California Association for Medical Laboratory Technology
Distance Learning Course

Update on West Nile Virus

By

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Course Number: DL-976
1.0 CE/Contact Hour
Level of Difficulty: Basic

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COURSE NAME: UPDATE ON WEST NILE VIRUS
COURSE #: DL-976

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1. a  b  c  d
2. a  b  c  d
3. a  b  c  d
4. a  b  c  d
5. a  b  c  d
6. a  b  c  d
7. a  b  c  d
8. a  b  c  d
9. a  b  c  d
10. a  b  c  d

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1. Overall, I was satisfied with the quality of this Distance Learning course.
   5  4  3  2  1

2. The objectives of this Distance Learning course were met.
   5  4  3  2  1

3. The difficulty of this Distance Learning course was consistent with the number of CE hours.
   5  4  3  2  1

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   5  4  3  2  1

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Update on West Nile Virus

OBJECTIVES:
After completing this course the participant will be able to:
1. Discuss the history of West Nile virus (WNV).
2. Explain the epidemiology of WNV in the United States.
3. Outline the range of clinical symptoms in humans infected with WNV.
4. State the classification of WNV.
5. Discuss methods of prevention of WNV spread and infection.
6. Outline the surveillance programs for WNV.

INTRODUCTION:
Yet again the international spread of a disease that was formerly restricted to a small area shows how vulnerable the entire world is to emerging diseases. We must be constantly prepared to investigate such new-to-us maladies. West Nile virus is an example of our shrinking globe and how a tipping point—a critical mass or condition—can start an epidemic.

West Nile virus has emerged in recent years in temperate regions of Europe and North America, presenting a threat to public, bird, and animal health. The virus was first identified in the United States in New York in 1999. Since then there has been rapid spread to include all the forty-eight contiguous states.

The most serious manifestation of WNV infection is fatal encephalitis in humans and horses, as well as mortality in certain domestic and wild birds. Although WNV can cause serious disease, less than 1% of those diagnosed with the infection suffer the more serious complications.

HISTORY:
West Nile virus was first isolated from a febrile adult woman in the West Nile district of Uganda in 1937 (1). The epidemiology was characterized in Egypt in the 1950s. The virus was recognized as a cause of severe human meningoencephalitis in elderly patients during an outbreak in Israel in 1957. In the 1990s there were several large human outbreaks in Africa, western Asia, and Europe as the virus spread to virgin populations. The 1996-97 epidemic in Bucharest, Romania was more deadly than usual with a 10% mortality rate in over 500 clinical cases. The Bucharest WNV outbreak reaffirmed that mosquito-borne viral diseases may occur on a mass scale, even in temperate climates. The first cases in the Western Hemisphere were found in New York City in 1999. Since then it has spread inexorably across the continental United States. Human disease finally appeared in the last state, Washington, in 2006.

Equine disease was first noted in Egypt and France in the early 1960s. Epizootics occurred in horses in Morocco in 1996, Italy in 1998, and the United States after 1999. In the U.S., 2002 was a peak year with 14,571 cases. WNV is more serious in horses than in any other host.

The Egyptian outbreak established that birds are the definitive host and that the virus is spread by migrating birds. Corvid birds—crows, jays, and magpies—are most susceptible to morbidity and mortality. Other birds become infected but are less seriously
infected. In California’s Central Valley the West Nile virus has caused a significant decline in the magpie population.

At present WNV is most commonly found in Africa, West Asia, Europe, the Middle East and North America. There is concern regarding the possible spread to the areas in South America where mosquitoes are abundant.

**ECOLOGY/EPIDEMIOLOGY**

Mosquitoes, largely bird-feeding species, are the principal vectors of West Nile virus. *Culex* mosquitoes, particularly *C. quinquefasciatus*, are the most common mosquito vectors in the United States but *Aedes, Anopheles*, and *Ochlerotatus* genera have also been implicated. In studying the spread and scope of the disease, mosquito pools from nature are tested for WNV.

Wild birds are the principal hosts of West Nile virus. The virus has been isolated from a number of wetland and terrestrial avian species in diverse areas. Infection has been identified in over 300 species. Corvids have the highest incidence of disease; the highest incidence in non-corvid birds occurs in sparrows and finches. High, long-term viremia, sufficient to infect vector mosquitoes, has been observed in infected birds. The virus has been found to persist in the organs of inoculated ducks and pigeons for 20 to 100 days. Therefore, migratory birds are primarily instrumental in the spread of the virus.

The first step in the transmission cycle occurs when a mosquito bites an infected bird. The virus primarily cycles between mosquitoes and birds, but infected female mosquitoes can also transmit WNV to humans and other incidental hosts through bites. With so many susceptible hosts to amplify the virus and so many types of mosquitoes to transmit it, WNV has spread rapidly across the United States (see Table 1).

**TABLE 1. ANNUAL INCIDENCE OF WEST NILE VIRUS IN THE U.S. SINCE 1999**

<table>
<thead>
<tr>
<th>Year</th>
<th># States with Human Cases</th>
<th># Human Cases</th>
<th># Deaths</th>
<th>State with Most Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>1</td>
<td>62</td>
<td>7</td>
<td>New York (62)</td>
</tr>
<tr>
<td>2000</td>
<td>3</td>
<td>21</td>
<td>2</td>
<td>New York (14)</td>
</tr>
<tr>
<td>2001</td>
<td>10</td>
<td>66</td>
<td>9</td>
<td>New York (15)</td>
</tr>
<tr>
<td>2002</td>
<td>39</td>
<td>4,156</td>
<td>284</td>
<td>Illinois (884)</td>
</tr>
<tr>
<td>2003</td>
<td>45</td>
<td>9,862</td>
<td>264</td>
<td>Colorado (2,947)</td>
</tr>
<tr>
<td>2004</td>
<td>40</td>
<td>2,539</td>
<td>100</td>
<td>California (830)</td>
</tr>
<tr>
<td>2005</td>
<td>43</td>
<td>3,000</td>
<td>119</td>
<td>California (935)</td>
</tr>
<tr>
<td>2006</td>
<td>41</td>
<td>3,011</td>
<td>94</td>
<td>Idaho (642)</td>
</tr>
<tr>
<td>2007</td>
<td>44</td>
<td>3,630</td>
<td>117</td>
<td>California (380)</td>
</tr>
<tr>
<td>2008</td>
<td>45</td>
<td>1,356</td>
<td>44</td>
<td>California (445)</td>
</tr>
<tr>
<td>2009</td>
<td>38</td>
<td>720</td>
<td>32</td>
<td>Texas (115)</td>
</tr>
<tr>
<td>2010</td>
<td>40</td>
<td>931</td>
<td>39</td>
<td>Arizona (157)</td>
</tr>
<tr>
<td>2011</td>
<td>43</td>
<td>712</td>
<td>43</td>
<td>California (158)</td>
</tr>
<tr>
<td>2012 (to 12/11/12)</td>
<td>48</td>
<td>5,387</td>
<td>243</td>
<td>Texas (1,739)</td>
</tr>
</tbody>
</table>
West Nile virus reached California in 2003, although there was an isolated human case in Los Angeles in 2002 without any other WNV activity (2). After 2003 the activity spread north from southern California (see Table 2). Surveillance of WNV activity, through testing mosquito pools, dead birds, and routine monitoring of sentinel chickens indicated the geographic spread of the virus before human cases appeared. There was rapid environmental spread to all 58 California counties in 2004 from just six counties in 2003. The number of human cases paralleled this advance. Starting in 2006 the activity and number of cases unexpectedly decreased. Except for an increase in 2008, there were fewer cases in 2009-2011 until this year when 468 human cases were reported (Table 2). The California Department of Public Health closely monitors the activity and some local jurisdictions have instituted mosquito spraying programs.

### TABLE 2. HISTORY OF WEST NILE VIRUS IN CALIFORNIA

<table>
<thead>
<tr>
<th>Year</th>
<th>Counties with WNV Activity*</th>
<th>Counties with Human Cases</th>
<th># Human Cases</th>
<th># Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>58</td>
<td>23</td>
<td>830</td>
<td>28</td>
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<td>2005</td>
<td>54</td>
<td>40</td>
<td>935</td>
<td>19</td>
</tr>
<tr>
<td>2006</td>
<td>54</td>
<td>36</td>
<td>272</td>
<td>7</td>
</tr>
<tr>
<td>2007</td>
<td>51</td>
<td>30</td>
<td>380</td>
<td>21</td>
</tr>
<tr>
<td>2008</td>
<td>49</td>
<td>27</td>
<td>445</td>
<td>15</td>
</tr>
<tr>
<td>2009</td>
<td>42</td>
<td>19</td>
<td>112</td>
<td>4</td>
</tr>
<tr>
<td>2010</td>
<td>35</td>
<td>17</td>
<td>111</td>
<td>6</td>
</tr>
<tr>
<td>2011</td>
<td>36</td>
<td>24</td>
<td>158</td>
<td>9</td>
</tr>
<tr>
<td>2012</td>
<td>42</td>
<td>31</td>
<td>468</td>
<td>18</td>
</tr>
</tbody>
</table>

* birds, animals, or mosquito pools positive

West Nile virus also occurs in other animals, particularly horses. The 2002 equine WNV epizootic was unprecedented given its geographic span and the number (14,571) of horses affected in the U.S. The development of immunization for horses has been instrumental in significantly decreasing the incidence of disease. In 2009, 276 cases were identified in the U.S. in horses. Because WNV-infected horses seldom develop viremias sufficient to infect feeding mosquitoes, they are unlikely to pose a risk to humans. However, equine epizootics reflect intense enzootic WNV activity in mosquitoes, which might place humans at increased risk.

Although infection in other animal species has been identified, they play little role in the spread of the disease. Recently WNV had been found in sick or dead tree squirrels in California, including Fox squirrels, the most common urban squirrel. The significance of squirrel infection is not known. The California Department of Health Services is following the incidence in these animals.

Cases of WNV occur mostly in the warm summer months. Warmer, moist areas favor the multiplication of mosquitoes. As the weather cools, the incidence of WNV illness decreases. The peak season is usually around the end of August to early September. Transmission can occur most of the year in the milder areas of the U.S.
As the disease has spread westward across the United States, fewer cases occur in the eastern and southern regions of the United States where it was first prevalent. It may be that many people in these areas became immune, particularly since most infected with the virus have no, or only mild, symptoms. This increase in herd immunity decreases the number of susceptibles and the incidence of infection decreases. This has been shown by fewer recent cases in the Eastern and Southern U.S. In Africa where the disease is endemic, it is a mild childhood malady that almost never develops serious consequences. This situation may come to pass in the U.S.

CAUSATIVE AGENT:
West Nile virus is a member of the Japanese encephalitis antigenic complex of the genus *Flavivirus*, family *Flaviridae*. Flaviviruses share a common size (40-60nm), symmetry (enveloped, icosahedral nucleocapsid), nucleic acid (positive-sense, single stranded RNA approximately 10,000-11,000 bases), and appearance in the electron microscope. All known members of the Japanese encephalitis antigenic complex (Alfuy, Cacipacore, Japanese encephalitis, Kokobera, Koutango, Kunjin, Murray Valley encephalitis, Rocio, St. Louis encephalitis, Stratford, Usutu, West Nile, and Yaounde viruses) are transmissible by mosquitoes and many of them can cause febrile, sometimes fatal, illnesses in humans.

DISEASE:
Most people who are infected with the WN virus have no symptoms whatsoever. About 20% of those infected develop a mild illness, West Nile fever (WNF). This is a febrile, influenza-like illness, characterized by an abrupt onset (incubation period is 3-14 days) of moderate to high fever lasting three to six days. Symptoms can include malaise, headache (often frontal), body aches, maculopapular or roseolar rash (in approximately half the cases, spreading from the trunk to the extremities and head), lymphadenopathy, anorexia, nausea, vomiting, and eye pain.

Severe West Nile infection resulting in neurological disease (WNND) occurs in about 1 in 250 people who are infected. Encephalitis is more commonly reported (fever and headache associated with alteration of consciousness, which may be mild and result in lethargy, but may progress to confusion or coma). Tremor of extremities, abnormal reflexes, limb paralysis, and cranial nerve palsies may be present. Meningitis is less common and usually involves fever, headache, and stiff neck. Changes in consciousness are not usually seen. West Nile poliomyelitis, a flaccid paralysis, is infrequent. It is associated with acute onset of asymmetric limb weakness or paralysis, sometimes with pain. It can occur without fever, headache, or other common symptoms.

Laboratory findings involve a slightly increased sedimentation rate and a mild leukocytosis; cerebrospinal fluid in patients with central nervous system involvement is clear, with moderate pleocytosis, usually with a predominance of lymphocytes, elevated protein, and normal glucose. Hyponatremia is sometimes present, particularly among patients with encephalitis. The virus can be recovered from the blood of immunocompetent febrile patients for up to 10 days, with an average duration of seven days. Peak viremia occurs four to eight days post-infection. It can be found as late as 22 to 28 days after infection in immunocompromised patients.
Recovery in non-fatal cases is usually complete (less rapid in adults than in children, often accompanied by long-term myalgias and weakness). Permanent neurological sequelae have been reported. The elderly are particularly susceptible to clinical illness. Most fatal cases have been recorded in patients over 50 years of age. Patients in this age category have rapid onset; morbidity is high and lingering in the nonfatal cases. There is no specific treatment for infection with WN virus, although supportive care is important.

Diagnosis of WNV infection is based on a high index of clinical suspicion and on obtaining specific laboratory tests. WNV, or other arboviral diseases such as St. Louis encephalitis, should be strongly considered in adults over 50 years who develop unexplained encephalitis or meningitis in summer or early fall. The local presence of WNV enzootic activity or other human cases should further raise suspicion. Obtaining a recent travel history is also important to the diagnostic process.

Note: Severe neurological disease due to WNV infection (WNND) has occurred in patients of all ages. Year-round transmission is possible in some areas. Therefore, WNV should be considered in all persons with unexplained encephalitis and meningitis. The timely identification of persons with acute WNV or other arboviral infection may have significant public health implications and will likely augment the public health response to reduce the risk of additional human infections.

Diagnostic testing: WNV testing for patients with encephalitis or meningitis can be obtained through local or state health departments. The most efficient diagnostic method is detection of IgM antibody to WNV in serum or cerebrospinal fluid (CSF) collected within 8 days of illness onset using the IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA). Since IgM antibody does not cross the blood-brain barrier, IgM antibody in CSF strongly suggests central nervous system infection. Patients who have been recently vaccinated against or recently infected with related flaviviruses (e.g., yellow fever, Japanese encephalitis, and dengue) may have positive WNV MAC-ELISA results.

OTHER METHODS OF TRANSMISSION:

Blood Transfusions: Transmission by blood transfusion was first reported in 2002 (3). That year there were 23 documented infections. Following this finding researchers and blood banks hastened to develop a test to use on donated blood. Screening using the Nucleic Acid Amplification test (NAT) was implemented nationally in July, 2003. Transfusion associated infections were significantly decreased; there were no cases in later years.

Blood banks report presumptive viremic donors to their local Public Health Department which in turn reports to ArboNET (a national electronic-based surveillance and reporting system under the Centers for Disease Control and Prevention). ArboNET monitors and investigates arthropod-borne (arbo) diseases.

National statistics for presumptive viremic donors (those whose NAT was positive) for the past three years are: 2010—144 cases; 2011—137 cases; 2012—597 cases.

In 2012 the states reporting the highest number of positives were California and Texas.

Solid Organ Transplant: The first evidence of transmission by solid organ transplant was also identified in 2002 with four cases. In 2005 one donor transmitted the infection to three recipients; one other recipient was not infected. Organ donors are screened only
to identify infectious risks. The NAT is not done because it would take too long and it has not been approved in the organ donor setting. Transplant recipients are treated with Omr-IgG-am, an intravenous immunoglobulin product with high titered neutralizing antibodies to WNV. Since 2002 there have been several sporadic cases of WNV transmitted to organ recipients.

**PREVENTION:**

There are several avenues of prevention and control: surveillance, environmental control, public education, and vaccine development.

**Surveillance:** The Centers for Disease Control and Prevention developed surveillance guidelines for states and other jurisdictions in 2003 (4). The guidelines state, “Appropriate and timely response to surveillance data is the key to preventing human and animal disease associated with WNV and other arboviruses. That response must include effective mosquito control and public education without delay if an increasing intensity of virus activity is detected by bird and mosquito based surveillance systems.” The following is based on their guidelines:

A. **ECOLOGIC SURVEILLANCE**

Detection of WNV in bird and mosquito populations helps health officials predict and prevent human and domestic animal infections. Surveillance to detect WNV should focus on the avian and mosquito components of the enzootic transmission cycle. Non-human mammals, particularly equines, may also serve as effective sentinels because a high intensity of mosquito exposure makes them more likely to be infected than people. Local (state and other jurisdictions) WNV surveillance networks collect and test for WNV antigens in tissue or antibodies in blood specimens from dead birds, captive sentinel animals (mostly chickens), wild-caught birds, mosquitoes and veterinary patients. Test results, including the county and the week of specimen collection, are entered into local electronic databases, and standardized summaries are forwarded weekly to CDC’s ArboNET database system.

The following information is based on California’s procedures (2):

1. **Avian:** Avian morbidity/mortality surveillance appears to be the most sensitive early detection system for WNV activity. This consists of testing dead wild birds, monitoring sentinel chicken flocks, and testing captured wild birds.

   **Dead Birds:** The public is asked to report a dead bird by sending a form available on the state’s website or by calling the Dead Bird Hotline. If the bird is picked up by public health personnel, it is tested for presence of WNV antigens in tissue or oral swab.

   **Sentinal Chickens:** Detection of transmission of arboviruses in bird populations can be accomplished by using caged chickens as sentinels and bleeding them routinely. About 200 flocks of ten chickens are maintained in various areas where mosquito abundance is high or where viral activity is known. They are bled every two weeks and the blood is tested for antibodies.

   **Live Wild Birds:** Trapping of wild birds, identifying, bleeding and testing the blood can be done. However it is costly and of dubious value because birds migrate and the location of infection cannot be determined, so it is not used in California.

2. **Mosquitoes:** Surveillance includes monitoring the numbers of immature and mature mosquito forms and testing for the virus in pools of adult mosquitoes.
Abundance: Larval numbers are determined by dipping a net into water sources and counting the larvae per dip; adult numbers are determined by using baited traps.

Infections: Female mosquitoes are trapped in CO$_2$ traps. The females are speciated and tested in lots of 50 for presence of WNV.

3. Equine surveillance: Equines appear to be important sentinels of WNV epizootic activity and human risk, at least in some geographic regions. In addition, equine health is an important economic issue. Therefore, surveillance for equine WNV disease should be conducted in jurisdictions where equines are present. Equines are highly conspicuous, numerous, and widely distributed in some areas. They may be particularly useful sentinels in rural areas, where dead birds may be less likely to be detected. All equine neurologic disease cases should be promptly reported; the equines should be tested for infection with WNV and other arboviruses as geographically appropriate, and for rabies. A licensed equine WNV vaccine has been available in the U.S. since 2001. Widespread use of equine WNV vaccines may decrease the incidence of equine WNV disease and therefore the usefulness of equines as sentinels.

B. HUMAN SURVEILLANCE

Because the primary public health objective of surveillance systems for neurotropic arboviruses is prevention of human infections and disease, human case surveillance alone should not be used for the detection of arbovirus activity, except in jurisdictions where arbovirus activity is rare, or resources to support avian-based and/or mosquito-based arbovirus surveillance are unavailable.

Physicians, hospital infection control personnel and laboratories are contacted regarding reporting possible WNV cases. Monitoring of encephalitis cases is the highest priority. The minimum human surveillance system is enhanced passive surveillance for hospitalized encephalitis cases of unknown etiology and for patients who have IgM antibodies to either WN or SLE virus in tests conducted in diagnostic or reference laboratories.

Reports of human WNV cases and other reports of WNV activity are reported to CDC by telephone, facsimile, or e-mail.

ENVIRONMENTAL CONTROL:

Primary control of mosquitoes is done by educating the public to reduce possible breeding sites by removing stagnant water. Use of mosquito fish, larvacides and spray programs for adult mosquitoes are done in other situations.

PUBLIC EDUCATION:

CDC’s recommendations include (4): “inform the public about WNV, promote the adoption of preventive behaviors that reduce disease risk, and gain public support for control measures. Health education/public information includes use of print materials (posters, brochures, fact sheets), electronic information (Web sites), presentations (health experts or peers speaking to community groups), and the media.

Address the multiple levels at which prevention can occur: personal protection (use of repellent on skin and clothing, use of protective clothing, awareness of prime mosquito-biting hours); household protection (eliminating mosquito breeding sites, repairing/installing screens); and community protection (reporting dead birds, advocating for organized mosquito abatement, participating in community mobilization).”
VACCINE DEVELOPMENT:
Although licensed WNV vaccines exist for horses, there are no specific vaccines or treatments for human WNV disease. Scientists and public health organizations have accelerated research on developing tools to prevent WNV disease. The National Institute of Allergy and Infectious Diseases (NIAID) supports research on a variety of vaccine approaches that could potentially lead to a safe and effective preventive vaccine for West Nile virus. These approaches include vaccines containing cocktails of individual WNV proteins and chimeric vaccines that combine genes from more than one virus into a single vaccine. A third approach involves DNA vaccines, in which DNA that codes for a particular virus protein is combined with bacterial DNA, and the combined product is injected directly into the skin of the person or animal being vaccinated. Several of the potential vaccines are in clinical trials.

CONCLUSION:
West Nile Virus, an emerging infectious disease, spread across the United States in seven years after it appeared in New York City in 1999. During that time procedures of testing for the virus antigens and antibodies were developed and instituted. These procedures are used to identify human cases, to protect the blood supply, to survey the spread of the disease, and to guide methods of control. Public education is an important component in this control. Research into vaccine development may result in protection of the public against West Nile Virus in the future.

REFERENCES:
1. www.cdc.gov/ncidod/dvbid/westnile/
5. National Institute of Allergy and Infectious Diseases, NIAID Research on West Nile Virus. www.niaid.nih.gov/topics/westnile/Pages/
REVIEW QUESTIONS
Course #DL-976
Choose the one best answer

1. The geographic spread of WNV is probably due to:
   a. transported horses
   b. traveling humans
   c. migrating birds
   d. flying mosquitoes

2. Which of the following viruses is not a flavivirus?
   a. yellow fever
   b. Japanese encephalitis
   c. western equine encephalitis
   d. West Nile virus

3. The bird species having the highest incidence of WNV infection is:
   a. house sparrow
   b. crow
   c. chicken
   d. finch

4. The majority of human infections with WNV are:
   a. asymptomatic
   b. in people who handle horses
   c. in people who pick up dead birds or squirrels
   d. in people bitten by ticks

5. WNV antibodies in serum are used in testing for virus activity in:
   a. adult mosquito pools
   b. sentinel chickens
   c. mosquito larvae
   d. dead birds

6. The most practical method of protecting people and animals from infection by WNV is:
   a. avoiding proximity to horses
   b. not going outside at dawn or dusk
   c. avoiding touching dead birds
   d. control of mosquito population

7. Arbovirus means:
   a. arthropod borne viruses
   b. viruses found in forested regions
   c. viruses found in arbors
   d. viruses transmitted only by ticks
8. Vaccination is presently available for:
   a. people
   b. dogs
   c. chickens
   d. horses

9. The most effective environmental control the general public can perform is:
   a. use of insect repellant
   b. removing any stagnant water on their property
   c. reporting dead birds
   d. installing a mosquito zapper

10. The most common severe WNV sequel in humans is:
    a. meningitis
    b. encephalitis
    c. flaccid paralysis
    d. influenza