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EPIDEMIOLOGIC STUDIES OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN ALMIRANTE, PANAMA[1]

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Grayson, M. A. and P. Galindo (Gorgas Memorial Laboratory, Panama, Republic of Panama). Epidemiologic studies of Venezuelan equine encephalitis virus in Almirante, Panama. *Amer. J. Epid.*, 1968, 88: 80–96.—Forty-three isolations of Venezuelan equine encephalitis (VEE) virus were made from specimens collected or exposed in a tropical rainforest area of Panama during 1961 and 1962. Six isolates were recovered from febrile patients, seven from field rodents and nine from at least six species of wild birds. Twelve strains of VEE virus were obtained from at least four species of mosquitoes, and nine litters of sentinel mice exposed to the bites of bloodsucking insects also yielded the virus.

Serologic evidence of VEE virus activity in the area before 1961 was acquired. Antibodies to VEE virus were detected in 25 species of vertebrates including humans, equines, bovines, canines and domestic fowl as well as several species of wild mammals, birds and reptiles. Human infections with VEE virus were widespread, occurred nearly uniformly in both sexes and were associated with length and place of residence in Almirante.

The accumulated evidence suggests that VEE is endemic in Almirante, Panama; that rodents, especially the cotton rat (*Sigmodon hispidus*), are important reservoirs; and that *Culex* (*Melanoconion*) mosquitoes, in particular, *C.* (*M.*) *taeniopus*, are the most efficient vectors of this virus in the area.

Introduction

The virus of Venezuelan equine encephalitis (VEE) was first isolated by Beck and Wyckoff during investigations of an equine epizootic which occurred in Venezuela between 1936 and 1938 (1). Since then the virus has been reported from Trinidad, Ecuador, Colombia, Brazil, Mexico and the United States (2–7).

In Panama, VEE virus was first recovered in April, 1961 from a fatal human case in Cañito, a small rural com-

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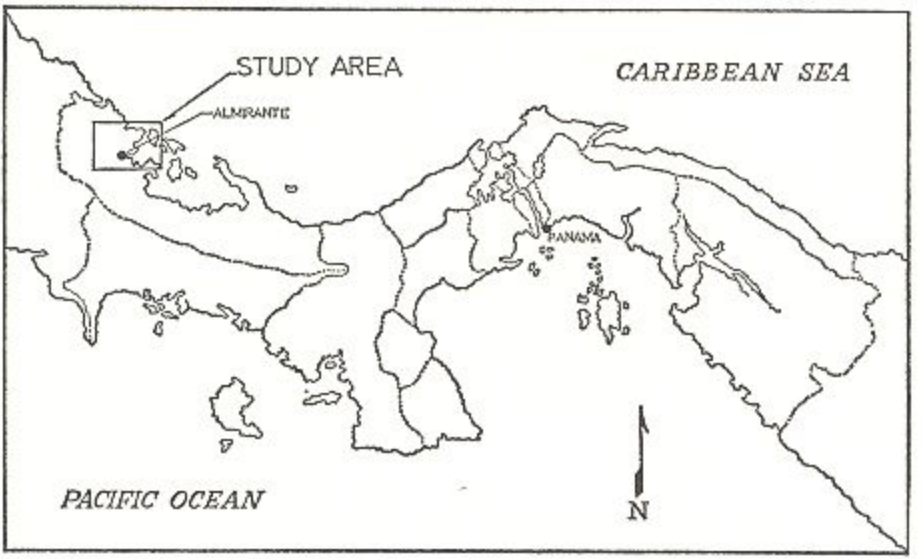

FIGURE 1. Map of the Republic of Panama showing location of study area.

munity some 20 miles northwest of Panama City (8). Shortly thereafter, the virus was detected in a tropical rainforest area near the Atlantic seacoast town of Almirante, where an extensive survey for arboviruses had been underway since September, 1959 (9).

The results of studies relating to the epidemiology of VEE virus in the vicinity of Almirante, Panama from September, 1959 to September, 1962 comprise the subject of this report.

Description of the study area

The study area, which covers a territory of some 620 square miles extending from sea level to 6,000 feet in elevation, is located in the province of Bocas del Toro in northwestern Panama (figure 1). It is bordered on the south and west by a series of complex forested ridges forming the Continental Divide, which at this point rises to considerable heights with several peaks of over 9,000 feet above sea level. To the north, the area is bounded by the Changuinola River and to the east by the Caribbean Sea. The hydrography of the area is dominated by the Changuinola River and its numerous tributaries. During periods of heavy rainfall, a considerable amount of water is spilled into the flood plains of these streams, forming the complex of ox-bow lakes and freshwater swamps which dominate the landscape of the lowlands. Part of this swampy land has been reclaimed for grazing pastures and for the cultivation of bananas and cacao.

According to available climatological information, a tropical rainforest climate prevails in this region which is covered by large tracts of broad-leaf evergreen forest. Within this almost continuous cover of evergreen forest, special orographic, hydrographic and edaphic factors have influenced the development of several distinct ecological associations of which the main categories recognized in the study area are: lowland tropical rainforest, upland tropical rainforest, cloud forest and open

freshwater marsh. In addition, human activity has produced a variety of domestic and peridomestic habitats in the region.

Field stations for the collection of arthropods and wild vertebrates and for the exposure of sentinel animals were established at ten separate localities representing the major types of ecological associations in the study area. A detailed description of the study area and collecting stations is presented by Galindo et al. (9).

Demography

According to the 1960 census of the Republic of Panama, the study area has a total of 2,921 dwellings inhabited by 11,452 persons (10). These individuals are distributed in two main population centers, namely, Changuinola and Almirante, which have very distinct demographic characteristics. Changuinola, a rural community, is inhabited largely by Indians of the Guamí tribe with a small percentage of the population consisting of Negroes, Caucasians and Cuna Indians. Almirante, a deep seaport on the Caribbean, is used almost exclusively by the Chiriquí Land Company for the shipment of bananas and cacao. Most of the inhabitants are Negroes of West Indian origin engaged in activities related to the export of fruit, although a few Caucasians are employed in administrative positions by the company.

The bulk of our epidemiologic studies were conducted in the town of Almirante and its environs (figure 2). In 1960 the population consisted of 4,920 persons distributed in 1,188 dwellings grouped in an urban community scarcely one mile long and a quarter of a mile wide, with a few scattered farmhouses on the outskirts of town. Almirante is bordered on the north by swamp forests and open marshes, on the east by the Chiriquí Lagoon, on the south by Almirante Bay and on the west by the slopes of Risco Ridge, part of the complex of hills and mountains constituting the Continental Divide. The town is divided approxi-

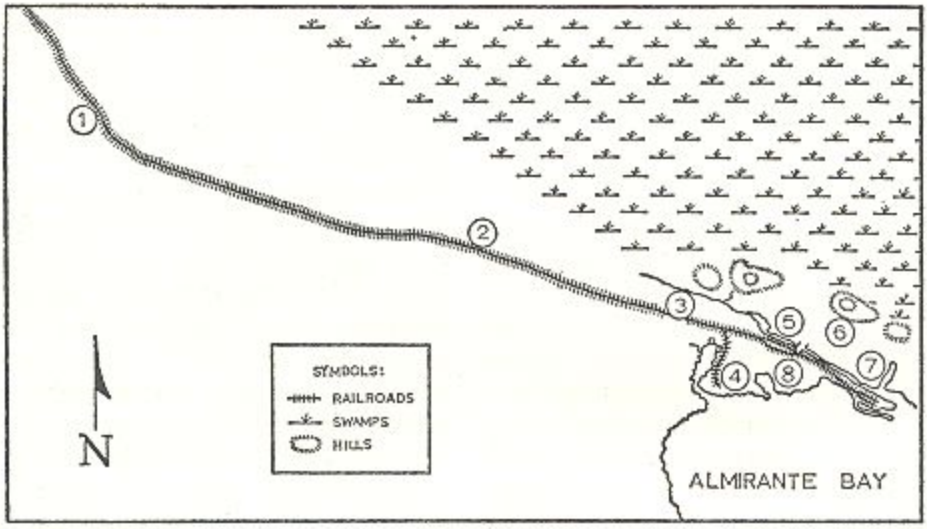

FIGURE 2. Almirante collecting area showing location of field headquarters and sectors of town: 1) field headquarters; 2) One-mile; 3) Half-mile; 4) Zegla; 5) Almirante; 6) Tampico; 7) Patoistown; 8) company compound.

mately north and south by the Chiriquí Land Company railroad which is accompanied part of the way by a slough of dark foul water called Quebrada del Cedro. The southern part of town, built against Almirante Bay, comprises Zegla and the company compound, which includes recreational facilities, housing for administrative personnel and a hospital with capacity for 200 bed-patients. Sanitary conditions in this area are good, with mosquito-proof screening, chlorinated running water and toilet facilities in all dwellings. The houses, which are built of wood with galvanized iron roofs, are elevated from the ground and usually shaded by luxuriant coconut palms. The northern part of town, bordering on extensive swamplands, is divided into five sectors known from west to east as One-mile, Half-mile, Almirante, Tampico and Patoistown. Houses in the first three sectors, even though of lower grade than those in Zegla, have satisfactory sanitary conditions. Tampico and Patoistown, however, with 160 houses and a population of 720 persons (1961), are veritable slums. Dwellings are constructed of very low grade lumber over swampy terrain, with no screening, electricity, private water supply or even pit latrines.

Materials and methods

The following methods were used to detect VEE virus infections in the study area: inoculation of field-collected materials for virus isolation attempts in mice; exposure of sentinel animals to the bites of hematophagous insects, with subsequent attempts to recover virus; and serum surveys of vertebrates for antibodies to VEE and related arboviruses.

Field techniques

Blood samples from humans with fever of unknown origin were obtained from patients reporting sick at the Almirante Hospital and from field personnel engaged in the collection of arthropods. Blood and tissue specimens were obtained from wild vertebrates captured by baited live-traps, mist nets or shooting. Recently dead, moribund or healthy animals were bled from the heart or jugular vein and samples of internal tissue (usually liver, spleen and kidney) were removed from sacrificed specimens. Aseptic techniques were employed for the collection of all blood and tissue specimens which were refrigerated and shipped twice a week by air freight to the laboratory in Panama City. Skins and/or skulls were prepared for those vertebrates not identified by the field technician or field ecologist and submitted to acknowledged authorities for definitive identification (9).

Diurnal and nocturnal collections of bloodsucking insects were made from a number of different habitats on the ground and in the forest canopy using various methods which included traps, attractants and hand capture from natural resting places. Engorged females were held at ambient temperatures for at least 24 hours. Live insects, placed in jars packed in wet ice, were shipped twice a week by air freight to the central laboratory where they were identified in lots according to species or species-group.

Four to six litters of suckling mice with mothers were exposed once a week for 9 to 12 hours overnight at specified stations in the study area and returned the following day to the laboratory for observation.

Human sera for antibody studies were

obtained in three successive yearly bleedings conducted in 1960, 1961 and 1962 from hospital patients, school children and other residents of Almirante. Domestic animals in the vicinity of Almirante and Changuinola were bled for these studies in August, 1961. Wild mammals, birds and reptiles from various localities in the region were sampled throughout the three-year study period.

Details of the field techniques utilized in this study are given elsewhere (9).

Laboratory techniques

Virus isolation attempts. All virus isolation attempts from field-collected specimens were made in Swiss white mice, two to four days of age, using 0.02 ml volumes of inoculum and the combined intracerebral (i.c.) and intraperitoneal (i.p.) routes of injection. Rabbit serum saline (RSS) solution was employed for processing and diluting all field materials. Sterilization of the diluent, which contained 10 per cent normal rabbit serum previously heated at 56 C for 30 minutes, was accomplished by means of vacuum filtration through a millipore filter with pore size of 450 mμ. Penicillin and streptomycin were added prior to use in final concentrations of 1,000 units and 4,000 μg, respectively, per ml.

Heparinized or clotted blood specimens, which were allowed to retract in the cold, were centrifuged at 1,500 rpm for 10 minutes at 4 C. The separated plasma or serum was usually clarified by spinning an additional five minutes at the same velocity. Sera from acutely ill febrile humans were injected without dilution. Serum or plasma specimens from domestic and wild animals were routinely diluted with an equal volume of RSS before injection.

Tissue samples from the same animal were usually pooled, triturated and suspended in diluent to 10 per cent (w/v). Clarification was carried out in the cold at 4,000 rpm for one hour. The supernatant liquid was separated and injected without further dilution.

Bloodsucking insects were usually pooled by species or species-group and triturated with 1 to 2 ml of diluent. During the first two years of study, the number of insects in each pool ranged from 1 to 150 and centrifugation of suspensions was carried out in the cold at 2,000 rpm for 30 minutes. In the third year, most mosquito pools contained from 30 to 60 specimens and suspensions were clarified at 4,000 rpm for 60 minutes. The undiluted supernatant fluid was employed for virus isolation attempts except in the case of proved toxic suspensions when the supernate was diluted with one to three volumes of RSS.

Sentinel mice, as well as those injected with field-collected materials, were kept under observation for a period of 15 days during which time mice exhibiting signs of illness were sacrificed for serial passage. The brains of sick, moribund or recently dead mice were harvested aseptically, weighed and triturated with nine volumes of RSS. The resulting 10 per cent (w/v) suspension was centrifuged at 2,000 rpm for 15 minutes in the cold. The supernatant liquid was then inoculated i.c. in suckling and/or weanling mice, using 0.02 ml of inoculum per mouse. Tests for sterility of passage materials were performed routinely, using blood agar medium.

Reference viruses. Locally isolated strains were employed as reference viruses in this study. Dr. Pauline Peralta of the Middle America Research Unit (MARU) furnished us with VEE (№3880) and Una (BT 1495–3) viruses

whereas the eastern equine encephalitis (EEE #3847) agent was obtained from the Panama Veterinary Laboratory through the courtesy of Dr. Chester Gleiser.

Reagents. Stock virus pools for both test and reference strains were prepared from infected suckling mice. The brains of sick or moribund animals were triturated with RSS to make a 20 per cent (w/v) suspension which was centrifuged at 10,000 rpm for one hour in the cold. The separated supernatant liquid constituted the stock virus suspension.

Crude antigens were prepared from the brains of infected suckling mice, using slightly modified techniques of Clarke and Casals (11). Hemagglutinins were made from tissue triturated with four volumes of borate-saline, pH 9.3. The resulting 20 per cent (w/v) suspensions were incubated at 4 C approximately 48 hours prior to centrifugation. In the case of complement-fixing antigens, homogenization was carried out at pH 9.0 and suspensions were refrigerated for about 24 hours before clarification. Protamine sulfate treatment was not employed. Antigens for suspected VEE virus isolates were prepared from second to fourth passage mouse brains. Reference antigens were made from brains of infected mice at the sixth to tenth passage level. Tissue control antigens for use in complement-fixation (CF) tests were prepared from the brains of normal suckling mice after several serial blind passages.

Immune sera for test and reference strains of VEE virus were prepared as follows: Swiss albino mice, seven to eight weeks of age, were injected i.p. with 0.2 ml each of a 10 per cent (w/v) suspension of infected suckling mouse brain in normal saline solution containing 0.3 per cent formalin, the inoculum previously incubated for approximately 24 hours at 4 C. Specific immune serum for use in CF identification procedures was obtained from mice bled from the heart 10 to 12 days later. Antisera used in cross neutralization (N) tests were prepared in mice given three i.p. injections of virus administered 10 days apart. The first inoculation, employing formalin-treated virus, was carried out as described above. Inocula for the second and third injections consisted of 10 per cent (w/v) infected suckling mouse brain in physiological saline solution administered in 0.2 ml doses. Mice were bled out for immune serum by cardiac puncture 10 to 12 days after the third injection. Single and multiple injection sera for use as normal controls in CF and N tests, respectively, were obtained from mice treated as described above with the exception that inocula were prepared from the brains of normal, rather than infected, suckling mice.

All reagents were tested for sterility on blood agar medium prior to storage at −20 C or −65 C.

Serologic tests. Hemagglutination-inhibition (HI) tests were performed in lucite plates according to the techniques described by Clarke and Casals (11). All sera were treated with kaolin for the removal of non-specific inhibitors. Tests were incubated and read at room temperature after the addition of goose erythrocytes. Sera reacting with eight units of viral antigen at dilutions of 1/20 or greater were considered positive. Sera inhibiting more than one hemagglutinin were considered positive only for the antigen reacting at the highest serum dilution. HI antibody titers were adjusted, when necessary, to those expected with eight units of antigen.

A modification of the CF technique described by Kerr (12) was used in

these studies. Veronal buffered saline solution was employed as diluent (13). Two exact units of complement were used. Sheep cell suspensions were adjusted spectrophotometrically to contain approximately 5×10^8 erythrocytes per ml. Sensitization was obtained by incubation with an equal volume of hemolysin containing two to four units for 15 minutes at room temperature. All sera were inactivated in fourfold dilution at 56 C for 30 minutes. Grid titrations were utilized for the identification of virus isolates, titers being expressed as the reciprocal of the highest serum or antigen dilution giving three or four plus fixation of complement with any dilution of antigen or serum, respectively. Paired human sera were tested with two to four units of VEE antigen. In addition to the usual anticomplementary controls, all sera and antigens were tested for non-specific reactivity. Appropriate hemolytic, cell and complement controls were also included in each test.

N tests, performed in weanling mice three to four weeks of age by the extranecural route of inoculation, were executed according to the method of Lennette and Koprowski (14) with some modifications. All sera were inactivated at 56 C for 30 minutes. Each serum-virus mixture was inoculated, without prior incubation, in six mice by the i.p. route of injection, employing 0.02 ml of inoculum per mouse. Stock virus suspensions were routinely diluted in RSS. The constant-serum varying-virus dilution technique, in which serial tenfold dilutions of virus were mixed with equal volumes of undiluted sera, was employed in all virus identification procedures. This technique was also utilized for the detection of antibody conversions in human infections. Tests of paired human sera were conducted simultaneously, using normal rabbit serum for the control virus titration. Differences of more than one log in N indices of sera under comparison were considered significant. In surveys of vertebrates for neutralizing antibodies to VEE virus, undiluted sera were screened for ability to neutralize approximately 100 median lethal doses (LD_{50}) of virus, the doses in these tests ranging from 25 to 316. Control virus titrations were performed in the presence of normal rabbit serum. Test sera protecting 6/6 or 5/6 mice were considered positive; 4/6, 3/6 or 2/6 equivocal; and 1/6 or 0/6 negative. In all N tests, titration end-points, based on the number of mice surviving on the fourteenth day, were calculated by the method of Reed and Muench (15). Deaths occurring before the fourth day of the test or as a result of cannibalism were considered non-specific and, therefore, excluded from the results.

Results

Virus isolations

Twenty-two of 2,008 vertebrates examined during this study yielded isolations of VEE virus (table 1). This agent was also recovered from 12 of 2,098 insect pools tested (table 2) and from nine litters of sentinel mice exposed to the bites of bloodsucking arthropods. Further information on the types of insects and vertebrates examined during the survey with negative results can be found in a report by Galindo et al. (9). Details concerning isolations of VEE virus from specimens collected or exposed in the study area are presented here.

Humans. Isolations of VEE virus were made from 6 of 157 febrile humans examined during the study period (table

TABLE 1

VEE virus isolates from vertebrates of Almirante, Panama

Class	Species	No. of specimens	No. of isolates
Mammals:			
Human	*Homo sapiens*	157	6
Cotton rat	*Sigmodon hispidus*	170	7
Other species		103	0
Birds:			
Green heron	*Butorides virescens*	20	2
Groove-billed ani	*Crotophaga sulcirostris*	16	1
Social flycatcher	*Myiozetetes similis*	17	1
Gray-capped flycatcher	*Myiozetetes granadensis*	16	1
Undetermined flycatcher	*Myiozetetes* spp.	21	1
Black-cowled oriole	*Icterus prosthemelas*	2	1
Scarlet-rumped tanager	*Ramphocelus passerinii*	201	2
Other species		951	0
Reptiles		331	0
Amphibians		3	0
Totals		2,008	22

1). Four of these were obtained from patients admitted to the Almirante Hospital or attended at the outpatient clinic during June and July, 1961. Analysis of the case histories of these individuals indicated that all had probably acquired infection in one of the two slum sectors of Almirante, namely, Tampico and Patoistown. The other two isolations were made in July and August, 1962 from persons residing or working in Patoistown. All isolates were recovered from blood sera of patients with acute febrile disease characterized by sudden onset, headache, chills, malaise and, occasionally, vomiting. Duration of fever ranged from two to eight days, the highest recorded temperature being 105.5 F in a severely ill patient.

Four of the patients exhibited fourfold or greater rises in HI and CF antibody titers to VEE virus during convalescence, and significant increases in N antibody levels were observed in two of these. Although the quantity of acute-phase serum from the other two patients was not sufficient for comparison by N technique, specimens collected during the recovery phase neutralized $10^{3.4}$ or more LD_{50} of virus. Unfortunately, serum obtained during the acute phase in the remaining two cases was not available for serologic testing. However, high levels of HI, CF and N antibodies to VEE virus were observed in these patients four to six weeks after onset of illness.

TABLE 2

VEE virus isolates from bloodsucking insects of Almirante, Panama

Species	No. of pools	No. of specimens	No. of isolates
Aedes (Ochlerotatus) angustivittatus	39	3,724	1
Aedes (Ochlerotatus) spp.	220	22,686	1
Culex (Culex) pipiens quinquefasciatus	95	2,246	2
Culex (Melanoconion) taeniopus	160	7,669	5
Culex (Melanoconion) vomerifer	152	10,594	3
Other species	1,423	148,594	0
Totals	2,098	195,513	12

Reisolation of VEE virus from acute-

phase serum, stored 1 to 30 months at −65 C, was obtained in five of the cases. Although attempts to reisolate the virus from one positive specimen stored 17 months at −65 C were unsuccessful, serologic conversion during convalescence of this patient was detected by HI, CF and N techniques.

Rodents. Seven of 170 cotton rats (*Sigmodon hispidus*) tested in this study yielded VEE virus (table 1). These isolates were obtained from blood sera of rodents captured during May and June, 1961 in the grassy edge of an open freshwater marsh approximately two miles northwest of Almirante.

The virus was reisolated from each of the positive specimens after more than two years of storage at −65 C.

Birds. VEE virus was obtained from 9 of 1,244 avian blood and tissue specimens examined (table 1). Infections were found in at least six species of birds nesting between March and September, 1962 in the margins of open freshwater marshes, fruit orchards and abandoned fields covered by early second-growth. With the exception of one agent from an adult female black-cowled oriole (*Icterus prosthemelas*), all virus isolates were recovered from nestling and fledgling birds. Eight of these strains were isolated from pools of liver, spleen and kidney tissue. One avian isolate came from the plasma of a sick nestling green heron (*Butorides virescens*) captured on August 15, 1962 in the grassy edge of a freshwater marsh near field headquarters. This bird was shipped back to the central laboratory where it was serially bled and kept under observation until its death 13 days later. HI antibody titers to VEE virus of 1/20 and 1/80 were detected in plasma specimens collected 6 and 13 days, respectively, after the recovery of virus from the blood.

Reisolation of the virus was accomplished from each of the positive avian specimens after 7 to 16 months of storage at −50 C to −65 C.

Mosquitoes. Isolations of VEE virus were made from 12 of 2,098 insect pools, comprising 195,513 specimens, tested in this study (table 2). At least four species of mosquitoes in two genera yielded virus. Eight of the 12 isolates were obtained from two closely related species, *Culex* (*Melanoconion*) *vomerifer* and *C.* (*M.*) *taeniopus*. Eleven isolations were produced by insects collected between March and November, 1961 and one by a pool of *C. taeniopus* captured in July, 1962. Naturally infected mosquitoes were found in swamp forests and open marshes as well as in domestic and peridomestic habitats of Almirante.

Three of the *C. taeniopus* isolates came from mosquito pools recovered on sentinel animals. One of these pools was collected on June 27, 1961 from three litters of sentinel mice exposed in a silica palm swamp. Four suckling mice in one of the litters also yielded virus. Another positive pool was taken from three sentinel mouse groups exposed in the edge of an open freshwater marsh on October 3, 1961. VEE virus was subsequently recovered from the mother of one of these litters.

The two isolations from *Culex pipiens quinquefasciatus* were made from insects collected late in June, 1961 from dwellings in Patoistown. One isolate came from specimens taken in the home of an individual who was viremic at the time of collection. The other was recovered from a pool of mosquitoes captured in the domiciles of two Patoistown resi-

dents, one of whom had experienced infection with VEE virus about two weeks before.

Seven of 11 attempts to reisolate VEE virus from positive mosquito suspensions held from 3 to 29 months in dry-ice cabinets were successful. The four unsuccessful attempts were made from suspensions which had been stored 16 to 29 months at the temperature of dry ice. Reisolation attempts were not made from one of the positive pools because of insufficient material.

Sentinel mice. Between December, 1960 and October, 1962, 436 litters of sentinel mice were exposed to the bites of bloodsucking arthropods in the study area. Although nine litters yielded 30 isolations of VEE virus, multiple recoveries of this agent from the same group of sentinel animals are considered as a single isolation in this report. All virus isolates were obtained from sentinel mice exposed nocturnally between the hours of 6:30 P.M. and 6:30 A.M. Infections were detected in five sentinel mouse groups exposed between June and October, 1961 and four exposed in July and September, 1962. All of the isolates except one from a sentinel mouse mother were recovered from suckling mice. Exposure sites of positive sentinel animals were located either in swamp forest or open freshwater marsh associations.

Virus identifications

In CF screening procedures employing polyvalent and hyperimmune reference sera or ascitic fluids prepared for certain arboviruses in groups A, B and C, several of the early isolates were found to be antigenically related to VEE virus. Upon serial passage of these agents, average survival times of 1.5 and 3 days were observed in suckling and weanling mice, respectively. Subsequently, all new virus isolates which produced similar rapidly fatal infections in mice were directly compared, by CF technique, with the reference strain of VEE virus, omitting the usual preliminary serologic procedures.

Antigens prepared for five of the isolates from sentinel mice were tested in CF grid titrations with a specific VEE immune serum. The remaining 38 strains from all sources were identified by means of cross CF grid titrations. The results of these tests, shown for six of the isolates in table 3, indicate that all of the Almirante strains are closely related to the MARU №3880 strain of VEE virus.

The identities of six of the agents, previously shown to be related to VEE virus by CF technique (table 3), were confirmed by means of cross N tests in mice. Neutralization of at least $10^{7.6}$ LD_{50} of virus was obtained in each test, observed differences in N indices of homologous and heterologous sera not exceeding one log.

Antibody surveys

HI tests. A total of 2,468 vertebrates representing at least 122 species, 57 families and 28 orders of mammals, birds, reptiles and amphibians were examined for HI antibodies to three group A arboviruses, namely, VEE, EEE and Una. Seventeen (less than 1.0 per cent) of the samples tested were positive for EEE or Una virus. However, 842 (34.1 per cent) of the specimens were positive for VEE virus. Of these, only 37 (4.4 per cent) reacted at lower titers with the EEE and/or Una antigens. HI antibody titers of VEE positive sera ranged from 1/20 to 1/2,560, the highest titers in

TABLE 3

Results of cross CF grid titrations with VEE virus and six isolates from Almirante, Panama

Antigen	Antiserum						
	VEE (#3880)	Crespo	BT 1383	BTS 4713	BTNP 173-B	BTNP 2070-B	BTSM 104-A
VEE (#3880)	32/64*	32/64	32/64	16/64	16/64	16/64	16/16
Crespo[a]	32/64	64/64					
BT 1383[b]	16/64		64/64				
BTS 4713[c]	32/16			32/32			
BTNP 173-B[d]	32/64				32/64		
BTNP 2070-B[e]	32/128					32/128	
BTSM 104-A[f]	16/32						32/64

* Serum titer/antigen titer.

[a] Isolated from blood serum of febrile human.

[b] Isolated from pool of *Culex taeniopus* mosquitoes.

[c] Isolated from blood serum of a cotton rat (*Sigmodon hispidus*).

[d] Isolated from tissues of a social flycatcher (*Myiozetetes similis*).

[e] Isolated from blood plasma of a green heron (*Butorides virescens*).

[f] Isolated from a sentinel suckling mouse.

TABLE 4

Distribution of VEE seropositive individuals by length of residence in Almirante, Panama, 1960

Years of residence	Individuals examined for HI antibodies to VEE virus		
	No. tested	No. positive	% positive
<1	11	2	18.2
1–9	97	20	20.6
10–19	117	48	41.0
20–39	127	62	48.8
40–59	65	39	60.0
60–	39	24	61.5
Totals	456	195	42.8

mammals, birds and reptiles being 1/2,560, 1/160 and 1/20, respectively.

Humans: Antibodies to VEE virus were detected in 416 (36.6 per cent) of 1,136 single serum samples obtained from Almirante residents in 1960, 1961 and 1962. The distribution of serologic positives by sex was not significantly different, prevalence rates in 613 males and 523 females being 34.7 per cent and 38.8 per cent, respectively.

The distribution of positives by length of residence in the area is shown in table 4 for 456 individuals, including 387 indigenous residents of Almirante, examined in October, 1960. Seropositivity and length of residence in the area are positively correlated. A similar trend was observed when the 69 immigrants in this group were examined separately.

The distribution of seropositive individuals by place of residence in Almirante is shown in table 5 for 431 school children, 6 to 12 years of age, examined in July and August, 1961. Children from the slum sectors of Tampico and Patoistown exhibited significantly higher rates of VEE seropositivity than those from the other more sanitated parts of town. Since the six groups shown in table 5 are similar to one another in age and sex composition, seropositivity also appears to be related to place of residence in Almirante.

A number of families from Tampico and Patoistown were included in the group tested for VEE antibodies in

1961. Although several households had two or more seropositive members, evidence of clustering of positive families within the slum area was not acquired.

Blood specimens from 131 of the persons examined in 1961 were collected for antibody studies in September and October, 1962. Four (8.5 per cent) of the 47 members of this group who were serologically positive for VEE virus in 1961 became negative in 1962, whereas eight (9.5 per cent) of the 84 negative individuals converted to positive the following year.

Domestic animals: Fifty-two (71.2 per cent) of 73 domestic animals examined in August, 1961 exhibited HI antibodies to VEE virus (table 6). Serologic positives were widely distributed in these animals, occurring in equines, bovines, canines and gallinaceous birds.

Wild animals: A total of 758 mammals in 21 species, 9 families and 3 orders were examined by HI technique for antibodies to VEE virus. Three hundred and sixty-three individuals (47.9 per cent) in 11 species and 7 families of rodents, marsupials and bats gave positive reactions (table 7).

Serologic positives were encountered with the greatest frequency in rodents, occurring in 320 (54.1 per cent) of 592 specimens examined. More than two-thirds (67.1 per cent) of 231 spiny rats (*Proechimys semispinosus*) tested had antibodies to VEE virus and nearly half (46.6 per cent) of 324 cotton rats examined throughout the study period were serologically positive for this virus. Of the latter, positive reactions were observed in 40.4 per cent, 56.1 per cent and 31.4 per cent of the samples collected in 1960, 1961 and 1962, respectively. Other rodent species in which VEE antibodies were detected included

TABLE 5

Distribution of VEE seropositive children, age 6 to 12 years, by place of residence in Almirante, Panama, 1961

Place of residence	Individuals examined for HI antibodies to VEE virus		
	No. tested	No. positive	% positive
Zegla	81	7	8.6
Almirante	69	7	10.1
Half-mile	76	13	17.1
One-mile	77	18	23.4
Tampico	55	27	49.1
Patoistown	73	36	49.3
Totals	431	108	25.1

TABLE 6

Distribution of HI antibodies to VEE virus in domestic animals of Almirante, Panama, 1961

Species	No. tested	No. positive
Horse (*Equus caballus*)	22	19
Mule	34	28
Cow (*Bos tauros*)	2	2
Dog (*Canis familiaris*)	2	2
Chicken (*Gallus gallus*)	13	1
Totals	73	52

the spiny rat (*Hoplomys gymnurus*), rice rat (*Oryzomys caliginosus*), common rat (*Rattus rattus*), paca (*Agouti paca*), red squirrel (*Sciurus granatensis*) and variegated squirrel (*Sciurus variegatoides*).

Forty-two (30.2 per cent) of 139 marsupials examined were positive for VEE virus. Antibodies were demonstrated in 16 (33.3 per cent) of 48 common opossums (*Didelphis marsupialis*) and 26 (31.0 per cent) of 84 *Philander* opossums.

Among the 27 chiropterans tested for HI antibodies, 1 of 12 big fruit-eating bats (*Artibeus lituratus*) gave a positive reaction for VEE virus.

TABLE 7

Distribution of HI antibodies to VEE virus in wild animals of Almirante, Panama, 1960–1962

Class	Species	No. tested	No. positive
Mammals:			
Cotton rat	*Sigmodon hispidus*	324	151
Spiny rat	*Proechimys semispinosus*	231	155
Spiny rat	*Hoplomys gymnurus*	4	1
Rice rat	*Oryzomys caliginosus*	12	7
Common rat	*Rattus rattus*	8	1
Paca	*Agouti paca*	1	1
Red squirrel	*Sciurus granatensis*	7	3
Variegated squirrel	*Sciurus variegatoides*	1	1
Common opossum	*Didelphis marsupialis*	48	16
Four-eyed opossum	*Philander opossum*	84	26
Big fruit-eating bat	*Artibeus lituratus*	12	1
Other species		26	0
Birds:			
Scarlet-rumped tanager	*Ramphocelus passerinii*	67	4
Catbird	*Dumetella carolinensis*	3	1
Keel-billed toucan	*Ramphastos sulfuratus*	13	1
Swainson's toucan	*Ramphastos swainsonii*	4	1
Collared aracari	*Pteroglossus torquatus*	15	1
Black vulture	*Coragyps atratus*	4	1
Other species		293	0
Reptiles:			
Cayman	*Caiman fuscus*	6	1
Common iguana	*Iguana iguana*	16	1
Other species		78	0
Amphibians		2	0
Totals		1,259	374

A total of 399 serum or plasma samples representing at least 80 species, 34 families and 17 orders of birds were examined for seropositivity. Of these, only nine reacted with VEE virus. These positive specimens belonged to six avian species in four families and three orders (table 7). Four (6.0 per cent) of 67 scarlet-rumped tanagers (*Ramphocelus passerinii*) had antibodies to this virus and each of the following species included one positive individual: catbird (*Dumetella carolinensis*), keel-billed toucan (*Ramphastos sulfuratus*), Swainson's toucan (*Ramphastos swainsonii*), collared aracari (*Pteroglossus torquatus*) and black vulture (*Coragyps atratus*).

One hundred reptiles representing at least 15 species and 9 families of lizards, snakes, turtles and crocodilians were examined by HI technique. Low-titered antibodies to VEE virus were encountered in one of six caymans (*Caiman fuscus*) and 1 of 16 common iguanas (*Iguana iguana*) tested (table 7).

Two amphibians included in these tests were serologically negative for VEE virus.

N tests. A total of 206 sera representing 20 species of vertebrates sam-

TABLE 8

A comparison of HI and N antibodies to VEE virus in vertebrates of Almirante, Panama, 1960–1962

Class	Species	No. tested	HI positive			HI negative		
			N positive	N equivocal	N negative	N positive	N equivocal	N negative
Mammals:								
Human	*Homo sapiens*	108	62	16	6		1	23
Horse	*Equus caballus*	14	11			3		
Mule		11	6			2	3	
Cow	*Bos tauros*	2	2					
Dog	*Canis familiaris*	1	1					
Cotton rat	*Sigmodon hispidus*	12	5	1				6
Spiny rat	*Proechimys semispinosus*	12	6					6
Spiny rat	*Hoplomys gymnurus*	4	1					3
Rice rat	*Oryzomys caliginosus*	5	1	2		1		1
Common rat	*Rattus rattus*	7		1				6
Agouti	*Dasyprocta punctata*	3					1	2
Paca	*Agouti paca*	1	1					
Red squirrel	*Sciurus granatensis*	4			1			3
Variegated squirrel	*Sciurus variegatoides*	1		1				
Common opossum	*Didelphis marsupialis*	6	1	2				3
Four-eyed opossum	*Philander opossum*	8	2	2	1			3
Birds:								
Scarlet-rumped tanager	*Ramphocelus passerinii*	1						1
Catbird	*Dumetella carolinensis*	2	1					1
Reptiles:								
Cayman	*Caiman fuscus*	2			1			1
Common iguana	*Iguana iguana*	2			1			1
Sub-totals			100	25	10	6	5	60
Totals		206	135			71		

pled throughout the study period were tested for N antibodies to approximately 100 LD_{50} of VEE virus. The results of these tests with 135 HI positive and 71 HI negative sera are shown in table 8. N antibodies to this virus were encountered in 13 of 20 species examined and 74.1 per cent of the HI positive individuals were also unequivocally positive in the N test. It is noteworthy that the 10 HI positive sera in which N antibodies were not detected presented HI titers of 1/80 or less. A high degree of correlation was also obtained in these tests with HI negative sera, 84.5 per cent of which had no detectable N antibodies to VEE virus.

DISCUSSION

The interpretation of results of antibody surveys in vertebrate populations with respect to virus etiology is at best difficult. However, the evidence presented here suggests that the majority of positive HI reactions to VEE virus encountered in our study represented infection with this agent rather than with some other related arbovirus. At present,

only three other group A arboviruses are known to occur in the study area, namely, EEE, Una and Mayaro. Although numerous isolations of VEE virus have been made in Almirante from insects, vertebrates and sentinel animals, other group A arboviruses have been recovered there only from mosquitoes on very few occasions (9, 16). In our surveys of vertebrates for HI antibodies to group A arboviruses, the low rates of positive reactions to EEE and Una virus contrasted sharply with the results obtained with VEE virus, in which approximately one-third of all vertebrates examined were positive. The detection of specific N antibodies to VEE virus in more than 70 per cent of the HI positive individuals examined by both techniques lends further support to this hypothesis.

The recovery of VEE virus from six febrile patients and the detection of serologic conversions in eight individuals examined consecutively in 1961 and 1962 indicate that the virus was active in the human population during this period. Serologic evidence of human infection acquired before 1961 was also obtained. The results of serum surveys of Almirante residents conducted in 1960, 1961 and 1962 revealed that VEE virus infections were widespread among populations sampled, occurred nearly uniformly in both sexes and were associated with length and place of residence in the town. VEE seropositivity was encountered more frequently in children from Tampico and Patoistown than from four other sectors of Almirante. All of the acute febrile cases detected in this study were individuals residing or working in one of these slum sectors, and case history studies of four patients yielding the virus in 1961 indicated that the infections were acquired there. In a population that is approximately 85 per cent indigenous, the correlation of VEE seropositivity with length of residence in the area points to the probable endemicity of this agent in Almirante.

Although antibodies to VEE virus were prevalent in many species of domestic and wild mammals, our findings suggest that rodents are the most important reservoirs of this virus in the study area. Antibodies were encountered in 8 of 11 species tested and several of these, such as the spiny rat, rice rat and cotton rat, exhibited high rates of seropositivity. However, the cotton rat was the only wild mammal from which the virus was recovered in our study. In 1961, viremias were detected in 4 per cent of the cotton rats tested, and nearly half of the specimens examined from 1960 to 1962 had antibodies to this virus. The cotton rat is also the most common species of field rodent in the study area, inhabiting the fringes of forests and often occurring near human habitations bordering on swampy, forested areas.

The prevalence of VEE virus infections in two closely related *Culex* (*Melanoconion*) mosquitoes, *C. taeniopus* and *C. vomerifer*, suggests that these two species, which were frequently found feeding on sentinel mice, cotton rats and other wild rodents, are instrumental in transmitting the virus among rodents. These insects are among the most common mosquito species in Almirante, occurring in the study area throughout the year. Population peaks seem to vary from year to year depending on the rainfall pattern. In 1961 and 1962, they were predominant from April to October, the months during which almost all of the isolations of VEE virus were obtained. They are nocturnal, sylvan mosquitoes which inhabit both the forest

canopy and floor, occasionally invading peridomestic habitats. Although they do not enter houses, large numbers of these mosquitoes have been observed biting man at dusk on the porches of dwellings in Patoistown. These two species exhibit similar feeding habits, being readily attracted to humans, equines, birds and rodents, the latter apparently being the preferred hosts (9). The frequency of virus isolations from these mosquitoes, coupled with their feeding habits, particularly their association with rodents, suggests that they are among the most important vectors of VEE virus in the study area. Recently, transmission studies employing wild-caught mosquitoes have indicated that *C. taeniopus* may prove to be more significant in this respect than *C. vomerifer* (17). The former is known to breed in freshwater swamps such as those surrounding Tampico and Patoistown and was found in abundance there, together with the cotton rat, during the peak months of VEE virus activity in 1961.

The possibility of man to man transmission of this agent by domestic *Culex pipiens* mosquitoes is also worthy of consideration. On two separate occasions, natural infections of these mosquitoes were found in close association with human infections.

Although birds have previously been shown to be susceptible to infection with VEE virus in the laboratory (18), the isolation of this agent from wild birds in Almirante represents the first unequivocal report of naturally infected avians. It is noteworthy that one of the avian isolates was recovered from the peripheral blood of a green heron. Since this specimen was taken from a nest in the study area in August, 1962, it was obviously a member of a resident population, probably *Butorides virescens maculatus*. A close relative, *Butorides virescens virescens*, which is a common winter visitor in Panama from October to April, breeds from Canada south through the eastern United States to Mexico (19). Certainly, the potential role of viremic birds, not only in local transmission cycles, but in the geographic dissemination of VEE virus merits further investigation.

It is evident that the epidemiologic aspects of VEE virus infections in an endemic tropical area such as Almirante present a complex picture involving many species of insect and vertebrate hosts. Most likely several cycles of virus transmission occur, some or all of which may operate in the same area at the same time. Both terrestrial and arboreal animals appear to be involved as well as several different types of habitats. The transmission cycles which prevail in a given area during a given period of time are probably determined to a great extent by such factors as vector potential, immunological status and population densities of available host species.

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