EASTERN ENCEPHALITIS VIRUS FROM VIRGIN FORESTS OF NORTHERN BRAZIL[1,2]

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The following contribution describes isolation of eastern encephalitis (EE) virus from sentinel hamsters exposed to wet tropical forest at an isolated settlement in Brazil's Amazon region. This discovery of EE virus activity, together with indications that human exposure to EE virus in the test area was slight, provides further evidence of a truly sylvatic EE transmission cycle outside man's sphere of influence.

Introduction

In conjunction with studies carried out by the Phelps Ornithological Collection, under the auspices of the Joint Brazilian-Venezuelan Border Demarcation Commission on Mount Urutani along the Venezuelan-Brazilian frontier, the senior author was able to carry out preliminary arbovirus investigations employing sentinel hamsters at the Commission's base camp at Uaica on the Uraricoera River in northern Brazil. This article presents the results of those field investigations, which were made during March and April 1977.

Materials and Methods

Study Site and Field Techniques

Uaica, the main supply camp for the Commission, was located in wet virgin tropical forest along the Uraricoera River, at an elevation of about 260 meters (Figure 1). The camp was serviced by a Venezuelan military helicopter that shuttled supplies in from Boa Vista, Brazil, to working camps of surveyors on Mount Urutaní. When the Uaica base was established in December 1976-January 1977, large trees of the genus *Cecropia* (that had grown up since missionaries abandoned the camp 10 years before) were left standing; all shrubs were removed. The forest floor within the camp was swept at least twice a week. The staff, which ranged in size from 30 to 100 men, made use of toilet facilities located on cleared paths a short distance inside the undisturbed forest bordering the camp.

Syrian golden hamsters from the Lakeview Hamster Colony in New Jersey were exposed, when about 5 weeks old, in open wire mesh cages suspended about a meter above ground under protective roofs within the forests surrounding the Uaica camp and the adjacent landing strip. The hamsters had food and water available *ad libidum*. Animals that did not become

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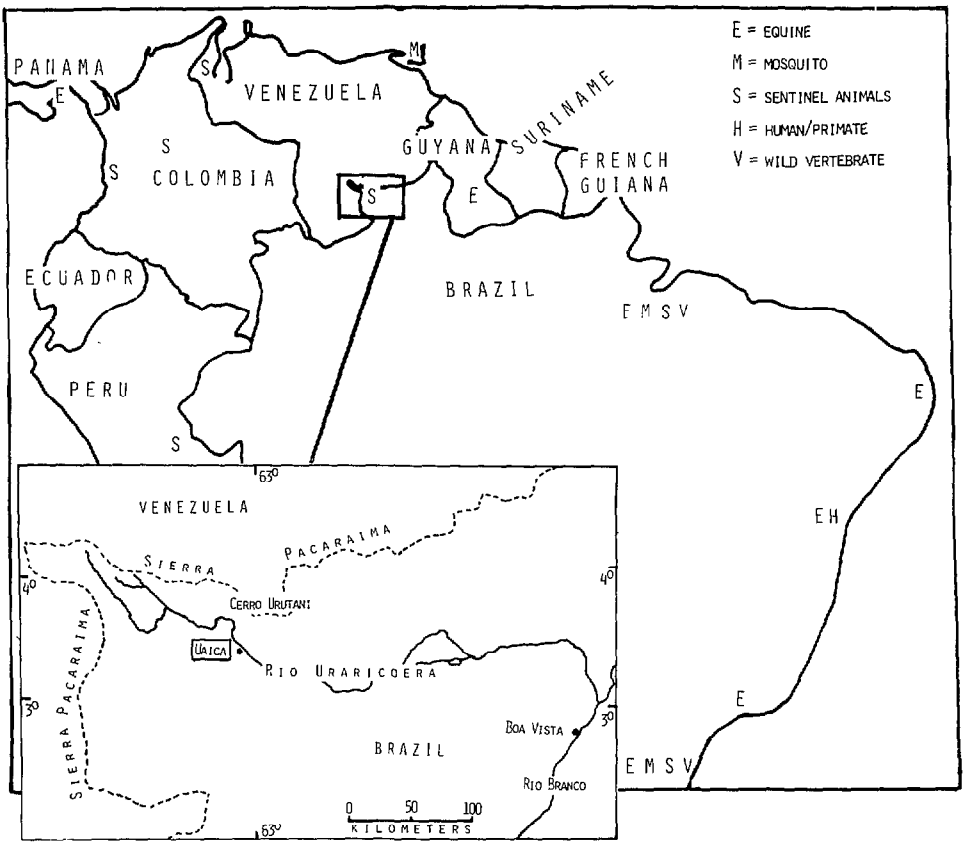
Figure 1. A map showing the location of the Uaica, Brazil, study area and places in northern and central South America (indicated by letters) from which EE virus has been isolated, by source of the isolate.



moribund or die during the study period grew normally and developed extensive deposits of fat. Moribund and dead hamsters were labeled and stored at −5 to 5°C until autopsied, or were autopsied immediately—with tissue pools being frozen in vapor over liquid nitrogen. Three tissue samples were obtained under sterile conditions from each animal. These consisted of brain tissue alone; pooled heart, lung, liver, and kidney tissues; and pooled tissues from both the brain and the four organs.

Variations in conditions of preservation resulted from recall of the supply helicopter for emergency transport and exhaustion of the supply of liquid nitrogen. A small amount of dry ice arrived in the field as the study was being terminated, but this did not last until the specimens reached Caracas. Consequently, whole hamsters or tissue samples from 10 animals were stored at temperatures ranging from −5 to 10°C for 6 to 13 days, while samples from 14 animals were frozen and thawed two to four times before reaching New York. Specimens were transported to Cornell University Medical College (New York, N.Y.) from Caracas packed in dry ice.

Virus Isolation

At Cornell, 10 per cent suspensions of brain tissue samples were prepared in Hanks' balanced salt solution containing 1 per cent bovine albumin, and were inoculated intracranially into suckling albino white mice 1 to 4 days of age that had been obtained from Taconic Farms in Germantown, N.Y. Similar preparations were made from all mice that died after being inoculated with the hamster brain suspensions. Aliquots of first mouse passages of strains isolated at Cornell, as well as all unopened combined pools of brain and organ tissues from the field, were packed in dry ice and shipped to the Evandro Chagas Institute in Belém, where all were tested for the presence of virus by similar procedures employing suckling mice.

Virus Identification at the Evandro Chagas Institute

Both hemagglutination-inhibition (HI) and neutralization (N) tests were used for virus identification. The HI test procedure employed has been described before (*1, 2*). The N test was performed in 96 microplate wells (Linbro Chemical Co., New Haven, Conn.) using Vero cells, as described earlier (*3*). Basically, virus and heat-inactivated serum were mixed in the microplate wells and were incubated at 37°C for 1 hour. At this point, approximately 2 x 10^4 cells were added to the contents of each well. Medium 199—containing 5 per cent fetal bovine serum and buffered with HEPES, sodium bicarbonate, and sodium hydroxide—was used to dilute the reagents and provide nutrients suitable for cell growth. Fresh rhesus monkey serum was added to the medium at a final dilution of 1:8. On some occasions the constant serum-varying virus method was used, whereas at other times fixed amounts of virus were mixed with several serum dilutions. The presence of a cytopathic effect was taken as an indicator of virus growth.

Antibody Survey

Serum samples collected from 48 Yanomama Indians in August 1977 were tested against eastern encephalitis (EE) virus by the HI and N tests. The procedures for these tests were similar to those cited above.

Virus Strain Identification

Four strains isolated at Cornell were sent to the Bureau of Laboratories, Vector-borne Disease Division, of the U.S. Center for Disease Control in frozen 10 per cent brain suspensions from the first or second passages in suckling mice. There they were tested with the short incubation hemagglutination test, using previously described methods (*4*).

Results

Viruses from Hamsters

Twenty-one hamsters were exposed in wet tropical forest at the base camp or along the landing strip for a total of 424 hamster-days between 7 March and 11 April 1977. Of these, 17 became moribund or died. The first animal died on the fourth day following placement in the field; seven hamsters died 4 to 10 days after exposure. The mean time between exposure and death for the 17 animals dying after exposure along the edge of the forest was approximately 19 days. No hamsters died during a total of 106 hamster-days of exposure in the middle of the camp area.

Sixteen virus strains were isolated at Cornell from brain tissues of the 17 stricken hamsters, and 13 strains were isolated at the Belém laboratory from the pooled samples of brain, heart, lung,

kidney, and liver tissues. No virus was isolated from the tissues of one hamster.

All 29 of the isolated virus strains were indistinguishable in HI tests from EE virus Belém prototype BeAn 7526. Also, two strains from Uaica (77U43 and 77U48) were found to be identical to Belém strain BeAn 7526 when compared by complement fixation (CF) and cell culture N tests (Table 1).

Human Antibodies

As noted before, serum samples from 48 Yanomama Indians collected in August 1977 were tested for antibodies to EE virus by HI and N tests (Table 2). These Indians had lived in the Uaica site up to approximately 10 years before collection of the samples. At the time of bleeding they had resided for several years at the village of Parimiu near the headwaters of the Uraricoera River. Serum from one subject over 15 years of age yielded HI and N test results positive for antibodies to EE virus.

CDC Tests

Four isolates from Uaica test by the short incubation HI test appeared similar if not identical to one another and two other South American EE isolates, while yielding results different from two North American EE isolates (Table 3).

Table 2. HI and N antibodies to EE virus (Uaica strain 77U48) found among Indians who formerly lived at Uaica.

Sex	Age (years)	No. positive/ No. tested
M	< 15	0/4
M	> 15	1/19*
F	< 15	0/1
F	> 15	0/24
Total		1/48

*Subject positive for both HI and N antibodies.

Discussion

The discovery of EE virus at Uaica, an isolated site in northern Brazil, adds to the data documenting the extremely widespread distribution of EE virus in northern South America (see Figure 1). EE virus is known to have caused human disease in Brazil (*5*) and Trinidad (*6*, not shown in figure), as well as equine disease in Panama (*7*), Venezuela (*8*), Guyana (*7*), Argentina (*9*), and the Brazilian States of

Table 1. HI, CF,[a] and N[b] tests comparing EE virus strains from Uaica (77U43) and Belém (BeAn 7526), Brazil.

	Test serum	Virus strains		
		BeAn 7526	77U43	77U48
HI	BeAn 7526	1:160	1:160	1:160
	77U43	1:160	1:160	1:160
	77U48	1:80	1:80	1:80
CF	BeAn 7526	16/64	16/16	16/64
	77U43	32/64	32/16	32/64
	77U48	8/16	16/16	16/64
N	BeAn 7526	4.25	4.37	5.5
	77U43	4.25	4.0	4.0
	77U48	4.25	4.37	4.0

[a]Serum/antigen CF titers.
[b]The N (neutralization) tests employed the constant serum-varying virus dilution method.

Table 3. Results of short incubation tests comparing four EE virus strains from Uaica with each other, two other South American strains, and two North American strains.

Strain and source	Antibody units	Antigen units			
		NJO	Arth 167	TR24443	BeAn5122
Uaica, Brazil					
77U19	8	16	8	64	≥ 128
77U25	8	8	16	256	64
77U34	8	16	16	64	64
77U49	8	8	16	64	8
New Jersey					
NJO	4	16	16	2	1
Louisiana					
Arth 167	2	8	16	1	1
Trinidad					
TR24443	2	< 1	1	≥ 32	16
Belém, Brazil					
BeAn5122	4	1	4	32	32

Pará (*10*), São Paulo, Rio de Janeiro, Bahia, and Pernambuco (*11*). It has been isolated from sentinel animals (hamsters, mice, monkeys, or chickens) in Colombia (*12, 13*), Peru (*14*), and Venezuela (*8*); from sentinel animals, wild vertebrates, and mosquitoes in the Brazilian States of Pará (*10, 14, 15*) and São Paulo (*16*); and from mosquitoes in Trinidad (*7*).

Although many of these isolations have been made in disturbed habitats, where human and domestic animal populations have impinged upon wild areas, numerous isolates have also been obtained from little-disturbed forests near Belém. Shope et al. (*15*) found evidence of enzootic forest cycles of EE virus, the virus being isolated from mosquitoes and sentinel animals, with involvement of birds being infrequent. Also, EE virus has been isolated from *Culex* (*Melanoconion*) *taeniopus* mosquitoes collected in 1975 in virgin forests of Aripuana in the north Brazilian State of Mato Grosso (*17*).

Within this context, identification of an intensely active focus of EE virus at Uaica, inside a region of hundreds of square kilometers of virgin forest, further indicates a truly sylvatic cycle in the absence of human influence. Especially interesting is the high rate of EE isolation from sentinel rodents—indicating active transmission of this usually avian-based arbovirus by mammalian-feeding vectors, a situation similar to that found by Shope et al. (*15*). No avian studies were carried out at Uaica.

Casals (*18*) and more recently Calisher et al. (*5*) have demonstrated that timed HI tests can be used to distinguish Central and South American EE virus strains from North American strains. In addition, published studies by Walder, Jahrling, and Eddy (*19*) have employed hydroxylapatite chromatography to substantiate and enlarge upon this work. Although, as noted above, EE virus has caused disease in humans and equine animals in South America, the relative virulence of the two groups of strains (Central-South and North American) is unknown.

It is interesting to observe that despite intensive mosquito-rodent transmission around the Uaica camp, we observed no human disease (nor was any reported by the camp physician) that might have been caused by EE virus. It is possible that the vectors involved were not anthropophilic—

a theory supported by the low incidence of EE antibody among Indians who previously lived at Uaica. A further possibility is that the twilight-nocturnal flight ranges of the mosquito vectors may have restricted them to the confines of uncleared forest containing dense tree cover and abundant litter. (In contrast, human twilight and nocturnal activity was mostly restricted to the cleared open airstrip and camp areas.) In general, however, there is little doubt that as Brazil and Venezuela extend their highway systems into unopened regions of the Amazon River Basin, ever-increasing numbers of people and domestic animals will be exposed to the risk of infection by this widely distributed enzootic pathogen.

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SUMMARY

Eastern encephalitis (EE) virus was successfully isolated from 16 sentinel hamsters exposed to a wet tropical forest environment in the far-northern reaches of the Brazilian Amazon. The place of exposure, a settlement named Uaica near the Brazil-Venezuela border, is surrounded by hundreds of square kilometers of virgin forest.

Tests run on sera from Yanomama Indians who had lived in the area indicated local human exposure to the virus was very slight. Therefore, the detection of intense EE virus activity in this area provides further evidence of a truly sylvatic EE cycle outside the present sphere of human influence.

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MOTOR ACCIDENT DEATH RATES*

According to a recent study of 26 developed and 4 developing countries reported in WHO's *World Health Statistics Quarterly* (volume 32, number 3, 1979), mortality rates from motor vehicle accidents are continuing to climb for all age groups. But the increases are disproportionately high among the 15- to 24-year old age groups, with rises of more than 50 per cent among females in three-fourths of the countries. The abuse of alcohol is blamed for much of the increase.

Thus far, public health authorities generally consider auto accidents as "acts of God," and confine their responsibility to the treatment of victims. The study urges a shift of concern to accident prevention.

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