REX HEALTHCARE

Laboratory Bulletin...

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West Nile Virus

West Nile virus is a member of the Flaviviridae family, genus Flavivirus, whose natural reservoir and host is the avian population. The virus survives in nature via a mosquito-bird-mosquito transmission cycle and is indigenous to Africa, Asia, Europe and Australia. Recent large epidemics have occurred in Romania, Russia, Israel and the United States. The West Nile virus contains a single-stranded RNA genome within a viral membrane, and proteins that confer many of the important factors related to the host range, tissue tropism, replication and B and T- cell immune responses. Nucleic acid sequencing has demonstrated two distinct viral lineages, in which the first lineage has a worldwide distribution including west Africa, the Middle East, Europe, North America and Australia. The second lineage has a more restricted distribution to enzootic strains in Africa. Although the virus is maintained in nature by primarily the Culex species mosquitoes-bird-mosquito cycle, twenty-nine mosquito species have harbored the virus in the United States alone. Both hard and soft ticks have been found to harbor the virus in the Eastern Hemisphere, but are not considered important vectors in epidemics. Birds are the natural reservoir for the virus and act as the amplification step in the cycle. West Nile virus has infected at least 111 species of birds in North America and is particularly virulent in crows and jays. These birds have been critical in the dead-bird-based surveillance programs that are used in the detecting and tracking the virus in a particular region. Many different mammals are susceptible to natural or experimental infection, but naturally acquired disease has been conclusively confirmed only in human beings and horses. The role of mammals in the transmission cycle is unknown at this time and experimental studies suggest that horses are dead-end hosts for West Nile virus.

Geographical Distribution and Epidemiology

West Nile and Kunjin (a subtype of West Nile) viruses are distributed worldwide. The virus was first detected in North America in New York City during the first wave of viral transmission. The Middle East was the likely source of the virus, but the mode of introduction is unknown. From 1999-2002, the virus has extended its penetration into North America from Maine and south central Canada to the Florida Keys and the Caribbean islands with westward extension to North Dakota. In any given region, the distribution of the virus has been multifocal and discontinuous due to complex ecological relationships. Although the mosquito-bird-mosquito transmission cycle remains the most important, there have been recent documented cases of human-human transmission. Human infections occur most frequently from summer to early fall in the temperate and subtropical areas, but infections have been known to occur as late as December. Figure 1 shows the number of West Nile meningoencephalitis or West Nile fever cases reported by month. All age groups (both male and female) appear equally susceptible to infection, but the incidence of severe complications (encephalitis) and death increase with advancing age. Risk factors in recent urban epidemics include length of time spent outdoors, failure to regularly apply mosquito repellant and living in an apartment building with a flooded basement. Other risk factors for developing meningoencephalitis with West Nile or St. Louis virus infection have not been identified to date. The frequency of particular clinical symptom complexes will vary according to previous West Nile virus activity in the population with resultant background immunity, age distribution, and the sophistication of surveillance studies. In endemic areas in Africa, the prevalence of background immunity in the population is 50% in children and roughly 90% in adults. In contrast, European immunity is most likely very low and in the United States essentially nonexistent.

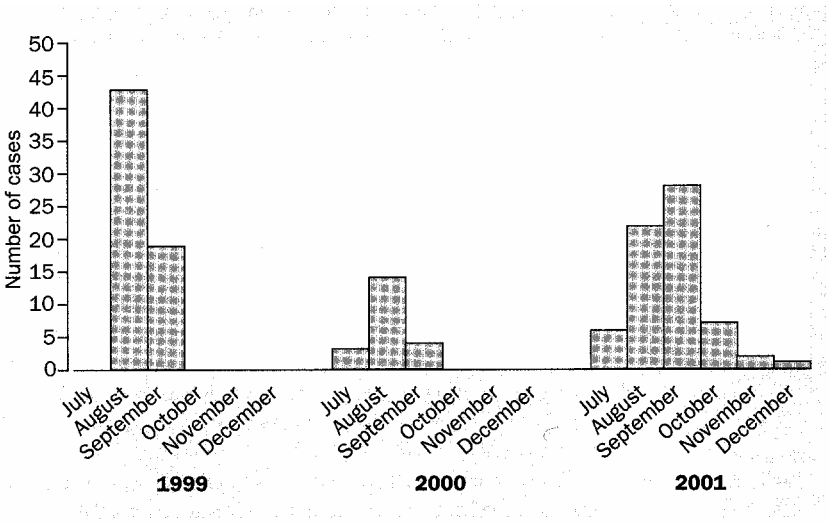

Figure 4. Number of WN meningoencephalitis or WN fever cases reported by month of illness onset, 1999–2001, USA. Data are from the ArboNET surveillance system, Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention.

In 1999-2000 in the United States, 78 cases of West Nile virus meningoencephalitis were reported and all were within the greater New York City metropolitan area during August and September. In 2001, a much greater degree of geographic and temporal dispersion was evident with 64 cases reported from 38 counties (10 states), from July to December. As of October 2, 2002, the number of West Nile virus cases reported to the CDC increased to 2,530 with 125 deaths from 32 states and Washington, DC. Ongoing investigation by the CDC, FDA, Health Resources and Services Administration in conjunction with blood collection agencies and local health departments has identified West Nile virus infection secondary to organ transplantation and blood product transfusion. Fifteen patients from 10 states became infected with the virus within one month following blood transfusion, but not all of these represent viral transmission by transfusion. All of the individuals were from areas of active West Nile virus activity and most likely reflects infection from mosquito bites. Only three instances of West Nile viral infection secondary to blood product transfusion have been confirmed. The CDC and the Michigan Department of Community Health are investigating a case of West Nile virus infection in a woman, who became ill after receiving a blood product transfusion and while breastfeeding her infant. While viral culture of the breast milk is pending, IgM antibodies to West Nile virus have been identified in the infant, which supports infection of the infant. This infant represents the youngest infected patient, since the start of the epidemic in the United States since 1999. The NC State Public Health Laboratory has recently documented the second and third human infections in North Carolina. The total number of infected birds documented to date is 117 from 35 of the state's 100 counties. Of note, LaCrosse viral infection appears to be increasing at pace in North Carolina to set a new record.

Clinical and Pathologic Features

The majority of West Nile viral infections are asymptomatic. Approximately 20% of infected persons will develop mild illness characterized by a febrile illness of sudden onset often with malaise, anorexia, nausea, vomiting, headache, eye pain, myalgia, rash and lymphadenopathy. The incubation period is thought to be from 3 to 14 days with the duration of symptoms lasting 3 to 6 days. One in 150 patients may develop severe infection, including encephalitis more often than meningitis. Neurological abnormalities include ataxia, extrapyramidal signs, cranial nerve abnormalities, myelitis, optic neuritis, polyradiculitis and seizures. Some patients develop severe muscle weakness or flaccid paralysis. A minority of patients with severe infection will have a maculopapular or morbilliform rash involving the neck, trunk, arms or legs. Initial replication of the virus is thought to occur in the skin in the area of the mosquito bite and regional lymph nodes with a subsequent seeding of the reticuloendothelial system by a primary viremia. Depending on the level of replication in the reticuloendothelial system, a secondary viremia may develop with subsequent potential seeding of the central nervous system. The viral envelope protein appears to be a primary virulence factor by mediating cell attachment and neuroinvasiveness. The changes in the

central nervous system are due to viral proliferation in neuronal and glial cells, cytotoxic immune response to the infected cells, diffuse perivascular inflammation and glial nodule formation. These changes resolve in the majority of patients, who survive West Nile meningoencephalitis. Some individuals, however, do have permanent neurologic sequela, though the reasons for this are not understood.

Laboratory and Radiographic Findings, Diagnosis and Treatment

Laboratory and radiologic findings in infected persons include:

* Normal or elevated WBC with lymphocytosis and anemia
* Hyponatremia (especially with encephalitis)
* CSF pleocytosis, including a predominance of lymphocytes.
* CSF protein increased with normal glucose
* Enhancement of the leptomeninges and/or periventricular area by MRI.

Laboratory diagnostic testing for suspected West Nile virus encephalitis or meningitis:

* IgM antibody to West Nile virus in serum and cerebrospinal fluid collected within 8 days of illness onset. The presence of IgM antibody in cerebrospinal fluid is strong presumptive evidence of central nervous system infection. A second useful test in identifying possible West Nile virus infections is the IgG antibody to West Nile virus. Results are usually available within 5 days from receipt to the State Public Health Laboratory. All cases of CSF testing must be accompanied by a serum sample collected on the same day as the CSF and submitted with the appropriate State Laboratory forms available on the the web site http://slph.state.nc.us. The following information must be included: onset date, specimen collection date and signs/symptoms. Additional useful information includes: history of travel, military service, arboviral vaccination or transfusion.
* Patients recently infected or vaccinated against other related flaviviruses may show positive West Nile virus antibody results. Additional testing for so-called false positive results involves the use of the plaque-reduction neutralization test (PRNT), the most specific test for distinguishing between the different flavivirus species. This test may take up to 8 days or longer for completion depending on the virus.
* Acute and convalescent serum samples are recommended, since antibodies from a previous infection with West Nile virus may persist for years. In this situation, the PRNT is used to identify a four-fold or greater increase in antibody titer between the acute and convalescent phase paired samples.
* Viral detection by cell culture or laboratory animals and genomic amplification by various reverse transcriptase-polymerase chain reaction (RT-PCR) are considered less useful due to lower sensitivity and are inappropriate for sole testing of possible West Nile virus infection.
* Antigen detection assays for viral proteins developed in a dipstick format are used only for testing in bird and mosquito tissues.

Treatment for West Nile virus infection is generally supportive. Some activity against West Nile virus has been shown in in-vitro studies using ribavirin in high doses and interferon alpha-2b. However, there are no controlled studies available on their use in patients.

The Future and Prevention Measures:

Based on the migratory patterns of birds and the West Nile virus transmission cycle, authorities project the disease will spread to the western parts of the United States and Central and South America over the next several years. In the upcoming decades**,** an ecological/epidemiological balance will occur, most likely resembling St. Louis encephalitis (SLE) virus with modest numbers of sporadic cases and occasional difficult to predict outbreaks. From 1964-2000, a median of 26 St. Louis encephalitis cases per year were reported in the United States and in the summer and fall of 1975, approximately 2000 human SLE cases with nearly 170 deaths were documented. Prevention will center around local comprehensive arboviral surveillance with mosquito control programs in areas

of virus activity. A human West Nile virus vaccine is not currently available, although some are in development. A widespread vaccination program is not expected, due to the small numbers of West Nile disease in most areas. Equine vaccines have been developed for use, but their efficacy is unknown at the current time. Mosquito control programs should emphasize the larval control and adult chemical spraying control programs reserved for emergency application after viral activity has been demonstrated in the community. Physicians rapidly become an important link in any program with case reporting to health departments and patient education to avoid or decrease the risk of being bitten by infected mosquitos. Education programs should include general advice concerning limiting activity where mosquitos are common, especially during the peak mosquito biting activity (from dusk to dawn), wearing of protective clothing and application of repellants containing DEET (N,N-diethyl-m-toluamide) as an active ingredient to exposed skin and clothing. For additional information regarding reporting requirements, please refer to your local and state health departments: www.cdc.gov/ncidod/dvbid/westnile/city **states.htm** . West Nile virus is on the list of nationally notifiable arboviral encephalitides.

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I would like to thank Dr. Lou Turner (Director of the North Carolina State Laboratory of Public Health) and Dr. J. Todd McPherson (Head, Virology/Serology Unit, North Carolina State Laboratory of Public Health) for sharing timely updates of West Nile Virus activity in the United States and in North Carolina.

References:

1. West Nile virus. Campbell, G.L. et al. The Lancet Infectious Diseases. 2(9):519-529. September 2002.

2. CDC Communique: West Nile Virus Infection. Information for Clinicians. August, 2002.

3. North Carolina State Laboratory of Public Health Communiques for Arbovirus Surveillance, Methods for West Nile Virus Laboratory Testing and West Nile Updates. July-October, 2002.

Lab Notes

On Saturday, September 28 2002, eleven Rex Laboratory employees volunteered to assist in the Prostate Cancer Awareness Screening Clinic held at the Rex Senior Center. A total of 396 patients were seen. Of note, 5 phlebotomists (Sonia A., Nga M., Dee R., Dawn W, and Mae W-B) averaged drawing one patient every 4-5 minutes for 5 hours – a new indoor world record.

The Laboratory recently underwent successful inspection and accreditation by the College of American Pathologists. In addition, both the Food and Drug Administration and the American Association of Blood Banks recently subjected the Blood Bank and Rex Blood Plan to inspections. No deficiencies were found and Dr. Carter was released unharmed.

For further information, call the Laboratory (784-3040). Telephone extensions are: Pathologists' Direct Line (3201), Sharon Logue (Lab Director 2400), Robin Ivosic (Microbiology Lab Manager 3053), Elaine Patterson (Core Lab Manager 3054), Jackie Okoth (Core Lab PM Manager 4248), Diane Young (Anatomic Pathology Manager 3888), Nga Moore (Customer Service Manager 3396)