



UNIVERSITÀ  
DEGLI STUDI  
DI TERAMO



IZSAM G. CAPORALE  
TERAMO

# Interaction of West Nile and Usutu viruses in a murine model

I risultati della ricerca corrente condotta dall'Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise - anno 2020

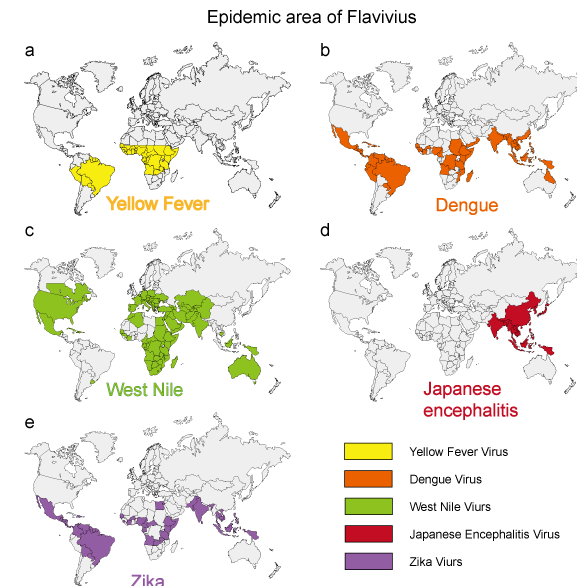
Lucija Jurišić,  
June 17, 2021, Teramo, Italy

N° Project/year	IZS AM 04/18 RC
Responsible researcher	Dr. Alessio Lorusso



# Flavivirus genus

- Dengue virus
- Zika virus
- **West Nile virus (WNV)**
- **Usutu virus (USUV)**



- Clinical neuro-invasive USUV infections in humans
- Two the most widespread mosquito-borne flaviviruses in Italy
- Co-circulation in the same geographical areas



Original article

Usutu virus infections in humans: a retrospective analysis in the municipality of Modena, Italy

A. Grotola<sup>1,2</sup>, M. Marcacci<sup>4</sup>, S. Tagliacucchi<sup>2</sup>, W. Gennari<sup>2</sup>, A. Di Gennaro<sup>4</sup>, M. Orsini<sup>4</sup>, F. Monaco<sup>4</sup>, P. Marchegiano<sup>3</sup>, V. Marini<sup>4</sup>, M. Meacci<sup>2</sup>, F. Rumpianesi<sup>2</sup>, A. Lorusso<sup>4</sup>, M. Pecorari<sup>2</sup>, G. Savini<sup>4,\*</sup>

<sup>1</sup> Dipartimento di Medicina Diagnostica, Clinica e di Sanità pubblica, Università di Modena e Reggio Emilia, Modena, Italy

<sup>2</sup> S.C. Microbiologia e Virologia, Modena, Italy

<sup>3</sup> Servizio di Prevenzione e Protezione, Azienda Ospedaliera, Università Policlinica, Modena, Italy

<sup>4</sup> UE Reference Laboratory for West Nile Fever, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise, Teramo, Italy

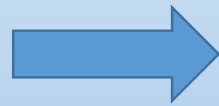
Source: <https://www.sciencedirect.com/science/article/pii/S1198743X16304256?via%3Dihub>

# Flavivirus virulence in murine model

**1. Comparative analysis of USUV and WNV strains  
in CD1 mice**

**2. Genome manipulation by reverse genetics**

**3. Virulence factors ( E protein and 5'UTR)**

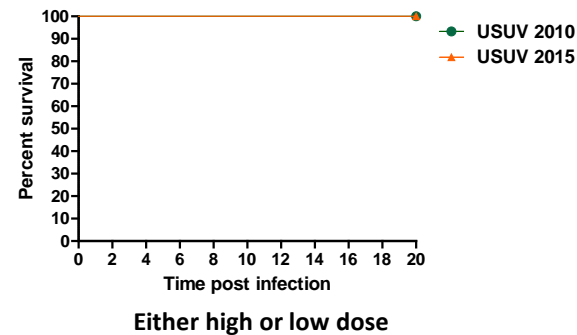
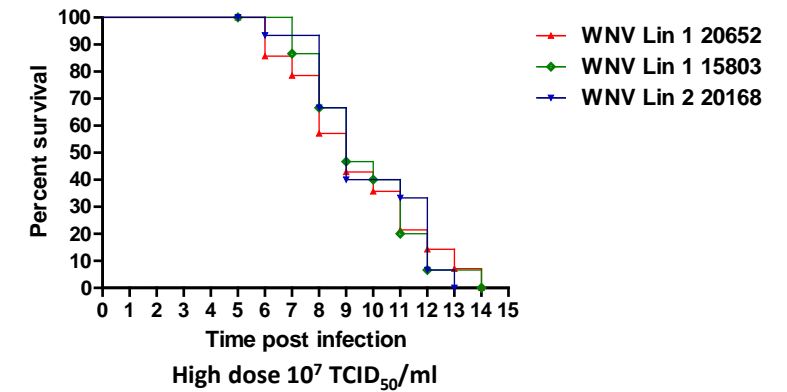
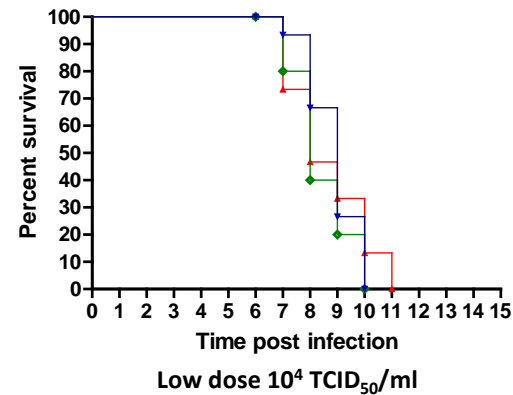


**4. Sequential infections with USUV and WNV**

**5. Role of pre-existing immunity**

## 1. Comparative analysis of USUV and WNV strains in CD1 mice

- Intra-peritoneal infection
- High or low dose of WNV (3 isolates) and USUV (2 isolates)
- qPCR from several tissues

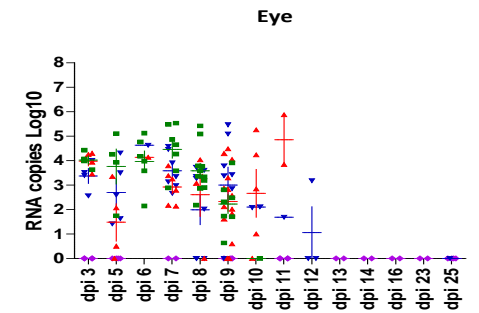
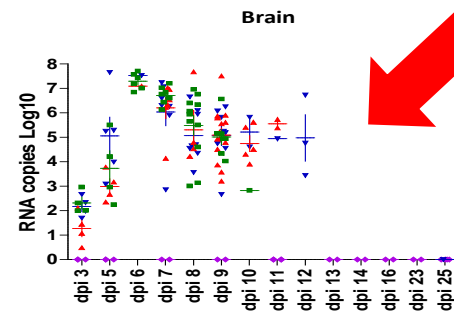
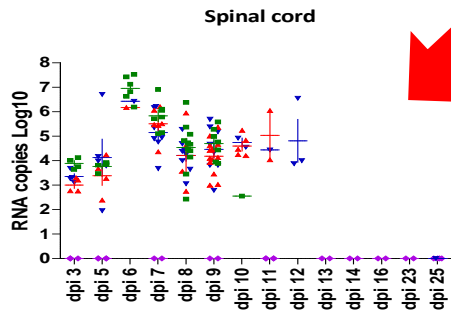
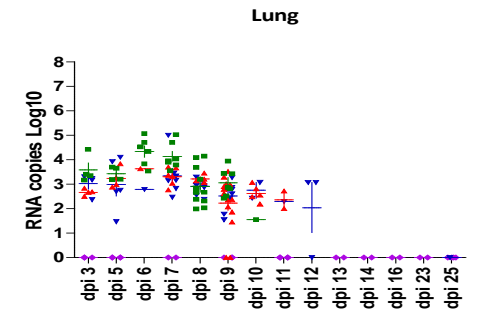
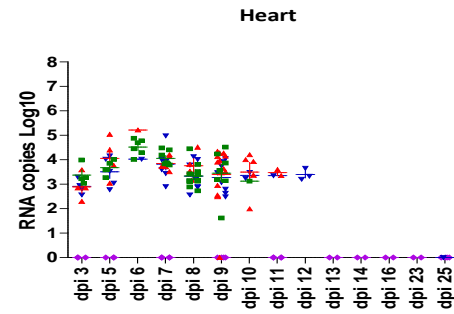
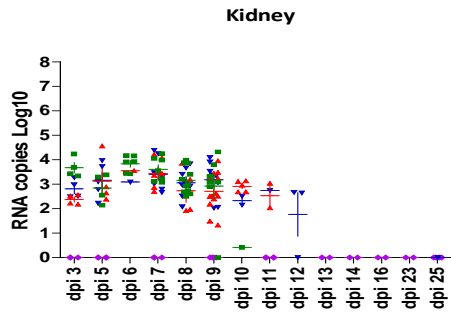
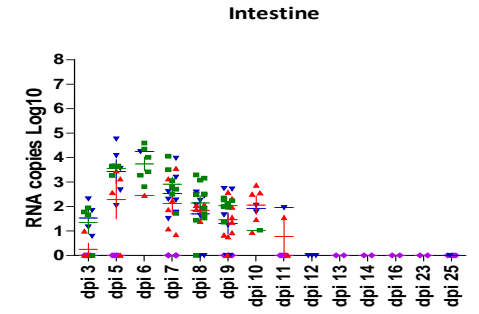
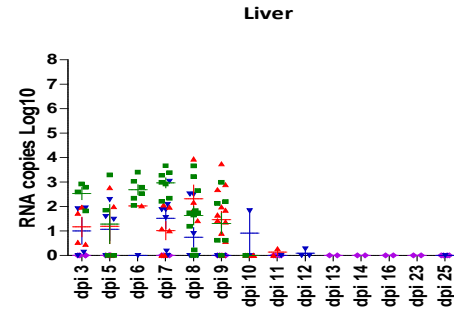
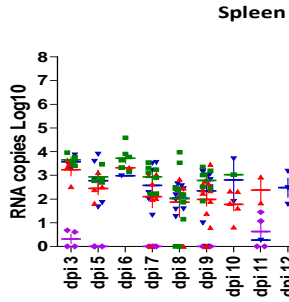


# HIGH WNV RNA TITRES IN SPINAL CORD AND BRAIN. NO USUV RNA in organs



WNV L1 15803  
WNV L2 20168  
WNV L1 20652 Livenza  
USUV 2010

WNV L1 15803  
WNV L2 20168  
WNV L1 20652 Livenza  
USUV 2010

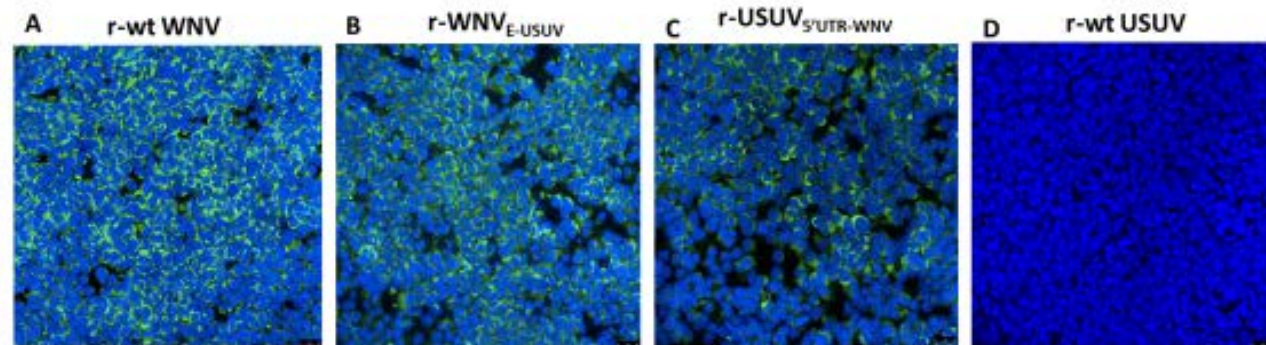


## 2. Genome manipulation by reverse genetics



95, 2462–2467

DOI 10.1099/vir.0.068023-0



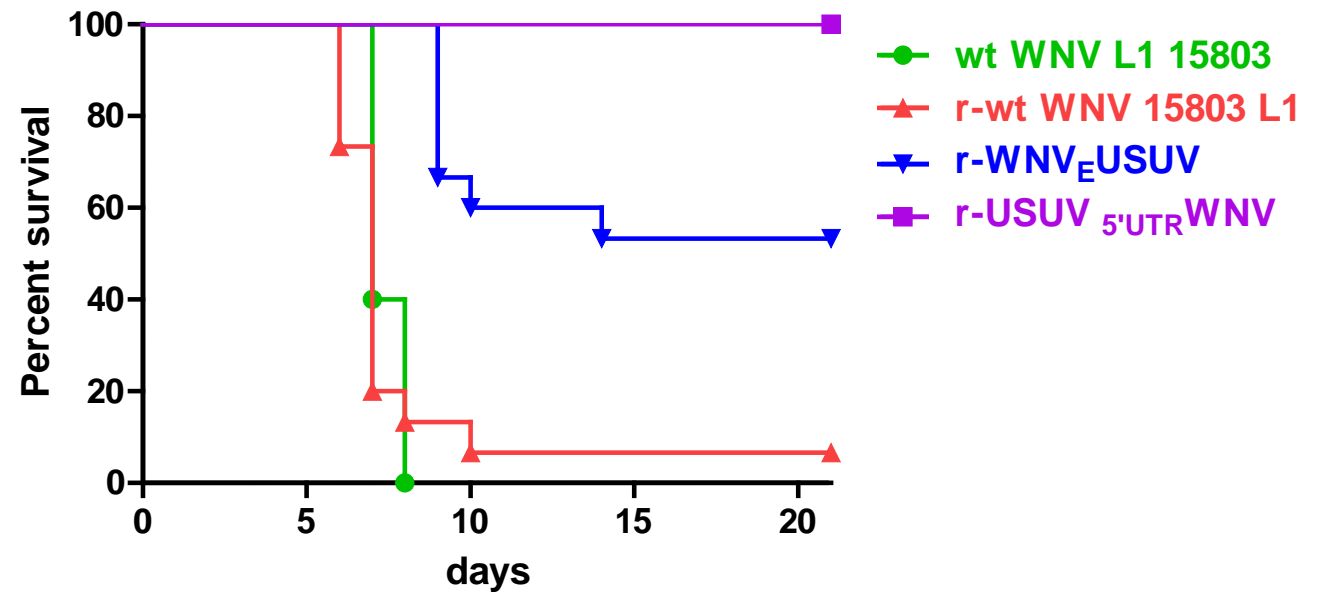
Single-stranded positive-sense RNA viruses generated in days using infectious subgenomic amplicons

Fabien Aubry, Antoine Nougairède, Lauriane de Fabritus, Gilles Querat, Ernest A. Gould and Xavier de Lamballerie

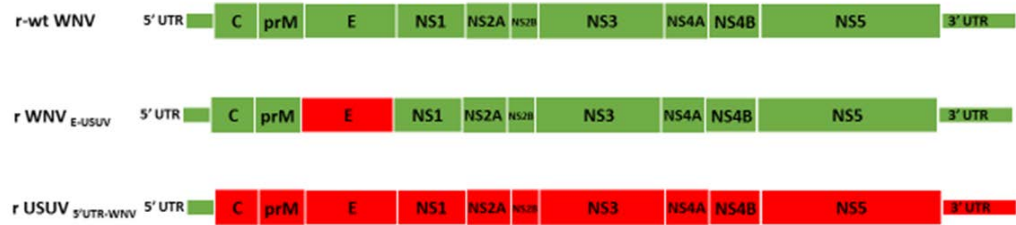
Aix Marseille Université, IRD French Institute of Research for Development, EHESP French School of Public Health, EPV UMR\_D 190 'Emergence des Pathologies Virales', 13385 Marseille, France

## 3. Virulence factors ( E protein and 5'UTR)

- Intraperitoneal infection
- Recombinant virus
- qPCR from several tissues



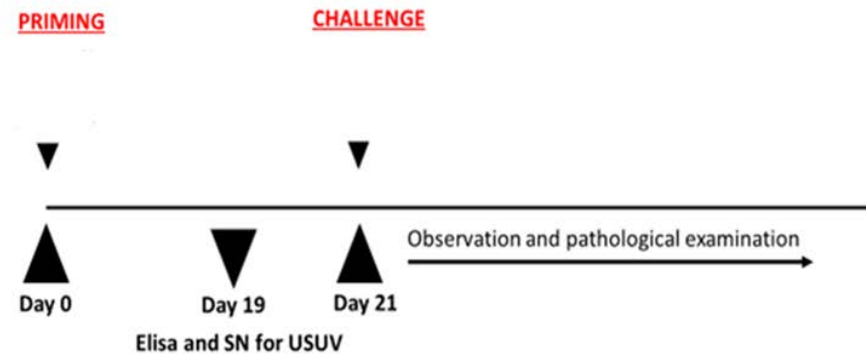
Source: [https://www.123rf.com/photo\\_87892048\\_white-laboratory-mouse-in-a-glass-jar.html](https://www.123rf.com/photo_87892048_white-laboratory-mouse-in-a-glass-jar.html)





## 4. Sequential infections with USUV and WNV in CD1 mice

- Intra-peritoneal infection
- Either low or high dose of USUV
- Challenge with either low or high dose of WNV
- qPCR from several tissues



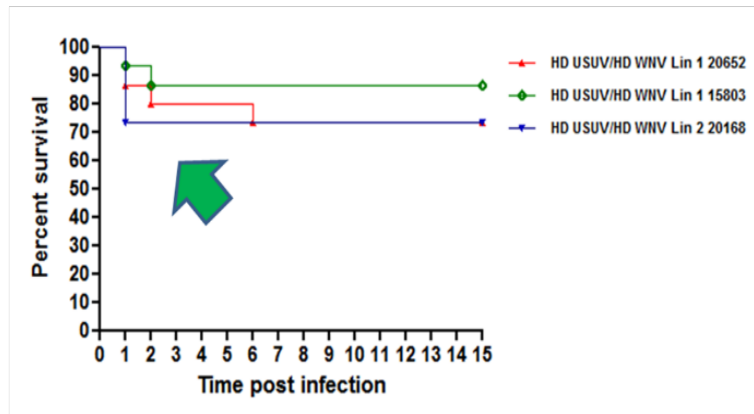


# Sequential infections with in CD1 mice: USUV HD/ WNV HD

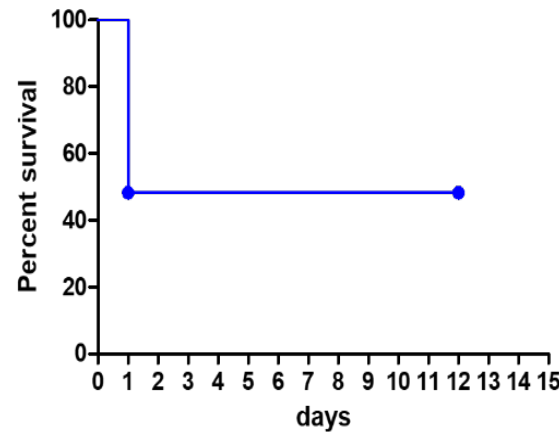
## Survival curves and microscopic lesions

HD USUV 2010 + challenge HD WNV, 21 dp USUV infection

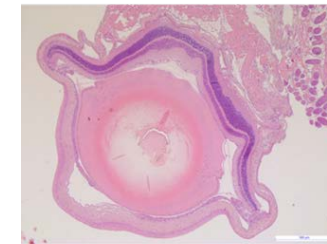
HD USUV 2010 + challenge HD WNV L2, 21 dp USUV infection



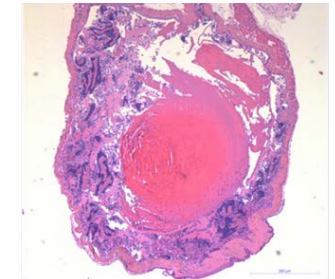
First experiment



Second experiment



Control, 6 dpi WNV  
normal structure



USUV + WNV, 1 dpi  
Abnormal structure, immune-mediated uveitis?

- More than the 50% of animals succumbed day 1 postchallenge

- Bleeding, abnormal histopathological lesions (eye)
- Absence (or very low titres) of WNV RNA in the internal organs

# Sequential infections with USUV and WNV/ BTV/ MHV in 2 mice lines

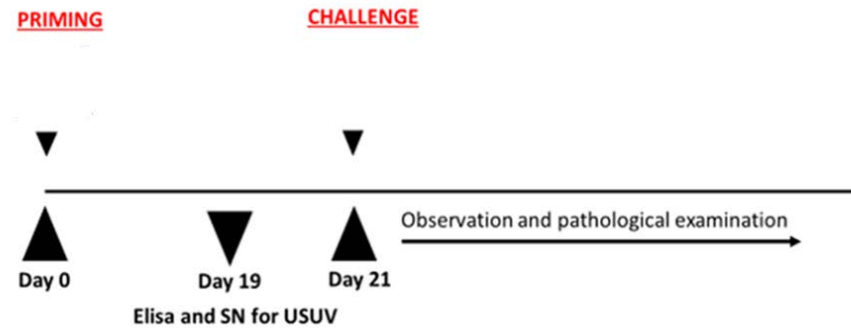
## ➤ USUV/ WNV-Lin2



## ➤ USUV/ BTV-1



## ➤ USUV/ MHV-A59



CD1 mice, Charles River Inc.  
Source: <https://www.criver.com/products-services/find-model/cd1-elite-sopf-mouse?region=27>

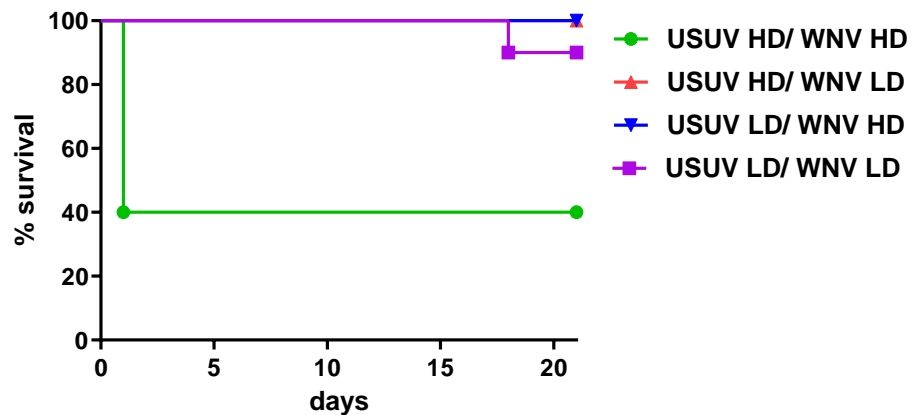


BALB/c mice, Charles River Inc.  
Source: <https://www.criver.com/products-services/find-model/balbc-mouse?region=27>

# Sequential infections with USUV and WNV

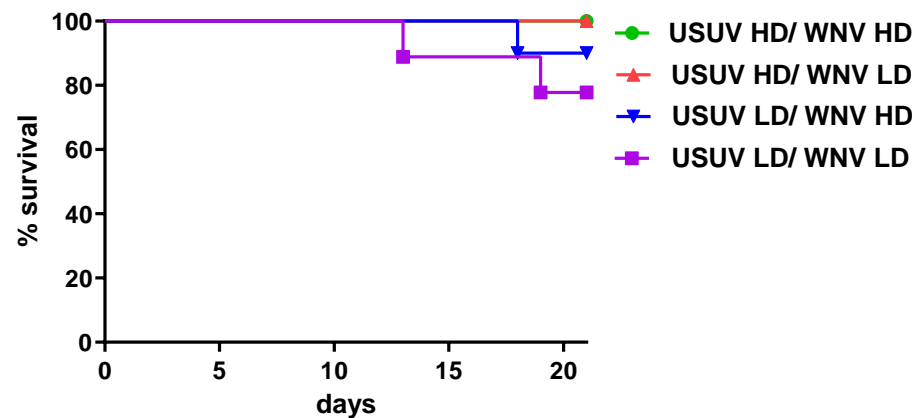
## Survival curves – differences in mice line

CD1 mice



Source: <https://www.criver.com/products-services/find-model/cd1-elite-sopf-mouse?region=27>

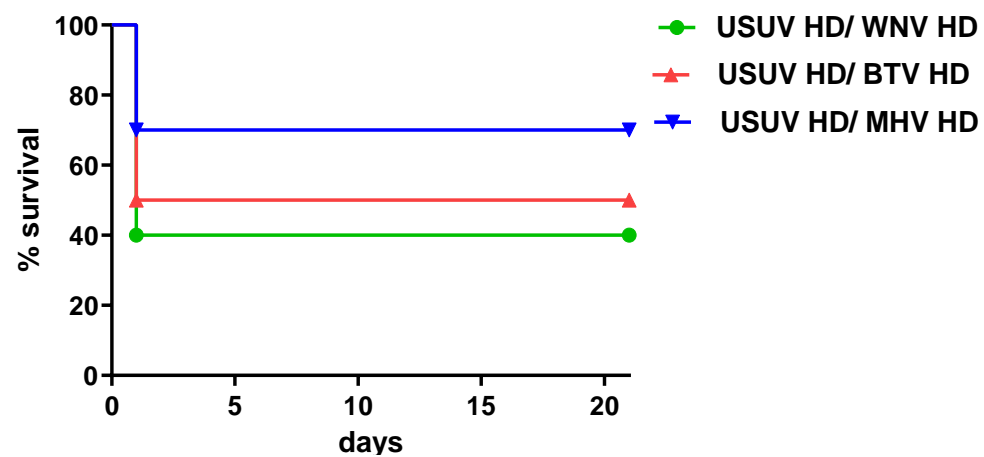
Balb/c mice



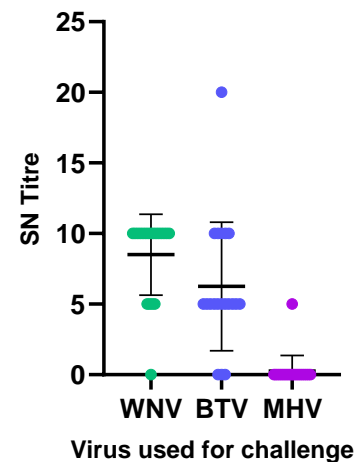
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# Sequential infections with USUV and WNV HD/ HD trials in CD1 mice

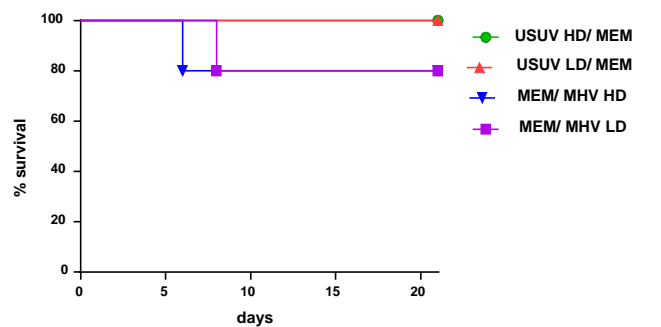
CD1 mice



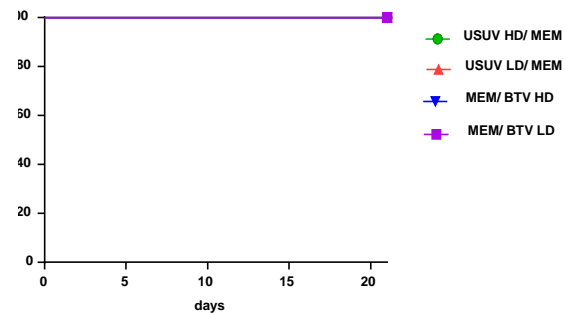
CD1 USUV HD SN titres before challenge



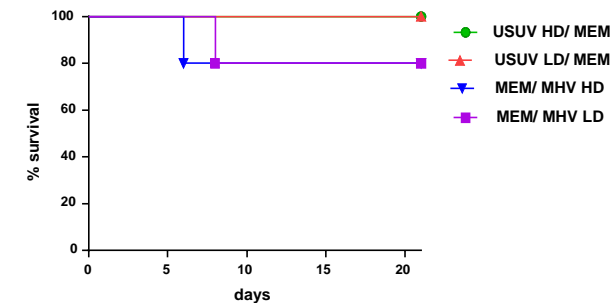
CD1 Control



CD1 Control

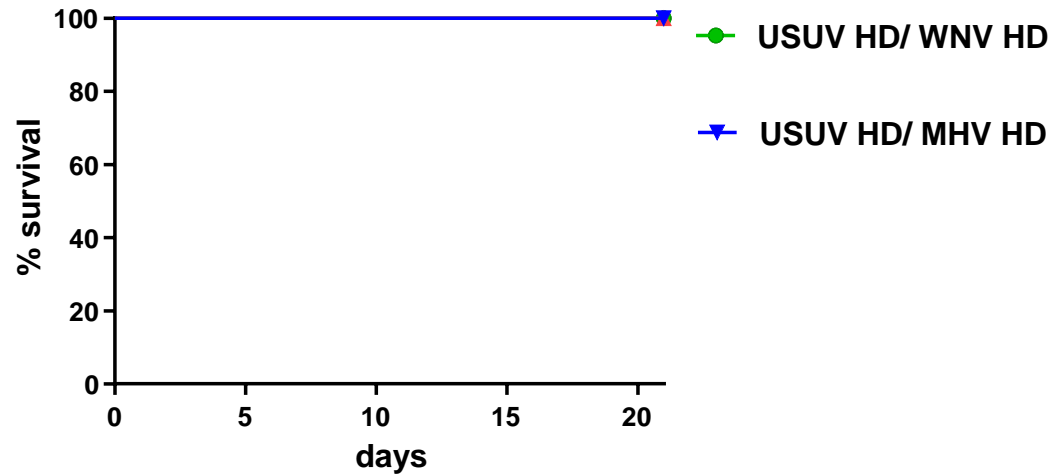


CD1 Control

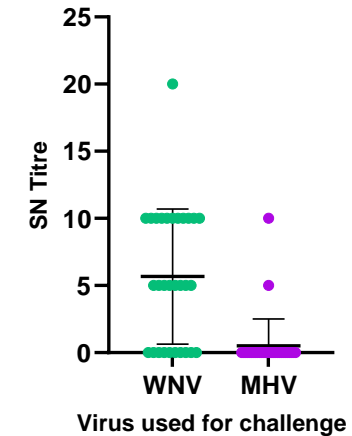


# Sequential infections with USUV and WNV HD/ HD trials in BALB/C mice

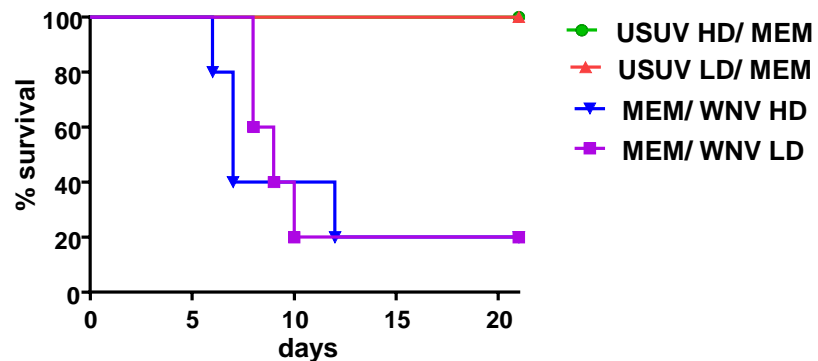
Balb/c mice



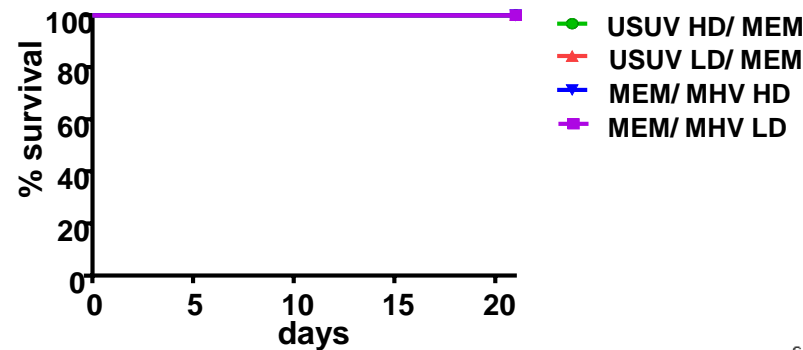
Balbc USUV HD SN titres before challenge



Balb/c Control



Balb/c Control

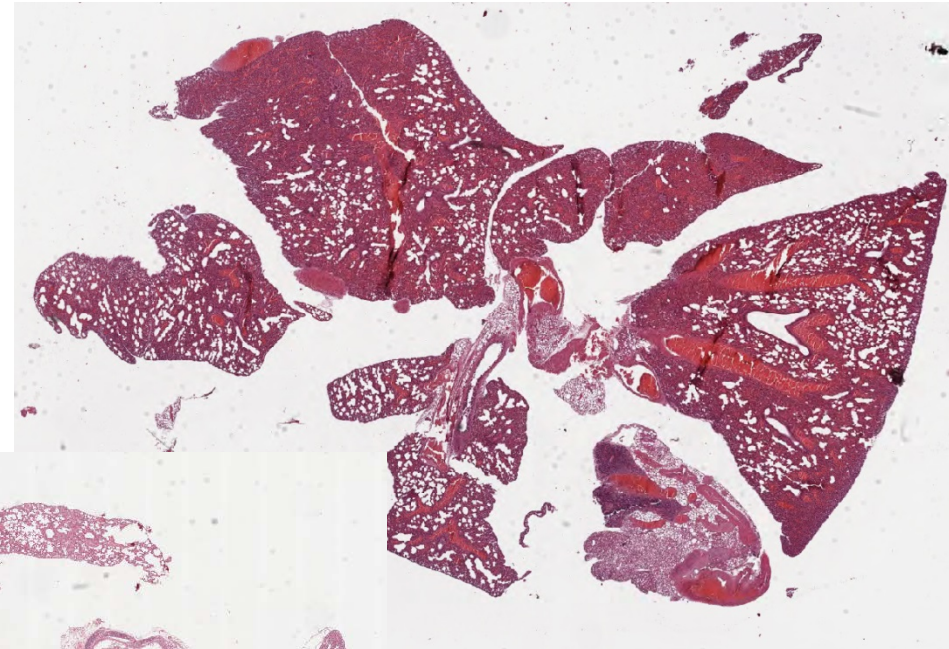
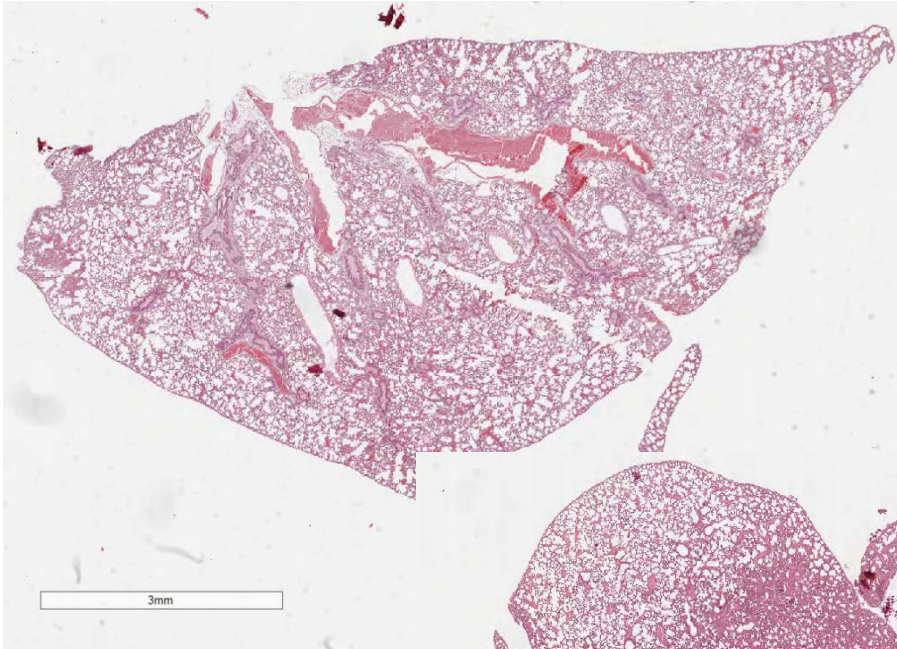




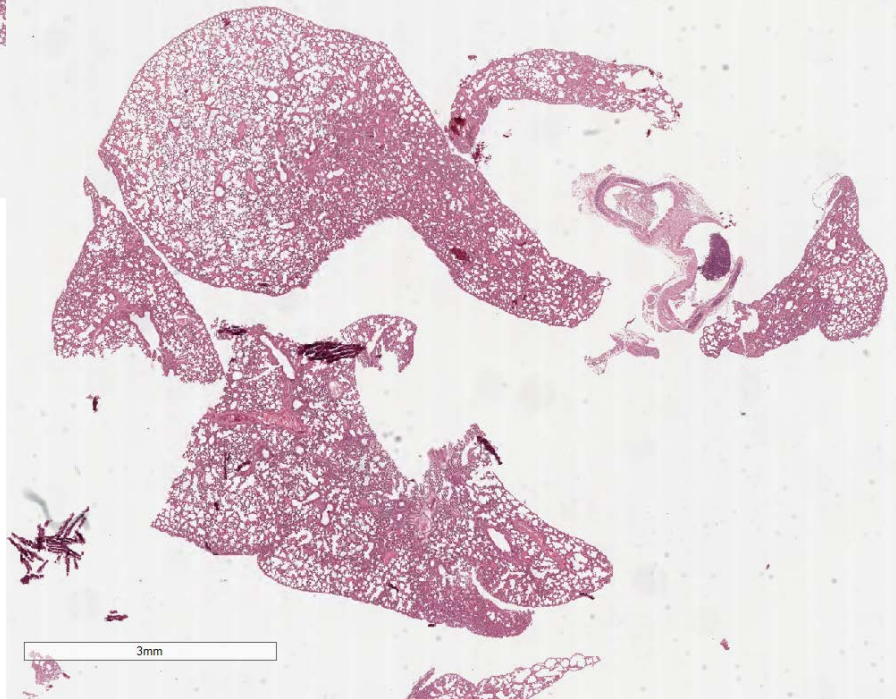
# MICROSCOPICAL FINDINGS HD/ HD trials in CD1 mice

## Lung tissue

Lung tissue  
CD1 mice that  
survived  
USUV HD/ WNV HD  
trial



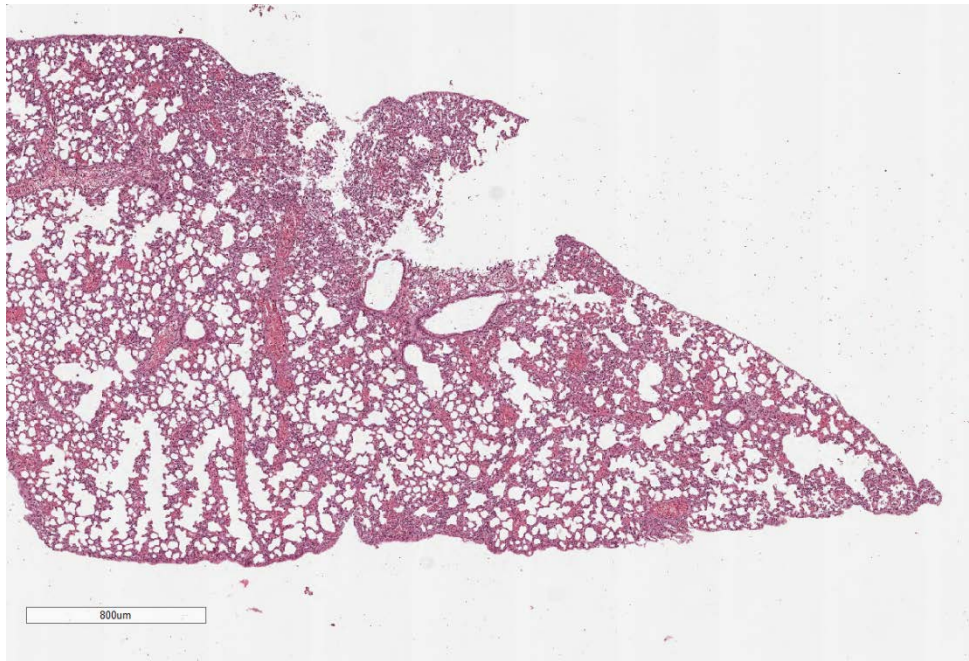
Lung tissue  
CD1 mice that died on  
day 1 postchallenge  
of USUV HD/ WNV HD  
trial



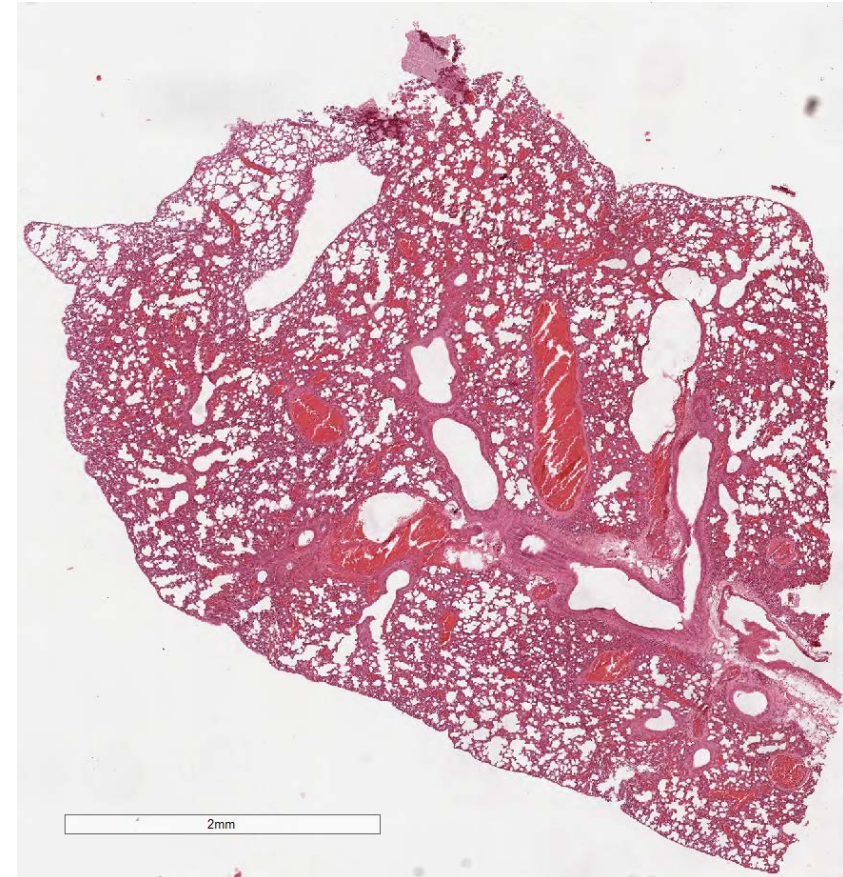
Lung tissue  
CD1 mice that died on day 1  
postchallenge of  
USUV HD/ BTV HD trial



# Histology HD/ HD trial in CD1 mice lung tissue



Lung tissue  
CD1 mice that survived USUV HD/ MHV HD trial



Lung tissue  
CD1 mice that died on day 1 postchallenge of USUV HD/ MHV HD trial



# Flavivirus virulence in murine model

- **Inflammatory factors**
- **Differences in immune response in CD1 and BALB/c mice**

## 4. Role of pre-existing immunity



# ACKNOWLEDGMENTS



**Guendalina  
Zaccaria**



**Shadia Berjaoui, Ilaria Puglia**

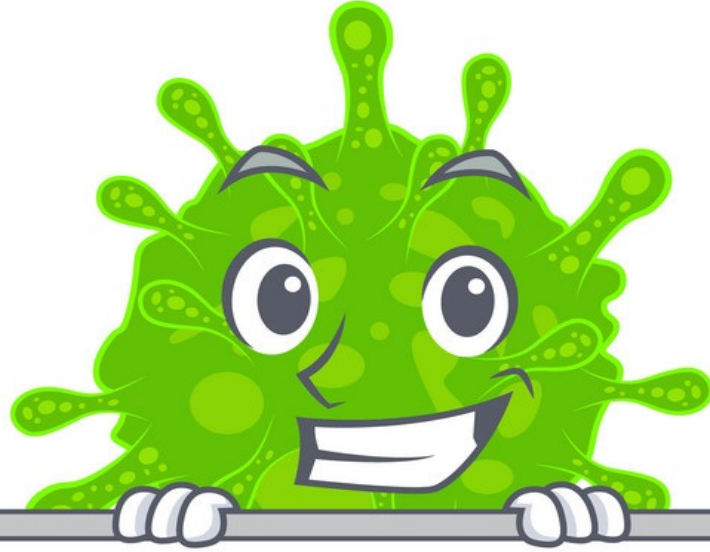


**Daniela Malatesta, Giovanni Di Teodoro**



**Alessio Lorusso**

- ✓ **Virologia e Colture Cellulari, IZSAM**
- ✓ **Diagnostica Specialistica, IZSAM**
- ✓ **Centro di Riferenza Nazionale per Sequenze Genomiche di microrganismi patogeni, IZSAM**



**THANK YOU  
FOR YOUR  
ATTENTION**