sciensano

WOAH Reference Laboratory for Lumpy skin disease

Reference Centre World Organisation for Animal Health Founded as OIE

EU Reference Laboratory for Capripox viruses

Funded by the European Union

Food and Agriculture Organization of the United Nations

Lumpy skin disease virus: diagnostics

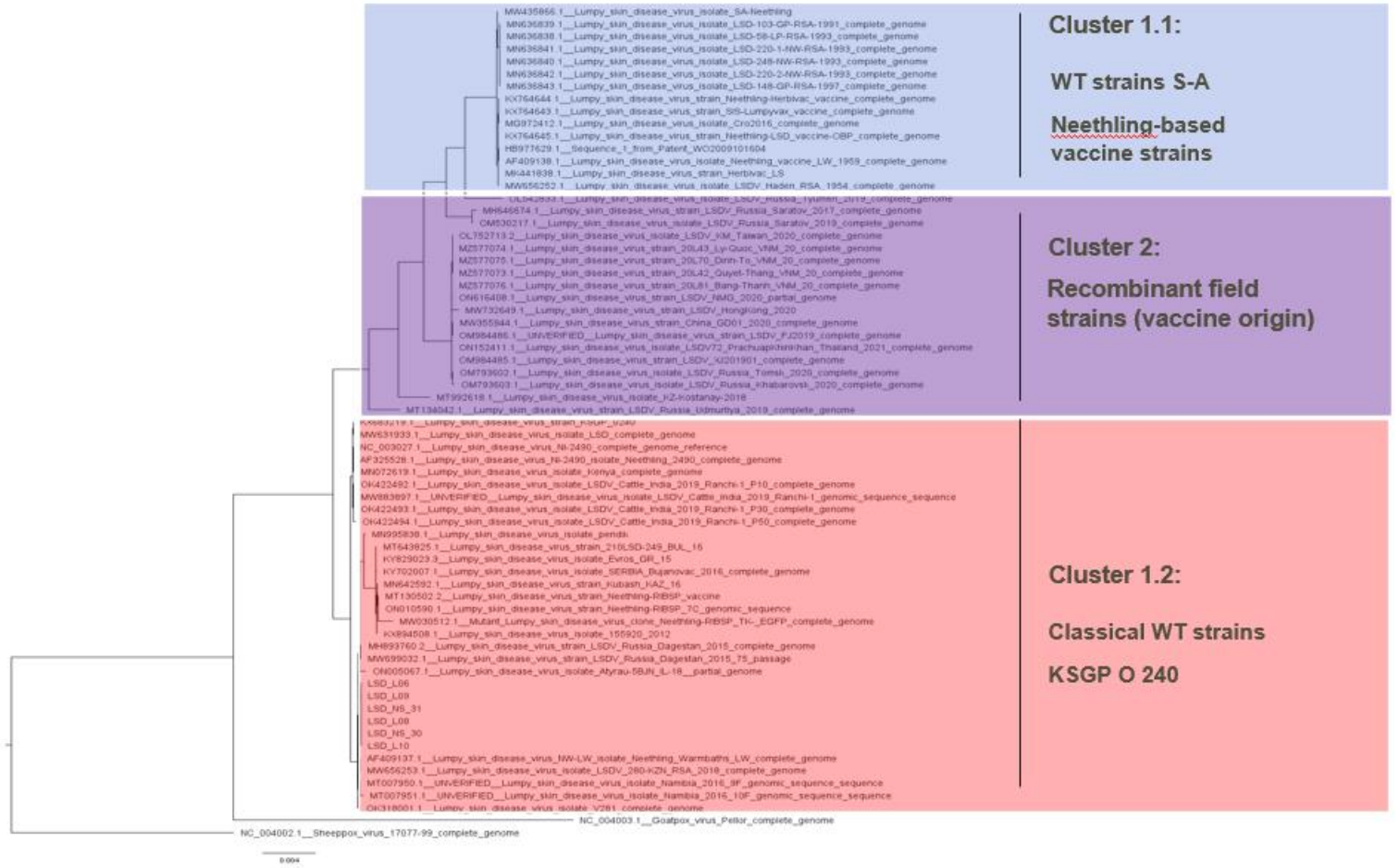
Nick De Regge

4th LSD coordination meeting for South-East Asia

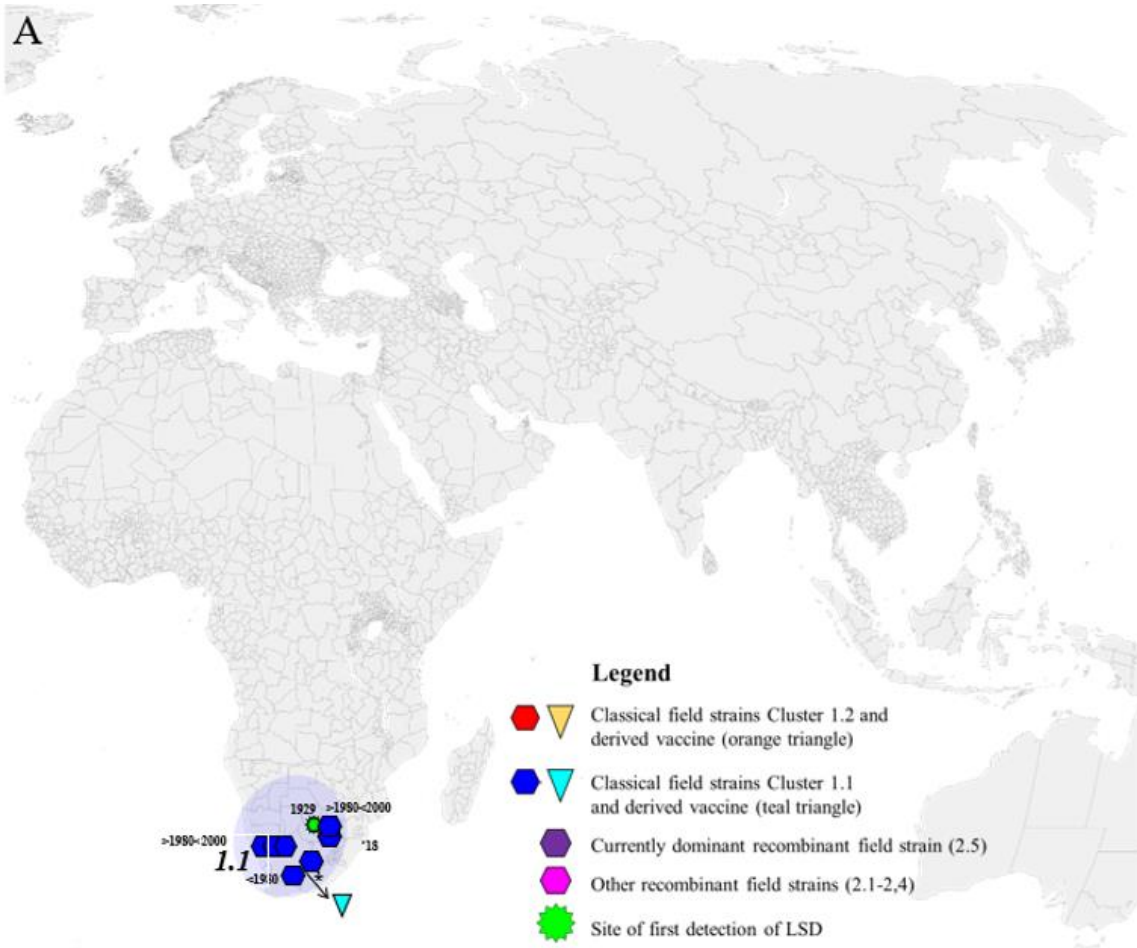
28-29 November 2023, Bangkok, Thailand

.be

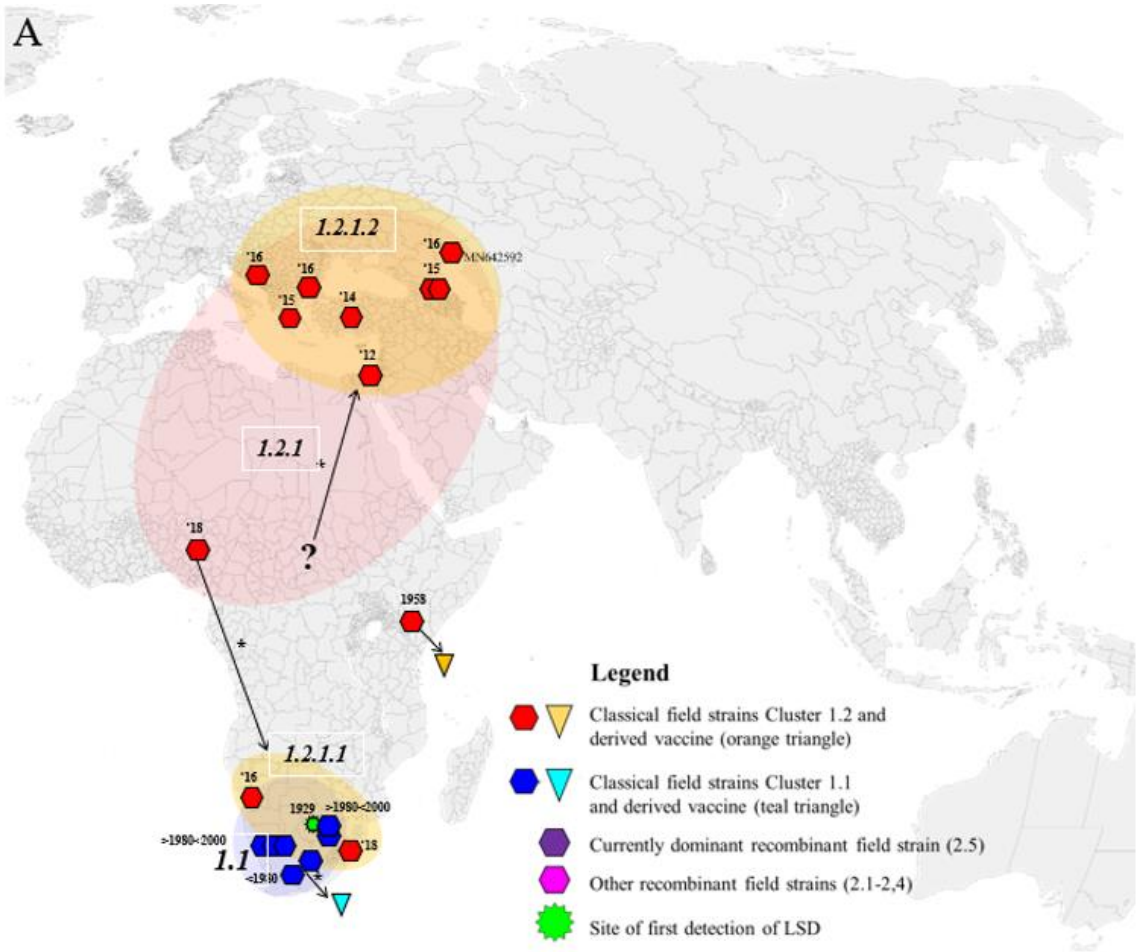
LSDV strains around the world



LSDV strains around the world



LSDV strains around the world



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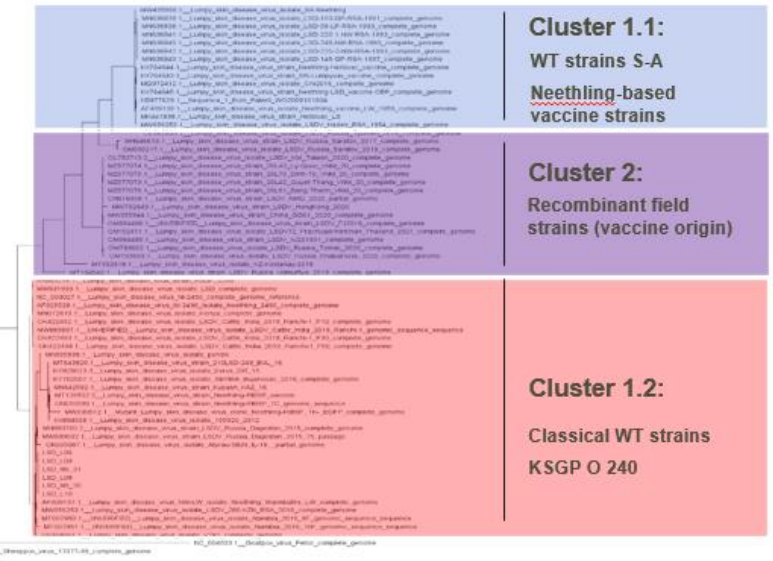
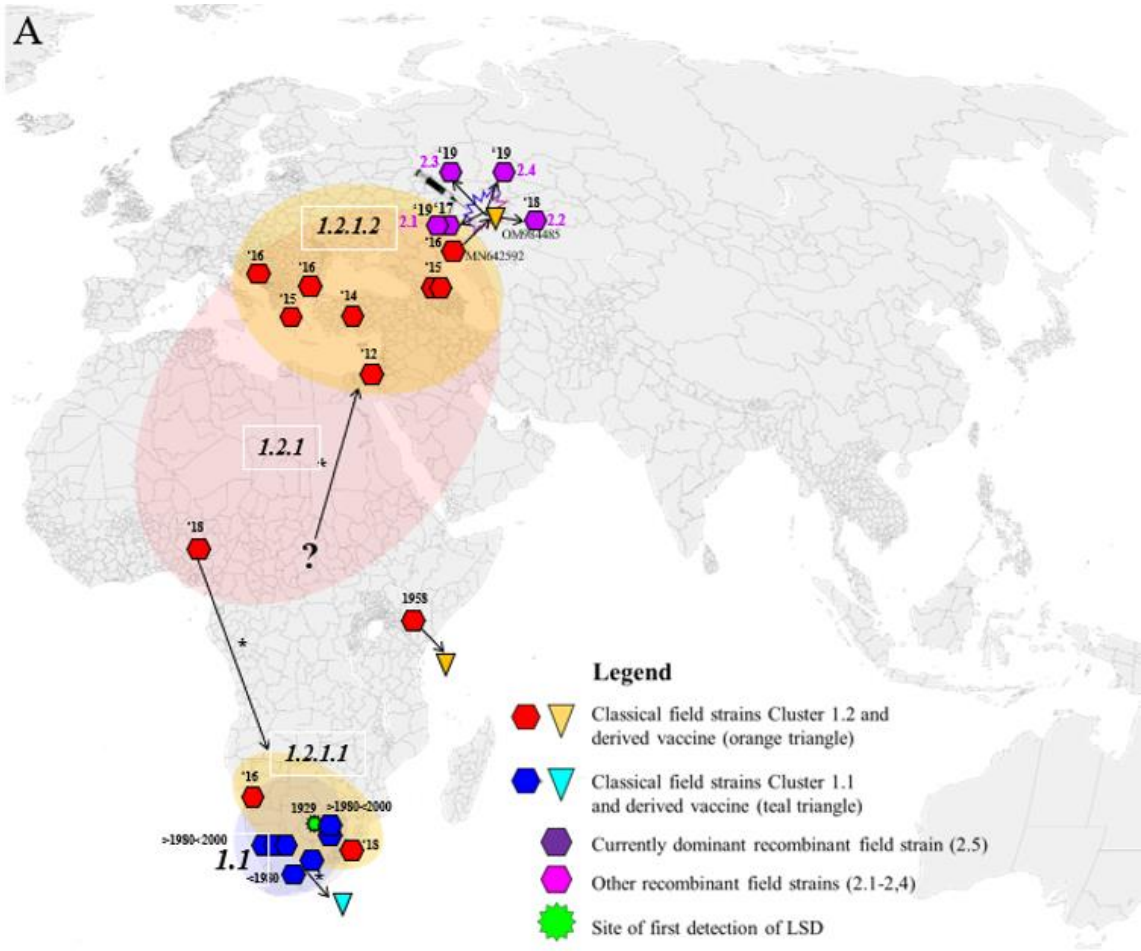
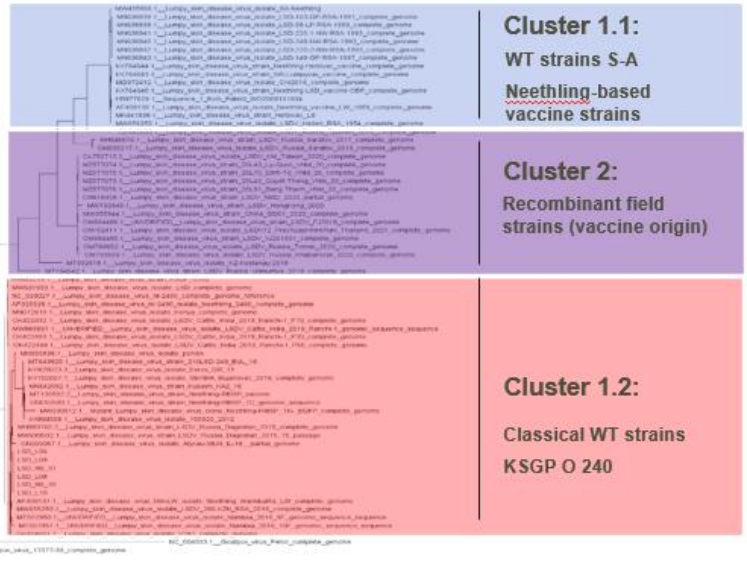
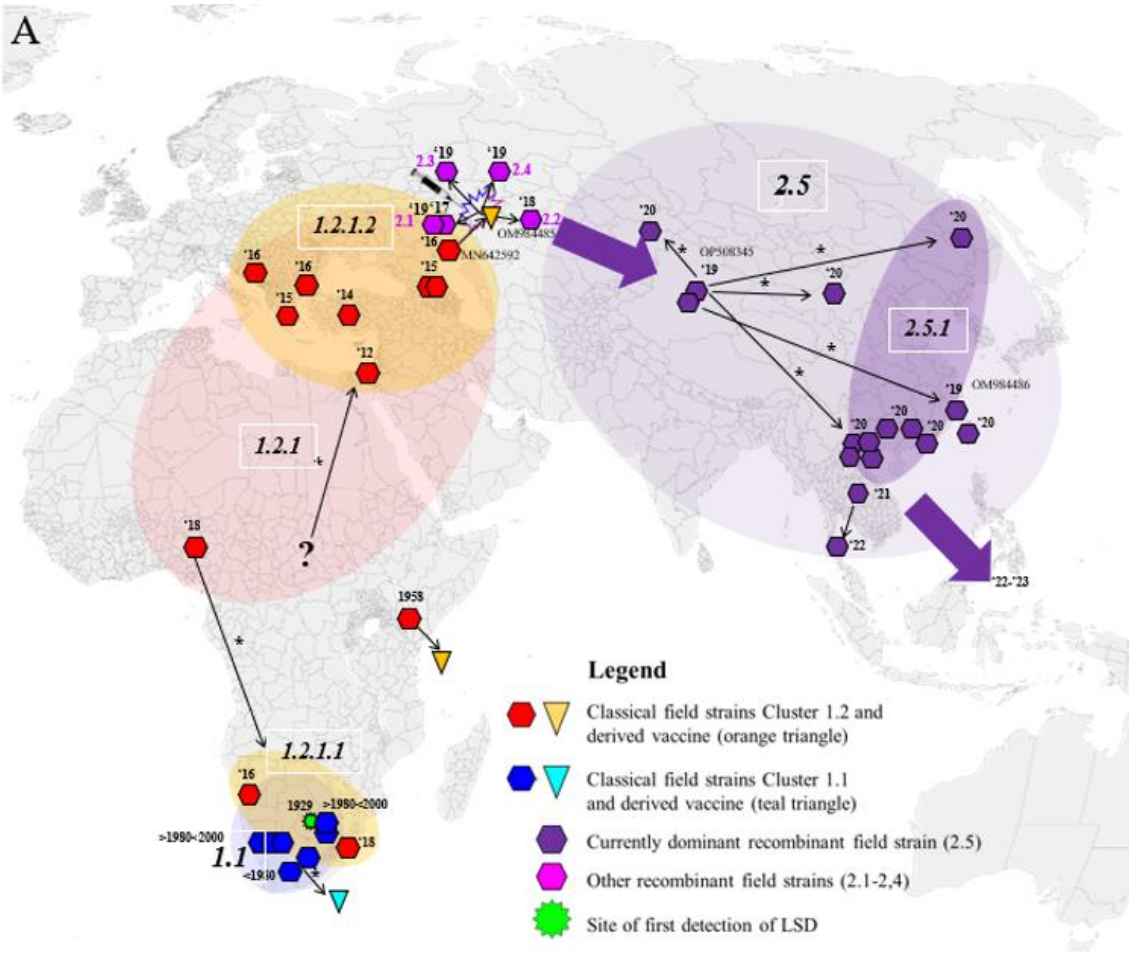




LSDV strains around the world



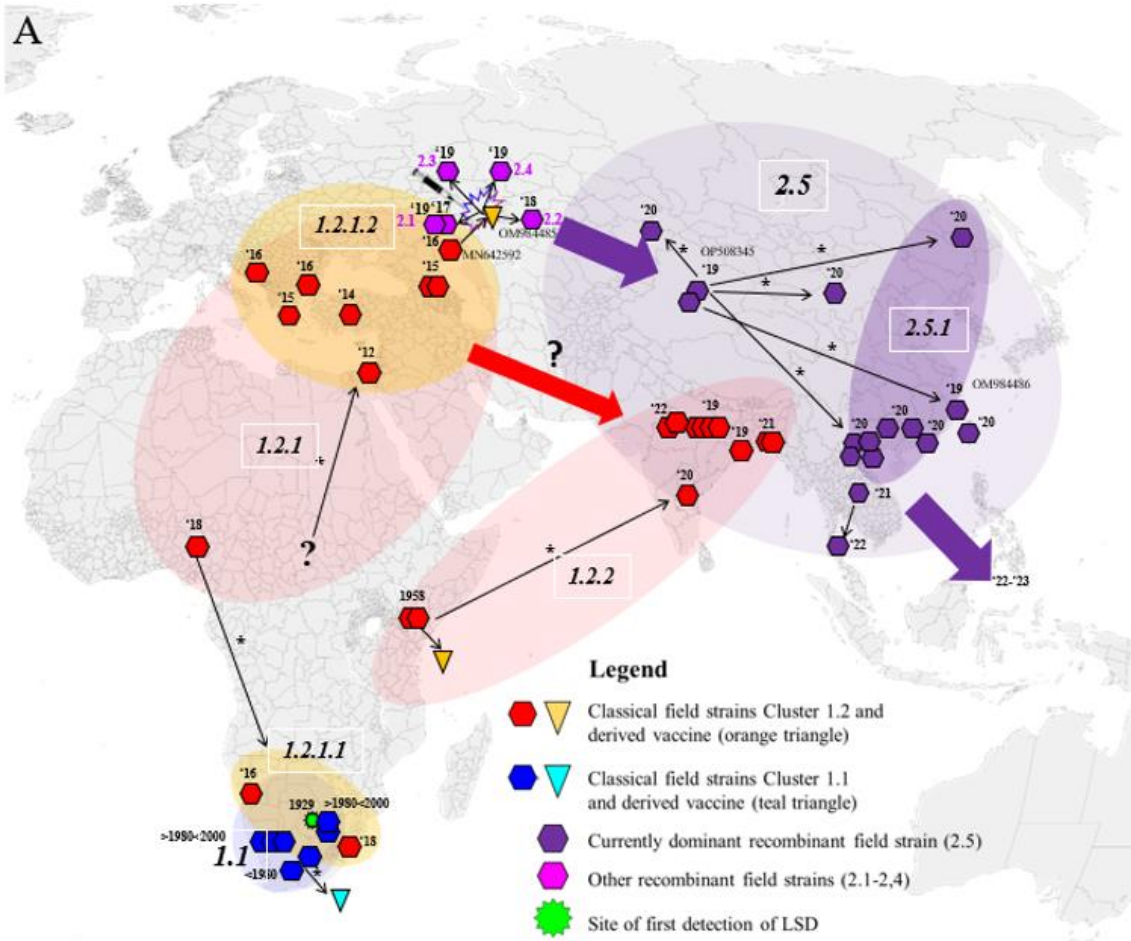


LSDV strains around the world

viruses
MDPI
Article

Lumpy Skin Disease Virus Genome Sequence Analysis: Putative Spatio-Temporal Epidemiology, Single Gene versus Whole Genome Phylogeny and Genomic Evolution

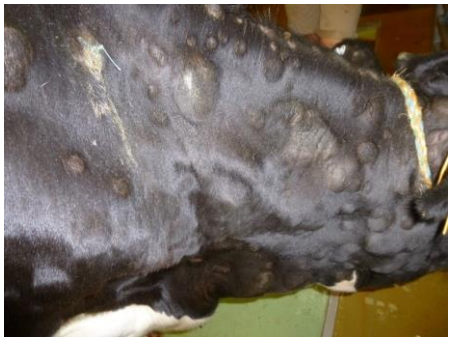
Floris C. Breman *, Andy Haegeman, Nina Krešić, Wannes Philips and Nick De Regge



Pathogenesis

- Disease severity varies between animals

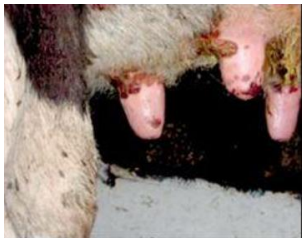
severe LSD cases – mild LSD cases – subclinical animals – no productive infection



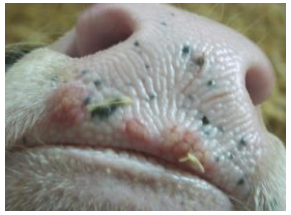
- 30 to 50% diseased animals in experimental settings
- Morbidity: 10 to 45%; Mortality: 1-5%
- Self limiting disease
- No-carrier status, no latency

Differential diagnosis

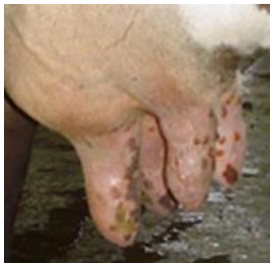
- Bovine herpes mammillitis (=pseudo-lumpy skin disease) (bovine herpesvirus 2)
- Bovine papular stomatitis (parapoxvirus)
- Pseudocowpox (parapoxvirus)
- Vaccinia virus and cowpox virus (orthopoxvirus)
- Dermatophilosis
- Demodicosis
- Insect bites, tick bites
- Besnoitiosis
- Rinderpest
- Hypoderma bovis infection
- Photosensitization
- Urticaria
- Onchocercosis



Bovine herpesvirus 2



Bovine popular stomatitis



Pseudocowpox

- Clinical diagnosis: passive and active surveillance; not always straightforward, mild cases, asymptomatic cases, production system, other pathogens, …
- Importance of laboratory diagnosis

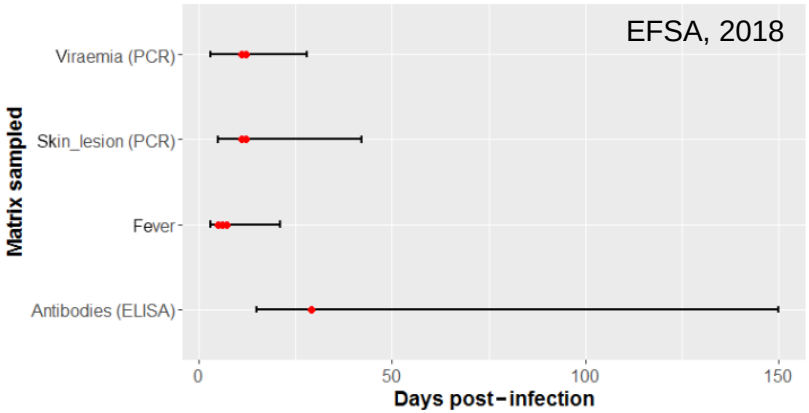
LSDV diagnosis: sample selection

Diagnostic samples: blood, saliva, ocular-nasal discharge, nodules, scabs, crusts, semen

✓ Wound crusts → **Ideal to confirm clinical picture**

- High viral load, long time positive
- Suited for virus isolation and PCR
- Easy to collect
- No need for the most sensitive test

- High risk of contamination
- Extra care is needed in sampling and handling
- Change gloves, also during field collection



✓ Nodule and skin lesion

- Similar remarks as wound crusts
- Need to take a biopt, change biopsy needles

✓ EDTA blood

- Suited for virus isolation and PCR
- Easy to collect

- Viremia is limited in time and often low
- More sensitive tests are needed

✓ Swabs (buccal, nasal)

- Similar remarks as for blood
- Potential for environmental contamination
- Risky to determine individual status

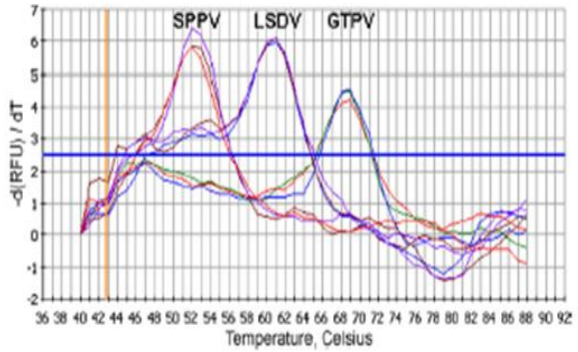
poxvirus infection

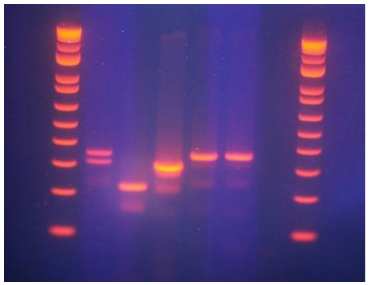
Live virus can be isolated	Time
Skin nodules	39 days
Dried scabs	Several years if kept at -20°C
Blood	5-16 days
Saliva and nasal discharge	At least 21 days
Eye discharge	Duration of infectivity not known
Semen	22 days to 42 days
Milk	Duration of infectivity not known
Urine and feces	Duration of infectivity not known

(Babiuk, 2008, Dietze *et al.*, 2018, Irons *et al.*, 2005, Tuppurainen *et al.*, 2005, Weis, 1968)

Laboratory diagnosis - virology

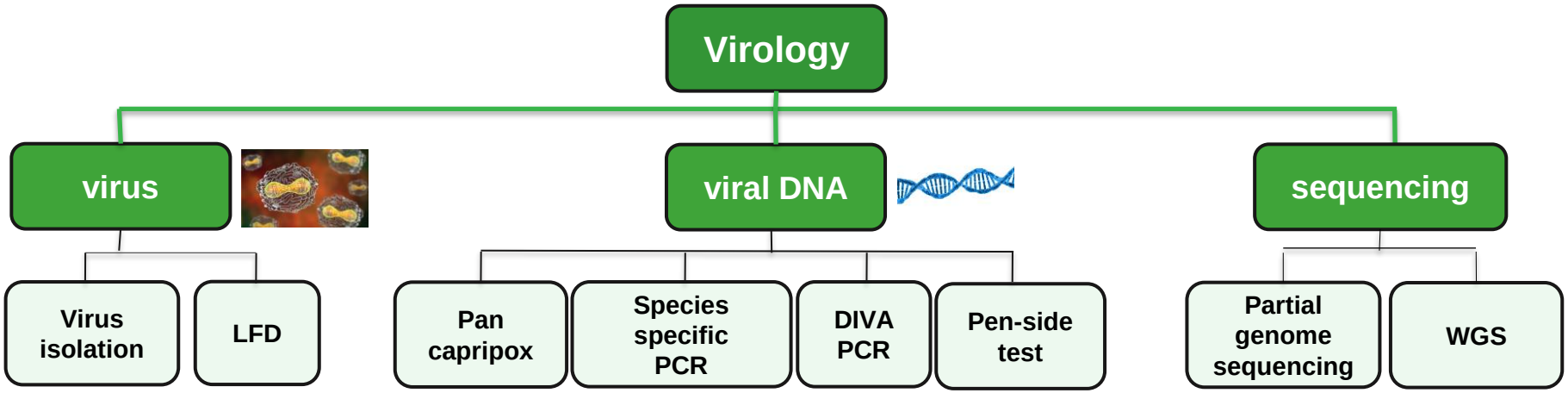
- **Virology**
  - **virus** 
    - Virus isolation
    - LFD
  - **viral DNA** 
    - Pan capripox
    - Species specific PCR
    - DIVA PCR
    - Pen-side test
  - **sequencing**
    - Partial genome sequencing
    - WGS





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

Laboratory diagnosis - virology



pan capripox PCR assays

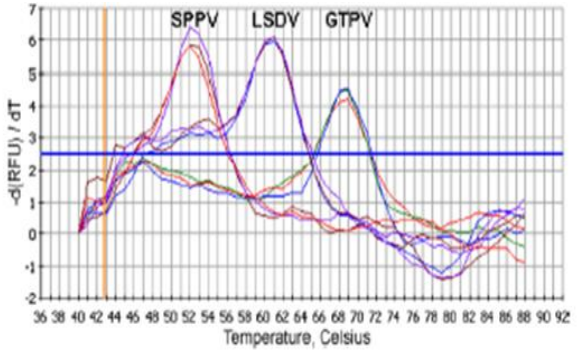
- Detect LSDV, SPPV, GTPV

- Bowden et al, 2008
- Haegeman et al, 2013
- ID.Vet capripox triplex assay

Species specific PCR assays

- Differentiates between LSDV, SPPV and GTPV

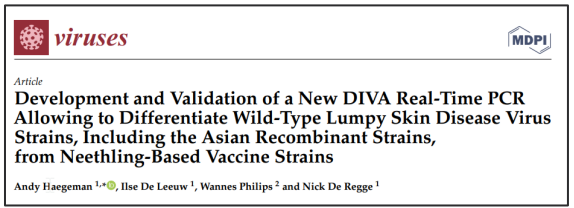
- Lamien et al, 2011
- Wolff et al, 2021
- Galaye et al, 2015 & 2017



Laboratory diagnosis – DIVA PCRs

DIVA PCRs are important to differentiate adverse reactions after vaccination from clinical disease induced by virulent field strains:

- Multiple DIVA PCRs exist
- All have specific set-up, fit for purpose in specific epidemiological context
- DIVA test selection depends on knowledge of locally circulating strains
- 2.5 recombinant strains seem predominant in SE Asian epidemic →

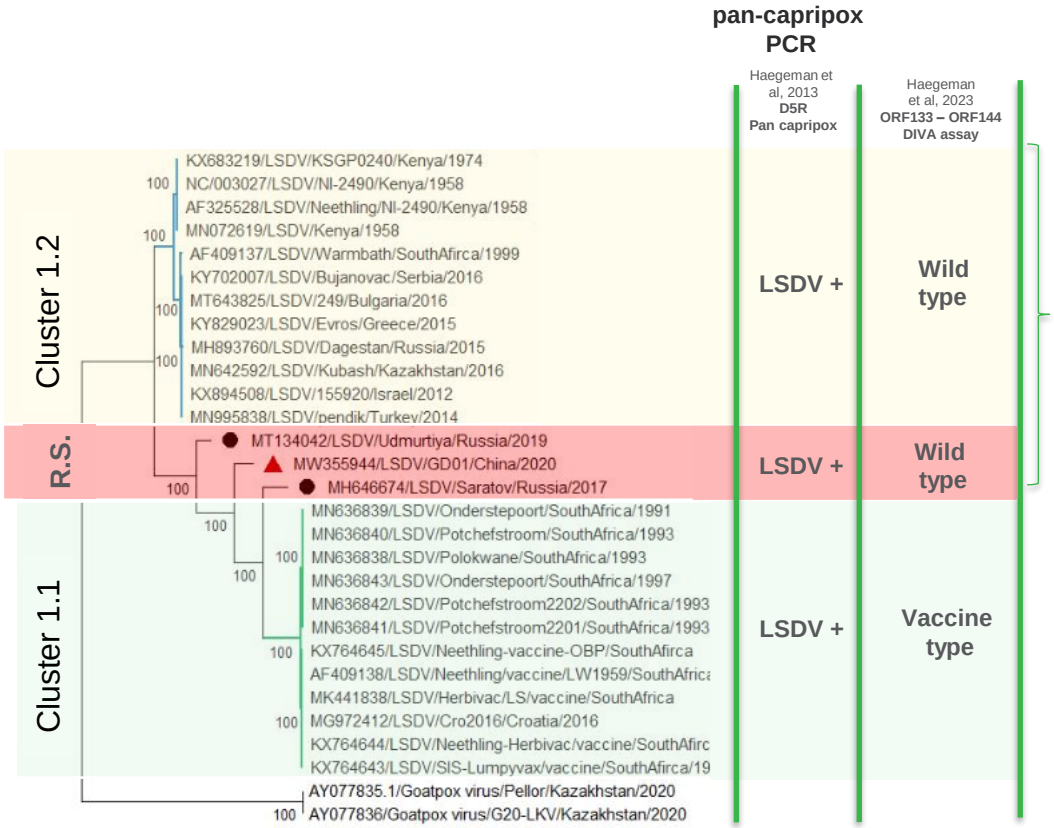

viruses MDPI

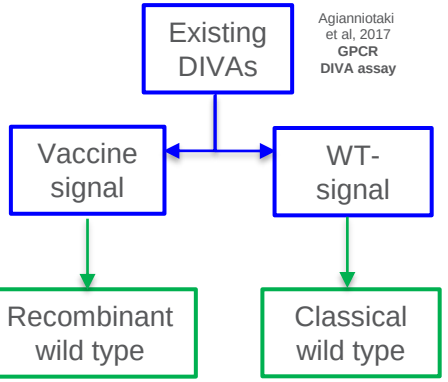
Article

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 [1,*], Ilse De Leeuw [1], Wannes Philips [2] and Nick De Regge [1]

Laboratory diagnosis – DIVA PCRs





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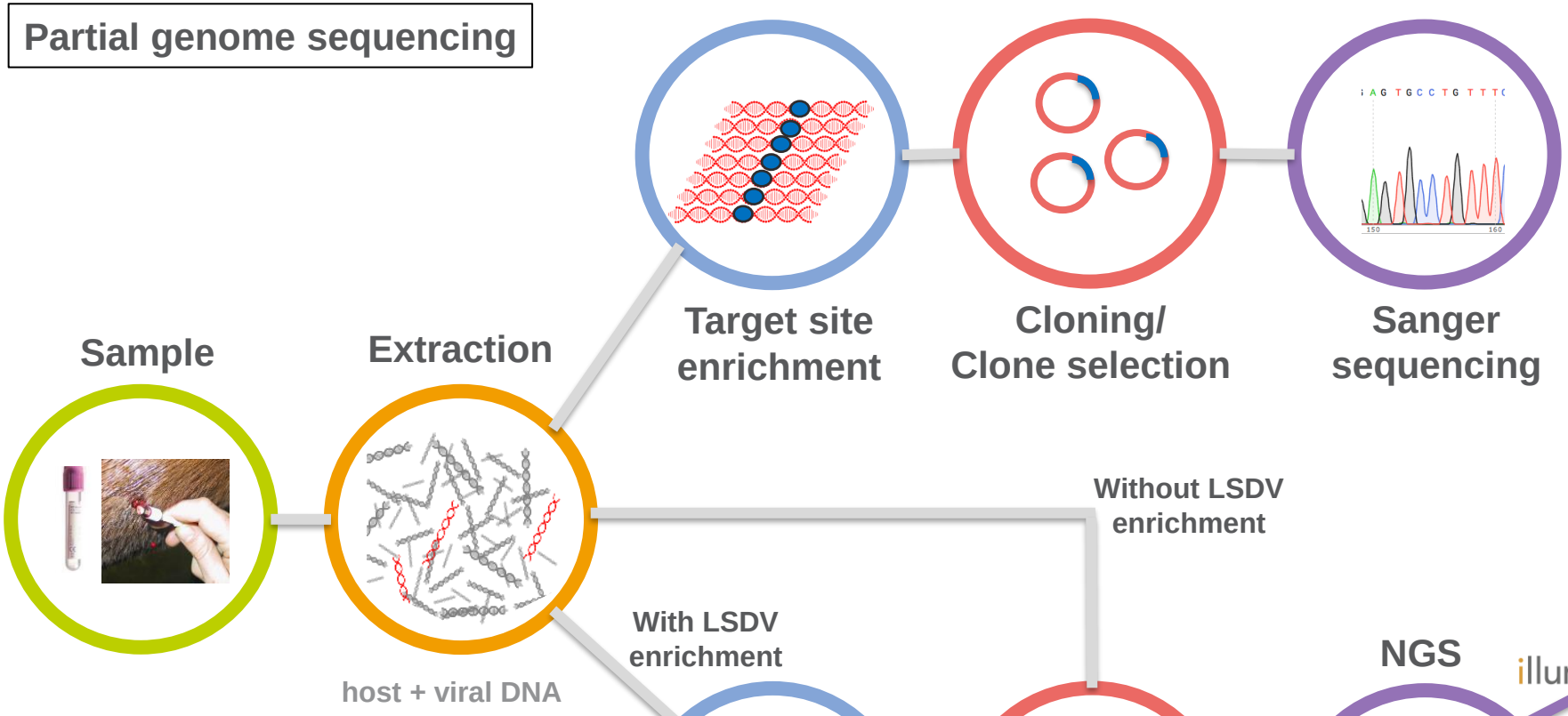
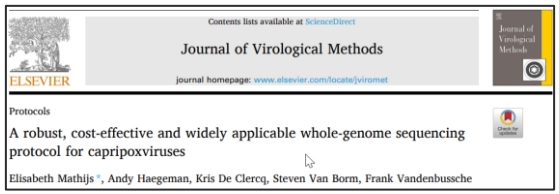
LSDV genome sequencing

Partial genome sequencing





Contents lists available at ScienceDirect

Journal of Virological Methods

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

Protocols

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

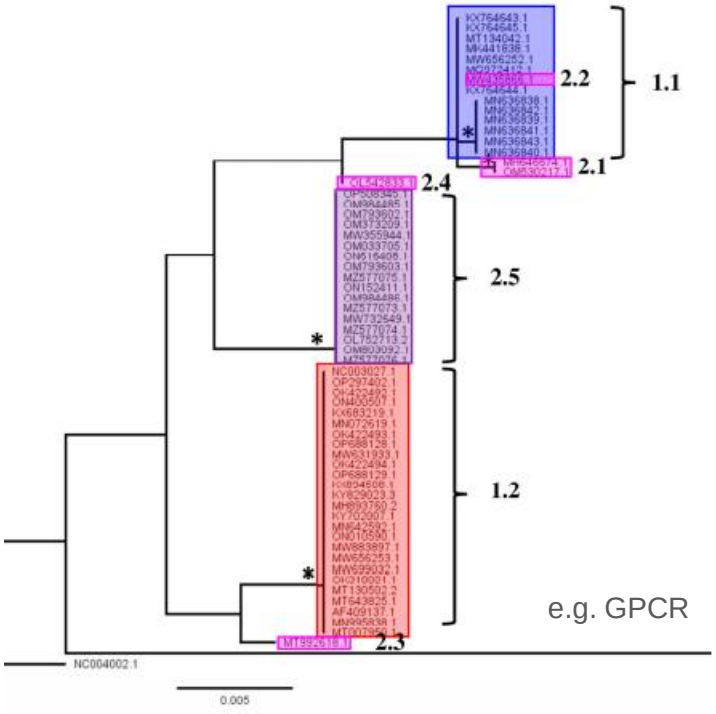
Elisabeth Mathijs *, Andy Haegeman, Kris De Clercq, Steven Van Borm, Frank Vandenbussche

Whole genome sequencing

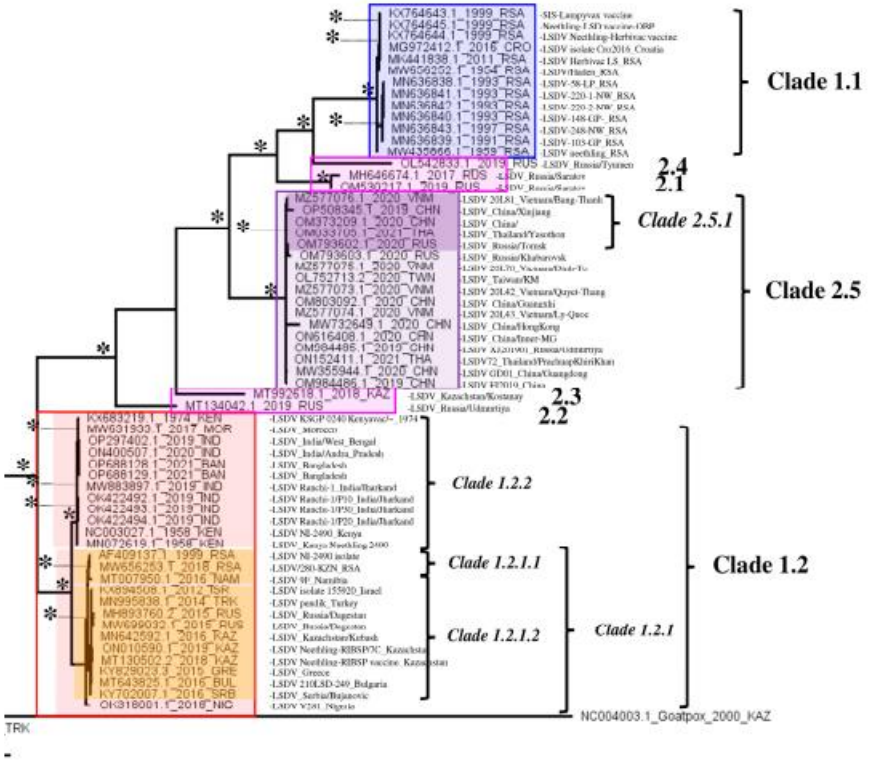
LSDV genome sequencing

Partial genome sequencing

- GPCR
- RPO30
- DNA_pol
- P32
- RPO132



Whole genome sequencing



Advisable to use WGS for monitoring of emerging LSDV strains

Virological workflow at WOAH RL

Sample

Pan Capx RT-PCR panel – Haegeman et al. 2013

Species differentiation
- Wolff et al, 2021
- Lamien et al, 2011

DIVA PCR
- Haegeman et al, 2023
- Agianniotaki et al, 2017 & 2021
- Vidanovic et al, 2021

Virus isolation

Next generation sequencing (WGS)
- With enrichment
- Without enrichment

Phylogenetic sequencing (Sanger)
- Haegeman et al, 2021

phylogeny

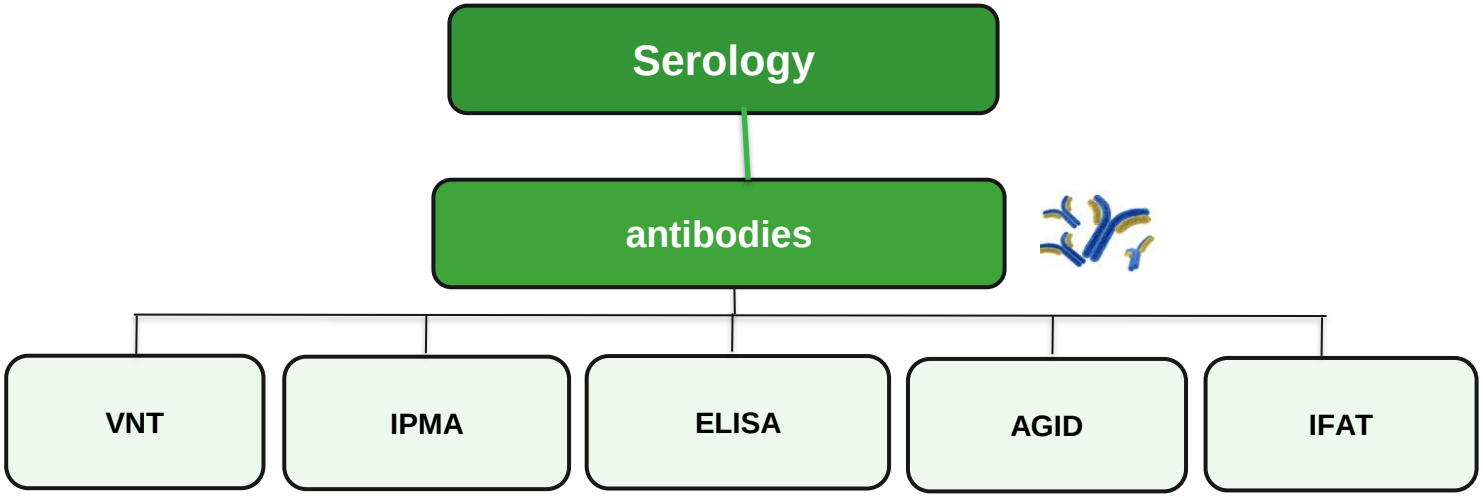
Laboratory diagnosis - serology

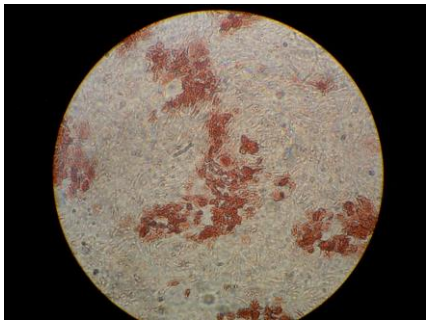
✓ Serum/serology

- Easy to collect
- Antibodies can remain present for a prolonged period of time
- Suitable for screening/surveillance/post-vaccination monitoring

- Not suitable for early detection
- Can take multiple weeks before seroconversion is detected
- Depends on the method used

Laboratory diagnosis - serology



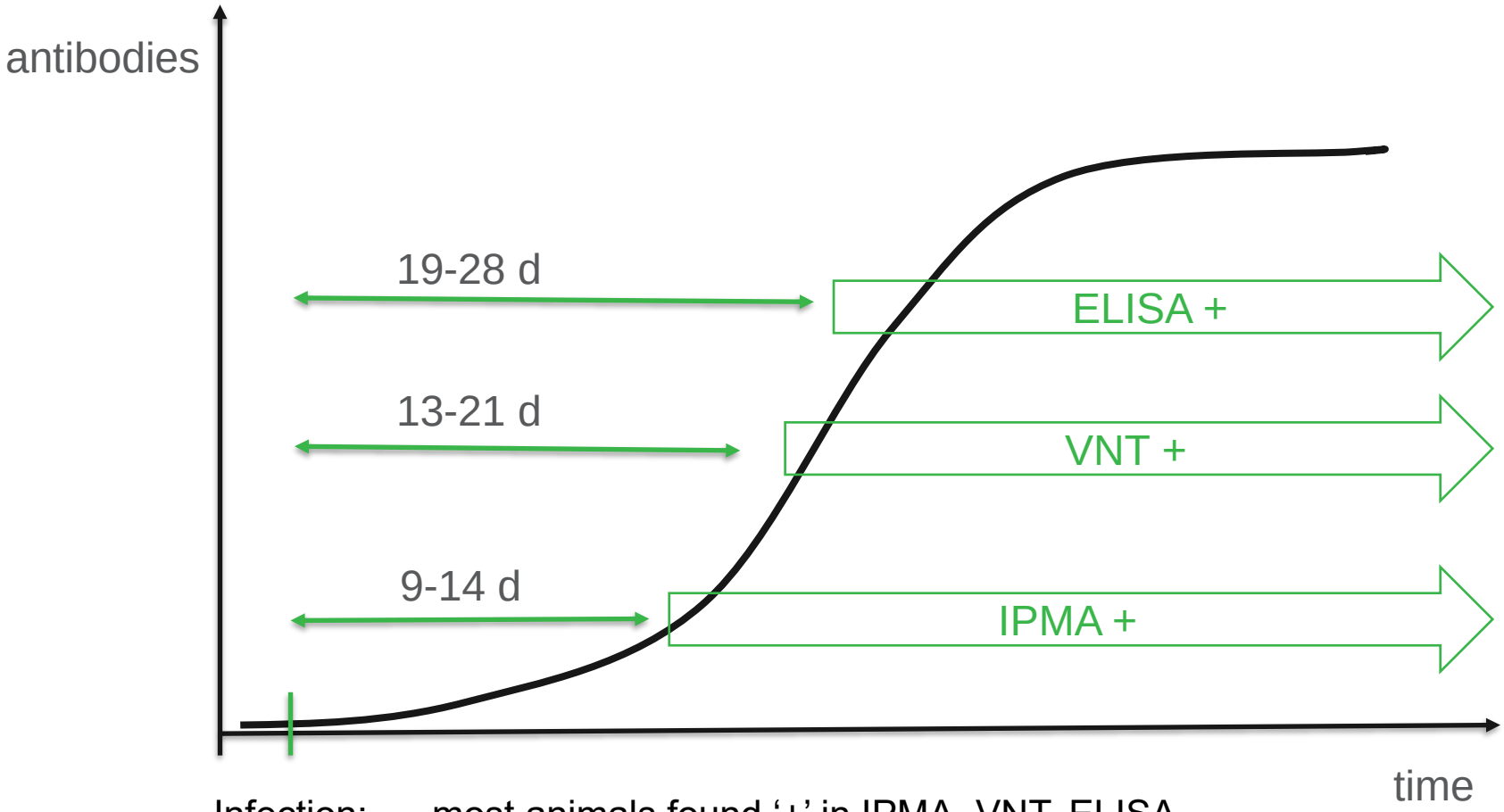


Laboratory diagnosis - serology

Test	Advantages	Disadvantages
VNT	- Reliable (gold standard) - 2 approaches	- Live virus - Time and costs - CPE effect ! Not virus specific
IPMA	- Early detection - Fast results if plates prepared - Highly sensitivity	- Experience - Live virus - More sensitive to sample quality
ELISA	- Commercially available - Easy to do - High throughput - Good sensitivity / specificity	- Less sensitive then VNT and IPMA, not for early detection
Western Blot	- High specific - High sensitive	- Difficult - Expensive
IFAT	- High sensitivity - Flexible (other dyes/microscopes)	- Cross reaction - Time and costs

LSDV diagnostics: serology

Virus neutralization tests – IPMA – ELISA



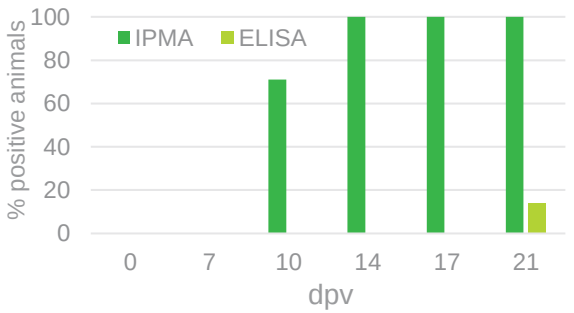
Infection: most animals found '+' in IPMA, VNT, ELISA

Vaccination: most animals found '+' in IPMA, VNT; only +/- 50% '+' in ELISA

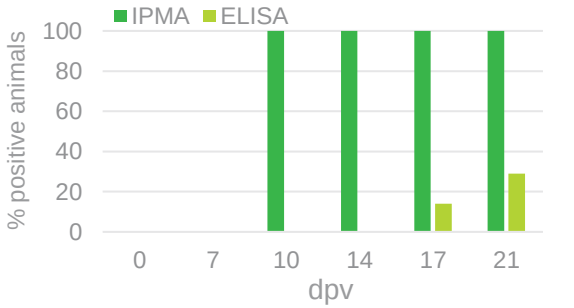
LSDV diagnostics: serology

vaccination

	LAV1-1		LAV1-2		LAV1-3		LAV1-4		LAV1-5		LAV1-6		LAV1-7	
	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA
0 dpv	NEG	3,667	NEG	3,771131	NEG	1,482445	NEG	6,63199	NEG	0,338	NEG	1,638	NEG	-0,858
7 dpv	NEG	-0,494	NEG	-0,026	NEG	-4,447	NEG	-6,112	NEG	0,286	NEG	4,811	NEG	0,182
10 dpv	50	1,014	50	1,118	50	-0,442	50	-0,234	50	-0,962	NEG	-2,887	NEG	-0,754
14 dpv	300	1,534	300	-0,026	300	5,852	300	1,482	50	-0,234	50	3,043	50	0,650
17 dpv	300	13,342	300	12,926	300	15,319	300	0,234	50	0,702	50	3,303	50	2,159
21 dpv	300	20,50332	300	29,19278	300	32,90598	300	13,16239	50	0,493827	50	18,78443	50	3,941121

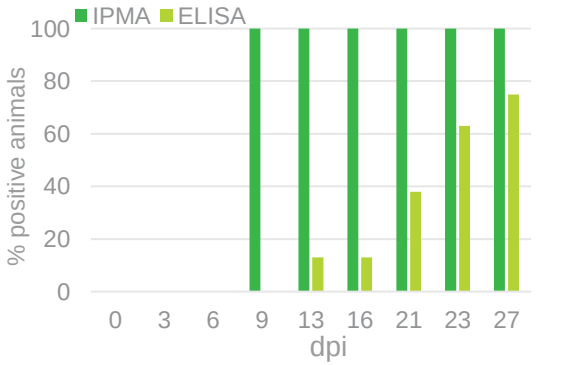


	LAV2-1		LAV2-2		LAV2-3		LAV2-4		LAV2-5		LAV2-6		LAV2-7	
	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA
0 dpv	NEG	2,003	NEG	-5,436	NEG	-2,627	NEG	-0,130	NEG	6,892	NEG	0,442	NEG	-0,182
7 dpv	NEG	-0,390	NEG	0,442	NEG	12,458	NEG	-1,118	NEG	-4,083	NEG	16,203	NEG	-5,384
10 dpv	50	-0,026	300	0,962	50	1,534	50	1,691	300	1,482	50	-0,494	300	9,753
14 dpv	300	0,078	300	4,863	300	-0,130	300	6,632	300	1,014	300	2,211	300	18,440
17 dpv	300	6,424	300	4,135	300	-1,795	300	17,971	300	-0,130	300	0,598	300	38,986
21 dpv	300	3,20038	300	14,79582	300	0,39886	300	55,64103	300	8,243115	300	0,588794	300	82,94397



infection

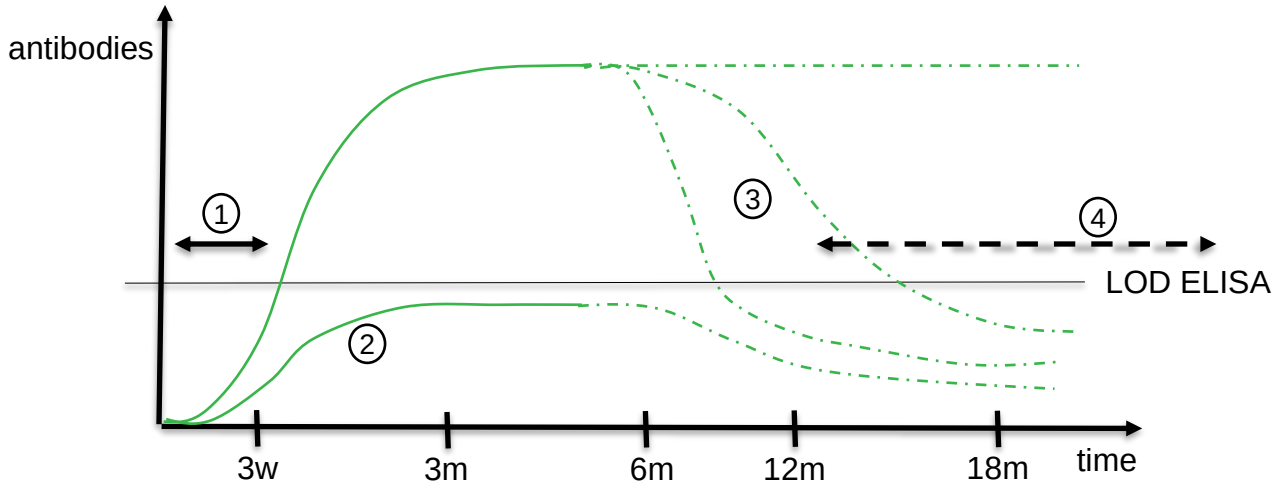
	INF1		INF2		INF3		INF4		INF5		INF6		INF7		INF8	
	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA	IPMA	ELISA
0 dpi	NEG	5,488	NEG	-0,026	NEG	0,910	NEG	-3,407	NEG	-0,130	NEG	-0,234	NEG	1,534	NEG	0,234
3 dpi	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A
6 dpi	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A	NEG	N/A
9 dpi	50	-0,962	50	0,650	300	-0,026	300	-2,367	50	0,650	50	0,078	50	0,172	50	0,302
13 dpi	300	9,222	300	33,053	300	0,732601	300	0,904977	300	4,266322	300	4,137039	300	0,818789	300	1,077354
16 dpi	300	17,19457	300	36,45766	300	3,792286	300	5,429864	300	9,696186	300	7,584572	300	6,377936	300	11,50614
21 dpi	300	65,84788	300	63,69317	300	2,58565	300	18,48739	300	27,36479	300	31,32945	300	9,954751	300	27,88192
23 dpi	300	113,2945	300	97,47899	300	4,567981	300	23,78798	300	62,44344	300	63,21913	300	10,2995	300	44,55936
27 dpi	300	225,8134	300	179,2286	300	8,015514	300	53,69532	300	75,37169	300	117,3454	300	18,83215	300	108,2094



Post vaccination monitoring

- No DIVA vaccines available – vaccinated and infected animals cannot be differentiated based on serology
- Important to keep good and complete records of vaccinated animals
- Important to implement passive and active LSDV clinical surveillance upon vaccination to ensure early detection of new cases
- Limitations for serological surveillance for post vaccination monitoring

Post vaccination monitoring



1. ELISA is most convenient test for PVM, but only around 50% of vaccinated animals develop antibody levels above the LOD of the commercial ELISA. 2 to 3 months post vaccination is best moment to measure efficacy of LSD vaccination

2. Vaccine induced antibodies might disappear over time although ELISA positive animals have been detected at 18m post vaccination under experimental conditions (unpublished EURL results)

3. Protection against challenge is not dependent on ELISA antibody detection in challenged animals, indicating role of CMI.

Acknowledgements

EUROPEAN COMMISSION