



CO-CIRCULATION OF FLAVIVIRUSES AND VACCINATION WITHIN EUROPE

EVALUATION OF SEROLOGICAL DIAGNOSTIC TOOLS IN HORSES

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TABLE

1-INTRODUCTION

- Flavivirus : the structure
- Flavivirus : co-circulation in Europe
- WNV diagnosis in horses

2-FLAVIVIRUS EPIDEMIOLOGICAL SURVEY

- Kits commercially available for IgG diagnostic
- Evaluation of WNV competitive ELISA kits (ILPT 2013 and 2016)

3-WNV OUTBREAKS

- Kits commercially available for IgM diagnostic
- Evaluation of WNV IgM ELISA kits (ILPT 2013 and 2016)

4-VACCINATION and WNV DIAGNOSIS

- The vaccines available
- Vaccination following the 2015 outbreak in France
- DIVA test

5-CONCLUSIONS

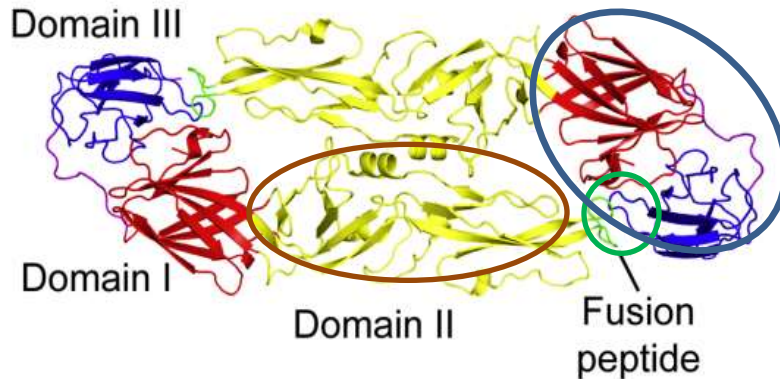
1-INTRODUCTION

➤ Flavivirus: the structure



Glycoprotein E : highly immunogenic

Top view sE



(Heinz, J Clin Virol 2012)

- Domain I and III : virus specific epitope

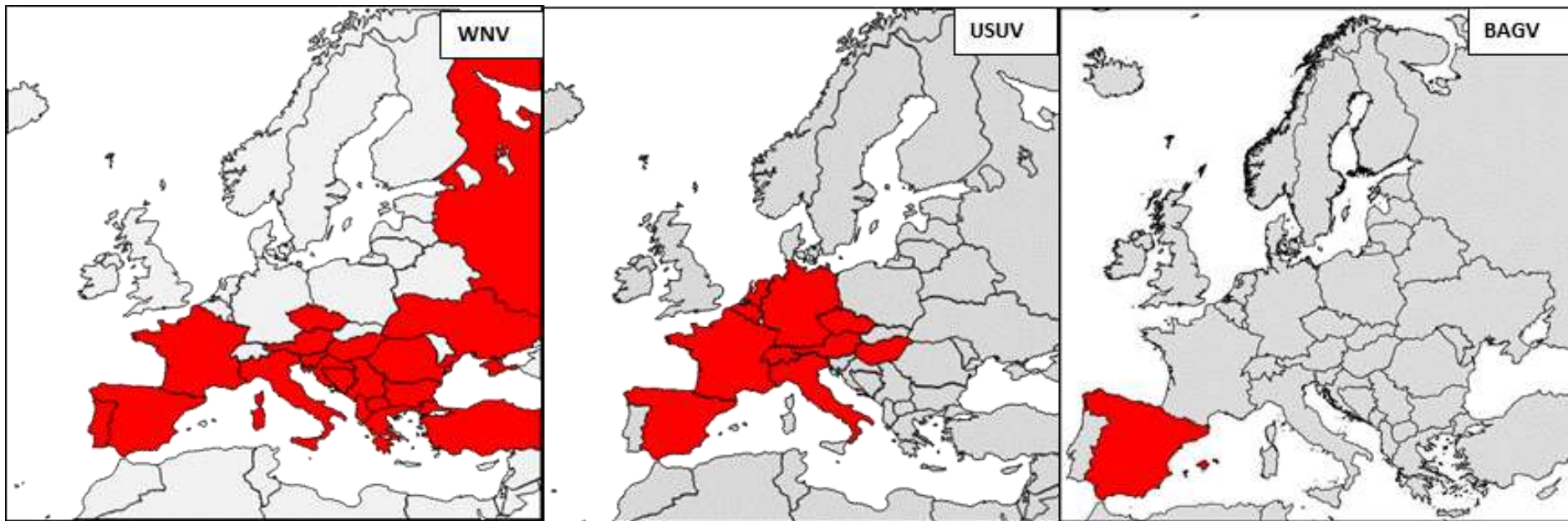
- Domain II with fusion peptide: cross serological reaction

➤ Frequent cross reactions among flaviviruses, especially in rapid serological tests like ELISAs

1-INTRODUCTION

➤ Flavivirus : co-circulation in Europe

Vector : mosquitoes



Map of the main flaviviruses circulating in Europe (2003-2016)

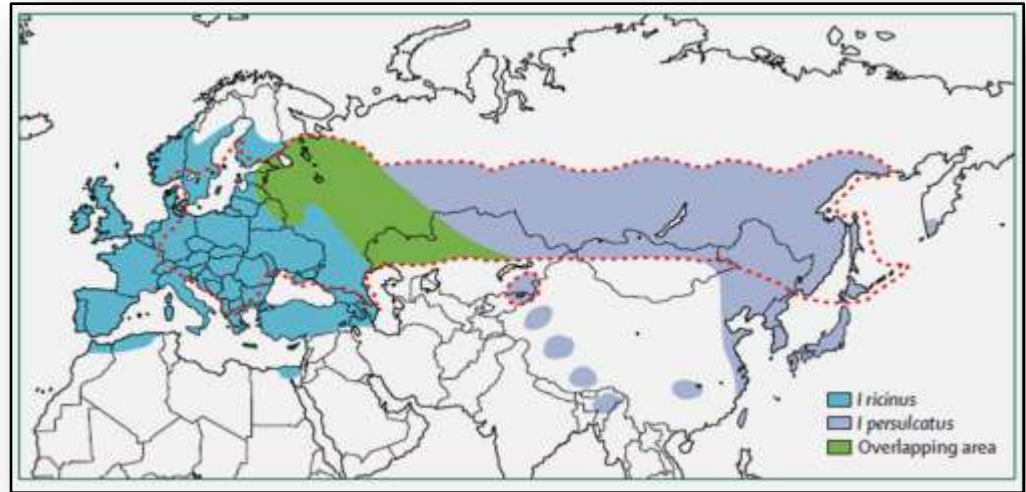
1-INTRODUCTION

➤ Flavivirus : co-circulation in Europe

Vector : ticks



LIV: ovine cases (2016)



Adapt of Lindquist , Lancet 2008

TBEV: human cases - - - - Risk of TBEV infection

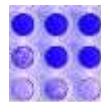
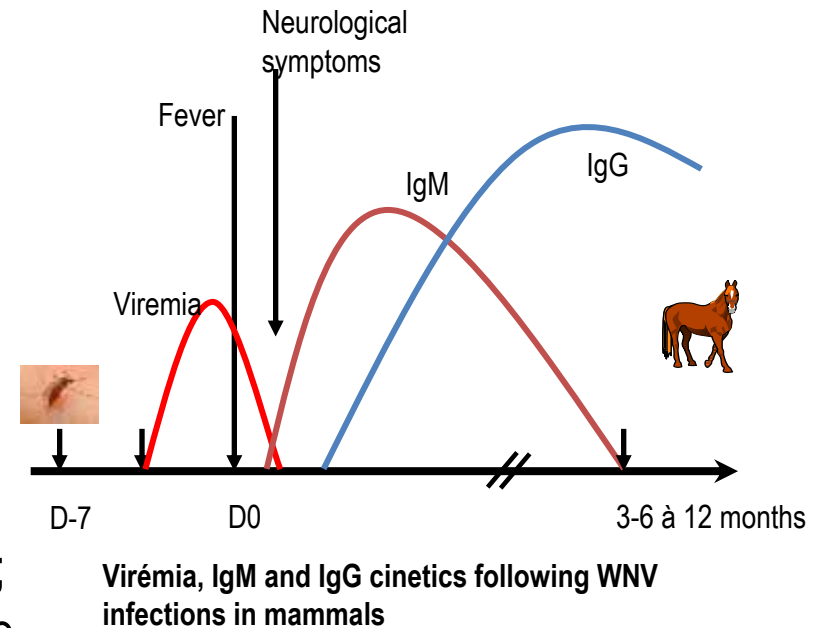
➤ Overlapping of area : interpretation of serological diagnostics with care

1-INTRODUCTION

➤ WNV diagnosis in horses

Short duration and low level of viremia

- **primary tools used to diagnose WNV are serological tests**
- Many serological diagnosis tools are available;
The most used in veterinary diagnostics are
- IgM ELISA (early detection) and IgG ELISA tests (epidemiological survey)
- VNT (gold standard)



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4 –VACCINATION and WNV DIAGNOSIS

- The vaccines available
- Vaccination following the 2015 outbreak in France
- DIVA test

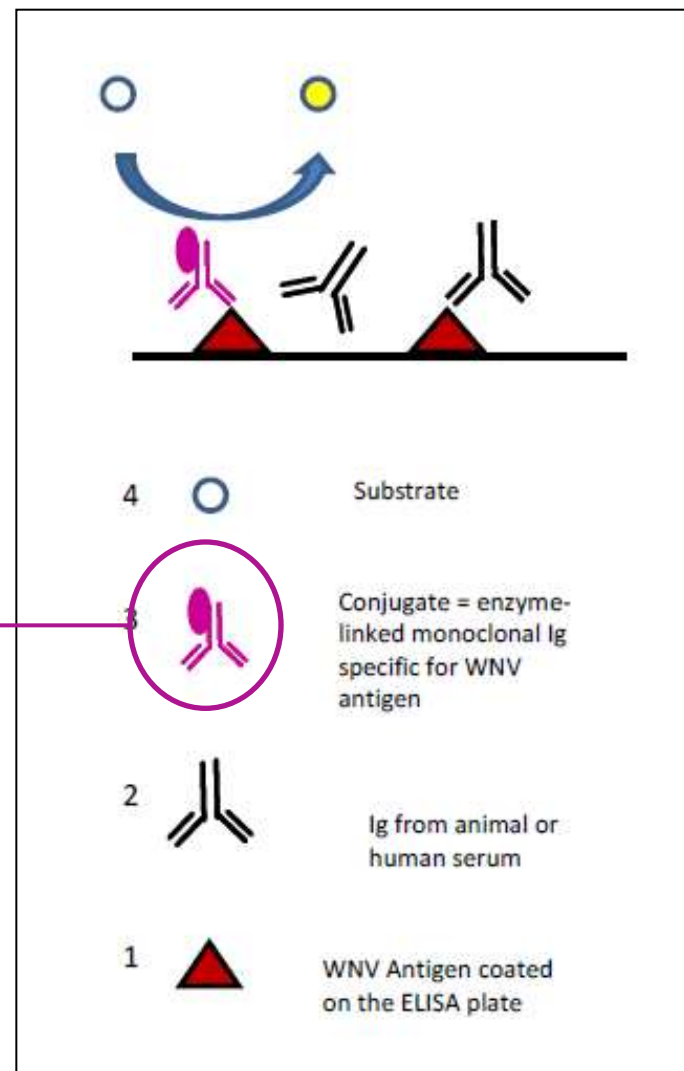
5- CONCLUSIONS

2- FLAVIVIRUS EPIDEMIOLOGICAL SURVEY

➤ Kits commercially available for IgG diagnostic

Competitive ELISA kits	ID screen®WNV competition kit (IDVet)
	Ingezim®WNV compaq kit

- Anti E in ID screen®WNV competition kit
- Anti E domain III in Ingezim®WNV compaq kit



2- FLAVIVIRUS EPIDEMIOLOGICAL SURVEY

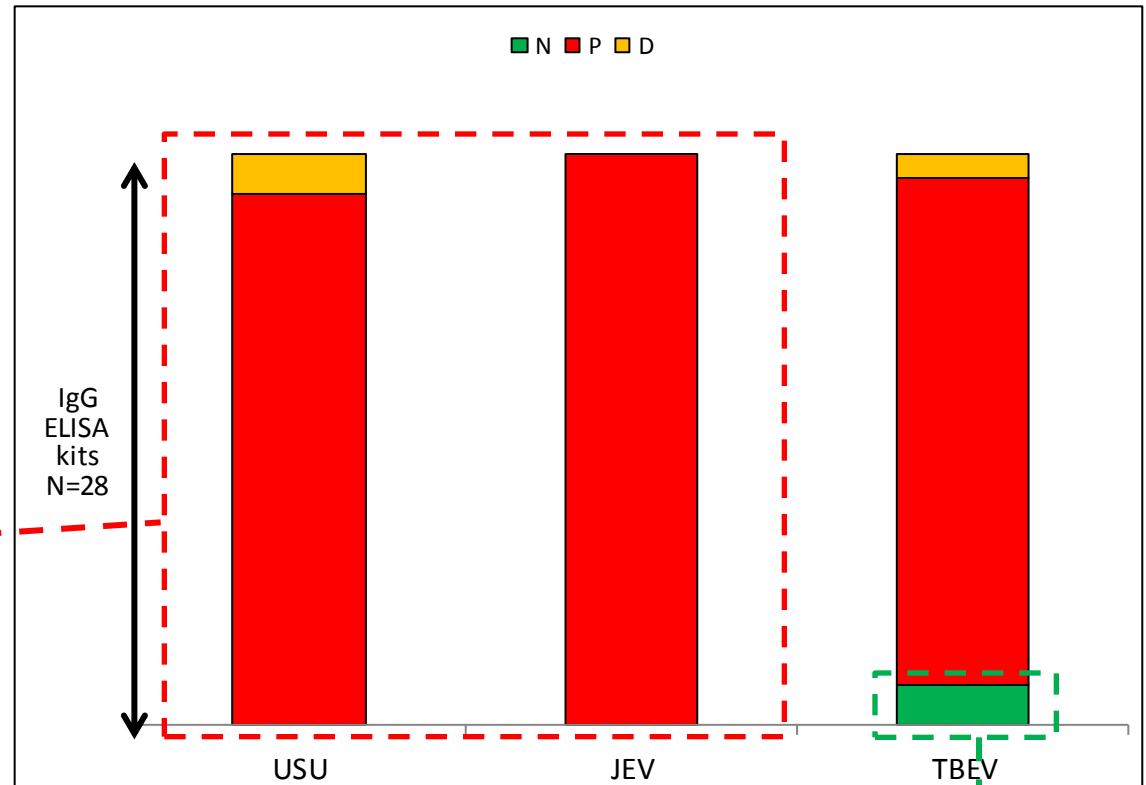
➤ Evaluation of WNV competitive ELISA kits/diagnostic specificity (ILPT 2013)

Experimental infection of horses with related flaviviruses (USUV, JEV and TBEV)

- ID Vet : 25 assays
- Ingezim : 3 assays

IDVet and Ingezim

- For horses infected with flaviviruses belonging to the same serocomplex (USUV and JEV) horses found positive or doubtful whatever the ELISA kit used



Ingezim
anti-TBEV Abs not detected

➤ Serological cross reactions underlined with related flavivirus

2- FLAVIVIRUS EPIDEMIOLOGICAL SURVEY

➤ Evaluation of WNV competitive ELISA kits (ILPT 2016)

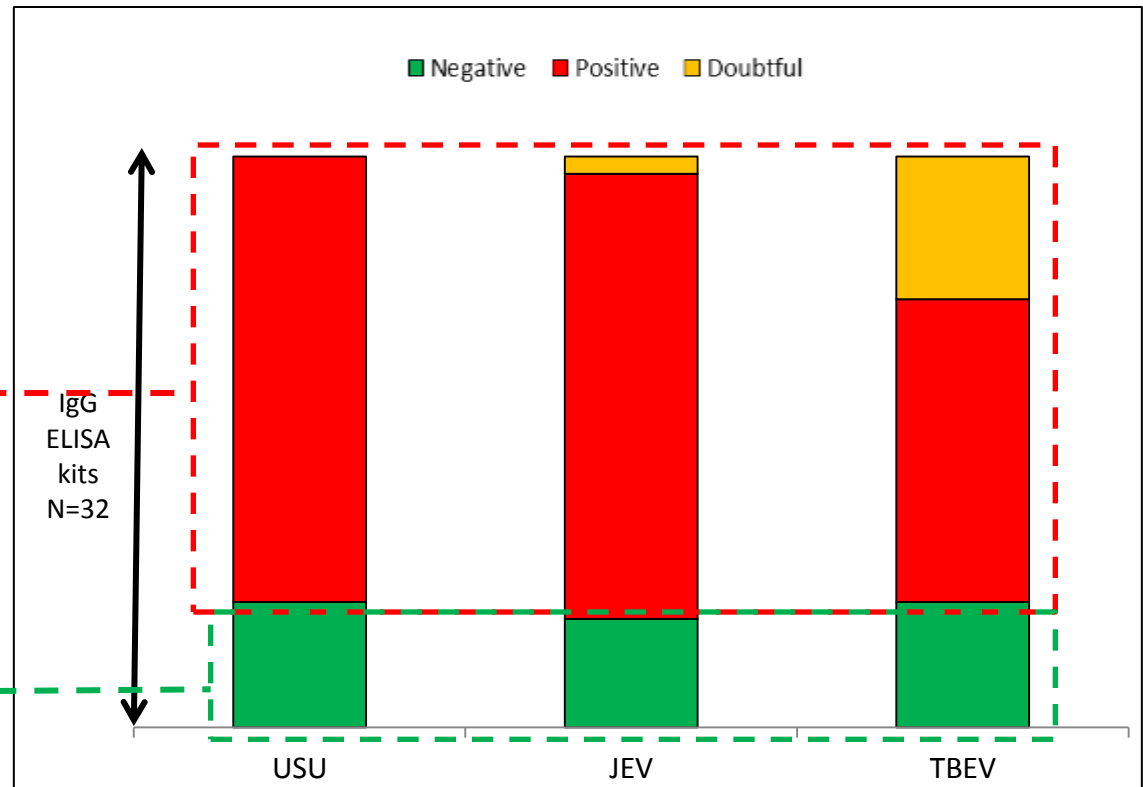
Experimental infection with related flaviviruses (USUV, JEV and TBEV)

- ID Vet : 26 assays
- Ingezim : 6 assays

IDVet only

- Horses found positive or doubtful for USUV, JEV and TBEV (>95% of participants)

Ingezim WNV compac kit
anti- USUV, JEV and TBEV Abs not detected (100% of participants)



- Improvement of the diagnostic specificity of the Ingezim WNV compac kit between the 2 ILPTs
- This data should be validated with more related flaviviruses samples



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- **Kits commercially available for IgM diagnostic**
- **Evaluation of WNV IgM ELISA kits (ILPT 2013 and 2016)**

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3- WNV OUTBREAKS

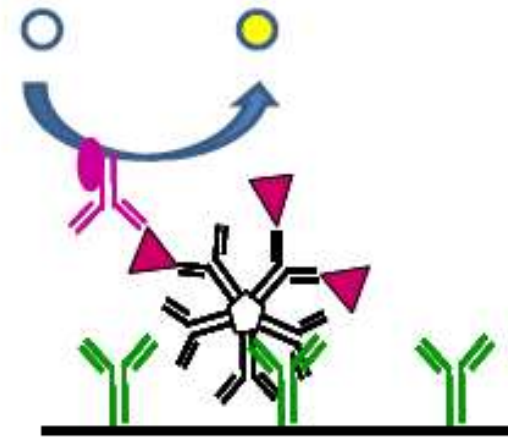
➤ Kits commercially available for IgM diagnostic

Capture ELISA kits

ID screen® WNV IgM capture kit

Ingezim® WNV IgM kit

IDEXX® IgM WNV Ab kit



5



Substrate

4



Conjugate =
enzyme-linked Ig
specific for WNV
antigen

3



WNV Antigen

2



IgM from animal or
human serum

1



Anti-IgM antibodies
coated on the ELISA
plate

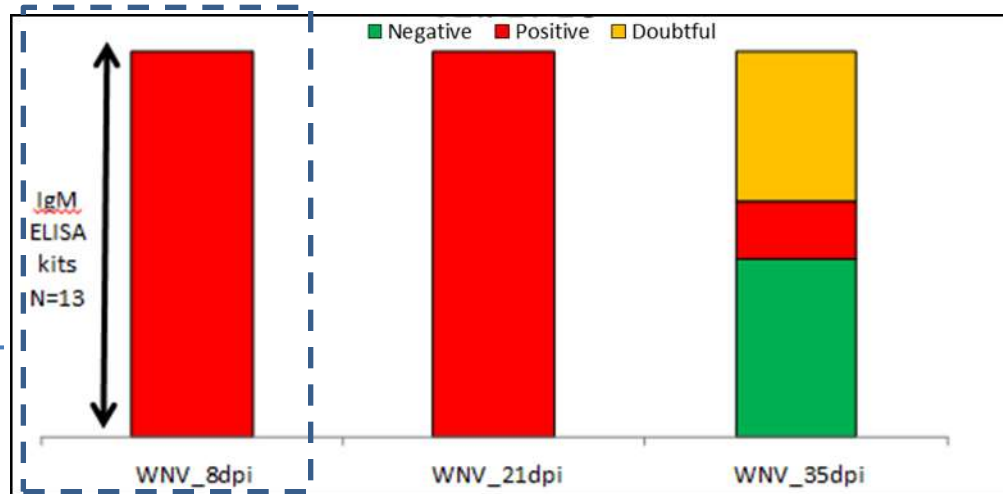
3- WNV OUTBREAKS

➤ Early detection

Experimental infection and kinetics of anti-WNV antibody response (ILPT2013)

Evaluation of MAC ELISA kits (2013 ILPT)

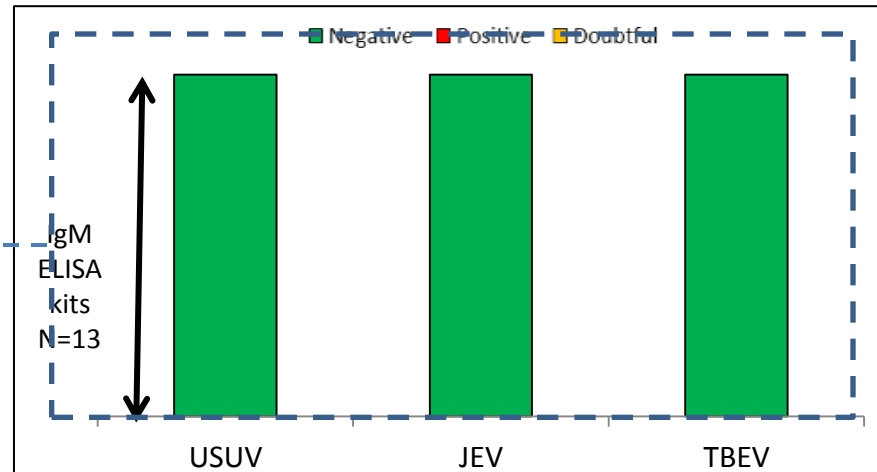
- Animals recently infected by WNV were correctly detected as early as 8 dpi for the WNV lineage 1 infected pony whatever the kits used



➤ Diagnostic specificity

Evaluation of MAC ELISA kits (2013 and 2016 ILPT)

- No cross reaction was observed in particular on sera from horses belonging to JEV serocomplex whatever the kits used



➤ WNV MAC ELISA kits specifically detected WNV antibodies

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4- VACCINATION AND WNV DIAGNOSIS

➤ The vaccines available

Inactivated vaccine with adjuvant (WNV et JEV)

- WNV : Zoetis Equip WNV ® (MA in Europe)
- JEV : Kakektusen Encevac ® and Nisseikein ® (MA in Japan)



WNV
inactivated

Recombinant vaccine (WNV)

- Intervet, Equilis West Nile ® (MA in Europe)
- Merial, Proteq West Nile ® (MA in Europe)



YFV



YFV (prM-E WNV)
inactivated



Canarypox



Canarypox+transgen
(prM-E WNV)

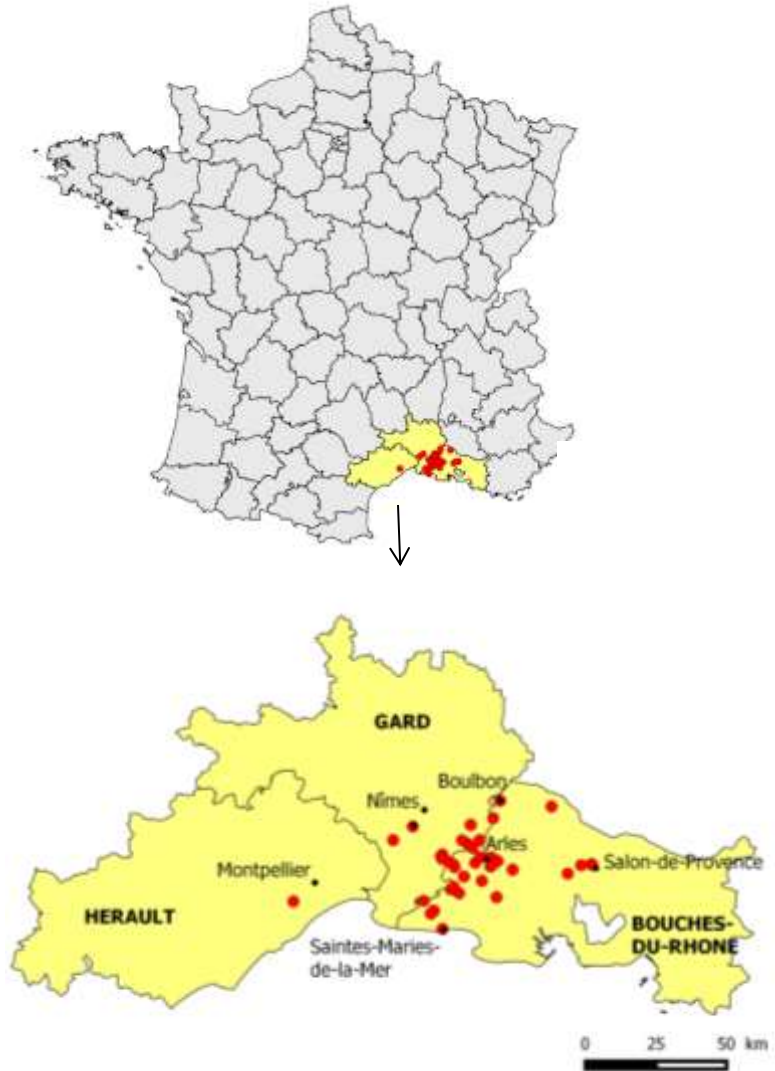
4- VACCINATION AND WNV DIAGNOSIS

➤ Vaccination following outbreaks in France (2015)

49 cases with 41 neuroinvasive forms

Localisation : South of France (Camargue area)

Following this outbreak, vaccination against WNV in some stables



4- VACCINATION AND WNV DIAGNOSIS

➤ Vaccination following outbreaks in France (2015)

Serosurvey was carried out in the camargue area on:

- ✓ 235 horses not vaccinated
- ✓ 54 horses vaccinated

▲ 36 horses vaccinated : Equip WNV® ZOETIS



● 18 horses vaccinated : Proteq West Nile® MERIAL



Agnès Leblond and Alain Sandoz

Test with ID screen®WNV competition kit on these samples :
Infected horses and mostly (49/54) of vaccinated horses were found positive

Objective : Implement a DIVA test for WNV (Opium project)

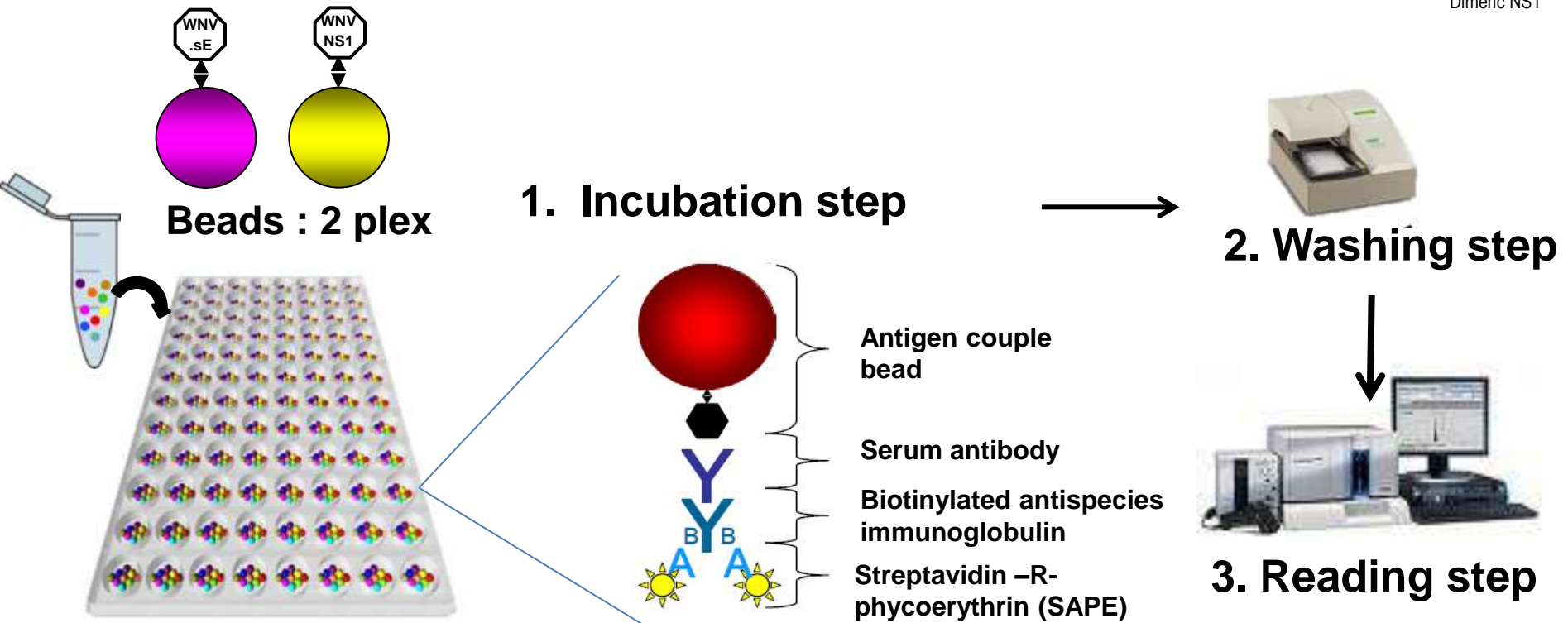
4- VACCINATION AND WNV DIAGNOSIS

➤ DIVA test

Microsphere immunoassay. 2 WNV antigens used : envelope glycoprotein of WNV (WNV.sE) and NS1 (WNV.NS1) antigens (collaboration N. Cleton, Erasmus medical Centre NL)



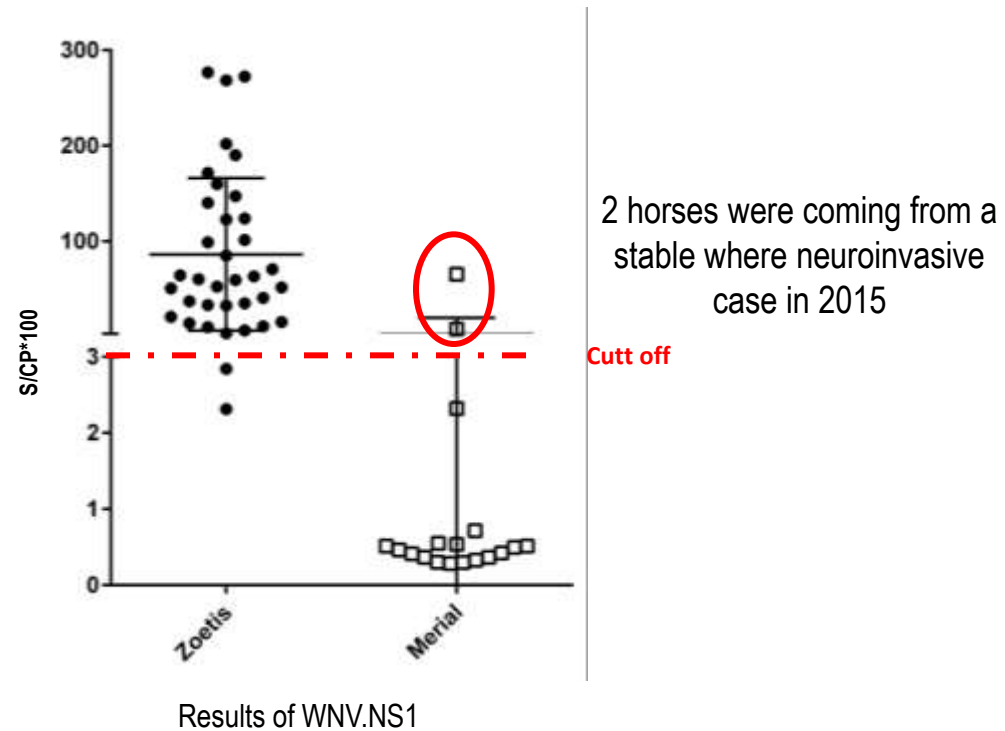
Dimeric NS1



4- VACCINATION AND WNV DIAGNOSIS

vaccinal statut	Awaited results	
	WNV.sE	WNV.NS1
infected	+	+
Vaccinated	+	-

➤ DIVA test : results



- Inactivated vaccine from Zoetis is not well purified and contains NS1 antigens.
- DIVA test only for Merial vaccine

5- CONCLUSIONS

Variable specificity of competitive WNV ELISA kits (Ingezim > IDVet)

Thanks to its high specificity : Mac ELISA kits could be used for the confirmation of recent WNV infection in horses (alternatives to VNT)

DIVA test only with Proteq West Nile vaccine from Merial

Acknowledgements



Stéphan zientara
Sylvie Lecollinet
Steeve Lowenski
Céline Bahuon
Benoit Durand

Edouard Guitton
Philippe Desprès
Paolo Cordioli (IZLER Institute)
Miguel Angel Jimenez Clavero (Cisia INIA Institute)
Norbert Nowotny (University of veterinary Medicine Vienna)

- NRLs network and PT participants
- DG SANTE 
- Producer of ELISA Kits

Thank you for your attention