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SHORT REPORT: SEROLOGICAL EVIDENCE OF WEST NILE VIRUS ACTIVITY IN EL SALVADOR

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Abstract. Epizootics of encephalitis in El Salvador killed 203 equines between November 2001 and April 2003. During an investigation of the outbreaks, 18 (25%) of 73 serum samples collected from stablemates of deceased animals in 2003 had antibodies to West Nile virus. Ten of these infections were confirmed by plaque reduction neutralization tests, suggesting West Nile virus has extended its range and spread to Central America.

Since its introduction into North America in 1999, West Nile virus (WNV) has caused disease in thousands of humans, equines and birds.[1–3] By the end of September 2004, the WNV epizootic/epidemic had spread to most of the continental United States and Puerto Rico.[1] Recent reports also indicate that WNV is now widely distributed in Mexico.[4–7] In March 2002, anecdotal reports of equine deaths in Berlin, Usulutan, El Salvador, prompted a field investigation by the El Salvador (Central America) Ministry of Health and Welfare's Field Epidemiology Training Program (FETP). We report preliminary findings of the ongoing investigation of these epizootics.

Information gathered from horse owners confirmed the occurrence of equine deaths beginning in November 2001. By the second week of March 2002, equine deaths had been reported in 11 of the 19 villages of the municipality of Berlin, in the State of Usulutan, (Figure 1). A few weeks later, another outbreak was reported and investigated in Jutiapa, El Salvador. An estimated 10% of the equines in this area succumbed to encephalitis during the outbreak. Clinically, at least 70% of the equine deaths and the few reports of illness among surviving animals described physical signs consistent with ataxia (stumbling, staggering, incoordination), head tilt, muscular fasciculation, circling, or paralysis. El Salvador's Ministry of Agriculture (MOA) initially suspected anthrax, whereas the Ministry of Health (MOH) suggested a viral etiology such as Venezuelan equine encephalitis virus (VEEV) as the cause of these deaths, because VEEV caused epizootics in Central America in 1969–1972.[8] Because of the lack of confirmed equine or human VEEV infections (i.e., lack of virus isolation, demonstration of viral RNA, or serological evidence), the FETP obtained 60 serum samples from horses and 18 from humans exhibiting acute fever in Berlin at the time of the outbreak investigation and sent these to the University of Texas Medical Branch for analysis.

Forty-three equine serum samples were assayed by hemagglutination inhibition (HI) tests for antibodies to VEEV, eastern equine encephalitis virus (EEEV), and WNV. Eight (19%) of these had HI antibody titers, suggesting they were infected with WNV or another closely related virus. All but one of these serum samples also had lower levels of HI antibodies to Saint Louis encephalitis (SLEV) and Ilheus viruses. Two of those eight samples had titers $\geq$ 320 by 90% plaque reduction neutralization tests ($PRNT_{90}$) with the NY99 WNV (strain 385-99); the other six had titers ranging from 20 to 160. No virus was isolated from the equine sera, nor from brain tissue from a recently deceased horse from the area. Five samples had HI antibodies to dengue virus serotypes 1–4 (< 1:160).

Surveillance activities were subsequently enhanced and weekly reports of equine deaths and field research results were prepared by FETP and MOA teams. By April 2003, 203 equine deaths were reported from 6 states. All occurred between November 2001 and March 2002 and between November 2002 and April 2003. According to estimates of the MOA, 60,000 equines were present in El Salvador in 2000. Reports were entered into a geo-referenced database (Epi2000) and analyses were performed using different layers (humidity, land use, etc.). Eight of the nine foci of equine deaths occurred in three areas: Berlin, Jutiapa, and near Tamanique (Figure 1). Entomological evaluations conducted by El Salvador MOH entomologists reported the presence of *Culex nigripalpus* and *Culex quinquefasciatus* in the premises where equines had died in Berlin. At the time of this report, no testing for WNV infection in mosquitoes had been conducted.

Equine encephalitis deaths occurred in only 23 of the 2079 townships across El Salvador. Equine deaths formed 12 separated and discrete geographic foci ranging from 1 to 36 deaths each (Figure 1). All were either within ($N = 10$) or adjacent to ($N = 2$) steeply sloped (15–50%) terrain broken by deep gullies of temporary watercourses. The most affected area was Berlin, Usulutan (25,000 inhabitants) (area A in Figure 1). In one village of Berlin, San Felipe, 90 horses were enumerated in 2002 during a census carried out on behalf of the MOA. By April of 2003, 54 horses had died.

Samples taken from stable mates of equines that had died of neurologic illness were sent to the Centers for Disease Control and Prevention in Ft. Collins, Colorado, and tested by enzyme-linked immunosorbent assay (ELISA) for IgG and IgM antibodies to WNV and VEEV and by $PRNT_{90}$ for antibody against WNV and SLEV.[9] Twenty-three samples (from a total of 30 tested) with positive results in the initial screen are shown in Table 1; these sera were further tested to elucidate the likely infecting flavivirus by $PRNT_{90}$ against a panel of flaviviruses considered most prevalent in Central America (Table 2). Ten of the serum samples had results suggestive of WNV infection with the $PRNT_{90}$ antibody titers 4-fold or more greater for WNV than for SLEV or other

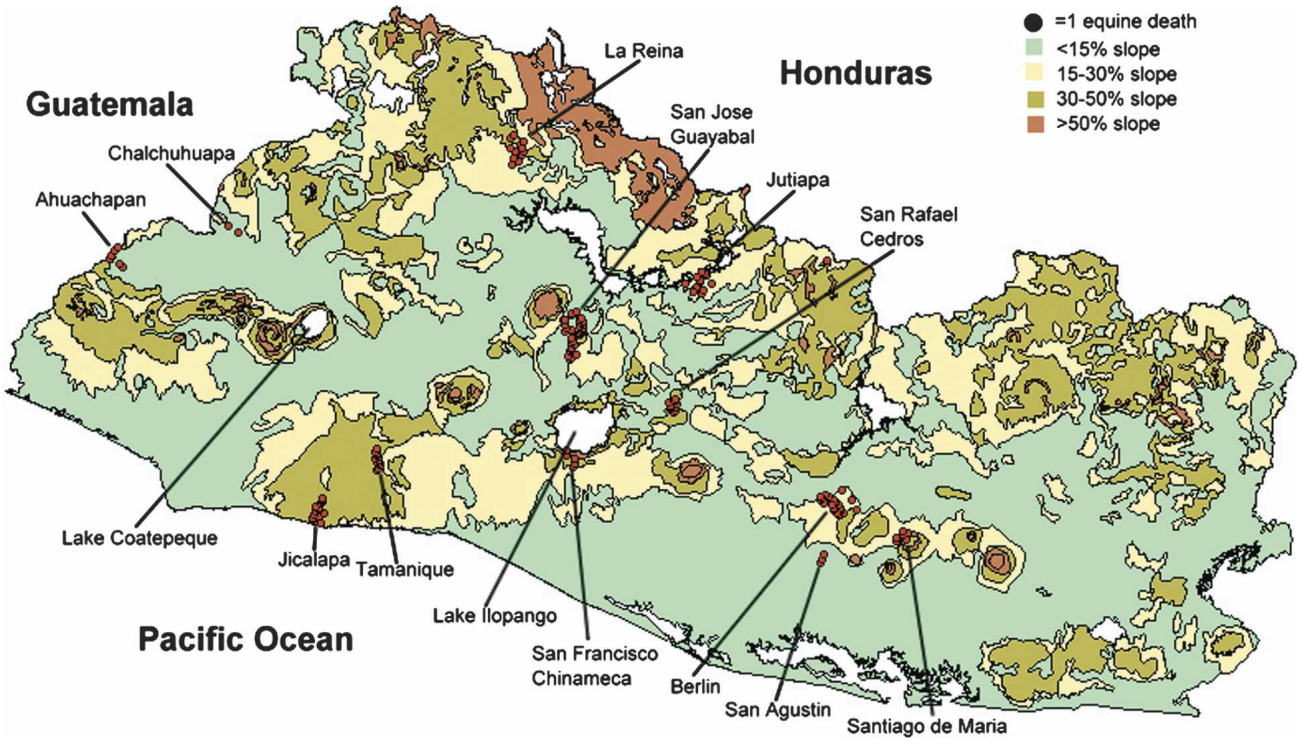

FIGURE 1. Equine West Nile encephalitis deaths by place of occurrence and percent slope of areas, El Salvador, 2001–2003. Equine encephalitis deaths occurred throughout El Salvador in 12 separated and discrete geographic foci ranging from 1 to 36 deaths each. All were either within (10) or adjacent to (2) steeply sloped (15–50%) terrain broken by deep gullies of temporary watercourses. This figure appears in color at www.ajtmh.org.

flaviviruses. However, none of the WNV-positive samples was IgM positive. In humans, IgM antibodies to WNV have been shown to have a half-life 6-8 months, but it is possible they do not persist long in infected horses.[10] All VEEV positive samples were IgG but not IgM positive (Table 1).

To look for evidence of human infection with WNV, field investigations included limited serologic household surveys, including 26 serum samples taken from acutely ill fever patients residing in affected villages identified through door-to-door surveys, and examination of emergency room visits and hospital discharge records. However, these limited inquiries failed to identify CNS disease among humans as having been caused by WNV infection. Further human studies will be required to assess human WNV infection patterns in El Salvador.

The surveillance and serologic data presented herein suggests epizootic transmission of WNV in El Salvador. The pattern of occurrence of this epizootic is consistent with a seasonal component limited to the dry season, when *Culex* mosquitoes are believed to be more abundant in El Salvador. The discrete geographic foci and November to March season indicate the possibility of introduction of WNV by migratory birds from North America to a limited number of points scattered throughout a characteristic ecological zone. The isolation of WNV from a dead bird from southern Mexico in May 2003 indicates that it was present nearby.[6] Our data represent a rapid response to a potential public health emergency and accordingly have several limitations. First, the data and specimens were collected at the end of the epizootics, mainly from unaffected equines. Virus isolation or antigen and IgM antibody detection would have been more productive during the initial rise and peak of the outbreak. Similarly, human disease and vector populations in March may not represent the situation earlier in the season. We also have serologic studies from only 3 of the largest foci of equine encephalitis outbreaks the others may not have given similar results. Although we demonstrated 4-fold or greater differences in 90% PRNT titer between WNV and the other flaviviruses (including members of the JE complex known to circulate in Central America), the remote possibility exists that another untested flavivirus was responsible for the reactivity to WNV. Finally, we were not able to obtain follow-up sera from the equines that we sampled to show changes in antibody titers. The low prevalence of IgG VEEV antibodies would not be unexpected since VEEV occasionally appears in Central America and Mexico and since the vaccine has been used in El Salvador. Eventually, the confirmation of WNV transmission in Central America will rest on antigen detection from appropriate human, equine, avian, or mosquito specimens collected during the peak, November–February season.

Epizootics of WNV have occurred in Europe and the United States without recognized disease in humans.[11–13] Although human surveillance was extremely limited during the equine outbreak in 2003 in El Salvador, the apparent absence of human cases of WNV encephalitis could be due to multiple factors. First, there may be a lack of awareness and familiarity with the clinical picture caused by WNV. Another explanation might be that humans living in this region are not often bitten by the local WNV vector(s); in contrast, horses that roam freely in the fields of villages such as San Felipe, in Berlin, Usulutan, in the hills of El Salvador could be bitten by mosquitoes that do not normally feed on humans. Further, immunity to other flaviviruses present in Central America may play a role in preventing severe human disease as sug-

TABLE 1

Results of testing for antibody against West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), and Venezuelan equine encephalitis virus (VEEV) in equine serum samples from San Felipe, Usulutan, El Salvador, 2003

Sample no.	IgG P/N		IgM P/N		90% Neutralization		Interpretation
	WNV	VEEV	WNV	VEEV	WNV	SLEV	
12–1A	14.95	(–)	(–)	(–)	40	40	undetermined
2–S2	13.42	(–)	(–)	(–)	320	< 10	WNV
10–S2	11.99	(–)	(–)	(–)	≥ 320	≥ 320	undetermined
13–1A	11.16	7.82	(–)	(–)	320	80	WNV
17–1A	10.87	(–)	(–)	(–)	80	< 10	WNV
7–S2	9.59	11.48	(–)	(–)	160	≥ 320	undetermined
5–1A	9.37	(–)	(–)	(–)	320	≥ 320	undetermined
1–S2	9.03	(–)	(–)	(–)	320	40	WNV
10–1A	8.93	(–)	(–)	(–)	160	160	undetermined
9–1A	8.37	(–)	(–)	(–)	160	< 10	WNV
8–1A	8.10	(–)	(–)	(–)	160	40	WNV
8–S2	7.22	(–)	(–)	(–)	≥ 320	20	WNV
3–S2	7.06	(–)	(–)	(–)	320	< 10	WNV
18–1A	5.81	(–)	(–)	(–)	80	< 10	WNV
5–S2	5.49	(–)	(–)	(–)	80	< 10	WNV
4–1A	5.12	(–)	(–)	(–)	< 10	10	undetermined
16–1A	4.79	(–)	(–)	(–)	80	80	undetermined
4–S2	4.64	(–)	(–)	(–)	80	80	undetermined
6–S2	4.21	(–)	(–)	(–)	20	< 10	undetermined
3–1A	(–)	(–)	(–)	(–)	< 10	10	undetermined
6–1A	(–)	25.52	(–)	(–)	< 10	10	undetermined
14–1A	(–)	6.25	(–)	(–)	< 10	< 10	undetermined
15–1A	(–)	(–)	(–)	(–)	10	< 10	undetermined

Cutoff values (reactive) for IgG and IgM are $P/N = 3$; all P/N values less than 3 are considered negative and are denoted as (–). Any titer of 10 or more is considered positive for neutralization.

gested by one experimental study in hamsters.[14] Although it is not certain that human infections did not occur, further investigations into the WNV ecology, pathogenesis, and education of local clinicians are warranted. At this point, it is unclear why these epizootics seem to have been limited to hilly terrains.

In summary, serologic results strongly suggest the spread of WNV infection to Central America by 2003. Our preliminary findings underscore the need for further field investigations, isolation of virus from vectors, equines, birds, and humans, enhanced regional WNV surveillance, and assessment of the potential impact to humans in tropical America.

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TABLE 2

Results of 90% plaque reduction neutralization assays against flaviviruses known to exist in Central America for 10 equine sera with antibody to West Nile virus (WNV), San Felipe, Usulutan, El Salvador

Sample/set	Neutralization titers against					
	WNV	SLEV	DENV-2	YFV	BSQV	ILHV
13/1A	320	40	(–)	(–)	(–)	(–)
3/S2	320	(–)	(–)	(–)	(–)	(–)
8/1A	160	20	10	(–)	(–)	(–)
8/S2	160	10	(–)	(–)	(–)	(–)
1/S2	80	20	(–)	(–)	(–)	(–)
9/1A	80	(–)	(–)	(–)	(–)	(–)
17/1A	80	(–)	(–)	(–)	(–)	(–)
18/1A	80	(–)	(–)	(–)	(–)	(–)
2/S2	80	(–)	(–)	(–)	(–)	(–)
5/S2	80	(–)	(–)	(–)	(–)	(–)
Median titer	80	<10	(–)	(–)	(–)	(–)

WNV, West Nile virus; ILHV, Ilheus virus; YFV, yellow fever virus; SLEV, Saint Louis encephalitis virus; BSQV, Bussuquara virus; DENV-2, dengue virus type 2; (–), neutralizing antibody < 10.

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