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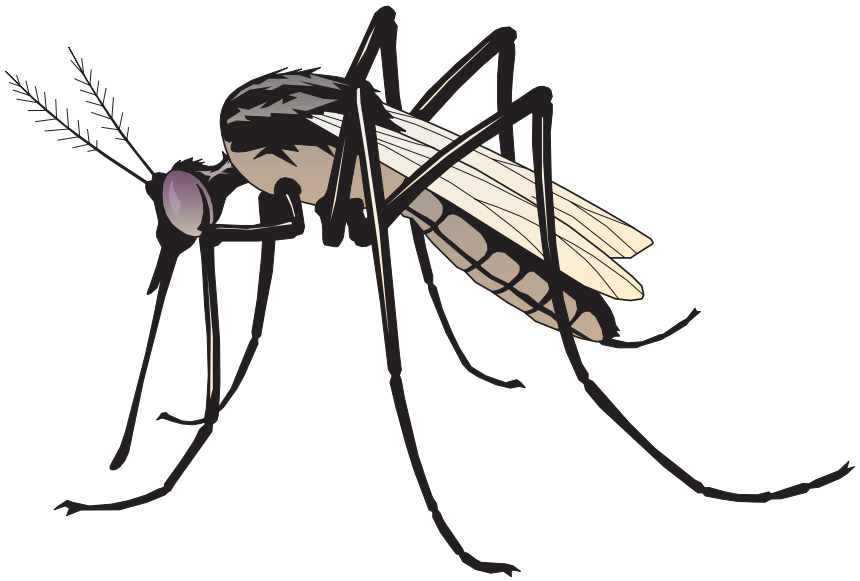

West Nile Virus Infection

A Review for Clinicians

by Matthew J. Bankowski, PhD, D(ABMM), and Steven M. Anderson, PhD

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West Nile virus (WNV) infections in the United States have demonstrated major outbreaks during the 2002 and 2003 arbovirus season. The WNV can truly be viewed as an emerging infectious disease agent that has rapidly spread throughout the mosquito, bird, animal and human populations in the United States. Information on the virus, clinical presentation, epidemiology, laboratory testing, control and prevention is the focus of this article.

Virology

WNV is an arthropod-borne virus first isolated in 1937 from the West Nile region of northern Uganda.[1] The virus belongs to the Japanese encephalitis serocomplex of the family Flaviviridae, within the genus *Flavivirus*. The *Flavivirus* genus contains approximately 70 different viruses, including human pathogens such as yellow fever virus, dengue, Japanese encephalitis (JE) virus, St. Louis encephalitis (SLE) virus and Murray Valley encephalitis virus. The members of the JE serogroup are closely related both antigenically and genetically.

WNV is a small, single-stranded, positive-sense RNA virus with a genome size of approximately 11,000 nucleotides.[2] The viral genome has a short 5´ noncoding region, followed by a single open reading frame coding for three structural and seven nonstructural proteins.[2] The viral genome is wrapped in a nucleocapsid and surrounded by a host-derived lipid membrane. A key structural protein is the viral envelope glycoprotein, which plays an important role in processes such as host cell binding, tissue tropism and host range.[2] The envelope glycoprotein is also the most immunologically important structural protein.Viral-neutralizing antibodies are most often directed at the envelope glycoprotein.[3]

Phylogenetic analysis of various WNV isolates has shown that they can be divided into two distinct lineages. Lin-

Learning Objectives

On successful completion of this module, the participant will be able to:

1. Outline the biological background of West Nile virus.
2. Relate the natural history of West Nile virus.
3. Enumerate the salient features of West Nile virus epidemiology.
4. Discuss West Nile virus as an example of an emerging pathogen.
5. Review clinical features of West Nile virus infections.
6. Provide an overview of laboratory methods for the identification of West Nile virus in clinical specimens.

eage one strains have been described from various locations around the world (e.g., Africa, Europe, United States) and are associated with human disease. Lineage two strains have not been linked to human disease and have been found mainly in Africa. The WNV isolate described from New York in 1999 is from lineage one and is most closely related to a strain first described from Israel. Studies of sequence variations from isolates collected in the United States since 1999 have shown very few genetic changes within the population of viruses circulating in this country.[4]

The natural transmission cycle of WNV includes mosquitoes (mainly Culex species) and birds (i.e., crows and blue jays). Humans and other animals (e.g., horses) serve as incidental hosts. Several bird species (e.g., crows, jays, blackbirds, sparrows and finches) are considered amplifying hosts and important reservoirs for WNV since they develop high levels of transient viremia. Humans and animals normally do not transmit the virus to biting mosquitoes because of low viremia levels and, consequently, are considered dead-end hosts for the virus.

Surveillance programs used in the United States to monitor the presence and spread of WNV have shown a broadening of vector and reservoir populations since the virus was first introduced in this country. WNV has been isolated from more than 29 different species and 10 genera of mosquitoes and in more than 140 different avian species.[5] In addition, an increasing number of animal species has shown evidence of infection with WNV.

In areas where WNV is endemic, human infections are common but typically mild or subclinical. Severe disease is usually found in the elderly or immunocompromised population. WNV infections are associated with significant mortality in horses and certain domestic and wild birds. Members of the Corvidae family (e.g., crows, bluejays) are particularly susceptible to WNV infections and demonstrate high levels of mortality. For this reason, surveillance for dead birds of these species can be important for monitoring WNV activity in a particular area.

Historically, WNV has been found primarily in Africa, Asia, southern Europe and Australia. In endemic areas, there is very little evidence of WNV-associated disease because of high-level background immunity. In such areas, as many as 50 percent of children and 90 percent of adults show potential immunity to WNV.[2,6] WNV has been associated with several epidemics, such as those in Israel, South Africa, France, Romania and, most recently, with outbreaks in the United States.[1] Three trends related to these outbreaks have been noted:

1. an increase in the frequency of outbreaks in horses and humans;
2. high avian death rates accompanying human epidemics; and
3. an apparent increase in the severity of human disease.[1]

Emergence of WNV in the United States

WNV was first observed in the New York metropolitan area during the summer of 1999. During 1999 and 2000, there were more than 70 cases of confirmed WNV-associated disease and nine fatalities in the New York area.[7] Since that time an extensive surveillance network has been implemented involving the evaluation of avian mortality, equine disease, mosquito pools and human specimens for the presence of WNV.[8]

In 2001, 66 cases of human West Nile disease were reported, including nine fatal cases. During the summer of 2002 there was a dramatic increase in WNV activity in the United States with evidence of WNV reported in approximately 40 states. More than 4,000 laboratory-confirmed cases were reported to the CDC, with 284 deaths attributable to WNV (Table 1).[9]

Most human infections with WNV occur in the summer or early fall. In the United States, peak times have been between mid-August to mid-September. With the expansion of the geographic range of WNV, however, cases have been detected as early as May and as late as December. The highest incidence of West Nile cases in this country has been in urban environments. Risk factors for these recent outbreaks have included length of time spent outdoors, lack of regular use of mosquito repellant and observation of dead birds in one's neighborhood.[10]

Clinical Aspects of West Nile-associated Disease

In humans, most WNV infections are subclinical. Febrile disease is the most common clinical presentation in young adults.[11] The incubation period ranges from two to 14 days. West Nile fever (WNF) often presents with sudden onset of acute flu-like symptoms. Because of the nonspecificity of the presentation, WNV infections may be highly under-recognized. Other symptoms may include fatigue, headache, weakness, nausea, vomiting and altered mental status.[12]

Neurological involvement, which occurs in a small percentage of infected individuals, may pro- ▶

Table 1
HISTORY OF WEST NILE VIRUS CASES IN THE UNITED STATES

Year	No. Cases/Deaths	No. States	Dates of Onset
1999	62/9	1	8/2–9/24
2000	21/0	3	7/10–9/27
2001	66/9	10	7/13–12/7
2002	4156/284	39	5/19–12/14

Table 2
WNV PERSON-TO-PERSON TRANSMISSION

Type of Transmission
Blood Transfusion
Organ Transplants
Mother-to-infant Transmission
-Breast Milk (?)
-Transplacental Transmission
Laboratory-acquired Infection

duce both encephalitis and meningitis—known as West Nile meningoencephalitis (WNME). In such cases, approximately two-thirds are considered consistent with encephalitis and one-third with meningitis. Symptoms indicating potential central nervous system involvement usually develop after a febrile prodrome (approximately one to seven days) and may include confusion, nuchal rigidity and loss of consciousness.[11]

Approximately one in five individuals infected with the virus demonstrates symptoms of WNF, and one in 150 shows central nervous system involvement.[1,11,12] Mortality rates between 4 percent and 14 percent have been reported in individuals who demonstrate neurological disease.[1,11,12] Risk factors for mortality include profound weakness, deep coma and other underlying health conditions such as hypertension and diabetes. Recently, a new syndrome involving acute flaccid paralysis (also known as West Nile polio) was described and linked to WNV infections.[13]

Treatment

To date, there are no specific treatments for WNV-infected individuals. Patients with suspected WNME are hospitalized for observation, supportive care and to rule out other treatable central nervous system diseases (i.e., HSV, bacterial meningoencephalitis). Currently being evaluated for treatment potential are several antiviral agents that have shown some success in cell lines and animal models. These include agents such as the purine analog, ribavirin and interferon-alpha.[14] So far, these agents have not yet been demonstrated to be effective in humans via rigorous clinical trials. A vaccine has been developed for use in horses, and research is under way regarding the development of a human vaccine.[15]

Survivors of WNV disease may also need continued medical support and evaluation. Prospective studies have shown that as many as 50 percent of such individuals have a functional deficit (e.g., muscle weakness, cognitive dysfunction) at discharge and approximately one-third continue to show such deficits one year later.[12,16]

Person-to-person Transmission of WNV

During the year 2002, several examples of person-to-person transmission of WNV were documented. These are listed in Table 2 and include blood transfusion, organ donation, intrauterine transmission and laboratory exposure.[17,18]

Also, a suspected case of mother-to-child transmission via breast milk has been investigated. The risk of transmission via blood transfusion has been estimated at 1.46-12.33 per 10,000 donations. Because of the high level of risk associated with blood transfusions, recommendations have been made to screen the U.S. blood supply using molecular technologies.[19]

Diagnostic Laboratory Testing

WNV testing in the clinical laboratory was not a part of infectious disease test considerations up to the year 1999. Other members of the Arbovirus group, such as SLE virus or LaCrosse encephalitis virus, were more prevalent and were part of testing algorithms indicated during Arbovirus season. In 1999, however, New York City experienced numerous cases of WNV disease, including encephalitis and death. In the following four years we have seen a dramatic increase in WNV cases, culminating in more than 4,000 last year. WNV infection is seasonal and from the trend observed in 1999-2002, the number of reported cases may continue to increase exponentially.[20-22]

Laboratory testing for any infectious agent can be categorized as indirect or direct. The most common indirect test is a measure of the antibody response: IgM or IgG. Alternatively, culture, animal inoculation, antigen detection and molecular methods provide direct support for

Table 3
LABORATORY TESTING FOR HUMAN WNV

Laboratory Testing	Description	Specimen(s)
Serology	IgM and IgG Antibody -ELISA, IFA, Multiplex Bead Assay	Blood (Serum) CSF
Molecular Diagnosis	Nucleic Acid Amplification -RT-PCR, Real-time RT-PCR, -TaqMan, NASBA	Brain tissue CSF
Virology	Virus isolation and detection -Culture (Monoclonal antibody IFA) -Animal inoculation (Neonatal mice) - Plaque Reduction Neutralization Test (PRNT) -Immunohistochemistry (IHC)	CSF Brain tissue Blood (heart)

infection. Most of these methods are available in reference laboratories, large research-based medical center laboratories, state public health laboratories and the CDC.

Current testing for WNV is summarized in Table 3. Laboratory testing is an important part of any clinical differential diagnosis. Testing serves as support for the clinical history and other patient procedures. Tests should always be carefully selected and based on the pathology of the infectious agent. For instance, in most infections it appears that WNV is present in very low numbers in the blood (viremia) before seroconversion (i.e., appearance of IgM); therefore, molecular detection or virus isolation may be most useful only in IgM-negative cases.

Consequently, serological testing of acute and convalescent specimens often provides the most useful support for infection. Likewise, if the patient has neurological involvement (e.g., meningoencephalitis) along with or rather than a WNV fever syndrome, molecular diagnosis (e.g., real-time reverse-transcription polymerase chain reaction [RT-PCR]) testing of cerebrospinal fluid (CSF) may provide strong support for infection.

No matter what laboratory test is performed, correct interpretation of the results in the context of the entire clinical picture is most important. This may also include additional testing based on clinical consultation with the laboratory for support of WNV infection.

Serology (i.e., IgM and IgG) testing is the most common, noninvasive method available for support of WNV infection. Several test formats are in use, such as immunofluorescent antibody (IFA) (Table 3) or enzyme-linked immunosorbent assay (ELISA) (Table 4). Test performance (i.e., sensitivity, specificity, predictive values, accuracy and precision) varies among the assays.[23]

Generally, ELISA testing is more sensitive and specific than IFA.[24] Some of the reasons for higher values for ELISA are based on both the technology and the use of antibody capture formats (e.g., MAC-ELISA).[25] Due to the extensive cross-reactivity and persistence of WNV IgM among the Flaviviruses, however, one should be cautious in the interpretation of serological tests.[26]

Different approaches have been used to eliminate or identify nonspecificity. In one study using an IgM ELISA on samples also found reactive by the CDC and state public health laboratories, a background subtraction technique was used to eliminate nonspecific reactions.[27] Even with this approach, nonWNV Flaviviruses (i.e., 7.8 percent dengue and 2.0 percent SLE) and other non-WNV reactivity (15.7 percent) were present. In the true WNV reactive samples (74.5 percent), however, the index value for the test ranged from moderate to high and had a high positive predictive value. An approach used by the CDC has been to use a MAC-ELISA with three antigens (i.e., WNV, SLE and JE).[28] Specificity in this assay format was 92 percent, and the test showed less reactivity to dengue.[28]

Standard tissue culture or animal inoculation can accomplish virology testing for isolation of WNV (Table 3). A BSL-3 facility should be used for this type of testing. Standard tissue culture is performed using mammalian or mosquito cell lines. Specific detection and confirmation of the virus can be done using either monoclonal antibodies, molecular methods or virus neutralization. Culture is usually performed on postmortem specimens such as brain tissue, CSF or heart blood. It should also be noted that the WNV is easily inactivated at ambient temperature in both serum and CSF.[29]

The complete genome sequence of multiple WNV strains has been determined.[30] This information on the molecular blueprint of the various virus strains aids the development of highly specific molecular-based diagnostic tests. Both classical PCR and real-time-based tests (TaqMan, NASBA and LightCycler) have been successfully applied to human samples (Table 3).[31]

In a study by the CDC, both the TaqMan assay and RT-PCR were applied to brain tissue, CSF and serum.[32] The authors found RT-PCR to be much less sensitive than the real-time TaqMan assay.[32] The specimen with the highest positive percentage was brain tissue (100 percent), followed by CSF (57.1 percent) and serum (14.3 percent). Ranges of WNV detection (using molecular assays) for various specimen types are shown in Table 5.

Conclusion

WNV is an excellent example of an emerging infectious disease agent. It is now an established Arbovirus appearing seasonally in ever-increasing numbers of cases. To ▶

Table 4
PERFORMANCE FOR THE SEROLOGICAL DETECTION OF WNV IGM USING ELISA AND IFA

Test	Sensitivity	Specificity
ELISA (IgM)	89 – 95	88 – 100
IFA (IgM)	72 – 96	83 – 100

Table 5
WNV REAL-TIME MOLECULAR PCR TESTING

Specimen	Positive (%)	Comments
Blood	0 – 14	Short-lived viremia mostly in IgM neg WNV cleared with IgM seroconversion (IgM-positive)
CSF	30 – 57	Higher detection may be seen in cases of greater morbidity and in cases of mortality
Brain Tissue	80 – 100	Highest specimen test sensitivity

date, it has been found in animals, birds and humans in approximately 40 states. Control is multifactorial and relies on effective education of the population at large; efficient vector elimination; rapid, high-performance laboratory testing; and research for agents that can provide treatment for the diseased. ■

Dr. Bankowski is the director of Infectious Disease Testing at ViroMed Laboratories, a wholly owned subsidiary of LabCorp, and Dr. Anderson is chief scientific officer of ViroMed Laboratories.

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West Nile Virus Infection

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SELF-ASSESSMENT QUESTIONS

1. West Nile virus was first isolated from
   A. the United States.
   B. Israel.
   C. Australia.
   D. Uganda.

2. Which virus is closely related to WNV?
   A. Human immunodeficiency virus
   B. Human papillomavirus
   C. St. Louis encephalitis virus
   D. Adenovirus

3. Which of the following groups are part of the natural transmission cycle of WNV?
   A. mosquitoes and horses
   B. horses and birds
   C. mosquitoes and birds
   D. horses and humans

4. Most WNV infections
   A. are acute.
   B. are mild or subclinical.
   C. result in meningoencephalitis.
   D. cause West Nile polio.

5. The first cases of WNV infection in the United States were reported in
   A. 1937.
   B. 1975.
   C. 1999.
   D. 2002.

6. Which is not considered a risk factor for WNV infection?
   A. Time spent outdoors
   B. Recent travel to Africa
   C. Lack of regular use of mosquito repellant
   D. Observation of dead birds in one's neighborhood

7. Approximately what fraction of individuals exposed to WNV develop some symptomology?
   A. 1 in 2
   B. 1 in 5
   C. 1 in 50
   D. 1 in 150

8. Evidence of neurological involvement may include
   A. diagnosis of meningoencephalitis.
   B. confusion or altered mental state.
   C. loss of consciousness.
   D. all of the above.

9. The most commonly used laboratory method to support a diagnosis of WNV is
   A. cell culture.
   B. the polymerase chain reaction.
   C. detection of viral antigen.
   D. detection of IgM serology response.

10. Polymerase chain reaction testing for WNV has the highest sensitivity in
   A. blood.
   B. CSF.
   C. serum.
   D. brain tissue.

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1. The article met the stated objectives.
   A. Strongly agree
   B. Agree
   C. Uncertain
   D. Disagree
   E. Strongly disagree

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9. The level of instruction in the article was appropriate.
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   E. Strongly disagree

10. I would recommend this article to my colleagues.
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   C. Uncertain
   D. Disagree
   E. Strongly disagree

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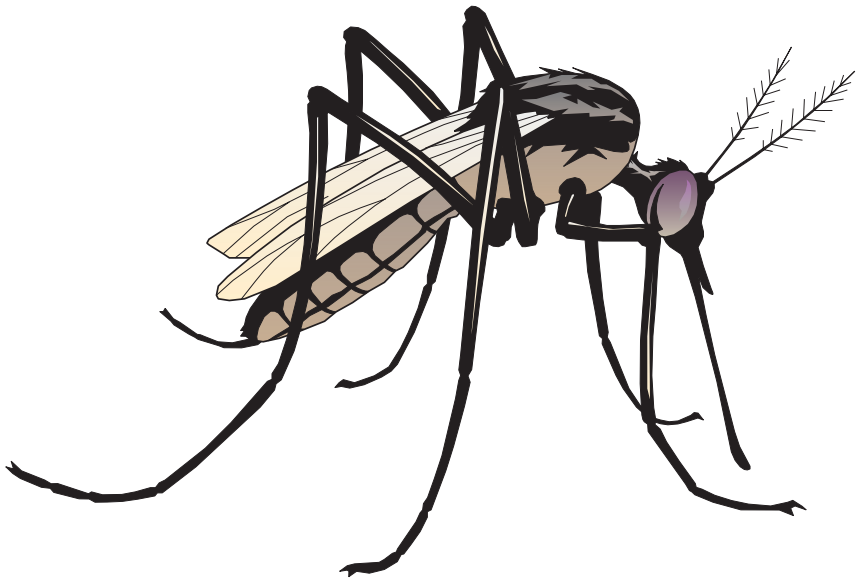
6.	○	○	○	○	○
7.	○	○	○	○	○
8.	○	○	○	○	○
9.	○	○	○	○	○
10.	○	○	○	○	○

WE APPRECIATE YOUR COMMENTS.

HOW LONG DID IT TAKE YOU TO COMPLETE THIS ACTIVITY, INCLUDING THE SELF-ASSESSMENT QUESTIONS AND EVALUATION?
_____ MINUTES

Signature _____



LEARNING SCOPE 2

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