

A Survey to Assess Potential Human Disease Hazards Along Proposed Sea Level Canal Routes in Panamá and Colombia. IV. Arbovirus Surveillance in Man

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As part of the 1966-68 sea level canal feasibility studies conducted by the Office of the Field Director, Atlantic-Pacific Interoceanic Canal Study Commission (A-PICSC), Gorgas Memorial Laboratory (GML) conducted certain medico-ecological investigations along the two proposed canal routes, in addition to the ecological studies described elsewhere in this series.^{1,2} Under a grant from the US Army Medical Research and Development Command, GML initiated a study for disease surveillance in individuals engaged in the feasibility studies, both those indigenous to the proposed canal routes and those entering from other areas.

This communication describes our findings on arbovirus infections in man during the course of this disease surveillance.

Materials and Methods

The plan of study was to examine all non-indigenous individuals, including visitors, before they entered the route areas, periodically while there, during any illness, and upon the termination of their work. Indigenous workers were studied similarly. It was thus possible to determine what infections had been acquired. Individuals were grouped for purposes of this study according to several factors. The majority of workers designated as Group I are US citizens and mostly from outside the Canal Zone and Panamá City. It consisted mainly of Department of Army civilians, Panama Canal Company employees, and US contractors and subcontractors. Group II was composed of Panamanian citizens hired by the Office of the Field Director from personnel living in the Canal Zone and Panamá City, and personnel locally hired on the routes and residents of the two areas being studied. Group III consisted of indigenous workers from Route 17, and Group IV indigenous workers from Route 25. These two groups also included certain isolated populations of natives who are permanent residents of the route areas. Whenever indicated, medical teams were sent to investigate specific outbreaks of disease in native

populations living in the vicinity of the proposed canal routes.

Study Areas

The two proposed sea level canal routes (Routes 17 and 25) which represent the areas of exposure for the study subjects are described in detail in earlier papers of this series.^{1,2}

Collection of Samples

Blood specimens were obtained from all persons participating in the feasibility study. To detect arbovirus infections contracted during the study period, serial bleedings were scheduled for subjects in Groups I and II at three to six month intervals. Unfortunately, second and subsequent bleedings were not obtained for many of the subjects (Table I). This was particularly true for visitors, consultants, and subcontractors, many of whom spent as little as a week or so on the routes. Single serum samples of Groups III and IV were studied for the prevalence of arboviruses in their respective areas. A small number of second samples were obtained from certain families of local employees. In addition, blood specimens for virus isolation were collected from people working in the areas who came down with febrile illness. Convalescent-phase serum samples from these cases were also obtained for serological studies.

Collections of the first blood specimens in Group I were initiated early in 1966, when the personnel of the Office of the Field Director, A-PICSC, began to arrive in the Canal Zone for work. The Medical Support and Research Division of the Office of the Field Director was responsible for sample collections and other pertinent data needed for baseline examination. Most of the specimens collected as part of the medical survey of workers on Route 17 were obtained during 1967. In the original concept, collection of field data on the two routes was to be performed concurrently. Since collection of data on Route 17 started well ahead of Route 25, however, some individuals included in the survey of Route 17 in Panamá were shifted to work on Route 25 in Colombia.

Virus Isolation Attempts

Blood sera obtained from febrile patients during the acute phase were inoculated intracerebrally (IC) into groups of seven newborn (two to three day) Swiss mice[‡], and also

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‡In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

into paired culture tubes of African green monkey kidney cell line (Vero). The mice and culture tubes were observed for any sign of illness and cytopathic effect (CPE) over periods of one to two weeks. When isolation of viruses were obtained, reagents were prepared for antigenic identification.

Serological Techniques

Sucrose-acetone extracted antigens³ were used for hemagglutination-inhibition (HI) and complement-fixation (CF). Periodic serum samples were examined for antibodies to arboviruses known to occur in Panamá. HI testing antigens included the following: Group A: Venezuelan equine encephalitis (VEE), Eastern equine encephalitis (EEE), Una (UNA) and Mayaro (MAY); Group B: Ilheus (ILH), Yellow fever (YF), St. Louis encephalitis (SLE) and Bussuquara (BSQ); Group C: Ossa (OSSA), Madrid (MAD) and Nepuyo (NEP); Group Patois: Patois (PAT) and Zegla (ZEG); and Phlebotomus fever group: Chagres (CHG). Sera were treated with kaolin for removal of non-specific inhibitors. Screening tests of sera at dilutions of 1:10 and 1:20 and titrations of the positive samples were conducted in a microtitration system. Upon detection of differences in titers of the serial bleedings from an individual, tests were repeated for all periodic samples in lucite plates. Single serum titers of 1:40 or more against four units of antigen and antibody conversions of more than four-fold differences were considered positive only to the highest reacted antigen in the same group.

For CF tests, seven antigens, i.e., Guama (GMA), Cache Valley (CV), Guaroa (GRO), Wyomyia (WYO), Melao (MEL), Changuinola (CGL), and Vesicular stomatitis-Indian type (VSI), were included. Serum titers of 1:4 or higher were considered positive. Neutralization tests were performed in Vero cell cultures, using a constant-virus (approximately 100 TCID₅₀), varying-serum dilution technique. The serum-virus mixtures were incubated at 4°C overnight before inoculation into culture tubes.

Results

Clinical Case Studies

A total of 58 febrile patients were examined. Blood samples drawn during the acute phase were inoculated, both into baby mice and Vero cell cultures. Viruses were isolated from serum specimens obtained from four patients as follows:

Case 1. A male Cuna Indian (A-PICSC No. 1035), age 29 years, was ill with a fever of 103°F, headache and vomiting. He was locally employed on Route 17. A group C arbovirus was isolated from his blood sample drawn on April 19, 1967. Just before his illness, he had worked in Coredo, Rio Sabana area of Route 17.¹ The patient's infection subsided within a few days. This strain was identified as Ossa virus which had never been found before in the Darién Province of Panamá. Reisolation attempts from acute-phase serum, after two-year storage at -65°C, were successful.

Case 2. A male Caucasian US employee (A-PICSC No. 0214), age 26 years, was ill with a fever of 103.8°F, severe headache, nausea and vomiting on the first night of illness. A group A virus was isolated from his blood sample taken on August 3, 1967. At

TABLE I
CLASSIFICATION OF BLOOD SAMPLES INCLUDED
IN SEROLOGICAL STUDIES

Serial Bleeding	Group I Foreign Personnel	Group II Local hire Personnel	Group III Indigenous Rt. 17	Group IV Indigenous Rt. 25	Total
First	519	209	176	61	965
Second	194	82	3	0	279
Third	96	35	1	0	132
Fourth	61	0	0	0	61
Fifth	28	0	0	0	28
Sixth	9	0	0	0	9
Seventh	1	0	0	0	1
Total	908	326	180	61	1,475

the time of his illness and for two weeks earlier he had worked in the Rio Atrato area (near Hacienda Sautatá) on Route 25.¹ He recovered without sequelae after a few days of illness. The virus was identified as VEE and a significant rise in antibody titer was shown in his convalescent-phase serum obtained about three weeks after the onset of symptoms.

Case 3. A male Caucasian US employee (A-PICSC No. 0239), age 34 years, was ill with a fever of 104°F and severe headache, joint pain, muscular pain, backache and pain in the eyes without nausea nor vomiting. Fever occurred during September 27 and 28, 1967. A virus was not isolated from his blood drawn on the second and third day of illness, but VEE was recovered from a throat swab washing obtained on the second day (September 28). The patient apparently contracted VEE infection when he worked at Loma Teguerré on Route 25.¹ His illness lasted for a few days and his recovery was uneventful. VEE antibody rise was shown in his blood sample obtained three weeks after the onset of fever.

Case 4. A male Colombian (A-PICSC No. 2047), age 20 years, was ill with mild fever, a chilly sensation, headache and diarrhea. He worked temporarily on a cattle farm at Santa Fé on Route 17.¹ VSI virus was isolated from his blood drawn on September 16, 1967, the first day of his illness. During convalescence, he was not available and we were unable to secure blood samples for serological studies. Reisolation attempts from acute-phase serum after two months storage at -65°C were unsuccessful. Result of virus isolation from this case was also included in the report of the overall VSI virus studies at GML.⁴

Of the remaining 54 febrile cases from whom no viruses were isolated, only 25 convalescent-phase sera were obtained for serological tests. No significant increases in antibody titers were observed in the 21 arboviruses tested.

Serological Studies

A total of 965 individuals were included in the serological studies for arbovirus antibodies. The number of persons in each of the four groups tested are shown in Table I. Serial bleedings within each group are also shown. Altogether, 1,475 serum samples were tested with 21 antigens.

Base-line Findings. The results of HI tests of samples taken initially to determine what antibodies were present in persons (usually group I) about to go into the route areas, and those in the native populations as shown in Table II. Of the first group of samples, 519 sera were tested. Six were positive for group A and 313 for group B. Most of the group B reactivities probably resulted from YF-vaccination which

was required prior to entering the study areas. If this is the case, then, any infections by group B viruses in these individuals were obscured.

The local-hire and indigenous native Indian (Groups II, III, IV) sera showed different results. Approximately 56 per cent showed antibodies to one or more viruses. There was evidence of past infections with VEE, MAY and/or other group A viruses. As for group B reactions, there were no records of YF vaccination having been given to the indigenous populations in this area. The relatively high reaction rate of these people for group B viruses could have been due to YF which has been present sporadically in the area³ since 1948, or to other group B viruses which have been isolated from man in Panamá (i.e., ILH,⁶ SLE⁷ and BSQ⁸).

Similar results were obtained from these baseline samples when tested by CF with GMA, CV, GRO, WYO, MEL, CGL, CHG and VSI antigens. All sera from Group I personnel were negative for the eight antigens. Five persons in Group II were positive (three with CGL and two with VSI). Approximately 10 per cent of both Groups III and IV had CF antibodies to one or more arboviruses. Positive reactions in order of frequency were: CGL, MEL, WYO, VSI and GRO.

In order to confirm the finding of VSI in man, neutralization tests were performed with 132 sera collected from indigenous people on Route 17 (group III). Twenty-two per cent were positive for neutralizing antibodies to VSI virus.

Antibody Conversions. Consecutive serological examinations were performed on sera from Group I, who were presumably the group most susceptible to the local viruses. A total of 389 specimens from serial bleedings were tested. Table III lists all individuals who probably contracted virus infections in the field. During the period covered by these data, most of these persons were working on Route 17. Where tests were positive to more than one antigen in the same serological group, antibody titers are shown only to the highest reacted antigen.

TABLE II
RESULTS OF HEMAGGLUTINATION-INHIBITION TESTS
ON BASE-LINE SAMPLES*

Antibodies to	Group I	Group II	Group III	Group IV
Group A				
VEE	2	25	4	22
Mayaro	0	6	14	7
Other Gr.A	4	6	3	0
Group B	313	114	78	28
Groups C and Patois	0	5	0	1
Group Phlebotomus	0	4	0	0
Total positives**	313	121	89	41
Numbers tested	519	209	176	61

*Tests were performed with the following antigens:

Group A: VEE, EEE, Una, Mayaro

Group B: Ilheus, SLE, YF, Bussuquara

Groups C and Patois: Ossa, Madrid, Nepuyo, Patois, Zegla

Group Phlebotomus: Chagres

**Only positive individuals were counted. More than one virus may have been present in the same subject.

In Group I personnel, eight persons developed significant increases in viral antibody titers. One subject was positive for Group A, MAY virus. The remaining seven positives were due to Group B infections, ILH, BSQ and/or SLE viruses.

Of 82 persons in Group II from whom serial samples were obtained, four showed significant rises in antibody titers. Two cases probably contracted multiple infections, both by one of the viruses in group A and one in group B. The other two persons showed CF antibody conversions to CGL, a recently recognized human pathogen in Panamá.⁹

Discussion

Prior to the present study, most studies of arbovirus ecology in the Republic of Panamá were made in the north-western part of the country, especially in Almirante.⁹ At least 17 virus types have been isolated from that area. In contrast, little was known regarding the occurrence of arboviruses in Eastern Panamá or the Darién area. Only YF, SLE and WYO¹⁰ had been recovered there. The engineering feasibility studies for a sea level canal in that area provided an opportunity to gather considerable information regarding presence of viruses in areas east of the present Panama Canal.

The clinical course of human infections with arboviruses varies from mild febrile illness to severe headache, with or without signs of central nervous system involvement. Certain of these viruses have caused hemorrhagic manifestations.¹¹ Often the infection is subclinical or unrecognized, and is detectable only through the subsequent appearance of specific antibodies in the blood. The presence of viruses along the two proposed canal routes was revealed by serological findings in indigenous population groups and by the occurrence of clinical viral infections in A-PICSC survey personnel, from whom virus isolations were made and/or significant rises in antibody titers were observed. As a result of this study, at least eight arbovirus types, i.e., VEE, MAY, BSQ, ILH, SLE, OSSA, VSI and CGL, were shown to be present in the proposed route areas during 1966 and 1967. Except for MAY and VSI, all other viruses have previously been isolated from man in other areas of Panamá. VSI and CGL viruses have been isolated from phlebotomine sand flies, while the six other viruses mentioned above have been repeatedly recovered from culicine mosquitoes in various parts of the country.

It should be emphasized at this point that several arbovirus infections in non-indigenous personnel did occur, despite the screened buildings, bed nets etc. and that a greater preventive effort would be necessary to prevent cases and/or outbreaks during the canal construction.

In regard to the potential health hazards posed by any of the viruses found in the area, no known epidemics of these viruses have occurred in Panamá, although VEE virus was widespread among human, wild vertebrate and mosquito populations in the Almirante area.^{9,12} This virus also has been responsible for several cases of febrile illness in man in the village along the present Panama Canal,⁸ including one fatal case.¹³ It has caused severe outbreaks with

many cases in Colombia and Venezuela.^{13,14} Usually, however, it has been a relatively mild uncomplicated febrile illness.

Although MAY virus never has been isolated from man in Panamá, serological findings in this study indicate that this virus or a closely-related one probably exists there. Different strains of MAY virus have been responsible for outbreaks of febrile illness in Trinidad and South America¹⁶

TABLE III
RESULTS OF ANTIBODY CONVERSION STUDIES

Personnel Group	Number	Dates of Samples Obtained	Virus	Titers
I	0028	2-4-66, 12-3-66	Mayaro	0-1:160
	0057	3-21-66, 5-23-67	Bussuquara	1:80-1:640
	0197	5-9-67, 11-6-67	Bussuquara	0-1:1280
			SLE	0-1:1280
	0212	11-30-66, 5-2-67	Ilheus	0-1:160
			Bussuquara	0-1:160
	0220	12-1-66, 5-2-67	SLE	0-1:320
	0223	12-8-66, 3-14-67	SLE	0-1:640
	0242	1-5-67, 5-2-67	Ilheus	0-1:160
	0302	3-8-67, 6-26-67	Bussuquara	0-1:80
II	1154	6-5-67, 8-27-67	VEE	0-1:1280
			Ilheus	0-1:1280
	1160	5-25-67, 7-29-67	Mayaro	1:320-1:1280
			Ilheus	0-1:160
	1011	2-27-67, 6-1-67	Changuinola*	0-1:16
1014	2-27-67, 9-11-67	Changuinola*	0-1:8	

By CF tests, 0 = 1:4; others by HI tests, 0 = 1:40 (the lowest dilution tested)

since 1954. This virus was once recovered from mosquitoes in Almirante, Panamá.⁹

Because SLE virus has been the causative agent of several serious outbreaks of systemic febrile disease with encephalitis in the United States, it is significant that the virus was found in the Route 17 area in Panamá, and especially important that it was identified in human cases. Two isolates have been made from field workers and numerous strains have been obtained from several genera of mosquitoes.⁷ However, although no SLE virus was isolated during the present study on Route 17, antibody conversions were shown in at least two or three cases, indicating the existence of this virus in the area.

Several isolates of the OSSA virus have been recovered from man, sentinel and wild vertebrates and mosquitoes in the Almirante area, ever since the first discovery in 1961 of the virus in man in that area of Panamá. Isolation of this virus from a fever case in the present study represents the first recovery of Ossa virus in eastern Panamá.

The presence of other viruses in the area being surveyed, (BSQ, ILH, VSI and CGL) is probably of minor importance, since no outbreaks caused by any of these viruses have ever occurred in man. It is difficult to say at this time whether or not a particular virus has any practical or public health significance, especially when only a single isolation has been made. However, it is possible that any of these

viruses might cause outbreaks of disease in non-immune populations when epidemiological conditions are favorable. For example, an epidemic of Oropouche virus involving more than 7,000 human cases occurred in Brazil about six years after the first and only isolation of this virus in Trinidad.¹⁷

It is noteworthy that other investigators have reported human cases of MAD, CHG and PT infections in Panamá. MAD was discovered in man in Almirante⁹ and was found later in sentinel and wild vertebrates in Colombia¹⁸ on Route 25 of the proposed canal routes. The other two viruses were isolated from students at the Jungle Warfare Training Center, located on the Atlantic side of the Canal Zone.^{19,20} Two additional strains of CHG virus were obtained from febrile patients in the villages around Gatun Lake.⁸ No sources other than man have been demonstrated for CHG and PT viruses, which are antigenically classified in the *Phlebotomus* fever group of arboviruses.

In the present study, results of virus isolation attempts and serological findings indicated that transmission of certain arboviruses occurred during the rainy season of 1967. Only a few isolated groups of Indians inhabit the route areas under study, and they are so remotely located that virus infections have not been investigated. It is quite possible that upon movement of large numbers of personnel into the area for large scale construction activities, any of these viruses which are now known to occur there could take on new significance as potential health hazards.

Summary

Arbovirus disease surveillance studies were performed on blood specimens from personnel engaged in engineering surveys for a proposed sea-level canal in Panamá and Colombia. A total of 519 US and other foreigners (Group I) and 209 Panamanian citizens from the Canal Zone and Panama City and on-route locally hired personnel (Group II) participated in this study. Two hundred and thirty-seven people indigenous to the route areas (Groups III and IV) also were included. Of 58 febrile cases studied, four virus isolations—VEE (2), Ossa (1) and VSV-Indiana (1)—were made. Serological tests with initial blood samples of foreigners showed that very few were positive for any of the 21 arboviruses known to occur in Panamá. In contrast, 56 per cent of samples from locally-hired individuals and indigenous populations were positive.

Tests of serially collected blood samples from Group I personnel, working mostly on Route 17 in Panamá, showed eight individuals with significant rises in viral antibody titers, one as a result of Mayaro, Group A virus and the others with one or more Group B arboviruses. Of 117 second or third samples obtained from Group II personnel, four cases were positive for antibody conversions and showed two with mixed infections of groups A and B and the other two with Changuinola virus. The latter is a newly recognized virus in humans in Panamá. These studies revealed at least eight arboviruses, some of which might be potential health hazards for non-immune or large populations moved into the two route areas for construction of the proposed interoceanic canal.

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