European Union Reference Laboratory for Equine Diseases

French National Reference Laboratory West Nile

Japanese encephalitis virus

Camille Migné

French National Reference Laboratory for West Nile virus

(Head of NRL : G. Gonzalez)

European Union Reference Laboratory for Equine Diseases (Director : S. Zientara)

WNV and Exotic equine encephalitis virus

ANSES, Animal Health Laboratory (France)

Virology Unit

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UMR Virologie 1161 INRAe - Anses - EnvA

Flavivirus - Generalities

Family: *Flaviviridae*
Genus: *Flavivirus*

Reservoir:
avifauna
Meningo-encephalitis

Reservoir:
rodents
Meningo-encephalitis

Japanese encephalitis
serocomplex

Clade Culex

Clade Aedes

Reservoir:
primate
Haemorrhagic fevers

Unknown vector

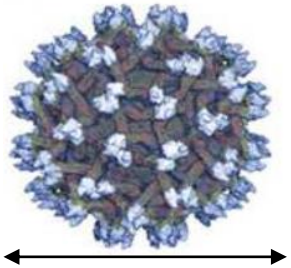
Hard ticks group

Soft ticks group

Beck et al, IJERPH, 2013

Flavivirus structure

Enveloped virus

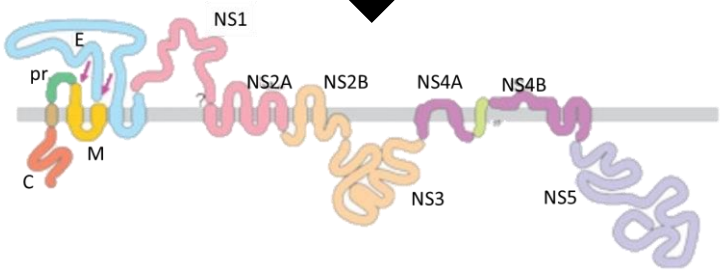


Single stranded RNA +
≈ 11 kb

3 Structural proteins

7 Non-structural proteins

Structure (packaging of viral RNA)
Entry and exit of the virus from the cell
Antigenicity



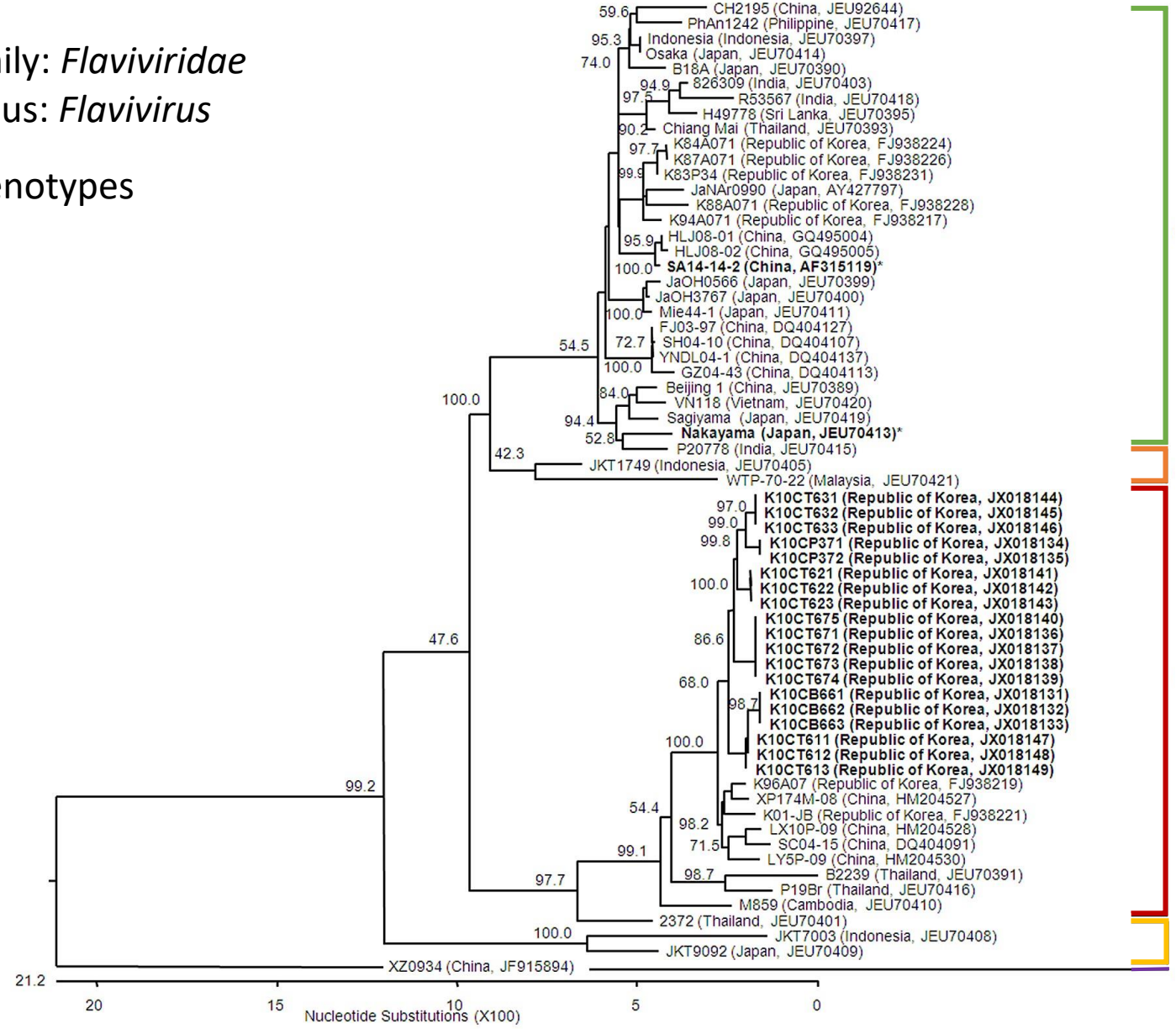
Virus replication
Host immune response
Pathogenesis

(Tiwari S, *et al.*, 2012)

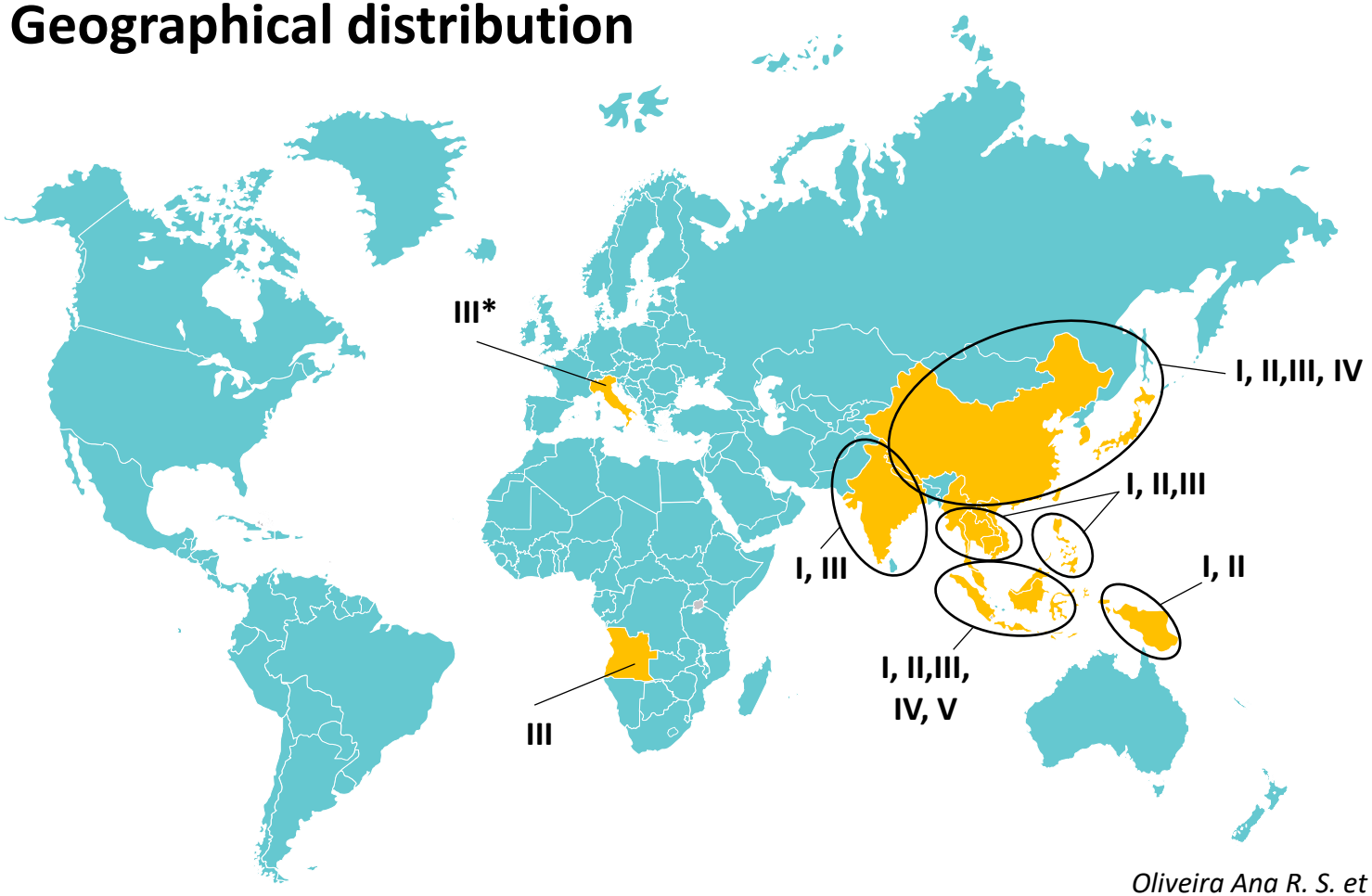
Japanese encephalitis virus (JEV)

Family: *Flaviviridae*
Genus: *Flavivirus*

5 genotypes



JEV – Geographical distribution



Oliveira Ana R. S. et al., 2020

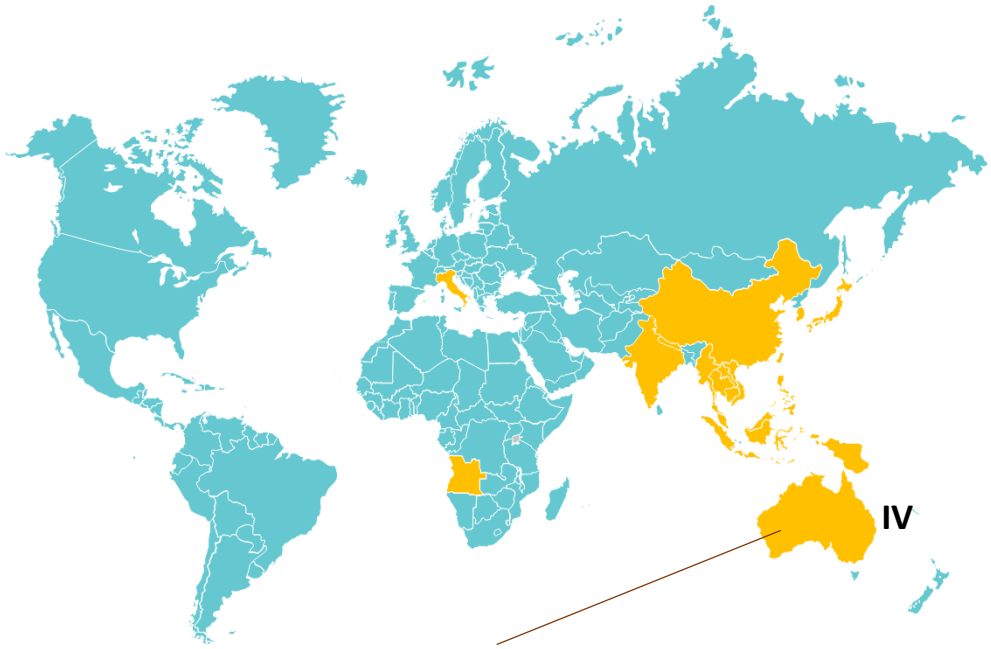
<u>Until 2021 :</u>

→ Southeast Asia

→ *Italy : JEV detected in mosquitoes and birds collected in Northern Italy (no human case reported) *(Platonov AE, et al., 2012; Ravanini P, et al., 2012)*

→ Angola : Autochthonous JE with yellow fever co-infection in March 2016 *(Simon-Loriere E., et al., 2017)*

JEV – Geographical distribution



JEV outbreaks in Australia in **2022**

★ Outbreaks in pigs

Mackenzie John S. et al., 2022

<u>Since 2022:</u>

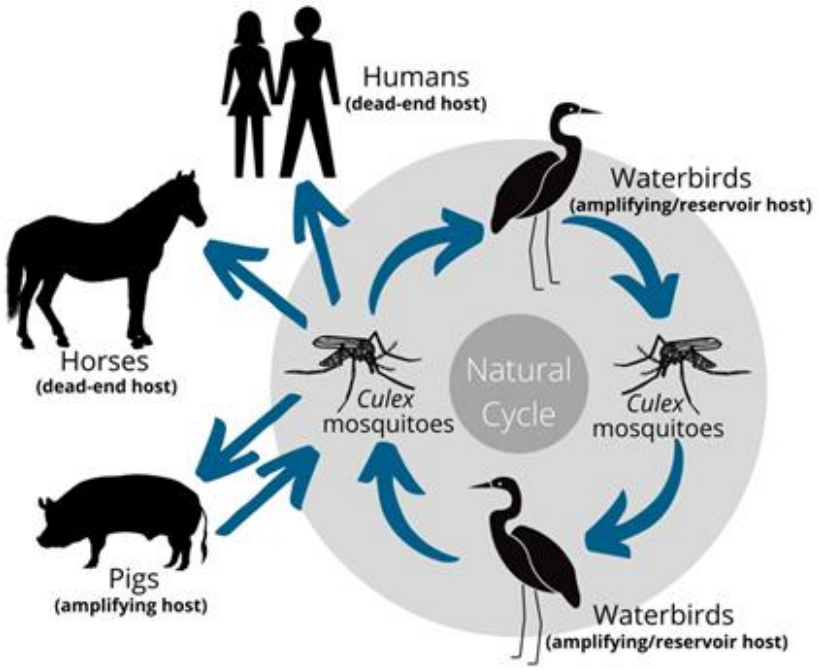
Outbreaks in pigs reported from February 2022

→ March 30: Alpaca outbreak reported

→ May: Suspicion of equine infection (30)

No cases have been definitively confirmed despite neurological signs and tests results

JEV – Transmission cycle



https://www.environment.act.gov.au

Pearce James C. et al., 2018

Mosquitoes vectors :

***Cx tritaeniorhyncus* (1st vector)**
Cx annulirostris (Australia)
Cx annulis Potential
Cx bitaeniorhyncus
Cx epidesmus
Cx (Lu) fuscanus
Cx fuscocephela
Cx gelidus
Cx infula
Cx orientalis
Cx pipiens
Cx pseudovishnui
Cx quinquefasciatus
Cx rubithoracis
Cx sitiens
Cx vishnui
Cx whitmorei

Ma annulifera
Ma indiana
Ma uniformis

Ar subalbatus

Ae albopictus
Ae assamensis
Ae butleri
Ae japonicus
Ae lineatopennis
Ae togoi
Ae vexans

Oc detritus

An annularis
An barbirostris
An pallidus
An peditaeniatus
An sinensis
An subpictus
An tessellatus
An vagus

Cx: Culex, Ma: Mansonia, Ar: Armigeres, An: Anopheles, Ae: Aedes, Oc: Ochlerotatus

Potential/Regional vector

Competent vector in laboratory

JEV – Disease in pigs

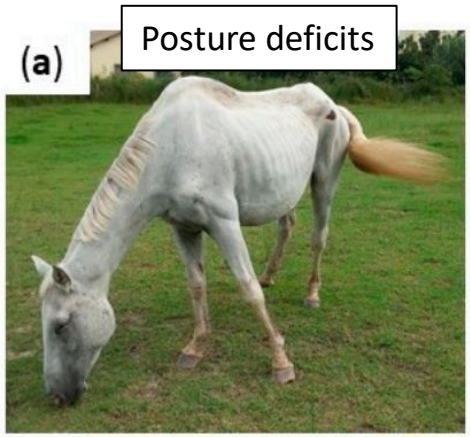
- Generally asymptomatic
- Reported cases with :
  - Pyrexia and anorexia
- In breeding animals
  - Abortion and mummification in pregnant sows
  - Orchitis, aspermia and infertility in boars
  - Neurological signs in piglets

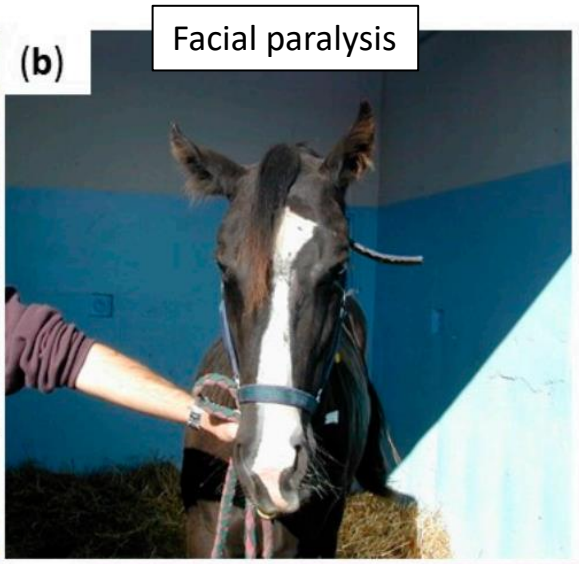
5 stillborn piglets and 2 smaller ones mummified from a sow with JE

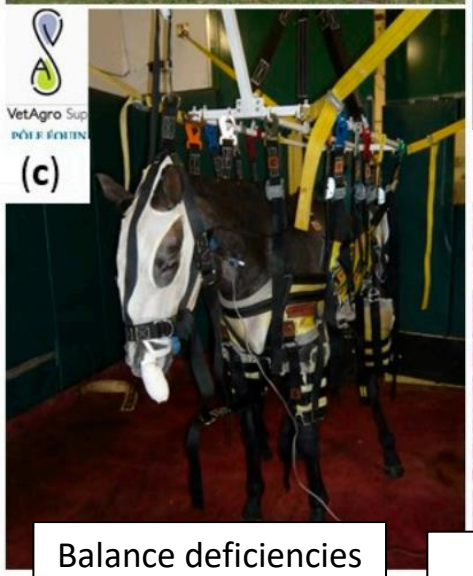
Mansfield Karen L. et al., 2017
https://www.woah.org/fr/document/japanese_encephalitis-2/

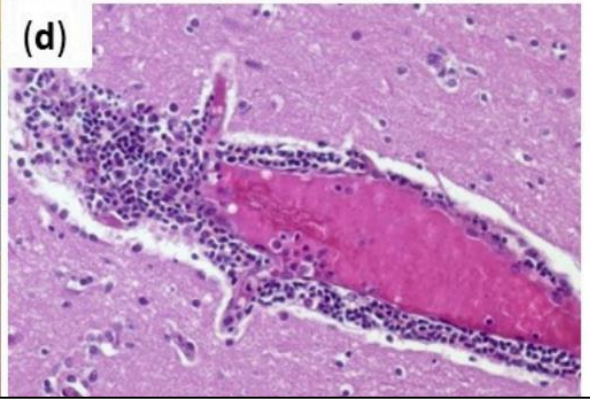
JEV – Disease in horses

- Most infections appear to be subclinical
- Fever, jaundice, lethargy, anorexia
- Neurological signs: ataxia, paralysis, collapse
- Death reported within 1–2 days
- **5-30% lethality** in horses









Brain lesions non-specific
→Perivascular infiltration of inflammatory cells

Mansfield Karen L. et al., 2017
Lecollinet Sylvie et al., 2020

JEV – Treatment in horses

No specific antiviral treatment, supportive treatment

Symptomatic and comfort treatment

→ perfusion, anti-inflammatory, protection and care against self-harm...)

JEV – Vaccines

- In animals (available in Asia)

Nisseiken (Japan)

https://www.jp-nisseiken.co.jp/en/products/vaccine/index.html

JEV

JEV and Getah virus

JEV, Equine Influenza and Tetanus

KM Biologics (Japan)

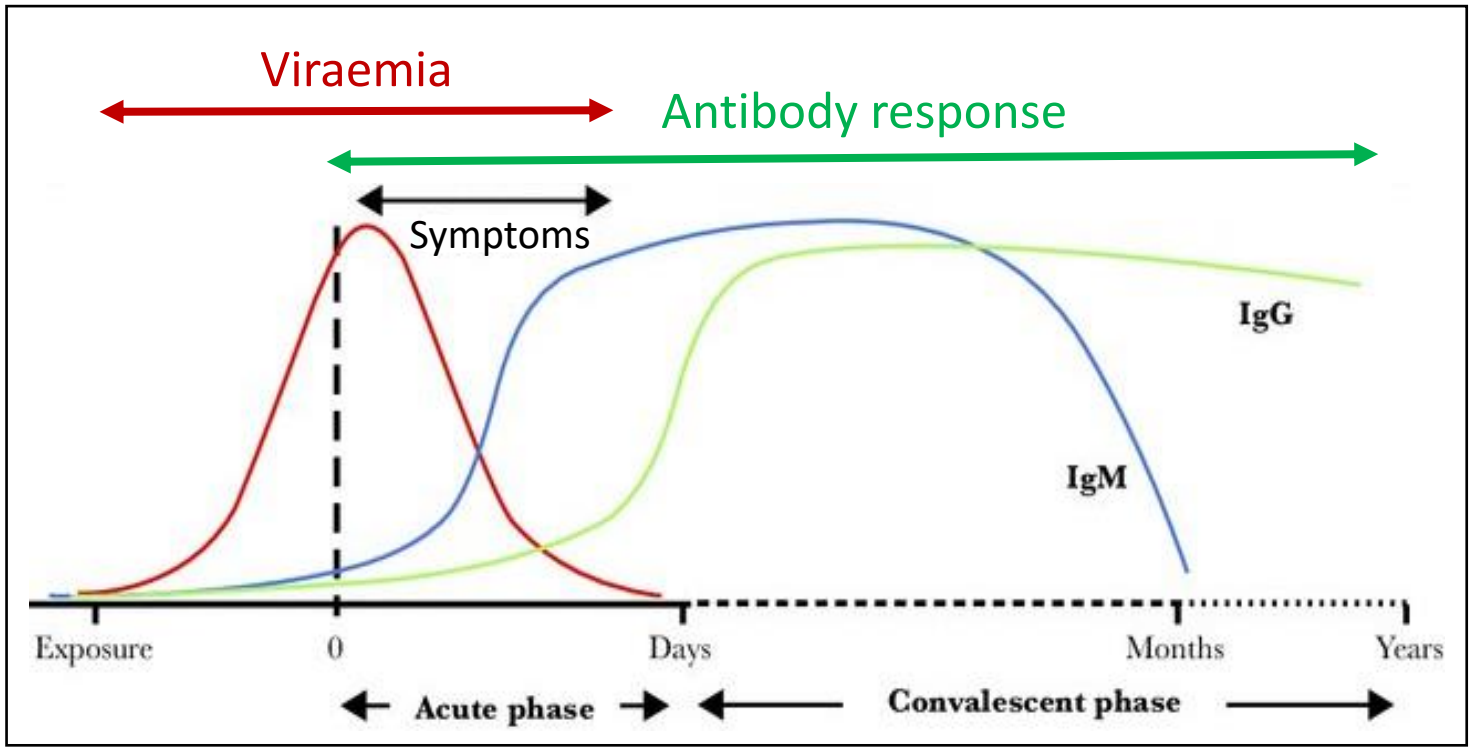
https://www.kmbiologics.com/en/products/equine.html

JEV

JEV, Equine Influenza and Tetanus

JEV – Disease in horses

- Short viraemia



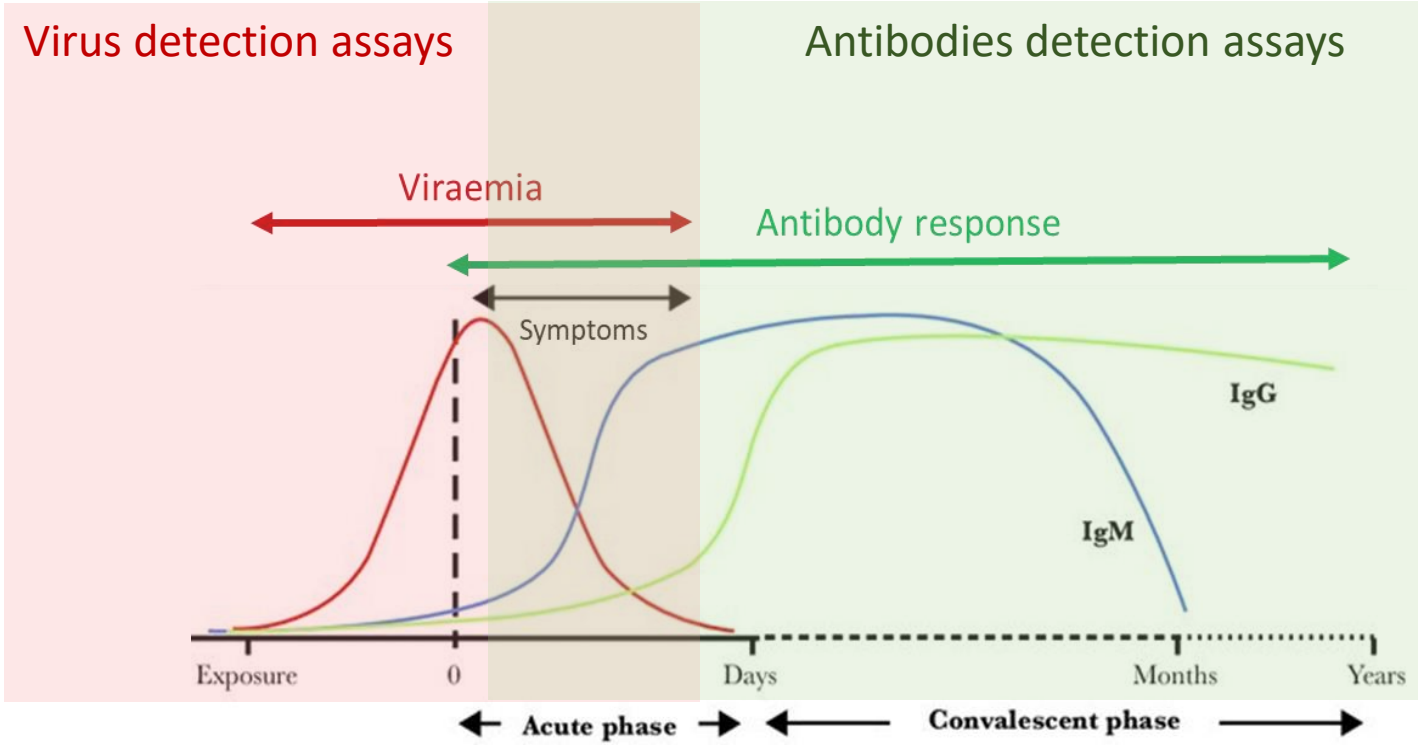
Schematic representation of the typical kinetics of flaviviral infections

(adapted from Goncalves A. et al., 2017)

JEV – Diagnosis

Diagnostic tools in laboratory

→ to be **adapted according to the kinetics of infection**



(adapted from Goncalves A. et al., 2017)

JEV – Diagnostic tools in laboratory

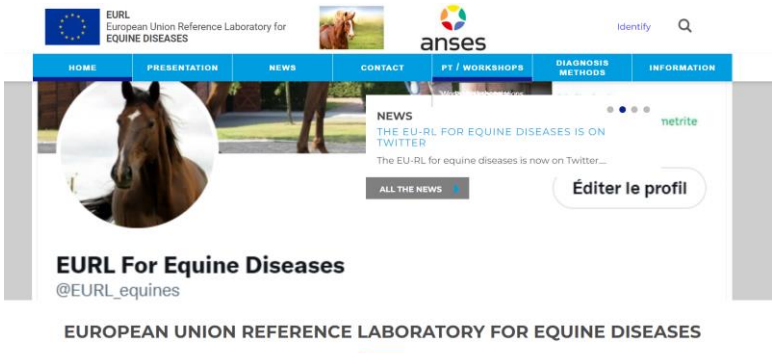
Test methods available for the diagnosis of Japanese encephalitis

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[1]						
Virus isolation	–	–	–	+++	–	–
Antigen detection	+	+	+	+	+	–
Real-time RT-PCR	++	++	++	+++	++	–
Detection of immune response						
HI	++	+++	++	+++	+++	+++
CFT	+	+	+	+	+	+
ELISA	++	++	++	++	++	++
VN (PRNT)	+	++	+	+++	++	++

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
RT-PCR = reverse-transcription polymerase chain reaction; HI = haemagglutination inhibition; CFT = complement fixation test;
ELISA = enzyme-linked immunosorbent assay; VN = virus neutralisation; PRNT: plaque reduction neutralisation test.

WOAH 2022, chapter 3.1.10, Japanese encephalitis

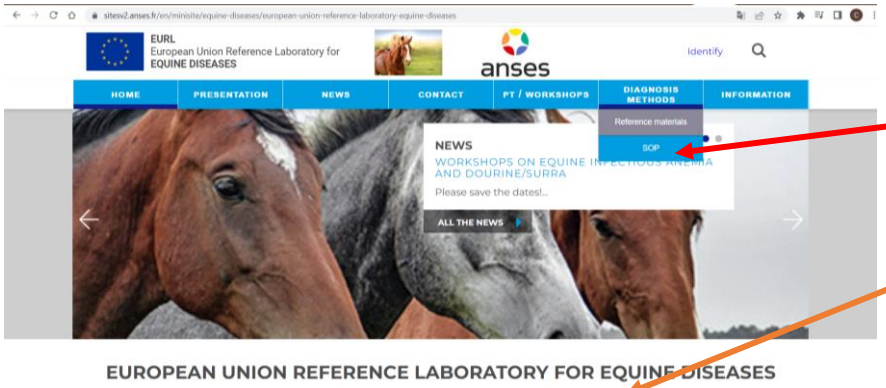
European union Reference laboratory for equine diseases



website : https://eurl-equinediseases.anses.fr/

11 equine diseases:

- Virological diseases: EVA, EIA, WNV, JEV, EEEV, VEEV, WEEV
- Bacteriological diseases: glanders/melioidosis and contagious equine metritis
- Parasitological disease : Dourine and Surra



Providing Standard Operating Procedure (SOP)

anses

STANDARD OPERATING PROCEDURE

Detection and titration of West Nile Virus specific antibodies using virus neutralization methods

Writer(s) : C.Beck	**Reviewer(s) :** G. Gonzalez and M Dumarest

This SOP is an OIE-based method used at the EURL; all OIE-PRNT/MNT based methods validated and used successfully in the PT can be used for this essay.

anses

STANDARD OPERATING PROCEDURE

JEV rtRT-PCR (adapted from Yang et al., 2004)

Writer(s) : C.BECK	**Reviewer(s) :** G. Gonzalez and Marine Dumarest

This SOP is an -based method used at the EURL, all RT-PCR based methods validated and used successfully in the EURL PT can be used for this essay

JEV – Diagnostic tools in the EURL

European Union Reference Laboratory for Equine Diseases

➢ **In horses *(ante mortem)* :**

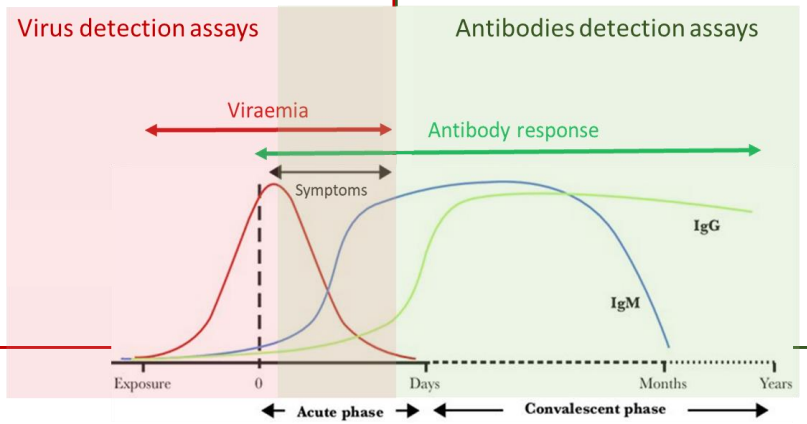
Tools/Techniques

Virus isolation

Virus genome detection by RT-qPCR

Samples

Whole blood (EDTA)
Cerebrospinal fluid



(adapted from Goncalves A. et al., 2017)

Tools/Techniques

ELISA:
IgG detection
ID Screen® West Nile Competition Multi-species
VNT

Samples

Serum (+++)
Collect serum samples (2–4 weeks apart)
↳ Evidence of a primary infection
Plasma (+/-)

Direct diagnostic
Specific
Only in a CL3

Indirect diagnostic

ELISA
Faster than VNT
Cross-reaction among flaviviruses

VNT
More specific than ELISA
Tedious, time-consuming
Only in a CL3

JEV – Diagnostic tools in the EURL

European Union Reference Laboratory for Equine Diseases

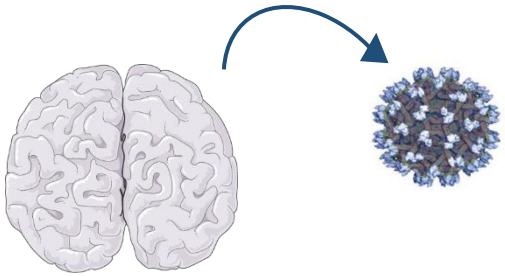
- **In horses *(post mortem)* :**

Virus detection (RT-qPCR) and isolation from :

Organs : Brain +++, spleen, liver

Whole blood

Cerebrospinal fluid



Take home messages

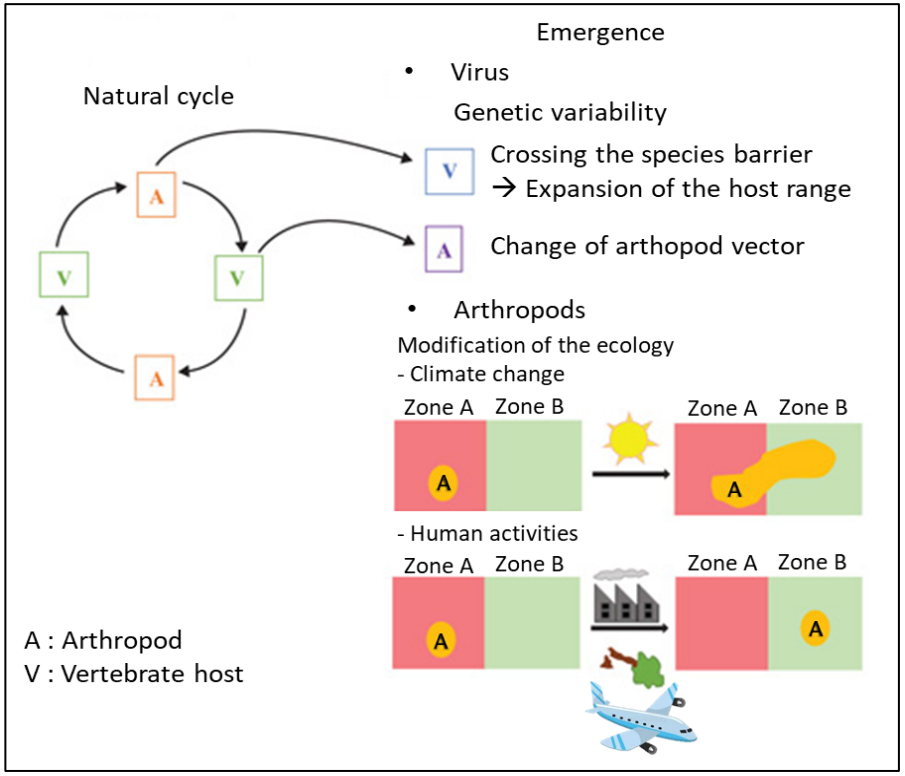
✓ Asia : endemicity of JEV
→ Risk of emergence of **more virulent** strains

✓ Co-circulation with other flaviviruses
→ Cross reaction
→ Attenuation effect

Need to be able to detect JEV early

→ Sensitive and specific diagnostic tools
→ Monitor JEV in each compartment of its transmission cycle

Take the necessary health measures rapidly



Schematic representation of a viral emergence *(adapted from Migné CV, et al., 2021)*

Optimisation of diagnostic tools for JEV with EU partners

→ To be prepared for the emergence of JEV in Europe

CoVetLab.org
Veterinary Public Health Institutes supporting each other
https://www.CoVetLab.org

- Virus genome detection : RT-qPCR and Amplicon-based Sequencing
- Antibodies detection : ELISA tests
  → IgM and IgG JEV detection

SVA NATIONAL VETERINARY INSTITUTE
Animal & Plant Health Agency
CENTRAL VETERINARY INSTITUTE WAGENINGEN UR
Technical University of Denmark DTU
anses

→ Shared to the scientific community
→ Training sessions / collaborations /advices → Happy to share

Aknowledgements

Virology Unit
Stephan Zientara

ZEN team
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