



ISTITUTO G. CAPORALE  
TERAMO

# Diagnosis of West Nile

OIE Reference Laboratory for WND  
Istituto G. Caporale – Teramo

Teramo 20 May 2011

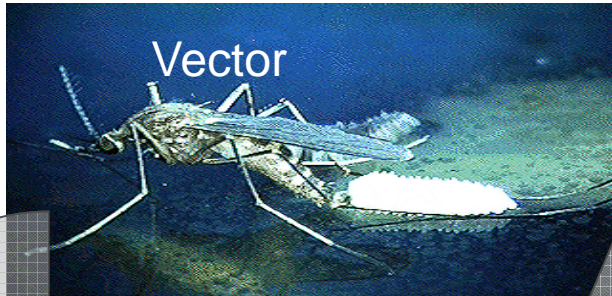




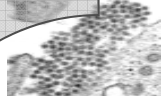


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# WNV life cycle

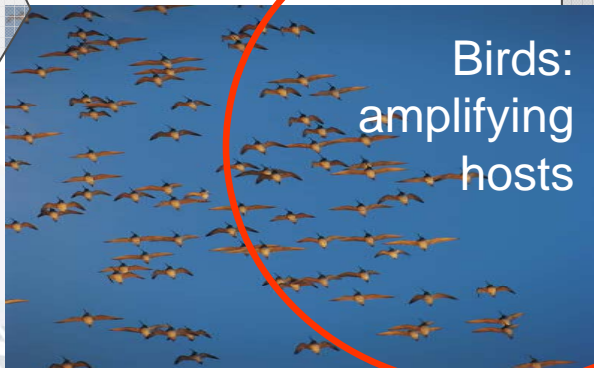
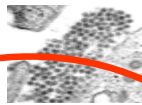
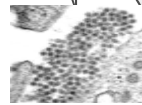


Vector

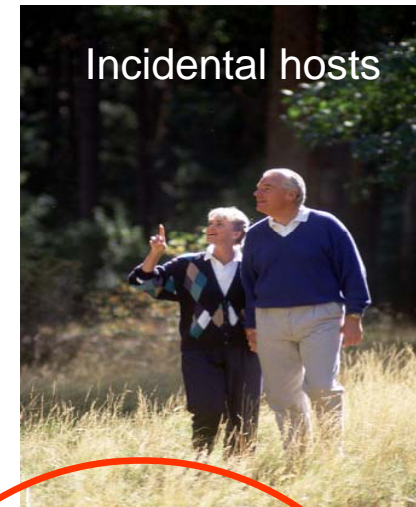


**Virus  
della  
West  
Nile**

**Virus  
della  
West  
Nile**



**Birds:  
amplifying  
hosts**



Incidental hosts



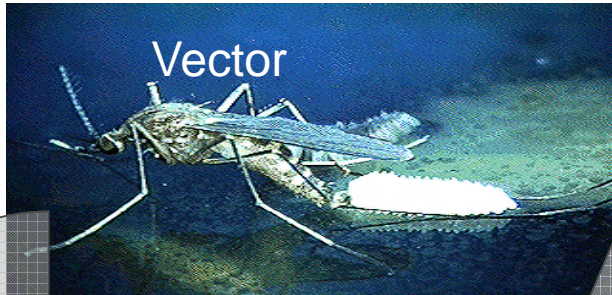
Incidental hosts





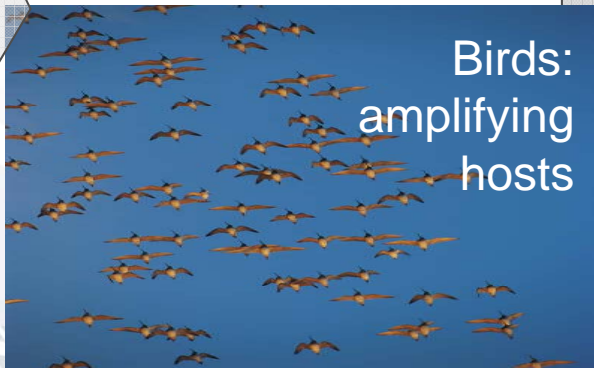


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Vector

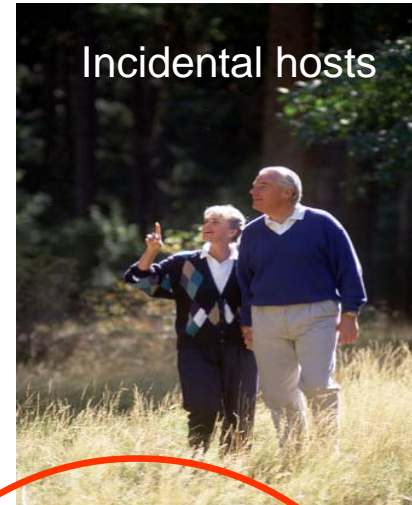
**Virus della West Nile**  
**Virus della West Nile**  
**Virus della West Nile**



Birds:  
amplifying  
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# WNV life cycle

Incidental hosts




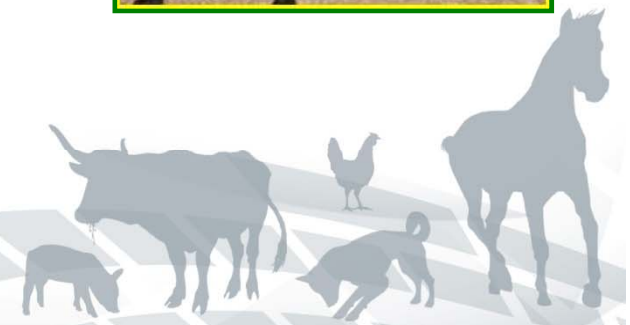
Incidental hosts





# Clinical signs in horses

- 
- ❑ Incubation period: 3-15 days
  - ❑ The infection is mostly (70%) asymptomatic
  - ❑ In 20% of cases animals show mild clinical signs:
    - ✓ fever, weakness, ataxia, muscle rigidity
  - ❑ In 1 to 10% the neurological form could be observed:
    - ✓ When the virus invades the SNC (medulla, rhombencephalon, mesencephalon, spinal cord)





# Clinical signs in horses

- ❑ Animals could recover in 5-15 days
- ❑ Some animals affected by the nervous form might die or be euthanised
- ❑ Mortality rate: 38% - 57,1%





# Clinical signs in horses



✓ Most common neurological signs observed during the 2009 WND Italian outbreak:

- ☐ muscle fasciculations 66.67%
- ☐ ataxia/incoordination 62.96%
- ☐ limb paresis or paralysis (particularly of the hind limbs) 44.44%
- ☐ depression 48.15%
- ☐ disturbed proprioception 25.93%
- ☐ recumbency 22,22%
- ☐ lower lip ptosis or paresis of facial and labial muscles 14,81%
- ☐ walking in circles 7,41%
- ☐ blurred vision 11,11%.





## Altri sintomi



- ☐ Weakness
- ☐ Altered behaviour
- ☐ Hypertermia
- ☐ Fasciculations
- ☐ Anorexia
- ☐ Cranial nerve altered
- ☐ Grinding







## Other symptoms

Altered behaviour:

- ☐ minimal or no response to stimuli
- ☐ Lethargia





# Clinical signs in horses

hyperaesthesia, ataxia, lethargia



Head tremors



Fort Dodge





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TERAMO

Nervous symptoms,  
seasonal incidence lab  
confirmation is required

## Differential diagnosis

- Protozoan encephalomyelitis
- Herpes virus encephalitis
- Japanese **encephalitis**
- Eastern equine **encephalitis**
- Western equine **encephalitis**
- Venezuelan equine **encephalitis**
- Dourine
- Borna Disease
- Lameness

Presence of swelling of  
genitalia and cutaneous  
plaques

Chronic disease with long  
incubation period and no  
seasonal incidence

Anamnesis and no nervous  
syptoms



Nervous symptoms, no seasonal incidence but lab confirmation is required

## Differential diagnosis

Anamnesis, no seasonal incidence

Anamnesis, no seasonal incidence, Classical clinical signs characterised by spasms and stiffness

Anamnesis, no seasonal incidence, classical clinical signs characterised by flaccid paralysis

Anamnesis, no seasonal incidence, but lab confirmation is required

Rabies

Tetanus

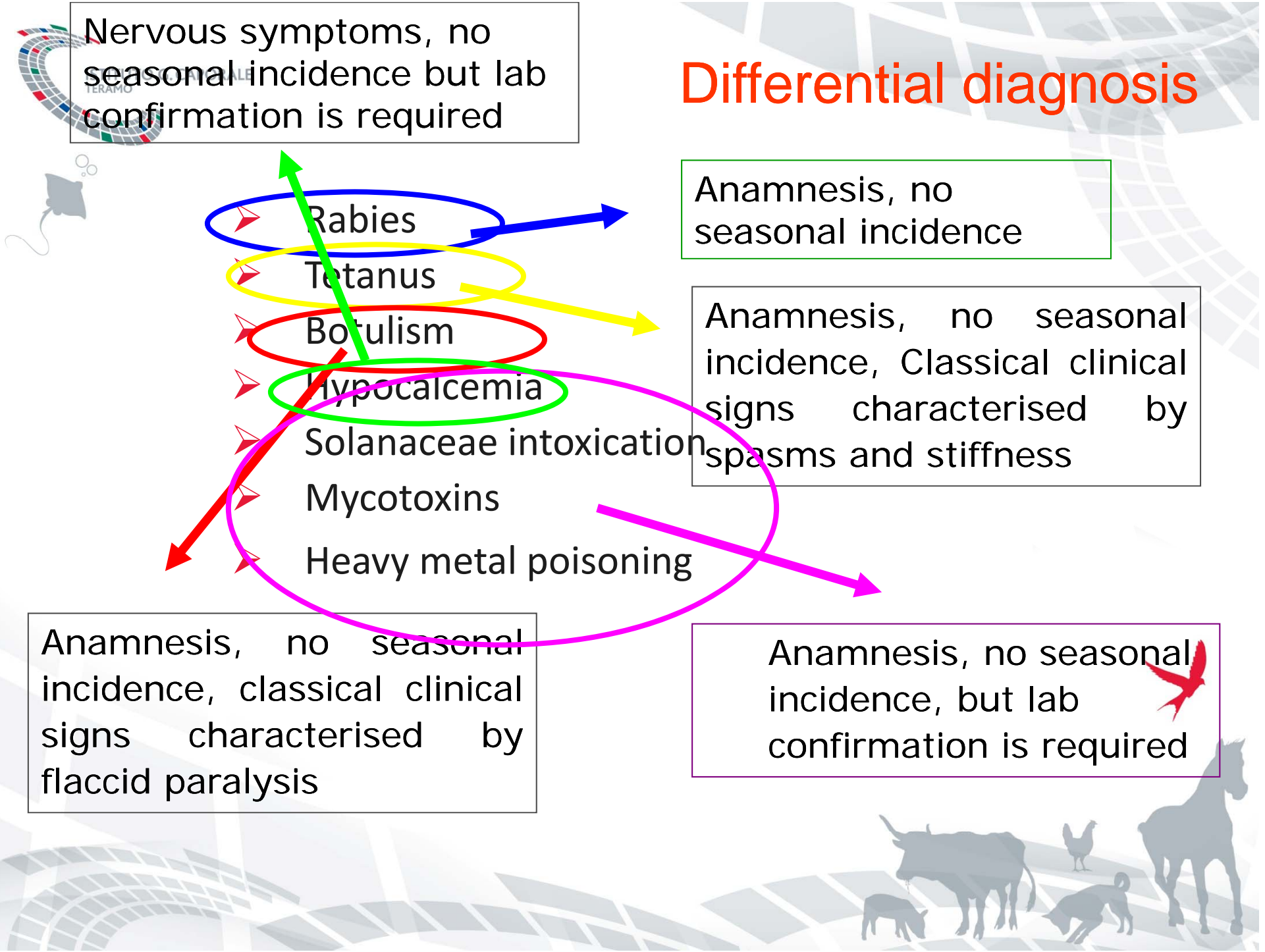
Botulism

Hypocalcemia

Solanaceae intoxication

Mycotoxins

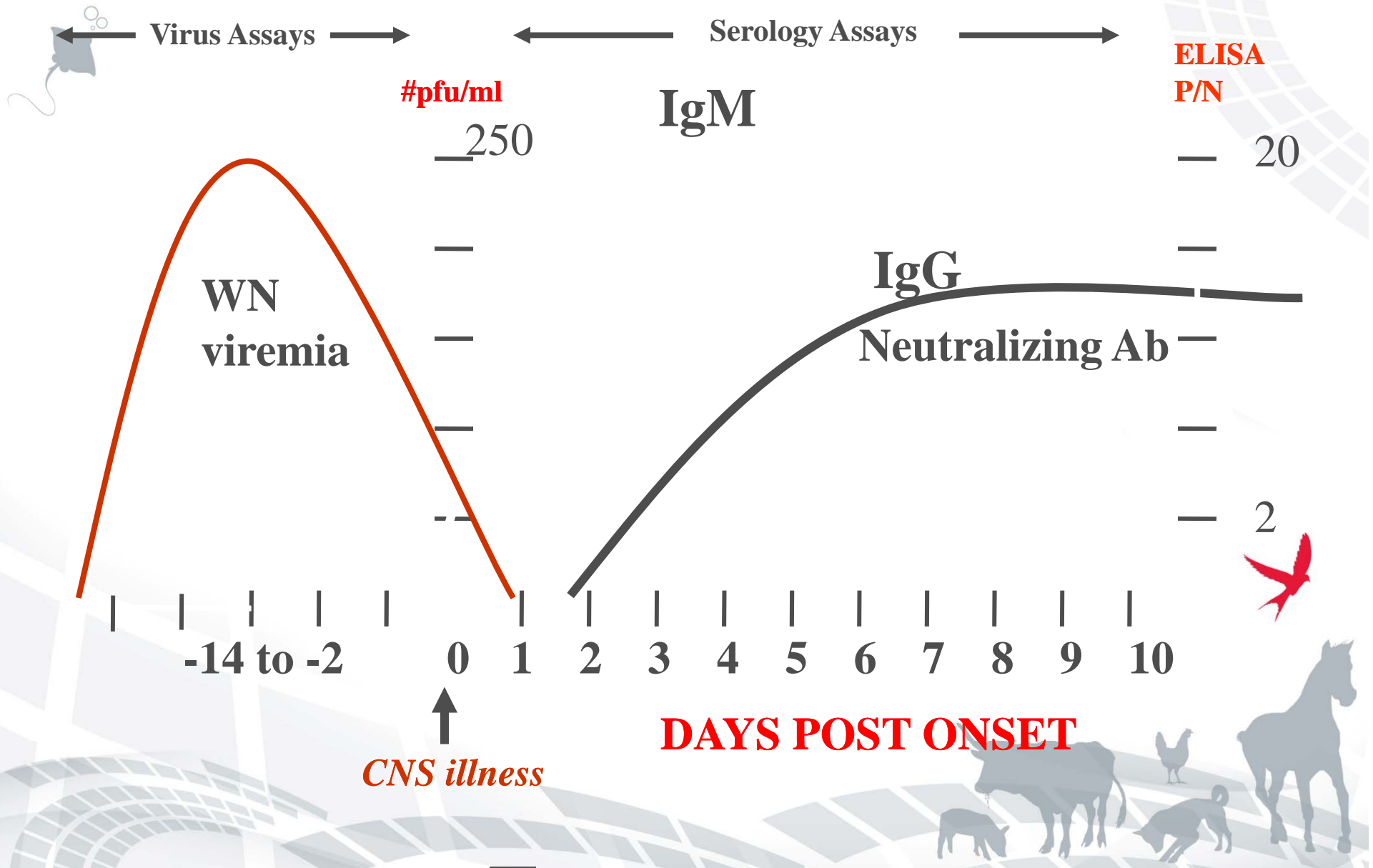
Heavy metal poisoning







# Theoretical Depiction of WNV Human Viremia & Immune Response

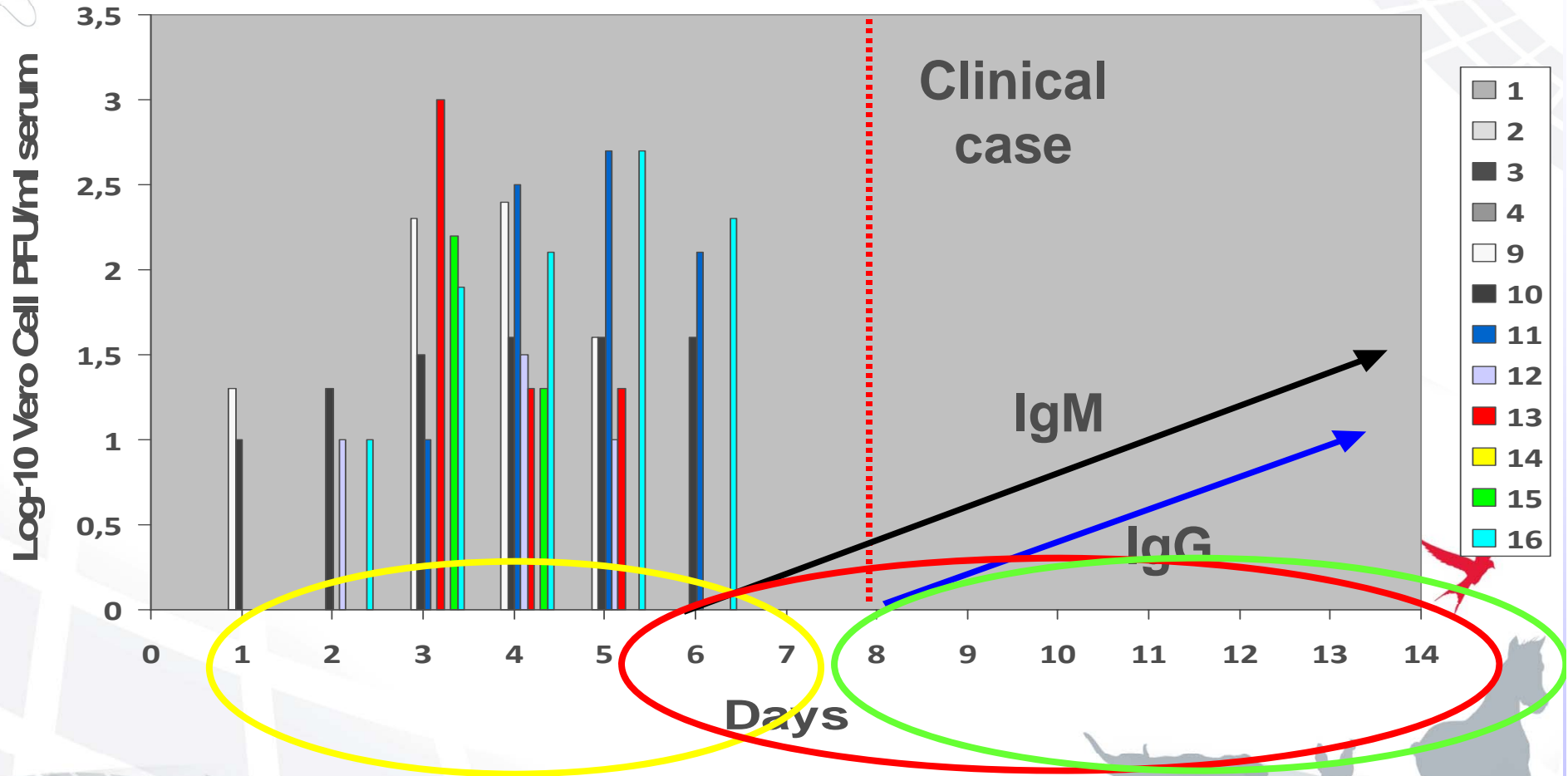






ISTITUTO G. CORBELLINI  
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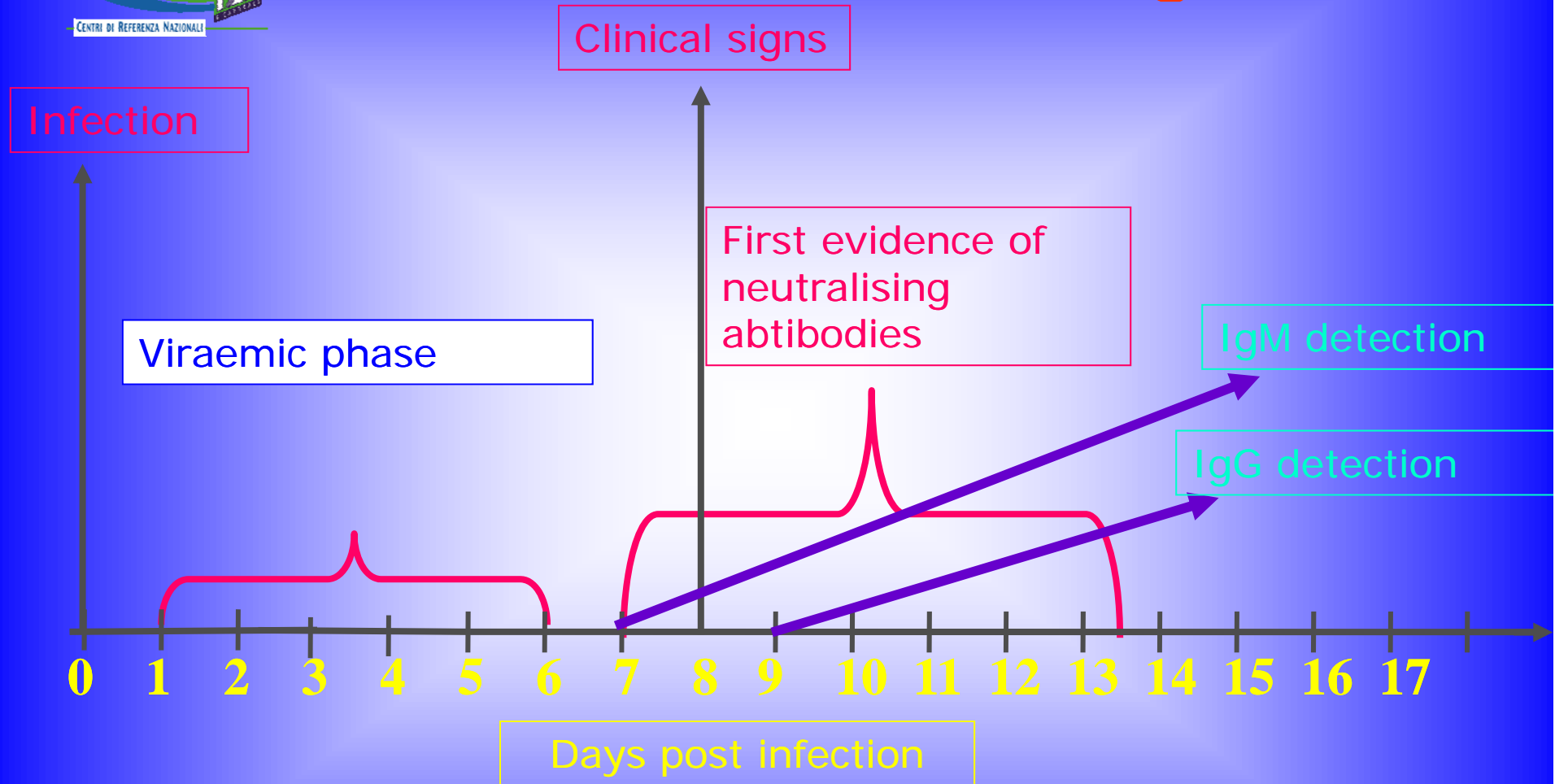
# Serological and virological response in horses following infection







# Serological and virological response in horses following infection



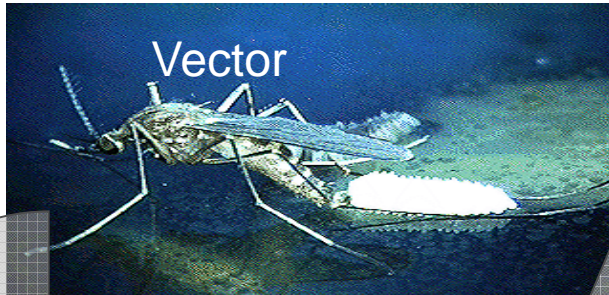
- Viraemic phase occurs before clinical sign appearance
- Viraemic animals only rarely show clinical signs





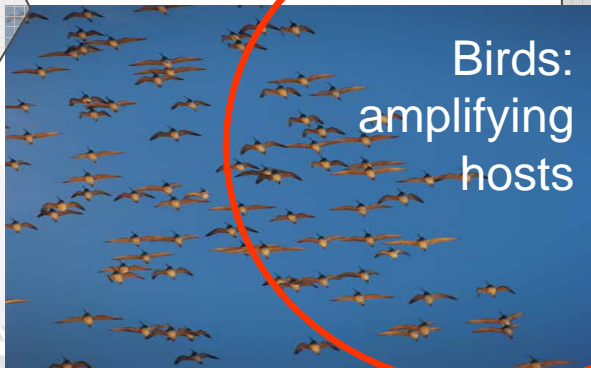
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# WNV life cycle

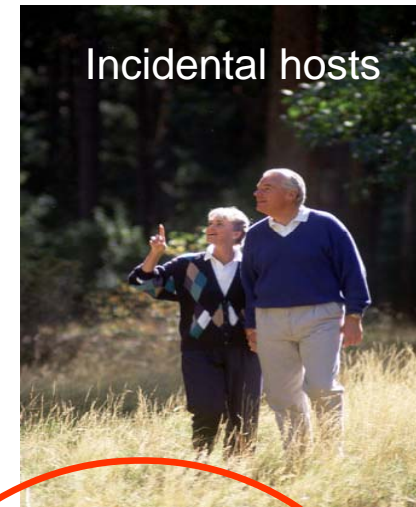


Vector

**Virus della West Nile**  
**Virus della West Nile**  
**Virus della West Nile**



Birds:  
amplifying  
hosts



Incidental hosts



Incidental hosts







"Of course this unknown flu that you and everyone else has caught is harmless, Mr Bell."

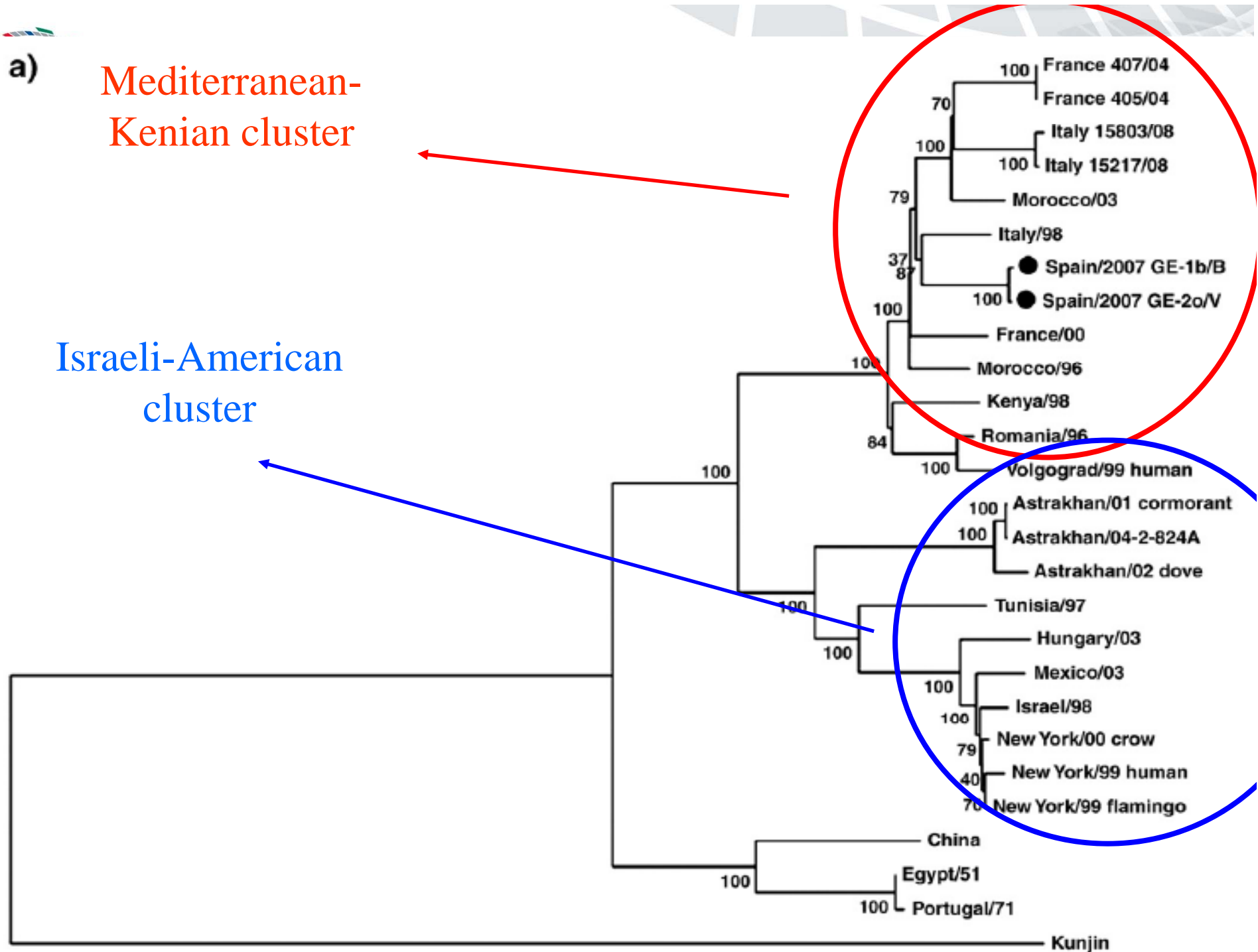




a)

Mediterranean-Kenian cluster

Israeli-American cluster





# Clinical signs in birds

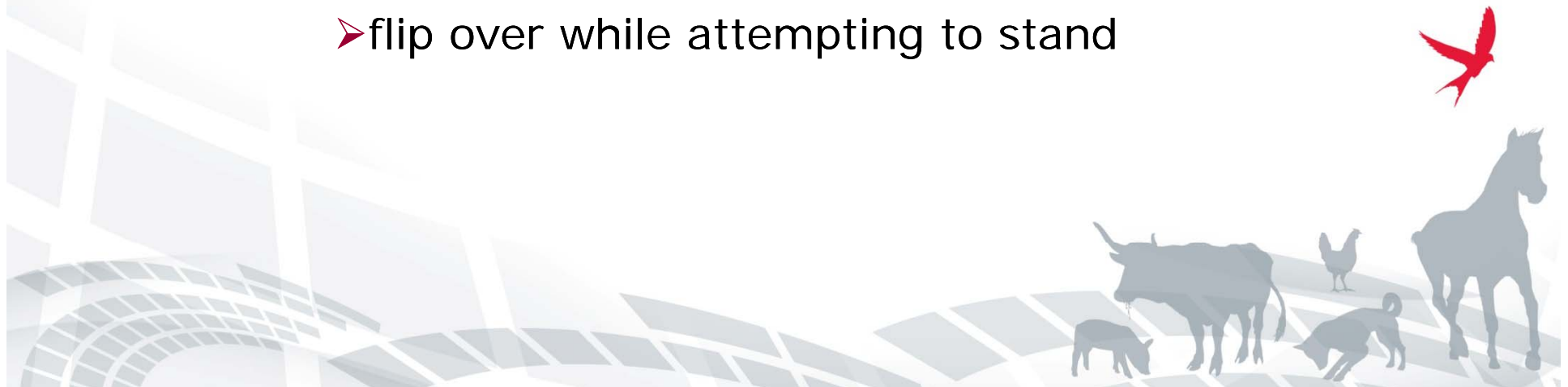
- ❑ Incubation period: 3-4 days
- ❑ In most cases the infection does not cause clinical signs
- ❑ Clinical signs are often associated to species and WND strains (1999 US epidemic: crows)





# Clinical signs in birds

- ❑ Affected birds may:
  - be totally recumbent (unable to stand)
  - have head tremors or show leg and/or wing paralysis
  - be reluctant or unable to move when disturbed
  - be uncoordinated
  - flip over while attempting to stand







# Clinical signs in birds

- ❑ Birds tend to show neurologic clinical signs [depending on strains and geographic areas]
- ❑ Mortality rate: 25% - 40% (for some authors: 60-100%).
- ❑ Death occurring within 24 hours from the beginning of nervous symptoms







# Clinical signs in birds

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# Testing for West Nile Virus

	<b>Bird Surveillance</b>	<b>Mosquito Surveillance</b>	<b>Veterinary Diagnostic</b>	<b>Human Diagnostic</b>
<b>Test Target</b>	Virus	Virus	Antibody	Antibody
<b>Sample Type</b>	Tissues, oral swabs	Mosquito pools	Serum, CSF	Serum, plasma, csf tissues
<b>Available Tests</b>	Lin1 & 2 RT-PCR TaqMan qRT-PCR RT-PCR Isolation in Vero	TaqMan RT-PCR RT-PCR Isolation in Vero	IgM ELISA VN Plaque Reduction Neutralization	IgM ELISA IgG ELISA Plaque Reduction Neutralization IgA ELISA IFA
<b>Comments</b>	Birds have high viremia; $10^6 - 10^9$	Mosquito pool titers vary;	Tissues from fatal equine cases tested by RT-PCR	Tissues from fatal human cases tested by RT-PCR. Plasma/serum/csf can be tested by NAT.



**Blood, Serum, Tissues, Insects**

**Real time RT-PCR** (Lanciotti et al., 2000 modified)

**NEG**

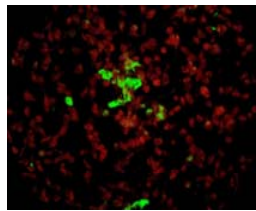
**STOP**

**POS**

**VI (Vero, RK<sub>13</sub>, C6/36)**

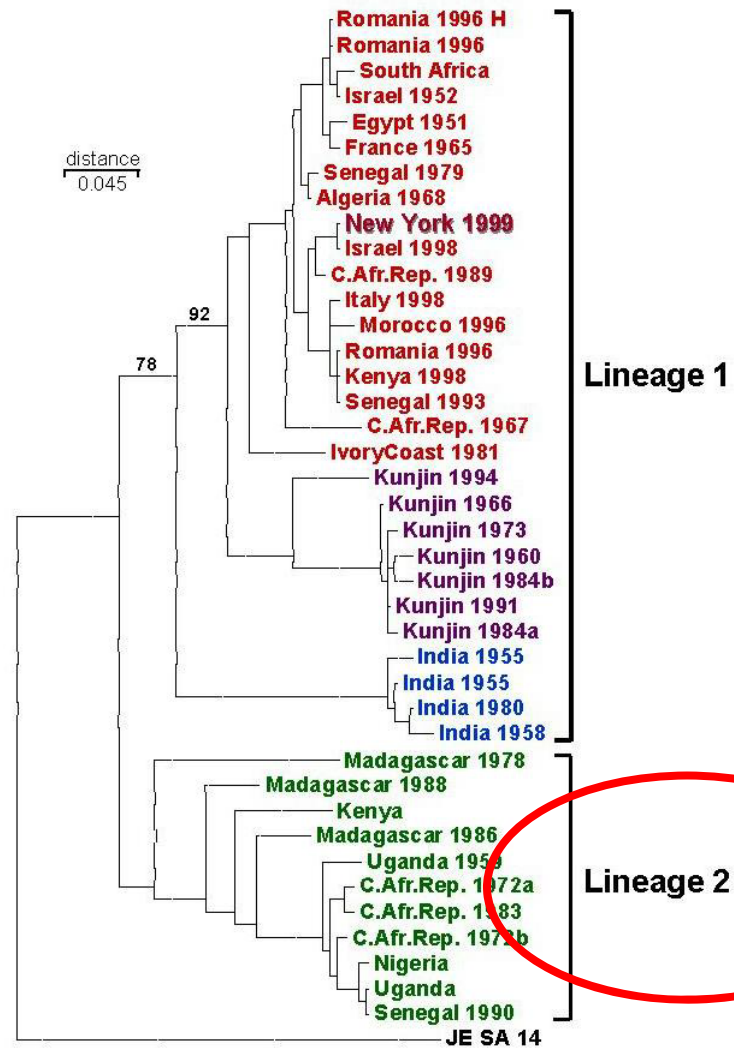
**Virus Identification (IF  
and RT-PCR)**

**Genome sequencing**





## Phylogenetic Tree Based on Envelope Glycoprotein Sequence Data



MEGA, distance tree, Kimura 2-parameter, neighbor-joining

**CDC**  
CENTERS FOR DISEASE CONTROL  
AND PREVENTION





# Molecular Amplification Assays

## 1. *RNA Extraction*

RNA extraction from:  
serum, csf, tissues, & mosquito pools

## 2. *Amplification*

## 3. *Detection*

**Standard  
RT-PCR**

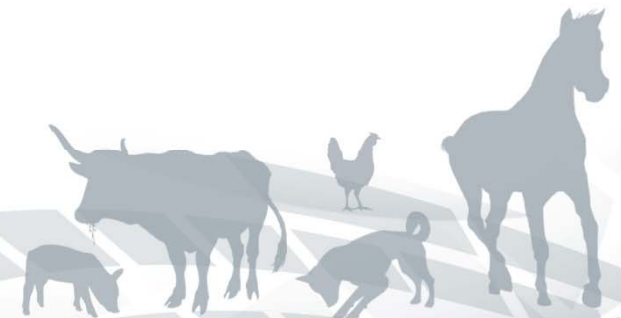
**TaqMan  
RT-PCR**

**Lin 1 & 2  
RT-PCR**

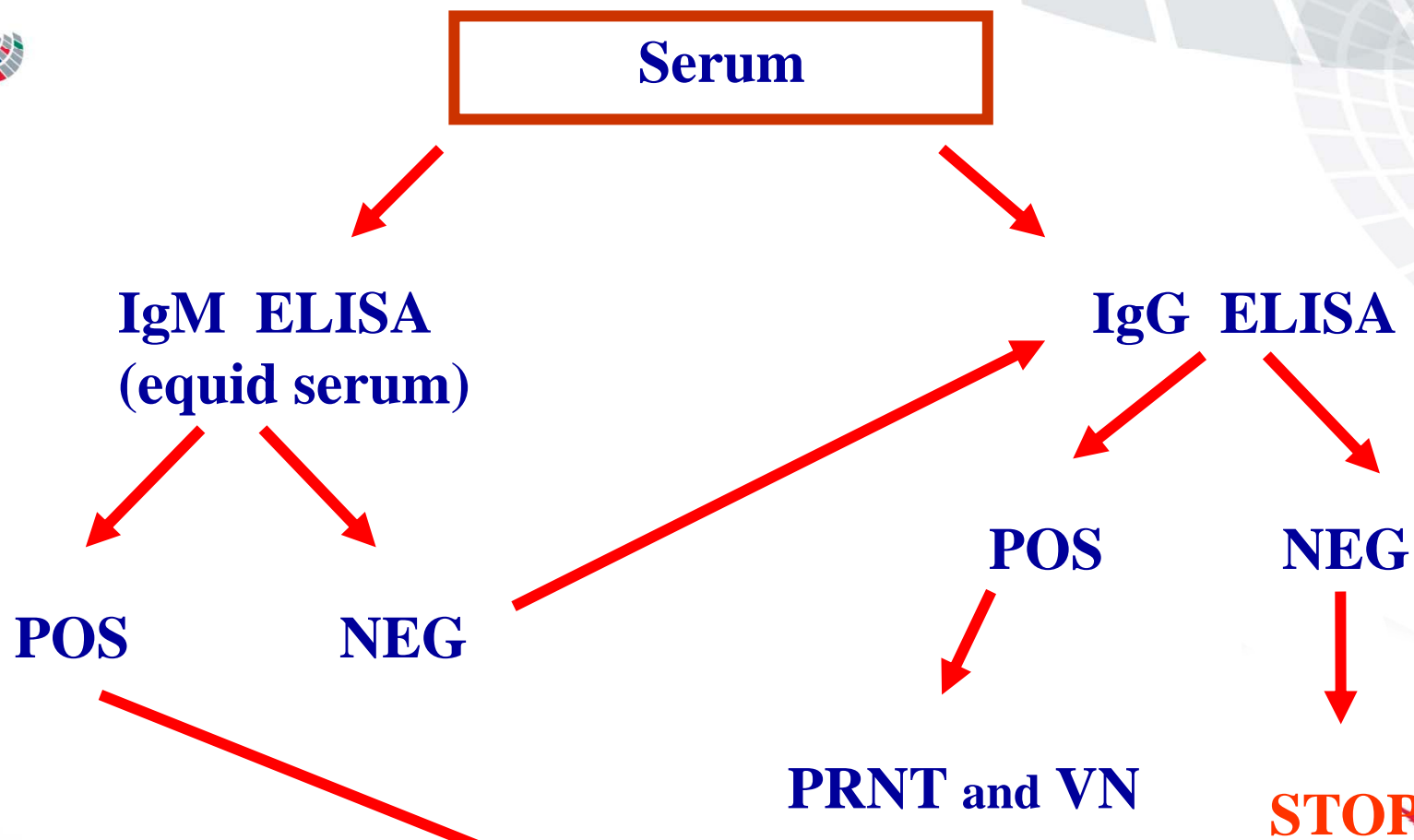
Agarose gel

TaqMan probe

TaqMan probe

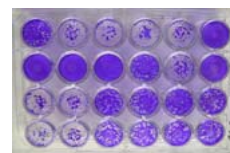






**PRNT and VN**

(OIE, 2008)



**WND**  
**Usutu**  
**TBE**

**STOP**





# Example of assay validation

		SN		
		Positivi	Negativi	Totale
ELISA	Positivi	277	52	329
	Negativi	0	118	118
	Totale	277	170	447





# Example of assay validation

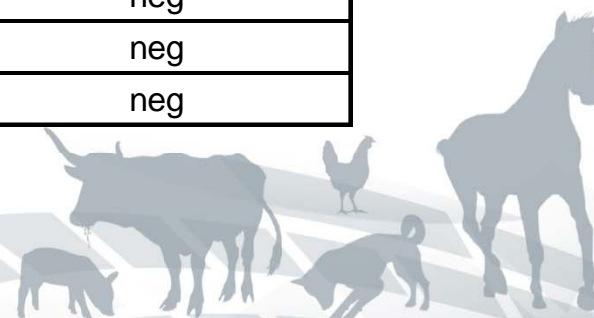
Indice	Valore	IC 95%
Agreement	0,883668904	85,1-91,0
Sensitivity	1	98,9-100
Specificity	0,694117647	62,1-75,8
Predictive value of positive	0,841945289	79,8-87,7
Predictive value of negative	1	97,5-100
Kappa	0,738	





# Example of validation

Dilution	USUV	WNDV
tq	pos	pos
1:10	pos	pos
1:20	pos	pos
1:40	pos	pos
1:80	pos	neg
1:160	pos	neg
1:320	neg	neg
1:640	neg	neg
1:1280	neg	neg
1:2560	neg	neg
1:5120	neg	neg
1:10240	neg	neg
1:20480	neg	neg
1:40960	neg	neg
1:81920	neg	neg

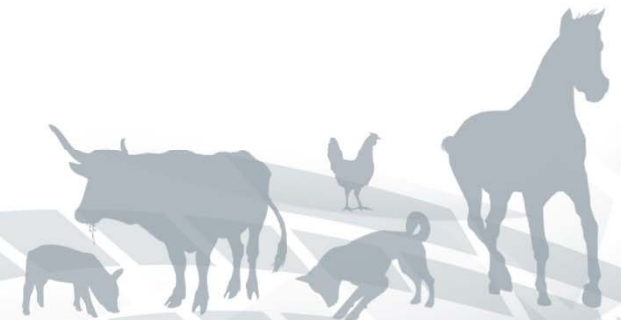






# Conclusions

- The low specificity value of the test was a consequence of **cross reactions with USUTU virus antibodies**
- Because of the very high PV(-) it is strongly suggested to use the test as **a screening test**







Thanks for  
your  
attention