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CONTRASTING SYLVATIC FOCI OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN NORTHERN SOUTH AMERICA

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Abstract. The ecology of Venezuelan equine encephalitis (VEE) virus transmission was compared at three enzootic foci: two forest sites in the Catatumbo region of western Venezuela that have yielded small numbers of virus isolates since the 1970s, and another focus in the middle Magdalena Valley of Colombia that has consistently yielded many VEE virus isolates. Our results demonstrated dramatic differences in VEE virus isolation rates from sentinel hamsters, as well as differences in mosquito species composition and captured mammals with antibodies to VEE virus, between the Colombian and Venezuelan study sites. The higher isolation rate of enzootic VEE virus in the Colombian site was associated with a more abundant fauna of spiny rats (*Proechimys* spp.), known reservoir hosts of enzootic VEE virus. Mosquito collections demonstrated that the Colombian forest had a higher mosquito diversity and species evenness than either of the Venezuelan forests. The Colombian focus was especially richer in its *Culex* (*Melanoconion*) spp. fauna, a subgenus that includes all proven enzootic vectors for VEE virus. Our results suggest that the greater abundance, diversity, and stability of enzootic vector populations, combined with the greater density of rodent reservoir hosts, explains the higher levels of VEE virus circulation in the Colombian focus compared with the Venezuelan forests.

INTRODUCTION

Venezuelan equine encephalitis (VEE) is an important re-emerging disease that affected hundred of thousands equines and humans in the Americas for much of the 20th century.[1–3] Venezuelan equine encephalitis virus, a mosquito-borne RNA virus belonging to family *Togaviridae* and genus *Alphavirus*[4] causes VEE. Venezuelan equine encephalitis virus strains can be classified into epizootic, comprising subtypes IAB and IC that are pathogenic for equines, and enzootic, which are generally avirulent for equines (subtype I, varieties D through F, subtypes II through VI).[5] Subtypes IAB and IC of VEE virus are responsible for all major epizoodemics, have been isolated only during outbreaks, occur in agricultural settings with the involvement of distinct mosquito vectors and hosts, and do not generally overlap in their geographic range with the equine avirulent, enzootic viruses.[5–7] The latter viruses have a widespread, generally allopatric distribution in the Americas, occur in inundated lowland tropical forests and swamps, and cycle between *Culex* (*Melanoconion*) spp. mosquito vectors and rodents. Thus, epizootic and enzootic VEE viruses have very distinct transmission cycles. Furthermore, there is strong evidence showing that epizootic VEE virus emerges as mutants of from a single enzootic subtype ID lineage that occurs in Venezuela, Colombia, and northern Peru.[8–11] This underscores the importance of understanding the geographic distribution, genetics, and ecology of enzootic VEE virus foci representing this epizootic progenitor lineage.

Enzootic subtype ID VEE virus lineages are distributed into five non-overlapping geographic ranges: 1) northern Peru, Colombia, and western Venezuela; 2) Panama and the Amazon basin of Peru; 3) Miranda State in northcentral Venezuela; 4) Florida (Everglades virus, genetically an ID-like variant); and 5) southwestern Colombia and coastal Ecuador.[11,12] However, only the first ID genotype is linked to epizootic emergence, which is probably related to the genetic background of the enzootic precursors and their ability to generate appropriate combinations of epizootic mutations.[11]

Enzootic subtype ID VEE virus circulating during the past 30 years in the Catatumbo region of Venezuela and the middle Magdalena Valley of Colombia are members of the ID lineage with a history of generating epizootic subtype IAB or IC VEE virus via small numbers of mutations.[9,13] These VEE virus enzootic foci include lowland forests of the Catatumbo (Venezuela) and Magdalena (Colombia) River basins (350 km apart), and viruses isolated there are closely related to the epizootic subtype IAB and IC VEE virus emergences that affected both countries since the early part of the 20th century. Two slightly different genetic VEE virus variants within this ID lineage have been found co-circulating in the same forests of the Catatumbo area, and one of these also occurs in the Magdalena Valley of Colombia.[13] The strongest genetic and geographic link between enzootic and epizootic viruses is represented by one of these variants, which is found in two different forests of the Catatumbo region and is closely related to the epizootic IC VEE emergence of 1992–1993 in western Venezuela.[10,13] Contrasting the transmission dynamics of subtype ID VEE virus between the Colombian and Venezuelan foci could contribute to our understanding of the emergence of new epizootic VEE virus variants.

Subtype ID VEE virus has been routinely isolated from sentinel hamsters in the lowland forests of the Magdalena Valley in Colombia since the 1970s,[14] but is comparatively more difficult to isolate in the forests of the Venezuelan Catatumbo area.[13,15–17] Differences in virus diversity and isolation rates between the two areas may be related to variations in weather, and ecologic differences in vectors and hosts involved in the natural transmission cycle. A detailed comparison of the Colombian versus Venezuelan foci is pertinent because the landscapes in both areas appear similar upon superficial examination, with equivalent land-use patterns (cattle ranching, oil extraction), vegetation (pastures, patches of tall lowland tropical forests, limited agriculture), drainage (close to major rivers), and terrain slope. Therefore, we undertook a comparative study to contrast VEE virus isolation rates, meteorologic variables, mosquito communities, and

small-mammal populations between enzootic foci in the Catatumbo and the middle Magdalena River basins. Our results indicate that differences in the populations of reservoir hosts and mosquito vectors probably explain the contrasting rates of VEE virus circulation.

MATERIALS AND METHODS

Study areas. The study was designed to compare three enzootic foci, one in Colombia and two in Venezuela, where subtype ID VEE virus had been isolated: 1) Monte San Miguel forest (6°23′ 30”N, 74°21′41”W), Cimitarra District, Santander, Colombia, henceforth referred to as CO-San Miguel, 2) Río Claro forest (9°0′44.5”N, 72°41′53”W), henceforth referred to as VZ-Rio Claro, and 3) Las Nubes forest (9°3′39”N, 72°37′12”W), henceforth referred to as VZ-Las Nubes, both in the Semprúm District, Zulia State, Venezuela (Figure 1). The Venezuelan study sites are 10 km apart, and the distance between the Colombian and Venezuelan sites is approximately 350 km. The landscape in both areas is remarkably similar, and characterized by extensive pasture created by deforestation, isolated shade trees, and highly fragmented patches of forest (Figure 2). The main economic activities are cattle ranching, oil extraction, and little agriculture, except for the extensive groves of African palms in the Catatumbo area.

Relatively cloud-free Landsat 5 Thematic Mapper images for the study sites in Venezuela (path 7, row 54, September 6, 1996) and Colombia (path 8, row 56, August 14, 1991) were used to identify forests and water on the ground around the study sites. All six bands of reflected radiation (bands 1–5, 7) were used to perform a supervised classification using the FEATURE MAPPING capabilities of TNTmips software (MicroImages, Inc., Lincoln, NE). Ground control points within forests were gathered with differential global positioning system units. Forests and water were readily separated and identified in the 20 × 20 km^2 sub-scenes (Figure 2). The area surrounding San Miguel Forest contained 26.1% forests and 6.3% water in 1991 (Magdalena River, small lagoons; Figure 2), whereas the Venezuelan study sites were comprised of 14.7% forest and 3.7% water in 1991 (Catatumbo and Socuavo Rivers). The length of forest boundaries per unit area in the San Miguel study area was about double (6,024 km) that in the Catatumbo study area (3,213 km). The images also indicate the larger size of the San Miguel forest existing in the 1991 image than of the Catatumbo forests in 1996.

Forests in the Catatumbo area are remnant forests (altitude = 40–70 meters) on the tropical plains of the Catatumbo River. The area undergoes frequent flooding due to high precipitation and low slopes (< 0.05%). Reticular soil erosion (Moment “II”[18]) produces small mounds and canals of varying dimensions on the ground that fill with water and become the main pre-adult mosquito habitats in the forests. Original vegetation is lowland tropical forest that has been cleared extensively for cattle grazing. Remaining forests in the area are either on poorly drained soils or on areas with high reticular erosion where soil leveling is costly. Río Claro forest is situated on gentle rolling hills crossed by small streams, and much of the lowland portions flood throughout the year. The main tree species are *Jacaranda copaia*, *Protium sp., Licania arborea*, *Attalea maracaibensis,* and *Ficus* sp. Surrounding pastures are composed of *Echinocloa spectabilis*, *Panicum* sp. *Mimosa pigra*, and several Cyperaceae species. The VZ-Las Nubes forest is situated on level terrain that floods intermittently throughout the year. The main tree species are *Copaifera publifora*, *Coriaiana pyriformis*, *Jacaranda copaia*, *Attalea maracaibensis*, and *Licania arborea*. Inundated pastures and tall, aquatic vegetation (*Hymenachne amplexicaulis*, *Heliconia marginata*, *Thalia geniculata*) surround the forest. Agriculture is severely restricted because of acid soils and floods.

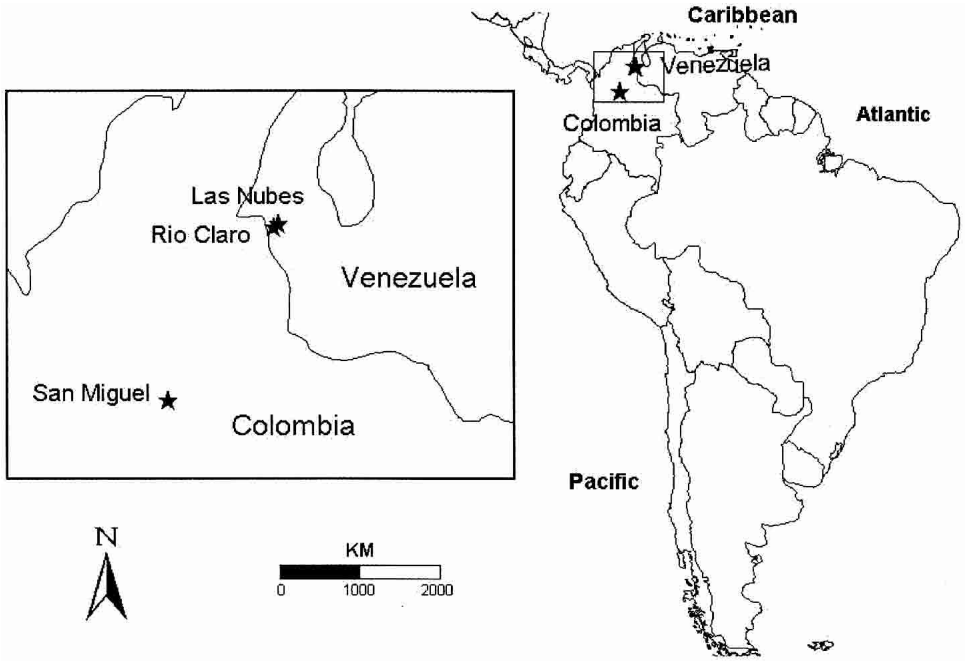


FIGURE 1. Location of the study sites in Colombia (San Miguel forest) and Venezuela (Las Nubes and Río Claro forests) in South America.

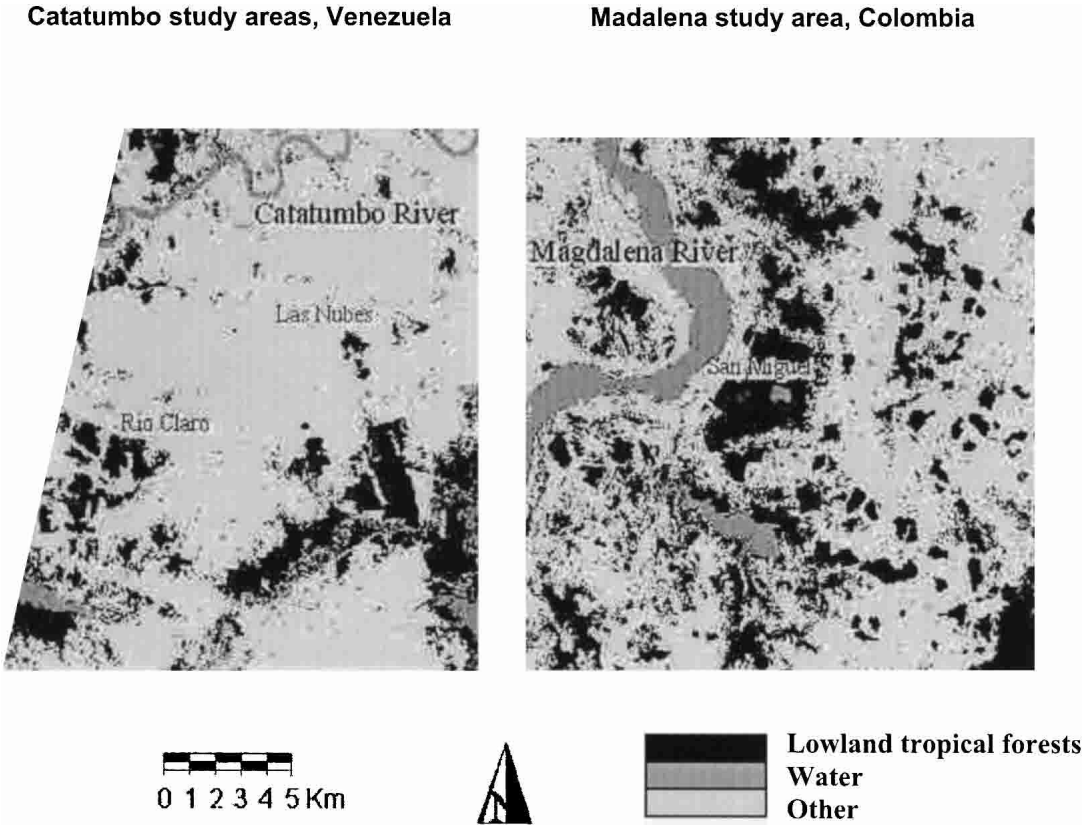


FIGURE 2. Location of lowland tropical forests, rivers, and other bodies of water in the Catatumbo (Venezuela) and Magdalena (Colombia) River basins as derived from Landsat 5 Thematic Mapper satellite images. Images are at the same scale to show the relative extent of the landscape features.

The San Miguel forest is a tropical humid forest located in the middle Magdalena Valley of central Colombia. According to Espinal,[19] pastures are populated by grasses such as foxtail *(Andropogon bicornis*) and by *Heliconia* sp.; many ponds are covered with water *lettuce (Pistia stratiotes*) and *Polyrrhiza spirodela*, and in their inundated borders are populated by vines and cattails (*Typha angustifolia*), para grass (*Brachiaria mutica*) and water hyacinth (*Eichhornia crassipes*). The flora of the forest and ecotone include the following species: *Pithecellobium* sp, *Hura crepitans*, *Trichillia aff pallida*, *Coccoloba* sp., *Ficus aff popenoi*, *Sorocea cfafinnis*, *Casearia corimbosa*, *Tabebuia rosea*, *Inga* sp., *Sanchezia pennelli*, *Calathea inocephalla, Calathea lutea*, and *Heliconia latispatha*.

Precipitation in 1998 was high and comparable in both study areas: 2,740 mm (annual mean = 2,957 mm from 1977 to 1999) in the Catatumbo and 2,691 in the middle Magdalena Valley of Colombia (annual mean = 2,405.6 mm from 1981 to 1999). In both areas, the lowest seasonal precipitation occurs between December and March (Figure 3), whereas during the remainder of the year precipitation usually exceeds 100 mm per month. The mean annual temperature is also comparable: 27.2°C (1978–1984) in the Catatumbo and 28.6°C in the middle Magdalena Valley (1981–1999).

Field studies. Surveillance for VEE virus was conducted in each of the study areas on four field trips in Venezuela (February, May, June, and October) and Colombia (March, May, August, and November) during 1998.

Sentinel animals. Syrian golden hamsters obtained from colonies at the Instituto Nacional de Higiene in Caracas and the Instituto Nacional de Salud in Bogota were exposed to mosquito bites in *coquito* cages[20] for seven days in each of the surveillance sites. Cages were suspended 1.2–1.5 m above the ground and placed in transects at 20–25-meter intervals. Hamsters were inspected and fed carrots daily. Blood samples were collected by cardiac puncture from moribund hamsters and from those surviving the one-week exposure; the hamsters were then humanely killed. Heart and spleen samples were dissected from hamsters found moribund dead during the exposure period and preserved in liquid nitrogen. A total of 546 (467 in Venezuela and 79 in Colombia) hamsters were exposed in the field during this study. The maintenance and care of animals complied with the guidelines of the National Institute of Hygiene (Caracas, Venezuela) or the National Institute of Health (Bogota, Colombia).

Collection of small mammals. Parallel to the transect where sentinel hamsters were exposed, we placed 40–45 Sherman (H. B. Sherman Traps, Inc., Tallahassee, FL) and 20–30 Tomahawk (Tomahawk Live Trap Co., Tomahawk, WI) traps for 5–7 days. Bait for the Sherman traps was replaced every day and consisted of a mixture of sardines, corn flour, corn grains, bird food, peanut butter, vanilla extract, and vegetable oil. Ripe plantains, cassava, and fresh fruits were used as bait in the Tomahawk traps. Captured animals were bled by cardiac puncture, and those that could not be readily identified were preserved with formaldehyde. Blood samples and organs were collected as described earlier in this report. A total of 3,640 trap-nights was sampled in Venezuela and 1,848 in Colombia. Mammals were identified with the aid of taxo-

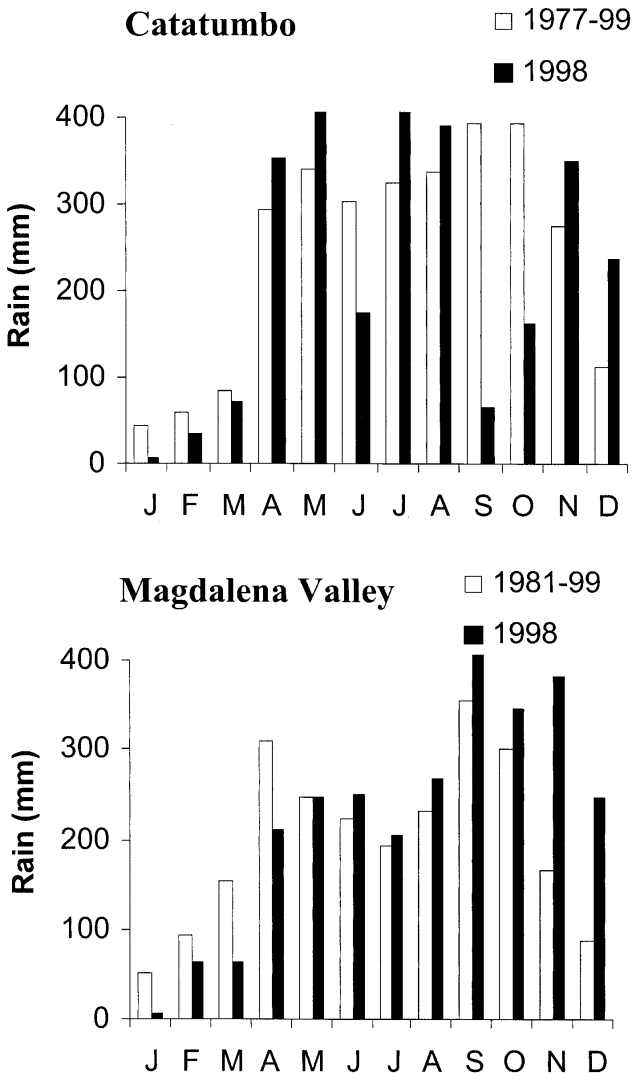


FIGURE 3. Monthly rainfall (mm) patterns from the closest meteorologic stations in the Catatumbo and Magdalena study sites from Venezuela and Colombia, respectively.

nomic keys.[21,22] All mammals were tested for antibodies to VEE virus using hemagglutination inhibition (HI) or plaque-reduction neutralization tests.

Collection of mosquitoes. Mosquitoes were collected using miniature Center for Disease Control (CDC) light traps[23,24] baited with light and CO_2 (approximately 250 grams of dry ice) and suspended approximately 1.5 meters above the ground. Dry ice was suspended near the trap opening and replaced every 12 hours. One CDC trap was placed outside the forest in an open pasture area 100–150 meters from the edge of the forest, and two were placed at 10 meters (ecotone) and 200 meters inside the forest, respectively. We operated the CDC traps for 3–4 days, collecting during the day (6:00 AM to 6:00 PM) and night (6:00 PM to 6:00 AM). The total sampling effort was 52 traps per 12-hour sampling interval in VZ-Rio Claro, 59 in VZ-Las Nubes, and 90 in San Miguel (Colombia). Mosquitoes were identified with the aid of taxonomic keys[25–28] and our own reference collections.[29]

Statistical analyses. Reported values are means and standard deviations. Mosquito species evenness, species diversity, and their variances were calculated following Bulla's indices.[30] Confidence intervals (99%) were used to test for differences between diversity indices at an overall error rate lower than 0.05.

RESULTS

Isolation of VEE virus. Of 20 VEE virus isolates from sentinel hamsters, 16 came from San Miguel forest (Colombia), none from VZ-Las Nubes, and only four from the Río Claro site (Venezuela; Table 1). Viruses were isolated only in February in Venezuela, but on every field trip in the Colombian study forest (two in March, one in May, four in August, and nine in November, 1998). Resulting isolation percentages (virus isolates/exposed hamsters × 100) were higher in Colombia (20.3%) than in Venezuela (combined 0.9%). All viruses were analyzed antigenically and classified into subtype ID, and genetic analyses indicated that they were all closely related to subtype IAB and IC epizootic VEE virus strains.[13]

Mosquito captures. Because mosquitoes outside the subgenus *Culex* (*Melanoconion*) may be involved in movement of VEE virus outside of the forest foci, we studied the entire mosquito fauna. A total of 49 mosquito species in 12 genera was collected in the combined study sites (Table 1): *Aedes* Meigen (1 spp.), *Aedomyia* Theobald (1 spp.), *Anopheles* Meigen (4 spp.), *Coquillettidia* Dyar (3 spp.), *Culex* L. (18 spp.), *Mansonia* Blanchard (2 spp.), *Limatus* Theobald (2 spp.), *Psorophora* Robineau-Desvoidy (6 spp.), *Runchomyia* Theobald (1 spp.), *Wyeomyia* Theobald (1 spp.), *Johnbelkinia* Zavortink (1 spp.), and *Uranotaenia* Lynch Arribalzaga (6 spp.). The CO-Monte San Miguel and VZ-Las Nubes forests showed higher species richness (32 species each) than the VZ-Río Claro forest (24 species). However, species diversity (evenness × species richness) was significantly higher ($\alpha < 0.05$) in the Colombian study site (99% confidence interval = 14.5 ± 0.2; Table 2) than in either of the Venezuelan sites (Las Nubes = 11.3 ± 0.1, Río Claro = 11.6 ± 0.3). Species diversity in Las Nubes study site (Venezuela) was relatively low despite its high species richness (32) due to the numerical dominance of a few species (e.g., *Cx. nigripalpus* Theobald, *Ps.*

TABLE 1

Venezuelan equine encephalitis (VEE) virus surveillance using sentinel hamsters in Venezuela (Río Claro and Las Nubes forests) and Colombia (San Miguel forest) during 1998, showing the sampling effort and virus isolations per field visit

Locality, month	Exposed	Moribund or dead	Lost	VEE virus isolations
Venezuela - Río Claro, February	60	5	0	4
Venezuela - Las Nubes, February	60	1	1	0
Colombia - San Miguel, March	35	5	0	2
Venezuela - Río Claro, May	65	18	0	0
Venezuela - Las Nubes, May	57	56	1	0
Colombia - San Miguel, May	10	10	0	1
Venezuela - Río Claro, July	40	3	0	0
Venezuela - Las Nubes, July	38	9	0	0
Colombia - San Miguel, August	10	10	0	4
Venezuela - Río Claro, October	74	20	0	0
Venezuela - Las Nubes, October	73	7	0	0
Colombia - San Miguel, November	24	17	0	9
Total, Venezuela	467	119	2	4
Total, Colombia	79	42	0	16
Total	546	161	2	20

TABLE 2

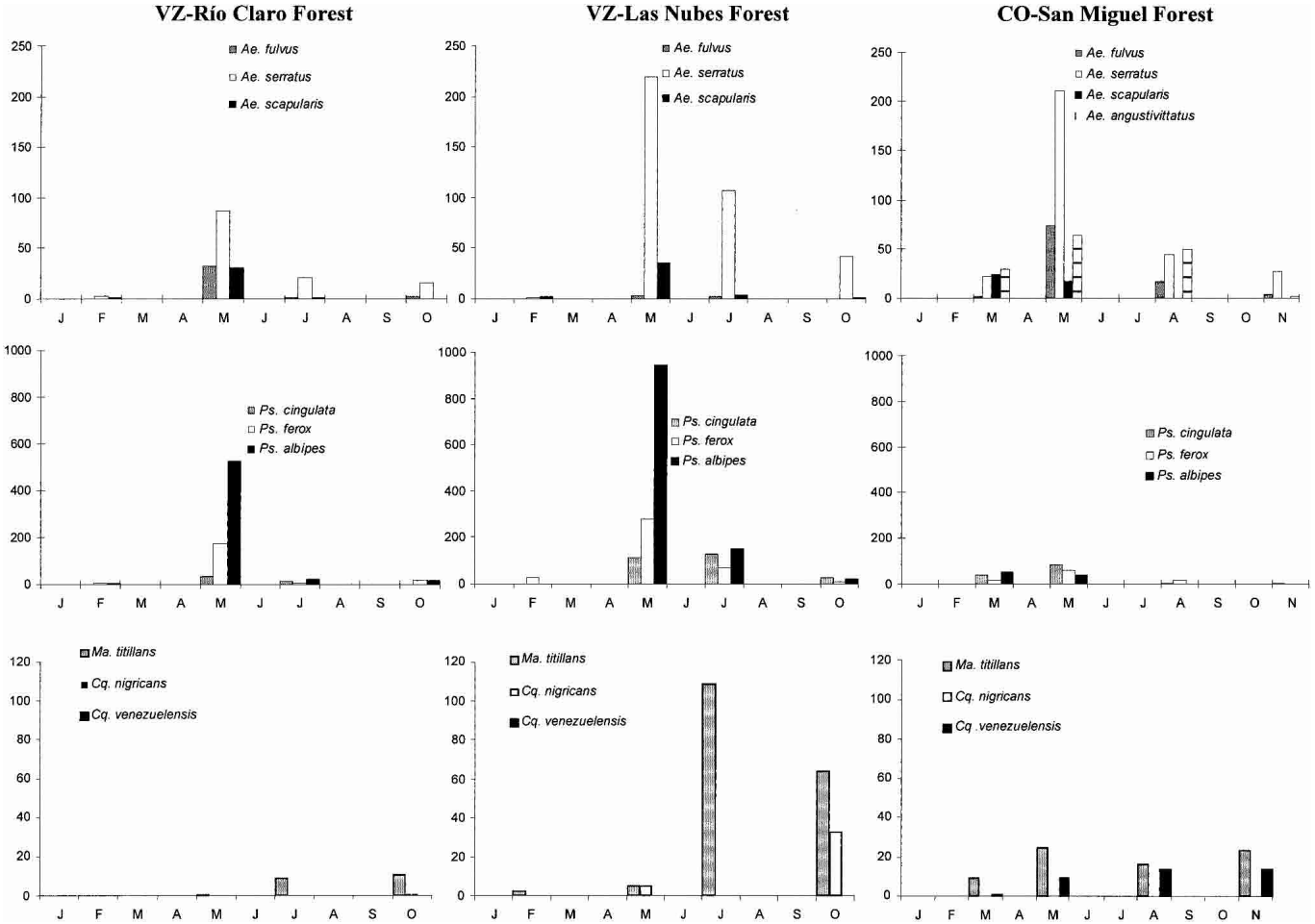
Mean mosquito abundance, species richness, and indices of evenness and diversity of collections from Centers for Disease Control miniature, light/CO_2-baited traps per 12-hour in the enzootic foci of Venezuelan equine encephalitis virus investigated in Colombia (San Miguel forest) and Venezuela (Río Claro and Las Nubes forests) during 1998*

Mosquito species	Río Claro forest		Las Nubes forest		San Miguel forest	
	Mean abundance	±SE	Mean abundance	±SE	Mean abundance	±SE
Ad. (Ady.) squamipennis	0.00	0.00	0.00	0.00	0.23	0.08
Ae. (Och.) angustivittatus	0.00	0.00	0.00	0.00	29.74	7.33
Ae. (Och.) fulvus	4.21	1.85	1.05	0.33	16.87	4.61
Ae. (Och.) hortator	1.56	0.31	0.03	0.02	0.01	0.01
Ae. (Och.) scapularis	3.33	1.91	9.64	3.22	8.07	3.37
Ae. (Och.) serratus	21.19	4.83	85.73	17.88	65.18	14.24
An. (Ano.) guarao	0.00	0.00	0.03	0.03	0.00	0.00
An. Rangeli/An. nuneztovari	0.00	0.00	3.46	1.37	0.00	0.00
Anopheles sp.	0.00	0.00	0.00	0.00	1.18	0.38
Cq. (Rhy.) juxtamansonia	0.04	0.04	0.39	0.20	0.00	0.00
Cq. (Rhy.) nigricans	0.37	0.31	8.88	4.17	0.00	0.00
Cq. (Rhy.) venezuelensis	0.00	0.00	0.00	0.00	11.64	2.65
Cx. (Ads.) accelerans	0.00	0.00	0.00	0.00	3.49	1.18
Cx. (Ads.) amazonensis	0.21	0.21	5.08	1.62	28.14	7.44
Cx. (Cux.) mollis	17.42	5.09	72.32	23.12	0.00	0.00
Cx. (Cux.) nigripalpus	84.02	31.41	337.78	83.26	152.94	29.86
Cx. (Cux.) sp.	24.25	11.27	33.14	11.06	0.00	0.00
Cx. (Cux.) sp4	0.00	0.00	0.14	0.14	0.00	0.00
Cx. (Mel) crybda	0.00	0.00	0.00	0.00	67.18	23.87
Cx. (Mel) spp	0.00	0.00	0.00	0.00	20.42	7.99
Cx. (Mel) vomerifer	0.00	0.00	0.00	0.00	58.12	13.37
Cx. (Mel.) caudelli	4.33	3.03	1.51	1.02	0.00	0.00
Cx. (Mel.) dunni	11.56	2.52	102.80	31.37	30.23	7.72
Cx. (Mel.) ocossa	0.40	0.13	2.34	0.77	4.48	2.08
Cx. (Mel.) pedroi	8.75	1.66	15.49	4.71	193.13	43.06
Cx. (Mel.) sp6	0.00	0.00	2.51	2.23	0.00	0.00
Cx. (Mel.) sp7	0.00	0.00	0.83	0.59	0.00	0.00
Cx. (Mel.) spissipes	13.69	2.76	65.29	14.82	189.40	38.30
Cx. (Mel) adamesi	0.00	0.00	0.00	0.00	13.52	3.15
Jb. longipes	0.00	0.00	0.00	0.00	1.60	0.34
Li. asulleptus	1.10	0.87	3.15	3.12	0.00	0.00
Li. durhami	0.79	0.17	0.46	0.16	0.00	0.00
Ma. (Man.) pseudotitillans	0.02	0.02	0.32	0.14	0.00	0.00
Ma. (Man.) titillans	6.63	3.05	44.93	23.88	14.71	4.17
Ps. (Gra.) cingulata	8.50	3.92	63.80	20.55	25.96	7.27
Ps. (Gra.) confinnis	0.12	0.12	0.08	0.04	2.59	1.33
Ps. (Jan.) albipes	65.40	30.42	251.34	80.25	22.36	5.31
Ps. (Jan.) ferox	24.56	11.44	88.17	22.07	18.04	5.77
Ps. (Pso.) cilipes	0.00	0.00	0.12	0.07	0.02	0.01
Ps. (Pso.) lineata	0.00	0.00	0.41	0.24	0.00	0.00
Ru. magna	0.00	0.00	0.02	0.02	0.00	0.00
Ur. (Ura.) geometrica	0.04	0.03	0.08	0.04	0.00	0.00
Uranotaenia sp1	0.00	0.00	0.00	0.00	2.54	1.13
Uranotaenia sp2	0.00	0.00	0.00	0.00	4.43	1.81
Uranotaenia sp3	0.00	0.00	0.00	0.00	0.91	0.27
Uranotaenia sp4	0.00	0.00	0.00	0.00	0.59	0.40
Uranotaenia sp5	0.00	0.00	0.00	0.00	0.02	0.01
Wyeomyia spp	0.00	0.00	0.00	0.00	1.97	0.67
Mean total	302.48		1201.32		989.70	
Species richness (species)	24		32		32	
Evenness index (0–1) ± CI 99%	0.48 ± 0.06		0.35 ± 0.02		0.45 ± 0.04	
Diversity index (species) ± CI 99%	11.55 ± 0.27		11.26 ± 0.11		14.53 ± 0.21	

* *Ad. = Aedomyia; Ae. = Aedes; An. = Anopheles; Cq. = Coquillettidia; Cx. = Culex; Jb. = Johnbelkinia; Li. = Limatus; Ma. = Mansonia; Ps. = Psorophora; Ru. = Runchomyia; Ur. = Uranotaenia; CI = confidence interval.*

albipes (Theobald)) that lowered the evenness. The following 13 species were common to all three forests, and accounted for most of the individuals (77–89%) in the captures: *Ae. serratus* (Theobald), *Ae. scapularis* (Rondani), *Ae. fulvus* (Wiedmann), *Cx. amazonensis* (Lutz), *Cx. nigripalpus*, *Cx. dunni* Dyar, *Cx. pedroi* Sirivanakarn & Belkin, *Cx. spissipes* (Theobald), *Ma. titillans* (Walker), *Ps. cingulata* (F.), *Ps. confinnis* (Lynch-Arribalzaga), *Ps. albipes*, and *Ps. ferox* (Humboldt).

The VZ-Las Nubes forest showed the highest mean total mosquito captures per trap interval (12-hour), followed by the CO-San Miguel and VZ-Río Claro forests (Table 2). Mosquito abundance also varied during the year, with greatest captures during May in most locations (Figure 4). May cor-


VZ-Río Claro Forest
VZ-Las Nubes Forest
CO-San Miguel Forest
Ae. fulvus
Ae. serratus
Ae. scapularis
Ae. angustivittatus
Ps. cingulata
Ps. ferox
Ps. albipes
Ma. titillans
Cq. nigricans
Cq. venezuelensis
J F M A M J J A S O
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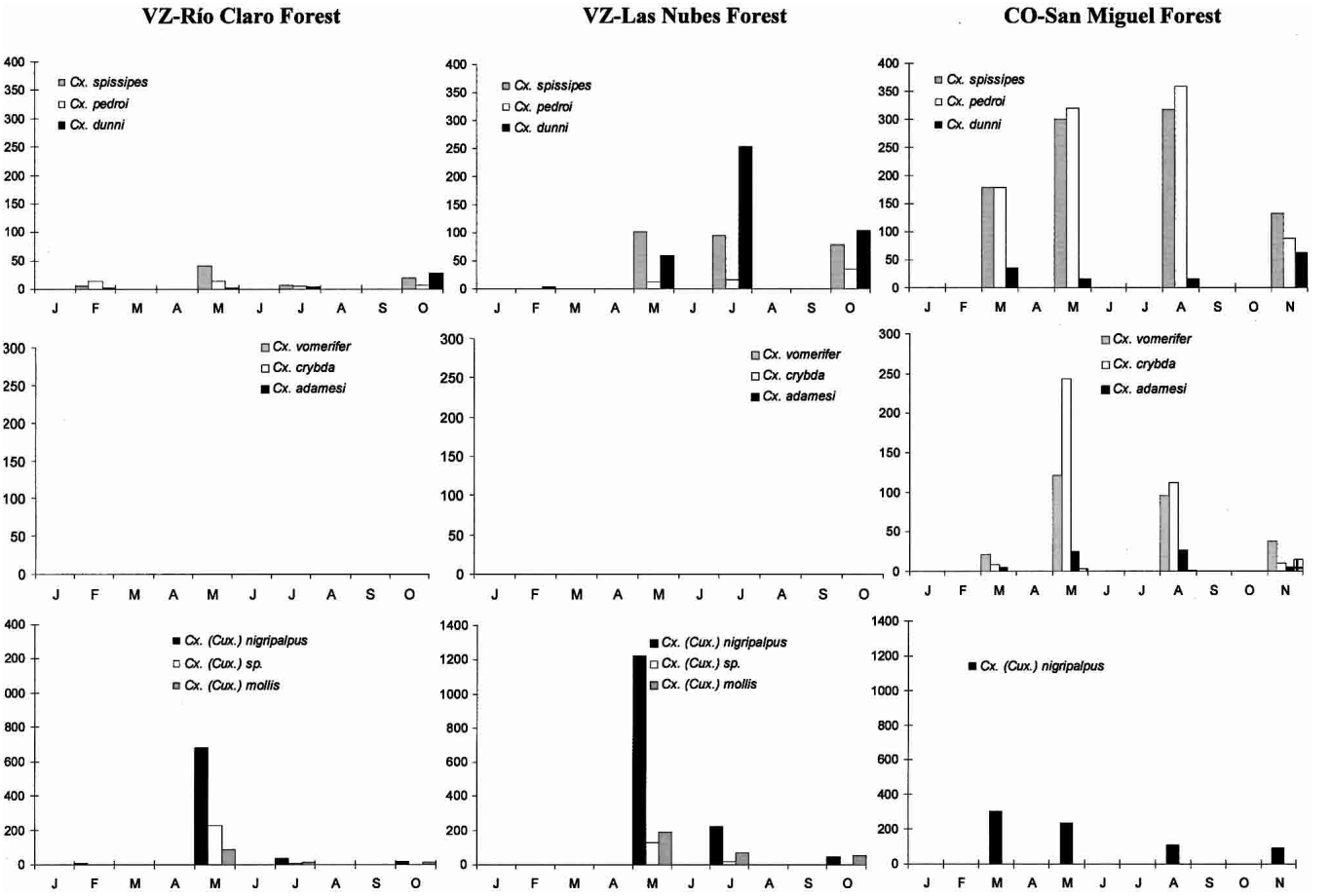
VZ-Río Claro Forest
VZ-Las Nubes Forest
CO-San Miguel Forest
Cx. spissipes
Cx. pedroi
Cx. dunni
Cx. vomerifer
Cx. crybda
Cx. adamesi
Cx. (Cux.) nigripalpus
Cx. (Cux.) sp.
Cx. (Cux.) mollis
J F M A M J J A S O
J F M A M J J A S O N

responds to the beginning of the rainy season, when ground pools are filled with water. The following species were most abundant in May: *Ae. serratus* (Theobald), *Ae. angustivittatus* Dyar & Knab, *Ae. fulvus*, *Ps. albipes*, *Ps. ferox*, *Ps. cingulata*, *Culex nigripalpus*, and other *Culex* (*Culex*) species (Figure 4). Mosquito captures were low in the drier months (February-March), although two *Culex* (*Melanoconion*) species (*Cx. spissipes, Cx. pedroi*) were relatively abundant in San Miguel (Colombia) at that time.

The most characteristic difference between mosquitoes of the Magdalena (Colombia) and Catatumbo (Venezuela) study sites was the richer fauna of *Culex* (*Melanoconion*) species in the former forest (Table 2 and Figure 4). Particularly abundant *Melanoconion* species were *Cx. dunni* and *Cx. spissipes* in all forests, and *Cx. crybda* Dyar, *Cx. vomerifer* Komp, *Cx. pedroi*, and *Cx. adamesi* Sirivanakarn & Galindo in Colombia. Those species were common in CO-San Miguel year round and in Venezuela these species were abundant during the rainy season.

Very few mosquitoes were collected during the day in the open pasture areas outside the forests (Figure 5), including only a few specimens of *Ae. scapularis* and *Cx. nigripalpus* in Venezuela. The most diurnal activity of mosquitoes was observed inside VZ-Las Nubes forest, where *Ps. albipes*, *Ps. ferox*, *Ae. serratus*, and *Cx. nigripalpus* where abundant (Figure 5). A few *Culex* (*Melanoconion*) spp. mosquitoes were also collected in the Venezuelan sites during the day. In general, there was more diurnal mosquito activity in Venezuela. However, the most abundant diurnal mosquitoes (*Ps. ferox*, *Ps. albipes,* and *Ae. serratus*) have not been implicated in enzootic VEE virus transmission cycles. The diurnal activity of *Culex* (*Melanoconion*) spp., including the probable enzootic vectors, was very low diurnally in both countries compared with the nocturnal activity of these species.

Captures at night in the open pasture areas in Venezuela were mainly represented by *Cx. nigripalpus*, *Ps. cingulata*, and *Cx. mollis* Dyar & Knab, with a few specimens of *Cx. spissipes*, *Cx. dunni*, *Ae. serratus*, *Ae. scapularis*, *Ps. ferox*, and *Ps. albipes* (Figure 6). In Colombia, *Cx. nigripalpus*, *Ae. angustivittatus*, *Ps. cingulata*, and *Ma. titillans* were the most abundant species in the open area at night, followed in abundance by *Ps. confinnis*, *Cx. pedroi*, *Cx. adamesi*, and *Cx. spissipes* (Figure 6). Although every mosquito species captured in the open was also captured in the ecotone and/or inside the forest, the following species were more abundant outside the forests: *Ps. cingulata*, *Ps. confinnis*, *Ma. titillans*, *Cq. nigricans*, *Cx. mollis*, *Cx. ocossa* Dyar & Knab, *Ae. angustivittatus*, and *Anopheles* spp.

At all three study sites, mosquitoes were generally more abundant at night and deeper inside the forest. The CO-San Miguel forest exhibited the greatest abundance of nocturnal mosquitoes, mostly represented by *Culex* (*Melanoconion*) spp. and *Cx. nigripalpus* (Figure 6). The main *Melanoconion* species were well represented in the forest ecotone and inside the forest. The dispersal pattern of *Cx. nigripalpus* varied with locality; it was uniformly abundant along the transect in VZ-Las Nubes, more abundant in the ecotone in CO-San Miguel, and more abundant inside the forest in Río Claro (Figure 6).

Mammal captures. Captures of small mammals in CO-San Miguel (Colombia) were more numerous (58 animals) than in Río Claro (Venezuela; no captures) or VZ-Las Nubes (Venezuela; four animals). Capture efficiency in the Colombian study site was 3.1%, and only 0.1% in the combined Venezuelan sites. Occasional checks of traps in the evening indicated that nearly all mammals were trapped during the night. All animals collected in Venezuela were *Didelphis marsupialis* (opossum), and all were negative for VEE virus and antibodies. Most mammals captured in Colombia were *Proechimys* sp. (spiny rats; 48 of 58), and 22 tested positive for antibodies to VEE virus (HI titer $\geq$ 1:40) but none for virus. An unidentified rodent also was positive for antibodies to VEE virus, which together with the positive *Proechimys*, gives an overall seroprevalence in the Colombian mammals of 40%. Other species captured in CO-Monte San Miguel, but with no antibodies, were *Didelphis* sp. marsupials and an unidentified rodent.

DISCUSSION

Our results showed dramatic differences in VEE virus isolation rates (four VEE virus isolations from 467 sentinel hamsters in Venezuela versus 16 of 79 in Colombia), mosquito species composition, and seropositive mammals between the Colombian and Venezuelan study sites. Venezuelan equine encephalitis virus was isolated on each field trip made to the CO-San Miguel forest (Table 1), even during the driest part of the year (Figure 3), but was isolated during only one of eight sampling trips in Venezuela. Similar differences have been noted in surveillance conducted since this study was completed (Barrera R, Ferro C, unpublished data). Also relevant is the smaller number of hamsters exposed on each occasion in CO-San Miguel forest compared with the Venezuelan forests. The relatively high VEE virus isolation rates in CO-San Miguel forest are consistent with observations made during the 1970s,[14] indicating the ease with which enzootic VEE virus could be isolated from forests in the middle Magdalena Valley of Colombia. The low rates of VEE virus isolation in the forests of the Catatumbo River basin in Venezuela are consistent with previous studies in the same area.[15,16]

The higher isolation rates of enzootic VEE virus in CO-San Miguel are associated with a more abundant fauna of *Proechimys* spp. rodents, and with a more diverse and abundant fauna of potential *Culex* (*Melanoconion*) mosquito vectors. The abundance of non-immune reservoir hosts (*Oryzomys* spp. rodents) has been shown to be associated with the level of virus (Mucambo) circulation in lowland tropical forests near Belem, Brazil.[31,32] More detailed ecologic studies are needed to understand why the CO-San Miguel forest is richer in rodents and potential vectors than the Venezuelan forests. In spite of the similar landscapes and land use of the two areas, there may be relevant differences in soil and plant species composition associated with a larger rodent population in the Colombian forest. The only detected differences between the two areas were the larger forest area and water surface around the CO-San Miguel study site (Figure 2). Forest fragmentation due to cattle raising in the Venezuelan

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FIGURE 4. Mean number of mosquitoes per trap/12-hour period collected during the months of study in 1998 from Río Claro, Las Nubes (Venezuela), and San Miguel (Colombia) forests. *Ae.* = *Aedes*; *Ps.* = *Psorophora*; *Ma.* = *Mansonia*; *Cq.* = *Coquillettidia*; *Co.* = ; *Cx.* = *Culex*.

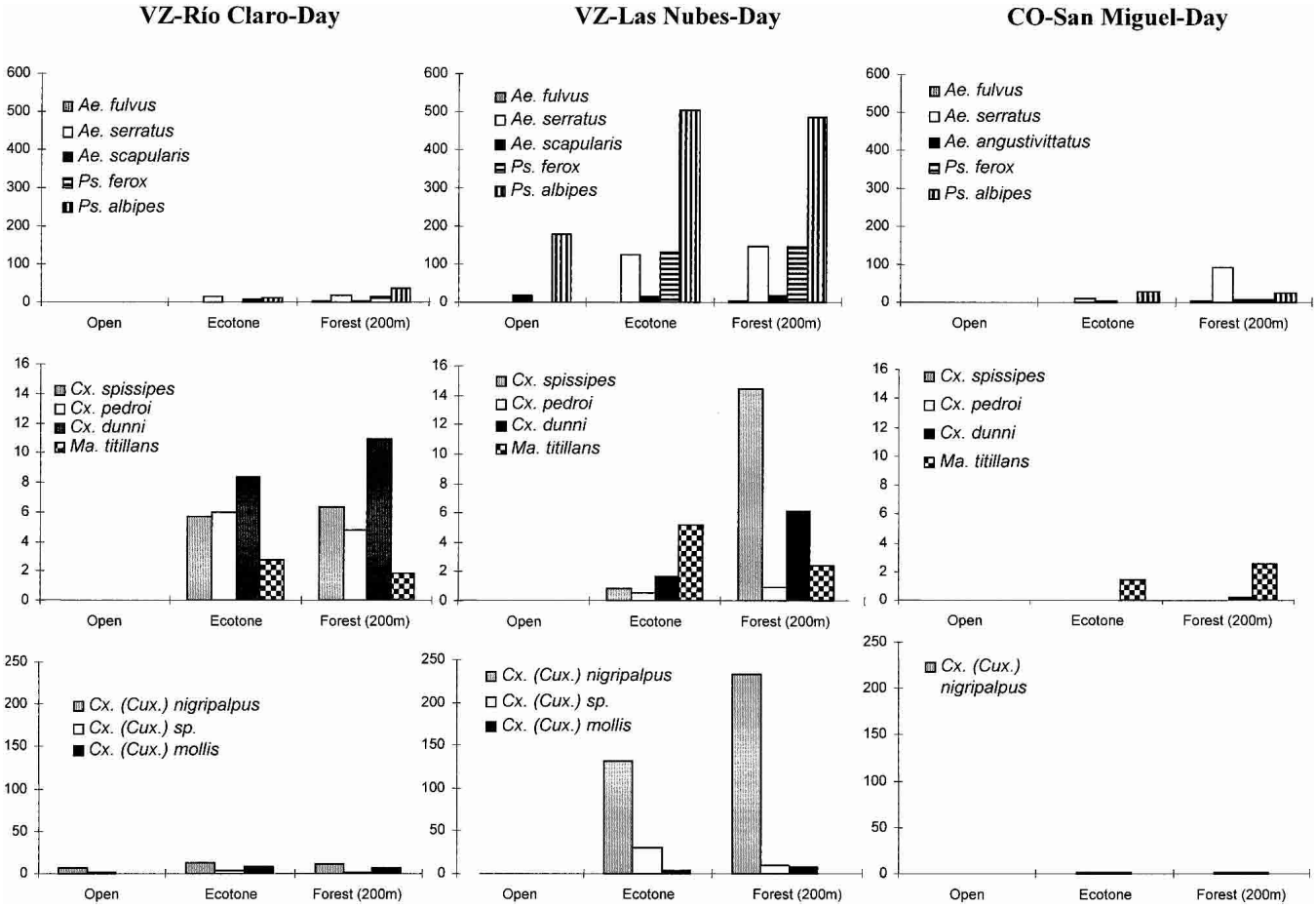


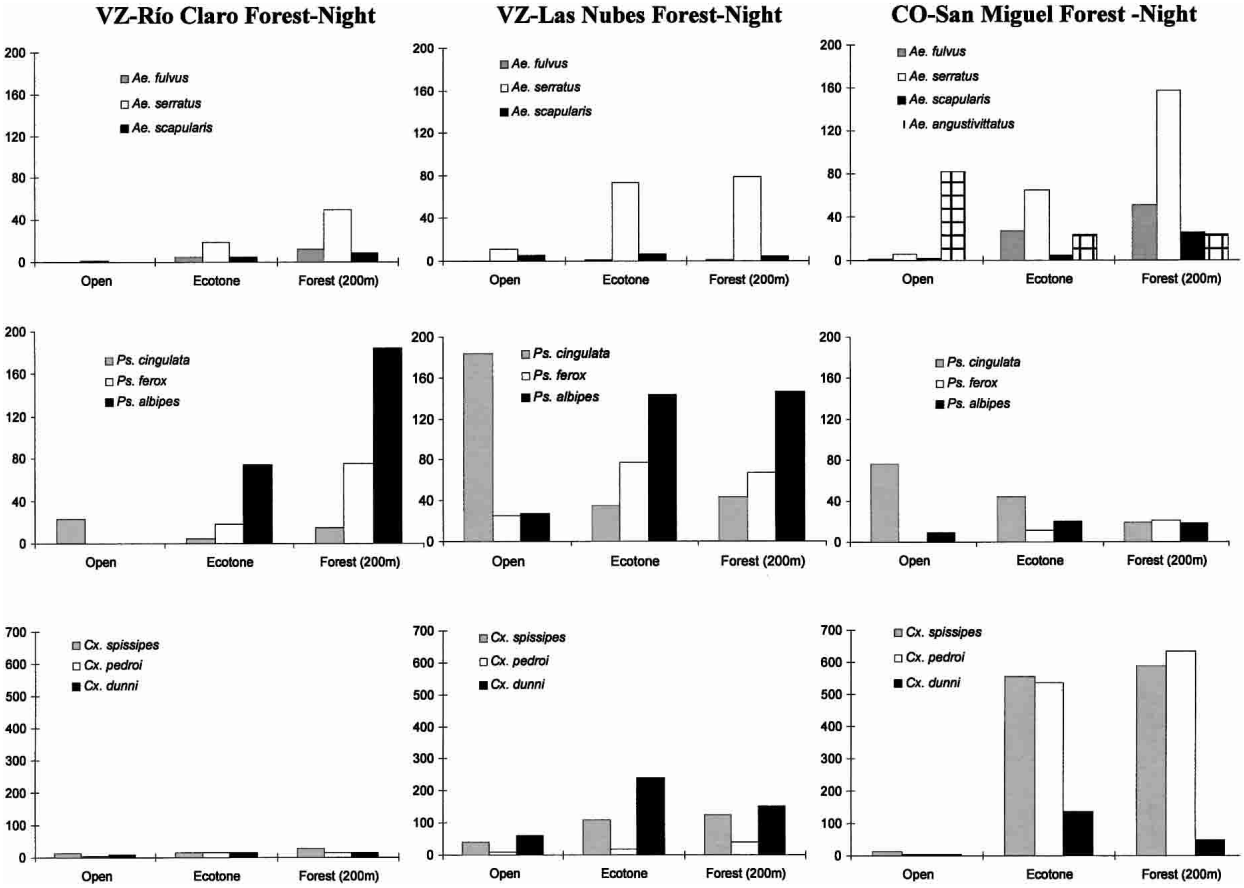
FIGURE 5. Mean number of more frequent mosquito species collected during the day (6:00 AM to 6:00 PM) along transects from the open area (200 meters) around the forest in the ecotone (0–10 meters within the forest), and inside the forest (200 meters) in Río Claro, Las Nubes (Venezuela), and San Miguel (Colombia) forests. For definition of abbreviations, see Figure 4.

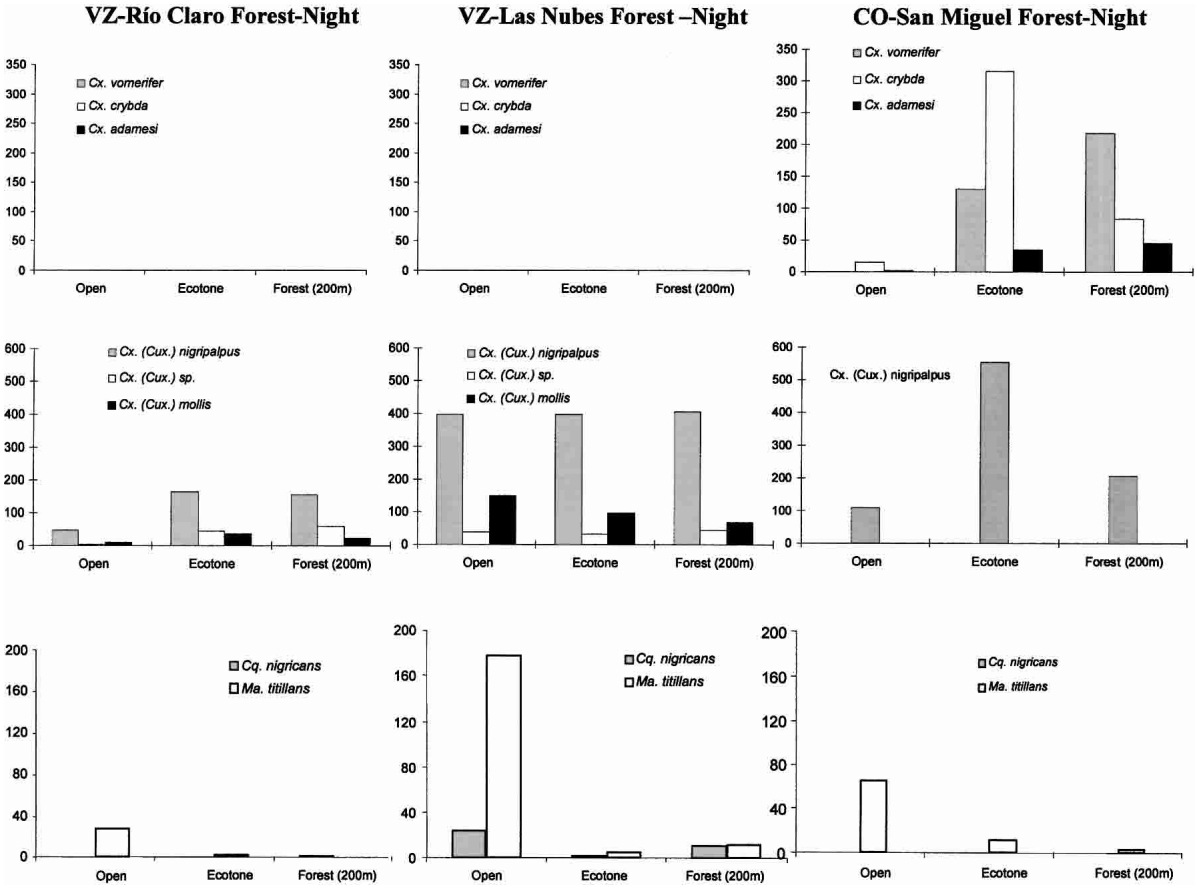
Catatumbo area has led to smaller forest stands. These data suggest that a larger forest fragment size in Colombia may increase the likelihood that reservoir mammal and/or vector mosquito faunas persist by recolonizing habitat patches when local populations become extinct. The larger size of the Colombian forests and the closer spatial proximity of forest patches could effectively maintain a higher diversity and abundance of hosts, mosquitoes and viruses than in the more sparse remnant forest of the Venezuelan Catatumbo.

It is interesting to note that although a similar enzootic ID VEE virus circulates in the Colombian and Venezuelan forests (350 km apart), a different, co-circulating genetic variant is also present in the forests of the Venezuelan Catatumbo.[13] Thus, the forests with a scarcer host and vector fauna, where VEE virus is more difficult to isolate, exhibited a greater genetic diversity of viruses than the Colombian forest where VEE virus circulates at higher levels. This could reflect greater fragmentation of the virus populations in the Venezuelan Catatumbo, providing more opportunities for allopatric divergence of virus lineages in different foci of transmission. One of the ID VEE virus variants present in the Catatumbo area is more closely associated with the subtype IC VEE virus that emerged in 1992 in nearby western Venezuela.[8,10]

The CO-San Miguel forest exhibited higher mosquito diversity than any of the Catatumbo forests, but particularly it showed a richer fauna of *Culex* (*Melanoconion*) species (Table 2). Several *Melanoconion* species were present in CO-San Miguel but absent in the Catatumbo forests (*Cx. vomerifer*, *Cx. crybda*, *Cx. adamesi*), and among the species common to both forests, *Cx. pedroi*, *Cx. ocossa*, and *Cx. spissipes* were more abundant in CO-San Miguel. Numerous studies point out that enzootic transmission of VEE virus involves *Culex* (*Mel.*) species vectors.[5] This virus has been recovered from *Cx.* (*Mel.*) *vomerifer*, *Cx.* (*Mel.*) *pedroi*, and *Cx.* (*Mel.*) *adamesi* in CO-San Miguel forest (Ferro C, unpublished data) and from *Cx.* (*Mel.*) *ferreri* Duret (more likely *spissipes*) and *Ae. fulvus* in the Catatumbo forests.[16] A mixture of *Cx. gnomatos* Sallum, Hutchings & Faria and *Cx. vomerifer* was found infected with VEE virus in a forest near Iquitos, Peru.[33]

FIGURE 6. Mean number of more frequent mosquito species collected at night (6:00 PM to 6:00 AM) along transects from the open area (200 meters) around the forest, in the ecotone (0–10 meters within the forest), and inside the forest (200 meters) in Río Claro, Las Nubes (Venezuela), and San Miguel (Colombia) forests. For definitions of abbreviations, see Figure 4.


VZ-Río Claro Forest-Night
VZ-Las Nubes Forest-Night
CO-San Miguel Forest -Night
Ae. fulvus
Ae. serratus
Ae. scapularis
Ae. angustivittatus
Ps. cingulata
Ps. ferox
Ps. albipes
Cx. spissipes
Cx. pedroi
Cx. dunni
Open
Ecotone
Forest (200m)


VZ-Río Claro Forest-Night
VZ-Las Nubes Forest –Night
CO-San Miguel Forest-Night
Cx. vomerifer
Cx. crybda
Cx. adamesi
Cx. (Cux.) nigripalpus
Cx. (Cux.) sp.
Cx. (Cux.) mollis
Cq. nigricans
Ma. titillans
Open
Ecotone
Forest (200m)

That mixture of mosquitoes was also demonstrated to transmit VEE virus experimentally.[33] However, the titers of virus in the viremic hamsters used in those infection studies are far higher than those reported after experimental infection of natural reservoir hosts.[34,35] Our results suggest that the greater abundance, diversity, and constancy of suspected enzootic vectors (subgenus *Melanoconion* species) in CO-San Miguel site partly explain the regularity and higher rates of VEE virus isolations. It is currently difficult to explain the contrasting differences in mosquito fauna between the Colombian and Venezuelan forests, particularly because little is known about the immature, aquatic habitats of many of the *Melanoconion* species.

It was surprising that in Venezuela, the only documented VEE virus transmission (sentinel hamster isolates; see Table 1) occurred during the dry season in February rather than during the rainy season. These VEE virus isolates occurred during periods of low mosquito density, as measured by our trap counts. Although these results could represent sampling variance, there could be greater transmission in Venezuela for several reasons. 1) Mosquito infection rates may peak during the dry season because of continued circulation by smaller numbers of vectors when larval habitats disappear and adult survival may decrease. Transovarial transmission, not demonstrated for VEE virus, could enhance dry season virus maintenance, 2) The average age of adult mosquitoes and rates of mosquito parity may be higher in Venezuela during the dry season, resulting in more transmission. 3) The breeding seasonality of the *Proechimys* spp. reservoir hosts may result in the presence of more non-immune individuals during the dry season, resulting in greater amplification of VEE virus. 4) Our data indicate that in the Venezuelan sites, circulation may not be continuous and reintroduction may occur periodically. Perhaps reintroduction is more common during the dry season due to dispersal patterns of vectors or mobile hosts such as bats or birds.

Because epizootic VEE virus may be derived from enzootic ID strains,[9,11] we have emphasized studying the mosquito fauna along transects from forests to open areas in an attempt to understand how viruses may leave the enzootic foci.[12,36] Candidate mosquito species to export enzootic or epizootic VEE virus mutants out of the enzootic foci should exhibit the following criteria: 1) abundance in both open areas and forests, 2) a wide range of vertebrate host contacts, 3) ability to disperse over long distances, and 4) competent vectors of enzootic and/or epizootic VEE virus. The most common mosquito species captured in open areas and in the forests in this study were *Ae. angustivittatus* (only in Colombia), *Ps. cingulata*, *Cx. nigripalpus*, and *Ma. titillans*. *Aedes angustivittatus* is a species that breeds in small, temporary ground pools in open areas, and from which only epizootic VEE virus has been isolated in Colombia.[37] We are not aware of previous isolations of VEE virus from *Ps. cingulata*, although this species can range across a wide variety of habitats[38–41] *Culex nigripalpus* and *Ma. titillans* seem to comply with the above criteria for all three forests.[33,42–46] These species are also common in the forest and surrounding open areas in another enzootic foci of VEE virus in northern Venezuela.[36] Future studies should investigate the movement of VEE virus away from enzootic foci into open areas, and its interaction with equines and other potential amplifying hosts.

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