Phylogenetic Analysis of West Nile Virus, Nuevo Leon State, Mexico

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West Nile virus RNA was detected in brain tissue from a horse that died in June 2003 in Nuevo Leon State, Mexico. Nucleotide sequencing and phylogenetic analysis of the premembrane and envelope genes showed that the virus was most closely related to West Nile virus isolates collected in Texas in 2002.

The Study

Cerebellar tissue was taken from a dead 12-year-old stallion from a privately owned ranch in the municipality of Juarez in Nuevo Leon State, Mexico, approximately 240 km south of the Texas border. The horse was first observed with neurologic symptoms on June 20, 2003, and it was euthanized 7 days later. The horse had never been outside the state of Nuevo Leon and had not been vaccinated against WNV. The tissue sample was immediately placed on dry ice and transported to the biosafety-level-3 facilities at Colorado State University for processing. Although we were unable to isolate virus from the sample by passing brain homogenate in Vero cells, we successfully amplified viral RNA.

Total RNA was extracted from approximately 100 µg of cerebellar tissue with Trizol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions. The prM-E genes were amplified as two fragments by reverse transcription–polymerase chain reaction (RT-PCR) by using primers designed from the nucleotide sequence of the prototype WN-NY99 strain (GenBank accession no. AF196835). PCR amplifications were performed by using Ex Taq DNA polymerase (Takara Biomedicals, Shiga, Japan), which has 3′→5′ exonuclease activity. Amplification products were separated by agarose gel electrophoresis, visualized with crystal violet, and extracted by using the rapid gel extraction system (Invitrogen, Carlsbad, CA). The resulting DNA fragments were reamplified by PCR because of the low RNA copy number in the original material and purified by using the QIAquick PCR purification kit (Qiagen, Valencia, CA). Purified DNAs were sequenced on both strands with an ABI 377 DNA sequencer (Davis Sequencing, Davis, CA) and eight pairs of WNV-specific primers.

The nucleotide sequence of the prM-E genes of the WNV from Nuevo Leon State, Mexico (designated MexNL-03) was submitted to GenBank (GenBank accession no. AY426741). This region comprises 2004 nucleotides and corresponds to nucleotides 466 to 2469 of the genomic RNA of the WN-NY99 strain (8). Alignment of the MexNL-03 sequence with other known sequences in the GenBank database showed that it was most closely related to the homologous regions of three WNV isolates collected in Harris County, Texas in 2002 (13).
Table. Nucleotide and deduced amino acid differences in the premembrane and envelope genes of the West Nile virus from Nuevo Leon State compared with various other West Nile viruses

<table>
<thead>
<tr>
<th>Strain</th>
<th>Geographic origin</th>
<th>Nucleotide no.(^a)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WN-NY99(^a)</td>
<td>New York, NY</td>
<td>483 U 549 C 660 C 858 C 887 C 1137 C 1179 C 1356 C 1432 C 1442 C 549 C 1626 C 2328 C 2388 C 2466 C</td>
<td>8</td>
</tr>
<tr>
<td>MexNL-03(^b)</td>
<td>Nuevo Leon State, northern Mexico</td>
<td>549 C 660 U</td>
<td></td>
</tr>
<tr>
<td>TM171-03(^c)</td>
<td>Tabasco State, southern Mexico</td>
<td>1356 U 1432 C 1442 U 2466 C</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Harris Co., inland Texas</td>
<td>119 U</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Nucleotide numbers correspond to those of the prototype New York strain (WN-NY99).

\(^b\)Isolated from a Chilean flamingo (Phoenicopterus chilensis) (collection date: 06/01/09). GenBank accession no.: AF196835.

\(^c\)Amino acid changes are shown in parentheses. Gaps indicate no change in the nucleotide sequence.

\(^d\)Isolated from a horse (collection date: 06/27/03). GenBank accession no.: AY426741.

\(^e\)Isolated from Common Raven (collection date: 05/05/03). GenBank accession no.: AY371271.

\(^f\)Isolated from a bluejay (Cyanocitta cristata) and passaged once in Vero cells (collection date: 06/14/02). GenBank accession no. AY185908.
WNV isolates circulating in the United States and Mexico diverged from a common ancestor from the northeastern United States.

The trees generated by NJ, MP, and ML analyses showed the same overall topologic features to the Bayesian tree, except that all of the coastal Texas isolates were basal to the Israeli isolate (data not shown). The bootstrap support for this topologic arrangement ranged from 58% to 73%. Similar findings were also reported by Estrada-Franco et al. (5). Furthermore, the WNV isolates from Maryland and New Jersey, as well as most of the isolates from New York, occupy the basal positions of clade 1 of the NJ/MP/ML trees. As a result, we have not shown the bootstrap values for clades 1 and 2 of the Bayesian tree because their composition does not match exactly to the corresponding clades of the NJ/MP/ML trees. However, the Bayesian analysis provides a more robust and efficient phylogenetic tool compared to more conventional phylogenetic techniques (15). Additional sequencing and phylogenetic analyses will be necessary to clarify these issues.

Conclusions

The data presented here indicate that WNV was introduced into Nuevo Leon State, Mexico, from inland Texas. A likely mode of introduction was by infected birds traveling for short distances (16). Earlier studies have provided serologic evidence of WNV infection in horses or birds in the nearby Mexican states of Coahuila, Tamaulipas, and Chihuahua (4,5,17). Taken together, our sequence data and the findings from the serosurveys indicate that WNV activity is now widespread in northern Mexico, as well as in other regions in Mexico (5,6,18). The geographic distribution of WNV in the Americas will likely continue to expand; thus, enhanced WNV surveillance in Mexico is warranted.

Figure 1. Phylogenetic analysis of West Nile virus (WNV) from Nuevo Leon State, Mexico. Phylogenies were estimated by using the program MRBAYES, version 2.0 (15). Sampling of trees from the posterior probability distribution used the Metropolis-coupled Markov chain Monte Carlo algorithm to allow running of multiple Markov chains. A run with four chains was performed for 90,000 generations, under a general time-reversible model (all six types of substitutions occur at different rates) with parameter value estimation for base frequencies, substitution matrix values, and rate heterogeneity. Rate heterogeneity was estimated by using a $\gamma$ distribution for the variable sites and assuming a certain portion of sites to be invariable. The burn-in time was 70,000 generations. The phylogenetic analysis is based on the 2004-nt fragment encoding the complete prM-E genes of 49 WNVs. The tree is rooted by using the prototype WNV strain from Uganda in 1937 (GenBank accession no. M10103) as an outgroup. Values above some branches represent the percentage support by parsimony bootstrap analysis. Values below some branches represent the percentage support by distance bootstrap analysis. The bootstrap confidence estimates are based on 1,000 replicates. The WNV from Nuevo Leon State is encapsulated.
Acknowledgments

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References


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