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## Improvement of molecular and serological tools for the detection of Venezuelan Equine Encephalitis Virus (VEEV)

Marine Dumarest, Camille Victoire Migné, Manon Chevrier, Naomie Ramage, Teheipuaura Helle, Scott C Weaver, Nolwenn M. Dheilily, Sylvie Lecollinet, Stéphan Zientara, Cécile Beck, et al.

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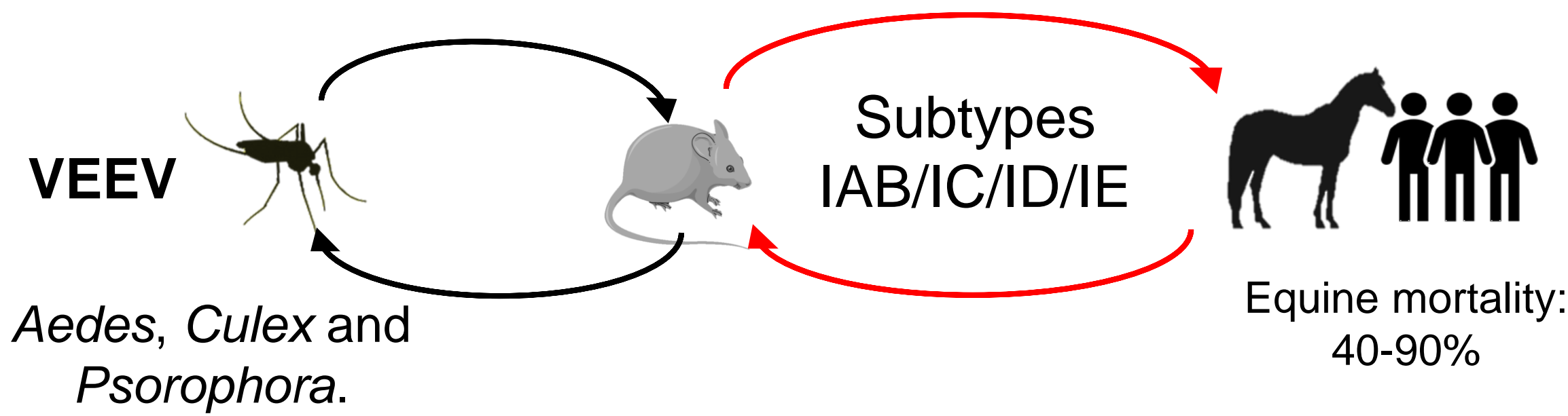
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## CONTEXT

VEEV belongs to *Alphavirus* genus within the *Togaviridae* family.

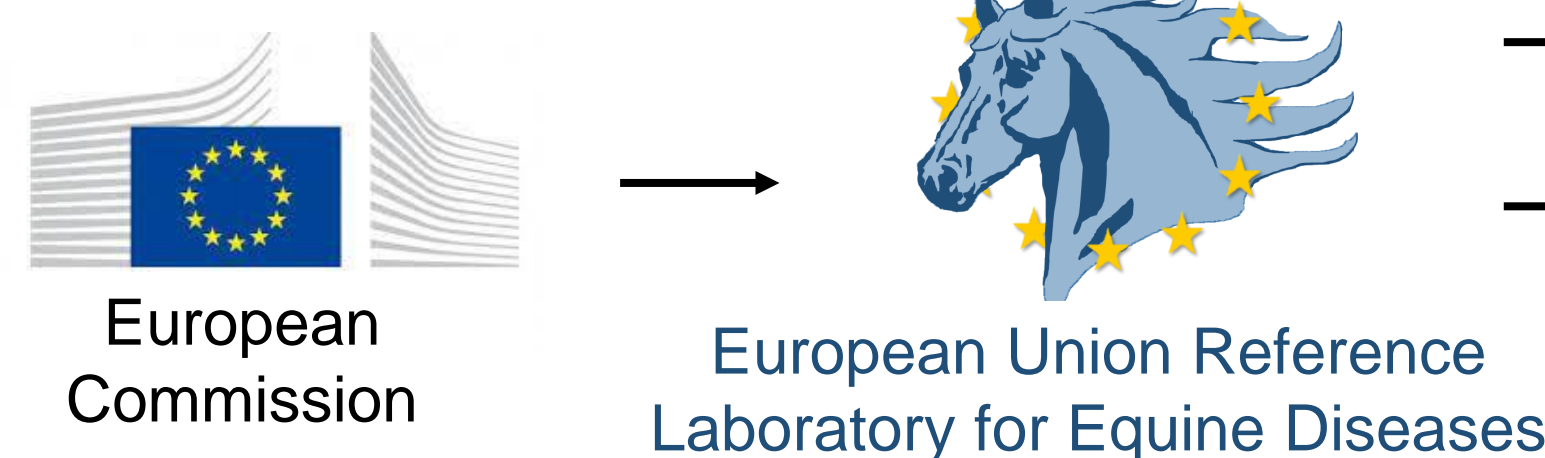


Distribution of VEEV and risk of emergence in Europe

- **Bioweapon:** easy aerosolization and low extremely infectious dose
- Classified in **categories D/E** (Animal Health Law 2021)

→ **Systematic control of horses imported from the 3<sup>rd</sup> countries**

## OBJECTIVES



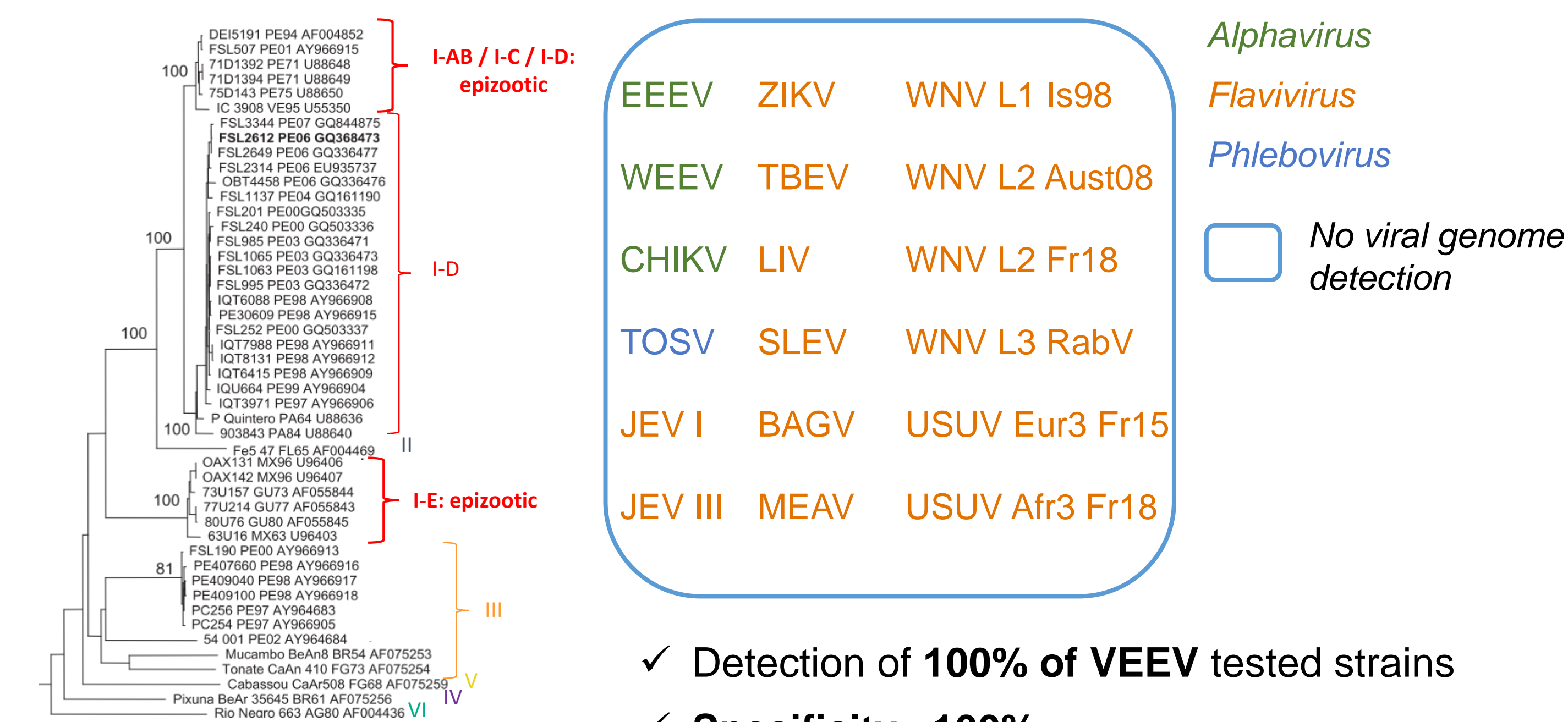
Surveillance  
Development of innovative diagnostic tools

- Optimization of **molecular** and **serological diagnostic** tools to detect rapidly and specifically VEEV
- **Transfer of knowledges**

VEEV RT-qPCR  
Virus neutralization test (VNT) in BSL2  
Indirect ELISA  
Hemagglutination inhibition assay

## RESULTS

### 1. Optimization of the sensitivity of VEEV RT-qPCR method

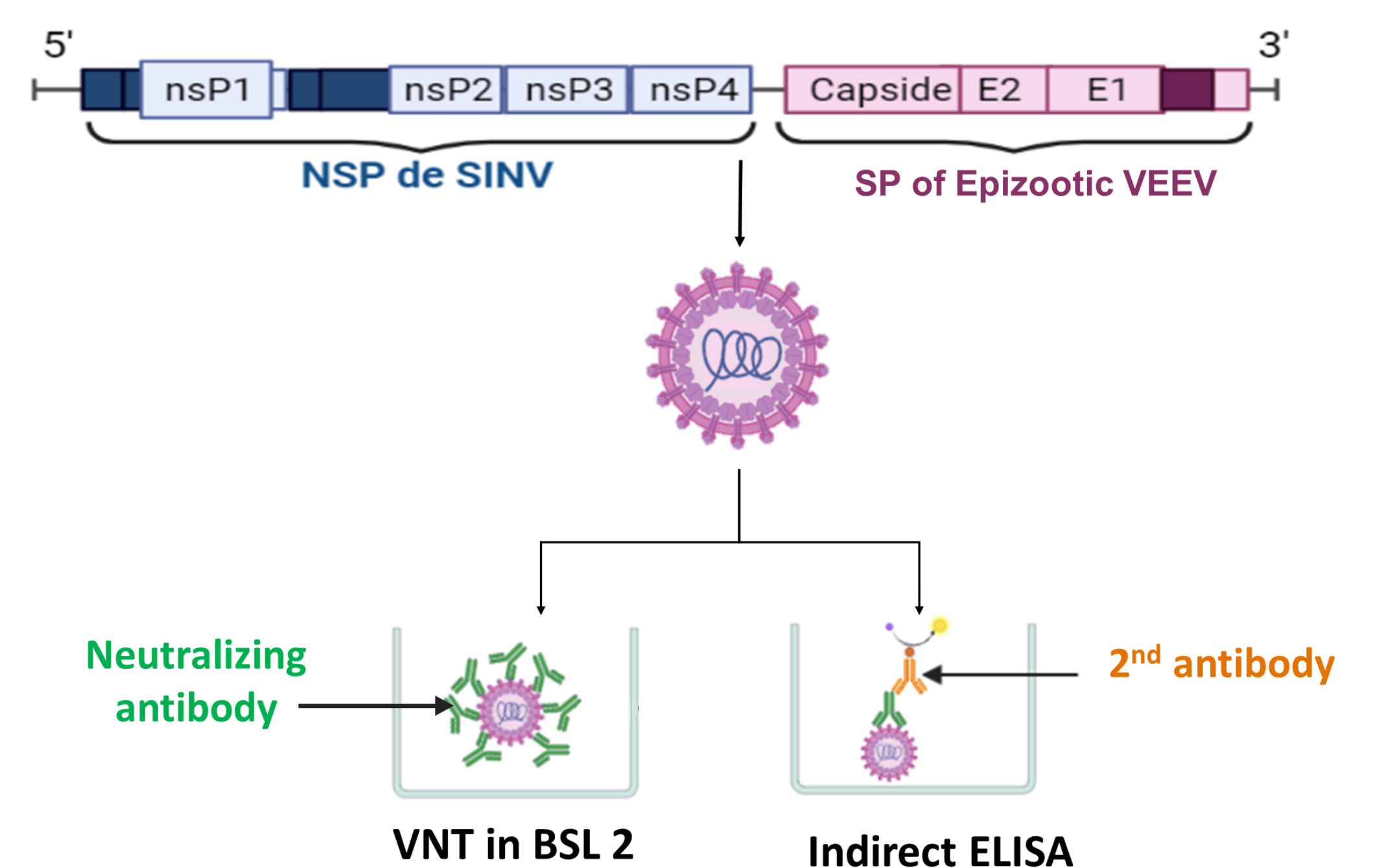


Phylogenetic tree of VEEV epizootic and enzootic strains

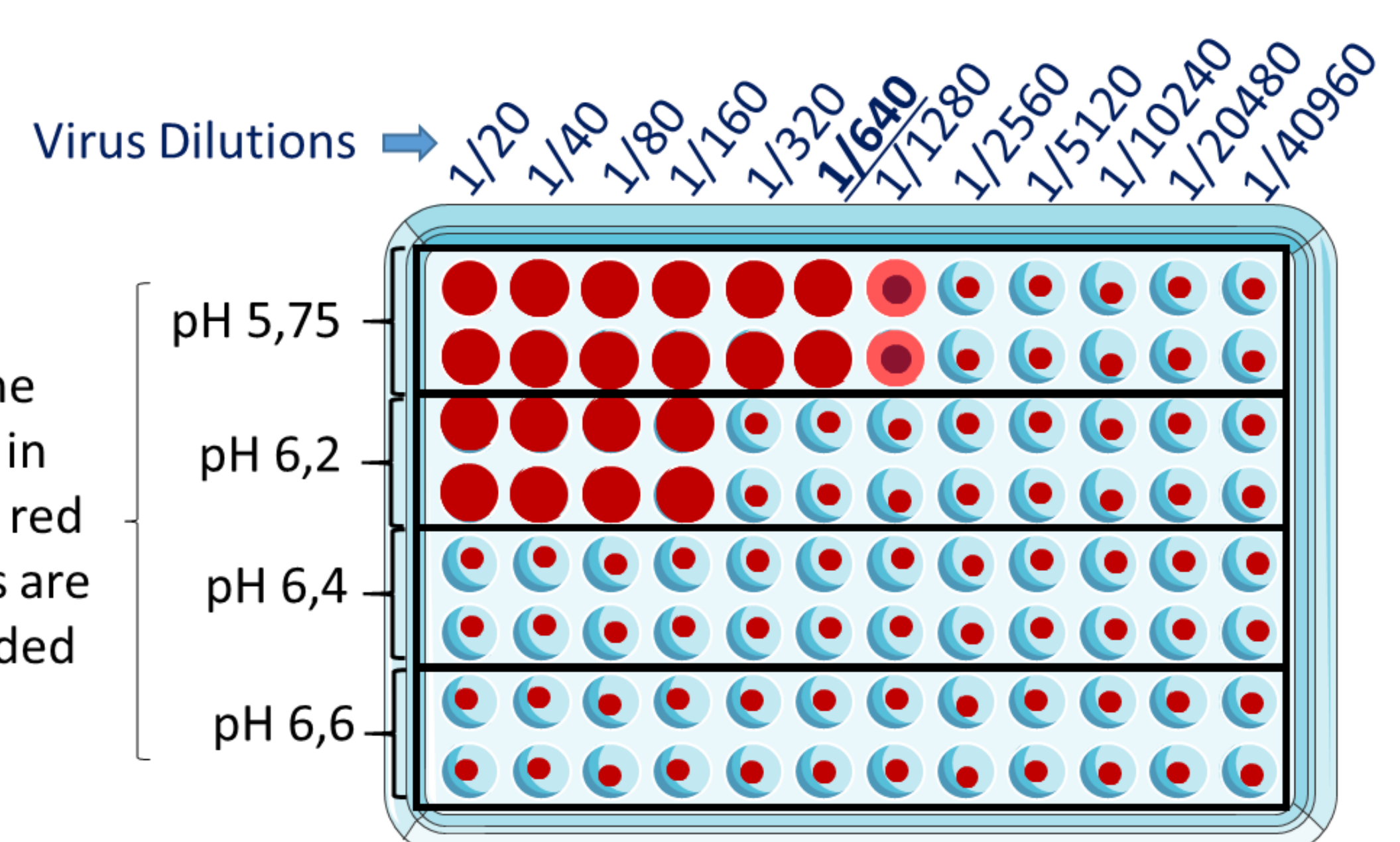
- ✓ Detection of **100%** of VEEV tested strains
- ✓ **Specificity : 100%**
- ✓ Sensitivity: estimated at **50 genome copies/μl**

### 2. Development of serological tools

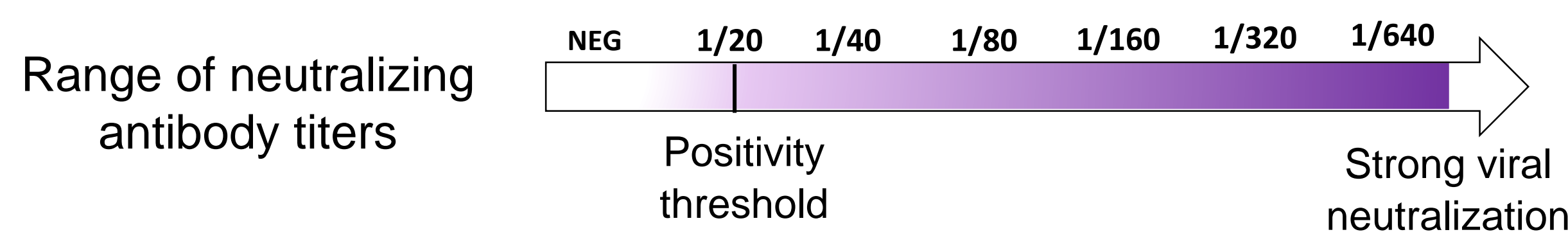
#### A. Use of Chimeric virus Sindbis/VEEV to perform VNT in BSL2



#### C. Hemagglutination inhibition assay (reference method)



#### B. VNT in BSL2 using chimeric SINV/VEEV



Sensitivity	Virus	Serum infected horse			
		Parental VEEV I-AB	SINV/VEEV/ I-AB	SINV/VEEV/ I-D	SINV/VEEV/ I-E
Cross-reactivity	18				
	17				
	20				
	22				
	23				
	POS EEEV/WEEV				

- ✓ **Natural neutralizing antibodies recognize SINV/VEEV chimeric viruses**  
→ Equal and Higher sensitivity using chimeric viruses
- ✓ **No cross-reactivity with other EEEV and WEEV**

#### Identification of critical parameters:

- Antigens concentration
- Use of goose red blood cells
- pH, incubation temperature

Development of an indirect « in-house » ELISA based on total inactivated particles

## CONCLUSIONS & PERSPECTIVES

- Improvement of molecular detection of VEEV by RT-qPCR
- Work in progress on « easy to use » serological tools

➔ **Europe needs to be prepared in case of emergence**

## REFERENCES

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