Pathological, Entomological, Avian and Meteorological Investigation of a West Nile Virus Epidemic in a Horse Farm


1 Institute of Comparative Medicine, College of Physicians and Surgeons, Columbia University, BB1810, 650 West 168 Street, New York, NY 10032
2 LDDC, University of Kentucky, 1429 New Town Pike, Lexington, KY 40511, USA
3 Department of Biology, University of Kentucky, Morgan Biological Science Building, Lexington, KY 40506, USA

Introduction

West Nile virus (WNV) a member of the Flaviviridae family (Calisher et al., 1989), related to Japanese Encephalitis, St Louis Encephalitis, and Murray Valley Encephalitis viruses, is an arthropod borne virus and was discovered in Uganda in 1937 (Smithburn et al., 1940). During 1999, WNV was responsible for the outbreaks of encephalomyelitis in humans and horses in New York (Nash et al., 2001). Most equine infections were thought to result in mild clinical disease or inapparent infections, with only occasional cases developing severe disease. Since the first outbreak of WNV in the continental United States, more than 24,791 horses (period, 1999–2006) were affected by WNV infection with many horses being euthanized because of grave prognosis indicating that several infections were fatal (http://www.aphis.usda.gov/vs/nahss/equine/wnv/wnv_distribution_maps.htm).

West Nile virus, like other members of the Japanese Encephalitis complex of viruses, typically circulates in nature between mosquitoes and reservoir hosts in sylvatic transmission cycles. Humans and horses are considered incidental hosts. Wild and domestic birds are the main reservoirs that may amplify the virus spread by migratory birds (Malkinson and Banet, 2002). Humans and horses become infected only during urban transmission cycle (Hurlbut, 1956; Hubalek and Halouzka, 1999). The United States WNV outbreak has been shown to infect numerous species of birds. A unique and currently unexplained aspect of the United States outbreak of WNV is that a large variety of bird species succumbed to fatal neurological disease (Nir et al., 1967; Ernek et al., 1977; Hubalek and Halouzka, 1996, 1999). During 2002, numerous wild birds in Kentucky infected with WNV died and many acted as transport and amplifying hosts for WNV (Roberts et al., 2003). The above mentioned information proves that wild birds and mosquitoes play an important role in the natural transmission of WNV in nature.

During the months of August and September, 2002, four horses in one farm that resided within a 500-m radius developed clinical signs of WNV infection. These
horses were euthanized because of grave prognosis. This report describes the pathological, the molecular biological and serological findings of affected horses, molecular biological findings of mosquitoes and wild birds collected from the horse farm epidemic, and correlates the environmental factors associated with the epidemic.

Materials and Methods

Study site

The total area of the farm in this study was approximately 2000 acres and 360 horses were kept in the farm. The horses stayed in the pasture year around. The farm had a small stream that is located near the geographic centre. During the period of July, August and September, there was not much running water in the stream except during rains. After every rain, water was retained in shallow pockets. These stagnant water collections served as a good breeding site for mosquitoes. The stream was located approximately 25 m from the pasture of horse #1 (located nearest to the stream) and approximately 300 m from horse #4 (located farthest to the stream). Horses #2 and 3 were located between these areas.

Avian and entomological investigation

At the time of the 2002 Kentucky WNV epidemic, the farm had an ongoing avian biology study in *Passer domesticus* (house sparrows) and blood was obtained on regular schedule by venipuncture from these birds. RNA was extracted from blood using Trio LS (Invitrogen Corporation, Carlsbad, CA, USA). The RT-PCR assay for WNV was carried out on the extracted RNA (Johnson et al., 2001).

Blood was obtained from 117 live birds for this study. During this same period, adult mosquitoes were trapped/collected from several sites in the farm. The adult mosquitoes collected were stored at −20°C until processed for RNA isolation. RNA was isolated from pooled samples of mosquitoes and was tested for WNV by the same procedure used on bird samples.

Meteorological data

Meteorological data of this farm were obtained from the University of Kentucky weather centre. These data included: monthly average temperature of June, July, August and September in 2002, normal monthly average temperature of the same 4 months during the last 16 years, monthly precipitation values of the same 4 months in 2002 and normal monthly average precipitation values for the same 4 months. The average monthly temperature values and precipitation values of the epidemic year were compared with corresponding values for each month during the preceding 16 years (Figs 1 and 2).

Equine studies

The four adult horses that developed severe clinical neurological signs (consisting of ataxia, head pressing, excitability, teeth grinding, in-coordination, muscle tremors of face or neck, blindness, inability to swallow, seizures, etc.) consistent with WNV infection were euthanized because of grave prognosis. Immediately following euthanasia
these four horses were submitted to the University of Kentucky, Livestock Disease Diagnostic Center for detailed pathological examination and related testing to confirm WNV infection. At necropsy, the entire brain and cervical spinal cord were grossly normal and were collected in 10% buffered formalin and were processed routinely. Sections were cut at 5 μm and stained with haematoxylin and eosin (HE) for light microscopic examination. Also a sample of brain stem and cervical spinal cord were collected for RT-PCR assay of WNV (Johnson et al., 2001). In situ hybridization for WNV nucleic acid was conducted on paraffin sections of brain stem from all the four horses (K. Latimer, personal communication). Prior to euthanasia, blood was obtained from three horses, and serum samples were tested for WNV antibody by IgM Capture ELISA (Weiss et al., 2000).

Results

Avian and entomological investigation

Of the 117 birds tested, 55 were RT-PCR positive for WNV, which accounted for 47% of the total tested for WNV. In all, 53 mosquito pools were collected, processed and tested for WNV. Culex pipiens accounted for more than 98% of the mosquitoes. All pools of C. pipiens were positive for WNV by RT-PCR assay.

Meteorological data analysis

The period of June through September during the epidemic year was drier than that of the preceding 16 years (Fig. 1). When compared with the preceding 16 years, monthly precipitation values in June, July and August of 2002 were lower (average of −2.7 for 3 months). The air temperature values in May and September were increased (by an average of +2.5°C). The average daily temperature was greater in the 4 months of June through September but was less in May.

Equine investigation

Serological examination of serum samples from three horses (horse #1, 2, 3) were positive for IgM capture ELISA. In three horses (horse #1, 2, 3), the RT-PCR for WNV was positive in the brain stem tissues. In situ hybridization of the brain stem tissue sections were positive for WNV in all the four horses. Histopathological findings were observed in the brain and spinal cord of all the four horses in the study and consisted of perivascular cuffing of lymphocytes and macrophages, mild gliosis and occasional neuronophagia in the ventral and dorsal horns of cervical spinal cord (Fig. 3), perivascular cuffing of lymphocytes within the perineuronal area of dorsal gan-

Discussion

The meteorological data during the period of June 2002 through September 2002 showed that there were several days of rain that resulted in times of intermittent running water in the stream located at the centre of the epidemic area. Mostly, however, the high temperature and drier days resulted in stagnation of water, forming water pockets in this stream bed. The stagnant water pools formed ideal sites for the multiplication of mosquitoes. Dry summers (less rainfall) have been associated with increased transmission and epidemics of St Louis encephalitis virus (SLE) (a virus related to WNV) in urban areas of the USA (Lumsden, 1958; Reiter, 1988). The primary urban vectors of SLE virus are members of the C. pipiens mos-
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model starts with the introduction of virus by the migratory birds. The virus may have entered a sylvatic cycle between \textit{C. pipiens} and birds. The virus may have moved horizontally involving \textit{C. pipiens} and birds via birds infected in the sylvatic cycle. The inclusion of the sylvatic cycle is based on the French epidemic of 1962 (Mouchet et al., 1970). Although we do not have direct evidence that such a cycle occurred in this particular farm, \textit{C. pipiens} is a common mosquito in the stream where the larva are often encountered in small water bodies with emergent vegetation (Nicolescu, 1998). \textit{Culex} sp may display a broad host range and readily attacks horses. The sylvatic population of \textit{C. pipiens} in Europe appears strongly ornithophilic and this species is likely more important in amplification of virus (Jaenson, 1990) among birds than in transmitting virus to humans and other species in natural areas. Transmission to horses may occur from mosquitoes infected in this cycle.

The present study is a classic example of increased presence of vectors and a favourable weather pattern with the presence of an amplifying host establishing a regular cycle, and non-immunized horses exposed to WNV leading to an outbreak of WNV infection in a small area.

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**References**


