

Figure. Temperature and heart rate relationship: scatter plot for patients with dengue fever and nondengue febrile illnesses.

the etiology of dengue-associated relative bradycardia.

**Aisha Lateef,\***

**Dale Andrew Fisher,\*†**

**and Paul Ananth Tambyah,\*†**

\*National University Hospital, Singapore; and †National University of Singapore, Singapore

## References

- Guzman MG, Kouri G. Dengue: an update. *Lancet Infect Dis.* 2002;2:33–42.
- Fisher D. To the vector borne...mosquito-transmitted diseases in Singapore. *Singapore Med J.* 2005;46:596.
- Wilder-Smith A, Earnest A, Paton NI. Use of simple laboratory features to distinguish the early stage of severe acute respiratory syndrome from dengue fever. *Clin Infect Dis.* 2004;39:1818–23.
- Guzman MG, Kouri G. Dengue diagnosis, advances and challenges. *Int J Infect Dis.* 2004;8:69–80.
- Ostergaard L, Huniche B, Andersen PL. Relative bradycardia in infectious diseases. *J Infect.* 1996;33:185–91.
- Cunha BA. The diagnostic significance of relative bradycardia in infectious disease. *Clin Microbiol Infect.* 2000;6:633–4.
- Khongphathanayothin A, Suesawalak M, Muangmingsook S, Bhattarakosol P, Pancharoen C. Hemodynamic profiles of patients with dengue hemorrhagic fever during toxic stage: an echocardiographic study. *Intensive Care Med.* 2003;29:570–4.

- Wali JP, Biswas A, Chandra S, Malhotra A, Aggarwal P, Handa R, et al. Cardiac involvement in dengue haemorrhagic fever. *Int J Cardiol.* 1998;64:31–6.
- Blatteis CM, Li S, Li Z, Feleder C, Perlik V. Cytokines, PGE2 and endotoxin fever: a reassessment. *Prostaglandins Other Lipid Mediat.* 2005;76:1–18.
- Fink J, Gu F, Vasudevan SG. Role of T cells, cytokines and antibody in dengue fever and dengue haemorrhagic fever. *Rev Med Virol.* 2006;16:263–75.

Address for correspondence: Paul Ananth Tambyah, Department of Medicine, National University of Singapore, 5 Lower Kent Ridge Rd, Singapore 119074; email: mdcpat@nus.edu.sg



## West Nile Virus, Venezuela

**To the Editor:** West Nile virus (WNV; genus *Flavivirus*; family *Flaviviridae*) has been perpetuating in North America since 1999 (1). However, its status as a self-perpetuating pathogen in South America remains uncertain. Infected horses and birds have been reported in various Caribbean Islands, Mexico, and northern Central America (2,3). In South America, isolated reports of infected dead-end hosts (horses) have come from northern Colombia and Argentina, but they lack evidence for infection in avian amplifying hosts (4,5). We report serologic evidence of establishment of WNV in South America.

Serum samples from birds and horses from 33 locations in Venezuela (Online Appendix Table, available from <http://www.cdc.gov/EID/content/13/4/651-appT.htm>) were screened for immunoglobulin G (IgG) antibodies against WNV antigen by ELISA (6) and confirmed by plaque reduction neutralization test (PRNT) as previously described (7). The flavivirus generating the IgG response was identified by using the following criteria: 90% inhibition of virus in serum diluted at least 1:40 and 4-fold greater neutralizing antibody titer compared with closely related flaviviruses. IgG antibody against flavivirus was detected by ELISA in 14 of 576 resident birds, including 5 *Turdus leucomelas*, 3 *Gallus gallus* (captive), 2 *Campylorhamphus trochilirostris*, and 1 each of *Elaenia flavogaster*, *Coereba flaveola*, *Thraupis palmarum*, and *Anisognathus flavinucha*.

WNV was confirmed as the etiologic agent of infection in 5 adult birds (3 *T. leucomelas* [pale-breasted thrush], 1 *C. flaveola* [bananaquit], and 1 *G. gallus* [domestic chicken] with the earliest collection date in February 2006); virus neutralization

titers ranged from 80 to 320. One serum sample cross-reacted with other flaviviruses tested, with equivalent titers to WNV, Saint Louis encephalitis virus (SLEV), and Ilheus virus (ILHV) and was thus considered infected with an undetermined flavivirus. Seven serum samples were negative (antibody titers <20), and 1 sample was not tested because of insufficient sample volume.

Antibody against flavivirus was detected by ELISA in 141 of 791 horses, and 34 (4.3%) were confirmed positive for WNV infection by PRNT; viral titers  $\geq 640$  occurred in half of these horses. The earliest collection date for a WNV-positive horse was February 2004 and the most recent was May 2006. Specific WNV-reactive equine serum samples were distributed in valley regions (prevalence 1.3%), savannah grasslands (2.4%), the western region of Zulia (0.4%) and the Central Lake Basin (0.3%). A total of 46 (5.8%) equine serum samples were positive for neutralizing antibody to SLEV, and 8 (1.0%) samples were positive for neutralizing antibodies to ILHV. Forty-nine samples neutralized at least 2 of the 3 viruses and were classified as undetermined flaviviruses. Serum samples from 2 horses were negative in neutralization assays; 2 others were not tested because of insufficient sample volume.

Detection of WNV-infected resident birds provides strong evidence of the establishment, rather than importation, of WNV in South America. We hypothesize that ornithophilic mosquitoes (such as some *Culex* spp.), which are present in the area in consistently high numbers, acquired the virus through hematophagous feeding on recently infected, migrating birds. Once introduced to local mosquitoes, virus is amplified among susceptible resident birds fed upon by ornithophilic mosquitoes. This pattern allows perpetuation and subsequent establishment of virus in a continuous transmission cycle, as opposed to

infection of dead-end hosts, e.g., horses. This is the first report of WNV infection in South American birds and definitive establishment of the virus in South America.

We observed varying WNV seroprevalence rates in birds and horses across regions in Venezuela (Figure). These differences reflect the focal and stochastic nature of arbovirus transmission, which depends upon many ecologic factors. One possible explanation for the greater seroprevalence in the central and eastern llanos (savannahs) and valley regions, compared with the coastal western region of Zulia State ( $p < 0.0001$ , by Pearson's  $\chi^2$  test) would be virus introduction by migrating birds by an eastern migration route.

Existence of several closely related flaviviruses in the American tropics (8–10) may convey cross-protection in animals (e.g., ILHV and SLEV) or humans (dengue viruses, yellow fever virus), thereby potentially diminishing disease caused by a newly introduced flavivirus such as WNV. Although ILHV infection has

not been detected in Venezuela, this flavivirus is prevalent in Brazil, Peru, French Guyana, Trinidad, and Colombia. Our study demonstrated widespread distribution of ILHV in Venezuela. Other South American flaviviruses, such as Bussuquara, Cacipacore, and Iguape, and as yet undiscovered viruses may also circulate in Venezuela.

We encourage those involved in the public and animal health systems in Venezuela to consider zoonotic flaviviruses in the differential diagnoses of encephalitis and to consider ecologic surveillance for zoonotic flaviviruses in mosquito and vertebrate host populations. We recommend monitoring blood and organ donations for flavivirus infections. Our study sheds light on flavivirus distribution in Venezuela. However, nothing else is known about the ecology of zoonotic flaviviruses in this country. Such knowledge will be essential for designing effective surveillance and control should these viruses be shown to cause human illnesses.

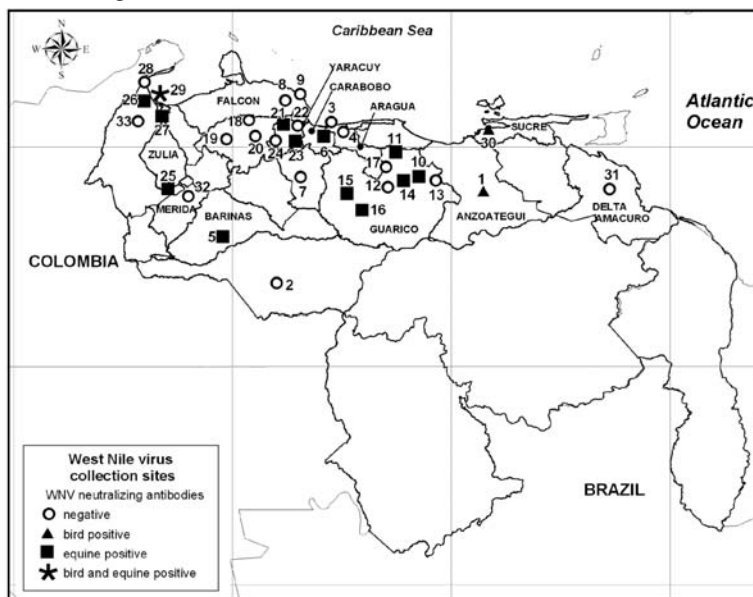


Figure. West Nile virus (WNV) collection sites in Venezuela, indicated by number (see online Appendix Table). Symbols represent results of tests for specific antibodies to WNV in serum samples of birds and horses (viral titers in a 90% plaque reduction neutralization test  $>40$  and a 4-fold differential inhibition in a neutralization assay to WNV compared with other related flaviviruses). Source: Instituto Geográfico de Venezuela Simón Bolívar, Caracas.

## Acknowledgments

We dedicate this work to the late Andrew Spielman, our coauthor and beloved mentor. We thank J. Rivero, I. Pérez, M. Méndez, I. Matheus, M. Aguiar de Bracho, I. Carreño, J.M. Hernández, A. Nagy, A. Suarez, N. Moncada, M. Kilpatrick, E. Rodríguez, E. Marquez, E. Marian, B. Hernández; C. Rivero-Blanco, M. Azar, J. Rodríguez, H. Montañez, F. Alfonzo, and G. Rangel for their contributions to this study; and the Centro de Investigaciones Biomédicas, Universidad de Carabobo and the Cell Culture Core of Wadsworth Center, New York State Health Department, for their support. We also thank ProFauna and the National Institute of Parks in Venezuela for permission to obtain mosquito and bird samples.

This study was supported by grant AI45440 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and an International Collaborations in Infectious Disease Research opportunity pool grant.

**Irene Bosch,\* Flor Herrera,†  
Juan-Carlos Navarro,‡  
Miguel Lentino,§ Alan Dupuis,¶  
Joseph Maffei,¶# Matthew Jones,¶#  
Ernesto Fernández,\*\*  
Nelson Pérez,†† Jorge Pérez-Emán,‡  
Anthony Érico Guimarães,‡‡  
Roberto Barrera,§§  
Nereida Valero,¶¶ Johanny Ruiz,†  
Glenda Velásquez,### Juan Martínez,‡  
Guillermo Comach,†  
Nicholas Komar,\*\*\*  
Andrew Spielman,†††<sup>1</sup>  
and Laura Kramer¶#**

\*University of Massachusetts Medical School, Worcester, Massachusetts, USA; †Universidad de Carabobo Biomed, Maracay, Venezuela; ‡Universidad Central de Venezuela, Caracas, Venezuela; §Colección Ornitológica Phelps, Caracas, Venezuela; ¶New York State Department of Health, Albany, New York, USA; #State University of New York at Albany, Albany, New York, USA; \*\*Universidad Central de Venezuela, Maracay, Venezuela; ††Instituto Nacional de Investigaciones

Agrícolas, Maracay, Venezuela; ‡‡Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; §§Centers for Disease Control and Prevention, San Juan, Puerto Rico, USA; ¶¶Universidad del Zulia, Maracaibo, Venezuela; ###Ministerio de Salud Insalud, Carabobo, Venezuela; \*\*\*Centers for Disease Control and Prevention, Fort Collins, Colorado, USA; and ††† Harvard School of Public Health, Boston, Massachusetts, USA

## References

- Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. Epidemiology and transmission dynamics of West Nile virus disease. *Emerg Infect Dis.* 2005;11:1167–73.
- Komar N, Clark GG. West Nile virus activity in Latin America and the Caribbean. *Rev Panam Salud Publica.* 2006;19:112–7.
- Morales-Betoulle ME, Morales H, Blitvich BJ, Powers AM, Davis EA, Klein R, et al. West Nile virus in horses, Guatemala. *Emerg Infect Dis.* 2006;12:1038–9.
- Mattar S, Edwards E, Laguado J, Gonzalez M, Alvarez J, Komar N. West Nile virus antibodies in Colombian horses. *Emerg Infect Dis.* 2005;11:1497–8.
- Morales MA, Barrandeguy M, Fabbri C, Garcia GB, Vissani A, Trono K, et al. West Nile virus isolation from equines in Argentina, 2006. *Emerg Infect Dis.* 2006;12:1559–61.
- Ebel GD, Dupuis AP II, Nicholas D, Young D, Maffei J, Kramer LD. Detection by enzyme-linked immunosorbent assay of antibodies to West Nile virus in birds. *Emerg Infect Dis.* 2002;8:979–82.
- Dupuis AP II, Marra PP, Kramer LD. Serologic evidence of West Nile virus transmission, Jamaica, West Indies. *Emerg Infect Dis.* 2003;9:860–3.
- Figueiredo LT. The Brazilian flaviviruses. *Microbes Infect.* 2000;2:1643–9.
- Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus *Flavivirus*. *J Virol.* 1998;72:73–83.
- Calisher CH, Monath TP, Karabatsos N, Trent DW. Arbovirus subtyping: applications to epidemiologic studies, availability of reagents, and testing services. *Am J Epidemiol.* 1981;114:619–31.

Address for correspondence: Irene Bosch, Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School, 55 Lake Ave North, Worcester, MA 01655, USA; email: irene.bosch@umassmed.edu

## Novel Extended-spectrum $\beta$ -Lactamase in *Shigella sonnei*

**To the Editor:** A 38-year-old French man with a history of chronic juvenile arthritis was referred to the Necker-Enfants Malades University hospital (Paris, France) with a dysenteric syndrome. The patient had returned the day before from a 1-month stay in Port-au-Prince, Haiti, where he spent most of his time in close contact with young children from an orphanage, most of whom had diarrhea. Clinical examination at admission showed fever (39°C), chills, diffuse abdominal pain, bloody diarrhea, and vomiting. The patient received ceftriaxone, which was stopped on day 4 because initial blood and stool cultures were negative for pathogens and clinical signs had completely resolved.

Ten days later, he reported the recurrence of diarrhea without fever. A novel stool culture grew *Shigella sonnei*. An extended-spectrum  $\beta$ -lactamase (ESBL) was detected by double-disk synergy test; the isolate was also resistant to aminoglycosides (except amikacin), tetracycline, and cotrimoxazole. The strain was susceptible to fluoroquinolones and fosfomycin. It also appeared susceptible to azithromycin (MIC 4  $\mu$ g/mL), although azithromycin MIC for *Shigella* spp. should be interpreted with caution (1). The patient was successfully treated with azithromycin at a dose of 500 mg/day for 5 days. Azithromycin was preferred to fluoroquinolones to avoid the risk for tendinopathy because of the patient's history of chronic juvenile arthritis and because this antimicrobial agent was shown to be effective in the treatment of shigellosis caused by multidrug-resistant strains (2).

To identify the molecular basis of this ESBL, a series of PCR primers

<sup>1</sup>Deceased.

Online Appendix Table. Locations in Venezuela sampled for West Nile virus

ID	State	County, Location	Regional ecosystem*	No. serum samples tested (no. positive)†	
				Birds	Equines
4	Aragua	Guirardot, Portachuelos	Coast	96 (0)	
8	Falcón	Manuere, Guayabal	Coast		7 (0)
25	Zulia	Francisco J. Pulgar	Coast		6 (1)
26	Zulia	Mara	Coast		9 (1)
33	Zulia	La C. de Urdaneta	Coast	12 (0)	
27	Zulia	Maracaibo	Coast		327 (1)
28	Zulia	Paez, Goajira	Coast		6 (0)
2	Apure	Romulo Gallegos	Llanos		25 (0)
10	Guarico	Rivas	Llanos		43 (1)
11	Guarico	Monagas, Altgr. Orituco	Llanos		6 (3)
13	Guarico	Ortiz, Zaraza	Llanos		5 (0)
14	Guarico	Infante, V. de la Pascua	Llanos		18 (3)
15	Guarico	Miranda, Guarico Dam	Llanos		50 (3)
16	Guarico	Miranda	Llanos		13 (5)
21	Yaracuy	Bolivar	Valley		27 (2)
23	Yaracuy	S. Felipe, Guaquira farm	Valley	75 (0)	24 (8)
22	Yaracuy	Veroes	Valley		18 (0)
5	Barinas	Pedraza	Llanos		9 (4)
7	Cojedes	Pao	Llanos		4 (0)
12	Guarico	Las Mercedes	Llanos		3 (0)
17	Guarico	Monagas	Llanos		4 (0)
18	Lara	Urdaneta	Valley		1 (0)
19	Lara	Torres	Valley		3 (0)
20	Lara	Iribarren	Valley		3 (0)
24	Yaracuy	Sucre	Valley		1 (0)
32	Merida	Libertador, Merida	Mountain		5 (0)
3	Aragua	Girardot, Cata farm	Coast	52 (0)	1 (0)
6	Carabobo	Valencia, Lake	Valley	16 (0)	173 (2)
29	Zulia	Paez, Sinamaica Lagoon	Coast	142 (1)	
30	Sucre	Sucre, Cumana	Coast	7 (1)	
1	Anzoategui	Miranda, F. El Tigre	Llanos	56 (3)	
9	Falcón	Iturriza, Cuare Reserve	Coast	48 (0)	
31	Delta Amacuro	Tucupita	Delta system	72 (0)	
	Total			576 (5)	791 (34)

\*Llanos, savannah.

†Positive by 90% plaque reduction neutralization test.