SEROLOGIC SURVEY FOR ANTIBODIES TO VE VIRUS IN WESTERN AND NORTHCENTRAL MEXICO [1]

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Venezuelan encephalitis virus was apparently absent from northwestern and northcentral Mexico during 1960-1965, and only suggestively active on the central Pacific coast. Thus during periods of rainfall and mosquito breedings, these regions could be susceptible to invasion by this mosquito-borne virus which is endemic in southeastern Mexico and throughout much of Central America. This article presents the results of the serologic survey for antibodies to the virus in domestic animals, humans and wild birds collected during 1960-1965 in the central Pacific coastal, northwestern and northcentral regions of Mexico.

Introduction

Venezuelan (equine) encephalitis (VE) virus and its antibodies have been found in southeastern and eastern Mexico from the Yucatan peninsula to Tampico, Tamaulipas (*1-4*). During 1963-1967, the virus maintained endemic cycles between vector mosquitoes and amplifying vertebrates in several locations in the State of Veracruz, but there was very little evidence of virus activity across the Isthmus of Tehuantepec, although antibody tests of pig sera suggested virus endemicity along the southeastern Pacific coast of Chiapas State (*5*). The virus has also been found throughout much of Central America where it produced an extensive epidemic and equine epizootic in 1969 (*6*).

To explore further the geographic distribution of VE virus in Mexico, antibody tests were done with domestic animal and human sera and wild bird plasma from the central Pacific coast (State of Nayarit) and the northwestern and northcentral regions in the States of Sonora and Coahuila (Figure 1). This article records the results of this serologic survey.

Materials and methods

Description of study region

The northern two-thirds of Mexico is dominated by broad arid to semi-arid valleys and plans interrupted by usually low, but occasionally high mountain ranges. The vegetation of these low to moderately elevated plains ranges from sparse cresote brush in their driest aspect in Sonora and some areas of Coahuila, to dense low desert chaparral where rainfall or soil conditions permit, to dense low thorn forest on the Pacific coastal lowlands south of Sonora. On the Pacific coastal lowlands in Nayarit there is a localized area of somewhat more mesic vegetation. *Hermosillo, Muzquiz* and *Nueva Rosita* (Figure 1) are all located in areas of extreme dryness, with cresote brush and other xeric desert vegetation dominating the surrounding countryside. Each city is located

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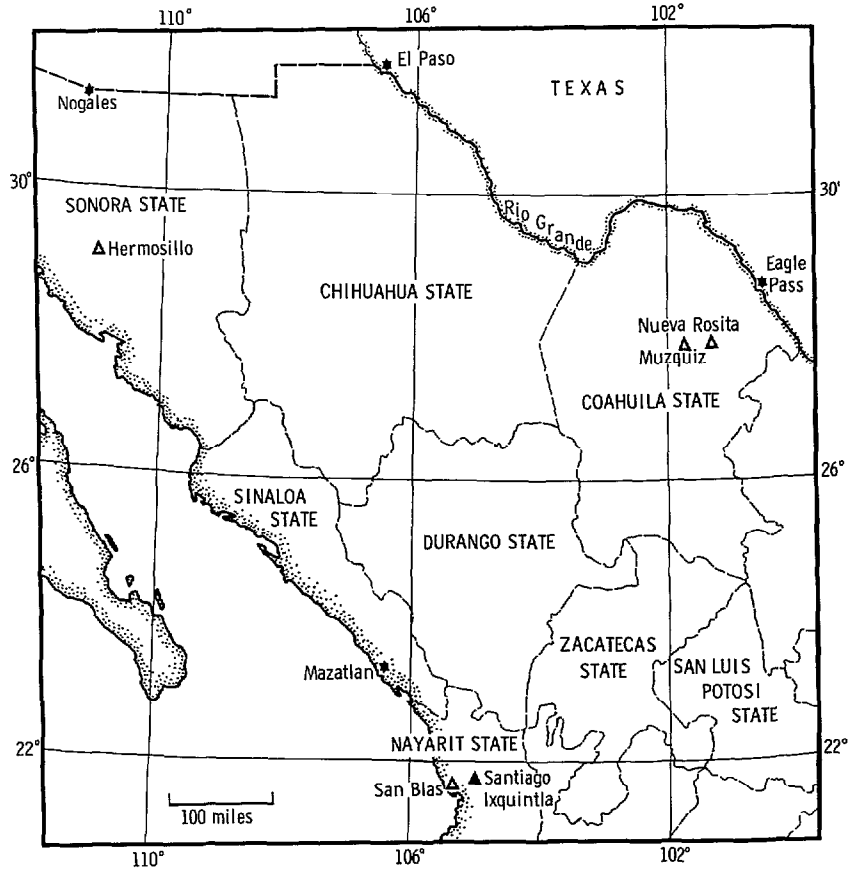
FIGURE 1—Map of study sites in northwestern Mexico during 1960-1965. Solid triangles indicate location where VE virus antibody was present, open triangles indicate sites without detectable antibody. Stars indicate places shown for orientation only.



on an intermittently flowing river, and each has in the immediate environs, locally developed irrigation farming. *Santiago Ixquintla* is located in the more mesic region of Nayarit, that supports extensive farming without irrigation in the rainy season. *San Blas* is a small coastal village surrounded by extensive seasonally flooded mangrove flats that are transected by tidal rivers and estuaries. Several species of herons nest in a large colony in these mangrove flats during July-October 1962-1965 (*7, 8*).

Collection of sera

Pig, cattle and goat sera from Nayarit and Coahuila were collected at slaughter houses as described elsewhere (*9*). Horses were bled by jugular venipuncture. Nestling anhingas, cormorants and herons 14 to 30 (mode about 20) days of age were caught by hand and bled by jugular venipuncture as described elsewhere (*10*). The sera from Sonora were kindly provided by Dr. W. C. Reeves, University of California and Dr. Harald N. Johnson, Rockefeller Foundation and California Department of Health, Berkeley, California; they represented residual volumes from a previous serologic survey in 1960 for antibodies to eastern, (EE) western (WE) and St. Louis encephalitis (SLE) viruses, Powassan, dengue types 1 and 2, Rio Bravo and Modoc arboviruses (*11*). Sera were stored at −20°C until tested.

TABLE 1—Results of VE virus HI and N antibody tests of pig and cattle sera collected at Santiago Ixquintla, Nayarit, on the central Pacific coast of Mexico.

Domestic animal	Dates bled	Ages in years	No. animals with detectable VE antibody in serum/tested			
			HI [1]	N-CEC [1] a	N-CEC [1] b	N-WMip [1]
Pig	Oct. 1964	<3	2/19 [2]	4/15	0/5 [3]	1/5
	Oct. 1965	<1	0/10			
		1-3	0/13		1/12	
Cattle	May 1962	adult	0/1		0/7	
	Oct. 1964	"	1/10 [4]		4/8 [5]	2/4
	Oct. 1965	"	0/10		5/10 [6]	

[1] HI positive indicates titer $\geq$ 10 for pigs and $\geq$ 20 for cattle. N positive = LNI > 1.6 in chicken embryonic cell cultures: a—undiluted, unheated serum in 13 mm wells in leucite plates and b—1:4 serum heated 60°C 20 min in 16 mm wells in plastic plates. N positive in weanling mice inoculated intraperitoneally (WM-ip) also signifies LNI > 1.6 with undiluted, unheated serum.
[2] HI titers = 10
[3] Includes the 4 sera which were positive undiluted and unheated.
[4] HI titer = 40, N titer $\geq$ 4.
[5] 3 titers = 4 and 1 titer = 20.
[6] 4 titers = 4 and 1 $\geq$ 100.

Antibody tests

Hemagglutination-inhibition (HI) and neutralization (N) antibody tests were performed with Mexican strain, 63U2, of VE virus (*2*) by microtechnics described elsewhere (*12, 13*).

Results

At Santiago Ixquintla, Nayarit, on the central Pacific coast of Mexico, a few pig and cattle sera had VE virus HI and N antibodies in significant titers during 1964 and 1965 (Table 1, Figure 1). Some of the

TABLE 2—Results of VE virus HI antibody tests of plasmas from nestling wild birds 14-30 days of age, collected at San Blas, Nayarit on the central Pacific coast of Mexico, during September and October 1962-1965.

Species	Year				Total
	1962	1963	1964	1965	
Anhinga (*Anhinga anhinga*)	0/3	0/2		0/1	0/6
Olivaceous Cormorant (*Phalacrocorax olivaceus*)	0/6				0/6
Green Heron (*Butorides virescens*)	0/3	0/4	0/22	0/39	0/68
Common Egret (*Egretta alba*)	0/6	0/2	0/4	0/40	0/52
Snowy Egret (*Egretta thula*)	0/5	1/14 [1]	0/23	0/22	1/64
Little Blue Heron (*Egretta coerulescens*)	0/12	0/7	0/18	0/23	0/60
Louisiana Heron (*Egretta tricolor*)	0/10	0/2	0/7	0/10	0/29
Reddish Egret (*Egretta rufescens*)				0/1	0/1
Yellow-crowned Night Heron (*Nyticorax violacea*)				0/10	0/10
Boat-billed Heron (*Cochlearius cochlearius*)	0/4	0/14	0/20	0/13	0/51
Totals	0/49	1/45	0/94	0/159	1/347

[1] Titer = 20 *vs* VE, 80 *vs* EE and 10 *vs* WE.

positive cattle sera may not represent specific virus antibody since cattle sera can contain non-specific arbovirus inhibitors (*14*). Nevertheless one serum had an HI titer=40 in 1964 and another, an N titer≧100 in 1965 which probably indicated specific VE virus antibody. The few positive results with pig sera reinforced this interpretation of cattle sera since pigs are known to develop specific antibodies to arboviruses (*15*). Since the numbers of sera tested were small, antibody prevalences could not be accurately determined, but they probably did not exceed .10 for pigs and .50 for cattle. The cattle of course were older than the pigs and thus had more opportunity to become infected.

At San Blas, Nayarit VE virus HI antibodies were found in only one of 347 plasmas from nestling cormorants, anhingas and herons 14-30 days of age collected in September-October 1962-1965 (Table 2). The one plasma that had an HI titer to VE of 20 also had a titer of 10 to WE virus and 80 to EE virus, and probably represents cross reactive HI antibody from infection with EE virus.

At Hermosillo, Sonora, in northwestern Mexico, no VE virus antibodies were found in sera of horses, chickens or humans except for two university students with low titers of HI antibody, but no detectable N antibody (Table 3). One of these sera also reacted with EE hemagglutinin (Table 3).

Likewise at Nueva Rosita and Muzquiz, Coahuila on the northern central plateau, no VE virus HI or N antibodies were found in pig, cattle, goat or horse sera except for one goat serum positive by N, but not HI antibody test (Table 4). The absence of positive tests with these cattle sera strengthened the interpretation of the positive tests with Pacific coast cattle as indicative of VE virus antibody.

TABLE 3—Results of VE virus HI and N antibody tests of horse, chicken and human sera collected in 1960 at Hermosillo, Sonora in northwestern Mexico.

Host	Ages in years	No. hosts with detectable VE antibody in serum/tested		
		HI	N-CEC [2] a	b
Horses	3-25	0/26	0/41	0/11
Chickens	adults		0/10	0/26
Humans	13-55	2/27 [1]	0/11	0/47

[1] Two university students 21-28 years of age with VE HI titers = 10. Both < 10 *vs* WE, Patois and Nepuyo viruses and one *vs* EE and SLE. The other was ≧ 20 *vs* SLE and 10 *vs* EE.
[2] See footnote [1] in Table 1 for meaning of a or b.

Discussion

Although during the period of this serologic survey, VE virus was widespread and endemic in southeastern Mexico (*1-3, 5*) and has been found along the Gulf coast as far north as Tampico, Tamaulipas, (*4*) this limited serologic survey for VE virus HI and N antibodies in wild bird, domestic animal and human sera showed that VE virus is active at most at a very low level on the central Pacific Coast (Nayarit State) and probably is non-existent in the northwestern (Sonora State) and northcentral

TABLE 4—Results of VE virus HI and N antibody tests of pig, cattle, goat and horse sera collected at Nueva Rosita or Muzquiz, Coahuila, on the northern central upland plateau of Mexico.[1]

Domestic animal	Dates bled	Age in years	No. animals with detectable VE antibody in serum/tested			
			HI	N-CEC a	b	N-WMip
Pig	June 1964	1/2-1	0/2	0/3		
Cattle	Apr.-June 1964	1-10	0/12	0/5	0/6	0/4
Goats	April 1964	adults	0/25	0/9	1/2 [2]	0/1
Horses	June 1964	3-20	0/7	0/7		

[1] See footnote [1] in Table 1. Horses were at Muzquiz.
[2] LNI 1.9 with 1:4 serum dilution, 1.6 with 1:20, and 1.4 with 1:100. HI < 10.

(Coahuila State) regions. The two university students from Sonora in 1960 with low levels of HI, but not N substances in serum may represent cross-reactive antibodies to another group A arbovirus; one serum was also positive with EE virus hemagglutinin and Reeves *et al.* found one percent of these human sera from Hermosillo positive (titers $\geq$1:20) to another group A arbovirus, western encephalitis (*11*). Likewise the single bird plasma positive by HI test to VE also probably represented cross reaction with other EE virus, another group A arbovirus. The one goat serum positive by VE virus N, but not HI, antibody tests in Coahuila cannot be explained at the moment. Presumably antibodies to other group A arboviruses should not cross-*neutralize* VE virus, and the fact that a 1:20 dilution of heated serum (60°C, 20 minutes) yielded an LNI of 1.6, was compatible with the N substance being specific antibody. However the results still could represent other virus inhibiting materials since apparently, nonspecific arbovirus inhibitors can occasionally occur in goat sera (*14*).

Nestling herons develop detectable levels of HI antibodies to VE virus by the sixth day post-infection (*16*). Thus nestlings sampled at 14–30 days of age were indicators of VE virus transmission for 7–24-day periods during the season of peak mosquito activity late in the rainy season during the 4 years represented by the sample.

The absence of detectable antibody evidence of VE virus in the relatively dry, arid northwestern and northern regions of Mexico was not unexpected although other arboviruses were being transmitted to wild birds at San Blas during the period represented by specimens here reported (*16*). To date the known endemic cycles of VE virus between mosquitoes and vertebrates have been limited to tropical, moist habitats. However, the virus can penetrate ordinarily dry regions during wet seasons and cause epidemics and epizootics, such as occurred in the Guajira peninsula of Colombia and Venezuela in 1963 and in semi-arid regions of Guatemala, El Salvador and Nicaragua in 1969. Thus in northern Mexico, during periods of rainfall or in areas with surface water, such as from irrigation, VE virus could presumably begin cycling between mosquitoes and vertebrates, and since protective antibodies were absent from the region during 1960-1965, the virus could cause an epidemic and equine epizootic. This possibility must be kept in mind by public health and agricultural authorities both in Mexico and in adjacent regions of the United States of America.

Summary

A serologic survey of wild birds, domestic animals and humans during 1960-1965 revealed very little evidence of Venezuelan encephalitis virus activity in pigs, cattle and wild birds on the central Pacific coast of Mexico, and no evidence of the virus in northwestern and northcentral Mexico by tests of horse, chicken, pig, cattle, goat and human sera, and colonial nesting heron plasmas. □

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Encuesta serológica relativa a los anticuerpos de virus de encefalitis venezolana en México (*Resumen*)

Una encuesta serológica de aves silvestres, animales domésticos y seres humanos, realizada de 1960 a 1965, reveló una actividad muy escasa del virus de la encefalitis venezolana en ganado porcino y bovino y en las aves silvestres de la costa central del Pacífico de México. No demostró la presencia del virus en el noroeste de México ni en el sector septentrional del centro del país, en las pruebas practicadas en sueros de caballos, aves de corral, cerdos, bovinos, cabras y seres humanos y en plasma sanguíneo de garzas que anidan en colonias.

Pesquisa serológica de anticorpos em virus de encefalite venezuelana no México (*Resumo*)

A pesquisa serológica em aves selvagens, animais domésticos e sêres humanos no periodo 1960-1965 apresentou muito pouca evidência de atividade do virus de encefalite venezuelana em porcinos, bovinos e aves selvagens no costa central mexicana do Pacífico. Nenhuma evidência do virus no noroeste e norcentro do México nos testes de plasma de aves em colônia e de serum de equinos, galináceos, porcinos, caprinos e humanos foi demostrada.

Recherche, par examen sérologique, d'anticorps indiquant une réaction au virus de l'encéphalite vénézuelienne au Mexique (*Résumé*)

L'examen, effectué en 1960-1965, de sérums d'oiseaux sauvages, d'animaux domestiques, et d'hommes, n'a révélé que fort peu de traces d'activité du virus de l'encéphalite vénézuelienne chez les porcins, les bovins et les oiseaux sauvages dans la partie centrale de la cote pacifique du Mexique; par ailleurs, dans les régions Nord-ouest et Nord-centre, aucun indice de contagion par ce virus n'a pu être décelé sur des sérums de cheval, de poulet, de porc, de bovin, de chèvre et d'homme, ni sur des plasmas de héron nicheur vivant en colonie.

Influenza en el mundo, 1968-1969

Durante el período comprendido entre octubre de 1968 y septiembre de 1969, la mayoría de los brotes se debieron al virus A2/Hong Kong/68 aunque en países de todos los continentes se aisló el virus B. La propagación en forma explosiva ocurrió de julio a septiembre de 1968 y, a partir de esta fecha, sólo continuó en los Estados Unidos, donde se esparció rápidamente hasta principios de febrero de 1969. Para diciembre de 1968 se habían notificado brotes de influenza en casi todos los estados de este país. La gravedad de esta epidemia se consideró de la misma magnitud que la ocurrida en 1957-1958 cuando prevaleció el virus A2 original. Sin embargo, en otros países de clima templado de las Américas la influenza no fue tan pronunciada. Por ejemplo, en el Canadá sólo hubo un ligero aumento en la incidencia de la enfermedad y muy pocas defunciones.

Algunos de los países tropicales que no fueron afectados durante la primera ola en julio, agosto y septiembre de 1968 fueron atacados más tarde. Se registraron brotes en Kenia y epidemias en el Brasil, Ceilán, Fijí, México, Puerto Rico y Senegal. Para 1969, los brotes empezaron en marzo en Sudáfrica y para mayo ya se habían notificado brotes o epidemias en Argentina, Australia, Chile, Nueva Zelandia y Uruguay.

Si bien en varios países de Europa ocurrieron brotes leves de influenza, en Polonia se estima que ocurrieron más de cuatro millones de casos de una enfermedad parecida a la influenza. En los países del norte, de clima templado, los brotes cesaron para fines de abril.

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