VENEZUELAN EQUINE ENCEPHALITIS VIRUS ACTIVITY IN NORTHERN COLOMBIA DURING APRIL AND MAY 1983[1]

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INTRODUCTION

The source of equine-virulent strains of Venezuelan equine encephalitis (VEE) virus that sporadically cause epidemics and equine epizootics is unknown. This is the most important unanswered scientific and public health question relating to VEE virus. One possibility is that epizootic virions exist at low levels in populations of enzootic strains. These virions could then break out into a visible epizootic cycle through the combination of chance selection and fortuitous passage through susceptible mammals, including equine animals and man, that produce high levels of viremia capable of infecting inefficient vectors.

Arbovirus studies in northwestern Venezuela's Lake Maracaibo drainage area that have isolated VEE virus have previously been reported *(1, 2)*. We report here the isolation and identification of strains of VEE virus from sentinel hamsters exposed in an extensive region of tropical wet forest in Colombia adjacent to the Venezuelan border, as well as data on mosquito collections from several foci of VEE virus. This investigation was conducted as a joint program between Cornell University in Ithaca, New York, USA, and Colombia's National Institute of Health (Instituto Nacional de Salud) in Bogotá.

[1] This article will also be published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, 1987. The investigation reported here was carried out in collaboration with the Pan American Health Organization and was supported in part by National Science Foundation Cooperative Science Program Grant NSF-INT 8203076.

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MATERIALS AND METHODS

Study Areas

Santa Marta region. Sentinel hamsters were exposed at two forests, "Guachaca" and "Don Diego," that are respectively located 44 and 61 kilometers east of the port of Santa Marta (see Figure 1). We found Guachaca to be a swamp forest with abundant standing water, whereas the tropical wet forest encountered at Don Diego had well-drained soil, with water standing only in some drainage channels. VEE virus was isolated in the region from *Culex* (*Melanoconion*) mosquitoes during the period October 1973–July 1974 and from sentinel hamsters in December 1975 and March and April 1976 (Groot, personal communication).

Magangue region. Sentinel hamsters were exposed in a low, flood-plain forest approximately two kilometers northwest of Boquillas on a side-channel of the Magdalena River, about 30 kilometers southeast of Magangue. The water table was high, and standing water was abundant. The "Magangue" strain of VEE was identified in this region in 1969 *(3)*.

Río de Oro region. A large area of tropical wet forest investigated along the Río de Oro was contiguous with the "Cano Mocho" site in adjacent Venezuela *(2)*. Hamsters were exposed in dense virgin forest about two kilometers northeast of the village of Río de Oro. One strain of VEE virus had previously been isolated from a sentinel hamster exposed near Tibu in the Río de Oro region in 1970 (Groot, personal communication).

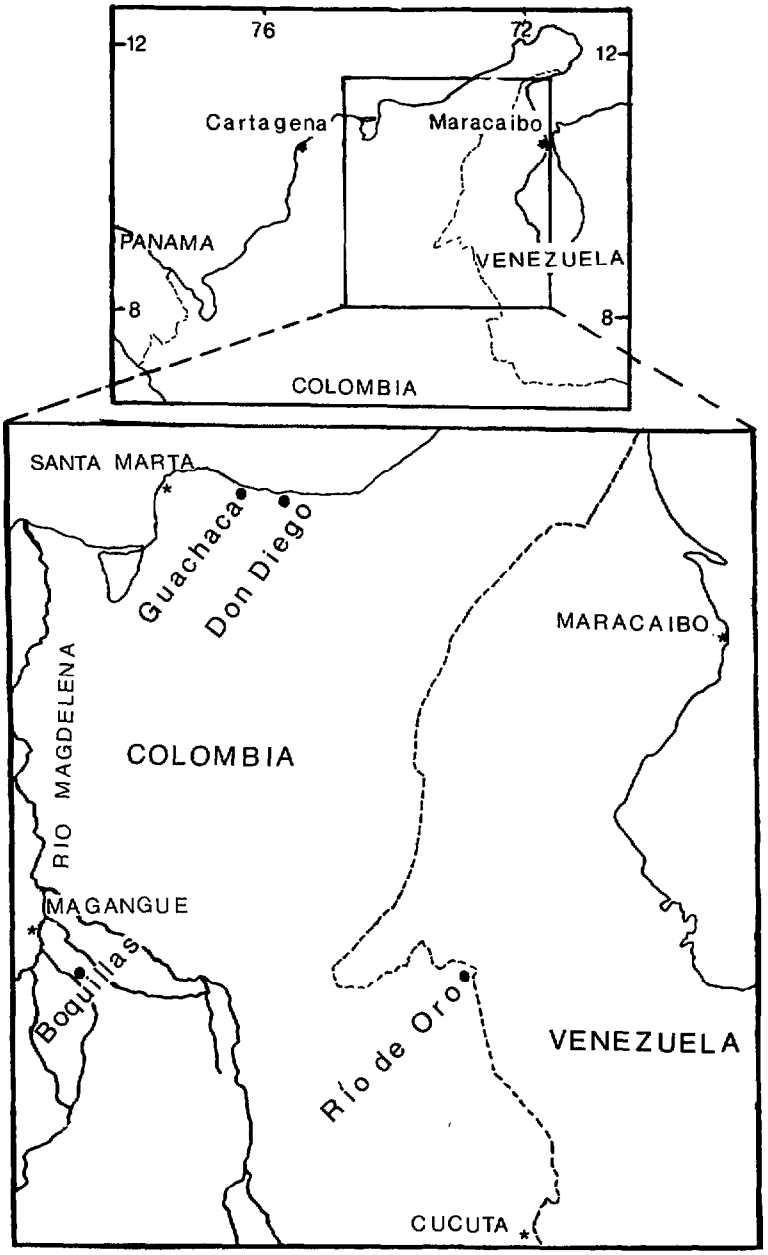
Field and Laboratory Techniques

Sentinel hamsters. Outbred Syrian hamsters from the colonies of the Instituto Nacional de Salud (INS) were exposed in sentinel cages as described previously for Middle America *(4)* or in plastic "coquito" cages. The latter were originally designed by Karl M. Johnson (then at the Middle American Research Unit, Panama) and modified and produced at the INS. They were made of two-liter plastic mixing bowls with tight snap-on covers. Most of the plastic of the cover was removed and replaced with half-inch metal mesh to form the floor of the cage when the bowl was suspended upside down from a hook inserted through the reinforced bottom of the bowl. The snap-on cover was easily removed later, permitting easy handling of the animal. This design also provided ready access to the animal for mosquitoes seeking a blood meal.

In addition, 14 hamsters were exposed in Trinidad No. 10 mosquito traps *(5)* to limited numbers of mosquitos at Guachaca (an average of 2.3 per hamster-night), Don Diego (an average of 2.8) and Río de Oro (an average of 3.5). Only the two hamsters exposed in No. 10 traps at Río de Oro were tested for virus.

Collection, processing, and testing of specimens from vertebrates. Randomly selected human subjects were bled by regional public health personnel using standard venipuncture techniques at Magangue and Río de Oro. Equine animals, wild birds, and mammals (including bats) were captured and bled as described previously *(6)*. The procedures

FIGURE 1. A map of Colombia and Venezuela showing the study areas.



used to process these blood specimens for serology and hamster tissues for virus isolation, as well as the techniques used for virus isolation, have previously been reported *(6, 7)*. The vaccine strain of VEE virus (TC-83) was used in plaque-reduction neutralization tests conducted with primary chick embryo cell (CEC) cultures for testing human and equine sera. A $\log_{10}$ neutralization index of 1.6 or greater was considered positive. Polyvalent VEE virus mouse ascitic fluid made by W. F. Scherer in 1966 against 12 strains of subtype I-E virus from Mexico was used in virus identification neutralization tests.

Mosquito collections. Adult mosquito collections were made within and proximal to forested sites near Río Guachaca, Río Don Diego, and Río de Oro using a D-Vac vacuum sweeper and CDC miniature light traps or human hosts as attractants. Female mosquitoes were pinned and shipped to the Department of Entomology at Cornell University for identification; *Culex* (*Melanoconion*) spp. males were dissected, and their genitalia were cleared and mounted on slides for later identification.

Hydroxylapatite chromatography. The methods of Jahrling and Eddy *(8)* were employed, except that virus was initially washed from the column with a 0.15 M phosphate solution at pH 7.0 before elutions with the phosphate gradient were begun. Virus in the fractions was assayed for plaque-forming units.

Results

Virus Isolation and Identification

There were no fatalities among the 52 hamsters exposed in tropical wet forests near Santa Marta for a total of 515 hamster-nights (Table 1). Fifteen of 27 hamsters exposed near Boquillas in the Magangue region were removed from the field moribund or dead. However, none yielded a plaque-forming virus in chick embryo cell cultures. Seven of the tissue suspensions were also tested by intracranial inoculation into suckling mice; all of these tests yielded negative results.

At Río de Oro, 36 of the 37 hamsters exposed in sentinel hamster cages or in Trinidad No. 10 mosquito traps were removed from the field moribund or dead. Tissues from 19 of these 37 hamsters (including both hamsters from the mosquito traps) were tested for virus; all yielded plaque-forming virus in chick embryo cell cultures that was neutralized by VEE virus-specific antibody. Of the 18 hamsters not tested, 10 were replacements put into cages where sentinel hamsters had died previously, and so their infections may have been caused by focally recycled virus. Six animals were spoiled when recovered from the field (see discussion), and two tissue samples were not found after returning to New York. The average number of hamster-nights exposure for animals that were probably not infected with focally recycled virus was 7.7. VEE virus isolations per 100 hamster-nights among this latter group of animals was 13.

Serology in Humans and Animals at Boquillas

No VEE virus was isolated from the tissues of 15 dead or moribund

TABLE 1. The numbers of hamsters exposed at four endemic foci of Venezuelan equine encephalitis virus in Colombia in 1983, together with the numbers becoming moribund or dying and the strains of virus isolated, by locality.

Locality	No. of hamsters exposed	Hamsters-nights of exposure	No. becoming ill or dying / No. exposed	No. of hamsters tested / No. of strains of VEE virus isolated
Santa Marta Region:				
Guachaca forest	22	279	0/22	None tested
Don Diego forest	32	236	2/32	None tested
Magangue Region:				
Boquillas	27	231	15/27	15/0
Tibu Region:				
Río de Oro	25 (12)[a]	164 (90)[a]	36/37	19/19[b]

[a] The numbers in parenthesis are the number of hamsters exposed and the number hamster-nights of exposure for animals moved to sites where a sentinel had died previously. Viral isolates from these animals could represent focally recycled virus

[b] The denominator includes two isolates that may represent recycled virus (as explained in footnote a above) and one isolate from the tissues of a seemingly healthy hamster that was killed at the end of the study.

hamsters exposed near Boquillas. However, 12 equine animals 2 to 10 years of age were sampled along the river near the forest where the hamsters were exposed, and 11 of these were positive for VEE virus-specific neutralizing antibodies. Twelve people 25 to 67 years of age (the average age being 47.9 years) were also positive for VEE neutralizing antibodies, while 23 people 1 through 64 years old (with an average age of 26.7 years) yielded negative results.

Mosquito Collections

About 3,600 mosquitoes were collected at the Río Guachaca and Río Don Diego sites, 60% of these coming from daytime collections made with human bait. *Psorophora ferox* was found to be the predominant diurnally active species at both locations. Females of this species readily attacked at the edge of the forest as well as along trails leading into the interior. *Aedes fulvus fulvus, Ae. angustivittatus, Ae. scapularis*, and members of the *Ae. terrens* group (near *Ae. whitmorei*) were collected in fewer numbers within the forest proper.

The species composition of twilight collections made with human bait at Río Guachaca differed markedly from the species composition of the daytime collections. *Culex (Mel.) crybda* was the predominant taxon, followed by *Cx. (Cx.)* species (principally *Cx. nigripalpus* and a few *Cx. corniger*) and by *Ae. fulvus fulvus*. D-Vac collections also contained numerous males later identified as *Cx. (Mel.) idottus*. Large numbers of *Deinocerites* mosquitoes (near *De. atlanticus*) were also collected in the vacuum sweeper by selectively sampling crabholes.

Though not as abundant because of drier conditions, the mosquito fauna at Río Don Diego was more diverse with regard to *Cx. crybda*, *Cx. spissipes*, and *Cx. pedroi*. As in the case of the Río Guachaca collections, *Ps. ferox* was the predominant diurnally active species.

Culex pedroi was a common anthropophilic species found in the wet forest habitat near Río de Oro. This mosquito was readily attracted to humans at

dusk and was also abundant in light-trap collections. Specifically, seven twilight collections made on successive evenings with human bait yielded collections of *Culex* (*Melanoconion*) mosquitoes that consisted 53% to 86% of *Cx. pedroi*, the average percentage of *Cx. pedroi* being 68%. *Culex spissipes* was also taken frequently in both types of collections but in considerably fewer numbers. As at the two sites near Santa Marta, *Ps. ferox* was again the predominant day-biting mosquito, far outnumbering any other species within the forest habitat.

Hydroxylapatite Elution Profiles of Río de Oro VEE Strains

The elution profiles were determined for three strains: 83U18, 83U78, and 83U434. Two strains eluted at a phosphate molarity of about 0.15, while more than 90% of the third strain (83U78) eluted at a phosphate molarity of less than 0.20. These three strains were therefore considered to represent an enzootic variety, supposedly 1D, rather than an epizootic variety—which would have eluted at a molarity in the range of 2.0–3.0 *(8)*.

Discussion and Conclusions

These studies demonstrate the continued existence of VEE virus in enzootic foci within tropical wet forests in northeastern Colombia along the Venezuelan border in the Lake Maracaibo drainage area. VEE virus activity has been demonstrated periodically in this area since 1971, when the Tibu strain was isolated. These records antedate by two years similar findings in the Catatumbo region of northwestern Venezuela *(1, 2)*.

In contrast, VEE virus was not isolated in the Santa Marta or Magangue regions after lapses of 9 and 16 years, respectively, despite "adequate" exposure periods exceeding 200 hamster-nights. In view of the perpetuation of VEE virus at other relatively unmodified sites over similar time spans, such as sites in the Tibu region of Colombia or at La Avellana in Guatemala *(9, 10)*, the failure to isolate virus in the Santa Marta and Magangue regions during this investigation must be considered a chance phenomenon. This failure does not prove the disappearance of the virus, but perhaps relates to local conditions—especially in the Magangue region, where children and young animals possessed VEE virus-specific neutralizing antibodies. The findings thus demonstrated further the need for more frequent long-term monitoring of enzootic situations.

The easily constructed, lightweight, compact, and stackable "coquito" sentinel hamster cages provided an inexpensive and utilitarian field tool. On the basis of our experience, minor modifications are recommended to further improve their design—specifically, to prevent the retention of urine in the groove where the cage and the floor meet. We found that the soiled cages attracted flies, which ovideposited on the moribund sentinels and caused rapid spoilage.

Findings from the vector studies, while principally qualitative, did not demonstrate any major differences between the mosquito faunas at the sites near Santa Marta and those at Río de Oro in the Tibu region. Of the species collected at Río Guachaca that have been circumstantially associated with the transmission of enzootic VEE viruses, only

Cx. crybda was noteworthy. This species has been placed in the Spissipes section of *Culex* (*Melanoconion*), a taxonomic grouping that contains several proven vectors *(11)*.

Interestingly, the forest near Río Don Diego contained four species belonging to the Spissipes section (*Cx. adamesi, Cx. crybda, Cx. spissipes*, and *Cx. pedroi*). Based on previous studies in Panama (revised by Galindo, *12*), all should be considered potential vectors. Of these, *Cx. pedroi* would be especially suspect because it is very closely related ecologically and taxonomically to *Cx. taeniopus (13)*, a widespread enzootic VEE vector in Middle America *(14)*. *Culex pedroi* in particular, as well as *Cx. spissipes*, were also abundant in light-trap and human bait collections made at Río de Oro, where virus transmission to sentinel hamsters occurred readily.

The role of *Ps. ferox* as an enzootic VEE vector in Colombia is problematic. While this species cannot be dismissed—because of earlier laboratory studies suggesting its susceptibility to VEE virus infection *(15)* and its pronounced abundance at each site—it is interesting to note that *Ps. ferox* did not yield a single VEE virus isolate in the Catatumbo region of Venezuela during an eight-year study *(2)*. Therefore, until further controlled laboratory and field studies can be conducted, this species should continue to be considered merely a potential vector.

Acknowledgments

We wish to acknowledge our legacy to our colleague and mentor, Dr. William F. Scherer, for his enthusiasm and enjoyment of field research and training and his fundamental contribution to the development of this program. The authors also wish to express their gratitude to Gilberto Torres, José Múñoz, José Botero, and Indalesio Amaya for their expert assistance in the field work; to Doña Castello for her excellent technical assistance in the laboratory; and to Dr. Sunthorn Sirivanakarn for confirming the identification of several *Culex* (*Melanoconion*) species.

Summary

Venezuelan equine encephalitis virus was isolated from sentinel hamsters exposed in the Río de Oro region of Colombia, in the Lake Maracaibo drainage area near the border with Venezuela, in 1983—13 years after the first strain was isolated from the region near Tibu.

Hydroxylapatite elution profiles of three isolates from the Río de Oro region were typical of enzootic strains. Relative abundance data and past isolations suggest that *Culex* (*Melanoconion*) *pedroi* is a probable enzootic vector at this focus.

The virus was not isolated from forests near Santa Marta and Magangue in the Río Magdalena drainage area in 1983—9 and 16 years, respectively, after the last previous isolations in those areas. However, equine animals 2 to 10 years of age and humans 25 to 67 years of age living near the Magangue field study area were positive for VEE virus-specific neutralizing antibodies.

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