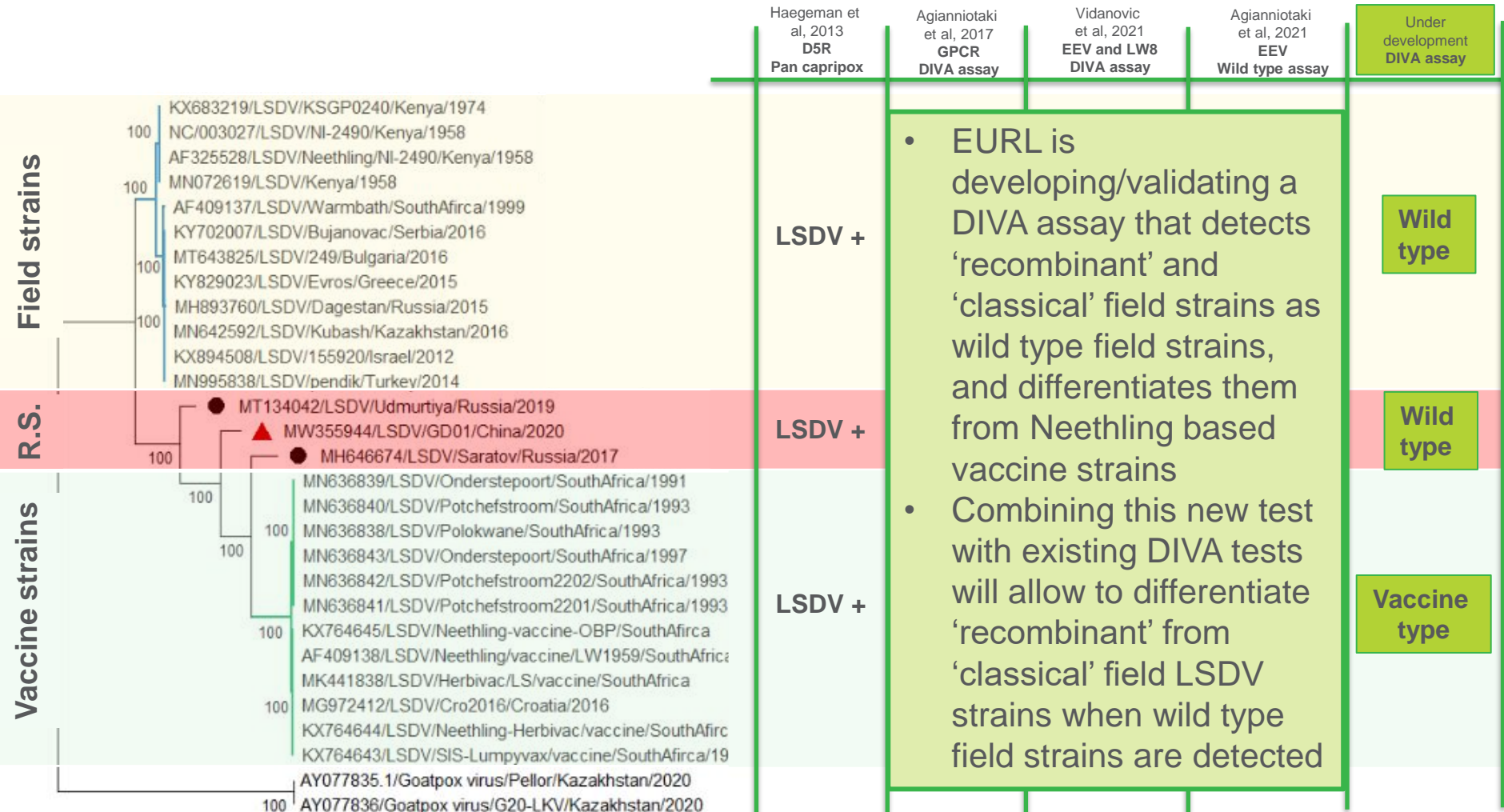
Part II: recombinant strains – DIVA PCRs

- Extensive in silico analysis by EURL-some examples

		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
Field strains	KX683219/LSDV/KSGP0240/Kenya/1974	LSDV +	Wild type	Wild type	Wild type	Not detected
	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						
R.S.	● MT134042/LSDV/Udmurtiya/Russia/2019	LSDV +	Vaccine type	Vaccine type - Saratov not detected	Not detected	Vaccine type
	▲ MW355944/LSDV/GD01/China/2020					
	● MH646674/LSDV/Saratov/Russia/2017					
Vaccine strains	MN636839/LSDV/Onderstepoort/SouthAfrica/1991	LSDV +	Vaccine type	Vaccine type	Not detected	Vaccine type
	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/Pellori/Kazakhstan/2020					
	AY077836/Goatpox virus/G20-LKV/Kazakhstan/2020					

Part II: recombinant strains – DIVA PCRs

- Extensive in silico analysis by EURL-some examples



Part II: recombinant strains – epidemiological implications

- EURL will try to obtain funding to perform studies:
 - on the pathogenesis of recombinant LSDV strains
 - efficacy of available vaccines against infection with recombinant strains
 - on the transmission routes of recombinant LSDV strains
- EURL is optimizing infection models for sheep pox and goat pox virus to perform vaccine evaluations



Thanks to the EC for the support

EU Reference Laboratory for Capripox viruses



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European Union