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EU Reference Laboratory
for Capripox viruses

Funded by the
European Union

levenslang gezond

THE IMPORTANCE OF QUALITY, SAFETY, EFFICACY TESTING FOR LUMPY SKIN DISEASE VACCINES

Andy Haegeman

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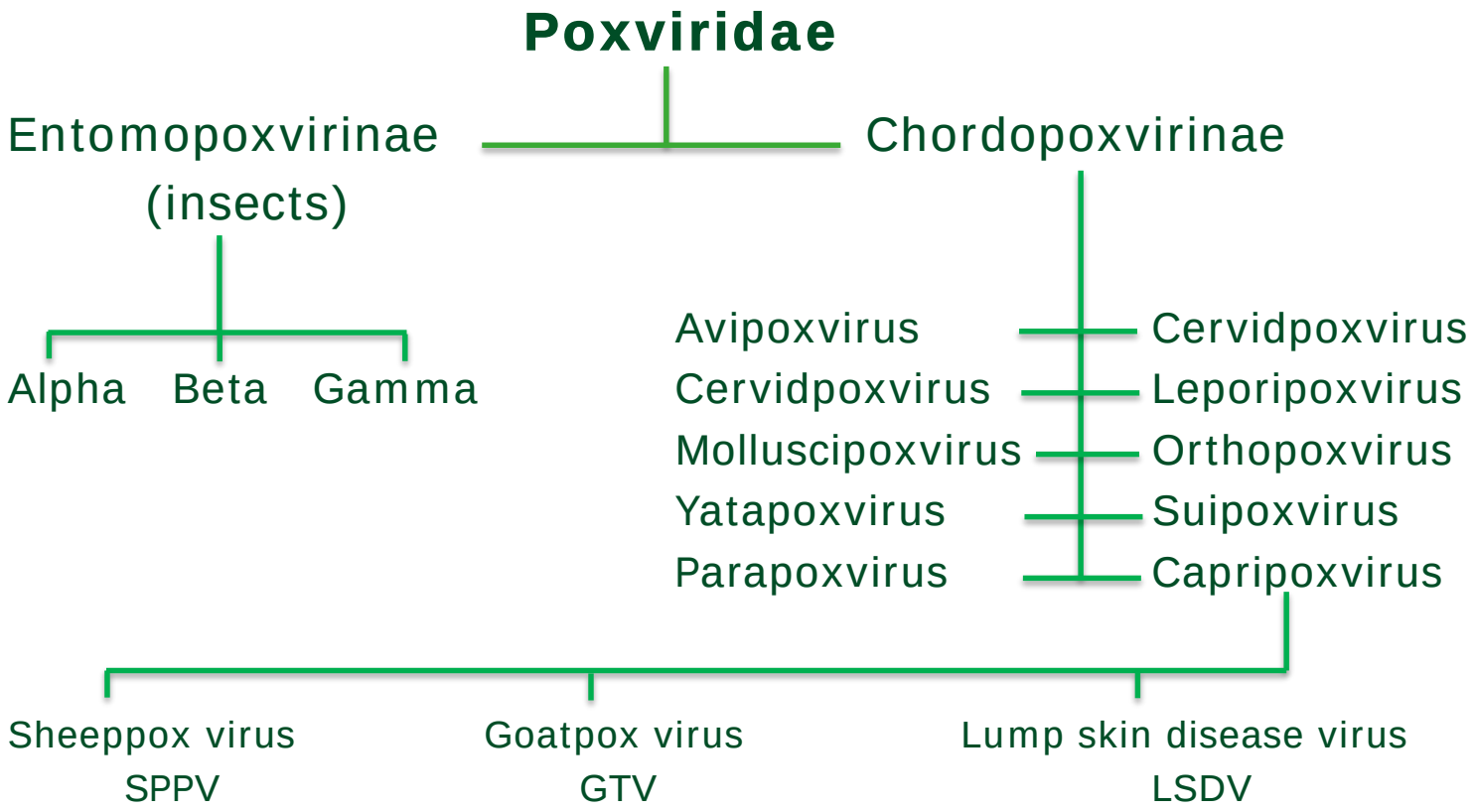
What is Lumpy skin disease ?

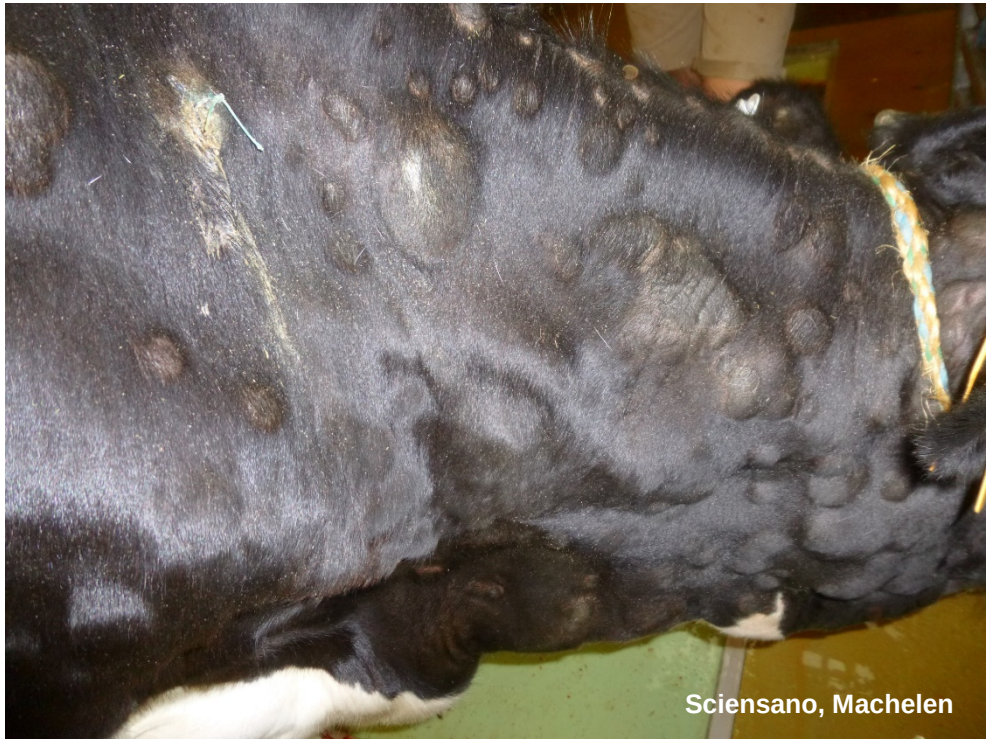
- Capripox
  - Classification



What is Lumpy skin disease ?

- Virus
  - Not segmented, dsDNA
  - Size: 150 to 160 kbp → +/- 147 putative genes
  - High nucleotide identity between SPPV, GTV, LSDV: +/- 96%

- Nodulaire dermatose (NL)
  Dermatose nodulaire contagieuse (Fr)
  Lumpy skin disease (Eng)
  Knopvelsiekte (Zuid-Afrikaans)



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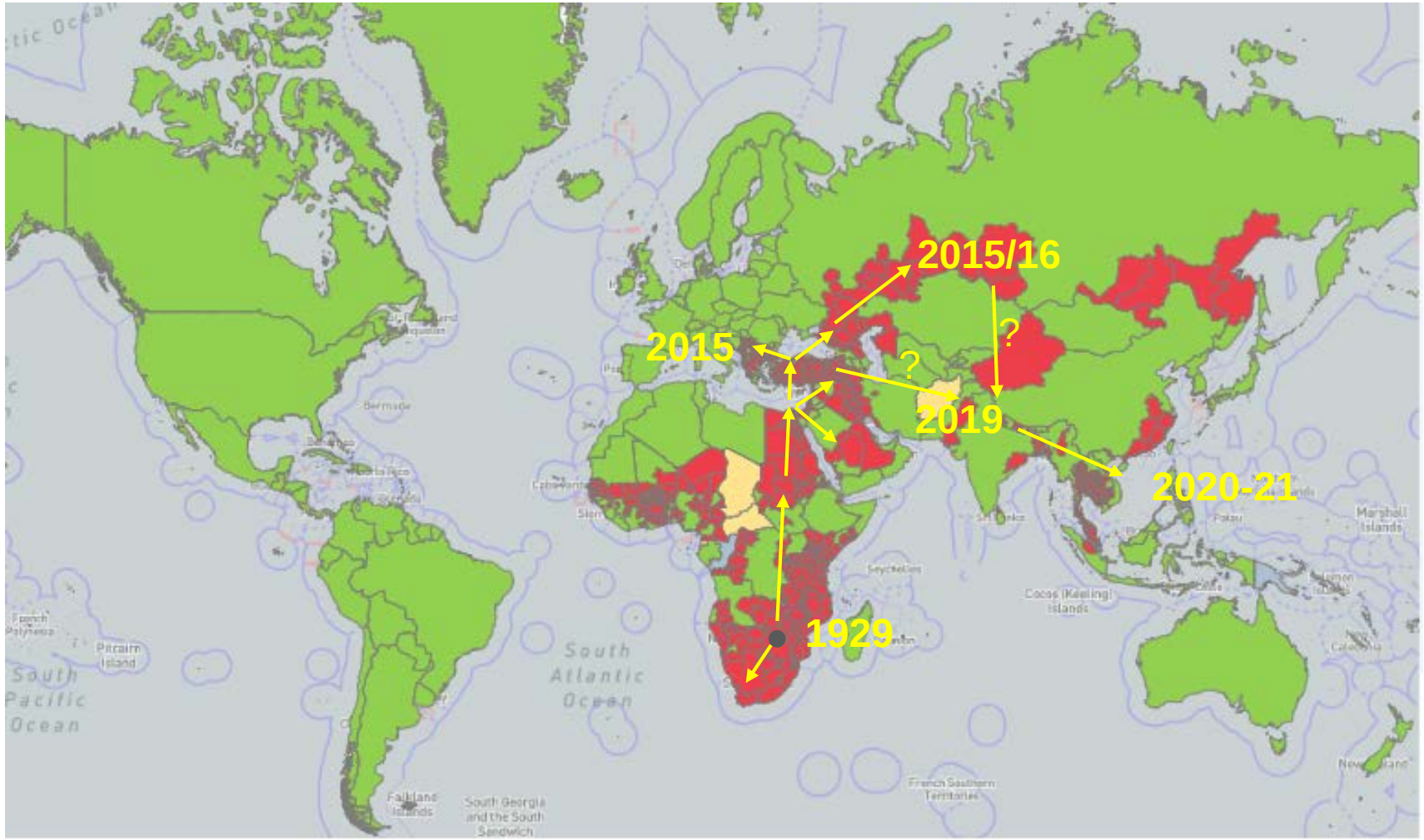
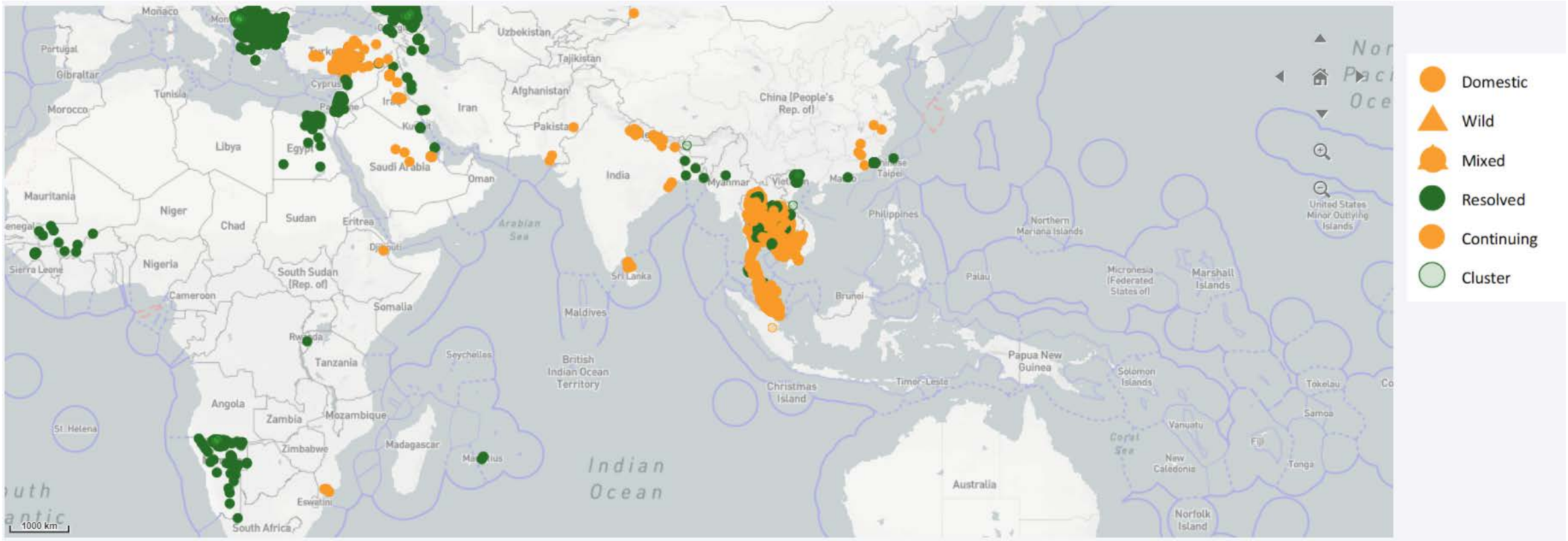
Where is LSDV?



Wahis db

Where is LSDV?



Wahis db 2007 - 2021

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Vaccine available ?

- Most commonly used: Live attenuated vaccines (LAV)
  - Based on LSDV: OBP, Lumpyvax, Herbivac, Kenyavac, Lumpivax, Lumpyvac, MCT (Neethling O)
  - Based on Sheeppox: Jovivac, Abic, Penpox-M, MCI (Romania)
  - Based upon Goatpox: Caprivac
- Recent: inactivated vaccines
  - Based upon LSDV: Bovivax

How good (efficacy) and safe are they ?

Not much data available under standardized conditions

Efficacy and safety

- Implementation of vaccination / challenge model

Israel field isolate
Titer 10^{6-7} $TCID_{50}$/ml
5ml intravenous
4x0,25ml intradermal

21 dpi monitoring

Clinical scoring/monitoring: fever, lnn swelling,local reactions, nodule development, feed uptake,…

Sampling: - EDTA blood, swabs (PCR, isolation)
- heparine blood (IFNg testing)
- serum (IPMA, VNT, ELISA)

Autopsy: biopts, organs (PCR, isolation)

Evaluation of different vaccines

Efficacy and safety

- Evaluation of different vaccines: some examples of data

Total Clinical scoring

1	2	3	7	9	14	22	23	24	27	28	29	Dpv
/	/	/	/	/	/	/	0,5	0,25	/	/	/	
/	/	/	/	/	/	/	1	0,25	/	/	/	
/	/	/	/	/	/	/	1	0,25	/	/	/	
/	/	/	/	/	/	/	0,5	0,25	/	/	/	
/	/	/	/	/	/	/	1	0,25	/	/	/	
/	/	/	/	/	/	/	1	/	/	/	/	
/	/	/	/	/	/	/	1	0,5	9	6	9	
6	3	6	4	4	9	>20	>20	>20	16	12	12	
10	10	12	12	8	16	>10	>10	10	16	16	14	
6	12	12	8	8	16	>10	>10	10	12	10	12	
6	8	12	10	8	32	>10	>10	>10	16	64	32	
20	20	16	16	16	16	>10	10	10	16	48	48	
5	8	16	12	24	12	>10	10	8	8	4	8	
4	1,5	3	3	1	/	1,5	9	2,5	12	10	12	

Some Neethling response in vaccinated animals

Challenge

Good candidate → limited local reaction. Did not protect against viremia after challenge

Not suited candidate → severe local reaction in all animals

LAV8

Control

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Efficacy and safety

type	strain	Vaccin	Company	Clinical signs		IPMA		IFNgamma	viremia		swabs	organs
				post vac	post chal	post vac	post chal	post vac	post vac	post chal	post chal*	post chal
LAV	LSDV	Lumpy Skin Dis Vac	OBP	A+	A++	+++	+	++	A+++	A+++	not tested	P-
	LSDV	LumpyVax	MSD Animal health	A+	A++	+	++	+++	A++	A+++	not tested	P-
	LSDV	KenyaVac	JOVAC	A++	A++	+	++	+++	A++	A+++	not tested	P-
	LSDV	Herbivac	Deltamune	P-	A+++	++	+	+++	P--	A+++	P-	P--
	LSDV	Neethling O	MCI	P--	A++	++	++	+++	A++	A+++	A++	P-
	LSDV	Lumpivax	Kevevapi	P-	A+++	+++	+	++	P-	A+++	not tested	P-
LAV	SPPV	Abic (10x)	Phibro	A++	P--	--	+++	+	A+++	P---	P--	P---
	SPPV	JoviVac	JOVAC	A+++	P-	-	++	++	A+++	P--	P-	P--
	SPPV	Penpox-M (3x)	Pendik	A+++	P--	+	++	++	A+++	P---	P--	P--
	SPPV	Romania (10x)	MCI	A+++	P--	--	+	+	A+++	P---	P--	P---
	GPV	CapriVac	JOVAC	A++	A+++	+++	+	+++	A++	A+++	not tested	A+++
INAC	LSDV	Bovivax (?)	MCI	A+	A++	+++	++	+++	A++	A+++	A+++	P-
	SPPV	Romania	MCI	A+++	P-	+	-	+	A+	P--	P-	P--

A: Absent; P: Present; *only viremic animals tested; +/-:weak; ++/--: intermediate; +++/---: strong

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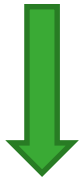
Quality control

- Vaccine properties: efficacy, safety, duration……



Passed

- Production phase → New series from challenges
  - Contamination issues
    - ✓ Used reagents (cell culture medium, fetal serum….)
    - ✓ Seeder cells (mycoplasmas,….)
    - ✓ Equipment failure
    - ✓ Equipment decontamination insufficient (multiple production processes)
  - Biological systems → Batch differences
    - ✓ Growing kinetics virus
    - ✓ Infection strength
  - Post production issues
    - ✓ Packaging, storage

Introduction foreign agents

Titer differences

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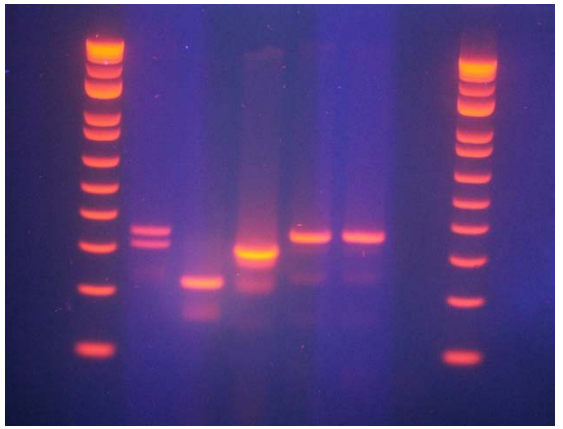
Case: Lumpivax, a Neethling-based Live attenuated vaccine against Lumpy Skin Disease

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

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Case: Lumpivax, a Neethling-based Live attenuated vaccine against Lumpy Skin Disease

4. Sequencing
   - Region 1
   - Region 2
   - Region 3

   → 1 clone sequenced → **Field type GTPV (Sudan)**

   Cfr DIVA 3 results

   - Region 6 → 1 clone sequenced → **LSDV**
   - Region 4 → 24 clone sequenced →

     58 % Field type GTPV (Sudan)

     29 % LSDV like

     57% Neethling like vaccine

     43% Field type LSDV (Evros, KSGPO240…)

     19 % Hybrid LSDV – GTPV: Recombinants?

Case: Lumpivax, a Neethling-based Live attenuated vaccine against Lumpy Skin Disease

4. 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 → **100 % LSDV like**
     55% Neethling like vaccine
     18 % Field type LSDV
     27% Hybrid LSDV Vaccine/Field Recombinants ?

Case: Lumpivax, a Neethling-based Live attenuated vaccine against Lumpy Skin Disease

4. 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**

Conclusion: Sequencing confirmed the presence of GTPV and field type LSDV sequences in addition to Neethling- like sequences

→ **Methodology – Linked?**

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Methodology check

Part 1 Sequencing

- OBP vaccine was used
- Region 1,2, 4, 5 and 6 → 20 to 25 clones sequenced → 100% Neethling
- No trace of GTPV or field like LSDV
- Minor variants present

PART 2: Hybrids due to PCR artifacts??

- Mixing equimolar quantities of Caprivac and OBP
- Region 4 → 18 clones sequenced → 50% OBP + 39% GTPV-like + **11% hybrids !**

Conclusion:

- The finding of GTPV and LSDV field like sequences not due to methodology
- Hybrid PCR fragments can be caused by PCR

Impact on vaccine quality: challenge study

Vaccine – challenge study (cfr Haegeman et al 2021b)

- Clinical findings:
  - 43% developed a severe local reaction at the site of vaccination
  - Neethling response in 3 animals
  - Good clinical protection after challenge

- Virological
  - 1 animal with a clear vaccine viremia + 2 animals with very limited detections
  - No virus detected after challenge

Impact on vaccine quality: challenge study

Vaccine – challenge study (cfr Haegeman et al 2021b)

- Virological:
  - Characterization of blood taken at 14 Dpv (vaccine viremia)

DIVA 1: Vaccine- like
DIVA 2: Field type Like } **Contradictory !!!**

DIVA 3: LSDV- Like → 18 clones sequenced → 100% Vaccine like

  - Characterization of biopsies taken during Neethling response

Impact on vaccine quality: challenge study

Time	Sample (a)	panCapx	DIVA-1	DIVA-2	DIVA-3
10 dpv	R3V biopsy vaccination site	15.97	WT + VAC	WT + VAC	LSDV *
14 dpv	R3V biopsy nodule	35.00	VAC	WT	SPPV/GTPV * + LSDV
14 dpv	R3V biopsy normal looking skin 1	NT	VAC	WT + VAC	LSDV
1				WT + VAC	no signal
1				Neg	SPPV/GTPV * + LSDV
1				WT + VAC	LSDV
1				WT	LSDV *
1				WT	LSDV *
1				NT	LSDV *
1				WT	SPPV/GTPV
1				WT + VAC	no signal
1				Neg	no signal
17 dpv	R3V biopsy normal looking skin	31,22	WT + VAC	WT + VAC	no signal

PCR repeated 4 times: fragments cloned
→ Neethling vaccine and field type LSDV each detected

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Impact on vaccine quality: challenge study

Vaccine – challenge study (cfr Haegeman et al 2021b)

- Serological and IFNg release
  - IPMA: 100% seroconversion at 14 Dpv
  - IFNg profile: medium responder (57% responsive animals)

- Overall conclusion animal trial:
  - Good clinical protection
  - Side effects
    - Neethling response more pronounced than OBP, Lumpyvax
    - Local reaction at the vaccine site was often observed
  - Vaccine viremia can occur
  - Serological and IFNg release comparable to other LSDV LAV

BUT

- GTPV and LSDV field like sequences found in biopsies and the latter also in blood
- Contradictory status seen in biopsies

OVERALL CONCLUSION

Vaccine efficacy: in general OK (some minor issues)

BUT What is really present in vaccine vial ???

GTPV and field type LSDV sequences were found: Different strains ??? Recombinants?? Combination of both ???

Also observed in vaccination animals !!!!

Wrong identification is possible !

In vitro + In vivo together:

Presence of recombinants explains best the observed data but are they alone ???

→ Additional confirmation (whole genome sequencing) is warranted

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TAKEAWAY MESSAGE

The heterogeneity observed in the vaccine vial combined with the impact on diagnostics and LSDV epidemiology is troublesome as it is unclear how this will vary, batch to batch, and evolve with the creation of new or additional recombinations. Therefore, quality control is not only warranted but this for each batch!

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