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53.


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Tsai T.F., Popovici F., Cernesur C., Campbell G.C., Nedelcu N.I.


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<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Collected</th>
<th>Tested</th>
<th>Positive</th>
<th>M</th>
<th>F</th>
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<td>American Kestrel</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>1</td>
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<td>0</td>
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</tbody>
</table>
Table No. 2: Order, species and sex-wise list of birds tested for WNV-associated

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Collected</th>
<th>Tested</th>
<th>Positive</th>
<th>M</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long eared owl</td>
<td><em>Asio otus</em></td>
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<td>1</td>
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<td>0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mourning dove</td>
<td><em>Zenaida macroura</em></td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Rock Pigeon</td>
<td><em>Columba livia</em></td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Trochiliformes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anna's hummingbird</td>
<td><em>Calypte anna</em></td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
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<tr>
<td><strong>Psittaciformes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cockatiel</td>
<td><em>Nymphicus hollandicus</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<tr>
<td><strong>Anseriformes</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Mallard duck</td>
<td><em>Anasplatyrhynchos</em></td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Accipitriformes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sharp-shinned hawk</td>
<td><em>Accipiter striatus</em></td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total birds</strong></td>
<td></td>
<td>198</td>
<td>172</td>
<td>83</td>
<td>57</td>
<td>26</td>
</tr>
</tbody>
</table>

Total Orders of dead birds tested : 8
Total Orders of birds found WNV positive : 7
Total birds collected : 198
Total birds tested : 172
Total birds found WNV positive : 83 (48.30%)
Total WNV positive male birds : 57 (68.68%)
Total WNV positive female birds : 26 (31.32%)
<table>
<thead>
<tr>
<th>Organ</th>
<th>Pattern of WNV antigen staining</th>
<th>Kind of cells</th>
<th>Frequency of WNV Antigen staining</th>
</tr>
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<tbody>
<tr>
<td>Heart</td>
<td>Faint, diffuse and scattered</td>
<td>Macrophages</td>
<td>Frequently</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myofibers</td>
<td>Occasionally</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endothelial cells</td>
<td>Occasionally</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pericardium showed intense staining of macrophages</td>
<td>Frequently and intense</td>
</tr>
<tr>
<td>Brain</td>
<td>Mostly focal, but sometimes multifocal</td>
<td>Purkinje cells</td>
<td>Frequently</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neurons</td>
<td>Occasionally</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glial cells</td>
<td>Occasionally</td>
</tr>
<tr>
<td>Liver</td>
<td>Intense and diffuse type</td>
<td>Kupffer cells</td>
<td>Frequently</td>
</tr>
<tr>
<td>Kidney</td>
<td>Multifocal and centered around collecting ducts</td>
<td>Macrophages</td>
<td>Frequently</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tubular epithelial</td>
<td>Frequently</td>
</tr>
<tr>
<td>Spleen</td>
<td>Intense and diffuse type</td>
<td>Reticuloendothelial cells</td>
<td>Frequently</td>
</tr>
<tr>
<td>Lung</td>
<td>Patchy and around airways and vessels</td>
<td>Macrophages</td>
<td>Frequently</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endothelium of vessels and airways</td>
<td>Frequently</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>Intense and focal</td>
<td>Payer patches</td>
<td>Frequently</td>
</tr>
<tr>
<td>Ovary</td>
<td>Focal and patchy</td>
<td>Parenchymal cells</td>
<td>Frequently</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interstitial cells</td>
<td>Frequently</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stromal cells</td>
<td>Occasionally</td>
</tr>
<tr>
<td>Testes</td>
<td>Focal and intense</td>
<td>Sertoli cells</td>
<td>Frequently</td>
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Table No. 6: Tissue wise distribution (%age positive) of West Nile virus antigen in different organs across a range of avian orders with special reference to family *Corvidae*

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<thead>
<tr>
<th>Order</th>
<th>Brain</th>
<th>Heart</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Testes</th>
<th>Ovary</th>
<th>Intestine</th>
<th>Lung</th>
</tr>
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<tbody>
<tr>
<td>Passeriformes (Family: <em>Corvidae</em>)</td>
<td>37.6</td>
<td>60</td>
<td>92</td>
<td>97.5</td>
<td>90</td>
<td>62</td>
<td>60</td>
<td>86</td>
<td>85.5</td>
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<td>0</td>
<td>0</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>ND</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Strigiformes</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>ND</td>
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<td>Columbiformes</td>
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<td>60</td>
<td>60</td>
<td>80</td>
<td>33</td>
<td>50</td>
<td>20</td>
<td>20</td>
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<td>Trochiliformes</td>
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<td>0</td>
<td>50</td>
<td>100</td>
<td>100</td>
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<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>ND</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Accipitriformes</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>0</td>
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ND: No distribution of WNV antigen was studied in this organ because all infected birds belonging to these Orders were of same sex
Table No. 1: History and characteristics of WNV related epidemics

<table>
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<th>Year</th>
<th>Country</th>
<th>Species involved</th>
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<td>1937</td>
<td>Uganda</td>
<td>Isolated in Human</td>
<td>Smithburn et al., 1940</td>
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<tr>
<td>1950s</td>
<td>Egypt, Upper Nile Delta</td>
<td>Humans</td>
<td>Taylor et al., 1956; Schmidt and Elmansory, 1963</td>
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<td>1951-54 1957</td>
<td>Israel</td>
<td>Humans</td>
<td>Weinberger et al., 2001</td>
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<tr>
<td>1970</td>
<td>Belarus, Ukraine</td>
<td>Humans</td>
<td>Hubalek and Halouzka, 1999</td>
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<td>1974</td>
<td>South Africa</td>
<td>Humans</td>
<td>McIntosh et al., 1976</td>
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<td>1994</td>
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<td>LeGuennno et al., 1996</td>
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<td>1996-97</td>
<td>Bucharest, Romania</td>
<td>Humans, Horses</td>
<td>Tsai et al., 1998</td>
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<td>1996</td>
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<td>Humans, Horses</td>
<td>Hubalek and Halouzka, 1999</td>
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<td>1997</td>
<td>Tunisia</td>
<td>Humans, Horses</td>
<td>Hubalek and Halouzka, 1999</td>
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<tr>
<td></td>
<td>Czechland</td>
<td>Humans</td>
<td>Hubalek et al., 1999</td>
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<tr>
<td>1998</td>
<td>Italy</td>
<td>Humans, Horses</td>
<td>Hubalek and Halouzka, 1999</td>
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<tr>
<td>1999</td>
<td>Russia</td>
<td>Humans, Horses</td>
<td>Platonov et al., 2001</td>
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<td>Israel</td>
<td>Humans</td>
<td>Lanciotti et al., 1999</td>
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<tr>
<td></td>
<td>USA</td>
<td>Horses, Birds</td>
<td>Pupo et al., 2006</td>
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<td></td>
<td>Cuba</td>
<td>Horses</td>
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<td></td>
<td>Cayman Islands</td>
<td>Humans</td>
<td>Komar and Clark, 2006</td>
</tr>
<tr>
<td></td>
<td>Argentina</td>
<td>Birds</td>
<td>Morales et al., 2006</td>
</tr>
<tr>
<td>2000</td>
<td>Israel</td>
<td>Humans</td>
<td>Weinberger et al., 2001</td>
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<td></td>
<td>France</td>
<td>Horses</td>
<td>Murgue et al., 2001</td>
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<td>USA</td>
<td></td>
<td>Weiss et al., 2001</td>
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<td></td>
<td>Trock et al., 2001</td>
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<tr>
<td>2001</td>
<td>Russia</td>
<td>Human</td>
<td>Zeller and Schuffnecker, 2004</td>
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<td>Caribbean basin</td>
<td>Birds</td>
<td>Komar and Clark, 2006</td>
</tr>
<tr>
<td>2002</td>
<td>Mexico</td>
<td>Humans, Horses</td>
<td>Komar and Clark, 2006</td>
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<td>Birds</td>
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<td>2004</td>
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<td>Spain</td>
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<td>Kaptoul et al., 2007</td>
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<td>Portugal</td>
<td>Humans</td>
<td>Connell et al., 2004</td>
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<td>2008</td>
<td>Hungary</td>
<td>Humans</td>
<td>Krisztalovics et al., 2008</td>
</tr>
<tr>
<td>2009</td>
<td>Italy</td>
<td>Humans</td>
<td>Rizzo et al., 2009</td>
</tr>
<tr>
<td>2010</td>
<td>Greece</td>
<td>Humans</td>
<td>Danis et al., 2011</td>
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Table No. 3: Demographic distribution and form of clinical presentation of human WNV illness cases in Riverside County, California, 2008-2010.

<table>
<thead>
<tr>
<th>Age group</th>
<th>WNV cases</th>
<th>WNND form (Fatalities)</th>
<th>WNF form</th>
<th>Asymptomatic</th>
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<tbody>
<tr>
<td>0-10</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
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<td>11-20</td>
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<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>21-30</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>31-40</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>41-50</td>
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<td>51-60</td>
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<td>61-70</td>
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</tr>
<tr>
<td>71-80</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>81-90*</td>
<td>8</td>
<td>6 (4)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total cases</td>
<td>69</td>
<td>49</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

**WNND**: West Nile neuroinvasive disease; **WNF**: West Nile fever; **Asymptomatic**: Cases detected as blood donors but clinical symptoms were never developed

* All the fatalities (n=4) occurred in this age group
Table No. 4: Distribution of clinical presentation and fatalities of WNV illness cases in humans, Riverside County, 2008-2010.

<table>
<thead>
<tr>
<th></th>
<th>WNND</th>
<th>WNF</th>
<th>Asymptomatic</th>
<th>Total No. of WNV cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>49</td>
<td>16</td>
<td>4</td>
<td>69</td>
</tr>
<tr>
<td>Rate of clinical presentation in %age</td>
<td>71</td>
<td>23</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Fatalities (% age in parenthesis)</td>
<td>4 (8.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (5.8)</td>
</tr>
<tr>
<td>Survival rate in % age</td>
<td>91.8</td>
<td>100</td>
<td>100</td>
<td>94.2</td>
</tr>
</tbody>
</table>

**WNND**: West Nile neuroinvasive disease; **WNF**: West Nile fever; **Asymptomatic**: Cases detected as blood donors but clinical symptoms were never developed.

Percentages are shown in parenthesis.
Table No. 5: Gender distribution of human WNV illness in Riverside County, California, 2008-10.

<table>
<thead>
<tr>
<th>Gender</th>
<th>WNV illness (% of total cases ie 69)</th>
<th>Fatalities (% of col. 2)</th>
<th>WNND (% of col. 2)</th>
<th>WNF (% of col. 2)</th>
<th>Asymptomatic (% of col 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>42 (61)</td>
<td>1 (2.48)</td>
<td>32 (76.2)</td>
<td>9 (21.43)</td>
<td>1 (2.38)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (39)</td>
<td>3 (11.11)</td>
<td>17 (63)</td>
<td>7 (25.93)</td>
<td>3 (11.11)</td>
</tr>
<tr>
<td>Total cases</td>
<td>69</td>
<td>4 (5.8)</td>
<td>49 (71)</td>
<td>16 (23.2)</td>
<td>4 (5.8)</td>
</tr>
</tbody>
</table>

WNND: West Nile neuroinvasive disease; WNF: West Nile fever; Asymptomatic: Cases detected as blood donors but clinical symptoms were never developed.

As compared to females, 50% more males fall ill with WNV because males spent more time outdoor due to their lifestyle thus chances of getting exposed to mosquito bites was much more as compared to females.
Fig No. 10: Percent-wise distribution of WNV antigen in different organs of birds belonging to different orders with special reference to Family Corvidae.
Fig No. 12: Antigenic distribution of WNV in liver

- Staining is diffuse type
- Stained cells are Kupffer cells
Fig No. 13: Antigenic distribution of WNV in kidney

- Staining is multifocal and centered around collecting ducts
- Stained cells are macrophages and tubular epithelial cells
Fig No. 11: Antigenic distribution of WNV in spleen

- Staining is diffuse
- Stained cells are reticuloendothelial cells
Fig No. 15: Antigenic distribution of WNV in lung

- Patchy staining around airways and vessels
- Stained cells are macrophages and endothelium of vessels and airways
Fig No. 14: Antigenic distribution of WNV in Small Intestine

- Staining is intense and focal
- Stained cells are payers patches
Fig No. 16: Antigenic distribution of WNV in heart

Explanation to Fig. 15 A and B:
A: Pericardium showed sections of intense staining of macrophages
B: Stained cells are macrophages, myofibers and occasionally endothelial cells
   Staining is faint, diffuse and scattered
Fig No. 17: Antigenic distribution of WNV in brain

- Staining is focal or multifocal
- Stained cells are Purkinje cells, Neurons and Glial cells
Stained cells are parenchymal, interstitial and stromal cells.
No WNV antigen was stained in the epithelium of the wall of oviduct and Zona pellucida (lower figure).
Fig No. 19: Antigenic distribution of WNV in testes

- Stained cells were Sertoli cells
- Staining pattern was focal
Plate 4: Sentinel Chicken flocks at fixed locations to test seroconversion for WNV

Plate 5: Collection of blood from jugular vein of live wild bird to test seroconversion for WNV
Fig No. 3: Helicopters were used for aerial adulticiding

Fig No. 4: Hovercrafts were used for larviciding of wetlands
Fig No. 9: Sub-dural hemorrhages, as grossly seen, in American crows
Plate 1: Necropsies of dead birds to collect tissue samples

Necropsy in progress to collect tissues -
1: American crow    2: Lesser goldfinch    3: House finch
4: Anna’s hummingbird    5: Researcher conducting necropsy
Fig No. 2: Study area, Riverside County on map of USA

Riverside County shown in red on map of California
Principle of Immunohistochemistry staining

Plate 2

Plate 3
Fig No. 1: West Nile Virus Transmission Cycle

Mosquito vector

West Nile virus

Bird - reservoir hosts

Incidental infections

Contd.
Clinical presentation of human WNV illness cases in Riverside County, California, 2008-2010

WNND: West Nile neuroinvasive disease (49/69)
WNF: West Nile fever (16/69)
Distribution of WNV illness cases and fatalities in different age groups of humans, Riverside County, California, 2008-2010

<table>
<thead>
<tr>
<th>Age group in years</th>
<th>Total WNV cases</th>
<th>Fatalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>11-20</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>21-30</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>31-40</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>41-50</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>51-60</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>61-70</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>71-80</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>81-90</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Number of cases
Gender distribution of human WNV illness cases in Riverside County, California, 2008-2010

- Female: 39%
- Male: 61%

Total: 69  Male: 42  Female: 27
Distribution of WNV illness and clinical presentation among different age groups of humans, Riverside County, California, 2008-2010

All fatalities (n=4) were due to WNND and were in 81-90 age group.