A Survey to Assess Potential Human Disease Hazards Along Proposed Sea Level Canal Routes in Panama and Colombia. III. Survey Methods

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THE objectives and philosophy of the medico-ecology survey of two proposed sea level canal routes in Panama and Colombia are discussed elsewhere.[6] It is appropriate to restate, however, that the survey methods described in this paper were chosen in the face of a two-year time limitation and a small budget. Table I shows, moreover, that the time eventually spent on the canal routes fell far short of two years. This paper will discuss the sources of information and field collection techniques upon which subsequent papers in this series are based.

Sources of Information

Existing Knowledge

There was little specific published medical information concerning the actual canal routes or the land immediately adjacent to them. A considerable amount of research had been done, however, on reservoir-vector-pathogen relationships in areas of Panamá and Colombia similar ecologically to the study areas. There was also some information available in reports of sickness and injury in workers involved in construction of the Panama Canal. In certain instances, information contained in the literature proved invaluable. This was especially true in the case of taxonomic literature on animals of medical importance, and in the many studies carried out in other parts of Panamá and Colombia on reservoirs and vectors of arthropod-borne diseases. Most of this information has been published over the years by the staffs of The Gorgas Memorial Laboratory (GML) and The Middle America Research Unit (MARU). Since few attempts were made in the present study to incriminate vectors of disease by the isolation of pathogens from arthropods, many conclusions in this area hinge on information contained in the literature.

Studies of Disease in Humans

Human Sentinel Survey.‡ Many of the individuals entering the study areas for the purpose of participating in the sea level canal feasibility studies had never before been to the tropics, let alone to Panamá or Colombia. In order to take advantage of this situation, it was decided to obtain blood samples of all study personnel before their initial trip to the study area. Included in this survey were all individuals not indigenous to the area and who were employed by the Atlantic-Pacific Interoceanic Canal Study Commission (A-PICSC) or any of its contractors. Subsequently, all individuals were re-bled at about three-month intervals or at the time of any febrile illness. In addition, stools were periodically collected from study personnel at irregular intervals. The handling and testing of these specimens will be discussed in greater detail in a subsequent paper.

Dispensary Reports of Disease. Personnel working on the routes received medical care from base camp dispensaries or from first aid stations located in field camps.[5] In addition, local individuals were provided emergency medical treatment. Dispensary and first aid station records furnished considerable information on disease occurring in the areas.

Special Human Disease Studies. At various times during the study, local human populations were studied intensively. This usually followed information received concerning large numbers of disease cases in a particular village or concerning a particular problem, such as the discovery of chloroquine-resistant falciparum malaria on the Pacific coast of Colombia in 1967.[1]

Studies of Potential Vectors and Reservoirs of Disease

The bulk of the medico-ecology information accumulated was obtained through the collection of vertebrate and invertebrate animals from the canal routes. Most of the vertebrates collected consisted of small rodents; lesser numbers of birds, reptiles, amphibians, and large mammals were collected. The principal invertebrates collected were arthropods: mosquitoes; sand flies; black flies; horse and deer flies; ticks, mites, triatomid bugs and other miscellaneous ectoparasites. The procedures used in making these collections comprise the remainder of this paper.

Field Collections

Areas of Collection

Collections were made only in and around a few locations on each route. Collection areas were chosen to be geographically representative of the routes and to furnish access to the major ecological associations present.[2] The collecting areas are shown in Fig. 1 (Route 17) and Fig. 2 (Route 25). A brief description of each area follows (Areas were numbered to facilitate machine data processing):

Area 1 (Santa Fé Camp, Route 17), on the Río Sabana,

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is located on the broad Pacific coastal plain. The climax vegetation here is classified as Transitional Tropical Moist/Dry Forest.[3] Well-drained soil is covered by a deciduous forest dominated by the cuipo (*Cavanillesia plantanifolia*). Several subclimax associations are also present. The area includes the farthest limit of tidal influence on the Río Sabana, thus the northern-most limit of mangrove swamps. Several extensive stands of mangrove are present, although they do not approach the extent of stands along the lower Sabana and other tidal estuaries adjacent to the Gulf of San Miguel. Much of the land in this area is subject to continual clearing and burning and to grazing by cattle. Where such land is no longer used for this purpose, it is gradually reverting to forest, and is covered by guarumo (*Cecropia sp.*), balsa (*Ochroma sp.*), and other secondary tree species. Although some hills having elevations in excess of 300 m occur within few kilometers of this area, all collections were made at elevations of 20 m or less.

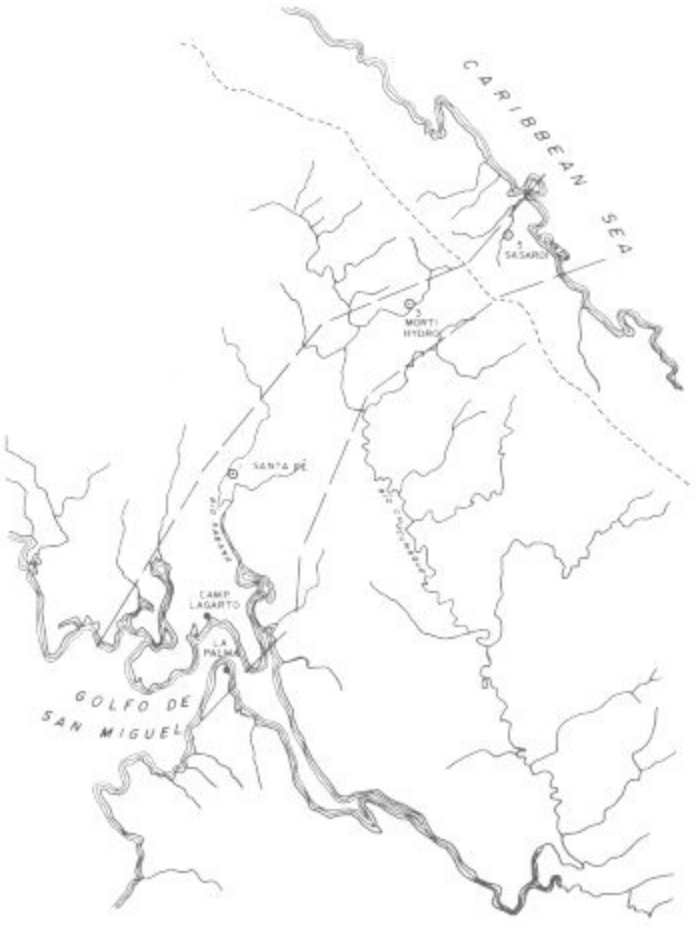

Fig. 1. Route 17, eastern Panamá.

Area 3 (Mortí Hydro Camp, Route 17) is located on the Pacific slope of the Serrania del Darién. It is crossed by the Río Mortí. The camp itself is approximately three airline kilometers upstream from the Indian village of Mortí Arriba. The area is marked by sharply rising hills with elevations ranging from 100 to 300 m. This area falls within the Transitional Tropical Moist/Dry Forest Life Zone,[3] although by casual inspection the zonal vegetation appears more hydrophytic than does that of Area 1. There is very little flat land in this area, and much of the collecting was done on steep slopes. Most of the land is covered by what appears to be primary forest. A few man-made clearings are present and some areas are apparently undergoing secondary succession.

Area 5 (Sasardi Camp, Route 17) is located at the junction of the steep northern (Atlantic) slope of the Serranía del Darién and the narrow coastal plain. The life zone is Tropical Moist Forest.[3] Clearing for agriculture has been very extensive, so that mature forests are almost completely gone from the coastal plain and are usually found only on the mountain slopes. On the plain, secondary successional stages from open field to secondary forest are present. There are also scattered poorly drained depressions supporting swamp forest (Fig. 3). Between the camp itself and the coast are extensive stands of coconut palm. Along the shores, a few narrow bands of mangrove grow at the mouths of streams. Several streams traverse this area, the most significant being the Río Cuadi. These streams drain the area of maximum precipitation on Route 17, and are subject to frequent wet season flooding. This factor, coupled with topographical conditions, have produced an area of great potential for the breeding of vector mosquitoes.

Area 2 (Teresita Camp, Route 25) is located across the continental divide from Area 4 in the eastern foothills of the Serranía de Baudó. The Río Truando, a major tribu-

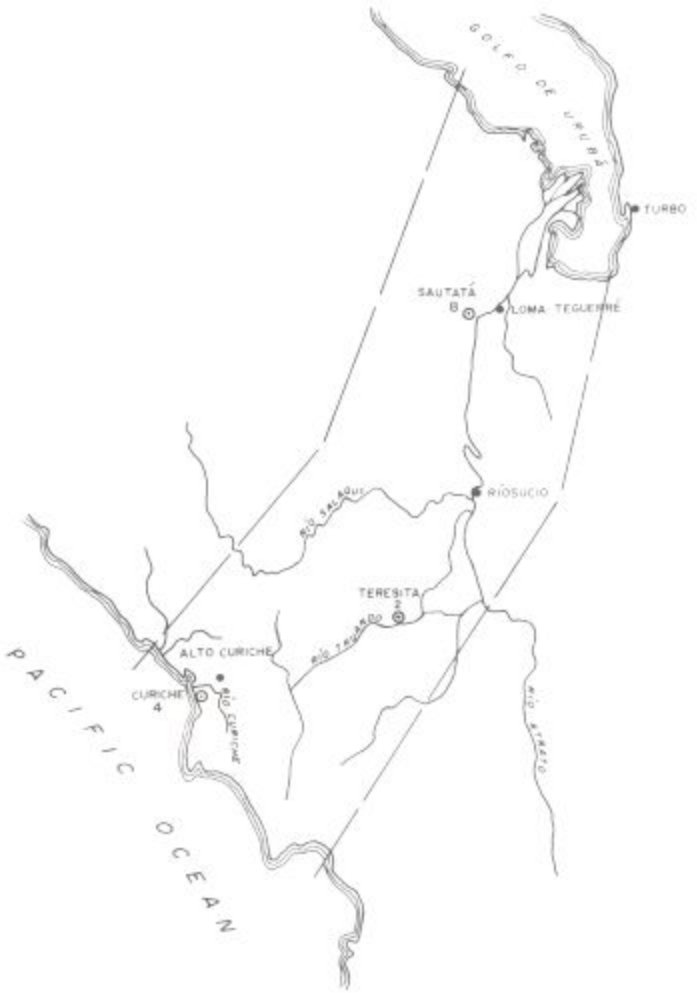

Fig. 2. Route 25, northwestern Colombia.

tary of the Río Atrato flows across this area, as does the Río Salado, a small tributary of the Truando. The land between these streams is subject to frequent flooding and supports considerable swamp forest. The area is included in the Transitional Tropical Moist/Wet Forest Life Zone.[10] Although considerable logging has occurred here, extensive stands of mature evergreen forest are still present, especially on mountain slopes and on land away from large streams. Human activity has been largely restricted to lands adjacent to the Río Truando; consequently, such lands now are either in cultivation or in various early successional stages.

Area 4 (Curiche Camp and Alto Curiche, Route 25) comprises the very narrow coastal plain between the western-most ridge of the Serranía de Baudó and the Pacific Ocean and between the villages of Guarín and Curiche. The area is within the Tropical Wet Forest Life Zone.[10] A series of edaphic-hydric associations is present beginning with narrow bands of mangrove swamp along tidal estuaries. Behind these, on slightly higher and less saline ground are narrow bands of alconorque (*Mora oleifera*), then wider bands of cativo (*Prioria copaifera*). On some well drained sites of the coastal plain and on the slopes and ridge behind the plain, a mixed evergreen forest is present which shows little evidence of disturbance. On most well-drained land lying close to the ocean shore, however, much clearing for agriculture has taken place and early successional stages are present. Some of this land is covered by dense stands of species of *Helicona* and *Calathea*. Alto Curiche Weather Station is located on the mountain ridge overlooking Camp Curiche on the Pacific Ocean, and is surrounded by mixed evergreen forest. All collections here were made at elevations ranging between 250 and 300 m.

Area 8 (Río Atrato Barge, Route 25) is located on the lower Río Atrato in the vicinity of Hacienda Sautatá, a large cattle ranch. The area falls into the Tropical Moist Forest Life Zone.[10] The ecology is completely dominated by the edaphic-hydric associations present on the enormous flood plain of the Río Atrato. Except on river levees, only grass and sedge marshes and palm swamps are present. The river levees support a well developed forest, although in some areas, for various reasons, it is reduced or even absent. At Hacienda Sautatá, the Río Atrato lies very close to the foothills of the Cordillera, and evergreen forest is present on slopes. The flatter land has been cleared for cattle grazing.

Occasional collections were also made at locations which were not included in the regular collection areas. Two areas which were frequently visited on Route 17 were the extensive swamp forests along the Río Chucunaque, between Areas 1 and 3, and the mangrove swamps surrounding Camp Lagarto, a survey camp on the shore of the Gulf of San Miguel directly across from the town of La Palma.

Collecting and Processing of Data

A variety of collecting methods were employed; some on a regular basis, others not. A collecting camp was maintained in each area, and was continuously operated throughout the collecting period for each camp. The periods of collection for each area are shown in Table I.

Within each collecting area, some permanent collecting sites were established where various collecting devices, such as Malaise traps and sentinel monkey platforms, were operated.§ Much of the collecting was also done on a random basis throughout these collecting areas.

TABLE I

PERIODS OF COLLECTION OF SPECIMENS FOR SEA LEVEL CANAL ROUTES 17 AND 25

Area	Route	Period Operated
1	17	Nov. 1966–Dec. 1967
3	17	Feb. 1967–Dec. 1967
5	17	Feb. 1967–Jan. 1968
2	25	Mar. 1967–Dec. 1967
4	25	Apr. 1967–Dec. 1967
8	25	Sep. 1967–Dec. 1967

A field laboratory, equipped with running water and electricity, was also operated on each route. These laboratories were used to support the collecting camps and to process material coming in from them. In each instance, they were located adjacent to an airplane landing strip, and all collected material was shipped to Panama City by air. Preliminary sorting was also accomplished at these laboratories.

Final identification, testing, and curating of material was accomplished at GML in Panama City. These procedures included:

1. Identification of arthropods, mammals, and birds.
2. Testing of specimens of whole-blood from wild-caught and sentinel vertebrates for antibodies against arbovirus diseases.
3. Identification of hemoparasites by examination and culture of specimens of whole blood and by examination of blood smears from wild-caught vertebrates.
4. Identification of intestinal parasites from wild-caught vertebrates.
5. Identification of pathogenic bacteria from wild-caught vertebrates.
6. Pathological examinations of tissues from sick sentinel animals.

Information pertaining to each collection was entered on a standard form in simple numerical code. When all collected material had been identified and coded, the information was transferred to punched cards for machine data processing. This, and subsequent sorting of cards and printing of information was done at the Middle American Research Unit, Balboa Heights, Canal Zone. Later processing was done at the Walter Reed Army Institute of Research, Washington, D.C.

Studies of Potential Disease Reservoirs

Collection of Wild Vertebrates. In order to determine relative numbers and types of small rodents present in the various ecological associations found within each area, a systematic program of trapping was employed. Each sampling was

§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.

done by dropping randomly 40 National Live Traps at 10-foot intervals. The traps were left in place for five consecutive nights. Traps were checked each morning, and trapped mammals were removed in their traps. In the evening, the trap line was again checked, all traps were freshly baited, and any traps removed in the morning were replaced. At the end of the five-day trapping period, the trap line was moved to a different ecological association. Trapping went on for five days at the new location and was again moved. In this manner, three or four associations were sampled in a fixed sequence, so that each association in an area was sampled at approximately monthly intervals.

In addition to systematic trapping, special habitats such as trees were also sampled at irregular intervals. Mist nets were used periodically to collect bats and small birds. Large mammals and large birds were shot and some were purchased from local inhabitants.

Reptiles were captured on a casual basis. A large collection of reptiles and amphibians was made in Area 3 by Dr. Charles Myers, a herpetologist now at the American Museum of Natural History.

Processing of Wild Vertebrates. When possible, identifications of vertebrates were made in the field. If a specimen could not be determined to species with certainty, however, a study skin and skull or the entire carcass in formalin was sent to GML for identification. In some instances, as in the case of an undescribed species of *Oryzomys* collected in Colombia, the specimen was shipped to authorities in the United States.

Fig. 3. Site 506, a freshwater swamp near Camp Sasardi, Comarca de San Blas, Panamá.

Whole blood samples were collected from all captured vertebrates. They were allowed to clot at room temperature and then stored in a household refrigerator (ca. 4°C). They were then shipped on wet ice in insulated containers to GML for serological and parasitological testing.

All collected vertebrates were carefully examined for ectoparasites. These were removed, sorted, and preserved for later identification. Most rodents were examined for intestinal parasitic worms by flushing the guts with distilled water.

Diptera. These insects are of greater medical importance than any comparable group of arthropods. Collections included mosquitoes, phlebotomine sand flies, black flies (Simuliidae), biting gnats (*Culicoides*), and horse and deer flies (Tabanidae). Collection methods used included Malaise trapping, light trapping, collections using humans and rodents as bait, collections of mosquito larvae from water, and aspiration of resting flies from tree buttressess and foliage. Malaise traps were constructed from plans published by Townes.[11] CDC miniature light traps[7] were used, without dry ice. Although the use of dry ice would have increased the volume of mosquitoes captured many fold, it was impossible to supply it to the collection camps on a regular basis. Rather than use it on an irregular basis, it was decided not to use it at all. Modified Shannon traps[4] were also used nocturnally with gasoline lanterns. Some collections were made of blow flies, flesh flies, and other nonbiting Diptera, mostly from Malaise traps.

Other Biting Arthropods. In addition to the ectoparasites obtained in connection with the vertebrates collection program, some triatomid bugs (Reduviidae) were collected, mostly in Shannon traps.

Sentinel Animal Studies

Laboratory-bred animals of known immunological status were exposed to the environment of the study areas. It was possible to detect active disease agents by isolating pathogens from the tissues of sick animals and by detecting antibodies in apparently healthy animals.

Several species of monkeys known to be susceptible to yellow fever were exposed at four study areas on platforms situated at heights of about 20 m above ground level (the likely vectors of jungle yellow fever are canopy-inhabiting mosquitoes). Temperatures of the monkeys were taken rectally daily, and daily captures of mosquitoes were made on the platform. These mosquitoes were shipped alive to GML for virus isolation attempts.

Golden hamsters, known to be susceptible to Venezuelan equine encephalitis and to many Group C arboviruses, were exposed at both ground and canopy levels. Hamsters are effective sentinels, even when transmission rates are low.[8] Cages were checked twice daily and any hamster showing signs of frank illness was necropsied. Tissues were shipped on wet ice to GML for pathological examination and attempted virus isolation. At the end of 30 days, all surviving hamsters were returned to GML and replaced with a fresh group.

Summary

A Medico-ecology survey was conducted of proposed sea-level canal routes in eastern Panama and northwestern Colombia. Information was gathered from a variety of sources, including published literature, unpublished reports, serological examinations of blood samples taken from nonindigenous personnel engaged in canal feasibility studies, dispensary disease records, studies of local human populations, and field collections of potential animal vectors and reservoirs of human disease.

A wide variety of vertebrate and invertebrate animals were collected, identified, and processed, using live animal traps, mist nets, shot guns, Malaise traps, light traps, Shannon Traps, human biting collections, larval collections, and other methods. Blood and other tissues were examined for pathogens and for antibodies against viral agents.

Acknowledgments

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