

# ISOLATION OF EASTERN ENCEPHALITIS VIRUS IN GUATEMALA FROM SENTINEL HAMSTERS EXPOSED DURING 1968<sup>1</sup>

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*Eastern (equine) encephalitis virus was isolated from sentinel hamsters exposed during 1968 in the Department of Petén in Guatemala. These isolations represent the first clear indication that the virus is probably endemic in northern Central America and raise important questions regarding the duration, mechanism of introduction, and public health importance of EE virus there.*

## Introduction

Eastern (equine) encephalitis (EE) virus is widely distributed throughout the Western Hemisphere (1), but prior to these studies it had only been isolated once in northern Central America from a migrant bird, an orchard oriole (*Icterus spurius*) caught in March 1964 in British Honduras (2). This isolation did not conclusively establish that EE virus was endemic in British Honduras because the migrant bird might have been infected elsewhere and merely have been carrying the virus as it flew through this region. No suspicious equine or human outbreaks of Eastern encephalitis have occurred in Central America, but intensive arbovirus investigations have not been carried out there until recent years. To the immediate north, isolations of EE virus from equines were recorded in 1941 on the Mexico-USA border along the Gulf coast during an

equine epizootic (3), and the virus has since been isolated in the Caribbean area (Jamaica, Dominican Republic, and Trinidad) and to the south in Panama, as well as throughout eastern and central USA in North America and in Guyana, Brazil, and Argentina in South America (1). This article reports three isolations of EE virus from sentinel hamsters in Guatemala during 1968, and records the probability that the virus is endemic there.

## Materials and Methods

*Study site.* Sayaxché is located along the Pasión River in the western part of the Department of Petén in Guatemala (about 16° 30' latitude and 90° 10' longitude). The Petén region lies on a limestone shield at the base of the Yucatán Peninsula, and thus in general is an area of permeable soil and underground drainage. However, in the Pasión River basin there are more mesic forests and abundant surface water. Several hundred people live in Sayaxché, many of them new settlers from the mountains or Pacific coast of Guatemala. This site was chosen for study because it was the only location in western Petén accessible by a commercial airline.

*Exposure of sentinel hamsters and recovery and identification of EE virus.* The methods used were similar to those used for Venezuelan encephalitis (VE) virus which have been described elsewhere (4), except that a liquid nitrogen tank with vapor phase canisters (-100°C) was employed for field storage of sick or dead hamsters, until they could be trans-

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ported to New York on dry ice (-60°C). During the months of this work in New York, no other strains of EE virus were handled in the laboratory. Viruses were isolated by intracranial and subcutaneous inoculation of hamster tissue suspensions into 1-4-day-old mice, and were identified by virus dilution plaque-reduction neutralization tests in 18 sq cm cultures of primary chicken embryonic cells made as already described (5, 6). Decimal dilutions of virus and one dilution of antiserum were incubated together at 37°C for one hour, and adsorbed to cells for one hour at 37°C before cells were covered with agar medium. The EE antiserum used was made in rabbits (four injections) versus strain 448, at the U.S. Army 406th Medical General Laboratory in Japan in 1955, and was kept at -20°C; its log<sub>10</sub> neutralization index (LNI) versus EE virus, strain Riche, was >1.9, and versus VE virus, strain 63U2, was 1.0. The VE rabbit antiserum was made at Cornell in 1963-1964 with strain 63U2 (four injections) and had a homologous LNI = 3.1, and an LNI versus EE virus = 0.

Western encephalitis virus rabbit antiserum was also made in Japan in 1955, using North Dakota strain (five injections).

## Results

Three adult hamsters exposed to mosquitoes at Sayaxché in the northern forested Petén region yielded three strains of EE virus during August 1968 (Table 1). These hamsters survived for only three to seven days before becoming ill or dying. Each virus was neutralized in a plaque-reduction test in primary cultures of chicken embryonic cells, both as virus from original hamster tissues and from first mouse passages. These viruses were not neutralized by VE virus antiserum, nor was one strain (68U230) by Western encephalitis antiserum.

Titers of EE virus were determined in the original sentinel hamster tissues which had been thawed and refrozen to -60°C only once or twice. For two hamsters, these titers ranged from 10<sup>3.6</sup> to 10<sup>7.1</sup> CEC pfu/0.2 gm of heart,

Table 1—Isolations, identifications, and titers of Eastern encephalitis (EE) virus from three of 20 sentinel hamsters during 117 hamster-days of exposure at Sayaxché, Petén, Guatemala, in August 1968.

Information concerning EE virus strains	Data by virus strain designation		
	68U1	68U230	68U231
Days of exposure in field	3	3	7
Days of illness or death	6 Aug.	7 Aug.	11 Aug.
Identification by neutralization test			
LNI versus EE antibody of virus from sentinel hamster <sup>a</sup>			
heart	>1.4		>2.0
brain			>3.0
liver		>1.7	
virus from first passage baby mouse brain <sup>a</sup>	>2.3	>3.2	>2.4
Virus titer as negative log <sub>10</sub> CEC pfu/0.2 gm hamster heart			
heart	4.7	<1.0	5.7
brain	b	b	7.1 <sup>b</sup>
liver	4.6	1.8	3.6
kidney	3.6	<1.0	5.8
first passage baby mouse brain	8.6	8.7	9.2

<sup>a</sup>LNI (log<sub>10</sub> neutralization index) of these virus suspensions versus VE antiserum <0.8, and of 68U230 mouse brain virus versus Western encephalitis antiserum ≤0.3.

<sup>b</sup>Original isolate came from hamster brain tissue.

brain, liver, or kidney, but for one hamster virus was detected only in brain and liver and not in heart or kidney. Perhaps the tissues of this animal had undergone some autolysis in the field before freezing. The titers of EE virus in tissues of hamsters 68U1 and 68U231 were similar to those found with VE virus in hamsters (7).

## Discussion

The isolation of EE virus in the remote, forested, northern region of Guatemala at Sayaxché adds to its geographic distribution in the Western Hemisphere, which includes the Caribbean islands of Jamaica, Dominican Republic, and Trinidad as well as southern USA, northern Mexico, British Honduras, and Panama on the periphery of the Caribbean (1-3). When and how the virus reached the region of Sayaxché is unknown, but its recovery from sentinel hamsters kept in wire-mesh cages about 2 meters above ground indicated that it was being transmitted by flying arthropods, presumably mosquitoes, which entered the cages. This fact, in turn, indicated that EE virus was cycling between arthropod vectors and vertebrate hosts at Sayaxché, and thus that it was probably endemic there as of 1968. Only a few hundred people were living in Sayaxché in 1968, but in recent years there has been an airstrip with at least weekly airplane contact with other areas of the Petén region and with Guatemala City. There is also a road with daily bus connections to Flores, the major city in central Petén. Flores, in turn, is connected by road to British Honduras and the Caribbean port there. There is also the possibility of introduction of EE virus into this region by birds migrating during inapparent infection and silent viremia (8). This possibility is exemplified by the isolation of EE virus from a migrant orchard oriole in British Honduras in March 1964 (2). Regardless of the mecha-

nism of introduction, and whether it occurred recently or years to centuries ago, cycling of the virus seemed to be active in Sayaxché in August 1968 since three of 20 sentinel hamsters promptly became infected. The habitats in Sayaxché also harbored other arboviruses, since other hamsters yielded two strains of VE virus (9), five strains of group C arbovirus (one Nepuyo strain by neutralization test), and six Patois group viruses including one strain of Zegla virus. Thus this wet region in western Petén seemed to be particularly rich in arboviruses. What effect these viruses will have on the development and settlement of this area with susceptible people from other parts of Guatemala remains to be seen.

## Summary

Three strains of Eastern encephalitis (EE) virus were recovered from sentinel hamsters exposed to flying arthropods during August 1968 in wet, forested, and marshy habitats near the Pasión River in Sayaxché, Department of Petén, in northern Guatemala. The viruses were identified by plaque-reduction neutralization tests in chicken embryonic cell cultures. Titers of virus in sentinel hamster tissues usually ranged between  $10^3$  and  $10^7$  pfu/0.2 gm. These isolations extended the geographic distribution of EE virus to Guatemala and represented only the second time that EE virus has been isolated in Central America between Panama and northern Mexico, and the first time that virus isolations strongly suggest an endemic cycle in northern Central America involving flying vector arthropods.

## Acknowledgments

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**Aislamiento de virus de encefalitis oriental en cricétidos  
centinela expuestos durante 1968, en Guatemala (Resumen)**

Se recuperaron tres cepas de virus de encefalitis oriental en cricétidos centinela, expuestos a artrópodos voladores en agosto de 1968, en habitat húmedos, forestales y pantanosos cerca del río Pasión en Sayaxché, departamento del Petén, al norte de Guatemala. Los virus fueron identificados mediante pruebas de neutralización en placa en cultivos celulares de embrión de pollo. Los títulos de virus en tejidos de cricétidos centinela oscilaban normalmente entre

$10^3$  y  $10^7$  pfu (unidades fijadoras de placa) /0.2 g. Estos aislamientos indican la extensión de la distribución geográfica del virus de encefalitis oriental en Guatemala, y sólo se habían obtenido en otra ocasión en Centroamérica, entre Panamá y el norte de México. Asimismo, son los primeros que sugieren firmemente la presencia de un ciclo endémico en la región septentrional de Centroamérica en el que intervienen artrópodos voladores vectores.

**Isolamento do virus da encefalite oriental na Guatemala  
em cobaias expostas durante 1968 (Resumo)**

Foram recuperados três espécies de virus de encefalite oriental de cobaias expostas aos artrópodos voadores em agosto de 1968 em "habitats" úmidos, florestais e pantanosos próximos do Rio Pasión, em Sayaxché, Departamento de El Petén, na Guatemala Setentrional. Os virus foram identificados por testes de neutralização em redução placa em culturas de células de embrião de galinha. Os títulos do virus nos tecidos das cobaias oscilaram geral-

mente entre  $10^3$  e  $10^7$  pfu/0,2 gm. Estes isolamentos ampliam a distribuição geográfica do virus da encefalite oriental na Guatemala e representam apenas a segunda vez que este virus foi isolado na América Central, entre o Panamá e o México setentrional, e a primeira vez que tais isolamentos sugerem um ciclo endêmico na América Central setentrional, envolvendo um vetor de artrópodes voadores.

**Au Guatemala, des chercheurs ont réussi à isoler le virus de l'encephalite orientale à partir d'hamsters exposés en 1968 (Résumé)**

Trois souches d'encephalite orientale ont été obtenues à partir d'hamsters exposés pendant le mois d'août 1968 à des arthropodes volants dans les régions humides, boisées et marécageuses où coule le Fleuve Pasión à Sayaxché (département d'El Peten) au nord du Guatemala. Ces virus ont été identifiés au moyen de tests de neutralisation par réduction des plaques, réalisés sur des cultures de cellules d'embryon de poulet. Les virus observés dans les tissus des hamsters soumis à l'expérience

titraient de  $10^3$  à  $10^7$  ufp/0,2 g. Cette expérience prouve que l'encephalite orientale sévit jusqu'au Guatemala; c'est seulement la deuxième fois que des chercheurs parviennent à isoler ce virus en Amérique Centrale, entre Panama et le Mexique septentrional, et la première fois qu'ils sont fortement tentés d'en conclure à l'existence, dans le nord de l'Amérique Centrale, d'un cycle endémique où les arthropodes volants jouent le rôle de vecteurs.

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