

Incontro sulla West Nile disease – Brescia, 20 maggio 2015



# West Nile Disease

Sorveglianza entomologica: l'attività del Reparto di Virologia

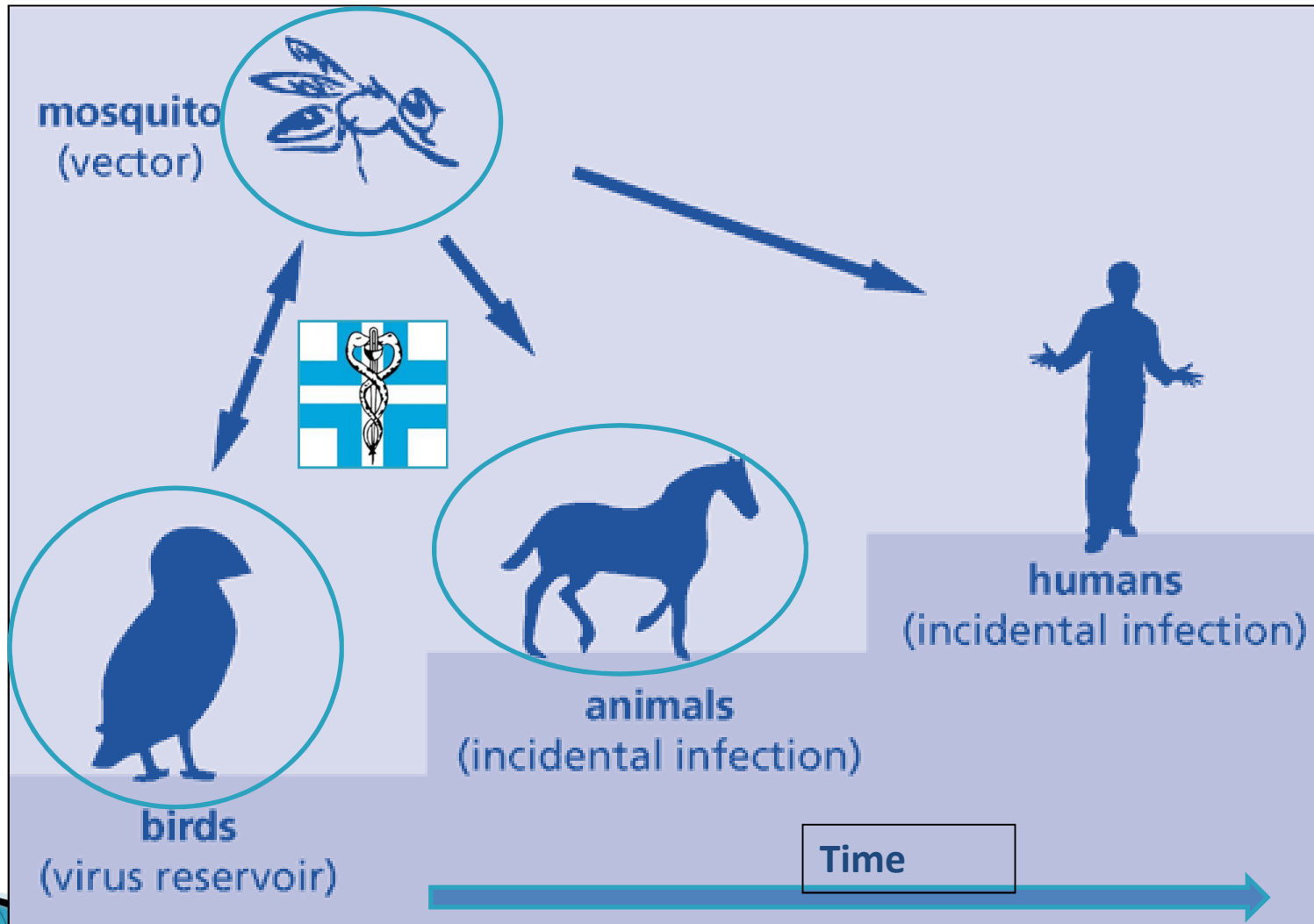
Davide Lelli



IZSLER, Istituto Zooprofilattico Sperimentale  
della Lombardia e dell'Emilia Romagna

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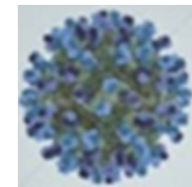
# Sorveglianza veterinaria



# Obiettivi della sorveglianza entomologica

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- 1. Identificare precocemente le aree con circolazione virale** per attivare la sorveglianza sull'uomo e le misure di prevenzione sulla popolazione
2. Conoscenza delle specie presenti sul territorio
3. Densità assolute e relative
4. Tasso d'infezione
5. Introduzione di nuove specie di vettori o patogeni
6. Caratterizzazione genomica dei virus identificati
7. Overwintering



Special Conference on West Nile Virus  
*Sorveglianza e prevenzione della trasmissione trasfusionale*

Roma, 27 marzo 2015

## Tempi medi tra raccolta campione entomologico e esito

### Tempi medi di risposta - zanzare

|                       |                     |
|-----------------------|---------------------|
| Veneto                | 7,2 gg (range 3-13) |
| Friuli Venezia Giulia | 7,2 gg (range 3-13) |
| Emilia-Romagna        | 6,3 gg (range 1-13) |
| Piemonte              | 7,3 gg (range 4-17) |
| Lombardia             | 5,67 gg (range 2-8) |



# WNV – Attività diagnostica

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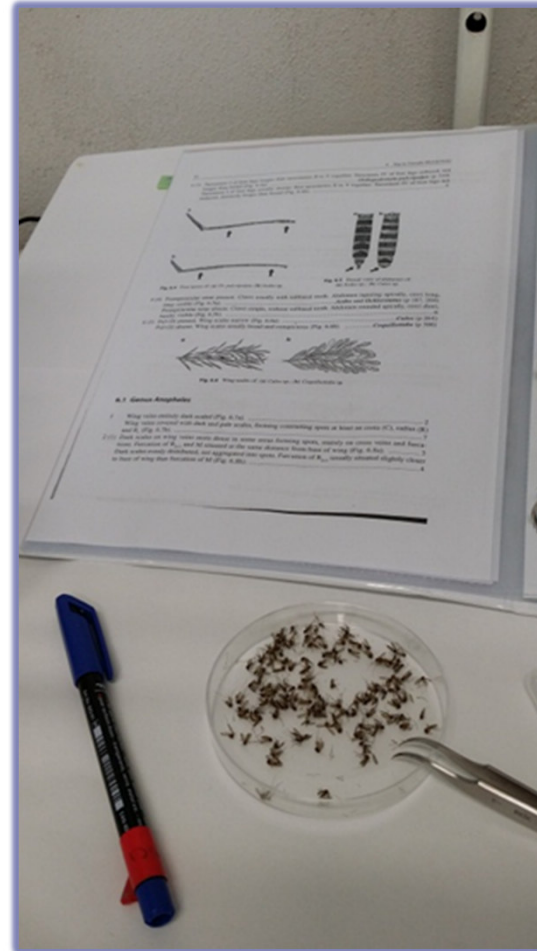
# Sorveglianza entomologica

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# Sorveglianza entomologica

## Identificazione morfologica

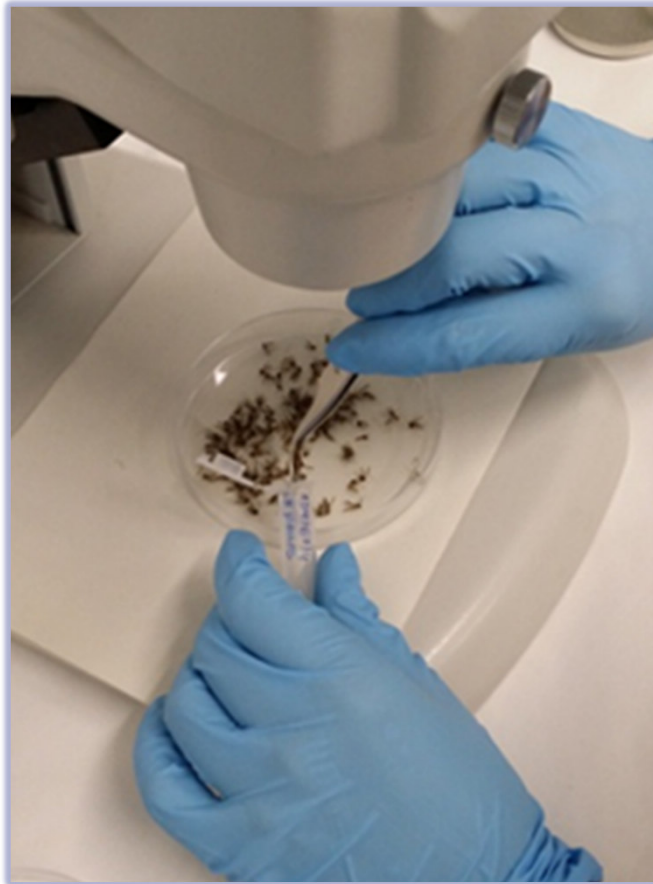




# Sorveglianza entomologica

## Preparazione del campione

allestimento dei pool e preparazione dell'omogenato



| N zanzare | PBS $\mu$ L |
|-----------|-------------|
| 1-30      | 500         |
| 31-60     | 900         |
| 61-100    | 1500        |
| 101-200   | 3000        |

Centrifugare a 3500 r.p.m. per 15 minuti a  $+5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ .



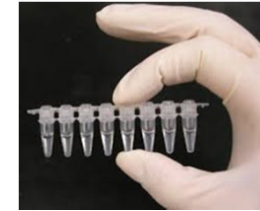


# Ricerca virale

## - Estrazione RNA e retrotrascrizione

## - PCR

- WNV NC 3' (Tang *et al.*, 2006. *J Clin Virol* 36:177–182)
- USUV NS5 gene (Cavrini *et al.*, 2011. *J Clin Virol* 50:221–223)
- Flivirus NS5 gene (Scaramozzino *et al.*, 2001)
- WNV NC 3' (Del Amo *et al.* 2013. *J Virol Methods*, 189:321– 327)



Highly sensitive TaqMan RT-PCR assay for detection and quantification of both lineages of West Nile virus RNA

Yanlin Tang\*, C. Anne Hapip, Bin Liu, Chyang T. Fang

*Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross,  
15601 Crabbs Branch Way, Rockville, MD 20855, USA*

A rapid and specific real-time RT-PCR assay to identify Usutu virus in human plasma, serum, and cerebrospinal fluid

Francesca Cavrini<sup>a</sup>, Maria Elena Della Pepa<sup>b</sup>, Paolo Gaibani<sup>a</sup>, Anna Maria Pierro<sup>a</sup>, Giada Rossini<sup>a</sup>, Maria Paola Landini<sup>b</sup>, Vittorio Sambri<sup>a,b,\*</sup>

<sup>a</sup> Unit of Clinical Microbiology, Regional Reference Centre for Microbiological Emergencies (CRREM), St. Orsola Malpighi University Hospital, University of Bologna, Via G. Massarenti 9, 40138 Bologna, Italy

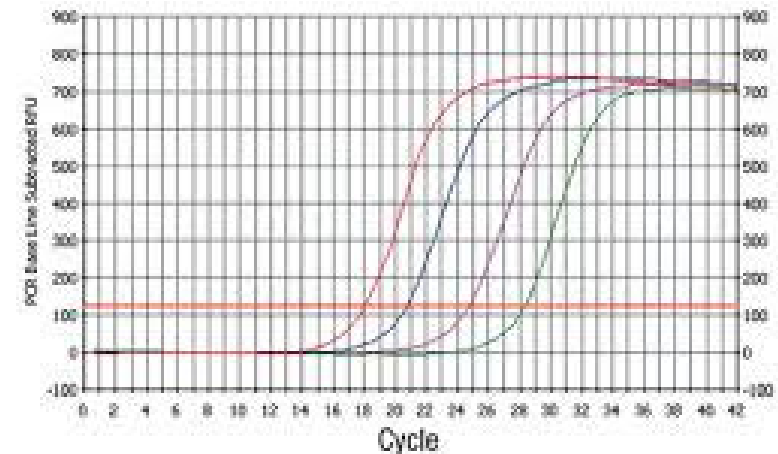
<sup>b</sup> Department of Haematology and Oncology, Section of Microbiology, St. Orsola Malpighi University Hospital, University of Bologna, Via G. Massarenti 9, 40138 Bologna, Italy

A novel quantitative multiplex real-time RT-PCR for the simultaneous detection and differentiation of West Nile virus lineages 1 and 2, and of Usutu virus

Javier Del Amo<sup>a</sup>, Elena Sotelo<sup>a</sup>, Jovita Fernández-Pinero<sup>a</sup>, Carmina Gallardo<sup>a</sup>, Francisco Lorente<sup>a</sup>, Montserrat Agüero<sup>b</sup>, Miguel Angel Jiménez-Clavero<sup>a,\*</sup>

<sup>a</sup> Centro de Investigación en Sanidad Animal, CISA (INIA), Valdeolmos, Spain

<sup>b</sup> Laboratorio Central de Veterinaria, MAGRAMA, Algete, Spain



# WNV – Attività diagnostica

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Conferme positività da parte del CESME





 **CESME**  
Centro di Referenza Nazionale

# CIRCUITO INTER-LABORATORIO

## WEST NILE DISEASE RT-PCR

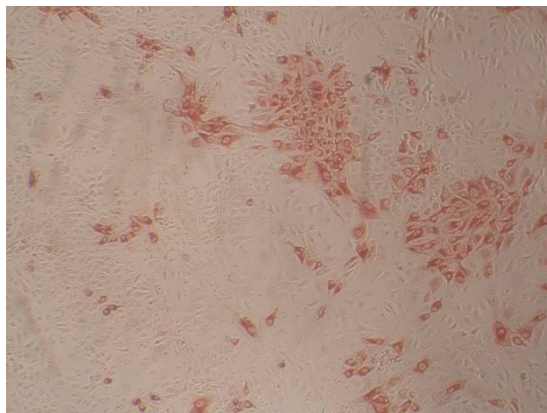
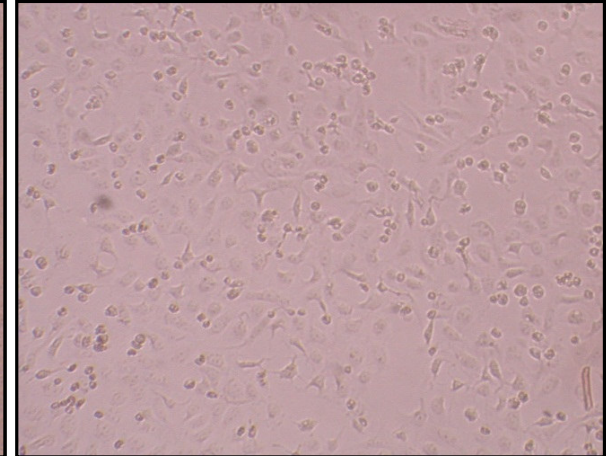
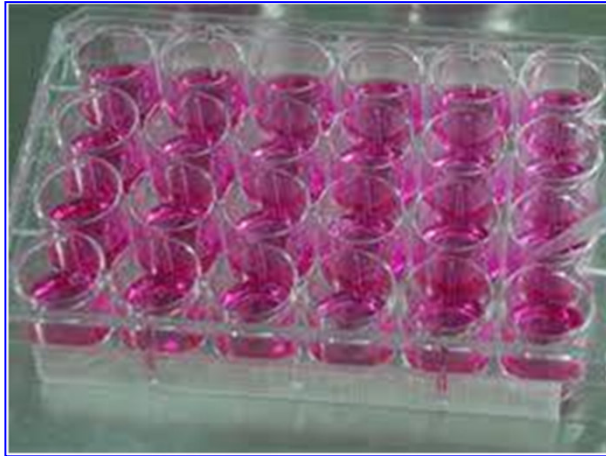
### Distribuzione 1/2014: ceppi virali e campioni

| N° campione | Virus/Ceppo         | Titolo (TCID <sub>50</sub> /ml)         |
|-------------|---------------------|---|
| 1           | WNV L2              | 10 <sup>6.49</sup>                      |
| 2           | WNV L2              | 10 <sup>5.49</sup>                      |
| 3           | WNV L2              | 10 <sup>4.49</sup>                      |
| 4           | WNV L1              | 10 <sup>5.88</sup>                      |
| 5           | WNV L1              | 10 <sup>4.88</sup>                      |
| 6           | WNV L1              | 10 <sup>3.88</sup>                      |
| 7           | USUV 939            | 10 <sup>3.22</sup>                      |
| 8           | USUV 939            | 10 <sup>2.22</sup>                      |
| 9           | WNV B956            | 10 <sup>3.61</sup>                      |
| 10          | WNV B956            | 10 <sup>2.61</sup>                      |
| 11          | WNV E101            | 10 <sup>5.14</sup>                      |
| 12          | WNV E101            | 10 <sup>4.14</sup>                      |
| 13          | WNV L1 - WNV L2     | 10 <sup>3.88</sup> - 10 <sup>4.49</sup> |
| 14          | WNV L1 - WNV L2     | 10 <sup>3.88</sup> - 10 <sup>4.49</sup> |
| 15          | WNV B956 - USUV 939 | 10 <sup>2.61</sup> - 10 <sup>2.22</sup> |
| 16          | WNV E101 - USUV 939 | 10 <sup>4.14</sup> - 10 <sup>2.22</sup> |



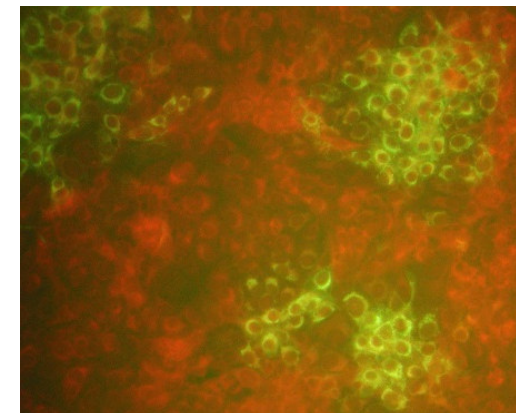
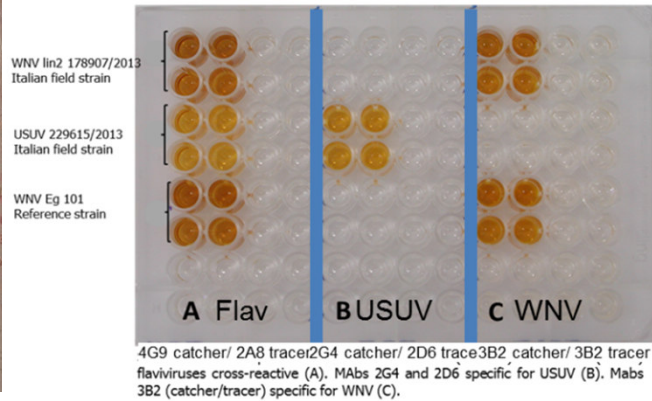


# Isolamento virale



Immunoperossidasi

Sandwich ELISA for WNV/USUV antigen detection



Immunofluorescenza



# WNV isolati IZSLER 2008-2014

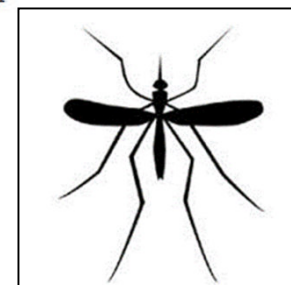
| WNV isolation: IZSLER 2008-2014 |                   |                   |         |                                   |                             |
|---------------------------------|-------------------|-------------------|---------|-----------------------------------|-----------------------------|
| N°                              | Year of isolation | Viral strains     | Lineage | Species                           | Place of origin             |
| 1                               | 2008              | WNV 203204/2008   | 1       | Magpie (Pica pica)                | Portomaggiore (FE)          |
| 2                               | 2008              | WNV 229892/2008   | 1       | Magpie (Pica pica)                | Voghiera (FE)               |
| 3                               | 2008              | WNV 242853/2008   | 1       | Jay (Garrulus glandarius)         | Mirandola (MO)              |
| 4                               | 2009              | WNV 204146/2009   | 1       | Crow (Corvus cornix)              | Codigoro (FE)               |
| 5                               | 2009              | WNV 205417/2009   | 1       | Horse (blood)                     | Roncoferraro (MN)           |
| 6                               | 2009              | WNV 208659/2009   | 1       | Horse (brain)                     | Noceto (PR)                 |
| 7                               | 2009              | WNV 208846/2009   | 1       | Magpie (Pica pica)                | Luzzara (RE)                |
| 8                               | 2009              | WNV 233184/2009   | 1       | Seagulls (Larus ridibundus)       | Codigoro (FE)               |
| 9                               | 2009              | WNV 225677/2009   | 1       | Jay (Garrulus glandarius)         | Ferrara                     |
| 10                              | 2009              | WNV 204913/2009   | 1       | <b>Mosquitoes (Culex pipiens)</b> | Montecchio Emilia (RE)      |
| 11                              | 2013              | WNV 178907/2013   | 2       | <b>Mosquitoes (Culex pipiens)</b> | Soresina (CR)               |
| 12                              | 2013              | WNV 205087/2013   | 2       | Crow (Corvus cornix)              | Cento (FE)                  |
| 13                              | 2013              | WNV 210912-1/2013 | 2       | Magpie (Pica pica)                | Castel Franco E. (MO)       |
| 14                              | 2013              | WNV 218203/2013   | 2       | <b>Mosquitoes (Culex pipiens)</b> | Parma                       |
| 15                              | 2013              | WNV 223278-3/2013 | 2       | Crow (Corvus cornix)              | Rivolta d'Adda (CR)         |
| 16                              | 2013              | WNV 230043/2013   | 2       | <b>Mosquitoes (Culex pipiens)</b> | Remedello (BS)              |
| 17                              | 2013              | WNV 244558/2013   | 2       | <b>Mosquitoes (Culex pipiens)</b> | Leno (BS)                   |
| 18                              | 2013              | WNV 225284/2013   | 2       | Upupa (Upupa epops)               | Ferrara                     |
| 19                              | 2013              | WNV 225303/2013   | 2       | Jay (Garrulus glandarius)         | Ferrara                     |
| 20                              | 2013              | WNV 225301/2013   | 2       | Blackbird (Turdus merula)         | Ferrara                     |
| 21                              | 2013              | WNV 231763/2013   | 2       | Magpie (Pica pica)                | Poggio Renatico (BS)        |
| 22                              | 2013              | WNV 225263/2013   | 2       | Assiolo (Otus scops)              | Ferrara                     |
| 23                              | 2013              | WNV 251643-1/2013 | 2       | <b>Mosquitoes (Culex pipiens)</b> | San Giovanni del Dosso (MN) |
| 24                              | 2013              | WNV 253901-9/2013 | 2       | Magpie (Pica pica)                | Novellara (RE)              |
| 25                              | 2014              | WNV 191997/4      | 2       | <b>Mosquitoes (Culex pipiens)</b> | Borgetto Lodigiano (LO)     |
| 26                              | 2014              | WNV 203929/6      | 2       | <b>Mosquitoes (Culex pipiens)</b> | Borgetto Lodigiano (LO)     |
| 27                              | 2014              | WNV 192173/1      | 2       | Magpie (Pica pica)                | Guastalla (RE)              |
| 28                              | 2014              | WNV 221558        | 2       | Crow (Corvus cornix)              | Milano                      |
| 29                              | 2014              | WNV 214495/5      | 2       | <b>Mosquitoes (Culex pipiens)</b> | Soresina (CR)               |
| 30                              | 2014              | WNV 221996/10     | 2       | Magpie (Pica pica)                | San Cesario sul Panaro (MO) |





Table 1. Number of collected (N) & tested mosquitoes (Nt), and number of pools (Np), for mosquito species with WNV and USUV positive pools.

| Species                        | N (%)        | Nt/Np        | Wnv+ | Usuv+ |
|--------------------------------|--------------|--------------|------|-------|
| <i>Ae. cinereus/geminus</i>    | 111(<0.1)    | 95/6         |      |       |
| <i>Ae. (Och.) cantans</i>      | 171(<0.1)    | 74/26        |      |       |
| → <i>Ae. (Och.) caspius</i>    | 44,108(5.8)  | 31,842/844   | 1    | 2     |
| <i>Ae. (Och.) detritus</i>     | 693(<0.1)    | 117/19       |      |       |
| → <i>Ae. (St.) albopictus</i>  | 4,659(0.6)   | 2,648/463    |      |       |
| <i>Ae. geniculatus</i>         | 214(<0.1)    | 130/9        |      |       |
| <i>Ae. (Hu.) koreicus</i>      | 26(<0.1)     | 6/5          |      |       |
| → <i>Ae. vexans</i>            | 14,436(1.8)  | 11,694/257   |      |       |
| <i>An. claviger/petragnani</i> | 16(<0.1)     | 10/8         |      |       |
| → <i>An. maculipennis s.l.</i> | 5,445(0.7)   | 2,704/156    |      |       |
| <i>An. plumbeus</i>            | 55(<0.1)     | 35/12        |      |       |
| <i>Cq. richiardii</i>          | 335(<0.1)    | 247/39       |      |       |
| <i>Cs. annulata</i>            | 246(<0.1)    | 203/82       |      |       |
| → <i>Cx. modestus</i>          | 2,231(0.3)   | 1,450/75     | 1    | 1     |
| → <i>Cx. pipiens</i>           | 684,660 (90) | 510,773/7256 | 176  | 238   |
| <i>Cx. territans</i>           | 42(<0.1)     | 39/5         |      |       |
| Other species§                 | 14(<0.1)     | 13/13        |      |       |
| Total                          | 757,461      | 562,079      |      |       |



97%

*Cx. pipiens*

*Och. caspius*

*Ae. vexans*

dati Nord 2013

Calzolari et al., subm

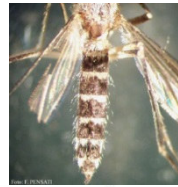




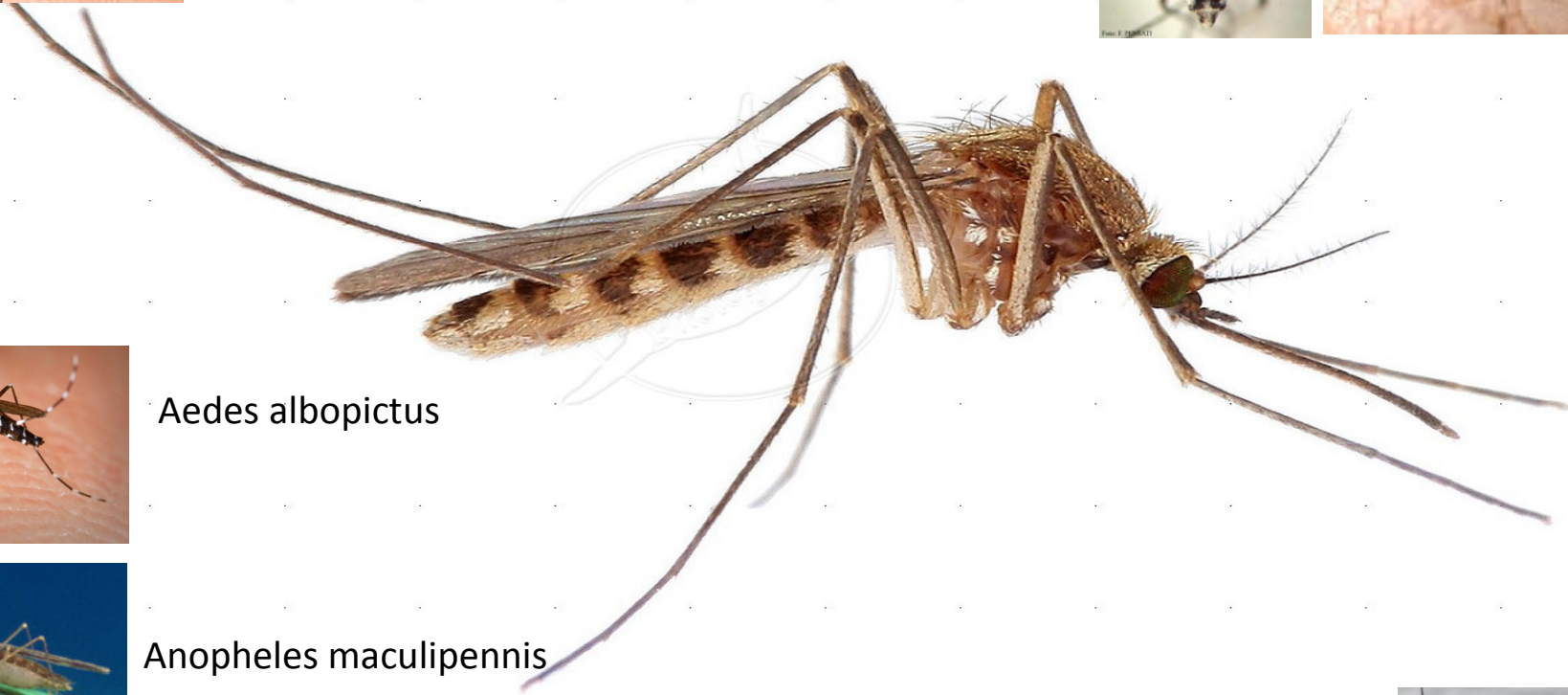
# Culex pipiens



Aedes vexans



Aedes (Ochlerotatus) caspius



Aedes albopictus



Anopheles maculipennis

Culex modestus



# Sorveglianza entomologica

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*Grazie per l'attenzione*





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# West Nile Disease

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