Mosquito based surveillance of Arboviruses in Northern Italy

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From 2010 to 2013 ECDC reported more than 600 human cases in Europe, in 2014 (until September) 55 human cases were reported.
Surveyed areas (I)
Since 2008, besides the activities supported by the Ministry of Health at national level, more comprehensive surveillance programs, including mosquito monitoring, are carried out at Regional level.
In 2013-2014 this surveillance was active in Emilia-Romagna (ER), Veneto (VEN), Friuli Venezia-Giulia (FVG) Lombardy (LOM), Piedmont (PIE) regions.
Surveyed areas (II)
Pianura Padana

Pianura Padana (or Pianura Padano-Veneta) is the wider plan of Italy (46,000 km$^2$)

It is an homogeneous territory with a characteristic morphology and hydrology

Pianura Padana is a densely populated area, i.e. 20 million inhabitants, 500 per km$^2$

Productive and urbanized areas are abundant, and environment is strongly influenced by human activity

The climate of this area is described as humid subtropical climate (Köppen climate classification)
In Northern Italy

- WNV was detected in 1998 at Padule di Fucecchio, Toscana (no Human cases)
- In Emilia-Romagna human cases were reported in 2008-2009 (Lineage I) and in 2013-2014 (Lineage II)
- In Veneto human cases were continuously reported in 2008-2014 period (Lineage I & II)
- In Lombardy human cases were reported in 2008 then in 2013-14
- In Friuli Venezia-Giulia in 2011-2012
- No human cases were detected in Piedmont
Usutu virus

Since 2009, both systems recorded the circulation of Usutu virus (USUV), a flavivirus closely related to WNV with a not yet defined pathogenic capacity.

Vectors: mosquitoes especially *Culex (Culex pipiens)*

Reservoirs: wild birds

Possible mass mortality in birds (blackbird)(Austria 2001).

WNV differential diagnosis (it can be cross-reactive also in PCR)

Human disease: 2 cases of encephalitis reported in immunosuppressed individuals in Italy in 2009 (Pecorari et al. 2009, Cavrini et al. 2009)

Anti-USUV response in healthy persons were recorded in ER, 4/359 in 2009; 14/6000 in 2010-11 (Gaibani et al. 2012, Pierro et al. 2013)
Surveillance targets

- Mosquitoes
- Birds, active and passive surveillance (mass mortality not recorded in Europe)
- Horses (not applicable after large vaccination campaigns)

Source: United States Centers for Disease Control and Prevention, US CDC
## Regional surveys features

<table>
<thead>
<tr>
<th>Region</th>
<th>Activity period</th>
<th>Stations</th>
<th>Sampling interval</th>
<th>Trap model</th>
<th>USUV_PCR</th>
<th>Max specimen s per pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emilia-Romagna</td>
<td>June-September</td>
<td>157*</td>
<td>2 weeks</td>
<td>CDC-CO2; GT</td>
<td>Yes</td>
<td>200</td>
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<tr>
<td>Lombardy</td>
<td>June-October</td>
<td>30</td>
<td>2 weeks</td>
<td>CDC-CO2</td>
<td>2013 Yes</td>
<td>200</td>
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<td></td>
<td></td>
<td>31</td>
<td></td>
<td></td>
<td>2014 No</td>
<td></td>
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<tr>
<td>Veneto</td>
<td>May-October</td>
<td>54</td>
<td>1 week</td>
<td>CDC-CO2</td>
<td>Yes</td>
<td>50</td>
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<tr>
<td>Friuli Venezia-Giulia</td>
<td>May-October</td>
<td>12</td>
<td>1 week</td>
<td>CDC-CO2</td>
<td>Yes</td>
<td>50</td>
</tr>
<tr>
<td>Piedmont</td>
<td>July-October</td>
<td>33</td>
<td>2 weeks</td>
<td>CDC-CO2; BG</td>
<td>Yes</td>
<td>50-200</td>
</tr>
</tbody>
</table>

286 204

* 52 sampled for 1-2 times

In 2014 in Emilia-Romagna and Lombardy only *Cx. pipiens* and *Cx. modestus* mosquitoes were tested
Sites for mosquito sampling in 2013/2014
## Sampled/Tested mosquitoes

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td><em>Ae. cinereus/geminus</em></td>
<td>111</td>
<td>95</td>
<td>6</td>
<td>0</td>
<td>111</td>
<td>95</td>
<td>6</td>
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<tr>
<td><em>Ae. rusticus</em></td>
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<td>2</td>
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<tr>
<td><em>Ae.(Och.)berlandi</em></td>
<td>1</td>
<td>1</td>
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<td>1</td>
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<tr>
<td><em>Ae.(Och.)cantans</em></td>
<td>171</td>
<td>74</td>
<td>26</td>
<td>11</td>
<td>182</td>
<td>74</td>
<td>26</td>
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<tr>
<td><em>Ae.(Och.)caspius</em></td>
<td>44,108</td>
<td>31,842</td>
<td>844</td>
<td>21,350</td>
<td>65,458</td>
<td>42,163</td>
<td>1,256</td>
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<td><em>Ae.(Och.)detritus</em></td>
<td>693</td>
<td>117</td>
<td>19</td>
<td>7</td>
<td>700</td>
<td>117</td>
<td>19</td>
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<td><em>Ae.(Och.)flavescens</em></td>
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<td><em>Ae.(Och.)sticticus</em></td>
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<td>4</td>
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<tr>
<td><em>Ae.(St.)albopictus</em></td>
<td>4,659</td>
<td>2,648</td>
<td>463</td>
<td>4,110</td>
<td>8,769</td>
<td>3,796</td>
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<td><em>Ae.geniculatus</em></td>
<td>214</td>
<td>130</td>
<td>9</td>
<td>131</td>
<td>345</td>
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<td><em>Ae.koreicus</em></td>
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<td><em>Ae.vexans</em></td>
<td>14,436</td>
<td>11,694</td>
<td>257</td>
<td>3,153</td>
<td>17,589</td>
<td>12,324</td>
<td>300</td>
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<td><em>Aedes spp.</em></td>
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<td>7</td>
<td>3</td>
<td>7</td>
<td>14</td>
<td>7</td>
<td>3</td>
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<td><em>An.claviger/petragani</em></td>
<td>16</td>
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<td>8</td>
<td>0</td>
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<td>10</td>
<td>8</td>
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<td><em>An.maculipennis s.l.</em></td>
<td>5,445</td>
<td>2,704</td>
<td>156</td>
<td>3,373</td>
<td>8,818</td>
<td>3,382</td>
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<td><em>An.patorium</em></td>
<td>55</td>
<td>35</td>
<td>12</td>
<td>56</td>
<td>111</td>
<td>47</td>
<td>22</td>
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<tr>
<td><em>Cq.richardii</em></td>
<td>335</td>
<td>247</td>
<td>39</td>
<td>63</td>
<td>398</td>
<td>247</td>
<td>39</td>
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<td><em>Cs. annulata</em></td>
<td>246</td>
<td>203</td>
<td>82</td>
<td>70</td>
<td>316</td>
<td>206</td>
<td>83</td>
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<td><em>Cs. subchorea</em></td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>2</td>
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<tr>
<td><em>Culiceta sp</em></td>
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<td>2</td>
<td>2</td>
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<tr>
<td><em>Cx.hortensis</em></td>
<td>1</td>
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<tr>
<td><em>Cx.modestus</em></td>
<td>2,231</td>
<td>1,450</td>
<td>75</td>
<td>1,054</td>
<td>3,285</td>
<td>2,361</td>
<td>117</td>
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<tr>
<td><em>Cx.pipiens</em></td>
<td>684,660</td>
<td>510,773</td>
<td>7,256</td>
<td>435,114</td>
<td>1,119,774</td>
<td>943,010</td>
<td>12,563</td>
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<tr>
<td><em>Cx.territans</em></td>
<td>42</td>
<td>39</td>
<td>5</td>
<td>0</td>
<td>42</td>
<td>39</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>757,461</strong></td>
<td><strong>562,079</strong></td>
<td><strong>9,268</strong></td>
<td><strong>468,506</strong></td>
<td><strong>1,225,967</strong></td>
<td><strong>1,008,142</strong></td>
<td><strong>15,304</strong></td>
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</tr>
</tbody>
</table>
Mosquito results

• In 2013-2014, 1,294,808 mosquitoes belonging to 23 species were sampled and the 77,9% were tested (1,008,142 sorted in 15,304 pools)
• The species *Culex pipiens* represent 90,3% of the collected mosquitoes
## Mosquito results

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>2014</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pools</td>
<td>WNV+</td>
<td>Pools</td>
</tr>
<tr>
<td>Ae.(Och.)caspius</td>
<td>844</td>
<td>1</td>
<td>412</td>
</tr>
<tr>
<td>Cx.modestus</td>
<td>75</td>
<td>1</td>
<td>42</td>
</tr>
<tr>
<td>Cx.pipiens</td>
<td>7256</td>
<td>177</td>
<td>5307</td>
</tr>
<tr>
<td>Total</td>
<td>9268</td>
<td>179</td>
<td>6036</td>
</tr>
</tbody>
</table>

- In 2013 WNV was detected in 177 pools (in ER, VEN, LOM)
- In 2014 WNV was detected in 129 pools in all surveyed regions (ER, VEN, LOM, PIE, FVG)
- WNV was almost exclusively detected in *Cx. pipiens*
- Possible active involvement of other mosquito tested positive for WNV need elucidation, particularly for *Aedes caspius*
Mosquito sites sampled and WNV-positive (red) in 2013
Mosquito sites sampled and WNV-positive (red) in 2014
Lineages

- Sequences obtained by field samples in 2013-2014 belonged mainly to lineage II
- Also lineage I sequences were detected in these period
- In previous outbreaks of WNV in Northern Italy only lineage I strains were detected
WNV neuroinvasive human cases, positive birds, positive mosquito pools in surveyed area

**WNND human cases**

**WNV positive birds**

**WNV positive mosquito pools**

Tested birds

Tested mosquitoes
Positive samples and human cases

Period of detection of WNV-positive pool of mosquito and WNV-positive birds respect to human cases for surveyed Regions

WNV detection in mosquitoes

WNV detection in birds

2013

2014

Lombardia
Piemonte
Friuli Venezia Giulia
Veneto
Emilia Romagna
Estimated cost for Emilia-Romagna region

- If The WNV circulate in an area, tests on the blood bags and on transplanted organs are required.
- Costs of these tests were estimated in 2,560,000 euro for the 2009-2013 period in the Emilia-Romagna region (test from 1 July to 30 November in provinces with viral circulation the previous year and provinces with human cases, according to the Surveillance National Plan).
- This sum is higher than the costs of the integrated surveillance system estimated in 2,093,000 euro in Emilia-Romagna for the same period 2009-2013, obtained by entomological surveillance and bird surveillance costs (582,215 euro), plus the blood screening surveillance linked to viral circulation detection at province level (1,512,226 euro).

From: Bellini et al. Eurosurveillance 2014
Added values of the surveillance

• Detection of other viruses by genus PCR and sequencing (Tahyna virus, Batai virus)
• Isolation of new viruses
• Definition of culicidic fauna of the surveyed area (*Anopheles maculipennis* complex, different sibling species with different vectorial competence for Malaria)
• Evaluation of influence of weather factors on the circulation of the detected viruses (in 2013 more dry and hot conditions were recorded in the WNV circulation area)
• Indication of possible reservoir and amplification role of tested birds
Conclusions

• Obtained data highlighted the role of *Cx. pipiens* as the main vector for WNV and USUV in Northern Italy.

• The systems provided epidemiological data and allowed to assess the geographical spread of the viruses, providing indication on the potential risk for humans and animals.

• Obtained results show a good performance of the two systems in term of sensitivity and early detection of viral circulation; confirming that, if mosquito trapping effort is intensive, these systems are useful and reliable to address the planning of public health policies.
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**Regione Veneto**
Russo Francesca, Cester Giorgio.

**Regione Friuli Venezia-Giulia**
Manlio Palei.
Map of the sites included in the survey and locations of polymerase chain reaction (PCR)–positive pools. Black, WN detections; gray, orthobunyavirus detections; lines, mosquito-only flavivirus detections.

Calzolari et al. 2010
Lombardy 2009-2011

<table>
<thead>
<tr>
<th>Virus</th>
<th>positive pools</th>
<th>species</th>
<th>years</th>
<th>month</th>
</tr>
</thead>
<tbody>
<tr>
<td>USUTU</td>
<td>3</td>
<td><em>Culex pipiens</em></td>
<td>2009-2011</td>
<td>August-September</td>
</tr>
<tr>
<td>BATAI</td>
<td>3</td>
<td><em>Culex pipiens</em> (2),</td>
<td>2009-2011</td>
<td>July</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Anophleles maculipennis s.l.</em> (1)</td>
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<tr>
<td>TAHYNA</td>
<td>2</td>
<td><em>Ochlerotatus caspius,</em></td>
<td>2009-2010</td>
<td>July</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Aedes vexans</em></td>
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</tr>
</tbody>
</table>

From these preliminary results detected viruses seem to have different seasonality:
August September for USUV (Flavivirus). More precocious circulation for BATV and TAHV (Orthobunyavirus)
Azure circle proportional to number of collected mosquitoes
Mosquito-only flavivirus

- The sequences obtained from flavivirus-positive PCR, not related to WNV or USUV, are due to the presence of three different mosquito-only flavivirus (identified different times) detected in *Aedes albopictus*, *Aedes vexans*, *Aedes caspius*

- Reports of the detection of these viruses, with no recognized pathogenic role in humans, are increasing in mosquitoes collected in Europe

- Application of other genus PCRs allowed the detection of other viruses. Tahyna virus was detected in 7 pools of mosquitoes during the survey (3 *Ae. vexans*, 3 *Ae. caspius*, 1 *Ae. albopictus*)
Detection of unknown viruses

- Two sequences (obtained by the flavivirus genus PCR) are more similar to the arthropod-transmitted flavivirus, and could be a clue of other viruses that are still unknown.

- Isolation of unknown viruses producing cytopathic effect on mosquito cell line.

Calzolari et al. PLoS ONE 2010

Calzolari et al. 2010 VBZD