

## STUDY OF VE VIRUS AND ISOLATION OF SLE, EE, GROUP C, AND GUAMA GROUP ARBOVIRUSES IN THE AMAZON REGION OF PERU, 1975<sup>1</sup>

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*The source of the Venezuelan encephalitis (VE) virus that has caused many of the human and equine disease outbreaks in Peru has not yet been determined. This article reports findings from a study in northern Peru consistent with the theory that the virus is silently enzootic in the Peruvian Amazon Basin, and that movements of virus out of this region are the cause of the periodic human and equine VE disease outbreaks along Peru's dry Pacific Coast.*

### Introduction

Venezuelan encephalitis (VE) virus has intermittently produced equine epizootics and human epidemics along the dry Pacific coastal plain of Peru for at least the last 35 years (1, 2). Equine epizootic disease, like that caused by VE virus, was recorded on the northern coastal plain of Peru in 1925, and VE outbreaks of proven etiology occurred from the early 1940s to the early 1950s and again in 1969 and 1973 (1-5).

The source of VE virus for these epidemics and equine epizootics has not been determined except in 1969, when the disease was limited to northwestern coastal

Peru adjacent to Ecuador. On that occasion the disease resulted from southward extension of a large Ecuadorian epizootic and epidemic (3, 6). But other Peruvian epidemics, the most recent recorded in 1973, have not bordered Ecuador.

The 1973 VE outbreak in northern Peru was described in Spanish by Madalengoitia and associates (4) and by Terry (5). The VE virus involved was isolated from both humans and equine animals, and its hemagglutination-inhibition (HI) subtype was established as I-B by the U.S. Center for Disease Control in Atlanta, Georgia, and by other studies (4, 7). Cases of disease in 93 human subjects 1 to 68 years of age were investigated. (Thirty per cent of those involved were under 10 years of age; 59 were males and 23 females, the gender of the remaining 11 not being recorded.) Central nervous system symptoms were present in about 25 per cent of the cases, although most patients were ambulatory.

A diagnosis of Venezuelan encephalitis was confirmed in 41 cases by isolation of the virus from blood and/or by VE antibody development in serum. A presumptive diagnosis was made in 28 cases, based on VE IgM antibodies or unusually high anti-

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body titers in single sera. Specimens from the other 24 cases were inadequate for diagnostic purposes. All of the 41 confirmed cases came from three departments along the Pacific Coast of northern Peru (4-9° south latitude), 1 coming from Lambayeque, 17 from La Libertad, and 23 from Piura (see Figure 1). The number of equine cases was estimated at over 2,500, equine mortality being estimated at about 40 per cent. The outbreak occurred during January-June 1973, but some equine cases (as indicated by clinical diagnoses) had been recorded in the region the previous year (in May 1972) following heavy rains. Inactivated VE vaccine was used to immunize equine animals in the early months of 1973, and use of attenuated live-virus vaccine from Mexico (about 50,000 doses) began in March 1973.

This epidemic drew renewed attention to an important question; namely, What is the source or sources of VE virus for such an epidemic when the disease first appears in an irrigated region on the dry Pacific Coast of northern Peru that is neither contiguous with Ecuador nor associated with concurrent disease in Ecuador? Two possibilities are either (a) that the virus comes from tropical rain forests in Peru's Amazon region or (b) that persistent small foci of virus, cycling between mosquitoes and vertebrates, exist in irrigated river valleys along the otherwise desert Pacific Coast of Peru. Studies of these possible sources of Peruvian VE epidemics and equine epizootics were carried out during 1970-1971 and have been reported (2, 8). However, because of the 1973 outbreak, further investigations were done in 1975. This article describes the results of these latter investigations.

As in 1970 and 1971, when arboviruses other than VE (including eastern encephalitis, group C, and Guama group viruses) were encountered in Peru's Amazon region (9), additional arboviruses were recovered from tissues of mosquitoes and sentinel hamsters in 1975. Information about those

isolations—of St. Louis encephalitis (SLE), eastern encephalitis (EE), group C (Marituba), and Guama group arboviruses—are included in this article.

## Materials and Methods

### *Study Sites and Field Techniques*

Two types of study sites were employed. These were: (1) in Loreto Department, tropical wet forests near Yurimaguas on the Huallaga River and Iquitos on the Amazon River, sites located at respective altitudes of 180 and less than 200 m; and (2) in Amazonas Department, tropical dry forest near Imacita on the Marañón River, a site situated at an altitude of 240 m and at 5°5' south, 78°22' west (see Figure 1). Only patches of forest remained in the environs of Yurimaguas, since most of the land there had been cleared for agricultural purposes. In contrast, the regions around Iquitos and Imacita were mostly forested in 1975.

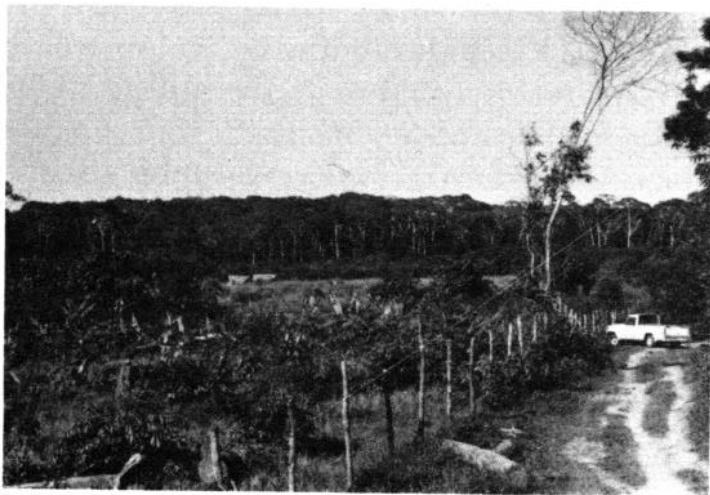
Mosquitoes were collected with Center for Disease Control (CDC) light traps at two forest habitats within 6 km of Yurimaguas (Muniches Airport Road and San Ramón), and sentinel Syrian hamsters (*Mesocricetus auratus*) were exposed in these areas (see Photographs 1-3). The site near Iquitos was the Quistococha forest previously studied in 1970-1971 (2, 9). The third site investigated was a small patch of forest within 1 km of Imacita (Photo 4) on the property of Benigno Chiozo (Photo 5).

The female mosquitoes collected, without further identification, were placed in paper candy cups. The cups were wrapped in aluminum foil, labeled inside and out, and stored in liquid nitrogen vapor at -100°C until they reached New York in May 1975. Subsequent storage was at -60°C. The sentinel hamsters came from the Lakeview Hamster Colony in New Jersey and were

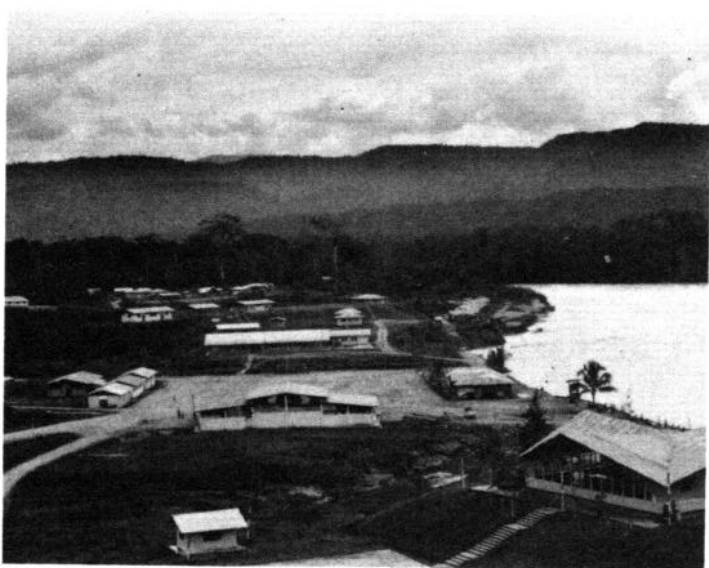


(1)

(2)



(3)

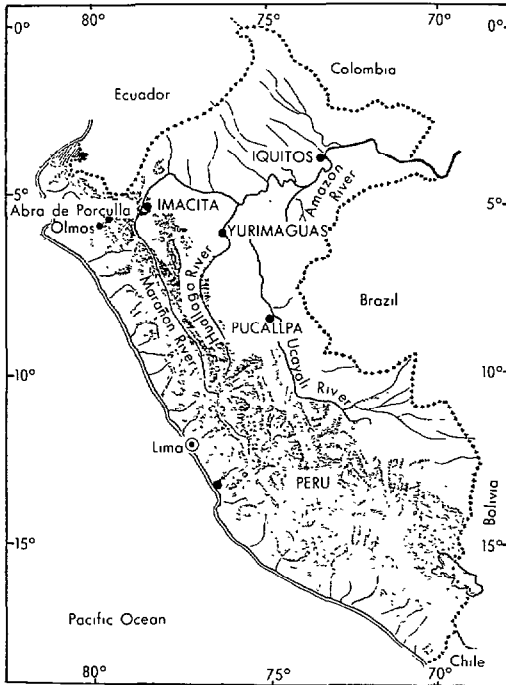


(4)



(5)

(1) The forest at the airport road site near Yurimaguas is seen in the distance to the left. The Yurimaguas airport is within a kilometer to the right of this road. (2) The forest at the San Ramón site, also near Yurimaguas, is seen in the distance across cleared fields. (3) A close-in view of forest at the San Ramón site. (4) The village of Imacita, located along the Marañón River in Amazonas Department. (5) The small forest studied near Imacita, on the Benigno Chiozo property.



**Figure 1.** A map of Peru showing the 1975 arbovirus study sites.

shipped by airplane to Peru. The hamsters exposed at Iquitos and Yurimaguas had been previously immunized in New York with group C and Patois arboviruses, since it was known from experience in Central America that these viruses could kill sentinel hamsters if transmitted to them by mosquitoes, and thus might interfere with VE virus isolations. This immunization was accomplished by inoculating the hamster with live Patois virus (Mexican strain 63A49) on day 0; then with killed (formalinized) Nepuyo virus (Mexican strain 63U11) with complete Freund's adjuvant on days 14, 20, and 27; with live Nepuyo virus on day 41; and with live Oriboca virus on day 47. Of the 156 hamsters 7 to 8 weeks old that were inoculated, 31 died after receiving Patois virus, 1 died after receiving inactivated Nepuyo virus, 1 died after receiving live Nepuyo virus, and 62 died after receiving live Oriboca virus.

This left 61 immune hamsters 14-15 weeks of age when their exposure began in Peru from late March to early May 1975. The normal unimmunized hamsters were 10 weeks of age when exposure began.

#### *Virus Isolation and Identification*

All tissue suspensions from mosquitoes and from sick or dead sentinel hamsters were tested by combined intracranial (ic) and subcutaneous (sc) inoculation of suckling Swiss albino mice 1-4 days of age that were obtained from Taconic Farms in Germantown, New York. Mosquito suspensions and sentinel hamsters yielding virus were tested in New York during the period May-July 1975. Complement fixation (CF) tests were performed as previously described (10) with saline-extracted antigens from infected suckling mouse brains.

Neutralization (N) tests were based on plaque-reductions—in primary chicken embryonic cell cultures for SLE and EE viruses, and in Vero African green monkey kidney cell cultures for group C and Guama group viruses (11). Some N tests for Guama group viruses were done with suckling mice inoculated ic (11).

As described previously, rooster antibodies were made and used to perform short incubation virus-dilution hemagglutination-inhibition tests to subtype VE virus isolates (7). All cell cultures were prepared and used as described elsewhere (8). Antisera used to identify viruses were as follows: VE—rabbit antisera obtained after two injections with Mexican strain 63U2; SLE—mouse ascitic fluid taken after four injections with Mexican strain 65V310; and EE—rabbit antisera obtained following three or four inoculations with Guatemalan strain 68U230. The U.S. National Institutes of Health (NIH) reference reagent mouse ascitic fluids used were: Group A: G209-701-567; group B: G216-701-567; Bunyamwera group: G205-701-567; group C: G201-

701-567; Guama group: G202-701-567; Ilheus: V509-701-562; Rio Bravo: V536-701-562; Bussuquara: V561-701-562; Modoc: V538-701-562; Cowbone Ridge: V533-701-562; Montana Myotis Leucoencephalitis (MML): V541-701-562; EE strain Massachusetts: V515-701-562; and Mayaro: V507-701-562 (12). Oriboca, Caraparu, and Marituba antisera were kindly supplied by Dr. C. J. Gibbs in 1965 after preparation in rabbits by Dr. W. Pond; and specific Guama, Moju, and Bimiti mouse ascitic fluids were generously provided by Dr. R. Shope.

### Antibody Tests

N and HI tests were done as previously described (11). The antigenic subtype designations used for VE viruses were based on the classification of Young and Johnson (13). For N tests, the strains used were VE strains 52/73, 71D1249, and 71D1252 from Peru (2, 4); SLE strain 75D90; and EE

strain 75U40. The N tests were performed by means of plaque-reduction in cultures of primary chicken embryonic cells prepared as described elsewhere—in plastic plate wells with an area of either 2 or 8 square centimeters (11). HI tests used hemagglutinins from Mexican VE strain 63U2, Guatemalan EE strain 68U230, Mexican SLE strain 65V310, and United States western encephalitis (WE) strain 1985-60.

### Results

#### VE Virus

As Table 1 indicates, a pool of 100 female mosquitoes collected on the night of 7-8 April 1975 at the Quistococha forest site near Iquitos yielded a strain of VE virus. All the inoculated mice in two litters died on days 3 or 6 following inoculation. The strain (75D143) was identified as VE virus by means of the plaque-reduction neutralization test; the  $\log_{10}$  neutralization index

Table 1. A listing of the VE, SLE, EE, group C, and Guama group arboviruses isolated from mosquitoes and adult sentinel hamsters in Peru's Amazon region from late March to early May 1975.

Location	Female mosquitoes					Sentinel hamsters				
	Numbers tested		No. of viruses isolated			Hamster-days of exposure		No. ill or dead over total No. exposed <sup>c</sup>	No. of viruses isolated	
	Individual mosquitoes <sup>a</sup>	Pools	VE	SLE	Guama group	Gr. C-Pat. immune hamsters <sup>b</sup>	Un-immunized hamsters		EE	Group C
Iquitos site (Quistococha)	25,436	245	1	0	2	702		2/18	0	0
Yurimaguas site (Muniches Airport Road)	4,774	44	0	1	0	270		0/10 1/5	0	0
Yurimaguas site (San Ramón)	8,980	56	0	0	0	297		0/11 87	1	1
Imacita site (Benigno Chiozo)	801	6	0	0	0		80	0/10		

<sup>a</sup>The respective numbers of CDC light-trap nights and average numbers of mosquitoes collected per trap night were as follows: Iquitos, 90 and 283; Yurimaguas (airport road site), 45 and 106; Yurimaguas (San Ramón site), 46 and 195; Imacita, 30 and 27. Day ice was only used with the light traps during 6 trap-nights at Iquitos.

<sup>b</sup>Gr. C-Pat. immune signifies hamsters immunized to group C viruses (Nepuyo and Oriboca) and to Patois virus, as described in the text.

<sup>c</sup>Intracranial inoculation of suckling mice with brain tissue and pooled heart, liver, and kidney tissue suspensions from the two dead Iquitos hamsters and one dead Yurimaguas (airport road) hamster did not yield any virus.

(LNI) with VE antiserum was greater than 2.5 and with EE antiserum was 0.5. In a one-hour, virus-dilution hemagglutination-inhibition test using early rooster antibodies, 75D143 hemagglutinin was inhibited 250-fold by antibody to a Colombian subtype I-D VE strain (V209A), 90- or 120-fold by antibodies to two 1971 Peruvian Amazon strains (71D1316 and 71D1249), 8-fold by antibodies to subtypes I-A (strain Kubes), I-B (strain 52/73), and III (strain BeAn8), and less than 8-fold by antibodies to strains of subtypes I-C, I-E, II, IV, and a possible new subtype V. Strain 75D143 (at the passage level of suckling mouse 2 and chicken embryonic cell culture 1) killed both of two adult English short-hair guinea pigs inoculated sc with 16,000 chicken embryonic cell culture (CEC) plaque forming units (pfu).

Sentinel hamsters exposed at Iquitos, Yurimaguas, and Imacita (see Table 1) yielded no VE virus. Plasmas obtained from 28 hamsters on 5 May 1975, at the end of their exposure at Yurimaguas, and from 10 other hamsters on 7 May, following

their exposure at Imacita, contained no detectable HI antibody to VE, EE, or SLE viruses at 1:10 dilutions. Plasmas from hamsters exposed at the Muniches Airport Road site near Yurimaguas yielded HI titers of 1:10 and  $\geq$  1:20 with western encephalitis virus. No CF antibodies to group Capim or Patois arboviruses were detected in 1:4 dilutions of plasma from 6 nonimmune hamsters exposed at Yurimaguas or from 10 exposed at Imacita.

N antibodies to VE were found in 21 per cent of the sera from 29 horses bled at Yurimaguas in April 1975. As Table 2 indicates, sera from animals as young as 2-4 years yielded positive results. Higher percentages of these sera were positive to strains 71D1249 and 71D1252 isolated during 1971 in the Peruvian Amazon region than were positive to epizootic strain 52/73 obtained from the outbreak on the northern Pacific Coast of Peru in 1973.

Sera obtained from people at Yurimaguas in April 1975 also had detectable VE N antibodies. In contrast to the results with

Table 2. Prevalences of VE, EE, and SLE viral neutralizing antibodies in sera from horses bled in Loreto Department, Peru, during 1970 and 1975.

Approximate age of horses (in years)	Proportion (No. positive <sup>a</sup> over No. tested) of horses with sera showing detectable N antibody to particular virus strains						
	Yurimaguas, April 1975				Iquitos, Sept. 1970	Pucallpa, Sept. 1970	
	VE HI subtypes and strains						
	I-B, strain 52/73	I-D, strain 71D1249	?V. strain 71D1252	EE strain 75U40	SLE strain 75D90	SLE strain 75D90	SLE strain 75D90
> 10	0/6	2/6	0/6	4/6	1/6	2/2	—
5-9 <sup>b</sup>	1/16	3/16	3/16	12/16	3/15	2/3	5/12
2-4 <sup>c</sup>	2/7	1/7	2/7	5/7	1/7	3/6	3/10
< 2	—	—	—	—	—	1/2	0/7
Total (percent)	3/29 (10%)	6/29 (21%)	5/29 (17%)	21/29 (72%)	5/28 (18%)	8/13 (62%)	8/29 (28%)

<sup>a</sup>Positive = LNI > 1.6 with 1:4 dilution of serum heated at 60°C for 20 minutes and tested by plaque-reduction against about 100 pfu (plaque forming units) in CEC (chicken embryonic cell culture).

<sup>b</sup>One serum gave a positive response to all three VE strains, while four others only responded positively to one strain.

<sup>c</sup>Two sera responded positively to both VE strains 52/73 and 71D1252, while one responded positively to only 71D1249.

horse sera, however, the highest percentage of positive results (38 per cent of 14 human sera) was obtained with epizootic strain 52/73; only 7 and 14 per cent of the human sera yielded positive results when tested with the Amazonian enzootic strains (see Table 3). Each of the three sera yielding a positive result with the Amazonian strains also yielded positive results with the epizootic strain, and all the people found to have antibodies were over 29 years of age.

VE N antibodies found in these horse and human sera did not correlate with EE N antibodies. Two horse and four human sera tested positively with VE and negatively with EE, while 13 horse and two human sera were negative with VE and positive with EE.

As Table 3 shows, none of the sera from 13 people bled at Imacita during April 1975 showed detectable levels of VE N antibody.

### SLE Virus

A suspension made from 60 female mosquitoes collected on 14-15 April 1975 at the Muniches Airport Road site near Yurimaguas contained SLE virus (see Table 1). All of eight suckling mice inoculated with the mosquito suspension died on day 5 after inoculation. Virus was reisolated from frozen mosquito suspension 6 months later when five more suckling mice were inoculated and died 9 days later. This strain of SLE virus (75D90) was identified by plaque-reduction neutralization tests. An immune mouse ascitic fluid made with SLE virus (Mexican strain 65V310) produced LNI of >2.4 and 3.1 in two tests. LNI obtained with immune mouse ascitic fluids made with Ilheus, Rio Bravo, Bussuquara, Modoc, Cowbone Ridge, and MML flavi-

**Table 3. Prevalences of VE, EE, and SLE viral neutralizing antibodies in sera from 27 human subjects bled in April 1975 at two locations in the Amazon Basin of central and northern Peru, just east of the Andes Mountains.**

Location	Age of subjects (in years)	Proportion (No. positive <sup>a</sup> over No. tested) of people with sera showing detectable N antibody to particular virus strains				
		VE HI subtypes and strains			EE strain 75U40	SLE strain 75D90
		I-B, strain 52/73	I-D, strain 71D1249	?V, strain 71D1252		
Yurimaguas	40-52	2/4	0/4	0/4	0/4	0/4
	30-39 <sup>b</sup>	3/8	1/8	2/8	2/8	0/8
	20-29	0/2	0/2	0/2	1/2	0/2
	Total	5/14	1/14	2/14	3/14	0/14
	(percent)	(36%)	(7%)	(14%)	(21%)	
Imacita	50-57	0/3	0/3	0/3	0/3	0/3
	30-39	0/1	0/1	0/1	0/1	0/1
	20-29	0/5	0/5	0/5	2/5	0/5
	10-19	0/4	0/4	0/4	0/4	0/4
	Total	0/13	0/13	0/13	2/13	0/13
	(percent)			(15%)		

<sup>a</sup>Positive = LNI > 1.6 with a 1:4 dilution of serum heated at 60°C for 20 minutes and tested by plaque-reduction against about 100 pfu (plaque forming units) in CEC (chicken embryonic cell cultures).

<sup>b</sup>One serum, from a malaria program field employee, responded positively to all three VE strains and EE. Another serum responded positively to both VE strains 52/73 and 71D1252.

viruses were in the range of 1.1-1.3. LNI obtained with polyvalent group A, Bunyamwera group, and group C mouse ascitic fluids—and with specific VE and EE rabbit antisera—ranged from 0 to 0.7.

As indicated in Table 2, N antibodies to SLE virus, strain 75D90, were detectable in sera from horses bled at Yurimaguas in 1975 and at Iquitos and Pucallpa (see Figure 1) in 1970. Small series of human sera from Yurimaguas and Imacita were negative for SLE N antibody, but sera from 2 of 13 people 15-53 years of age who were bled at Pucallpa in September 1970 showed detectable levels of SLE N antibody. The two subjects were 26 and 28 years old; the LNI obtained were  $> 2.4$  and 1.7 at fourfold serum dilutions.

#### *EE Virus*

A sentinel hamster died on 26 April 1975 after 18 days of exposure at the San Ramón site near Yurimaguas; brain tissue from this hamster yielded a strain of EE virus (75U40) upon inoculation into nine suckling mice, all of which died 2 days after inoculation (see Table 1). This strain was neutralized by polyvalent group A mouse ascitic fluid (LNI 3.5) and by each of three antisera to different strains of EE virus (Massachusetts, 448, and Guatemalan 68U230); in the three latter cases, each LNI was  $> 4.8$ . LNI obtained with VE rabbit antiserum (Guatemalan strain 69Z1), WE rabbit antiserum (North Dakota strain), and Mayaro mouse ascitic fluid (TRVL 15537 strain) were respectively 0.3, 0, and 0.

The 75U40 strain of EE virus thus obtained was classified by Dr. C. Calisher of the U.S. CDC facility at Ft. Collins, Colorado, as belonging to the South American subtype. Dr. Calisher also found two of the EE strains obtained from Pucallpa in 1970 (70U1104 and 70U1114) to be of the South American subtype.

Sera from 21 (72 per cent) of 29 horses bled at Yurimaguas during April 1975 had detectable levels of N antibody to the 75U40 strain of EE virus (see Table 2). In addition, sera from 3 (21 per cent) of 14 people bled at Yurimaguas and 2 (15 per cent) of 13 people bled at Imacita neutralized EE virus (see Table 3).

#### *Group C Virus*

One strain of group C arbovirus (75U41), tentatively identified as Marituba virus, was recovered from the brain of a nonimmunized sentinel hamster that died at the San Ramón site near Yurimaguas on 5 May 1975, following 27 days of exposure (see Table 1). All of the 11 suckling mice in two inoculated litters died 2 or 3 days after inoculation. Polyvalent group C mouse ascitic fluid produced an LNI  $> 2.3$  in a plaque-reduction neutralization test, and the LNI with Marituba antiserum was  $> 2.1$ . LNI obtained with antisera to other group C viruses (Oriboca and Caraparu) were 0.1 and 0.6 respectively, while those obtained with polyvalent group A, group B, and Guama group mouse ascitic fluids were  $< 0.4$ .

#### *Guama Group Virus*

As Table 1 shows, two pools of female mosquitoes from Iquitos yielded Guama group arboviruses. A pool of 100 mosquitoes, collected on the night of 8-9 April 1975 from the same light trap that had yielded the 75D143 strain of VE virus the night before, contained a virus (75D125) closely related to the Guama and Moju viruses; all of the eight suckling mice inoculated with this mosquito suspension became ill or died 9 or 10 days after inoculation. The virus was inactivated by shaking with chloroform ( $> 3.0$  log<sub>10</sub> reduction), and the virus produced plaques in cultures of Vero African green monkey kidney cell cultures. Guama group



mouse ascitic fluids and specific Guama and Moju mouse ascitic fluids neutralized 71D125 virus in suckling mouse tests, the respective LNI obtained being 1.8, 2.4, and 2.2. But the virus was not neutralized by antibodies to Bimiti, Bertioaga, Catu, or Mahogany Hammock viruses of the Guama group, respective LNI obtained in these cases being 0.7, <0.2, 0.3, and <1.7.

Another Guama group virus (75D235) was obtained from a pool of 100 female mosquitoes captured on 16-17 April 1975 in another light trap. This mosquito suspension killed two of eight suckling mice 7 days after inoculation; the virus involved was identified as Bimiti by means of plaque-reduction neutralization tests in Vero cell cultures. These tests yielded LNI >2.3 with Guama group immune mouse ascitic fluid and >2.1 with Bimiti mouse ascitic fluid.

## Discussion

Possible sources of VE epidemics and equine epizootics on the dry Pacific Coast of Peru were studied during 1970-1971 (2). The Amazon region was examined at two locations by testing mosquitoes for virus, testing sera for antibodies, and searching for VE virus activity with sentinel hamsters. Irrigated river valleys on the Pacific Coast, one in northern and one in southern Peru, were studied for evidence of VE virus activity by means of equine antibody tests and exposure of sentinel hamsters. Sentinel hamsters detected no VE virus activity in parts of north-coastal Peru bordering Ecuador during the dry season of 1970. However, VE virus was isolated from mosquitoes and sentinel hamsters across the Andes Mountains at the eastern extreme of Peru's Amazon region, near the city of Iquitos.

These findings were compatible with the possibility that the VE epidemics and equine epizootics on the Pacific Coast of Peru were caused by movements of virus carried by infected vertebrates traversing Andean passes

or carried in infected vertebrates or mosquitoes transported out of the Amazon region by airplanes. However, because the isolations made during 1971 were from a location about 750 km east of the Pacific coastal plain, it seemed advisable to examine regions of the Peruvian Amazon nearer the Andes Mountains for evidence of VE virus activity. Though simple in concept, this examination was difficult to perform. The two sites studied during 1970 and 1971 in the Peruvian Amazon (Iquitos and Pucallpa in Loreto Department) were chosen because they were among the few locations that could be reached by airplane; movement by roads or rivers was impractical because of the time required, the need for special vehicles, and the expense.

Another factor that had to be considered was how VE virus, if it were present just east of the Andes Mountains, might reach the Pacific Coast. As just noted, besides possible transportation in infected vertebrates and mosquitoes by airplane, there existed the possibility that infected vertebrates were traversing Andean passes. The lowest pass between the Amazonian and Pacific watersheds in the entire Andean system from southern Chile to eastern Colombia occurs between Olmos and Tambo in Peru's Piura Department. This place, known as the Abra de Porculla, is located at about 5°5' south, 79°30' west (see Figure 1). There are also other passes almost as low in nearby regions.

Still another factor to consider was that the likelihood of finding VE virus was greater in tropical wet forest than in tropical dry forest. Thus the desired study area would have been a region of tropical wet forest that could have been reached easily by airplane and that was situated just to the east of one of these low Andean passes in northern Peru. Unfortunately, no such area existed in 1975. Therefore, we were limited to two sites just east of the Andes Mountains; one, located in tropical wet forest at Yurimaguas, was accessible by air and was about 400 km east-southeast of the

Abra de Porculla; the second, situated in tropical dry forest at Imacita, was accessible by road through the Abra de Porculla, being about 200 km away from the Pacific side of the pass at Olmos by line-of-sight and 340 km away by road (see Figure 1). Iquitos, at the eastern extreme of Peru's Amazon region, was studied again in 1975 as a control site for evaluating the mosquito and sentinel hamster methods employed to isolate VE virus. Since VE virus was isolated there in 1971, it seemed likely that it could be found again in 1975.

Indeed, VE virus was recovered from mosquitoes collected near Iquitos. Closer to the Andes Mountains, at Yurimaguas, a location with only patches of tropical wet forest, N antibodies to VE virus were found in sera from both horses and people. The studies at Imacita, near the Andes Mountains but farther north than Yurimaguas, were made in tropical dry forest and were limited in time and small in scope; not unexpectedly, they revealed no evidence of VE virus activity.

These observations of VE virus in Peru during 1975 thus revealed virus activity in the eastern portion of the Amazon region near the Andes Mountains and also confirmed VE virus activity farther west near Iquitos. The VE antibodies found in horse sera from Yurimaguas were particularly informative, since most of these horses were born and raised in the region. Moreover, the existence of VE antibody in horses only 2-4 years of age indicated recent cycling of the virus. The VE antibodies found in human sera obtained during 1975, like those found in people on the eastern side of the Andes Mountains during 1965 (14), are harder to interpret as indicating local virus activity, since people travel periodically.

All knowledge to date of VE virus activity in Peru is compatible with the theory that the virus travels from tropical Amazonian rain forests to the irrigated valleys of the Pacific Coast to produce epidemics and equine epizootics. Before air travel between

these regions became extensive, the virus might have been carried by viremic people and equine animals moving through Andean passes. Nowadays, airplanes could easily move viremic people or infected mosquitoes.

This theory of origin, however, is currently difficult to prove. Comparisons made between VE strains from the Amazon region and those from coastal outbreaks show that they (a) exhibit similarities in their virulence for English short-hair guinea pigs inoculated sc with about 1,000 CEC pfu of virus and (b) exhibit both similarities and differences in virus-dilution HI tests. The VE strain obtained from Iquitos in 1975 (75D143) killed these guinea pigs, as did two strains obtained from Iquitos in 1971 (71D1249 and 70U1129) and one strain (52/73) from the 1973 coastal outbreak (15 and unpublished observations). The strains from both regions have the same HI subtype (subtype I) but have been reported to represent different varieties within that subtype. Iquitos strains 71D1249, 70U1129, and 75D143 have been related to variety D of subtype I, whereas coastal outbreak strains isolated in the 1940s—Piura and Hoja Redonda(Ica)—and in 1973 (52/73) are in variety B of subtype I (4, 7, 8). However, further HI tests using chicken antibodies have shown cross-reactions between Amazonian strains and variety B, especially Peruvian coastal strain 52/73, and also, as reported herein, between Iquitos strain 75D143 and variety A; cross-reactions have also been observed between Amazonian VE and subtype III (7). Thus the Amazonian strains are known to have HI antigenic relationships with coastal epidemic-epizootic strains. Nevertheless, their lack of complete identity in HI tests indicated that some change might have occurred, either before the virus left the Amazon region or after it arrived on the Pacific Coast and cycled through vector mosquitoes and vertebrate hosts to a point where it became amplified enough to cause

disease and thus be recognized. Such change could result from mutation and/or selection processes, but whether it actually does so remains to be determined.

The isolation of SLE virus from the Peruvian Amazon region is a new observation. This virus is widespread throughout the U.S.A. and has been recovered in Argentina, Brazil, Canada, Ecuador, Guatemala, Haiti, Jamaica, Mexico, and Trinidad (16, 17). The Brazilian isolations of SLE virus have occurred in São Paulo State, and near Belém on the eastern coast. In 1965 Madalengoitia, Flores, and Casals (14) tested sera from people residing in eastern Peru for arbovirus antibodies. Eleven sera—from both Indians and mestizos—had HI antibody patterns considered specific for SLE virus infection; these sera came from both the eastern foothills of the Andes and the lowland regions further east. Because of HI test cross-reactions among flavivirus antibodies, these data did not constitute unequivocal proof of the existence of SLE virus in eastern Peru. However, when these data are considered together with the isolation of SLE virus in 1975 and the finding of SLE N antibodies in sera collected during 1970 and 1975, they seem to establish the existence of SLE virus in the Amazon region

of eastern Peru. Whether the virus is actually causing human encephalitis has now to be determined by careful diagnostic studies of patients. Unfortunately, in such a remote region many patients may not receive medical help. Nevertheless, there are hospitals and clinics in Peru's Amazon region, and the physicians there should be alerted to send appropriate diagnostic specimens (especially acute and convalescent sera) to a diagnostic laboratory equipped to test for SLE antibodies.

With regard to other matters, the 1975 isolation of EE virus from a sentinel hamster just east of the Andes Mountains at Yurimaguas extends the distribution of this virus westward in the Peruvian Amazon from the locations (Pucallpa and Iquitos) where its activity was demonstrated in 1970-1971 (9). Likewise, the recovery of a group C arbovirus from a sentinel hamster at Yurimaguas and of Guama group viruses from mosquitoes at Iquitos fits in with previous isolations of these viruses near Iquitos in 1970 and 1971 (9). The extent to which these viruses cause disease in Peru now needs to be determined by close collaboration between clinicians of the Amazon region and a qualified diagnostic laboratory.

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#### SUMMARY

The studies reported here show that Venezuelan encephalitis virus (VE) was present in the Amazon region of Peru during 1975. The virus was isolated near Iquitos (Loreto Department) in eastern Peru; antibody evidence of recent infection without epidemics or equine epizootics

was found further west at Yurimaguas (Loreto Department) in lowlands near the Andes Mountains. These observations are consistent with the theory that VE virus is silently enzootic in the Peruvian Amazon Basin and that this region has served as a source of virus for the periodic

outbreaks of human and equine disease that have occurred in irrigated, inhabited locations on the dry Pacific Coast.

Historically, and most recently in 1973, the majority of these coastal epidemics and equine epizootics have taken place along Peru's northern coast—but in areas that (except in 1969) were not contiguous with Ecuador, where VE virus also causes disease. This fact fits in with the theory that VE virus may have traditionally moved through low mountain passes, which are

limited in Peru, to the northern portion of the Andes Mountains. Nowadays, of course, airplanes could also move viremic people or infected mosquitoes across the mountains.

Other mosquito-borne viruses, including St. Louis encephalitis (a flavivirus), eastern encephalitis (an alphavirus), and a group C bunyavirus were also isolated during 1975 near Yurimaguas; and two Guama group bunyaviruses were isolated near Iquitos in Peru's Amazon lowlands.

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### EDITORIAL PROFILE MISS CLAIRE HARLEY RETIRES

Bringing to a close her career of 29 years' service with the Pan American Health Organization, Miss Claire Harley, Chief of the Office of Publications, said good-bye to her co-workers on 30 April 1979.

Miss Harley's lively intellect, her facility for languages, and her formal education, which included schooling in Switzerland and at the Sorbonne, Columbia University, and the School of Economics in Rio de Janeiro, fitted her most singularly for her long and fruitful career with PAHO. She began her service with the Organization as a translator into English, having had the great advantage of proficiency in all four of its official languages. In 1958 she was placed in charge of the Special Publications Unit, thus beginning the work that was to lead to the position she held at the time of her retirement. In this capacity she was to establish a broad program of publications which over the years was to become one of the Organization's most important activities.

With an extraordinary editorial sense and a very special sensitivity for her task, Miss Harley worked devotedly to maintain the prestige of the *Boletín de la OSP*, improving the quality of this publication wherever she could, and she also founded the English-language *PAHO Bulletin*, of which she was editor. Always mindful of the Organization's priorities and its image, and of the value of its publications as support for its programs, she constantly insisted on the highest level of quality in the printed word—this in a time when emphasis on mass production, in detriment to the language, tends to prevail. Her determined effort and fine critical sense were reflected in all the results of her program—the periodic and scientific publications, the official documents, and the filmstrips, and in innovations in their distribution—so that she was able to see her ideas transformed into reality.

Miss Harley's absence will be keenly felt, especially by the staff who worked with her and who have benefited from her knowledge and experience, but she leaves a firm and solid basis for all future activities of the PAHO publications program.

