




IZSAM G.CAPORALE
TERAMO

L'adattamento del virus West Nile su linee cellulari di vettori artropodi e ospiti vertebrati modifica la virulenza del patogeno nel modello murino



Gian Mario Cosseddu,
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del
Molise «G. Caporale» - IZSAM
g.cosseddu@izs.it

Teramo, 13 Giugno 2019



Progetto ARBONET



ARBONET

The European arboviral disease network

WELCOME TO THE OFFICIAL ARBONET PROJECT WEBSITE

The proposed initiative "ARBONET" will be focused on WNF, CCHF and RVF infections, specifically on development of epidemiological models that can inform risk-based surveillance and interventions that can control disease outbreaks in Europe.

The major goal of this joint initiative is to create a multidisciplinary research network combining the expertise of veterinary and human epidemiologists, disease modelers, virologists and arthropod biologists to increase knowledge and understanding that will facilitate rapid control of disease outbreaks.

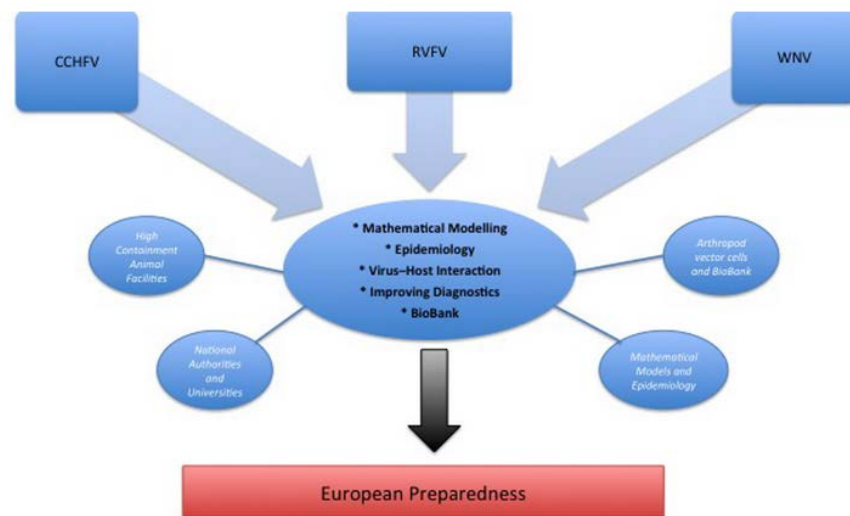




Objectives

1) To **assess** different **epidemiological models** that predict transmission of **WNV in a European context** to design proper early warning systems and select intervention strategies for lowering the risk of WNV transmission to equines and humans.

2) To evaluate the efficacy of using animals as early indicators of **CCHFV** presence and transmission in **early warning systems** for free but at-risk-of-introduction countries and for prompt recognition of seasonal re-emergence of the virus in infected countries.

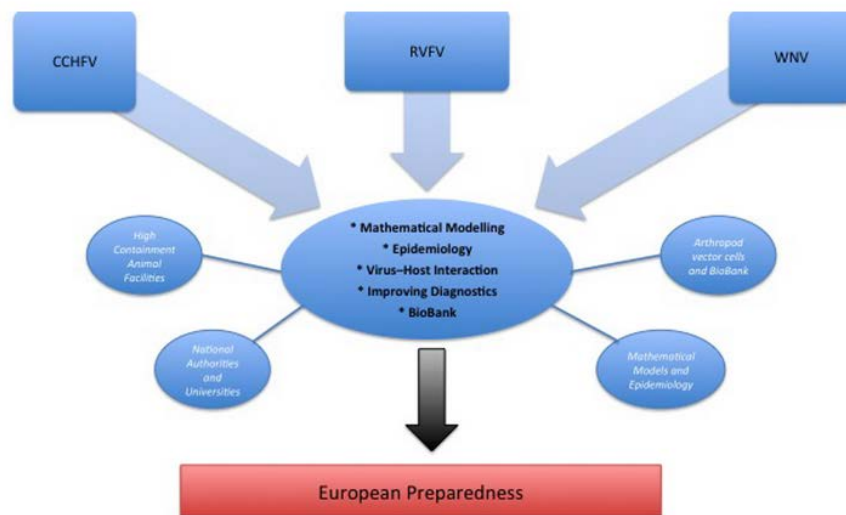




Objectives

3) To propose and assess an **early warning system** for possible scenarios of RVFV introduction into Europe.

4) To develop and use protocols and strategies to examine how **CCHFV**, **WNV** and **RVFV** interact with their arthropod vectors/hosts, information that can ultimately contribute to epidemiological modelling, risk-based surveillance and improved, targeted control strategies.





Workpackages

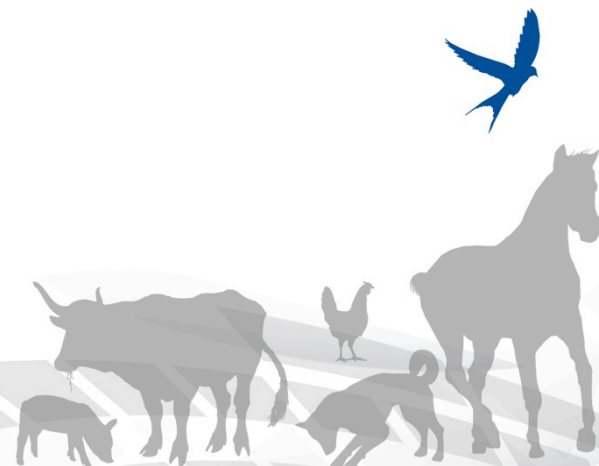
WP1. Coordination and dissemination

WP2. Epidemiological modelling of virus transmission in Europe

WP3. Biobank, diagnostics development and genomic characterization

WP4. Interaction with vertebrate hosts

WP5. Interaction with vectors





Participants

Karolinska Institutet

Kimron Veterinay Institute – Israel

**The Istituto Zooprofilattico
Sperimentale dell'Abruzzo e del Molise**

Institute Pasteur (Paris, France)



Friedrich-Loeffler-Institut – Germany

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses) - France

Institute of Infection and Global Health (IGH), University of Liverpool – United Kingdom



Institut Pasteur

FRIEDRICH-LOEFFLER-INSTITUT



Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

anses
agence nationale de sécurité sanitaire
alimentation, environnement, travail



THE
Pirbright
INSTITUTE



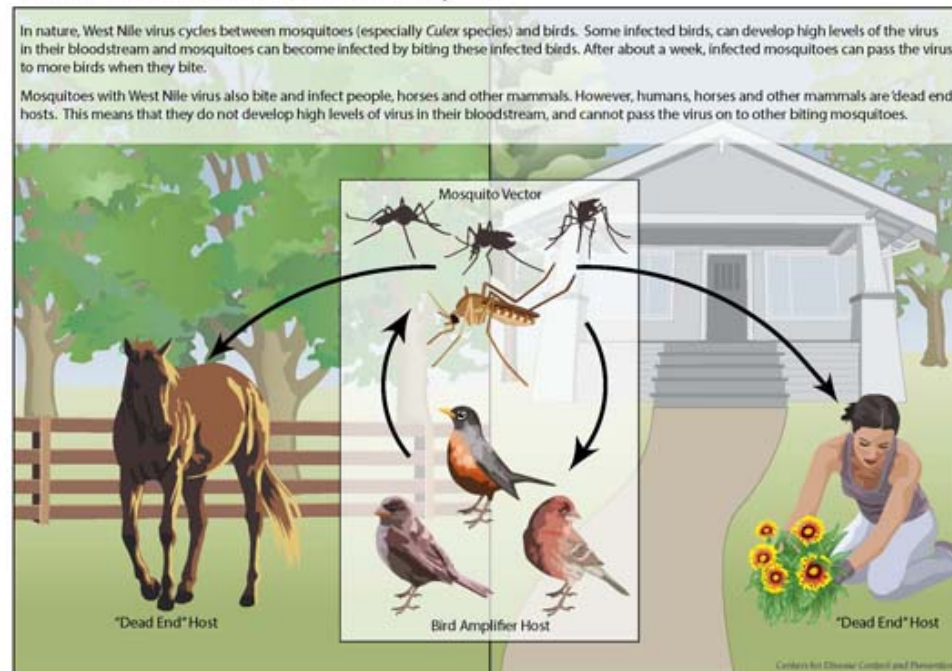
University of
Nottingham
UK | CHINA | MALAYSIA



West Nile Disease

West Nile Disease (WND) is a mosquito-transmitted viral infection. The disease may manifest itself with different symptoms, ranging from no symptoms at all or mild flu-like symptoms to brain damage that can lead to death in *birds*, *horses* and *humans*


West Nile Virus Transmission Cycle



<http://www.cdc.gov/westnile/transmission/>

West Nile Disease

The first time in Italy – 1998

- 
- ✓ WND was detected in Italy for the first time in **Toscana** (Padule di Fucecchio) in the late summer of **1998**.

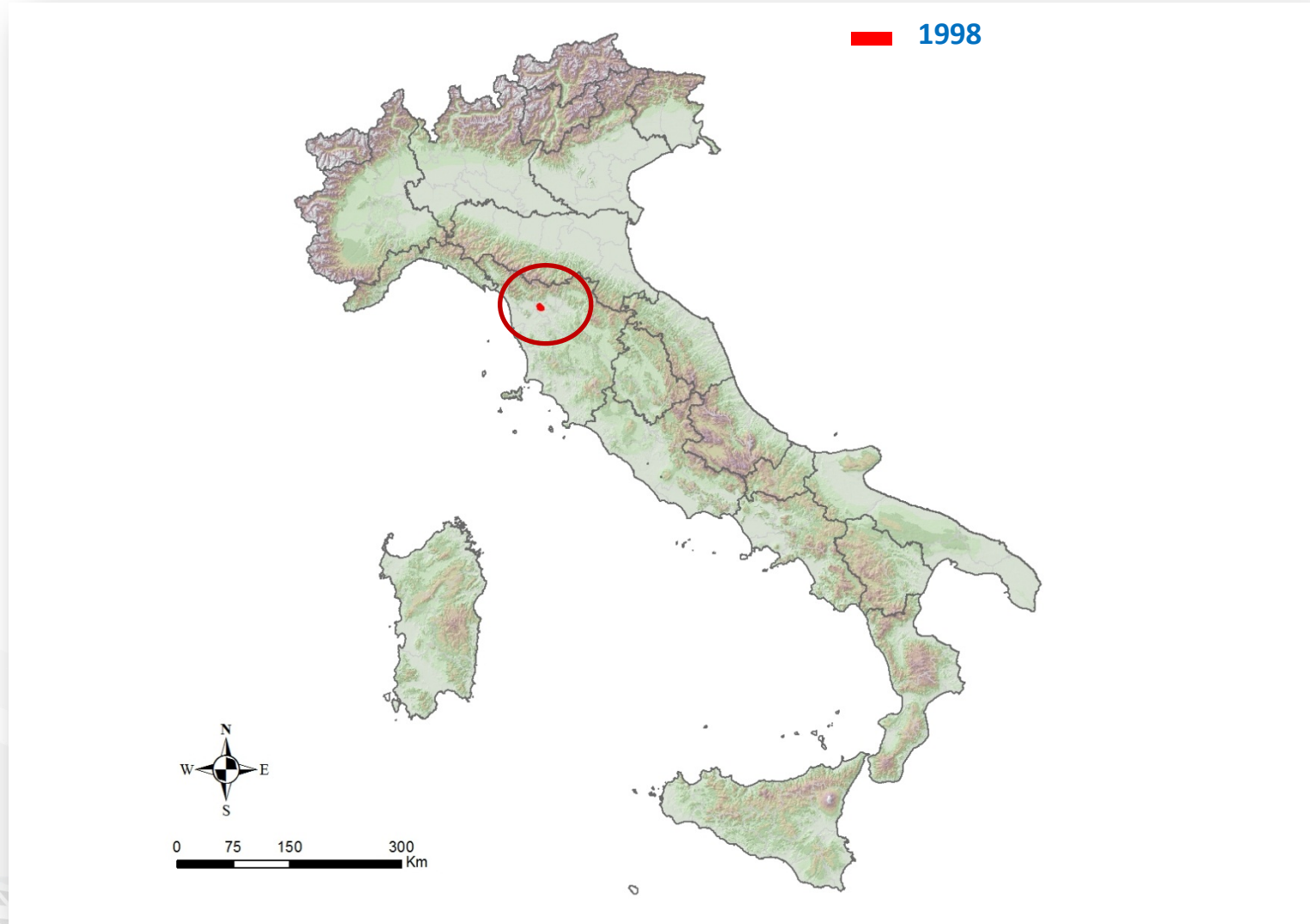


- ✓ The epidemic affected **14 horses** which developed **nervous** clinical signs.
- ✓ **No human cases** were observed.



West Nile Disease

The first time in Italy – 1998



West Nile Disease – 2008

- 
- ✓ **Ten years later** WND occurred again in Italy in the **Po river delta** in August 2008 .

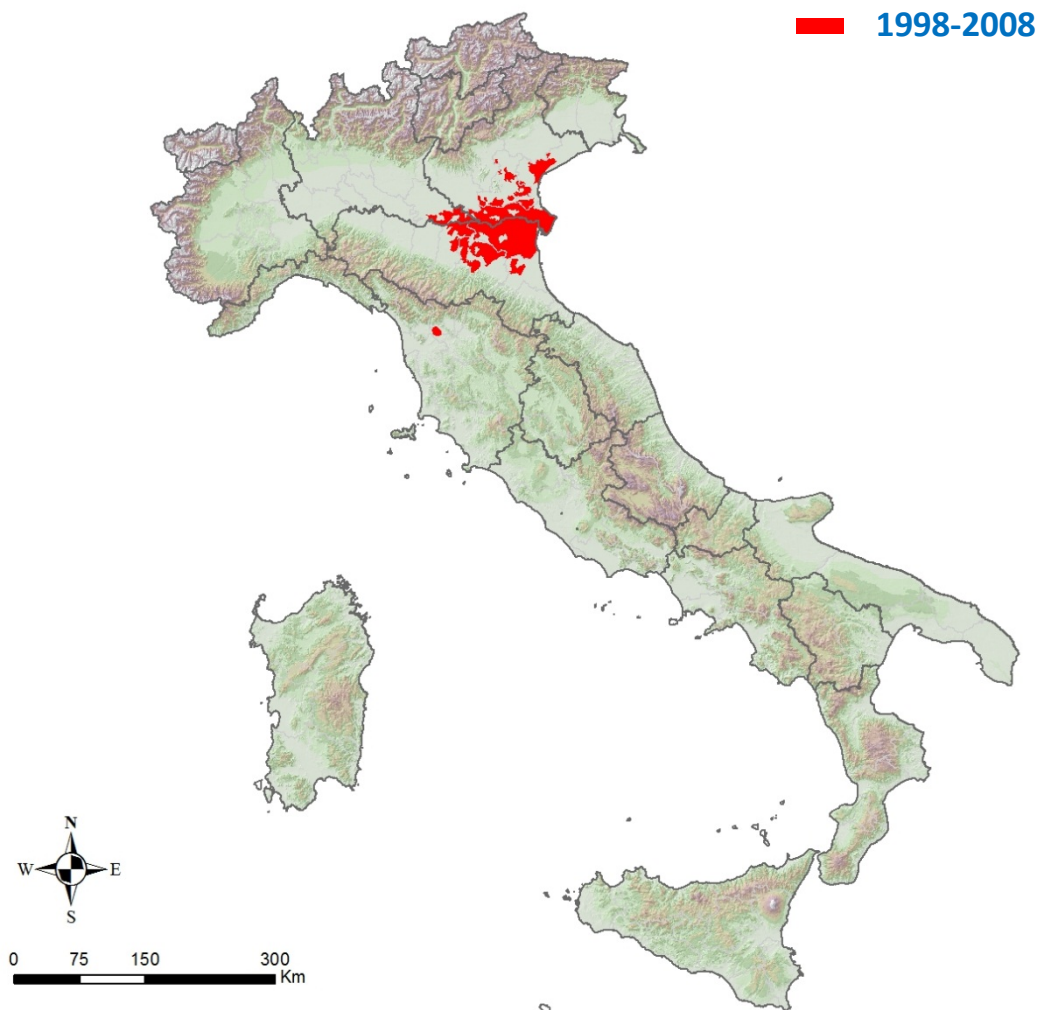


- ✓ The epidemic affected 3 Regions: **Emilia Romagna, Veneto** and **Lombardia**.

- ✓ **Nervous clinical signs** were observed in **horses** and **humans**.

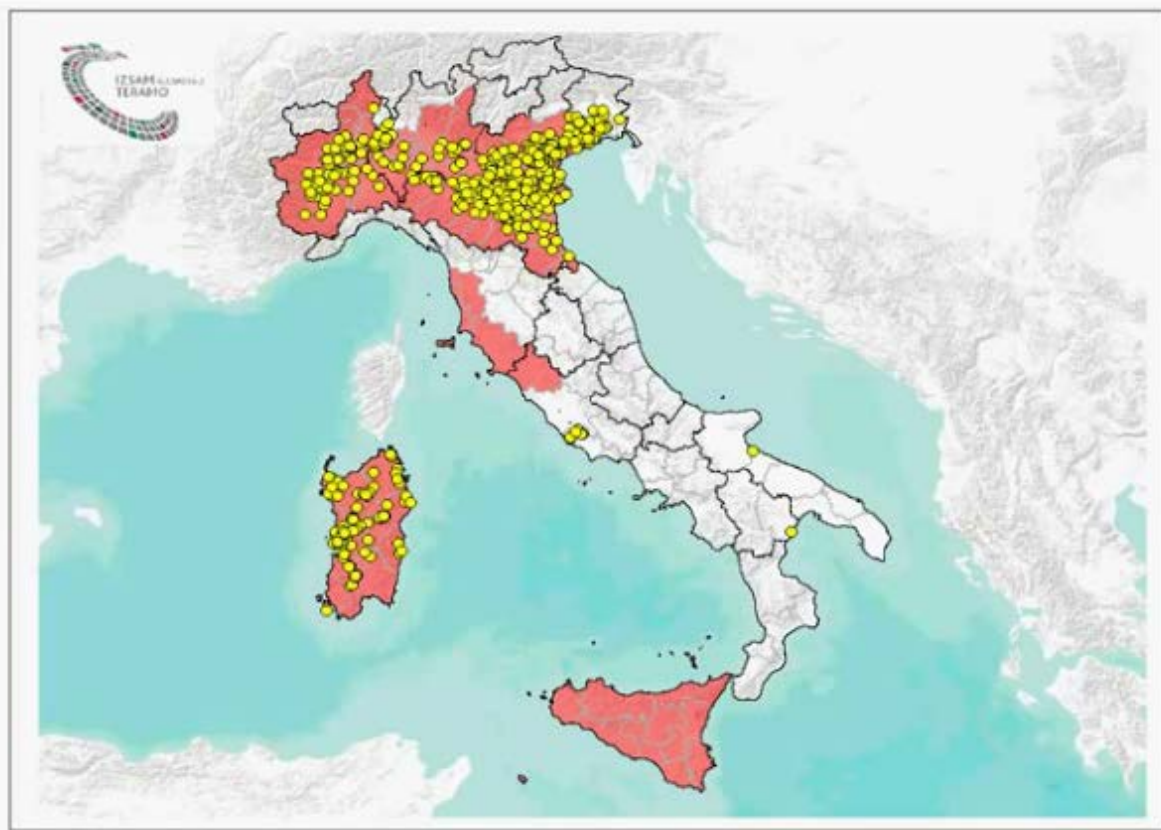


Progression of WND in Italy in animals: 1998 - 2008

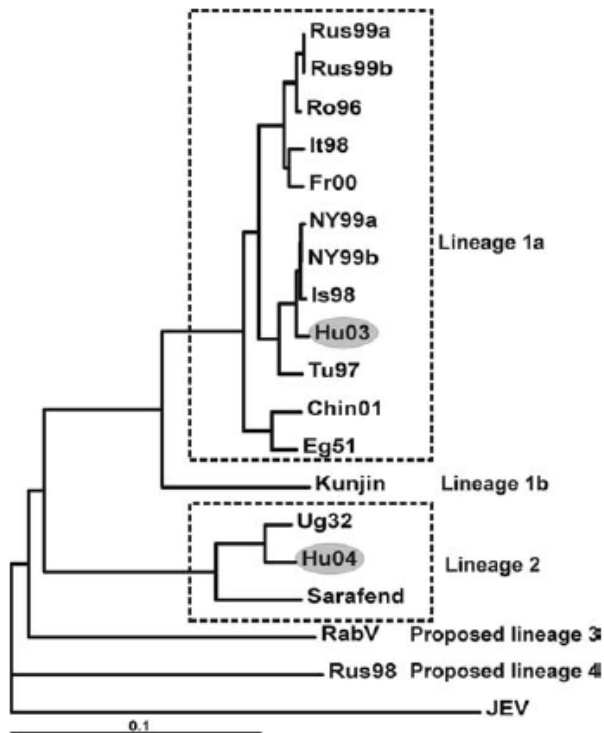


Distribution of outbreaks in 2018

West Nile season, in Italy



West Nile Virus distribution



Full genome sequence
(Bakonyi *et al.*, EID, 2006)

Two major genetic lineages of WNV have been reported. New additional **WNV lineages** have been proposed based on phylogenetic analysis. 8/9 lineages have been described up to now.

Lineage 1: worldwide distribution: northern and central Africa, Israel, Europe, India, Australia (Kunjin virus), North and Central America, Colombia and Argentina in South America.

Lineage 2: endemic in central and southern Africa and Madagascar. Hungary, Austria, Russia, Romania, Greece and Italy have recently also reported



West Nile Virus

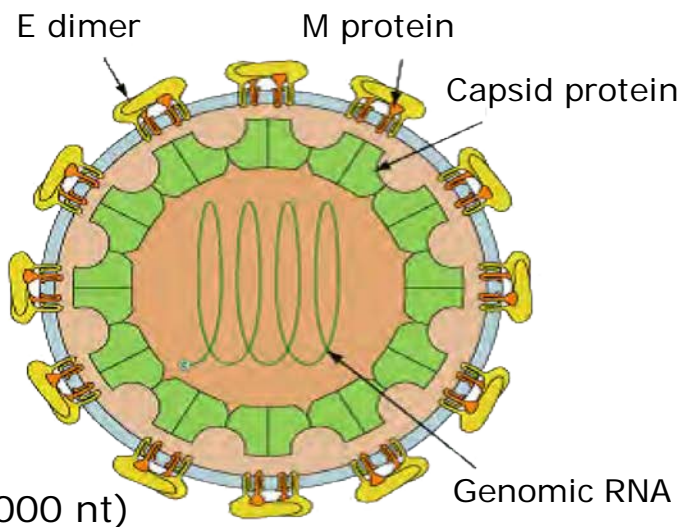
West Nile Virus (WNV)

Flaviviridae family

Flavivirus genus.

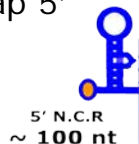
One single serotype

Multiple genetic lineages



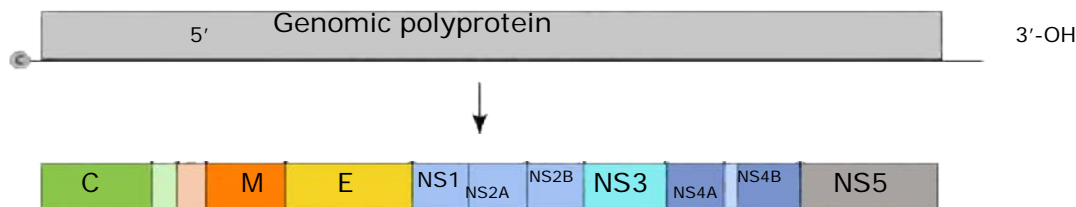
Genome (+ssRNA, 11000 nt)

Cap 5'



Structural proteins

Non structural proteins



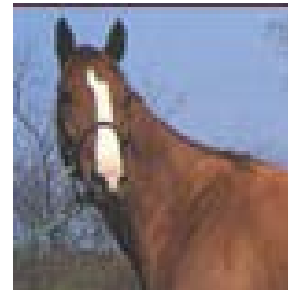
<http://www.viralzone>

Rationale for the study




Mechanisms of persistence and ability to become endemic rely on high plasticity of virus genome.

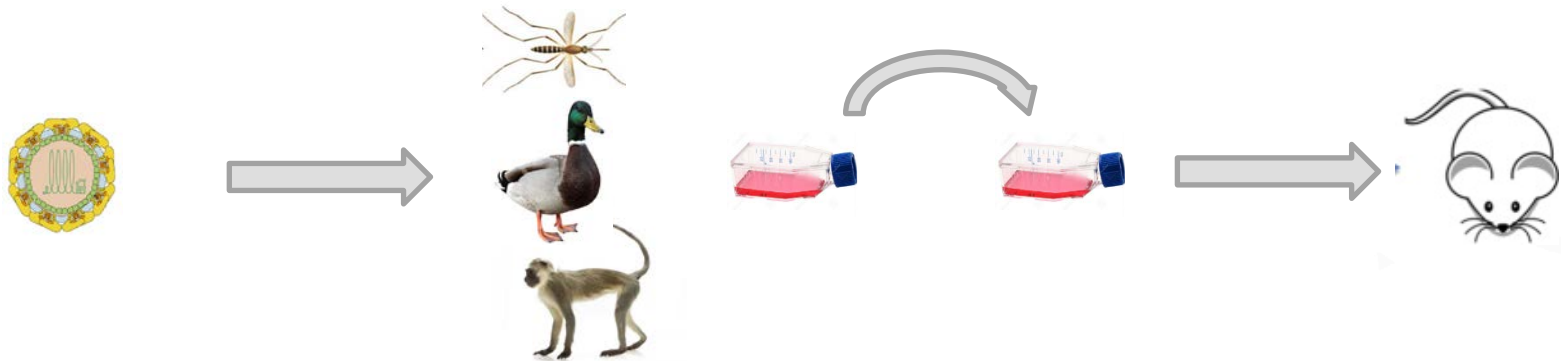
WNV **evolution strategy** improve maintaining adequate replicative fitness in two disparate hosts instead of gain a superior fitness in one single host (the **trade-off hypothesis**).



Aim of the study



The project aims at **investigating the changes in WNV genome** induced by serial passages in cell lines from different vectors and hosts and assess the **effect on virulence** using the mouse model



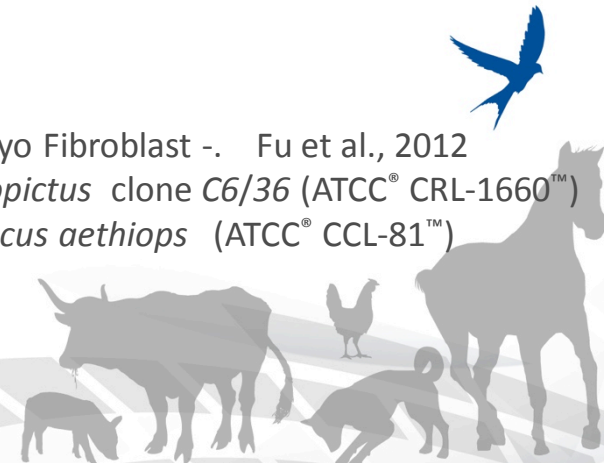
Selected virus strains

WND_L1 isolated from common magpie (*Pica pica*) in 2008 in Emilia Romagna

WND_L2 isolated from northern goshawk (*Accipiter gentilis*) in 2012 in Sardinia

Selected cell lines

- **DEF cells** - Duck Embryo Fibroblast -. Fu et al., 2012
- **C6/36 cells** *Aedes albopictus* clone C6/36 (ATCC® CRL-1660™)
- **Vero cells** - *Cercopithecus aethiops* (ATCC® CCL-81™)



Adaptation to cell lines

Single host



WNV1/WNV2

p10



p15



p20



⇒ Vero p20



⇒ C6/36 p20



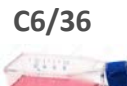
⇒ DEF p20



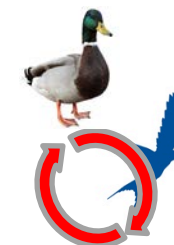
Host/vector alternation



WNV1/WNV2



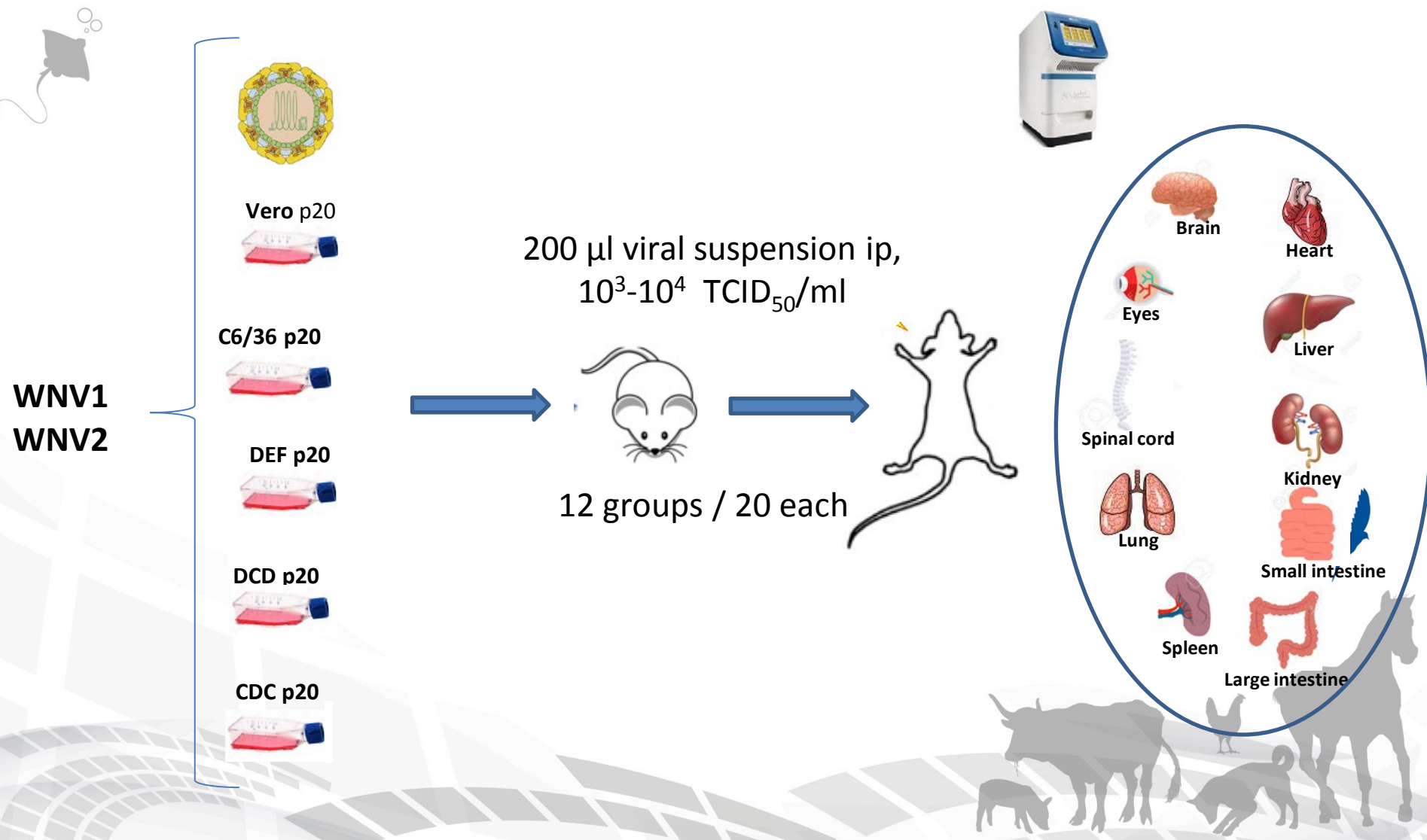
⇒ DCD p20



⇒ CDC p20

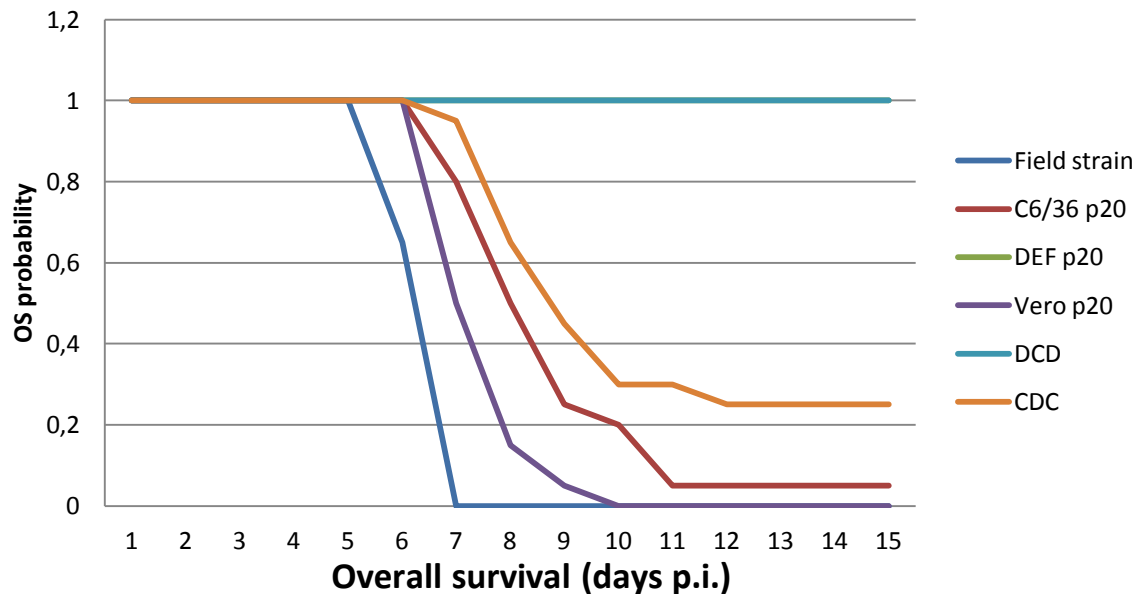


Experimental inoculation



Results : mortality

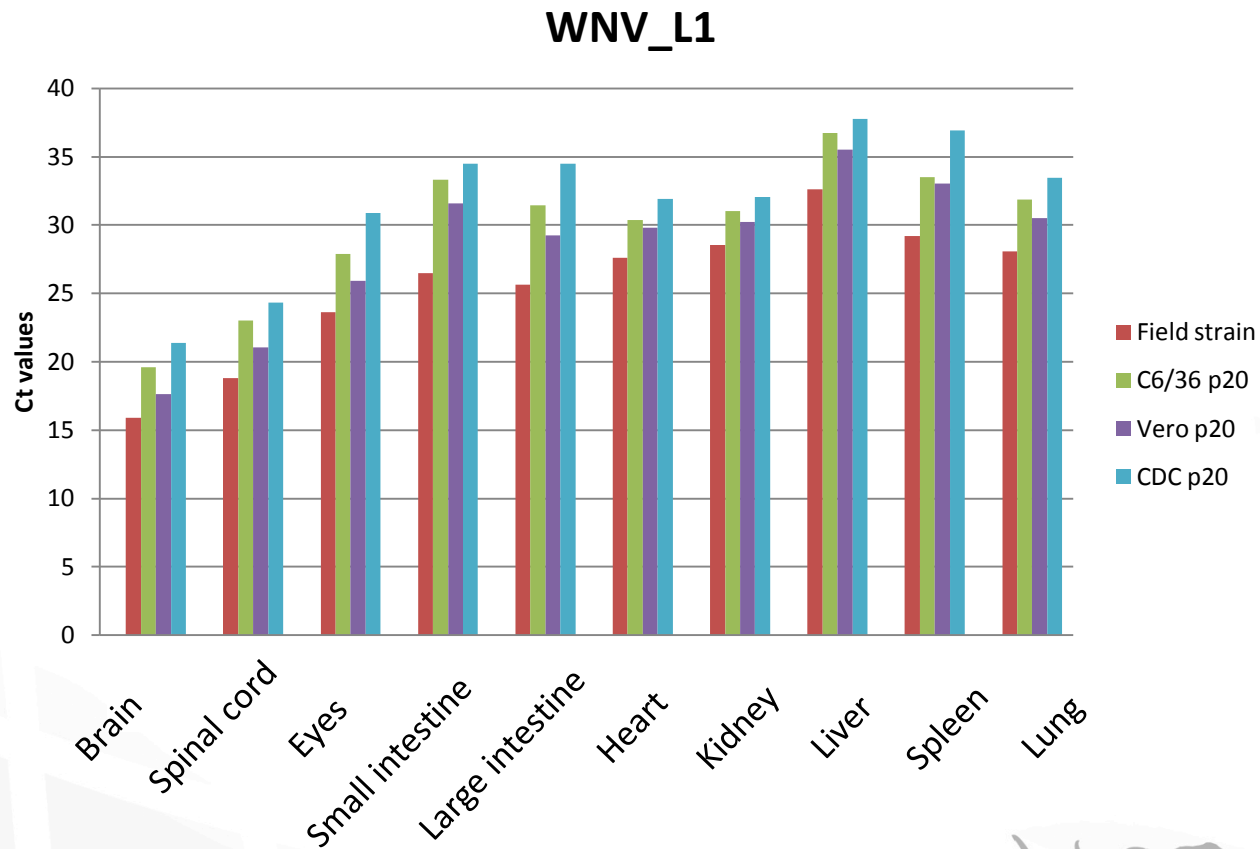
WNV_L1



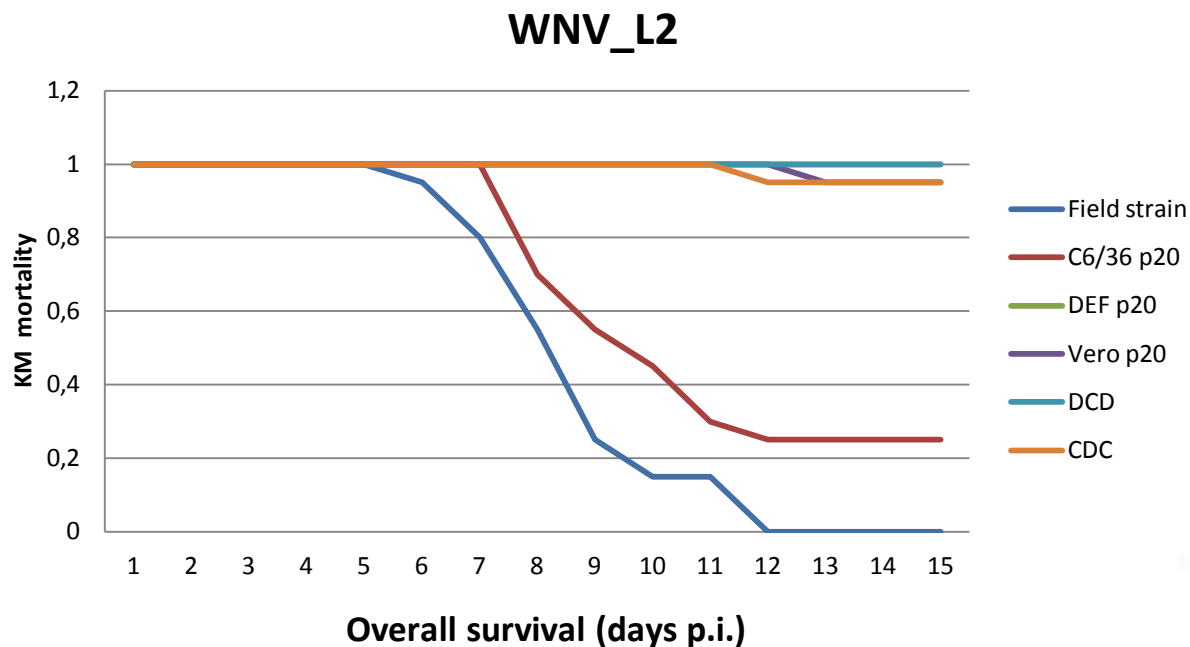
Inocula

	Field strain	C6/36 p20	DEF p20	Vero p20	DCD p20	CDC p20
Attack rate	20/20	19/20	0/20	20/20	0/20	15/20
Mean survival (dpi)	6,5	8,8	>15	7,7	>15	8,9
DS	0,49	1,65	nd	0,87	nd	1,25

Results : WNV distribution in tissues

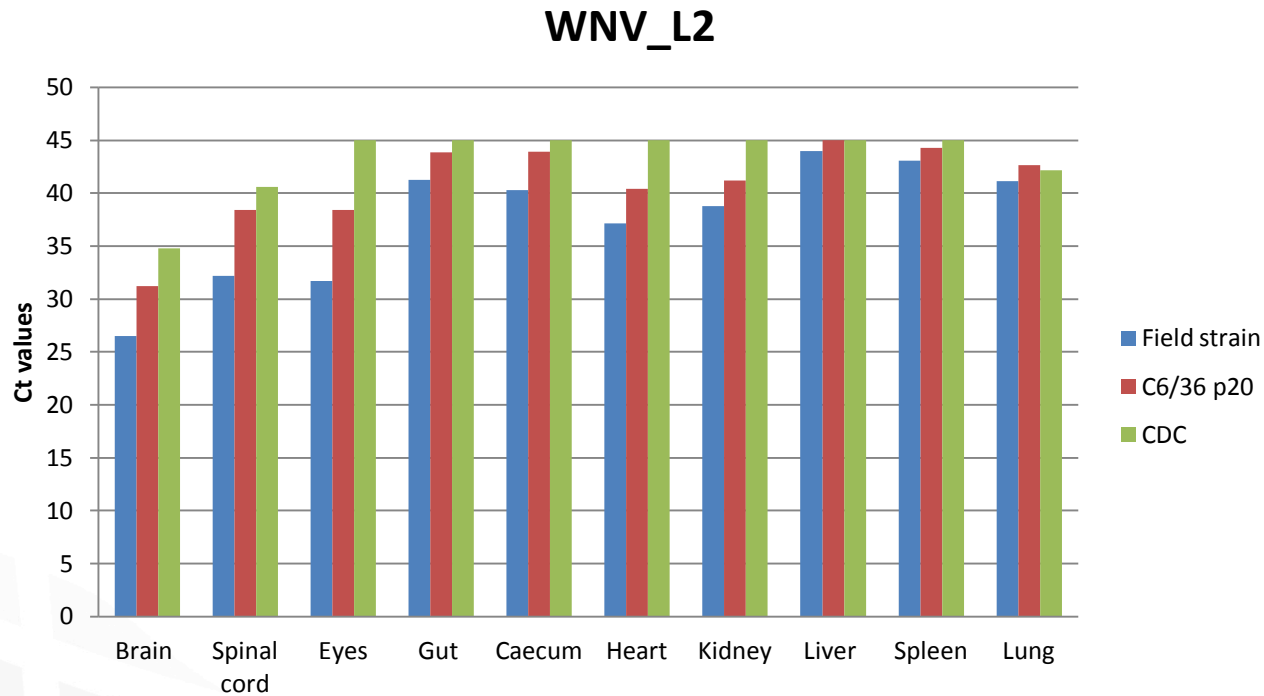


Results : mortality




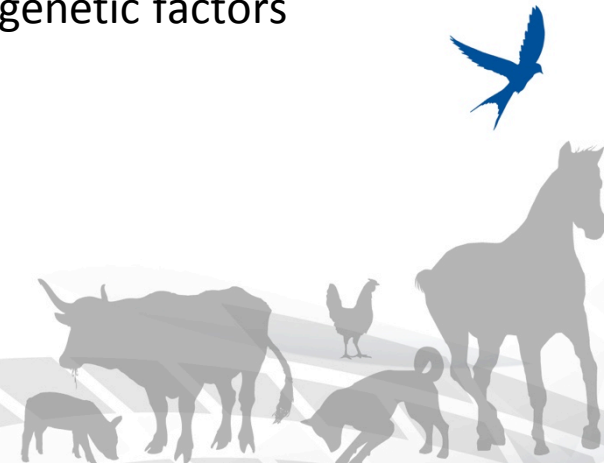
	Inocula					
	Field strain	C6/36 p20	DEF p20	Vero p20	DCD p20	CDC p20
Attack rate	20/20	19/20	0/20	2/20	0/20	2/20
Mean survival (dpi)	8,9	9,3	>15	14,0	>15	13,5
DS	1,7	1,4	nd	1,0	nd	1,5

Results : WNV distribution in tissues



Some conclusions

- 
1. The adaptation of WNV in different cells lines induced variation in virus virulence, in the mouse model
 2. mortality animals inoculated with WNV_L1_Verop20, WNV_L1_C6/36p20 and WNV_L2_C6/36p20, were very high similar to the unpassed control strains
 3. Serial passages in DEF cells completely attenuated the virus virulence.
 4. Analysis of the viral sequences is ongoing to identify genetic factors associated to WNV virulence in mice



Acknowledgment



Diagnosis and surveillance of viral diseases Unit

Chiara Pinoni
Andrea Polci
Letizia Di Biase
Fabrizia Valleriani
Elisa Mancuso
Elisabetta Di Felice
Andrea Capobianco Dondona

Federica Monaco

National Reference Center for Whole Genome Sequencing of microbial pathogens

Massimiliano Orsini
Maurilia Marcacci

Cesare Cammà

