Short Report: First Isolation of West Nile Virus in the Caribbean

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Abstract. A sentinel chicken program for West Nile virus (WNV) surveillance was initiated in July 2006 in eastern Puerto Rico, yielding the first seroconversions on June 4, 2007. WNV was isolated from sentinel chicken serum and mosquito pools (Culex nigripalpus, Culex bahamensis) for the first time in Tropical America. Preliminary sequence analysis of the prM and E genes revealed a 1-amino acid difference (V159A) between the Puerto Rican 2007 and the NY99. This mutation has been observed in the current dominant clade circulating in the United States. Sentinel chicken surveillance was a useful tool for the detection of West Nile virus in the tropics.

WNV has been spreading southward into the Caribbean Basin and Latin America since 2001 when the first human case was reported from the Cayman Islands. Serological evidence of WNV transmission has been accumulating, and cross-reactive WNV antibodies have been detected in humans from Mexico, The Bahamas, and Cuba, in horses from Guadalupe, Mexico, Central America, Cuba, Puerto Rico, Colombia, and Venezuela, and in resident birds from Jamaica, Dominican Republic, Cuba, Puerto Rico, and Venezuela. In November 2006, 4 serologically confirmed human WNV encephalitis cases were reported by Argentina. WNV may be following the same patterns of southward dissemination as other arboviruses into the Caribbean and Central and South America via migratory birds.

In spite of growing evidence for the presence of WNV in tropical America, no outbreaks have yet been reported from the region. It is also interesting to note that high bird mortality, as observed in the Nearctic, has not been reported. WNV has been isolated in tropical America from a dead raven in Tabasco, Mexico (18° N lat.), and in subtropical Argentina (23°49' S lat.) from dead horses, but no isolates have yet been reported from other areas in Central and South America or the Caribbean. One explanation for the paucity in WNV detection in tropical America may be the lack of active surveillance. One would hypothesize that the likelihood of identifying or isolating WNV would be maximized in the Caribbean Basin, given its location in the flight paths of infected migratory birds. Another plausible explanation is that WNV has not been able to establish persistent enzootic foci in the region, so that reported serological evidence may be caused by frequent but unsuccessful virus re-introductions.

Here, we report the first WNV isolation in the Caribbean, specifically from sentinel chicken serum and mosquitoes in natural and rural areas of eastern Puerto Rico.

To investigate the presence of WNV in Puerto Rico, a sentinel chicken program was established in July 2006 in Ceiba and Naguabo municipalities, near the place where a resident bird was previously found with WNV antibodies and close to the area where the Puerto Rico passive surveillance system detected 3 asymptomatic horses with WNV antibodies in 2004. The research protocol was approved by CDC’s Animal Care and Use Committee (06-012). Sixty sentinel chickens were pretested for flavivirus reactive antibodies and placed in 12 pens, each housing 5 chickens. The 12 pens were placed in 8 natural habitats (2 herbaceous wetlands, 2 mangrove forests, 2 deciduous forests, and 2 evergreen forests) and in 4 nearby human populated areas (2 rural and 2 urban areas). Blood samples were taken from all sentinel chickens every 2 weeks and tested by a chicken-specific MAC-ELISA; 1,247 blood samples were analyzed before initial seroconversion was detected (July–June 2006).

The first seroconversion of 7 chickens (12%) was detected in the sentinel chickens in 4 pens from natural areas (1 wetland, 1 mangrove forest, and 2 evergreen forests) in April, 2007. Following initial seroconversion, bleeding and sentinel replacement were performed weekly. Seroconversion increased to 40% on June 11, 2007, and was detected in chickens from 11 out of the 12 pens, covering all types of habitats including rural and urban areas. Chickens continued to seroconvert at high rates (max. 45%) throughout the rest of June and July, but the rate sharply declined during August 2007 (2%). Seroconversion has continued through October 2007 indicating a sustained low level of transmission (2–6%).

Plaque reduction neutralization tests (PRNT90) against WNV and Saint Louis encephalitis virus (SLE) were used to confirm that the infecting virus was WNV (Table 1). Serum samples were heat inactivated at 56°C for 30 minutes and serially diluted 2-fold in phosphate-buffered saline (PBS) with 30% heat-inactivated fetal bovine serum. One hundred microliters of 1:20 diluted serum was mixed with an equal volume of diluents containing ChimeraVax WNV or SLE virus and analyzed in duplicate (Acambis, Cambridge, MA). One hundred microliters of the serum–virus suspension was used to inoculate a confluent monolayer of Vero cells in 12-well plates and incubated at room temperature for 1 hour. Two milliliters of medium containing 10% M199 without phenol red, 1% essential amino acids, 1% vitamins, 1% glutamine, 5% inactivated FBS, 0.4% gentamicin, 4% sodium bicarbonate, and 0.6% agarose was added to each well and placed in a 37°C 10% CO2 incubator for 4 days for WNV and 5 days for SLE virus. Plaques were stained by adding 500 μL of PBS containing 3.2% neutral red to each well at 3 or 4 days post-infection for WNV and SLE virus, respectively. Plaques were counted 24 hours later, and endpoint titers were expressed as the reciprocal of serum dilutions yielding ≥ 90% reduction in the number of plaques. Controls included virus only and virus with normal serum.

Chicken serum specimens that were collected a week prior to seroconversion were tested by RT-PCR to attempt virus isolation from these samples. This led to positive identifi-
culation of WNV RNA in 1 of the sentinel chickens and subsequently WNV isolation from this chicken serum in Vero cells.

Mosquito captures were performed around the positive pen areas using CDC miniature light/CO₂ traps and CDC gravid traps. Captured mosquitoes were preserved on dry ice, sorted in pools (50 females per pool), and stored at -70°C until they were tested by RT-PCR. The following mosquito species were initially found infected with WNV: Culex nigripalpus (51 positive out of 101 pools tested; 50.5%), Culex bahamensis (3/8; 37.5%), and Culex quinquefasciatus (4/46; 8.7%). WNV isolations were made in both C6/36 and Vero cells¹⁶. 4 from Cx. nigripalpus pools and 1 Cx. bahamensis. Virus isolations were confirmed by RT-PCR and by specific immunostaining using West Nile/Kunjun 393 monoclonal antibody (CDC, catalog no. m28955A) and FITC-conjugated anti-mouse IgG (KPL, Inc., Gaithersburg, MD) in the presence of 4’,6-diamino-2-phenylindole, DAPI (Sigma, St. Louis, MO) according to previously published procedures in both cell lines.¹⁶ Preliminary sequence analysis of the prM and E genes (GenBank library accession number: EU394703) revealed one amino acid difference (V159A) between Puerto Rican 2007 and NY99. This mutation has been observed in the current endemic, will require careful interpretation of serologic tests and assiduous efforts to isolate the infecting virus. Our preliminary genetic analysis of the WNV Puerto Rico isolate from a sentinel chicken resulted in a 99.8% homology with NY99 strain, and further studies are being conducted to determine its pathogenesis.

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