THE FIRST ISOLATIONS OF EASTERN ENCEPHALITIS, GROUP C, AND GUAMA GROUP ARBOVIRUSES FROM THE PERUVIAN AMAZON REGION OF WESTERN SOUTH AMERICA[1, 2]

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The first isolations of eastern encephalitis, group C, and Guama group arboviruses from the Peruvian Amazon region of western South America are described. As this region develops and human and equine populations increase, diseases caused by these viruses may become important public health hazards.

Introduction

While studying Venezuelan encephalitis (VE) virus in the Amazon region of Peru during 1970 and 1971 (*1*), additional arboviruses were recovered from tissues of sentinel hamsters and mosquitoes by intracranial inoculation of suckling mice. This article describes those isolations.

Materials and Methods

Background Data

Study sites, field technics, and methods of virus isolation used in our investigation have been described elsewhere (*1*). Pucallpa, a town in Loreto Department, is on the Ucayali River in the Amazon region of Peru at 8° 30′south latitude, 74° 30′west longitude. Iquitos, the capital of Loreto, is located near the base of the Amazon River at 4° south latitude, 73° west longitude. Hamsters were flown to Lima, Peru, from the Lakeview Hamster Colony, New Jersey, in September 1970.

Identification of Virus Isolates

Complement-fixation (CF) tests were performed as previously described (*2*) with saline-extracted antigens from infected suckling mouse brains. Neutralization (N) tests were based on plaque-reductions in primary chicken embryonic cell cultures for Eastern encephalitis (EE) virus, and in vero African green monkey kidney cell cultures for group C and Guama group viruses (*3*). Some N tests for Guama group viruses were done with suckling mice inoculated intracranially (*3*). All the cell cultures were prepared and used as described elsewhere (*3*).

Results

Isolation and Identification of Eastern Encephalitis Virus

As shown in Table 1, EE virus was recovered from two of 21 sentinel Syrian hamsters

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TABLE 1–Isolation of eastern encephalitis, group C, and Guama group arboviruses in the Peruvian Amazon from adult sentinel hamsters exposed between mid-September 1970 and mid-February 1971, and from female mosquitoes collected during December 1970 and January-March 1971.

Location	Sentinel hamsters						Mosquitoes				
	Hamster-day exposure		Fraction ill or dead	No. of viruses isolated†			Numbers tested		No. of viruses isolated		
	Gr C-Pat immune*	Not immune		EE	Group C	Guama group	Individual mosquitoes	Pools	EE	Group C	Guama group
Pucallpa:											
Site IVe	2,330		11/36	0	0	0	1,000	27	0	0	0
		1,101	5/21	2	0	0					
Iquitos:											
Site QC	1,885		3/28	0	0	0	11,800	234	0	3	4
		155	10/10	0	9	0					
Site ZC	338		2/7	0	0	0	5,700	144	0	0	0
		38	2/2	0	2	0					
Site NB	580		0/10				none collected				
		174	0/3								

*Gr C-Pat immune signifies hamsters immunized to group C viruses (Nepuyo and Oriboca) and to Patois virus, as described elsewhere (*1*).

† Two strains of Venezuelan encephalitis virus were also isolated from two of the three "immune" hamsters that died at site QC, and one as yet unidentified virus (70U1084) came from one of the two "immune" hamsters that died at site ZC. Otherwise, the dead hamsters yielded only those viruses shown in this table.

exposed at an experimental agricultural station of San Marcos University's Veterinary Institute of Tropical and High-Altitude Research (site IVe). The station is situated about 60 kilometers southeast of Pucallpa.

One hamster, which yielded EE virus strain 70U1104, was six weeks of age when exposure began in mid-September 1970 at IVe. On 18 December 1970 this hamster was shifted to a wooded ravine nearly one km south of the highway and the main buildings of IVe; it died on 9 January 1971 after 22 days of exposure at the second location.

The other hamster, which yielded EE strain 70U1114, was initially kept in a laboratory at Lima on the dry Pacific coast. It was then flown to Pucallpa in December 1970 and was immediately placed in a forest on the southeast side of the agricultural station (see picture 1); it died on 10 January 1971 after 23 days of exposure.

These hamsters were both about 20 weeks of age when they died.

EE viruses were isolated from pooled suspensions of the hamsters' heart, brain, kidney, and liver tissues. The suspensions were initially inoculated intracranially into suckling mice in Lima during January 1971, and reisolations were done in New York during June 1971; final identifications were made with the reisolates.

PICTURE 1–Forest and a field cleared for livestock use at San Marcos University's Veterinary Institute of Tropical and High-Altitude Research, near Pucallpa in Loreto Department.

CF antigen titers of both isolates were a) 1:64 when tested against a 1:8 dilution of EE antibody (made as ascitic fluid in mice with the Massachusetts strain; NIH reference reagent V515-701-562) and b) 1:128 when tested against 8 units of guinea pig antiserum (made with strain Riche EE virus). No reactions occurred with 1:8 dilutions of antisera to Mayaro, western encephalitis, Simbu, and Patois arboviruses. Log_{10} neutralization indices (LNI) against EE virus rabbit antiserum (made with Guatemalan strain 68U230) were 2.7 for strain 70U1104 and 1.8 for strain 70U1114. LNI against VE virus rabbit antiserum (made with Mexican strain 63U2) were 0 and 0.1, respectively, for each of the two strains. The homologous LNI of the EE antiserum tested against EE virus (strain 68U230) was 3.3, and the heterologous LNI of the EE antiserum tested against VE virus (strain 63U2) was 0.6; the VE virus antiserum LNI were 0.5 against EE virus and >3.3 against VE virus.

No virus was detected in 1,000 mosquitoes tested as 27 pools from IVe (see Table 1).

Sera from horses at IVe contained N antibody to EE virus strain 70U1104; LNI were >1.6 with 1:4 serum dilution for 12 of 29 horses. However, there was no correlation between detectable N antibodies to EE and VE virus in horse sera from IVe (using strain 69Z1 of epizootic VE virus subtype I). VE virus antibody was found in sera from only seven of the 12 horses at IVe with EE virus antibody; and VE virus antibody was also present in three of 17 horses without EE virus antibody.

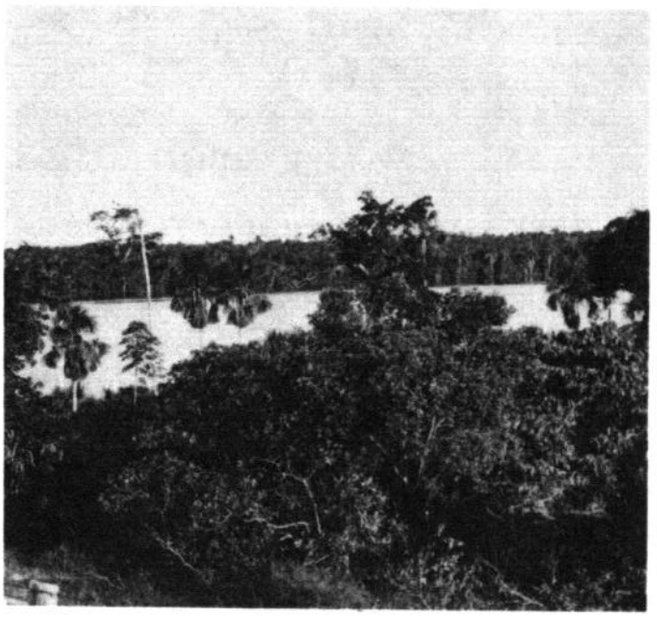
At Iquitos, three of 13 horses had antibody to EE virus (LNI >1.6 with a 1:4 serum dilution). Only one of the three horses with EE antibody had detectable VE virus antibody in its serum; and three of the 10 horse sera from Iquitos without detectable EE virus antibody also had VE virus antibody.

Isolation and Identification of Group C Arboviruses

Eleven strains of group C arboviruses were recovered from sentinel Syrian hamsters, 6-15 weeks of age, exposed at two sites near Iquitos (Quistococha and Zungarococha); three group C strains were also recovered from mosquitoes collected at one of the sites. Only nonimmunized hamsters yielded group C viruses; hamsters previously immunized against group C arboviruses survived exposure to the same habitats during the same time periods when normal hamsters were dying.

Nine of the group C virus strains obtained from the hamsters and all three strains recovered from mosquitoes came from a rain forest at the Quistococha (QC) study site (see pictures 2 and 3); the other two strains were recovered from hamsters that died in a rain forest at Zungarococha (ZC) (see picture 4). It is noteworthy, however, that three nonimmunized hamsters survived in a forest at a nearby Peruvian naval base (NB) for 174 hamster-days (Table 1).

At the first two sites all of the nonimmunized hamsters died rapidly. Exposures lasted only 5-34 days at Quistococha and 11-27 days at Zungarococha. The deaths at Quistococha occurred in the period 20 September-6 November 1970, after which no additional normal hamsters were exposed. Hamster exposures were begun at Zungarococha on 16 December 1970; the two nonimmune hamster deaths



PICTURE 2–A view of the research site at Quistococha on the outskirts of Iquitos, showing a nearby lake and surrounding forest.

PICTURE 3–The forest at Quistococha close to a fishery installation being built by the University of the Peruvian Amazon.

PICTURE 4–Tropical forest at the Zungarococha research site, on the property of the University of the Peruvian Amazon close to Iquitos.

there occurred on 27 December 1970 and 12 January 1971.

All virus strains from hamsters at Quistococha were initially recovered in Lima, Peru, by intracranial inoculation of hamster tissue suspensions into suckling mice. The strains were then subsequently reisolated and identified from frozen hamster tissue suspensions in New York. All strains of group C viruses from hamsters at Quistococha were obtained from suspensions of heart tissue; those from Zungarococha were obtained from pooled suspensions of heart, brain, kidney, and liver tissues. The three mosquito isolates of group C arboviruses were recovered from pools of 77, 50, and 58 female mosquitoes of unidentified species collected with CDC light traps on the respective dates of 24 February, 24 March, and 25 March 1971. Suspensions of material from these three pools were prepared and tested in New York on different days (8, 14, and 20 July 1971).

CF tests revealed that saline antigens from infected suckling mouse brains reacted to titers of 1:16-1:128 against polyvalent group C mouse antibodies (NIH reference reagent G201-701-567) and to titers of $<1:8$ with polyvalent group A or B antisera. Definitive identifications of group C arboviruses, made by plaque-reduction neutralization tests in vero cell cultures, revealed that nine of the group C strains were Caraparu-Ossa virus, four were Marituba and one was Oriboca-Itaqui (see Table 2).

Isolation and Identification of Guama Group Arboviruses

Four strains of Guama group arboviruses were recovered by inoculation of mosquito suspensions into suckling mice (Table 1). These suspensions were made from female mosquitoes captured at the Quistococha site on 11 and 17 February and on 17 and 24 March 1971 (the respective virus strains were given the designations 71D1117, 71D1393, 71D1342 and 71D1224). These strains were recovered from suspensions made during June and July 1971 from pools of 60, 50, 50, and 50 female mosquitoes unidentified as to genus or species.

CF antigens of the viral isolates reacted to titers of 1:64-1:128 with polyvalent Guama group mouse antibodies (NIH reference reagent G202-701-567) and to titers of $<1:8$ with polyvalent groups A, B, Bunyamwera, Capim, Simbu, Tacaribe, and VSV and with individual antisera to Naples and Sicilian Phlebotomus, Anopheles A and B, Chagres, Bwamba, Quaranfil, Colorado tick fever, Melao, Cali-

TABLE 2—Identification by plaque-reduction neutralization tests in vero African green monkey kidney cell cultures of 14 group C arboviruses isolated from sentinel hamsters or mosquitoes near Iquitos, Peru, during 1970 and 1971.

Viral isolate*	Log_{10} neutralization indices of antisera to group C viruses †										
	Caraparu			Madrid	Marituba	Marituba		Oriboca	Oriboca	Nepuyo	Gumbo Limbo
	Belem	*Ossa*	*Apeu*			*Murutucu*	*Restan*		*Itaqui*	*Trinidad*	
Site QC:											
70U1002	>*2.7*	>*2.7*	1.0	0.7	0.1	0	0	0.3	0.2	0.1	0
70U1006	>*2.9*	>*2.7*	0.6	0	0	0	0	1.4	0	0	0
70U1007	*1.7*	*2.2*	0.6	0	0	0	0	0	0	0	0
70U1008	0.6	0.6	0.3	0.3	*1.8*	0.3	0.3	0.3	0.3	0.3	0.3
70U1054	>*2.2*	>*2.2*	1.3	0	0	0	0	0	0	0	0
70U1055	>*1.8*	*1.8*	1.1	0	0	0	0	0.1	0	0	0
70U1056	*1.8*	*1.8*	0.6	0.1	0.1	0	0	0	0	0.1	0
70U1057	*1.8*	>*2.9*	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
70U1058	0.8	0.8	0.4	0.4	*1.1*	0.7	0.7	0.5	0.4	0.5	0.3
71D1191	0.6	0.6	<0.4	<0.4	*1.0*	0.4	0.4	0.4	0.4	0.4	0.4
71D1228	0.2	0.9	0	0	*1.9*	0	0.4	0.1	0	0	0
71D1238	0.1	0	0	0	0	0	0	>*2.5*	*2.2*	0	0
Site ZC:											
70U1139	*2.6*	>*2.9*	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
70U1154	>*1.6*	>*1.6*	0.3	0	0	0	0.3	0.4	0	0.1	0

*U signifies an isolate from a hamster and D an isolate from mosquitoes.

†Viruses in italics are currently classified as subtypes of the virus listed in regular type above. The antisera were kindly supplied by Drs. Karabatsos and Shope at Yale University. As reported by Karabatsos and Henderson (*4*), these antisera had Log_{10} neutralization indices of 2.7-4.6 against homologous viruses and LNI of 1.5-3.5 against related heterologous subtypes.

fornia encephalitis, and Patois viruses. N tests in vero cell cultures or suckling mice, made with antisera kindly supplied by Drs. Robert Shope and N. Karabatsos of Yale University, clearly identified three strains as Bimiti virus (see Table 3). The fourth strain (71D1342) was neutralized only by undiluted Bimiti virus antiserum from Yale and to a lesser degree by undiluted NIH reference Bimiti mouse ascitic fluid (V527-702-562) (Table 3). Mouse antiserum was made by Dr. R. Shope to each of the four strains, and each antiserum neutralized Bimiti virus with an LNI of 3.1-3.8 (Personal communication, Dr. R. Shope).

Discussion

EE virus has been recovered from North, Central, and South America, in areas extending from the northern United States of America to Argentina (*5*). Numerous isolates have come from the eastern Amazon region near Belém, on Brazil's Atlantic coast; in addition, a previous study of antibodies in human sera collected in 1965 from the Amazon lowlands of Peru had revealed evidence of infections with EE virus in that region (*6*).

Thus it was not surprising to isolate this virus during 1971 in the Amazon region of eastern Peru near Pucallpa. The historical absence of epizootic equine disease in that area probably reflects in part the low horse population there. However, the presence of EE virus antibodies in 20 to 40 per cent of the healthy horses tested at site IVe and Iquitos in 1970-1971 indicates that at least some of the EE virus strains existing there at the time were benign for equines. Presumably the Peruvian strains of EE virus are of the South American variety described by Casals on the basis of hemagglutination-inhibition tests (*5*).

Neither is it surprising to find group C and Guama group arboviruses in the Amazon region

TABLE 3–Identification of four Guama group arboviruses from the Peruvian Amazon by neutralization tests.

Known Guama group antisera	Homologous LNI*	Dilution used		LNI of Peruvian Guama group isolates†						
		Yale	Cornell	71D1117	71D1224	71D1342			71D1393	
				Test A	A	B	C	E	B	D
Guama	5.0	1:8	1:8	<0.9	0.9	<0.3		<2.3	1.0	<1.4
Bertioga	>2.6	1:2	1:8	0	0.2	<0.3			1.0	
"	2.6	–	1:2					<2.3		<1.4
Bimiti	>5.2	1:8	1:8	*>3.5*	*>2.5*	<0.3	<1.3	<2.3	<0.8	*2.0*
"	>5.2	–	undiluted				*1.9*			
"	>3.9	–	undiluted				1.5			
Catu	5.3	1:8	1:8	1.8	0.6	<0.3		<2.3	1.0	<1.4
Mahogany hammock	2.7	1:8	1:8	<0.9	1.4	<0.3		<2.3	<0.8	<1.4
Moju	4.2	1:2	1:8	<0.9	0.8	<0.3			<0.8	<1.4
"	4.2	–	1:2					<2.3		
BeAn 116382	nt	–	1:8	<0.9	0.2	<0.3			1.2	
"	nt	–	1:2					<2.3		<1.7

*Homologous LNI based on N tests in baby mice inoculated intracranially were kindly provided by Dr. Robert Shope of Yale University.

†All isolates were tested as brain suspensions from a second or third suckling mouse passage. Tests A and B were based on plaque-reduction in vero African green monkey kidney cell cultures. Tests C, D, and E were based on deaths of baby mice inoculated intracranially. Plaques were counted on day 11 at 37°C in test A and on day 14 in test B.

of eastern Peru–since they are also present at the Atlantic extreme of the Amazon River, and since antibodies to two group C arboviruses (Caraparu and Murutucu) were previously found in humans residing within Peru's Amazon lowlands in 1965 (*6*).

It is noteworthy that the Guama arboviruses came only from mosquitoes and not from sentinel hamsters. Complete information on the susceptibilities of hamsters to arboviruses inoculated intradermally or subcutaneously has never been assembled and published. However, a list was prepared during 1970 that was based upon our own experiences in Central America and Mexico, an article by Sunthorn and Johnson (*7*), and personal communications from J. Woodall of the Evandro Chagas Institute (Belém, Brazil), R. Shope of Yale University (New Haven, Connecticut, U.S.A.), and P. Galindo of Gorgas Memorial Laboratory (Panama Canal Zone). At that time, of the arboviruses known to exist in the Amazon region, only VE and EE viruses in group A, and Nepuyo, Itaqui, Ossa, and Madrid viruses in group C, had been detected by sentinel hamsters in nature.

Based on laboratory experiments showing that a particular virus would kill hamsters after subcutaneous or intraperitoneal inoculation, it was felt that the following viruses might also be detected by sentinel hamsters:

Group A: Mucambo (subtype III of VE) and Western encephalitis;
Group C: Caraparu, Oriboca, Restan, and some Murutucu strains;
Simbu group: Oropouche;
VSV group: Cocal;
Ungrouped: Ieri and Piry.

Four other viruses killed hamsters when inoculated intracranially, but peripheral routes had not been tested. These four were Capim and Acara viruses in the Capim group, Utinga virus in the Simbu group, and Pichinde virus in the Tacaribe group.

The following viruses did not kill hamsters inoculated subcutaneously or intraperitoneally:

Group A: Aura, Cayenne 508, Mayaro, Pixuna (subtype IV of VE), and Una;

Group B: Bussuquara, Ilheus, St. Louis encephalitis, and some strains of yellow fever;
Group C: Apeu and Marituba;
Bunyamwera group: Kairi, Maguari, Sororoca, Taiassui, and Tucunduba;
California group: BeAr 103645 and Melao;
Guama group: Catu, Guama, and Moju;
Phlebotomus group: Anhanga, BeAn 100049, Bujaru, Candiru, Icoaraci, and Itaporanga;
Tacaribe group: Amapari;
Ungrouped: Marco and Pacui.

In addition, of course, Patois and Zegla viruses of the Patois group kill sentinel hamsters in nature, but these viruses are not known to exist in the Amazon region.

As indicated by this list, the detection of Marituba-like group C arboviruses with sentinel hamsters in Peru created a conflict with data from laboratory experiments using prototypic Marituba virus (*7*; personal communication from J. Woodall). This discrepancy in results probably indicates strain variation.

The results with Guama viruses, however, were as expected, since the failure to isolate them from sentinel hamsters at Quistococha was in accord with their failure to kill peripherally inoculated hamsters. Indeed, one of the advantages of using hamsters as sentinels is that—either inherently or when immunized against viruses of lesser public health importance—they selectively detect arboviruses important to public health such as VE and EE.

Because EE, group C, and Guama group arboviruses cause febrile human disease, these viruses should now be considered in making differential diagnoses of fevers with unknown origin in the Peruvian Amazon region. Moreover, as additional settlers enter the area in future years, and as equine populations increase, these viruses may pose significant hazards to livestock and public health.

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SUMMARY

Two strains of eastern encephalitis (EE) virus were isolated in the Amazon region of Peru near Pucallpa, Loreto Department, using sentinel hamsters. EE virus antibodies were found in healthy horses at both Pucallpa and Iquitos in the same Department.

Fourteen group C and four Guama group arboviruses were recovered from sentinel hamsters and mosquitoes near Iquitos. The group C agents were Caraparu-Ossa, Marituba, and Oriboca-Itaqui viruses, and the Guama group agents were Bimiti virus. Besides providing a detailed account of these investigations, this article includes a current list of known arboviruses of the American tropics that can be detected with sentinel hamsters.

REFERENCES

(*1*) Scherer, W. F., J. Madalengoitia, W. Flores, and M. Acosta. Ecologic studies of Venezuelan encephalitis virus in Peru during 1970-1971. *Am J Epidemiol.* (In press, to appear in Vol. 101, No. 4, April 1975.)

(*2*) Scherer, W. F., and N. D. Lewis. Immunologic studies of Japanese encephalitis virus in Japan: VI. An evaluation of the direct complement-fixation test for detecting infection of swine. *Am J Vet Res* 23: 1157-1163, 1962.

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

(4) Karabatsos, N., and J. R. Henderson. Cross-neutralization studies with group C arboviruses. *Acta Virol (Praha)* 13: 544-548, 1969.

(5) Casals, J. Antigenic variants of eastern equine encephalitis virus. *J Exp Med* 119: 547-565, 1964.

(6) Madalengoitia, J., W. Flores, and J. Casals. Arbovirus antibody survey on sera from residents of eastern Peru. *Bulletin of the Pan American Health Organization* 7(4): 25-34, 1973.

(7) Srihongse, S., and K. M. Johnson. Hemagglutinin production and infectivity patterns in adult hamsters inoculated with group C and other New World arboviruses. *Am J Trop Med Hyg* 18: 273-279, 1969.