VENEZUELAN ENCEPHALITIS VIRUS ALONG THE CENTRAL AND NORTHERN GULF COAST OF MEXICO AS OF JULY-SEPTEMBER 1969 [1]

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VE virus is endemic in certain marshy and wet forested habitats in the coastal lowlands of southeastern and central Veracruz, Mexico. At Tampico, Tamaulipas, during 1969, there was residual antibody evidence of the 1966 equine outbreak though endemic virus activity was not found. Sizeable populations of susceptible equines and humans existed near Tampico and Matamoros, Tamaulipas. Thus spread of the disease to these areas is a real public health threat.

Introduction

Venezuelan (equine) encephalitis (VE) virus exists in endemic cycles involving vector mosquitoes and amplifying vertebrates along the Atlantic coast of Central America and the Pacific coast of Costa Rica and Guatemala (*1*). On the Atlantic lowlands, virus has been isolated by use of sentinel hamsters or from mosquitoes or wild vertebrates in Almirante, Panama; northeastern Nicaragua; Puerto Cortes, Honduras; Puerto Barrios, Guatemala; Belize, British Honduras; and several sites in the State of Veracruz, Mexico (*2-4*). During intensive studies of this virus in Veracruz from 1961 to 1967, virus was isolated only as far north as Sontecomapan on the Gulf coast near Lake Catemaco (*4*), and VE virus antibodies were found as far north as Tlacotalpan, about 100 kilometers west-northwest of Sontecomapan (Figure 1) (*5*). In 1966, an equine outbreak, identified by antibody tests as a VE virus epizootic, occurred in the vicinity of Tampico, Tamaulipas and adjacent northern Veracruz (*6, 7*). Further studies found antibody in serum of one of six bovines bled in October 1966 shortly after the epizootic as far north as Soto la Marina in central Tamaulipas (*7*). This epizootic was the first extensive outbreak of VE virus in animals or man north of Panama and at most, only the second recognized outbreak in Central America or Mexico, prior to 1969.[6]

The Tampico equine epizootic in 1966 raised the possibility of movement of VE virus along the Gulf coast of Mexico north from endemic habitats in southeastern Veracruz (*4*). It therefore seemed important to determine and record for future reference, the geographic distribution of VE virus along

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[6] During April-July 1962, a small human epidemic of encephalitis occurred in Campeche, Mexico on the Yucatan Peninsula. There were 12 cases, 3 with neurologic sequelae and 5 deaths in ages from 7 months to 47 years. Convalescent serum from one person with sequelae was tested and found to have VE virus HI antibody (*8*).

this coast, and to learn whether virus was still active, though clinically silent, in the Tampico region. Such a study was made during July-September 1969 using antibody tests of serum and isolations of virus from sentinel hamsters to map the virus.

Materials and methods

Study sites

Sentinel hamsters were exposed at 11, near sea-level locations along the Gulf of Mexico extending from Matamoros, State of Tamaulipas on the USA-Mexico border to Minatitlan, State of Veracruz a distance of about 1,000 kilometers (figure 1). Of this distance, approximately the northern 400 kilometers are in an arid to semi-arid zone (localities 1 and 2 described below), the next 400 kilometers are in a semi-humid region (localities 3-8) and the southern 200 kilometers are in a humid zone (localities 9-11). 1) *Matamoros,* a major port of entry in Mexico, has about 100,000 inhabitants, and is directly across the Rio Grande (Rio Bravo del Norte) from Brownsville, Texas. Sorghum and other irrigated crops are grown in the area. Hamsters were exposed in mesquite trees bordering the lower reaches of the Rio Grande in pasture land. 2) *La Pesca* was a small coastal village near the mouth of the Rio Soto la Marina in the arid zone of Tamaulipas. Commercial and sport fishing, some farming and raising of cattle and goats are the principal occupations. Mangroves reach their northern limit along the Gulf of Mexico coast at La Pesca. Inland from this narrow zone, the land is covered with dense, low, thorny-scrub forest. 3) *Tampico* and adjacent *Ciudad Madero* (about 200,000 total inhabitants) are located at the mouth of the confluence of the Rio Tamasi and the Rio Panuco. These cities are bordered by extensive freshwater marshes and more restricted brackish water marshes which become far more extensive in the latter part of the rainy season (May to September). Water depth at some hamster sites increased by 1½ meters in the latter part of August 1969. Hamsters were exposed in both fresh and brackish (non-tidal) marshes. 4) Near *Tuxpan,* hamsters were placed in mangroves and in thorn-brush adjacent to a freshwater cattail marsh at the mouth of Rio Tuxpan. 5) On the south side of the *Rio Tecolutla* hamsters were placed in mangroves along an estuary of the river. 6) Near *Miraflores* (32 kilometers south of Nautla) hamsters were exposed in a moist, moderately-grazed forest with tall canopy-forming trees adjacent to open pasture. 7) *Laguna la Mancha* (50 kilometers north of Veracruz City) was a tidal lagoon bordered by mangroves. 8) About one kilometer inland from *Barra de Chachalacas,* hamsters were placed in vegetation along a permanent freshwater marsh bordered by open, grazed swamp forest. 9) *Tlacotalpan* on the Rio Papaloapan is an area of savannah and extensive freshwater habitats. Hamster cages were hung in low willow trees and brush along the river. In September 1969 the entire area was inundated with nearly two meters of water in the village center. 10) At *Sontecomapan* the study site was a tropical wet forest of canopy-forming, largely second-growth trees. 11) Near *Minatitlan* hamsters were exposed in swamp forest bordering the extensive freshwater Coatzacoalcos marsh. The latter three study areas have been described and illustrated elsewhere; Tlacotalpan (*9*), Sontecomapan and Minatitlan (*10*).

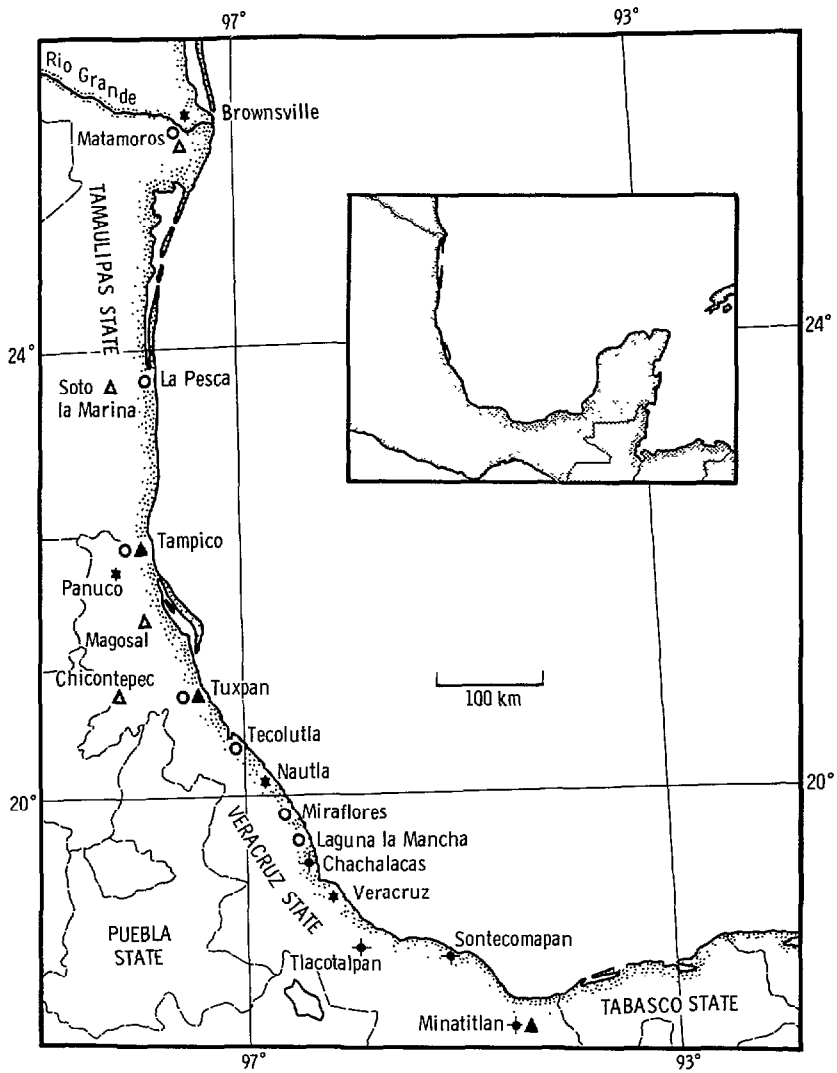
Collection of sera

Pig and goat sera were collected at slaughter houses from severed cervical vessels as described elsewhere (*11*). Horses, birds, humans and dogs were bled by venipuncture. Sera were stored at −20°C until tested.

VE virus antibody tests

Hemagglutination-inhibition (HI) and neutralization (N) antibody tests were per-

FIGURE 1.—Map of study sites of VE virus in Gulf lowland Mexico during July-September 1969. The region where VE virus is currently considered to be endemic is the shaded area of the inset.



✦—Locations of VE virus isolated from sentinel hamsters.
▲—Presence of VE virus antibodies.
○—Study sites without detectable VE virus by use of sentinel hamsters.
△—Sites with no detectable antibody.
★—Places shown for orientation only.

formed with Mexican strains of VE virus (63U2) (*3*), Nepuyo virus (63U11) (*10*) and Patois virus (63A49) (*12*) by microtechnics described elsewhere (*13, 14*).

Exposure of sentinel hamsters and recovery and identification of viruses

The methods used have been described (*10, 12, 15*) except that liquid nitrogen tanks with vapor phase canisters (−100°C) were used for field storage of sick or dead hamsters in locations remote from our field headquarters; otherwise dry ice boxes (−60°C) were used.

Results

VE virus antibody tests of sera from animals and humans

In April 1969, no VE virus N antibody was found in sera of 9 pigs and 12 cows from Matamoros, 16 pigs from Tampico, 3 pigs from Chicontepec, Veracruz and 7 pigs from Magosal, Veracruz; one additional pig serum from Matamoros had an LNI of 1.6 but the HI titer was <10. As a positive control for antibody tests of sera collected during July-September 1969, pig sera from an endemic region (Minatitlan, Veracruz) were collected in July 1969 and tested for VE virus HI antibody; 8 of 24 pigs less than 2 years of age had detectable HI antibody with titers ranging from 10 to 40. At various locations in northern Veracruz and southern Tamaulipas, VE virus HI and N antibodies were found in sera of pigs, horses and humans (table 1). However, in the Tampico region, no antibody was found in humans less than 3 years of age (*i.e.*, born since the 1966 outbreak), and in only one horse, 1-2 years of age and thus born since the outbreak. (The HI titer of this horse serum was 20 and N titer 4). Horses less than 1 year of age had HI and N antibodies, but the prevalence of N antibody was considerably higher than HI antibody (table 1). This observation plus the high prevalence of antibodies in horses over 2 years of age and the fact that horses nurse until at least 1 year of age, made it likely that these antibodies in young equines were maternal in origin. HI antibody titers in horses less than one year of age were 10 and N titers were 4 except for one horse with an N titer of 100. HI titers in horses over 2 years old ranged from 10 to 40 and

TABLE 1—Results of VE virus HI and N antibody tests of 579 vertebrate sera collected from northern Veracruz to northern Tamaulipas, Mexico during July-September 1969.

Host	Age	Fractions with detectable VE antibody in serum and numbers tested			
		Northern Veracruz southern Tamaulipas [a]		Central & northern Tamaulipas [b]	
		HI	N	HI	N
Pigs	½-2 yrs.		***.09*** 11		
Horses	<1 yr.	***.14*** 22	***.45*** 22	*.00* 7	*.00* 7
	1-2 yrs.	***.09*** 11	***.09*** 11	*.00* 6	*.00* 6
	>2 yrs.	***.40*** 78	***.61*** 78	*.00* 50	*.00* 50
Humans	<3 yrs.	*.00* 17	*.00* 17	*.00* 4	*.00* 4
	3-20 yrs.	*.00* 81		*.00* 38	*.00* 17
	>20 yrs.	***.10*** 122	***.20*** 73	*.00* 4	*.00* 4
Ardeidae	<6 days		*.00* 11 [c]		
	10-30 days	*.00* 24 [d]			
Icteridae	immature	*.00* 7			
	adult		*.00* 17		
Goats	<1 month				*.00* 46
Dogs	½-15 yrs.			*.00* 23	*.00* 23

[a] Pigs were from Tuxpan, Veracruz, horses, Icteridae and humans from Tampico, Tamaulipas and vicinity, and Ardeidae from Laguna la Mancha, Veracruz.
[b] Goats were from Cruillas and Soto la Marina, Tamaulipas. Others were from Matamoros, Tamaulipas and vicinity.
[c] 8 Cattle Egrets and 3 Louisiana herons.
[d] 23 Cattle Egrets and 1 Common Egret.

N titers from 4 to 100. The patterns of N antibody titers against three strains of VE virus suggested that these antibodies had been engendered by an endemic strain like Mexican 63U2; titers were $\geqq 20$ in 8 of 8 horses with 63U2 virus, in none of 8 with a 1969 Guatemalan epizootic-epidemic strain (69Z1) and in only 3 of 8 with the attenuated TC83 vaccine strain. Small samples of wild birds (Ardeidae and Icteridae) from northern Veracruz and southern Tamaulipas revealed no antibody in plasmas.

In central Tamaulipas suckling goats were without N antibody and in northern Tamaulipas near Matamoros, horses, humans and dogs were entirely negative for VE virus HI and N antibodies (table 1).

VE virus isolations from sentinel hamsters

Again, as positive controls, adult hamsters were exposed during July-August 1969 at previously documented endemic habitats of VE virus in southern Veracruz to reinforce the validity of possible negative results in more northern and arid regions along the Gulf of Mexico. Two strains of VE virus were recovered from 15 hamsters exposed at Minatitlan and 2 from the same number of hamsters at Sontecomapan (table 2). Isolations per 100 hamster-days at these locations were 2.0 and 1.7 which were similar to results at Minatitlan during 1964-1967 (average 2.4), but lower than the previous experience at Sontecomapan during 1963-1966 (average 4.0) (*15*). During July and August 1969, VE virus was isolated for the first time at Tlacotalpan, Veracruz, a location where antibody had been found as early as 1961 (*5*), but 216 hamster-days of exposure during 1964 and 1965 had failed to yield virus (*15*). VE virus was also isolated north of Veracruz City as Chachalacas near Cardel (table 2). This site seemed particularly active, yielding 5.0 VE virus isolations per 100 hamster-days. Four habitats between Chachalacas and Tampico, 5 near Tampico, one in low vegetation near the coast at La Pesca, Tamaulipas and sites in vegetation along the Rio Grande adjacent to open fields at Matamoros yielded no strains of VE virus during more than 1,900 hamster-days (table 2). No VE virus HI antibody was detected in serum of any of 3 surviving hamsters at Minatitlan, 6 at Sontecomapan, 15 at Tlacotalpan, 17 at Tampico or 14 at Matamoros.

Nepuyo virus in arbovirus group C was recovered from 5 additional hamsters dying or becoming ill at Minatitlan, from 1 at Sontecomapan, 1 at Chachalacas and 1 at

TABLE 2—Observations of 184 sentinel hamsters (5-11 weeks of age) on the Gulf coast of Mexico during July-August 1969.

State and location		No. of hamster-days	Fraction of hamsters yielding VE virus	VE virus isolation per 100 hamster-days
Veracruz				
Southern	Minatitlan	102	2/15	2.0
to	Sontecomapan	118	2/15	1.7
	Tlacotalpan	504	2/20	0.4
Central	Chachalacas	60	3/10	5.0
to	Laguna la Mancha (30 km N of Cardel)	64	0/8	0
	Miraflores(32 km S of Nautla)			
	Tecolutla	62	0/10	0
Northern	Tuxpan	133	0/9	0
	Near Tampico	435	0/26	0
Tamaulipas				
South	Near Tampico	611	0/27	0
Central	La Pesca	143	0/13	0
North	Matamoros	356	0/15	0

Tampico. CF antibody to Nepuyo virus was found in post-exposure serum of 1 hamster at Tampico and 1 at Matamoros. Patois group viruses were isolated or CF antibody found in post-exposure serum from 1 and 3 additional hamsters respectively at Minatitlan, 1 and 3 at Sontecomapan, 1 and 1 at Tlacotalpan, 2 and none at Chachalacas, none and none at Tampico and none and 2 at Matamoros. Thus apparently arboviruses other than VE virus were also active during July-August 1969 along the Gulf coastal lowlands of Mexico north to the USA border at Matamoros. Nepuyo and Patois group arboviruses were not previously known in Mexico north of southeastern Veracruz (*10, 12*).

Discussion

These studies were done to learn, as of July-September 1969, the geographic distribution of VE virus along the Gulf coast of Mexico north of its endemic locations near Minatitlan and Sontecomapan, Veracruz. Also the question was asked whether the virus was still present near Tampico, three years after it had produced an equine epizootic there. Historically the outbreak in 1966 was the first and only equine outbreak recognized to be due to VE virus north of Panama, prior to 1969 when these studies were done. Thus it raised the question as to whether the virus recently moved into the Tampico region and, if so, did it establish endemic cycles and remain there, or was the outbreak merely due to a temporary penetration of virus followed by retreat. In 1941, there was an equine encephalitis epizootic near Matamoros, Tamaulipas on the Mexico-U.S.A. border and in Texas, but the virus recovered from horse brain was identified as eastern encephalitis (*16*). Thus, although the region north from Tampico to Matamoros is fairly dry and arid much of the year, evidently arbovirus outbreaks can occur during rainy seasons and in the presence of irrigation such as exists in the Rio Grande valley near Matamoros. A third objective of these studies was therefore to learn whether equine and human populations on the northern Gulf coast of Mexico were susceptible or were partially immune to VE virus as of 1969.

The results showed that VE virus existed during July-September 1969 in marshy and wet, forested, lowland habitats in Veracruz as far north as Chachalacas north of Veracruz City. The virus was not present or sufficiently active to be recovered by the sensitive sentinel hamster technic (*17*) north of this point. Also by antibody tests of animal and human sera, there was no conclusive evidence of persistent VE virus activity near Tampico, and there were large populations of susceptible equines and humans without detectable neutralizing antibody in serum there and farther north at Matamoros. Thus these regions are open to future attack by this virus.

Of course, the precise northern limit of VE virus in the northern Veracruz region was probably not determined by these studies since not all available marshy or wet forested habitats were tested with sentinel hamsters. Moreover, in the Tampico region, though the usual large lakes were present there during July and August 1969, these months were relatively dry, and it was not until the latter part of August that intensive rains began and many fields became flooded to provide additional areas for vector mosquito breeding. Nevertheless, it can be stated that the limit of VE virus activity during July-September 1969 was somewhere in the Chachalacas to Tampico region of northern Veracruz State. With further, more detailed studies of this region, it should be possible to map the exact northern limit of virus activity, and to learn whether it fluctuates during wet and dry seasons and whether it is progressively moving northward.

Summary

During July-September 1969 VE virus was recovered from sentinel hamsters ex-

posed in marshy or wet, forested habitats along the Gulf coastal lowlands of Mexico as far north as Chachalacas (latitude 19° 26′ and longitude 96° 26′) north of Veracruz City. Hamsters north of this site were negative for VE virus although they yielded Nepuyo virus in arbovirus group C at Tampico, Tamaulipas and Patois group viruses at Chachalacas. Antibody evidence of sentinel hamster infections by Nepuyo and Patois group arboviruses also occurred as far north as Matamoros on the Mexico-U.S.A. border. By antibody tests of sera from horses, other animals and humans, evidence of VE virus infection was detected as far north as Tampico and vicinity, but the results did not conclusively indicate that VE virus cycled there since the equine epizootic in 1966. There was no evidence of VE virus activity at Matamoros, and thus large populations of equines and humans were susceptible there as well as near Tampico. □

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Virus de EV en la costa central y septentrional del Golfo de México, en julio-septiembre de 1969 (*Resumen*)

Durante los meses de julio a septiembre de 1969 se recobró virus de encefalitis venezolana (EV) en cricetos centinelas expuestos a habitat pantanosos o húmedos de las llanuras costeras del Golfo de México, a un punto tan septentrional como Chachalacas (19° 26′ de latitud y 96° 26′ de longitud) al norte de la ciudad de Veracruz. Los cricetos que habitaban al norte de este lugar resultaron negativos con respecto al virus EV aunque mostraban virus de Nepuyo en el grupo de arbovirus C en Tampico, Tamaulipas, y virus del grupo Patois en Chachalacas. También se obtuvieron pruebas de la presencia de anticuerpos de infecciones por arbovirus del grupo Nepuyo y Patois en cricetos centinelas, en puntos tan septentrionales como Matamoros en la frontera Mexicano-Estadounidense. Las pruebas de anticuerpos de sueros de caballo, otros animales y seres humanos demostraron la presencia de infecciones de virus EV en Tampico y sus inmediaciones, pero no se obtuvieron resultados concluyentes que indicaran manifestaciones de ciclos del virus EV en ese lugar desde la epizootia equina de 1966. No se demostró ninguna actividad de virus EV en Matamoros y, por consiguiente, una gran población equina y humana de este lugar, así como de las cercanías de Tampico, era susceptible a la enfermedad.

Virus da encefalite Venezuelana na costa central e do nordeste do Golfo do México, julho-setembro de 1969 (*Resumo*)

Durante julho-setembro de 1969 o virus VE foi isolado em cobaias expostas em habitat florestais pantonosos ou úmidos ao longo das terras baixas litorâneas do Golfo do México, tão ao norte como Chachalacas (latitude 19° 26′ e longitude 96° 26′), ao norte de Veracruz. As cobaias ao norte dêsse lugar eram negativas ao virus VE embora registrassem virus Nepuyo no arbovirus do grupo C em Tampico e Tamaulipas, e viroses do grupo Patois em Chachalacas. A evidência de anticorpos em cobaias infectadas por arboviroses do grupo Nepuyo e Patois foi registrada, também, tão ao norte como Matamoros, na fronteira do México-E.U.A. Os testes de anticorpos de serum de cavalos, outros animais e seres humanos localizaram uma infecção do virus VE bem ao norte, como em Tampico e suas vizinhanças; mas os resultados não indicaram em caráter definitivo a existência de um ciclo de virus VE alí, desde a epizootia eqüina de 1966. Não se verificou atividade do virus VE em Matamoros e, portanto, grandes populações eqüinas e humanas eram suscetíveis nessa localidade bem como nas proximidades de Tampico.

Virus de l'encéphalite vénézuelienne le long des parties septentrionale et centrale de la côte du Golfe du Mexique, Juillet–Septembre 1969 (*Résumé*)

Pendant la période Juillet-Septembre 1969, on a constaté des cas de contagion par virus de l'encéphalite vénézuelienne (EV) chez des hamsterstémoins exposés dans les régions boisées, et humides ou marécageuses, des basses terres de la côte du Golf du Mexique, y compris en des points aussi septentrionaux que Chachalacas (19° 26′ de latitude et 96° 26′ de longitude) au nord de Veracruz. Les hamsters postés au nord de cette région n'ont pas réagi au virus EV, alors qu'ils ont été touchés par le virus Nepuyo du groupe arbovirus C à Tampico, et par les virus des groupes Tamaulipas et Patois à Chachalacas. La présence d'anticorps a également révélé la contagion de certains hamsters par les arbovirus des groupes Nepuyo et Patois dans des régions aussi éloignées vers le nord que Matamoros, située entre la frontière Mexique-Etats-Unis. En testant les sérums d'équidés, d'autres animaux divers, et d'hommes pour la présence d'anticorps, on a pu déceler des cas d'infection par virus EV jusque dans le nord—Tampico et ses environs —mais depuis l'épizootie de 1966, on n'a trouvé aucune preuve indiquant de manière certaine la présence d'un cycle de virus EV dans cette région. A Matamoros, on n'a constaté aucune trace d'activité virale EV, ce qui signifie que de nombreux humains et équidés y sont exposés à la contagion.