West Nile Virus Neurologic Disease in Humans, South Africa, September 2008–May 2009

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We investigated West Nile virus (WNV) as a possible disease etiology in persons hospitalized in South Africa. Of 206 specimens tested, 36 had WNV neutralizing antibodies, significantly more than in similar earlier serosurveys. Seven probable acute WNV cases were identified, suggesting WNV may be overlooked as an etiology of severe disease in South Africa.

West Nile virus (WNV), a mosquito-borne flavivirus (1), is a reemerging pathogen of global concern (2). Febrile illness occurs in ≈20% of WNV-infected persons; neurologic complications (e.g., meningitis, encephalitis, flaccid paralysis) occur in <1% (3).

Detection of IgM in serum or cerebrospinal fluid (CSF) is the preferred method for diagnosing WNV infection; however, because of cross-reactivity between flaviviruses, positive results should be confirmed by virus neutralization assay. Early WNV infection can be diagnosed by PCR and virus isolation (4), but success has been limited in diagnosing more advanced disease with these techniques. WNV is endemic to southern Africa. In 1947, one of the largest WNV epidemics occurred in the Karoo region of South Africa (5), and another occurred in combination with a Sindbis virus epidemic in 1983–84 in the Witwatersrand–Pretoria region of South Africa. The most recent seroprevalence data for WNV in the Pretoria region of South Africa is from the 1970s (reviewed in [6]). To update that information, we determined whether WNV is being overlooked as a possible cause of disease in persons hospitalized in South Africa. The University of Pretoria Research Ethics Committee approved this study.

The Study

Serum and CSF samples were obtained from the National Health Laboratory Service, Thswane Academic Division, Thswane, South Africa, which serves public sector hospitals in northern South Africa. To select samples for testing, we reviewed laboratory submission requests for patients with clinical conditions consistent with WNV infection: fever, headache, rash, or neurologic signs (7,8). A total of 206 patient samples (15 CSF and 191 serum) were selected. During September 2008–May 2009, we screened samples for the presence of WNV by using real-time reverse transcription PCR (rRT-PCR) (9), virus neutralization assay, and IgM ELISA.

We detected WNV neutralizing antibodies in serum and CSF samples by using a modified method (10). In brief, we mixed 50% tissue culture infective doses of Kunjin virus strain MRM61C (100 U/mL) in 2% fetal bovine serum (Invitrogen, Carlsbad, CA, USA) with 2-fold dilutions of heat-inactivated patient serum (1:10–1:640) in equal volumes and incubated the mixture for 1 h at 37°C in 5% CO₂. We then added 1 volume of Vero cells (1 × 10⁶ cells/mL) in 2% fetal bovine serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin (Lonza, Basel, Switzerland) and incubated the mixture for 72 h at 37°C in 5% CO₂. Samples were considered positive for WNV neutralizing antibodies if <25% of the cells/well displayed cytopathic effect. Comparative testing with Wesselsbron virus, a closely related flavivirus, did not show cross-reactivity within the parameters of what we considered positive.

Of the 206 specimens, 40 (19.42%, 95% CI 14.02%–24.82%) were positive for neutralizing antibodies. Of these, 36/191 serum samples had antibody titers of <160. The positive CSF samples (4/15) had antibody titers of 4 (Table 1). Positive serum samples were also tested by WNV IgM capture ELISA (Panbio; Alere, Sinnamon Park, QLD, Australia); 2 had positive results (Table 1).

Of the 206 specimens, 190 were of sufficient quantity to be subjected to RNA extraction (QIAamp Viral RNA Mini Kit; QIAGEN, Valencia, CA, USA) and subsequent WNV nested rRT-PCR (9). The presence of lineage 2 WNV RNA was identified in 1 CSF specimen and confirmed by sequencing (GenBank accession no. JX974605) (Figure). To evaluate the sensitivity of these molecular and serologic tests for diagnosing WNV in humans, we used the same methods to test 9 archived sequential serum samples in parallel (Table 2). The samples were from a patient with WNV encephalitis who became infected with neuroinvasive lineage 2 WNV strain in 2003 after a needlestick injury (11). Samples were collected 0–30 days after exposure. Initial symptoms developed on postexposure day 7 and persisted for 19 days; the patient completely recovered by day 26 (11).

Conclusions

We conducted a retrospective investigation of patients hospitalized with febrile illness or neurologic disease of unknown etiology in the Pretoria region of South Africa
to determine whether some of the cases could be ascribed to WNV infection. Evidence of acute WNV infection was identified in samples for 7 patients (Table 1). For 2 of the patients, WNV infection was identified by the presence of IgM and neutralizing antibodies in serum samples; these patients had been hospitalized for febrile illness. For the other 5 patients, infection was identified by a WNV–positive (by PCR) CSF sample (1 patient) and by the presence of neutralizing antibodies in CSF samples (4 patients); these patients had been hospitalized for neurologic signs and symptoms.

The 4 patients with neutralizing antibodies in CSF all had severe neurologic complications (Table 1). Samples from these patients were insufficient for performing IgM testing; thus, WNV infection cannot be definitively determined. However, the presence of WNV neutralizing antibody in CSF samples plus acute clinical signs and symptoms of WNV infection provide a high index of suspicion for WNV infection in these patients. Factors such as increased blood–brain barrier permeability and the persistence of WNV antibodies long after infection may also serve as explanations for the presence of neutralizing antibodies in their CSF.

Nested rRT-PCR results and phylogenetic analysis confirmed the presence of lineage 2 WNV in the CSF sample from 1 patient (Figure); sequencing showed that the
African meningococcal disease, Culex univittatus univittatus, West Nile virus, neuroinvasive disease, seroconversion, enhanced prospective surveillance, more current serologic test used.

To evaluate the sensitivity of the 3 diagnostic methods used in our study, we conducted a time-trial experiment by using retrospective serum samples from a patient in whom WNV meningoencephalitis developed after a needlestick injury (11). Early samples (postexposure days 8 and 9) were positive for WNV by rRT-PCR only. Results for samples obtained ≥13 and ≥16 days after exposure were positive by neutralization assays and IgM ELISA, respectively (Table 2). Experiments with horses have indicated that WNV neutralizing antibody assays show a positive result earlier than IgM ELISAs; the reasons for this are undetermined (14).

Using virus neutralization assays, we identified the presence of WNV antibodies in 36/204 serum samples from patients with febrile and neurologic illness in South Africa. This finding indicates that the patients were exposed to WNV. In addition, results were negative for the patients in our study who were tested for herpes simplex virus types 1 and 2, measles, mumps, and enteroviruses, and no other etiologic agent was found. Thus, infection with WNV should be included in the differential diagnosis of patients in this region with neurologic disease, especially considering the frequent detection of severe neurologic disease in horses in the region (15).

Our findings confirm that WNV is being overlooked as a cause of severe neurologic disease in South Africa, and they suggest a need for increased clinical awareness, enhanced prospective surveillance, and a more current serosurvey of WNV infection in humans. PCR may be a useful diagnostic method during early infection, but after seroconversion has taken place, serologic tests (e.g., IgM ELISA in conjunction with virus neutralization) are more likely to yield accurate results.

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**References**

1. Ansis DS, Conetta R, Teixeira AA, Waldman G, Sampson BA. The West Nile virus outbreak of 1999 in New York: the Flushing Hospital experience. Clin Infect Dis. 2003;38:413–8. http://dx.doi.org/10.1086/373737
2. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science. 1999;286:2333–7. http://dx.doi.org/10.1126/science.286.5448.2333
3. Kulasekera VL, Kramer L, Nasci RS, Mostashari F, Cherry B, Trock SC, et al. West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000. Emerg Infect Dis. 2001;7:722–5.
4. Sampathkumar P. West Nile virus: epidemiology, clinical presentation, diagnosis, and prevention. Mayo Clin Proc. 2003;78:1137–44. http://dx.doi.org/10.1016/j.mayocp.2003.11.004
5. McIntosh BM, Jupp PG. Epidemiology of West Nile and Sindbis viruses in South Africa with Culex (Culex) univittatus Theobald as vector. S Afr J Sci. 1976;72:295–300.
6. Jupp PG. The ecology of West Nile virus in South Africa and the occurrence of outbreaks in humans. Ann N Y Acad Sci. 2001;951:143–52. http://dx.doi.org/10.1111/j.1749-6632.2001.tb02692.x
7. Hayes EB, Sejvar JJ, Zaki SR, Lanciotti RS, Bode AV, Campbell GL. Virology, pathology, and clinical manifestations of West Nile virus disease. Emerg Infect Dis. 2005;11:1174–9. http://dx.doi.org/10.3201/eid1108.050289b
8. Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellingar WC, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med. 2003;348:2196–203. http://dx.doi.org/10.1086/NEJMoa022987
etymologia

Ehrlichia

[är-lik’e-ə]

Named in honor of German scientist Paul Ehrlich, Ehrlichia is a genus of gram-negative bacteria of the family Anaplasmataceae. Although Ehrlich was not a bacteriologist and was primarily known for his work in hematology, immunology, and chemotherapy, the species Ehrlichia kuroi was proposed in 1937 by Russian rickettsiologist Sh. D. Moshkovsky.

In 1889, Mikhail Georgiyevich Kurloff, perhaps working with Ehrlich, published a description of atypical granules in guinea pig leukocytes. These granules were assumed to be normal; rickettsiae would not be discovered for several decades. In his 1937 paper, Moshkovsky recognized the guinea pig granules as rickettsial inclusion bodies and named the genus “in honor of Paul Ehrlich, since it was in his laboratory that the first representatives of this group were discovered, and because he has contributed so much to the study of the morphology of the blood and of the agents of infectious diseases.”

Sources
1. Dorland’s Illustrated Medical Dictionary, 32nd ed. Philadelphia: Elsevier Saunders; 2012.
2. Dumler JS, Walker DH. Ehrlichia chaffeensis (human monocytotropic ehrlichiosis), Anaplasma phagocytophilum (human granulocytotropic anaplasmosis), and other Anaplasmataceae. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practices of infectious diseases, 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 2531–8.
3. Moshkovsky SD. Sur l’existence chez le cobaye d’une rickettsiose chronique déterminée par Ehrlichia (Rickettsia) kuroi. CR Soc Biol. 1937;126:379–82.
4. The Nobel Prize in Physiology or Medicine 1908, Ilya Mechnikov, Paul Ehrlich. Nobel Lectures, Physiology or Medicine 1901–1921. Amsterdam: Elsevier Publishing Company; 1967.
5. Silverstein AM. On the naming of rickettsiae after Paul Ehrlich. Bull Hist Med. 1998;72:731–3. http://dx.doi.org/10.1353/bhm.1998.0218

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