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AN ECOLOGICAL SURVEY FOR ARBOVIRUSES IN ALMIRANTE, PANAMA, 1959–1962*

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The survey herein reported was initiated in September 1959 as the first part of a long-term project to study the ecology of arthropod-borne viruses (arboviruses) in a tropical rainforest area of Panama. Investigations in the field were carried out for three years during which time virus isolation attempts were made from blood-sucking insects, blood specimens from humans, blood and tissue samples from domestic and wild vertebrates and sentinel mice. In the first two years of work all blood-sucking insects collected were identified in lots according to species or species-group. Half of the insects in each lot were examined for virus at the Gorgas Memorial Laboratory (GML), the remainder being sent to the Middle America Research Unit (MARU) in the Canal Zone for virus isolation attempts. Insects collected during the third year of the survey were processed exclusively at GML. All virus isolation attempts from blood and tissue samples and from sentinel mice were made at GML.

Isolations of Ilhéus encephalitis virus and of two new group C arboviruses obtained during this survey have already been reported.[1,2] Viruses isolated and characterized at MARU are being reported elsewhere.[3–5] Isolations of Venezuelan equine encephalomyelitis (VEE) virus and certain group C arboviruses obtained at GML will be reported separately.[6,7] Some data from these manuscripts have been included here in order to present a more complete picture of results obtained in the survey.

Attempts to isolate viruses from the blood of human inhabitants of the study area were carried out throughout the study period with the help of the medical services of the Chiriqui Land Company, a subsidiary of United Fruit Company which operates in Almirante. Collection of wild vertebrates for virological and serological investigations was begun early in 1960 but work along these lines was not intensified until the following year. Weekly exposures of sentinel mice in the field were initiated in December 1960 and carried out without interruption until September 1962. Definitive identification of vertebrates was accomplished with the help of Mr. Eustorgio Méndez, GML; Drs. Alexander Wetmore (birds) and Charles O. Handley (mammals), Smithsonian Institution; Drs. Norman Hartweg and Charles F. Walker (lizards and snakes), University of Michigan; and Dr. John Legler (turtles), University of Utah.

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Description of the Study Area

The study area surrounds the town of Almirante in Bocas del Toro province, within the extreme northwestern quadrant of the Republic of Panama. A series of complex forested ridges, reaching elevations of over 9,000 feet, rises to the south and west of the study area, which is delimited on the north by the Changuinola River and on the east by the Caribbean Sea (Fig. 1).

Rainfall. The high mountain barrier formed by the continental divide produces an extreme uplifting of the moist air masses brought in by northeasterly trade winds, causing abundant precipitation throughout the year. Table 1 gives the monthly rainfall averages taken at five rain-gauge stations operated by the Chiriqui Land Company within the study area or in its immediate vicinity. It should be pointed out that all rain-gauges are located in the lowlands and that precipitation is even greater on the densely forested slopes of the continental divide. As may be noted in column two, the years in which the survey was conducted (1959–1962) were quite dry compared with the preceding 10 years, averaged in column one. One of the characteristics of the rainfall pattern of the study area is the great variation in the amount of monthly precipitation from year to year. However, September is generally the driest month with another relatively dry spell in March and April. The maximum amount of rainfall occurs in the month of December although July and November are also very wet.

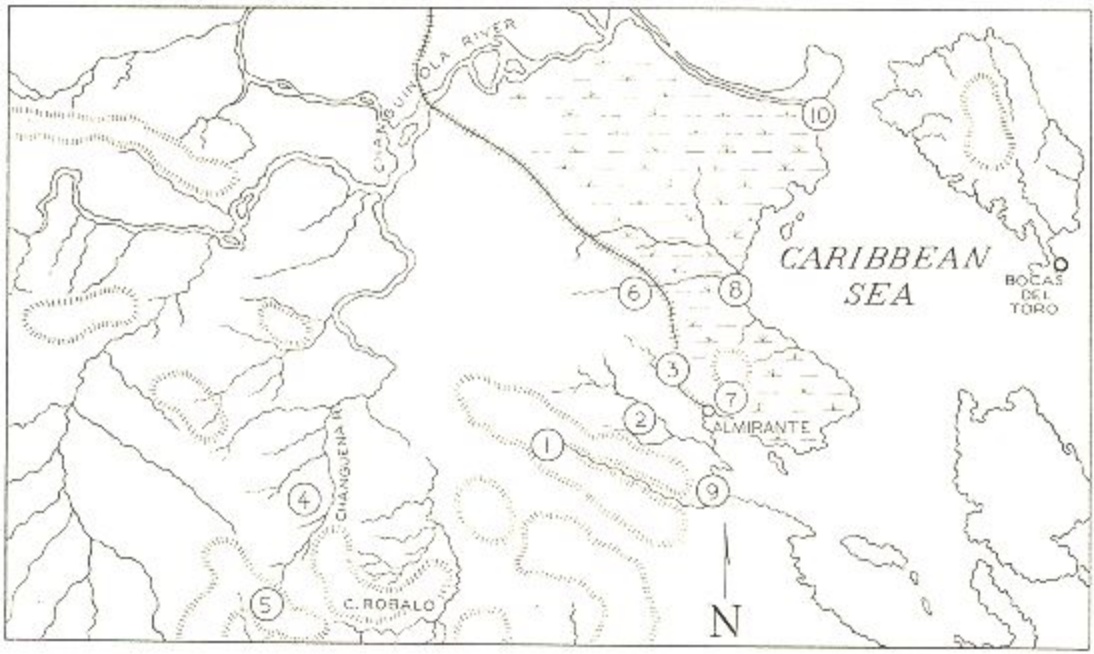

FIGURE 1. *Collecting stations within study area:* Station No. 1—Yellow Fever Station; Station No. 2—Nigger Creek Station; Station No. 3—Mile-2 Station; Station No. 4—Lower Changuena Station; Station No. 5—Upper Changuena Station; Station No. 6—Mile-5 Station; Station No. 7—Patoistown Station; Station No. 8—Banana River Station; Station No. 9—Western River Station; Station No. 10—Boca del Drago Station.

TABLE 1

Average monthly rainfall

Month	Ten-year average 1949–1959	Three-year average 1959–1962	1959–1960	1960–1961	1961–1962
September	4.21	3.43	3.52	4.10	2.67
October	6.32	3.72	2.52	3.16	5.47
November	12.36	7.79	8.66	6.85	7.85
December	15.39	9.66	7.98	12.33	8.67
January	8.45	7.08	8.52	4.93	7.80
February	7.50	3.67	6.41	1.46	3.14
March	5.56	5.99	6.52	8.08	3.39
April	5.98	6.92	3.74	7.25	9.76
May	9.04	7.38	4.77	9.55	7.82
June	8.36	9.26	8.06	11.93	7.79
July	12.04	8.89	6.92	11.18	8.58
August	9.09	7.04	7.87	8.97	4.28
Totals	104.30	80.83	75.49	89.79	77.22

Temperature and humidity. Temperatures during the period of surveying operations varied from a low of 65°F to a maximum of 93°F, while the monthly averages of the daily mean temperature varied from a low of 78.5°F in January 1962 to a high of 81.8°F in June 1960. Since recording thermographs were placed in the open, these data do not present a true picture of the microclimates to which most species of animals inhabiting the region are exposed. Pertinent data concerning the

ranges of ground and canopy temperature and relative humidity in the forested slopes southwest of Almirante during 1951 and 1952 have been presented by Trapido and Galindo.[8]

Available climatological information places the study area within the "tropical rainforest climate" of the Köppen system of climatic classification as defined by Trewartha.[9]

Winds. Although no data are available, strong winds are reported almost every year as damaging banana plantations. The area is not in the path of hurricanes.

Ecological associations. Since the region has a tropical rainforest climate it is mostly covered by large tracts of broadleaf evergreen forest. The trees never lose their leaves, thus forming an everpresent unbroken canopy which prevents passage of sunlight to the ground and inhibits herbaceous growth.

Within this almost continuous cover of broadleaf evergreen forest a number of distinct ecological associations have developed, influenced by special orographic, hydrographic and edaphic conditions or by human activity. The five main categories of ecological associations recognized in the study area are:

a) *Lowland tropical rainforest or swamp forest:* This type of forest grows on periodically flooded ground, which tends to form more or less permanent, deeply-shaded swamps. The trees, in general, do not reach the height observed in well-drained rainforests but the woody vegetation near the ground is much denser, sometimes forming almost impenetrable thickets. The chemical nature of the water (brackish or fresh), the type of soil and the permanency and depth of water on the ground influence the development of various ecological associations within this generic type of environment. Holdridge and Budowski recognized a number of these associations in the Almirante area as follows:[10]

Mangrove association—Composed mainly of red mangrove (*Rhizophora mangle*), black mangrove (*Avicennia marina*) and white mangrove (*Languncularia racemosa*). The vegetation cover ranges from very short red mangrove trees in deep saline waters to a mixture of tall red, black and white mangrove trees in brackish waters.

"Orey" association—This is a brackish-water association composed of almost pure stands of "orey" trees (*Campnosperma panamensis*). Its boundaries are established by the salinity of the water, giving way to mangrove in more brackish waters and to "cerillo" or "silica" swamps as the water loses its salt content. The association extends just back of the coastline in an almost continuous stand from Almirante north to Boca del Drago.

"Silica palm" association—Along the coastal plains north of Almirante and just back of the "orey" association, large stands of a handsome palm may be observed growing in swampy grounds almost always covered by fairly high levels of alluvial fresh water. These stands are composed entirely of "silica palm" (*Raphia taedigera*).

"Cerillo-sangrillo" association—Still further inland, covering large extensions of the flat alluvial plains north of Almirante, may be found a swamp forest association. The upper canopy, which reaches heights of 100 to 120 feet, is composed mainly of "cerillo" (*Symphonia globulifera*) and "sangrillo" (*Pterocarpus officinalis*) with a mixture of other species of trees such as "cedro bateo" (*Carapa slateri*), "guava" (*Inga* spp.), "cativo" (*Prioria copaifera*), etc. The second-story or lower canopy is usually composed of a thick growth of "coquillo palm" (*Manicaria saccifera*). As the swamp forests reach higher and better-drained ground the dominance of "cerillo-sangrillo" trees is diluted and a wider variety of species of trees may be observed. The latter type of forest is intermediate between a true swamp forest and the next category.

b) *Upland tropical rainforest:* This type of association covers the well-drained slopes of the continental divide. The forest is characterized by a very high and thick canopy and the ground is open and park-like. The climax association is a mixture of many species of trees with no apparent dominance by any species or species-group.

c) *Cloud forest or lower montane association:* This habitat is found in the study area above the 5,000 ft. contour. Greater precipitation, lower temperatures and higher humidity contribute toward the development of special features, such as the abundant growth of moss on tree trunks, the presence of numerous epiphytes, both in the canopy and near the ground, and a very wet forest floor.

d) *Open fresh-water marsh association:* One of the hydrographic features of the lowlands is the web of meanders formed by the streams and creeks which slowly flow through the region. These meanders are frequently cut off from the main stream by floods which sweep down from the mountains. The ox-bow lakes thus formed soon

become invaded by aquatic vegetation which eventually chokes them completely, forming open fresh-water marshes. A high water level throughout the year appears to be the most important factor preventing the eventual establishment of tree growth in these marshes. The fresh-water marsh association may be found spotted throughout the alluvial plains north of Almirante and from the air these areas give the appearance of large islands in the continuous thick forest growth covering the area.

e) *Domestic and peridomestic associations:* The actions of man have destroyed large stands of lowland and upland tropical rainforest for the construction of dwellings or for agricultural activities. The main agricultural practices engaged in by the inhabitants of the study area are the cultivation of bananas, plantains and cacao and the development of grazing pastures. As cultivated fields are abandoned they are quickly covered by second-growth which may be found in all stages of development, from primary, tall, weedy bushes through thickets of "platanillos" (*Heliconia* spp.) to a cover of pioneer trees, such as "guarumos" (*Cecropia* sp.), "balsos" (*Ochroma lagopus*), "jacarandas" (*Jacaranda copaia*) and "laureles" (*Cordia alliodora*). These changes produced by human activity have influenced the development of a number of ecological associations which we have grouped together under the generic terms of domestic and peridomestic habitats.

Demography. Most of the human population of the study area is concentrated in the community of Almirante which is located on the bay of the same name. It is a deep-sea port used almost exclusively by the Chiriqui Land Company for the shipment of bananas and cacao. In 1960 there were 1,188 human dwellings with a population of 4,920.[11] The dwellings are largely grouped in an urban community scarcely one mile long and a quarter of a mile wide. The town is bordered on the north by large stands of 'silica palm," "orey," mangrove and "cerillo-sangrillo" associations and by open fresh-water marshes; on the south by the quiet waters of Almirante Bay; on the east by the Chiriqui Lagoon; and on the west by the slopes of the Risco Ridge which forms part of the complex of hills and mountains that constitute the continental divide.

The town is divided into two main sectors by a slough of dark, foul water called Quebrada del Cedro. The southern sector, built against Almirante Bay, is inhabited by white workers of the Chiriqui Land Company. Sanitary conditions in this sector are good. A hospital, with capacity of 200 beds, is operated by the Company in this part of town and is the only medical center available for the inhabitants of the study area. The northern sector of town is occupied by colored workers of the Chiriqui Land Company as well as by natives not engaged in activities connected with the Company. This sector, built against extensive swamplands, is divided into five communities known from west to east as One-mile, Half-mile, Almirante, Tampico and Patoistown. Sanitary conditions in the first three communities, although of lower grade than those found in the southern sector, are satisfactory. Tampico and Patoistown, with approximately 160 houses and 720 persons (1961),[5] can be considered veritable slums.

Description of Collecting Stations

All captures of blood-sucking insects were made at five fixed stations. Vertebrates were gathered at many places within the study area, but the bulk of the collections also came from the stations where insects were captured. Following is a brief description of the collecting stations utilized during the survey. Figure 1 shows the relative positions of the different collecting sites.

Station No. 1 (Yellow Fever Station). Thus named because in 1951 a man died of yellow fever in this locality. The station is located some 12 kilometers southwest of Almirante on the slopes of the Risco Ridge. It is in a typical upland tropical rainforest association. Collections of insects and vertebrates were initiated here in September 1959 and continued until August 1961.

Station No. 2 (Nigger Creek Station). Located about three kilometers west of Almirante where water for the aqueduct is aired and pumped. This study plot includes the following ecological associations: domestic, peridomestic, swamp forest and open marsh. Insects were collected mainly in a cacao plantation with a swamp forest cover. Vertebrates were gathered in all habitats of the plot. Collections were started here in January 1960 and continued for the rest of the study period.

Station No. 3 (Mile-2 Station). Field headquarters for the survey were established here in a small wooden house on top of a grassy hill. This station is located along the railroad tracks two miles north of Almirante. There are a number of open fresh-water marshes bordering it. To the

west is a hill supporting a thinned-out cover of lowland-type rainforest. To the east may be found a small "silica palm" association. Limiting the station on the northwest is an untouched plot of well-drained lowland forest in which the "cerillo-sangrillo" dominance has been greatly diluted. Collection of vertebrates was begun at this station in March 1960, while capture of blood-sucking insects was not initiated until January 1961. Both were continued for the rest of the study period.

Station No. 4 (Lower Changuena Station). Located on the slopes of the continental divide at an elevation of 2,800 ft. above sea level, this station is in an upland tropical rainforest association. A field party was transported by helicopter to this collecting site in September 1961, and intensive collections of blood-sucking insects and vertebrates were made for a period of one month.

Station No. 5 (Upper Changuena Station). Located in a cloud forest association southwest of Station 4 at an elevation of 6,000 ft. above sea level. Vertebrates and blood-sucking insects were collected here during a period of two weeks in September 1961 for faunistic studies. Plans have been made to investigate arbovirus infections in animals inhabiting this area in the near future.

Station No. 6 (Mile-5 Station). This area was used exclusively for the collection of vertebrates. It is largely occupied by farm houses and fruit orchards with some open grassy marshes.

Station No. 7 (Patoistown). Intensive studies were carried out in this part of town for a period of two months during an outbreak of Venezuelan equine encephalomyelitis (VEE) in 1961.[8] These studies included collections of blood-sucking insects and of wild vertebrates for virus isolation attempts.

Stations Nos. 8, 9 and 10. These stations, located in the estuaries of Banana and Western Rivers and in an old canal at Boca del Drago, respectively, were utilized periodically for the collection of vertebrates. They are covered by mangrove and "orey" associations.

Field-collecting Methods

Collection of blood and tissue samples. Patients reporting sick to the Almirante hospital with a fever of short duration were bled from the median cubital vein. Likewise, personnel engaged in the collection of arthropods were bled by the field technician as soon as they exhibited symptoms of fever and malaise. Small mammals were routinely captured alive with baited traps, etherized and bled from the heart. Bats, collected by means of mist-nets, were also bled through heart puncture. Large mammals, such as primates, edentates and carnivores, and reptiles were usually shot and bled from the heart immediately.

Routine bleeding of wild birds, both native and migrant species and nestlings as well as adults, was carried out from March 1960 to the end of the study period. Birds were either mist-netted or shot depending on the species. Netted animals to be sacrificed were bled from the heart, while birds to be banded and released were bled from the external jugular vein.

Sacrificed specimens, which included all nestling birds and most reptiles, were autopsied and samples of internal tissues, mainly liver, spleen and kidney, removed for virus isolation attempts. Aseptic techniques were employed for the collection of all blood and tissue specimens which were refrigerated as soon as possible after collection. Materials, packed in thermos jugs filled with ice, were shipped twice a week by airfreight to Panama City where they were received at the laboratory on the day of shipment.

Exposure of sentinel mice. The use of sentinel suckling mice has been shown to be one of the more sensitive methods of detecting some arboviruses in the field.[12] Four to six litters of 2-day-old mice with mothers were shipped by air to Almirante once a week. At 6:30 p.m. on the same evening, the litters were transferred to exposure cages and hung under a Causey hood[12] at specified exposure stations. The following morning the mice were counted, transferred to their regular cages and flown to Panama City. Diurnal exposures from 9 a.m. to 4 p.m. were carried out for a period of four months during 1961 but were discontinued because of consistent negative findings. Mice were exposed in the Mile-2 station at the following four sites: a thinned-out lowland forest, the margin of an open marsh, the edge of a "silica palm" swamp and a bamboo grove at the edge of a well-drained lowland forest. Additional litters were exposed in a cacao plantation at the Nigger Creek station and in the forest canopy near the Yellow Fever station.

Collection of blood-sucking insects. Mosquitoes and sandflies were collected from a number of different habitats using the following methods:

a) Hand-capture with the collector acting as bait. Whenever possible, these collections were run simultaneously on the ground and in the canopy. Collecting periods ran from 9 a.m. to 4 p.m. and from 6 p.m. to 10 p.m. During a three-month period in 1961, all-night captures were instituted for special population studies.

b) Chicken-baited Bellamy-Reeves traps[13] exposed on the ground and in the canopy for all-night and, occasionally, diurnal periods.

c) Hand collections from a horse exposed in two daily periods, 12 noon to 4 p.m. and 6 p.m. to 10 p.m.

d) Modified Lumsden fan-traps[14] baited with small vertebrates, such as the cotton rat (*Sigmodon hispidus*), spiny rat (*Proechimys semispinosus*), Scarlet-rumped Tanager (*Ramphocelus passerinii*) and Buff-throated Saltator (*Saltator maximus*).

e) Collections from sentinel mice exposed under a Causey hood.[12]

f) New Jersey light traps with a bobinet cage fixed at the exit below the fan.

g) Modified Shannon trap with a kerosene pressure lantern as attractant.

h) Hand collections from diurnal resting places, such as tree-buttresses, edges of swamps and streams, accumulations of cacao pods, interiors of human dwellings, etc.

All captured mosquitoes were transferred alive to four-ounce screwcap jars lined with moistened plaster of Paris and shipped on ice by air to the central laboratory twice a week. Engorged females were held at environmental temperatures for 24 hours before they were transferred to jars.

Laboratory Methods

The techniques utilized for the recovery and characterization of certain virus isolates obtained during this survey are described elsewhere.[1-5, 7] A detailed description of the methods employed at GML for the isolation and identification of VEE virus strains will be presented, together with data confirming the validity of these isolations, in a forthcoming report.[6] Procedures followed for the isolation and characterization of the remaining viral agents are described below.

Primary isolation attempts were carried out in 2- to 4-day-old white Swiss mice, using 0.02-ml volumes of inoculum and the combined intracerebral (i.c.) and intraperitoneal (i.p.) routes of injection. 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 passage into fresh litters of suckling mice by the i.c. route, using brain tissue as a source of passage material.

Reference virus strains used for comparison of new isolates included 11 previously characterized virus strains isolated locally and 25 prototype strains obtained from the Communicable Disease Center in Atlanta, Georgia, and the Rockefeller Foundation Virus Laboratories in New York, New York, and Belém, Brazil.

Virus isolation attempts and reagents for type strains were made in entirely separate sections of the laboratory employing different personnel. Antigens were prepared by sucrose-acetone extraction of infected mouse brains by the technique of Clarke and Casals.[15] Immune serum for each type strain was prepared by inoculating a group of adult mice with four dosages of a 10% suspension of infected suckling mouse brain by the i.p. route. The first three injections were given at intervals of 10 days, the last inoculation being administered one month after the third injection. Bleedings to test antibody response were carried out 9 to 10 days after each individual injection. Ten days after the final injection, the mice were bled out for hyperimmune serum. Certain viruses, such as VEE, eastern equine encephalitis (EEE), western equine encephalitis (WEE) and St. Louis encephalitis (SLE), were formalinized prior to administration of the first injection. Polyvalent immune sera were prepared in adult mice and guinea pigs for arbovirus groups A, B and C. All immune sera were tested for specificity by the usual serological techniques.

Crude or extracted brain antigens of new isolates were tested for hemagglutinating (HA) activity at specified pH values ranging from 6.0 to 7.0. If HA activity was demonstrated, a hemagglutination-inhibition (HI) test was performed with antisera to viruses known or suspected to occur in the area. These antigens were also utilized in complement-fixation (CF) tests with a battery of antisera or with those indicated by results of HI tests. Neutralization and cross-challenge tests were also performed with some virus isolates. Whenever indicated, immune sera were prepared against unknown strains in order to confirm identities, using the technique described for type strains.

HI tests were executed according to the tech-

TABLE 2

Arboviruses isolated from vertebrates

Species of vertebrate	Class of vertebrate	No. spec. exam.	No. viruses isolated	Types of viruses											
				Group A	Group B		Group C						Guamá group	BT 436	Undetermined isolates
				VEE	Bussuquara	Ilhéus	Caraparu	Nepuyo	Ossa	Madrid	BT 4971	BT 5032			
Humans (*Homo sapiens*)	Mammal	157	9	6					1	1				1	
Cotton rat (*Sigmodon hispidus*)	Mammal	170	13	7							2	3			1
Spiny rat (*Proechimys semispinosus*)	Mammal	54	4					1							3
Sentinel Swiss mice	Mammal	436*	33†	9	2		3			2	1	2	12		2
Other species	Mammal	49	0												
Little Blue Heron (*Florida caerulea*)	Bird	14	1			1									
Green Heron (*Butorides virescens*)	Bird	20	2	2											
Groove-billed Ani (*Crotophaga sulcirostris*)	Bird	16	1	1											
Keel-billed Toucan (*Ramphastos sulfuratus*)	Bird	28	1			1									
Social Flycatcher (*Myiozetetes similis*)	Bird	17	1	1											
Gray-capped Flycatcher (*Myiozetetes granadensis*)	Bird	16	1	1											
Undetermined Flycatcher (*Myiozetetes* spp.)	Bird	21	1	1											
Black-cowled Oriole (*Icterus prosthemelas*)	Bird	2	1	1											
Scarlet-rumped Tanager (*Ramphocelus passerinii*)	Bird	201	4	2		2									
Clay-colored Robin (*Turdus grayi*)	Bird	43	1												1
Other species	Bird	866	0												
Reptiles	Reptile	331	0												
Amphibians	Amphibian	3	0												
Totals		2,444	73	31	2	4	3	1	1	3	3	5	12	1	7

* Refers to number of litters exposed.

† Refers to number of litters yielding arboviruses.

nique of Clarke and Casals.[15] Primary incubations were carried out at 4°C overnight and the tests were incubated at room temperature after the addition of goose erythrocytes. All immune sera were treated with kaolin for removal of nonspecific inhibitors. CF tests, utilizing two units of complement, were performed on plastic plates with drop quantities, following the techniques described by Fulton and Dumbell[16] and Kerr.[17] Neutralization tests were performed in mice, employing a constant-serum, varying-virus dilution technique. Infected suckling mouse brain served as a source of virus, and serum-virus mixtures were incubated for one hour at 37°C prior to i.c. injection in mice.

RESULTS

Virus isolation attempts from humans. Viral agents were isolated from nine of 157 human sera inoculated in suckling mice (Table 2). Five

agents, representing four viral types, namely, VEE, Ossa, Madrid and BT 436* were obtained from mosquito collectors. Four isolations of VEE virus were also made from patients at the Almirante hospital.

Virus isolation attempts from other mammals. Seventeen of 273 serum specimens from mammals other than man inoculated in suckling mice yielded virus isolates. As noted in Table 2, 13 of these were obtained from the cotton rat (*Sigmodon hispidus*) and four from the spiny rat (*Proechimys semispinosus*). Of the agents isolated from the cotton rat, seven were indistinguishable from VEE virus, five represented two new antigenic types of group C arboviruses (BT 4971 and BT 5012)† and one remains unidentified. From the spiny rat, one isolate has been identified as Nepuyo virus[18] and the other three have not yet been typed.

Sera from the following groups of mammals were inoculated in mice with negative results: 14 rodents, 2 bats, 27 opossums, 2 edentates, 2 carnivores and 2 equines.

Virus isolation attempts from sentinel mice. Of 436 litters of sentinel mice exposed in the field to the bites of blood-sucking insects, 33 yielded arboviruses (Table 2). The following viral types were recognized from this material: VEE, Bussuquara, Caraparu, Madrid, BT 4971, BT 5012 and Guamá.

Virus isolation attempts from birds. Fourteen isolations of arboviruses were obtained from 1,244 avian tissue and blood specimens examined. As may be noted in Table 2, Ilhéus virus was isolated from the Little Blue Heron (*Florida caerulea*), Keel-billed Toucan (*Ramphastos sulfuratus*) and Scarlet-rumped Tanager (*Ramphocelus passerinii*). VEE virus was obtained from the Green Heron (*Butorides virescens*), Groove-billed Ani (*Crotophaga sulcirostris*), Social Flycatcher (*Myiozetetes similis*), Gray-capped Flycatcher (*Myiozetetes granadensis*), Black-cowled Oriole (*Icterus prosthemelas*) and Scarlet-rumped Tanager. The Clay-colored Robin (*Turdus grayi*) yielded an agent which remains unidentified.

A total of 866 specimens from at least 101 species of birds belonging to 35 families was examined for arbovirus with negative results.

Virus isolation attempts from reptiles. Attempted isolations from 331 serum and tissue samples of at least 20 species of turtles, saurians, lizards and snakes were unsuccessful.

Virus isolation attempts from blood-sucking insects. A total of 400,552 blood-sucking insects was collected from all sources. Table 3 presents the number of insects collected under each of the major groups of attractants or baits used. Species or species-groups which yielded arboviruses are tabulated separately in this table in order to present preliminary data concerning the host-preferences of these arthropods.

Of the insects collected, 377,492 were inoculated in 3,439 pools for virus isolation attempts. Ninety-six viral agents representing at least 11 distinct arbovirus types were isolated from this material. Table 4 presents the number and types of arboviruses obtained from each species or species-group of insects.

Following is an analysis of each species or species-group which yielded virus.

Aedes (Ochlerotatus) spp.—Many specimens belonging to this subgenus could not be specifically identified because mesonotal scales were rubbed off during capture. However, it was estimated by lot-sampling that 90 to 95% of these lots were made up of *A. angustivittatus* D. & K. Other species identified in order of abundance were *A. serratus* Theob., *A. tormentor* D. & K., *A. oligopistus* Dyar and *A. hastatus* Dyar. These mosquitoes yielded 10 agents, representing the following arbovirus types: VEE, Una, Ilhéus and Guaroa. Species of this group are predominantly diurnal, although significant numbers appear in nocturnal collections. By hourly samples it was determined that activity after daylight was largely crepuscular, ceasing soon after darkness. This group showed a decided preference for large mammals such as man and equines, but was not infrequently attracted to avian hosts. Small rodent baits were seldom approached by these insects.

Aedes (Howardina) quadrivittatus Coq.—A single mosquito of this species collected in the canopy of the forest at the Lower Changuena station yielded a viral agent which has not yet been identified. *A. quadrivittatus* breeds in epiphytic bromeliads in upland tropical rainforests and cloud forests. It is common in the latter habitat.

Anophelini—A number of species was included under this heading. The most common in order of abundance were: *Chagasia bathana* Dyar,

* A new arbovirus to be named Changuinola virus.[5]

† These viruses will be named Patois and Zegla, respectively.[7]

TABLE 3

Number of blood-sucking insects captured with different attractants and baits

Type of attractant	Human bait		Avian bait		Equine bait		Rodent bait		At light and in resting places	Totals
Collecting period	Diurnal 9 a.m.–4 p.m.	Nocturnal 6 p.m.–5 a.m.	Diurnal 9 a.m.–2 p.m.	Nocturnal 6 p.m.–5 a.m.	Diurnal 12 M–4 p.m.	Nocturnal 6 p.m.–10 p.m.	Diurnal 9 a.m.–4 p.m.	Nocturnal 6 p.m.–6 a.m.		
Unit hours of collecting	6995 Man-hrs	6095 Man-hrs	512 Trap-hrs	15,612 Trap-hrs	160 Man-hrs	187 Man-hrs	546 Trap-hrs	4,992 Trap-hrs		
Aedes (Ochlerotatus) spp.	34,886	10,931	197	1,361	2,485	1,576	14	93	5,250	56,793
Aedes (Howardina) quadrivittatus	1	—	—	—	—	—	—	—	—	1
Anophelini	55	3,374	1	21	—	156	—	2	486	4,095
Culex (Culex) nigripalus	1,710	72,433	320	38,445	21	7,499	—	257	8,718	129,403
Culex (C.) quinquefasciatus	—	100	—	—	—	—	—	—	3,358	3,458
Culex (Melanoconion) spp.	34	548	20	546	6	139	—	271	3,853	5,417
Culex (M.) crybda	—	804	2	99	—	—	—	21	27	953
Culex (M.) taeniopus	25	7,568	175	1,614	4	213	5	2,704	533	12,841
Culex (M.) vomerifer	67	18,001	132	1,205	11	361	—	2,571	356	22,704
Mansonia venezuelensis	2,439	28,432	175	1,832	613	19,290	—	1,131	10,647	64,559
Psorophora (Janthinosoma) albipes	9,312	3,493	1	43	12	177	—	5	2,082	15,125
Psorophora (J.) ferox	8,681	999	15	26	252	114	—	3	1,169	11,259
Psorophora (J.) lutzii	656	14	1	1	6	1	—	—	282	961
Psorophora (Grabhamia) cingulata	532	1,876	—	113	23	478	—	67	239	3,328
Sabethes (Sabethoides) chloropterus	2,604	121	14	2	—	—	—	—	2	2,743
Trichoprosopon spp.	1,084	652	10	24	150	79	2	—	245	2,246
Phlebotomus spp.	108	24,788	—	17	1	354	1	157	1,134	26,560
Other species	15,777	7,893	101	2,698	2,150	3,236	0	72	6,179	38,106
Totals.....	77,971	182,027	1,164	48,047	5,734	33,673	22	7,354	44,560	400,552

Anopheles (Kerteszia) neivai H. D. & K., *A. (A.) apicimacula* D. & K., *A. (A.) punctimacula* D. & K., *A. (A.) vestitipennis* D. & K., *A. (Nyssorhynchus) oswaldoi* Peryassú and *A. (N.) albimanus* Wied. Three viral agents were yielded by mosquitoes of this tribe, namely, VEE, Ilhéus and Guaroa. As expected, most of the specimens were captured at dusk or at night from large mammals which proved to be the most attractive bait. Birds and small rodents were seldom attacked.

Culex (Culex) spp.—One pool of a mixed lot of *Culex (Culex)* mosquitoes, which included *C. coronator* D. & K., *C. corniger* Theob. and *C. declarator* D. & K., yielded an unidentified viral agent.

Culex (C.) nigripalpus Theob.—This is one of

TABLE 4

Arboviruses isolated from blood-sucking insects

Types of insects	Pools inoculated		Specimens inoculated		No. viruses isolated		Types of viruses																					
							Group A						Group B				Guamá group		Bunyamwera group						VSV (Indiana)		BT 436		Undetermined isolates	
							VEE		Una		Mayaro		Bussuquara		Ilheus				Cache Valley		Guaroa		Wyeomyia							
	A*	B†	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B					A	B
Aedes (Ochlerotatus) spp.	173	268	24,205	26,410	3	7		2	3	1						1						1								2
Aedes quadrivittatus	—	1	—	1	—	1																								1
Anophelini	22	32	2,219	2,296	2	1	1									1					1									
Culex spp.	8	11	693	353	—	1																								1
Culex nigripalus	438	484	64,098	63,479	1	3										2							1							1
Culex quinquefasciatus	1	95	53	2,246	—	2		2																						
Culex (Melanoconion) spp.	24	50	2,123	2,341	1	1												1											1‡	
Culex crybda	4	11	384	427	—	2								1				1												
Culex taeniopus	28	160	3,316	7,669	3	5	3	5																						
Culex vomerifer	68	152	9,145	10,594	9	9	1	3								1	8	4												1
Mansonia venezuelensis	210	258	30,186	29,507	—	1																1								
Psorophora (Janthinosoma) spp.	40	36	5,249	4,953	1	1																	1	1					
Psorophora albipes	21	32	2,597	3,024	—	1				1																				
Psorophora ferox	31	62	3,965	5,097	8	8			5	5		1			1	1							2	1						
Psorophora lutzii	4	8	324	373	—	1										1														
Psorophora cingulata	13	28	1,261	1,244	1	—													1											
Sabethes chloropterus	13	18	1,308	3,024	—	1																								1
Trichoprosopon spp.	11	24	918	983	—	1								1																
Phlebotomus spp.	68	102	14,706	14,945	10	11										1											4	1	6	9		
Other species	164	266	15,229	16,547	0	0																								
Sub-totals	1,341	2,098	181,979	195,513	39	57	5	12	8	7	—	1	—	2	1	8	8	6	1	—	1	2	4	2	4	1	6	9	1	7
Totals	3,439		377,492		96		17		15		1		2		9		14		1		3		6		5		15		8	

* Material processed at the Middle America Research Unit and reported by Peralta and Shelokov.[3]

† Material processed at Gorgas Memorial Laboratory.

‡ Characterized as a group-B agent.

the commonest mosquitoes in the lowlands of Almirante. Four pools of *C. nigripalpus* yielded arboviruses from which Ilhéus and Wyeomyia viruses were recognized. The species is decidedly nocturnal although, during periods of great abundance, significant catches were made during the day in the forest. These mosquitoes were frequently attracted to humans, equines and birds whereas the number of insects taken from rodent bait was erratic, being high during some periods and low during others, even though abundant captures were being made on avian bait nearby.

Culex (C.) pipiens quinquefasciatus Say—This species, which yielded two isolations of VEE virus, is very common in the slum sectors of Almirante.

Culex (Melanoconion) spp.—All *Culex* species of *Melanoconion* and allied subgenera that could not be identified to species, as well as lots of various known species represented by a few specimens only, were included in this category. Two viruses were isolated from mosquitoes grouped under this heading. One was identified as a Guamá-group agent and the other has been typed as a group B arbovirus but has not been specifically determined.

Culex (M.) crybda Dyar—This species is closely related to *C. taeniopus*. Two agents, Bussuquara and a Guamá-group virus, were obtained from these insects. A decidedly nocturnal species, these mosquitoes exhibited a predilection for human blood although a few specimens were also taken on avian and rodent bait.

Culex (M.) taeniopus D. & K.—This nocturnal species, which yielded eight isolations of VEE virus, has a wide host range, rodents and man being the preferred hosts followed by birds and equines.

Culex (M.) vomerifer Komp—Eighteen viral agents were obtained from this species during the survey period. VEE, Ilhéus and Guamá-group viruses have been identified from this material. Feeding habits are similar to those exhibited by *C. taeniopus*. In view of the intense arbovirus activity in the population of small rodents in Almirante, it is of interest to point out that these two species together with *Mansonia venezuelensis* are the only mosquitoes taken regularly in numbers on small rodents.

Mansonia venezuelensis Coq.—This is one of the most common mosquitoes in the study area. A single isolation of Guaroa virus was obtained from this species.

Psorophora (Janthinosoma) spp.—During the early phase of the survey, specimens of *P. albipes*, *P. ferox* and *P. lutzii* were pooled under the generic name *Psorophora* spp. Two pools of these mosquitoes yielded Wyeomyia virus. All species of the subgenus *Janthinosoma* exhibited similar host preferences. These insects were predominantly diurnal and crepuscular with a predilection for man and equines. They were seldom encountered in collections from small rodents, but large numbers of *P. ferox* have been observed by one of us (P. G.) feeding in nature on the common agouti (*Dasyprocta punctata*), a large diurnal rodent common in the forests of Panama. Collections with avian bait yielded very low catches of *Janthinosoma* mosquitoes either in the daytime, at dusk or during the night.

Psorophora (J.) albipes Theob.—Large populations of this species made sporadic and short-lived appearances during the study period. A single isolation of Una virus was obtained from these mosquitoes.

Psorophora (J.) ferox Humboldt—Sixteen isolates representing four viral types, namely, Una, Mayaro, Ilhéus and Wyeomyia viruses, were obtained from these insects.

Psorophora (J.) lutzii Theob.—A single isolation of Ilhéus virus was yielded by this species.

Psorophora (Grabhamia) cingulata Fab.—One isolate identified as Cache Valley virus was obtained from this species.

Sabethes (Sabethoides) chloropterus Humboldt—This diurnal, arboreal mosquito, which has been found naturally infected with yellow fever,[19] St. Louis[20] and Ilhéus viruses,[21] was common only in upper tropical rainforest associations. A single unidentified viral agent was obtained from this species during the study period.

Trichoprosopon spp.—A variety of species was pooled under this generic heading, namely, *T. (T.) digitatum* Rond., *T. (Rhunchomyia) longipes* Fab., *T. (Ctenogoeldia) magnum* Theob. and *T. (Isogoeldia) espini* Martini. A single viral agent, identified as Bussuquara, was isolated from these mosquitoes.

Phlebotomus spp.—Fairchild and Hertig have identified 43 different species of *Phlebotomus* from material collected in the study area.[22] No effort was made to identify *Phlebotomus* sandflies to species prior to virus isolation attempts. A few samples taken from lots prior to processing revealed that human collections were made up of the following species in order of abundance: *P.*

trapidoi F. & H., *P. yliphiletor* F. & H., *P. sanguinarius* F. & H., *P. pessoana* Barreto, *P. hansoni* F. & H., *P. panamensis* Shannon and *P. shannoni* Dyar. Samples from collections with rodent bait were made up mostly of *P. hansoni*, *P. vespertilionis* F. & H., *P. trapidoi* and *P. ovallesi* Ortiz. *P. panamensis* was the predominant species attacking horses. A total of 21 isolates was obtained from *Phlebotomus* sandflies. One agent was identified as Ilhéus, five were related to the Indiana type of vesicular stomatitis (VSV) and 15 were typed as BT 436 virus. Specimens taken showed a decided preference for human blood, although significant numbers were also found on horses and rodents. They were seldom captured on avian hosts.

Insect species which did not yield viruses. No viral agents were obtained from 31,776 specimens, processed in 430 pools, representing 18 species or species-groups, 10 genera and three families of nematoceran Diptera, namely, Culicidae, Psychodidae and Helcidae.

Identification of virus isolates. The degree to which isolates were characterized depended on the type of virus and availability of reference strains for comparison. Some incompletely identified agents were sent to the Belém Virus Laboratory (BVL) for further characterization. Following is a list of the viral types identified from the study area and the tests and reference strains used to arrive at identity of the GML isolates grouped under each type. Detailed data concerning the identification of certain virus isolates obtained during the survey are presented elsewhere.[1-7]

a) *Venezuelan equine encephalomyelitis virus:* Forty-eight isolations of this virus were made during the survey. The 43 GML virus isolates grouped under this type were compared with the MARU 3880 strain[23] of VEE virus by CF and, in some instances, by cross-neutralization test. The results of these tests indicate very close antigenic relationships between the 3880 strain and all GML VEE virus isolates from Almirante.[6]

b) *Una virus*: Fifteen virus isolates obtained during the survey period were typed as Una virus. Of the seven isolates from GML, two were compared with MARU strain BT 1495-3[3] by cross HI and CF test. Of the remaining five strains, one (BT 444) was sent to BVL where it was identified as Una virus. The other four were found to be antigenically indistinguishable from BT 444 by cross CF test.

c) *Mayaro virus:* The single isolate identified as Mayaro virus was compared by cross HI and CF test with BVL strain BeAr 20290.

d) *Bussuquara virus:* Four isolates were found to be closely related to BVL strain BeAn 4116 by cross HI and CF test.

e) *Ilhéus virus:* Thirteen isolates were referred to this virus type. Two of the 12 strains recovered at GML were previously described by Galindo and Rodaniche.[1] The remainder proved to be identical to the GML Honduran strain[24] of Ilhéus virus by cross CF, cross-neutralization and cross-protection tests in mice.

f) *Caraparu virus:* Three isolates obtained during the survey period were antigenically indistinguishable by cross HI and CF test from BVL strain BeAn 3994.

g) *Nepuyo virus:*[18] The single isolate referred to this virus type was indistinguishable by cross HI and CF test from BVL strain BeAn 10709.

h) *Ossa virus*: This group C agent, originally isolated from a human being in Panama, has been characterized by Rodaniche *et al.*[2]

i) *Madrid virus:* The original strain, also isolated from human blood, was characterized in Panama and Belém.[2] Two additional isolates obtained from sentinel mice were found to be indistinguishable from the prototype strain in cross HI and CF tests.

j) *BT 4971 and BT 5012:* These two types, represented by eight isolates from rodents, are considered to be new members of the group C arboviruses and will be described in a separate publication.[7]

k) *Guamá group:* Twenty-six isolates were referred to this group of arboviruses. Of the 18 isolates obtained at GML, one was found to be identical to MARU strain BT 640[8] in cross CF and hamster protection tests. Another strain, isolated from a pool of *Culex* (*Melanoconion*) spp. mosquitoes collected in upland tropical rainforest, was found to be related to but distinct from BT 640 by cross CF and cross-protection tests in hamsters. The remaining 16 strains obtained at GML were indistinguishable by cross CF test from each other. In cross CF and neutralization tests with BVL Guamá (BeAn 277), Catu (H 151) and Moju (BeAn 12590) strains, BT 640 and several representative GML isolates proved to be more closely related to Guamá than to Catu or Moju viruses. In summary, two distinct types of Guamá-group arboviruses have been found in Almirante. One of these occurs commonly in the lowlands in *Culex vomerifer*

mosquitoes and was frequently detected in sentinel mice exposed to the bites of these insects. The other type has been isolated only once from a pool of *Culex* (*Melanoconion*) spp., which was probably *C. elevator*, collected at the Yellow Fever station in upland tropical rainforest. The specific identity of the two types is still in doubt.

l) *Cache Valley Virus*: A single isolate was obtained at MARU and referred to this virus type at that laboratory.[3]

m) *Guaroa virus:* Three isolates have been assigned to this virus type. The two strains isolated at GML were compared with MARU strain BT 1122[3] by cross CF and hamster protection test and found to be closely related.

n) *Wyeomyia virus:* Six isolates were referred to this type of arbovirus. The two isolates obtained at GML were found to be identical to MARU strain BT 219[3] by cross CF and neutralization test. Thus far it can only be said that the Almirante strains belong to the Wyeomyia sub-group of arboviruses since additional work is necessary to type these strains within the Wyeomyia complex.

o) *Vesicular stomatitis virus (Indiana):* Five isolates obtained in the survey were assigned to this virus type. The single agent obtained at GML could not be distinguished from MARU strain BT 78[3] by cross-neutralization test.

p) *BT 436:* Fifteen isolates obtained from *Phlebotomus* sandflies and one from human blood have been assigned to this new virus type described elsewhere.[5] Of the 10 isolates obtained at GML, six were related to BT 436 by CF test at MARU. The remaining four were related to each other and to BT 436 by CF test at GML.

Annual cycle of virus activity: As would be expected in a tropical rainforest area, arbovirus activity was not greatly inhibited at any time of the year throughout the study period. This is due to the fact that there is no marked dry-season in Almirante and, as a consequence, there is continuous production of blood-sucking insects. However, there are very definite seasonal fluctuations in virus activity which probably depend on changes in population densities of the insect vectors. Table 5 presents the details by months of all arboviruses isolated in the study area from September 1959 to September 1962. As may be noted, those viruses, such as VEE, Una and Guamá, which are transmitted by mosquitoes were most active during or immediately after the months of peak mosquito production, namely, June, July and August. On the other hand, viruses which are *Phlebotomus*-borne, namely, VSV (Indiana) and BT 436 virus, seemed to be more active from October through March, with only two isolations recorded between March and October. In subsequent years, as data from additional studies are compiled, the pattern of activity of the different arboviral antigenic entities recognized in Almirante should become clearer.

The habitat and arbovirus activity: The first part of this paper presents a rather exhaustive description of the study area and of the collecting stations, discussing in detail the main ecological associations where collections of vertebrates and/or blood-sucking insects as well as exposures of sentinel mice were carried out for virus isolation attempts. In the case of indigenous vertebrates and sentinel mice the exact locality was noted, so that any specimen yielding an arbovirus could be traced back to the environment where it was taken or exposed. In collections of blood-sucking insects, the exact locality of each identified lot was also noted. However, occasionally, separate lots of one species collected in different habitats were included in a single pool for inoculation in mice, so that not all of the insects which yielded viruses could be traced back to the habitat in which they were captured.

Table 6 presents the total number of isolates from all sources, classified according to the ecological association in which the organism yielding the virus was collected or exposed. Following is an analysis by habitat of the data presented.

"Cerillo-sangrillo" swamp forest—This was the most productive of all habitats, as 66 of the 169 isolates obtained originated from material collected in it. Some viruses which were isolated several times from animals taken in this environment were also yielded by insects captured in an upland tropical rainforest association. These viruses were Una, Guaroa, Wyeomyia and BT 436. Most of the lowland material which yielded these four viruses came from a well-drained forest at Mile-2 where the "cerillo-sangrillo" dominance was greatly diluted. Such forests can be considered intermediate between swamp forest and upland tropical rainforest. It is thus possible that the hosts of these viruses may actually be primary inhabitants of the upland type of rainforest which secondarily invaded the intermediate type of forest found at Mile-2. The "cerillo-sangrillo" association appeared to be the preferred habitat of the hosts of two arboviruses common in the study

TABLE 5

Virus isolations by months from all sources (1959–1962)

Virus	Month												
	January	February	March	April	May	June	July	August	September	October	November	December	Totals
VEE			4	3	4	14	11	4	4	2	2		48
Una			1			2	4	5	1	2			15
Mayaro								1					1
Bussuquara					1				1	1	1		4
Ilhéus	2	2	2			1	1			1	4		13
Caraparu								2	1				3
Nepuyo						1							1
Ossa	1												1
Madrid			1				1	1					3
BT 4971						2	1						3
BT 5012						3	2						5
Guamá group	1				2	2	8	6	4	1	2		26
Cache Valley	1												1
Guaroa						1			2				3
Wyeomyia							3			2		1	6
VSV (Indiana)	1	1				1				1		1	5
BT 436	3	5	3			1				1	3		16
Undetermined isolates	1					2	6	1	3			2	15
Totals	10	8	11	3	7	30	37	20	16	11	12	4	169

area, namely, Ilhéus virus and the more prevalent of the two Guamá-group agents thus far recognized. Although VEE virus was not infrequently found in animals taken in "cerillo-sangrillo" forest it was more commonly isolated from inhabitants of more open environments.

Upland tropical rainforest—Twenty-eight viral agents were isolated from specimens collected in this environment and of these, 14 came from *Phlebotomus* sandflies. With the exception of Ilhéus virus, which appears to be quite ubiquitous, viruses found in hosts inhabiting this ecological association were generally localized there to the exclusion of all other types of habitats. Thus Ossa, VSV (Indiana) and an unidentified Guamá-group virus were found only in animals taken in upland forest. Although Una, Guaroa, Wyeomyia and BT 436 viruses were also isolated from specimens inhabiting a well-drained "cerillo-sangrillo" forest, as previously explained, that forest was of an intermediate type and cannot be considered a typical swamp forest association.

Cacao plantation—Numerous collections of vertebrates and insects were made in a cacao plantation at Nigger Creek in which the tallest trees of an original "cerillo-sangrillo" forest were being used as shade. Only six viral agents were isolated from animals taken in this plantation. Five of these were recognized as viral types typically found in inhabitants of swamp forest or open fresh-water marsh associations.

"Silica palm" swamp forest—This type of environment, which is almost always flooded to fairly high levels, yielded two isolations of VEE virus.

Edge of open fresh-water marsh—This habitat, characterized by a very tall cover of grasses and weeds and sustaining an abundant population of cotton rats and of *Culex* (*Melanoconion*) mosquitoes, was the most productive habitat for VEE virus and the group C agents. Of the six types of group C viruses thus far recognized in Almirante, five were recovered from material collected in this environment and two were found there exclusively. Three isolations of Ilhéus virus and four of the common Guamá-group viral type were also yielded by animals of this habitat.

Peridomestic and early second-growth habitats—Specimens from this generic type of environment

TABLE 6

Ecological associations as sources of arboviruses

Virus	Habitat								
	"Cerillo-sangrillo" swamp forest	Upland tropical rainforest	Cacao plantation	"Silica palm" swamp forest	Edge of open fresh-water marsh	Peridomestic and early second-growth	Domestic (intradomiciliary)	Undetermined habitat	Totals
VEE	6		1	2	18	14	2	5	48
Una	10	4						1	15
Mayaro	1								1
Bussuquara	3		1						4
Ilhéus	7	1	1		3	1			13
Caraparu					3				3
Nepuyo					1				1
Ossa		1							1
Madrid	1				1			1	3
BT 4971	1				2				3
BT 5012			1		4				5
Guamá group	19	1	1		4			1	26
Cache Valley	1								1
Guaroa	1	2							3
Wyeomyia	3	3							6
VSV (Indiana)		5							5
BT 436	7	9							16
Undetermined isolates	6	2	1		2	1		3	15
Totals	66	28	6	2	38	16	2	11	169

yielded 16 arboviruses, of which one was recognized as Ilhéus and 14 as VEE virus. However, it should be pointed out that three of the human VEE virus isolates included in this category may have originated in a domestic (intradomiciliary) habitat.

Domestic (intradomiciliary) habitat—This category included exclusively collections of blood-sucking insects made inside human dwellings at Patoistown. Two isolations of VEE virus were yielded by *Culex quinquefasciatus* mosquitoes taken in this habitat.

SUMMARY

A three-year survey was conducted to determine the arboviruses active in a tropical rainforest area of Panama. Materials for virus isolation attempts consisted of blood specimens from febrile patients, sera and tissues from domestic and wild vertebrates, blood-sucking insects taken on a variety of baits and sentinel mice exposed in the field. These specimens were collected or exposed in a variety of habitats which included upland tropical rainforest, swamp forest, open fresh-water marsh and domestic and peridomestic habitats. Following is a list of the arboviruses isolated and the sources from which they were obtained: *Venezuelan equine encephalomyelitis:* human blood, wild rodents, sentinel mice, adult birds, nestling birds, mosquitoes; *Una*: mosquitoes; *Mayaro*: mosquitoes; *Bussuquara*: sentinel mice, mosquitoes; *Ilhéus*: adult birds, mosquitoes, *Phlebotomus* sandflies; *Caraparu*: sentinel mice; *Nepuyo*: wild rodents; *Ossa*: human blood; *Madrid*: human blood, sentinel mice; *BT 4971* (new group C agent): wild rodents, sentinel mice; *BT 5012* (new group C agent): wild rodents, sentinel mice; *Guama group*: sentinel mice, mosquitoes; *Cache Valley*: mosquitoes; *Guaroa*: mosquitoes; *Wyeomyia*: mosquitoes; *Vesicular stomatitis (Indiana)*: *Phlebotomus* sandflies; *BT 436* (new arbovirus type): human blood, *Phlebotomus* sandflies.

The report concludes with a discussion of annual cycles of virus activity and considerations on the habitat in relation to arbovirus activity.

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