Bull Pan Am Health Organ 11(3), 1977.

SEROLOGIC SURVEYS FOR ANTIBODIES TO WESTERN, EASTERN, CALIFORNIA, AND ST. LOUIS ENCEPHALITIS AND DENGUE 3 ARBOVIRUSES IN MIDDLE AMERICA, 1961-1975[1, 2]

William F. Scherer,[3] Robert W. Dickerman,[4] and José V. Ordóñez[5]

A survey of sera and plasmas collected during 1961-1975 has shown that many people and animals of Middle America are susceptible to infection by dengue 3 virus and the viruses of eastern, western, St. Louis, and California encephalitis. Spread of any of these arboviruses in Middle America could cause serious epidemics, since vaccines are not currently available for these virus infections and mosquito vector control efforts are not always successful.

Introduction

Historically, the arboviruses which have produced significant human epidemics or equine epizootics in the Americas are yellow fever (YF), Dengue (DEN), and various encephalitis viruses—including western (WE), eastern (EE), St. Louis (SLE), Venezuelan (VE), and California (CAL) encephalitis. The presence or absence and the geographic distributions of these viruses are fairly well known in the United States of America, Canada, and the Caribbean area, but only YF and VE viruses have been mapped extensively in Middle America (*1-3*). EE virus has been isolated from horses in Mexico, from a sentinel hamster in Guatemala, and from a migrant bird in Belize; and SLE virus has been recovered from a bird and from mosquitoes in Mexico (*4-8*). On the whole, however, little is known of the geographic distributions of EE and SLE viruses in Middle America. No isolations of WE, CAL, or DEN viruses have been reported from Middle America, and published serologic surveys for antibodies to WE, EE, SLE, and DEN viruses have covered only limited regions (*9-18*).

Therefore, it seemed worthwhile to test sera from Middle America more extensively for antibodies to WE, EE, CAL, SLE, and DEN arboviruses, and in this way to seek areas with a high prevalence of infection which could subsequently be studied to isolate viruses and define disease incidences. Prior investigations of VE virus in Middle America during 1961-1971 provided us with sera to examine. These were tested for hemagglutination-inhibition (HI) antibodies to WE, EE, CAL, and SLE viruses,

[1]These investigations were performed in collaboration with the Pan American Health Organization and the Governments of Mexico, Guatemala, Belize, Honduras, El Salvador, Nicaragua, and Costa Rica. They were also sponsored in part by the U.S. Army Medical Research and Development Command, Washington, D.C. 20314, under contract no. DADA-17-72-C-2140, and in part by U.S. Public Health Service research grant AI-06248 from the National Institute of Allergy and Infectious Diseases.

[2]Also appearing in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, 1978.

[3]Professor, Department of Microbiology, Cornell University Medical College, New York, New York 10021, U.S.A.

[4]Associate Professor, Department of Microbiology, Cornell University Medical College.

[5]Professor, University of San Carlos Medical School, Guatemala.

and for neutralizing (N) antibodies to type 3 DEN, WE, EE, and VE viruses. This article reports the results of those tests.

Materials and Methods

Specimen Sources and Collection Methods

Sera or plasmas were obtained from the locations shown in Figure 1. These were collected in the manner previously described for investigation of VE virus (*19*) and were stored at -20°C.

The ages of human subjects and animals providing samples were as follows: Three per cent of the human subjects were 0 to 5 years of age, 11% were 6 to 10, 26% were 11 to 20, 35% were 21 to 40, 18% were 41 to 60, and 7% were over 60. Wild birds sampled at Tlacotalpan, Veracruz, Mexico, in 1963 and 1964 were juveniles and adults several months to several years of age. Other wild birds (mostly ardeids and associated marsh birds) were either nestlings less than a month old or (in a few cases) juveniles up to six months old. Samples were also taken from horses and burros ranging in age from 1 to 15 years; from pigs, some 1 to 6 months old but most in the 6 month to 2 year range; and from dogs 1 to 7 years of age. The ages of the wild mammals sampled are unknown.

HI Tests

These tests were performed with microtechnics previously described, using goose erythrocytes; serum was acetone-extracted twice (*20*). Pipettes (1 ml and 0.2 ml) were used to dilute sera 1:10 and to make subsequent two-fold dilutions. The virus strains employed were as follows: The WE virus was strain 1985-60, which had been isolated at the Rocky Mountain Laboratory in Montana (*15*). The EE virus used in most cases was strain 68U230 from Guatemala; however the human sera obtained from Sontecomapan, Veracruz, Mexico, in 1965 and pig sera collected in Belize in 1966 were tested with strain Riche from Louisiana (*5, 15*). The CAL virus used was strain BFS283 from California (*1*). The SLE strain employed was 65V310 from Mexico in most cases, but strain TRVL9464 from Trinidad was used for the sera tested with EE strain Riche (*7*). The VE virus used was strain 63U2 from Mexico (*21*).

Hemagglutinins were made from infected suckling mouse brains with the sucrose-acetone technic (*22*). For CAL virus, hemagglutinin was treated with 5 mg of trypsin (Difco 1:250) per 10 ml of hemagglutinin solution, was kept at 22°C for one hour, and was subsequently mixed with 5 mg of soybean trypsin inhibitor. Four to eight units of hemagglutinin were used in most cases. The pH of the HI tests was 6.2, except in the tests for SLE (pH 6.6) and for VE (pH sometimes 6.4). Serum-hemagglutinin mixtures were kept overnight at 5°C and were incubated at 37°C following addition of erythrocytes. All HI results recorded as positive in the accompanying tables were confirmed by a repeat test.

N Tests

N tests were accomplished by plaque-reduction in plastic plate wells with an area of two square centimeters, using methods similar to those previously described for VE virus (*21*). Tests for WE, EE, and VE virus N antibodies employed primary chicken embryonic cells produced as described elsewhere (*21*); tests for DEN type 3 antibody were done in LLCMK2 cultures as previously described (*23*). Virus strains utilized were as follows: WE strain 1985-60, EE strain 68U230, VE strain 63U2 or 69Z1, and DEN 3 strain 16562. Sera were tested at a 1:4 dilution after heating at 60°C for 20 minutes. Serum-virus mixtures containing about 100 plaque-forming units (pfu) were incubated at 37°C for one hour. Sera were considered positive for antibody if the log neutralization index (LNI) exceeded 1.6 (98% plaque reduction) in WE or VE tests, and if it

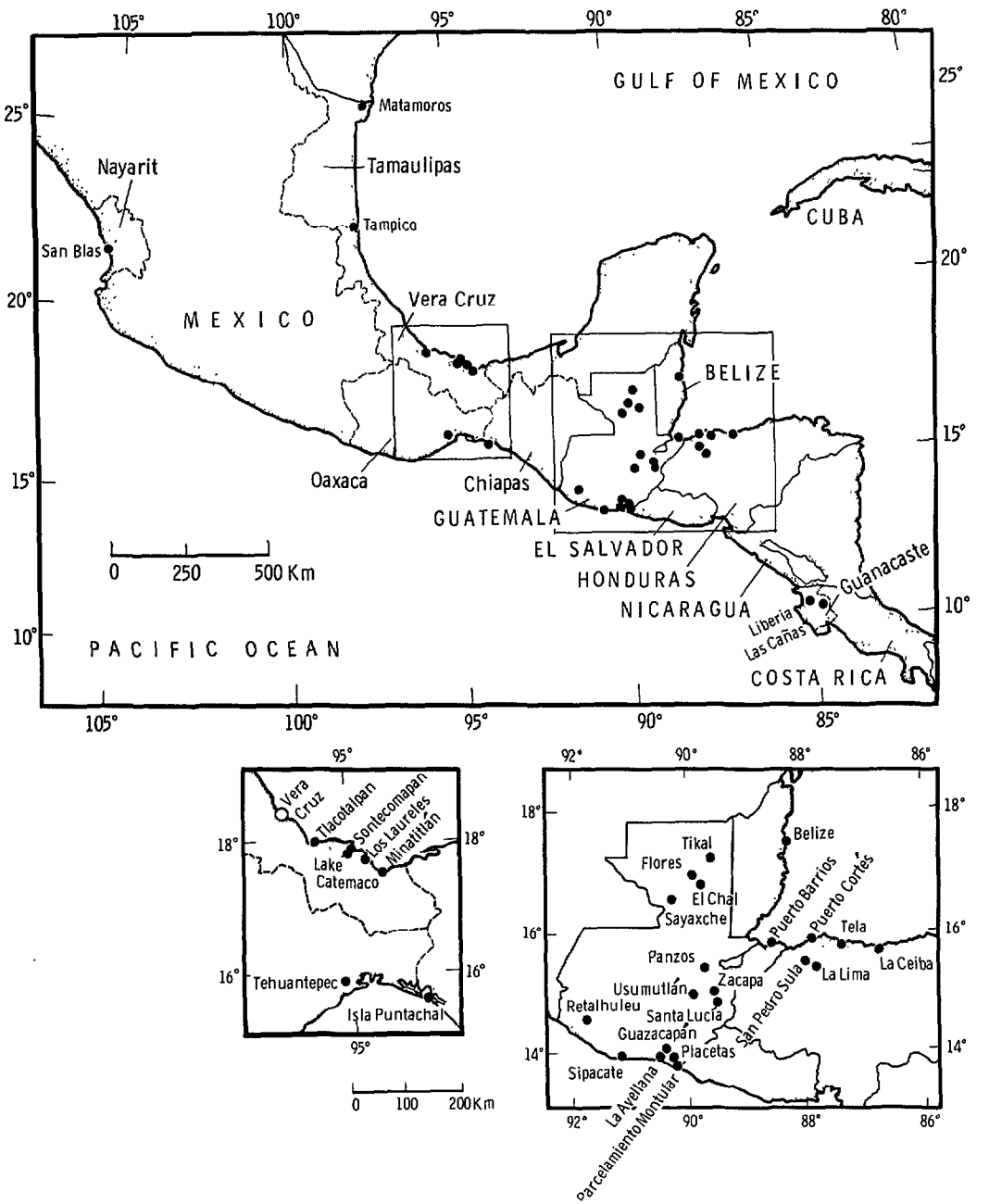

Figure 1. Maps showing the locales (black dots) where sera were collected.

exceeded 1.5 (97% plaque reduction) in DEN 3 tests.

Results

WE Virus

Numerous samples taken on the Atlantic side of Middle America yielded positive HI results for WE antibody. Positive findings were obtained with human sera from locations in Belize, Guatemala, Honduras, and the Mexican State of Veracruz (see Table 1). In addition, various animal plasmas collected in Veracruz reacted with WE virus hemagglutinin; these positive samples included 18 from wild terrestrial mammals, one from a bat, and four from wild nestling herons, egrets, or associated marsh birds. Positive results were also obtained with equine sera collected from northern Veracruz in 1971, from northern Tamaulipas in 1969, and from the Atlantic foothills of Guatemala in 1969. Some pig sera from Veracruz (Mexico), Belize City (Belize), Cortés (Honduras), and the Atlantic foothills of Guatemala also reacted positively, as did a few dog sera from Santa Lucía, Guatemala.

On the Pacific side, positive HI tests for WE virus occurred with human sera from Guatemala, with wild mammalian sera from Guatemala, with bird plasmas and pig sera from Mexico and Guatemala, with horse and dog sera from Guatemala, and with pig sera from Costa Rica (Table 2). Most sera were positive at dilutions in the 1:10 to 1:40 range. The few sera that reacted at higher dilutions are cited in footnotes to the tables.

On the other hand, N tests with WE virus showed that most of the positive HI reactions were not specific for WE (Table 3). In fact, out of 94 HI-positive sera, only one was N-positive and two others were possibly N-positive. Furthermore, all three of these sera came from the northern Atlantic coast of Mexico next to southern Texas, where WE virus is known to exist (*24*).

Another interesting point is that the positive HI results with WE virus were not entirely accounted for by coexistence in the sera of VE virus antibodies, since only 31 of 186 sera that were HI-positive with WE virus were also HI-positive with VE virus (Table 3). Only in a few places, where sera were obtained during or shortly after the Middle American VE outbreak, were sera found to be HI-positive with both WE and VE viruses. These places (not shown in Table 3) were in northern Veracruz near Tampico during 1971 (34 of 45 horse sera HI-positive with WE were also HI-positive with VE); the Atlantic foothills of Guatemala during 1969 (8 of 9 horse sera and 3 of 3 dog sera); La Avellana and Guazacapan in Santa Rosa, Guatemala, during 1970-71 (22 of 26 human sera); and Parcelamiento Montufar in Jutiapa, Guatemala, during 1969 (9 of 9 horse sera and 15 of 21 dog sera).

During 1972-1975, sera became available from horses on the Pacific coasts of El Salvador and Nicaragua. WE HI antibodies were not detected in any of 18 sera from El Salvador, but were detected in seven out of 63 Nicaraguan sera. However, six of the seven positive sera had HI titers of only 1:10, and no WE N antibody was found at a 1:4 dilution. One serum had a WE HI titer of at least 1:20 and a N titer of at least 1:4; this serum came from a three-year-old horse bled during May 1975 in Nicaragua's Chinandega Department. Whether these results indicated naturally-induced or vaccine-induced WE antibodies in this horse is unknown.

EE Virus

Only four of 1,255 human sera from Middle America gave positive HI results with EE virus (Table 4). These sera came from Guatemala and Honduras, not far

Table 1. Prevalences of serum HI antibodies reacting with WE virus in people and animals sampled on the Atlantic coastal lowlands of Mexico, Belize, Guatemala, and Honduras and the Atlantic foothills of Guatemala during 1961-1971.

Collection areas	Year(s) of collection	Sera with detectable HI antibody (No. positive/total No. tested), by source[a]					
		Humans	Wild mammals	Wild birds	Equine animals	Pigs	Dogs
ATLANTIC LOWLANDS:							
Mexico							
Tamaulipas:							
Matamoros	1969	0/46			32/40[b]		
Tampico	1969	0/25			0/39		
Veracruz:							
Near Tampico	1971				45/100[b]		
Tlacotalpan	1961			1/93			
Villages at Lake Catemaco	1965	8/90					
Sontecomapan	1963-66	25/63[b]	19/136[c]	0/58			
Los Laureles	1964	15/16					
Minatitlán	1965	0/14	0/16				
Minatitlán	1962-67			3/231		37/178[b]	
Belize							
Belmopán	1966-67	18/95[b]				11/45	
Guatemala:							
Izabal:							
Puerto Barrios	1970	21/87[b]					
Alta Verapaz:							
Panzos	1965	9/33					
Petén:							
Flores, El Chal	1966	3/42					
Sayaxche	1966	8/37					
Tikal	1966	3/37					
Honduras							
Cortés:							
Puerto Cortés, San Pedro Sula, La Lima	1967	22/128	0/29			1/40	
Atlántida:							
Tela, La Ceiba	1967	7/77				0/8	
ATLANTIC FOOTHILLS:							
Guatemala							
Zacapa:							
Santa Lucía	1969	10/26	0/18		9/21		3/6
Zacapa City	1969					15/25	
Usumutlán	1970	4/70					
Totals		**153/886**	**19/199**	**4/382**	**86/200**	**64/296**	**3/6**
% Positive		17%	10%	1%	43%	22%	50%

[a]Positive HI means a titer $\geq$ 1:10; Blank spaces = not tested.

[b]Sera from the following subjects yielded WE HI titers >1:40: eight horses from Matamoros, 11 horses from Tampico (1971), two people from Sontecomapan, two pigs from Minatitlán, one person from Belize, and four people from Puerto Barrios.

[c]Including bats = 1/43.

Table 2. Prevalences of serum HI antibodies reacting with WE virus in people and animals sampled on the Pacific coastal lowlands of Mexico, Guatemala, and Costa Rica during 1962-1971.

Collection areas	Year(s) of collection	Sera with detectable HI antibody (No. positive/total No. tested), by source[a]					
		Humans	Wild mammals	Wild birds	Equine animals	Pigs	Dogs
Mexico							
Nayarit:							
San Blas	1962-65			4/366		8/42	
Oaxaca:							
Tehuantepec	1965	0/42					
Chiapas:							
Islas Puntachal	1967			22/140			
Guatemala							
Retalhuleu:							
Retalhuleu	1967-68	14/112	0/20		0/19	5/95	
Escuintla:							
Sipacate	1970-71			10/29			
Santa Rosa:							
La Avellana	1970-71	23/47[b]	3/68	1/97			
Guazacapán	1970	3/41[b]					
Placetas	1969	15/26					
Jutiapa:							
Parcelamiento Montufar	1969	12/30[b]			9/18		21/22
Costa Rica							
Guanacaste:							
Liberia and Las Cañas	1967					14/20	
Totals		67/298	3/88	37/632	9/37	27/157	21/22
% Positive		22%	6%	6%	24%	17%	95%

[a]Positive HI means a titer $\geq$ 1:10; Blank spaces = not tested.
[b]The following human sera had WE HI titers > 1:40: five from La Avellana, one from Guazacapán and two from Montufar.

from the Petén area of Guatemala where EE virus was isolated in 1968 (*5*). A few wild terrestrial mammals and 40 nestling ardeids and associated marsh birds were positive in Veracruz, Mexico, and along the Pacific coasts of Mexico and Guatemala. Horse sera from Veracruz were positive (possibly due to administration of inactivated EE virus vaccine). Sera from pigs were negative except in Costa Rica and in portions of Belize and Honduras near areas where positive human sera were obtained. A few sera which were HI-positive to EE were also HI-tested with VE virus; positive results were found for 1 of 1 wild mammal serum from Veracruz, Mexico; 1 of 1 human serum from Izabal, Guatemala; 1 of 3 human sera from Cortés, Honduras; 1 of 34 bird sera from Nayarit, Mexico; 0 of 3 bird sera from Chiapas, Mexico; 0 of 2 bird sera from Escuintla, Guatemala; and 1 of 1 dog serum from Jutiapa, Guatemala. VE N tests confirmed the positive VE HI result from Cortés and the negative results from Chiapas and Escuintla.

EE HI antibodies were found in 0 of 18 and 1 of 63 horse sera collected in El Salvador and Nicaragua, respectively, during 1972-1975; the one positive serum (titer 1:10) was also positive to WE virus (HI titer $\geq$ 1:20) and VE virus (HI titer 1:640, N titer 1:2,916).

Table 3. A comparison of WE and VE antibodies detected in Middle American sera with HI and N tests.

Collection areas	Sources of sera	Sera with detectable HI or N antibody to WE or VE virus.[a]			
		WE HI + (No. +/No. tested)	WE N +/ WE HI +	VE HI +/ WE HI +	VE N +/ WE HI +
ATLANTIC LOWLANDS:					
Mexico:					
Tamaulipas	horses	32/40	1/10 [b]	0/32	0/32
Veracruz	humans	40/79	0/19	9/40	2/19
Veracruz	wild mammals	18/93	0/3	6/18	0/3
Veracruz	pigs	37/178	0/7	8/37	8/24
Belize:	humans	18/95	0/10	1/11	3/10
	pigs	11/45	0/6		3/6
Guatemala:					
Izabal	humans	21/87	0/7	7/21	1/7
PACIFIC LOWLANDS:					
Guatemala:					
Retalhuleu	humans	14/112	0/8	0/12	3/7
Santa Rosa	humans	15/26	0/10	0/15	1/10
Costa Rica:	pigs	14/20	0/14		2/14
Totals:		**220/775**	**1/94**	**31/186**	**23/132**
% Positive		28%	1%	17%	17%

[a] Positive HI means a titer ≥ 1:10; positive N signifies an LNI > 1.6 with a 1:4 dilution of serum. Blank spaces = not tested.
[b] Two additional sera had LNI = 1.4 and 1.5.

CAL Virus

Only seven of 1,109 human sera gave a positive HI response with CAL virus strain BFS283. These positive sera came from Veracruz, Mexico, and the Atlantic foothills and Pacific coastal lowlands of Guatemala (see Table 4). Of 737 plasmas from nestling marsh birds, only five from the Pacific coast of Mexico were positive. Similarly, of 410 pig sera, only four from Veracruz were positive. No sera had titers exceeding 1:40.

SLE, DEN, and Other Flaviviruses

In all, 23% of 1,197 human sera reacted with SLE virus hemagglutinin, and 20% of 405 sera responded positively in N tests with DEN 3 virus (see Table 5). However, only 1% of 299 wild mammals, 5% of 900 nestling marsh birds, 6% of 236 horses, 5% of 434 pigs, and 2% of 41 dogs gave a positive HI response when their sera were tested with SLE virus. The positive nestling marsh birds were captured in Minatitlán, Veracruz, where SLE virus had been isolated in 1965 (7), and at three colonies along the Pacific coasts of Mexico and Guatemala. Horse and pig sera from Veracruz were also positive to SLE. A few other positive pig sera came from Belize, Honduras, the Atlantic foothills of Guatemala, and the Pacific lowlands of Mexico and Costa Rica. The remaining positive samples—six horse sera and one dog serum—came from Jutiapa, Guatemala.

Table 4. Prevalences of serum HI antibodies to EE and CAL viruses in people and animals sampled along the Atlantic and Pacific coasts of Mexico, Belize, Guatemala, Honduras, and Costa Rica during 1961-1971.

Collection areas[a]	Year(s) of collection	HI test results: Numbers tested (and positive) for EE and CAL antibodies, by source[b]											
		EE antibody in sera from						CAL antibody in sera from:					
		Humans	Wild mammals	Wild birds	Equine animals	Pigs	Dogs	Humans	Wild mammals	Wild birds	Equine animals	Pigs	Dogs
ATLANTIC LOWLANDS													
Mexico:													
Tamaulipas	1969	71			79			70			79		
Veracruz	1961-71	210	152(14)	379(1)	99(12)[c]	177		155(4)	16	105		177(4)	
Belize.	1966-67	95				45(1)[c]		95				45	
Guatemala.													
Izabal, Alta Verapaz	1966-70	120(1)						120					
Petén	1966	115						115					
Honduras.													
Cortés	1967	126(3)	29			40(1)[c]		123	2			40	
Atlántida	1967	77				8		77				8	
Subtotals		814(4)	181(14)	379(1)	178(12)	270(2)		755(4)	18	105	79	270(4)	
ATLANTIC FOOTHILLS													
Guatemala:													
Zacapa	1969-70	96	18		21	25	19	96(1)	18		21	25	19
PACIFIC LOWLANDS													
Mexico.													
Nayarit	1962-65			366(34)[c]		23				366(4)[c]			
Oaxaca	1965	42											
Chiapas	1967			140(3)						140(1)			
Guatemala:													
Retalhuleu	1967-68	114	20		19	95		114	20		19	95	
Escuintla	1970			29(2)						29			
Santa Rosa	1969-71	114	97	97				114(2)	68	97			
Jutiapa	1969-70	75	9		18		22(1)	30	9		18		22
Costa Rica:													
Guanacaste	1967					20(1)						20	
Subtotals		345	126	632(39)	37	138(1)	22(1)	258(2)	97	632(5)	37	115	22
Totals		1,255(4)	325(14)	1,011(40)	236(12)	433(3)	41(1)	1,109(7)	133	737(5)	137	410(4)	41

[a]Specific locations within states or departments are the same as those listed in Tables 1 and 2.
[b]Positive HI means a titer $\geq$ 1:10.
[c]Sera with EE titers >1:40 were obtained from the following animals: 8 horses from Veracruz, 1 pig from Belize, 1 pig from Cortés (Honduras), and 10 birds from Nayarit, Mexico. Three birds from Nayarit yielded CAL titers >1:40.

DEN antibodies were found mostly in older persons; antibody was never detected in subjects under age 20 and was detected infrequently in those under 40. The percentages of various age groups yielding positive sera were as follows: 11-20 years, 0% of 24 persons; 21-40 years, 9% of 172; 41-60 years, 30% of 149; over 60 years, 40% of 60.

Plasmas from two sentinel hamsters exposed in Veracruz, Mexico, in 1969 yielded 1:10 HI titers with SLE virus hemagglutinin. The positive samples were taken after the animals had been exposed at Tlacotalpan and Sontecomapan for 28 and 34 days, respectively. These two hamsters were part of a sizable group used as sentinel animals in Middle America to detect arboviruses such as VE. Plasmas from 245 survivors exposed during 1967-1971 were available for antibody tests, but only the two just mentioned and five exposed at Minatitlán, Veracruz, in 1967 (which yielded 1:10 titers to WE virus) showed positive results in HI tests with SLE, EE, or WE viruses. The value of these hamster plasmas for serologic surveys was obviously limited because the exposures all occurred in one season (July-August) and only lasted for two to six weeks. Furthermore, one to two weeks need to be subtracted from this period because of the time required for detectable HI antibody to develop after an infection.

Table 5. Prevalences of serum antibodies to flaviviruses (SLE and DEN 3) in people and animals sampled along Atlantic and Pacific coastal areas of Mexico, Belize, Guatemala, Honduras, and Costa Rica during 1961-1971.

Collection areas[a]	Year(s) of collection	Sera with detectable HI antibody to SLE or N antibody to DEN 3, by source[b]						
		Flavivirus SLE antibody in sera (No positive/No tested) from:						Human sera with N antibody to DEN 3 (No positive/No tested)
		Humans	Wild mammals	Wild birds	Equine animals	Pigs	Dogs	
ATLANTIC LOWLANDS:								
Mexico:								
Tamaulipas	1969	1/74[c]			0/79			7/40
Veracruz	1961-71	65/244[c]	1/152	11/268[c]	8/99[c]	8/178		38/158
Belize:	1966-67	28/95[c]				2/45		14/36
Guatemala:								
Izabal, Alta Verapaz	1966-70	51/120[c]						8/73
Petén	1966	36/115[c]						6/12
Honduras:								
Cortés	1967	26/110[c]	0/13			1/40		2/56
Atlántida	1967	6/77				0/8		
Subtotals		213/835	1/165	11/268	8/178	11/271		75/375
ATLANTIC FOOTHILLS:								
Guatemala:								
Zacapa	1969-70	17/90	1/18		0/21	5/25	0/19	
PACIFIC LOWLANDS:								
Mexico:								
Nayarit	1962-65			3/366		1/23		
Oaxaca	1965	0/42						
Chiapas	1967			24/140				
Guatemala:								
Retalhuleu	1967-68	17/114[c]	1/20		0/19	0/95		5/16
Escuintla	1970			4/29				
Santa Rosa	1969-71	26/110[c]	0/87	0/97				3/14
Jutiapa	1969-70	1/6	0/9		6/18[c]		1/22	
Costa Rica:								
Guanacaste	1967					5/20		
Subtotals		44/272	1/116	31/632	6/37	6/138	1/22	8/30
Totals		**274/1,197**	**3/299**	**42/900**	**14/236**	**22/434**	**1/41**	**83/405**
% Positive		23%	1%	5%	6%	5%	2%	20%

[a]Specific locations within states or departments are the same as those listed in Tables 1 and 2

[b]Positive HI means a titer ≥1 10, positive N signifies an LNI >1 5 (97% plaque reduction) with a 1 4 dilution of serum heated to 60°C for 20 minutes and tested in LLCMK2 monkey kidney cell cultures

[c]The following human sera had SLE HI titers >1 40 1 from Tamaulipas, 21 from Veracruz, 10 from Belize, 5 from Izabal, 9 from Petén, 3 from Cortés, 2 from Retalhuleu and 3 from Santa Rosa Two birds and 1 horse from Veracruz and 1 horse from Jutiapa also yielded serum titers > 1:40.

Discussion

This serologic survey of Middle America revealed that numerous sera from the Atlantic coasts of Mexico, Belize, Guatemala, and Honduras, as well as many from the Pacific coast of Guatemala, contained HI antibodies that reacted with WE virus. However, N tests showed that these antibodies were not specific for WE virus. The only virus antibodies that appeared specific for WE were found in horses from Matamoros, Mexico, a town bordering portions of southern Texas where WE virus is known to be active (*24*). In other regions, WE HI antibodies did not always coexist with other alpha-virus (VE or EE) antibodies. Only in a few places that were heavily involved in America did positive WE HI tests correlate with positive VE HI tests. Thus in some regions the antibodies that reacted in HI tests with WE virus were probably

induced by another alphavirus which was not WE, EE, or VE. Future attempts should therefore be made to isolate such an alphavirus in Middle America and to learn whether it causes disease in people or animals.

Virtually no HI antibodies to EE virus were found in sera from north-coastal Middle America, except in sera obtained near the base of the Yucatan Peninsula in Belize, Guatemala, and Honduras. Since EE virus was isolated in the rain forest of Guatemala's Petén region in 1968 (*5*), this locale appears to be an enzootic focus. Health personnel diagnosing equine and human encephalitic disease there should consider EE virus as a possible etiologic agent. Also, the absence of EE virus antibody throughout much of north-coastal Middle America should alert health personnel to the susceptibilities of people and animals to EE virus diseases were the virus to invade new regions.

HI tests for CAL virus antibodies revealed no unequivocal CAL virus activity in Middle America. Since the CAL virus strain BFS283 hemagglutinin used for these surveys seems to detect antibodies to at least two other viruses of the CAL group (*1*), the negative HI results obtained with this strain indicate the probable absence of La Crosse virus, the major human pathogen of the CAL group in the Americas, and also the possible absence of Snowshoe Hare virus. However, nothing definite can be concluded about other CAL group viruses because HI tests with CAL strain BFS283 cannot be relied upon to detect their antibodies.

HI tests with SLE virus detected antibodies to SLE and other arboviruses of the flavivirus genus. The results with Middle American sera indicate moderate cycling of SLE or other flaviviruses along the Atlantic coasts of Mexico, Belize, Guatemala, and Honduras, and also along the Pacific coasts of Mexico, Guatemala, and Costa Rica. Some of these flavivirus antibodies probably resulted from infections with SLE, DEN, Jutiapa, or Ilheus viruses, but some may also have resulted from infections with as-yet-undiscovered flaviviruses of the region. Jutiapa virus was isolated from a cotton rat (*Sigmodon hispidus*) collected in the mountains of Guatemala near the southeastern Pacific coast during 1969, and Ilheus virus was recovered from mosquitoes on the Atlantic sides of Guatemala and Honduras in 1956 (*1*). It is noteworthy that SLE virus has recently caused a sizeable human epidemic in the central upland plateau of Mexico (*17*).

N tests revealed that DEN 3 virus antibodies were present only in older people, and that this virus had not cycled in tropical Middle America for many years. This observation fits with those of Rosen, who found that DEN 2 and DEN 3 viruses infected people in Panama between 1941 and 1954 (*18*). Suppression of DEN virus activity in Middle America has undoubtedly resulted from programs for control of the vector mosquito, *Aedes aegypti*. However, with DEN virus currently active in the Caribbean region, vigilance should be maintained in Middle America—so as to prevent recurrence of enough *Aedes aegypti* to transmit DEN viruses and cause human disease.

ACKNOWLEDGMENTS

We are grateful for the able technical assistance of F. A. Breakenridge, E. Du Casse, K. Anderson, J. Chin, and B. Pancake, and for the generous support of numerous persons in each country of Middle America who contributed to the collection of sera. In Mexico, studies were done in collaboration with personnel of the

National Institute of Virology and the Institute of Health and Tropical Diseases. In Guatemala, Belize, Honduras, and Nicaragua the studies were greatly facilitated by health and agriculture officials and personnel. Drs. G. Godoy and J. E. Navarro M. of the Medical School of the University of El Salvador participated in collecting sera in that country. Pig sera were collected in Costa Rica when Dr. Robert W. Dickerman participated in teaching work for the Organization for Tropical Studies. Permits to collect wild birds and mammals in Mexico and Guatemala were provided by appropriate authorities in those countries.

SUMMARY

A serologic survey for antibodies to dengue 3 and western, eastern, St. Louis, and California encephalitis arboviruses shows that coastal Middle America was relatively free of infections by these or antigenically related arboviruses during 1961-1975. Aside from pointing out a postulated but unidentified alphavirus that cross-reacts with western encephalitis virus, these findings provide no basis for further studies in the immediate future that seek to isolate these arboviruses from natural sources in Middle America. However, the presence of populations that are without antibodies and are therefore susceptible to these arboviruses should serve as a warning to health authorities. For example, eastern or St. Louis encephalitis viruses could conceivably extend their geographic range in Middle America and cycle between vertebrates and vector mosquitoes which bite humans or horses. Should this happen, the result could be human or equine encephalitis outbreaks like the recent epidemics of Venezuelan encephalitis in Middle America and St. Louis encephalitis in Mexico's central upland plateau.

REFERENCES

(*1*) Berge, T. O. (ed.). *International Catalogue of Arboviruses, Including Certain Other Viruses of Vertebrates* (Second edition). U.S. Department of Health, Education, and Welfare; DHEW Publication No. (CDC) 75-8301, Washington, 1975.

(*2*) Elton, N. W. Yellow fever in Central America: The imminent threat to Mexico and the United States. *Am J Public Health* 46:1259-1265, 1956.

(*3*) Trapido, H. Geographic distribution and ecologic setting. In: *Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus.* Pan American Health Organization. PAHO Scientific Publication 243, Washington, 1972, pp. 302-312.

(*4*) Tellez Girón, A., and O. Valdes Ornelas. La presencia de virus tipo Este de la encefalomielitis equina en la epizootia ocurrida en el Estado de Tamaulipas, México, durante el año de 1941. *Rev Soc Mex Hist Nat* 2:251-259, 1941.

(*5*) Ordoñez, J. V., W. F. Scherer, and R. W. Dickerman. Isolation of eastern encephalitis virus in Guatemala from sentinel hamsters exposed during 1968. *Bol Of Sanit Panam* 70: 371-375, 1971.

(*6*) Calisher, C. H., K.S.C. Maness, R. D. Lord, and P. H. Coleman. Identification of two South American strains of eastern encephalomyelitis virus from migrant birds captured on the Mississippi delta. *Am J Epidemiol* 94:172-178, 1971.

(*7*) Dickerman, R. W., W. F. Scherer, B. A. Pancake, and C. M. Bonacorsa. St. Louis encephalitis virus isolated from a nestling Common Egret in southeastern Mexico. *Bol Of Sanit Panam* (English edition) 6:26-30, 1972.

(*8*) Sudia, W. D., Z. Fernández L., V. F. Newhouse, B. Sanz R., and C. H. Calisher. Arbovirus vector ecology studies in Mexico during the 1972 Venezuelan equine encephalitis outbreak. *Am J Epidemiol* 101:51-58, 1975.

(*9*) Rosen, L. Observations on the epidemiology of dengue in Panama. *Am J Hyg* 66:45-58, 1958.

(*10*) Reeves, W. C., C. Ortiz Mariotte, H. N. Johnson, and R. E. Scrivani. Encuesta serológica sobre los virus transmitidos por artrópodos en la zona de Hermosillo, México. *Bol Of Sanit Panam* 52:228-230, 1962.

(*11*) Sosa-Martínez, J., M. Durán, and L. Benavides. St. Louis virus antibody survey on sera of residents of Yucatan, Mexico. *Bol Med Hosp Infant Mex* (English edition) ..37-42, 1963.

(*12*) Mucha Macías, J. de. Infecciones por

virus arbor: Estudios realizados en el Instituto Nacional de Virología de la Secretaría de Salubridad y Asistencia. *Gac Med Mex* 93:415-420, 1963.

(*13*) Mucha-Macías, J. de. Estudios epidemiológicos sobre virus arbor en el sureste de México. *Salud Pública Mex* 5:523-527, 1963.

(*14*) Sosa-Martínez, J., L. Benavides, and M. D. Vidaurri. Distribución de los virus arbor en las Américas. *Gac Med Mex* 8:855-867, 1964.

(*15*) Scherer, W. F., C. Campillo Sainz, J. de Mucha Macías, R. Rubio-Brito, T. Miura, R. W. Dickerman, D. W. Warner, and M. Dyer. Serologic survey for neutralizing antibodies to eastern equine and western equine encephalitis viruses in man, wild birds, and swine in southern Mexico during 1961. *Am J Trop Med Hyg* 15:211-218, 1966.

(*16*) Mucha Macías, J. de. Enfermedades por arbovirus: Anticuerpos para la encefalitis de San Luis en enfermos mentales. *Rev Inv Salud Pública* (México) 34:169-174, 1974.

(*17*) González Cortés, A., M. L. Zárate Aquino, J. Guzmán Bahena, J. Miró Abella, G. Cano Avila, and M. Aguilera Arrayo. St. Louis encephalomyelitis in Hermosillo, Sonora, Mexico. *Bull Pan Am Health Organ* 9:306-316, 1975.

(*18*) Rosen, L. Dengue type 3 infection in Panama. *Am J Trop Med Hyg* 23:1205-1206, 1974.

(*19*) Scherer, W. F., J. V. Ordoñez, P. B. Jahrling, B. A. Pancake, and R. W. Dickerman. Observations of equines, humans, and domestic and wild vertebrates during the 1969 equine epizootic and epidemic of Venezuelan encephalitis in Guatemala. *Am J Epidemiol* 95:255-266, 1972.

(*20*) Zárate, M. L., R. H. Geiger, R. E. Shope, and W. F. Scherer. Intergroup antigenic relationships among arboviruses manifested by a Mexican strain of Patois virus and viruses of the Bunyamwera, C, California, Capim and Guama groups. *Am J Epidemiol* 88:273-286, 1968.

(*21*) Scherer, W. F., C. Campillo-Sainz, J. de Mucha-Macías, R. W. Dickerman, C. Wong Chia, and M. L. Zárate. Ecologic studies of Venezuelan encephalitis virus in southeastern Mexico: VII. Infection of man. *Am J Trop Med Hyg* 21:79-85, 1972.

(*22*) Clarke, D. H., and J. Casals. Techniques for hemagglutination-inhibition with arthropod-borne viruses. *Am J Trop Med Hyg* 7:561-573, 1958.

(*23*) Scherer, W. F., F. A. Breakenridge, and R. W. Dickerman. Cross-protection studies and search for subclinical disease in New World monkeys infected sequentially with different immunologic types of dengue viruses. *Am J Epidemiol* 95:67-79, 1972.

(*24*) Sudia, W. D., V. F. Newhouse, L. D. Beadle, D. L. Miller, J. G. Johnston Jr., R. Young, C. H. Calisher, and K. Maness. Epidemic Venezuelan equine encephalitis in North America in 1971: Vector studies. *Am J Epidemiol* 101:17-35, 1975.