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DIAGNOSTICS LUMPY SKIN DISEASE

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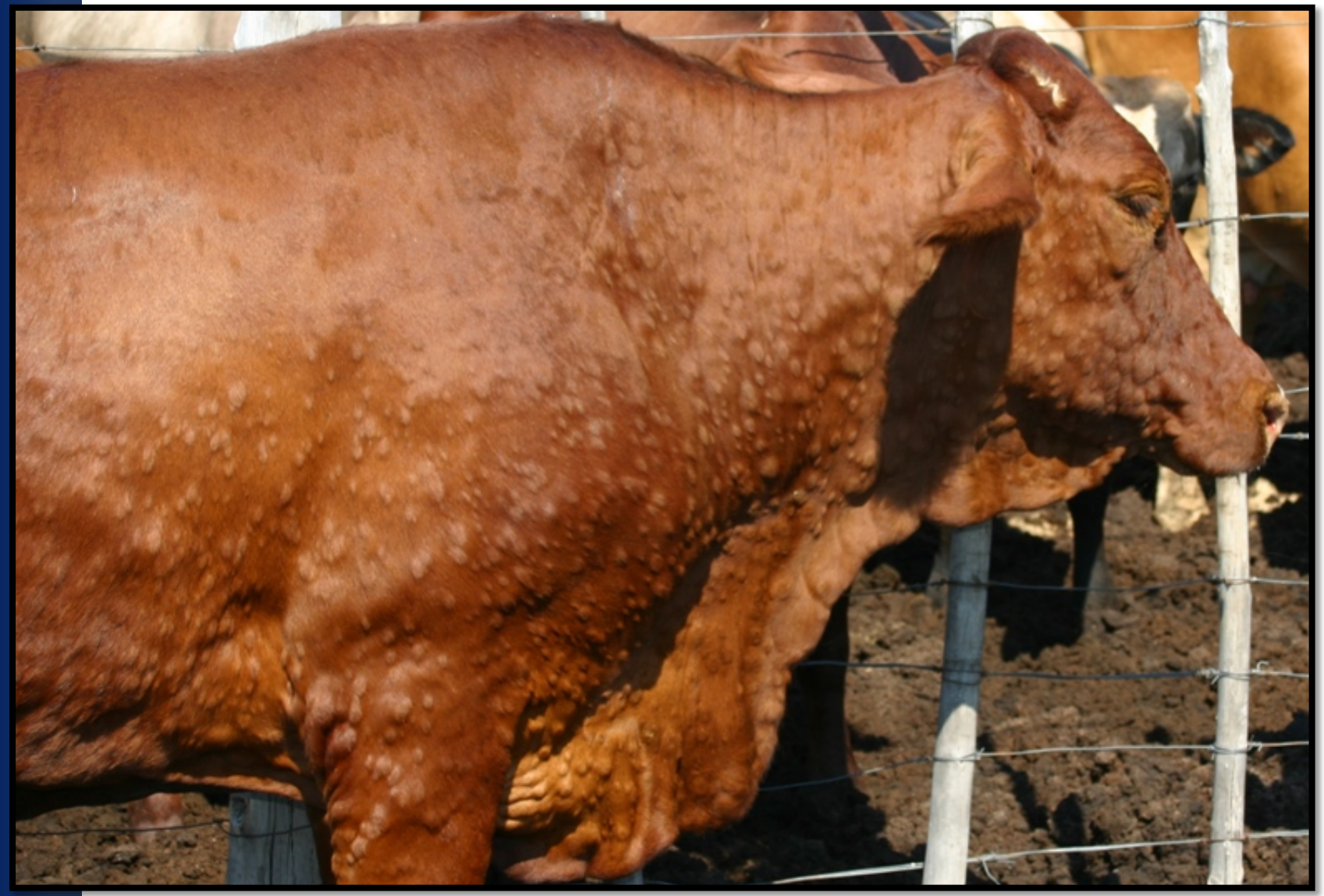
Lumpy skin disease: Characteristic clinical signs

- Fever
- Skin nodules
- Drop in milk production



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Lumpy skin disease: Laboratory confirmation

- Laboratory confirmation:

- "Gold standard" serological test: serum/virus neutralization test (SNT/VNT).

Not all animals either naturally infected or vaccinated develop LSDV neutralizing antibodies.

- Enzyme-linked immunosorbent assay (ELISA) by IDVet (France).
- Conventional and Real-Time Polymerase chain reaction (PCR) assays.
- Virus isolation on cell culture (Skin nodules).

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Lumpy skin disease: WOAH Manual: B. DIAGNOSTIC TECHNIQUES

Key:

+++ = recommended for this purpose

++ recommended but has limitations

+ = suitable in very limited circumstances

– = not appropriate for this purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent						
Virus isolation	+	++	+	+++	+	–
PCR	++	+++	++	+++	+	–
Transmission electron microscopy	–	–	–	+	–	–
Detection of immune response						
VNT	++	++	++	++	++	++
IFAT	+	+	+	+	+	+
ELISA	++	++	++	++	++	++

PCR = polymerase chain reaction; VNT = virus neutralisation test;
IFAT = indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay

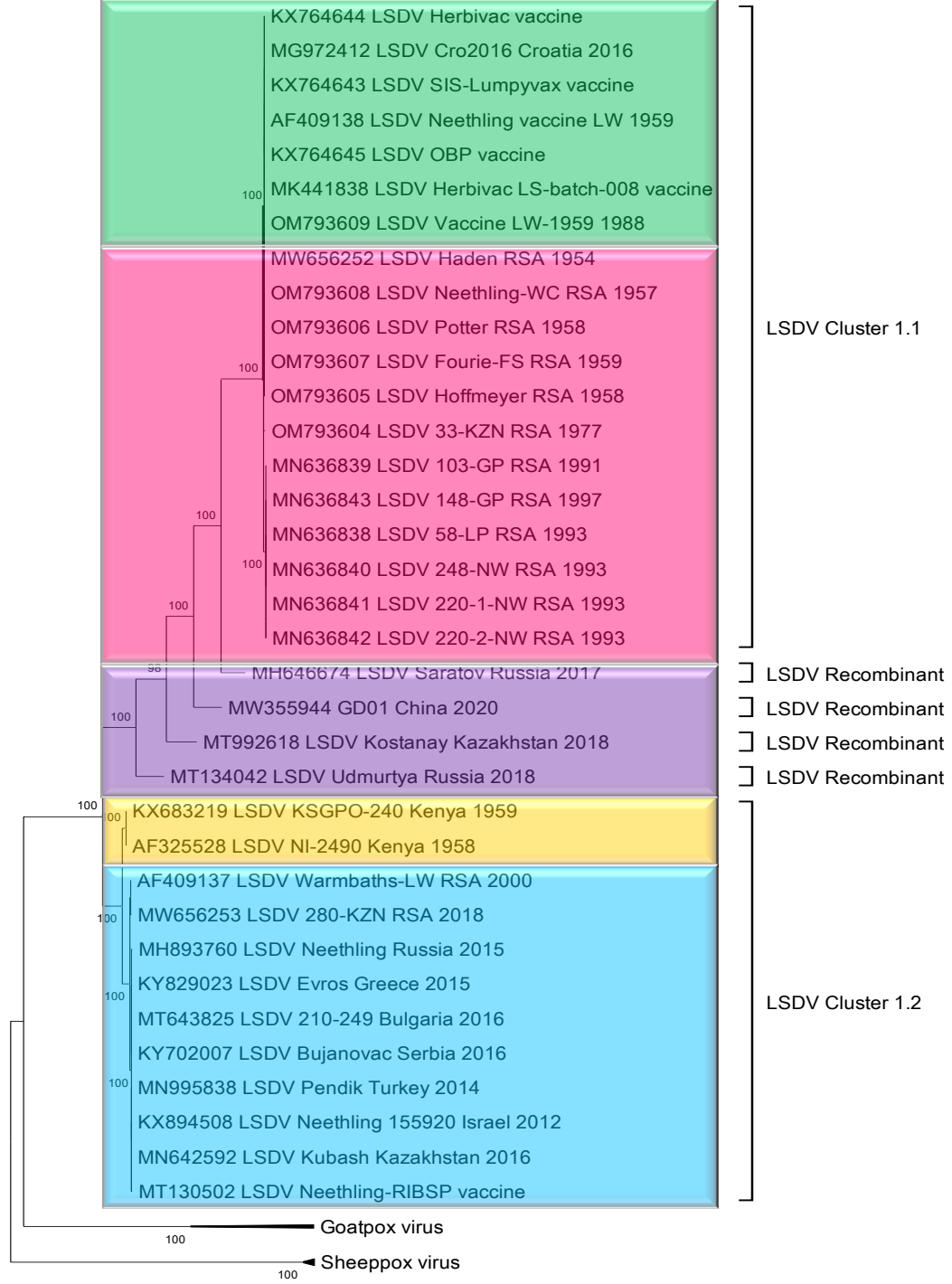
Lumpy skin disease: Molecular test to differentiate vaccine and wild-type LSDV

DIVA: Differentiation of Infected from Vaccinated Animals

Quantitative real-time PCR assays have been designed to differentiate between Neethling-based LSDV strains, which are often used for vaccination, and wild-type LSDV strains from cluster 1.2 (Agianniotaki et al., 2017; Pestova et al., 2018; Vidanovic et al., 2016).

Differentiate between Cluster 1.1 and 1.2

These DIVA assays (enable, for example, differentiation of “Neethling response” caused by vaccination with a LSDV Neethling vaccine strain from disease caused by infection with a cluster 1.2 wild-type virus.



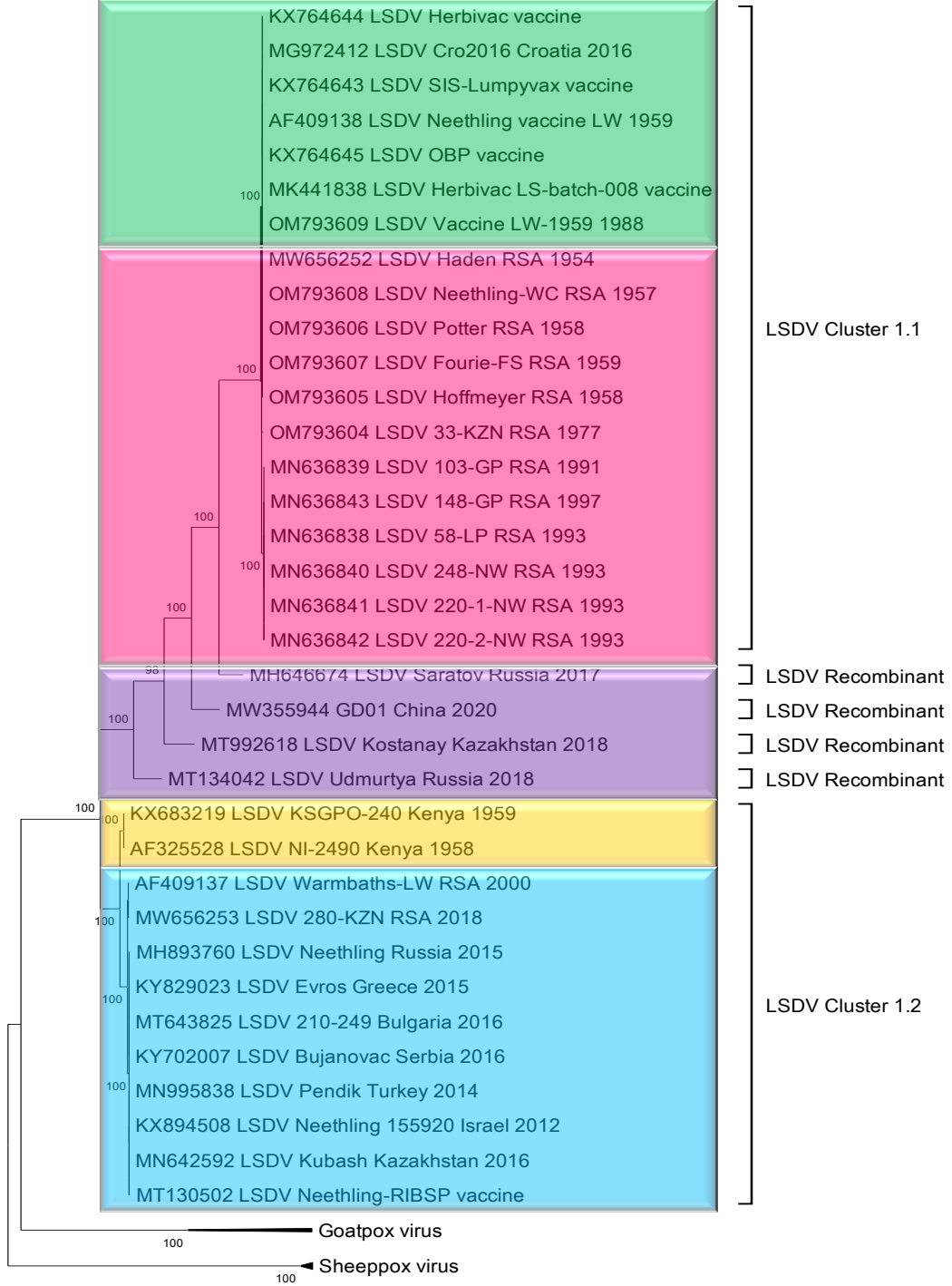
Lumpy skin disease: Molecular test to differentiate vaccine and wild-type LSDV

DIVA: **D**ifferentiation of **I**nfected from **V**accinated **A**nimals

CAN NOT **d**ifferentiate between vaccine and wild type isolates within Cluster 1.1

CAN NOT **d**ifferentiate between vaccines and novel recombinant strains (Cluster 2.1 – 2.5)

However, these DIVA PCR assays cannot distinguish between a LSDV Neethling vaccine strain and the novel recombinant LSDV strains recently isolated from disease outbreaks in Asia (Byadovskaya et al., 2021; Flannery et al., 2021). These DIVA assays are also not capable of discriminating between LSDV Neethling vaccine strains and recently characterised (historic) wild-type viruses from South Africa belonging within cluster 1.1 (Van Schalkwyk et al., 2020; 2021).



Lumpy skin disease: Epidemiology

Molecular markers

GPCR: TaqMan;
(Agianniotaki et al., 2017)

RPO30

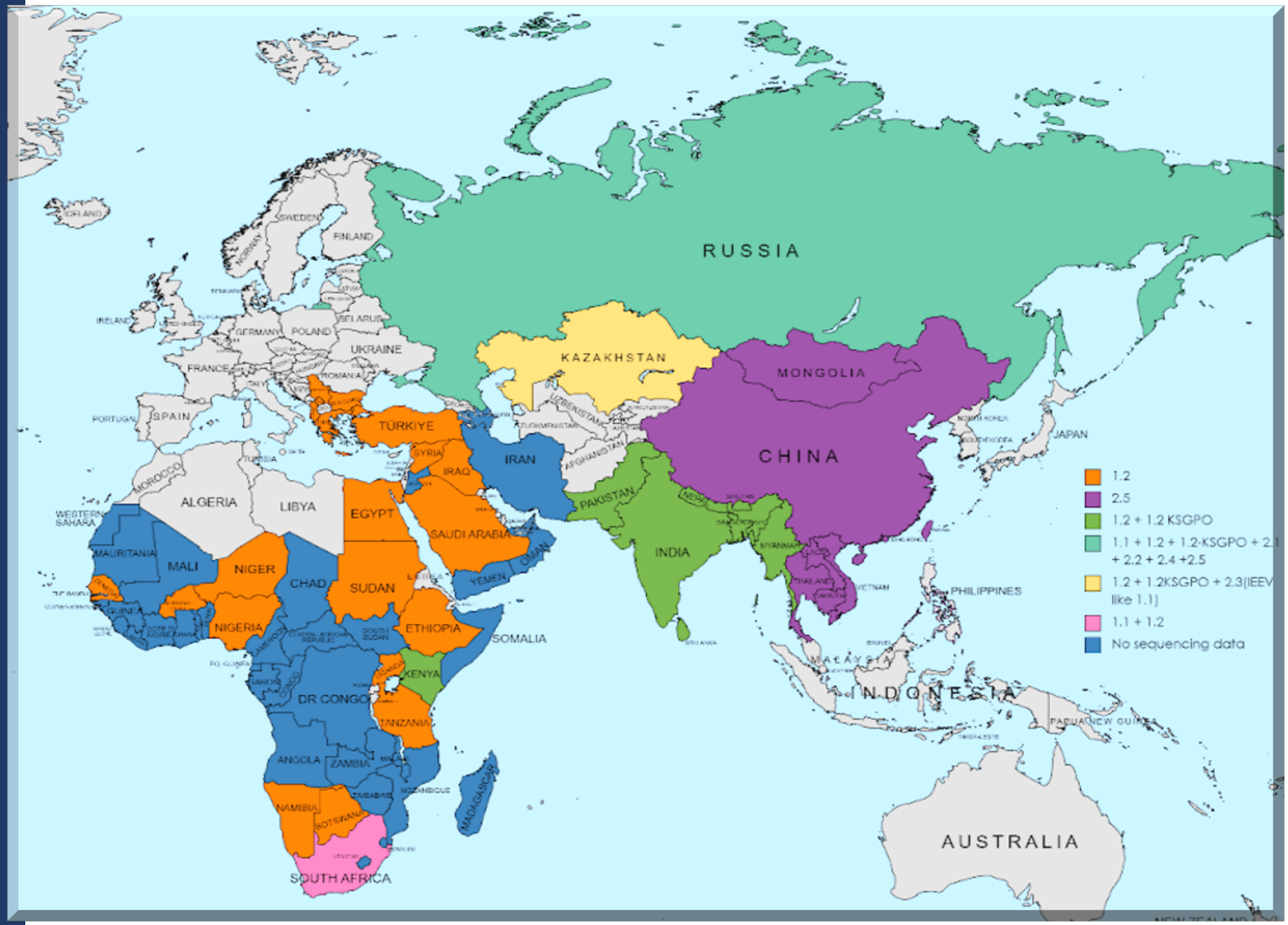
LW008: TaqMan;
(Vidanovic et al., 2021)

EEV
(Agianniotaki et al., 2021)

P32

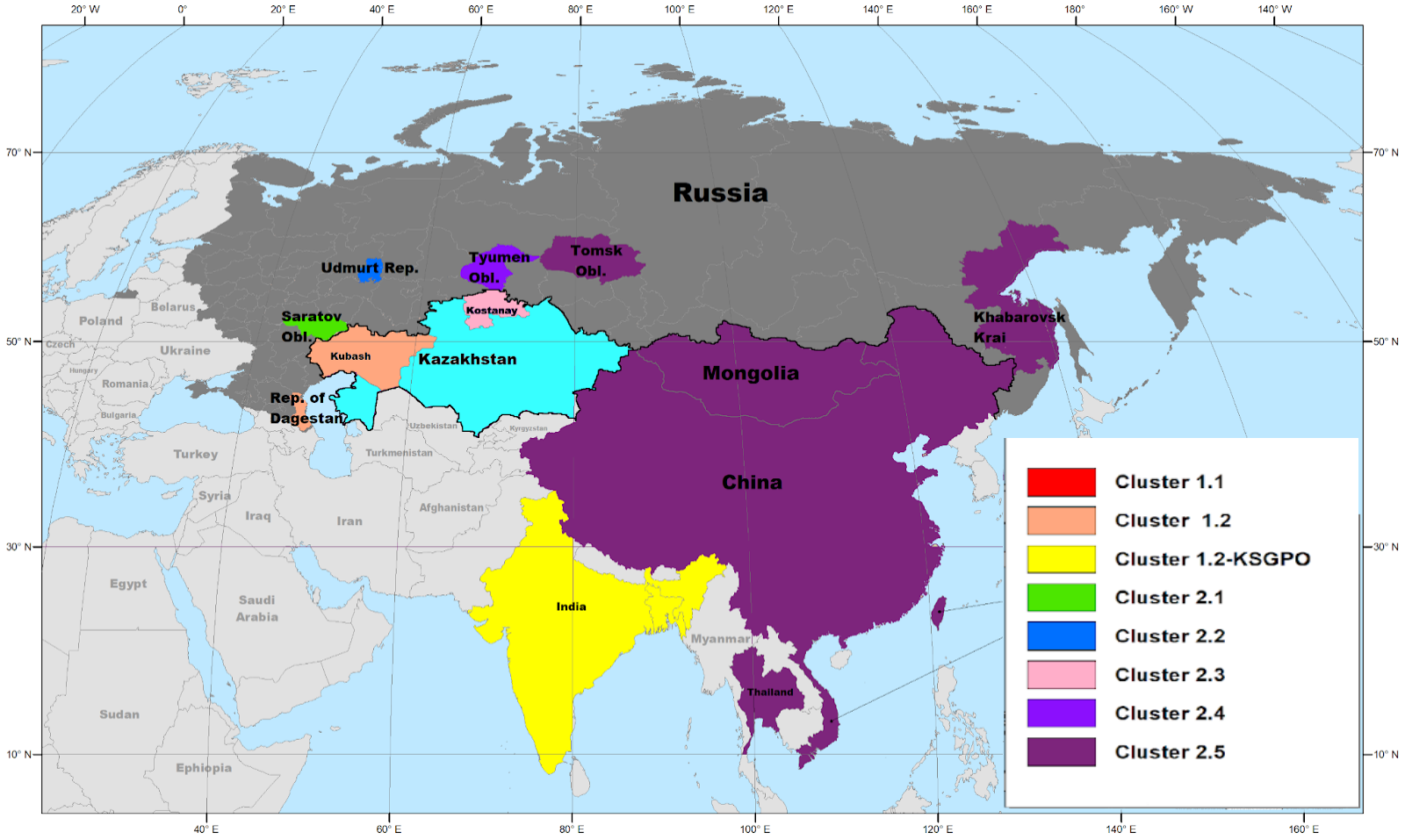
LW044: TaqMan
(Sprygin et al., 2019)

Complete genomes



Lumpy skin disease: Molecular test to differentiate vaccine and wild-type LSDV

Consequently, in regions where recombinant strains (currently Asia and possibly elsewhere) or wild-type cluster 1.1 strains are circulating (currently South Africa and possibly elsewhere) are circulating, these DIVA assays are not suitable for distinguishing vaccine and wild-type virus. Thus, in order to overcome these constraints, whole genome sequencing is recommended.



CAN NOT differentiate between vaccines and novel recombinant strains (Cluster 2.1 – 2.5)

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Lumpy skin disease: New Markers

Kumar et al., 2023	HRM-based gap-qRT-PCR: 801bp in terminal repeat region (ITR) • Vaccine: (Lumpi-ProVac^{Ind}) vs. Wild type: (LSDV/2019/India/Ranchi)
Haegeman et al., 2023	Duplex qRT-PCR: LW133 and LW144 • Vaccine (Neethling) vs. Wild type: Cluster 1.2 vs. Recombinant (Cluster 2.5)
Krotova et al., 2023	PCR and Sanger sequencing: 705bp in ORF LW134 • Vaccines (Neethling and KSGPO) vs. Wild type Cluster 1.2 vs. Recombinant (Cluster 2.1, 2.2, 2.3, 2.4 and 2.5)

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Lumpy skin disease: New Diagnostic tests

Nan et al., 2023	Triplex real-time PCR: • LSDV vs GTPV vs SPPV
Liao et al., 2023	CRISPR-Cas12a: • LSDV vs GTPV vs SPPV
Nandi et al., 2023	Isothermal PCR: 27bp in ORF LW126 • Vaccines (GTPV) vs. Wild type Cluster 1.2-KSGPO
Abdalhamed et al., 2022	Gold nanoparticle – lateral flow test
Sthitmatee et al., 2023	In-house ELISA using whole virus (LSD/THA/CMU/21/05)

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Questions?

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