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ORGANIZATION AND DEVELOPMENT OF A MULTIDISCIPLINARY RESEARCH PROGRAM
IN THE TRANS-AMAZON AREA

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1. INTRODUCTION

The Evandro Chagas Institute in Belém, Pará, Brazil, has been conducting epidemiologic and ecologic investigations in the Amazon area ever since construction of the Trans-Amazon Highway began in September 1970. The purpose of this research was to gather information on the infectious diseases—and their causative agents—indigenous to the area as well as those extraneous to it but which may have been introduced by the work force engaged in opening the Highway and by settlers coming from other parts of Brazil. Research support was derived from federal and state agencies in Brazil—Instituto Nacional de Colonização e Reforma Agraria (INCRA), Fundação Serviço Especial de Saúde Publica (FSESP), Superintendencia de Campanhas de Saúde Publica (SUCAM), and Superintendencia de Desenvolvimento da Amazônia (SUDAM). In 1971 the Pan American Health Organization (PAHO), in joint sponsorship with the Walter Reed Army Institute of Research (WRAIR), concluded an agreement with the Government of Brazil to carry out a program of infectious disease surveillance in the Amazon region. Under this agreement, which was renewed for 5 years in 1974, the Evandro Chagas Institute receives direct financial and technical support from WRAIR and assistance from PAHO in carrying out the international aspects of the program.

The first stages of the program, which was reviewed in June 1973 by the PAHO Advisory Committee on Medical Research at its Xllth meeting, included short (8-10 weeks) prospective visits to newly penetrated sites along the Trans-Amazon Highway to conduct initial epidemiologic and ecologic surveys.

The program was so successful that it drew a substantial increase in the support provided the Evandro Chagas Institute, so that work in progress might be continued and that an expanded program of longitudinal epidemiologic studies in human populations be developed along the new highways of the Amazon region.

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The present report describes the organization and development of the newly expanded program (Project Brazil 4311).

Under the terms of the agreement referred to above, WRAIR seconded to PAHO for appointment as consultants a team of investigators to conduct collaborative research with the Evandro Chagas Institute and provide it with technical support in the field and the laboratory.

The WRAIR staff transferred to Belém (USAMRU-Belém) comprise 2 epidemiologists, 1 microbiologist, 1 parasitologist, 1 entomologist, 1 wildlife ecologist, 1 epidemiology technician, and 1 administrative officer. Three additional persons joined the staff in 1975 and Walter Reed simultaneously increased its grant to the Evandro Chagas Institute.

II. OBJECTIVES OF PROJECT BRAZIL 4311

(1) To establish cohort studies for the surveillance of infectious disease in human populations along the new highways in the Amazon area.

(2) To develop the required field and laboratory capabilities to support these studies, principally in epidemiology, entomology, wildlife ecology, and isolation and serology of parasitic, viral, bacterial, rickettsial, and mycotic agents.

(3) To acquire the ability to identify and investigate rapidly outbreaks of disease in or near study areas.

The principal thrust of this program lies in the epidemiologic determination of incidence rates (the rate of infections over a specific period of time). In contrast, the previous program of the Evandro Chagas Institute produced essentially prevalence data (evidence of past or present infection at a specific point of time). Incidence studies are critical to assess the actual rate of infectious agent transmission and thereby define the risk of infection and disease to human populations.

There are, however, significant obstacles to the implementation of a program of this type in the Amazon area, namely, (a) the virtual absence of
laboratory diagnostic services in medical treatment facilities outside the major cities; (b) the vast distances involved—the present study areas encompass over 800 km; (c) the low density of human population—the colonist population in the study area is approximately 30,000 with a density of 1.46/km²; (d) the rudimentary logistical, transportation, and communication services; and (e) the need for local hiring and training of auxiliary field workers in study areas.

The Evandro Chagas Institute's reputation and its previous work in the present study areas have been of considerable assistance in overcoming many of these problems, as has the generous and enthusiastic support of INCRA, FSESP, SUCAM, and the Brazilian Army.

Long-range objectives include (1) the development of a model for regional epidemiologic surveillance programs; (2) the training of young Brazilian investigators; (3) the acquisition of data that may be used to develop rational plans for the delivery of medical care, disease control, and prevention programs; and (4) the use of data obtained to develop and evaluate priorities for research in tropical medicine.

III. APPROACH

Surveillance will be carried out for 24 months simultaneously by epidemiology, entomology, and wildlife ecology teams working in the same study areas. They will attempt to identify transmission of parasitic, bacteriologic, viral, rickettsial, and mycotic agents and obtain information about vectors, reservoirs, and ecologic conditions influencing disease agent introduction, transmission, and maintenance.

Specimens obtained are examined in field laboratories, in the Evandro Chagas Institute, and in other institutions as required, employing both routine and new techniques in serology and the isolation of infectious agents. Gathered data are forwarded to appropriate agencies of the Brazilian government at central, regional, and local levels.

Specific integrated studies being conducted within Project Brazil 4311 are described below.
A. Epidemiology*

The epidemiology section has initiated and now maintains a system of surveillance for acute diseases along the Trans-Amazon Highway. This system, designed to identify disease outbreaks as quickly as possible, is divided into two parts: (1) active surveillance of the colonists who live along the highway and in the "agrovilas", and (2) passive surveillance of persons examined in health units, persons admitted to the FSESP hospitals, and persons who have had blood smears examined for malaria by SUCAM.

At the present time, the program of active surveillance is functioning in two areas along the Trans-Amazon Highway: the stretch, west of Marabá to Arataú, and the stretch, west of the city of Altamira to the end of the Altamira jurisdiction. Each of the stretches is approximately 260 km long. The land on either side of the Highway is divided into "glebas", each of which is further subdivided into 10 roadfront lots and a variable number of interior lots. A sample of families living along the highway was randomly selected. Since many families in the Altamira jurisdiction do not live in roadfront lots, but in "agrovilas" further along the Highway, several of these "agrovilas" were chosen for supplementary studies.

Our field teams in Marabá and Altamira visit each of the sample families once every 2 weeks. Whenever they encounter a person who has been ill during the previous 2 weeks, they draw from that person 20 cc of blood for serologic tests, virus isolation, and malaria slides. At the same time, information on the symptoms and duration of the disease is collected.

The teams draw 20 additional cc of blood at the time of the original visit and at 6-month intervals thereafter. Serologic studies and virus isolation are being done by the Evandro Chagas Institute and our laboratory in Belém since last March.

Passive surveillance includes collection of information from persons admitted to the FSESP hospitals, those examined for malaria by SUCAM; and from patients treated at health units along the Highway.

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The most extensive part of the passive surveillance program takes place at the FSESP hospitals. There, the duration of disease and the symptoms are recorded for each person admitted to the hospital and blood tests are performed for every febrile patient. Whenever possible, another blood sample is obtained within 6 months to detect possible serologic conversions.

SUCAM workers visit each colonist once a month and prepare malaria slides for all persons with a history of recent fever. A review of their records reveals not only the number of malaria cases in an area at any given time, but also the number of persons with fever who did not have malaria.

Surveillance at the health units began in March and includes the names, ages, sexes, diagnoses, and treatments of all persons seen there.

At the end of each two-week period, the information obtained from each of these sources are summarized by the chiefs of the field teams in Marabá and Altamira and sent to our laboratory in Belém. If there is an unusual number of cases of any disease, an investigation is immediately started using the combined resources of Project Brazil 4311 and of the Evandro Chagas Institute.

This intensive surveillance program depends upon the continued cooperation of the colonists. In the past, in other programs, the cooperation of the study populations decreased over time, especially if no treatment was offered. At the present time, the only immediate benefit to the colonists is rapid treatment for malaria whenever blood smears are found positive. The refusal rate in the Marabá area at the time of the first 6-month visit was less than 10 percent. In the near future, we will offer immediate results of examinations for syphilis, blood type, Rh factor, hemoglobin level and perhaps other tests.

A program such as this, in which 2,000 persons are seen every 2 weeks, and data collected from an even greater number, can only exist with the cooperation and assistance of the other organizations working in the region. Whatever success our project may have will be caused in large part by the help we have received from SUCAM, FSESP, INCRA, and from our colleagues at the Evandro Chagas Institute.
B. Entomology

The entomology program of Project 4311 focuses primarily on surveillance for insects of medical importance along the Trans-Amazon Highway. Sites for routine collection were selected after preliminary reconnaissance around Marabá and Altamira, to provide geographic representation and comparative information about insect species and densities in various macrohabitats: cleared vs. forested areas, and well-drained vs. poorly drained terrain.

Routine surveillance is conducted at selected sites from the Araguaia River, east of Marabá, to Km 160, west of Altamira. This includes a total linear highway distance of almost 800 km. Eighteen sites have been established for surveillance. Each site is sampled for 2 days every 3 weeks with emphasis on man-biting collections. These collections are designed to provide information on the spatial and temporal distribution of the man-biting populations of insects at each site. Collections are made near the house, in the forest at ground level and 20 m above ground level at each site. Temporal distribution will be elucidated by making collections during the day and early evening throughout the year. Data on the distribution of man-biting species by habitat in conjunction with detailed floral and faunal descriptions of the various macrohabitats will be used to determine which habitats favor or do not favor the presence of various insect species.

In addition to the man-biting collections, captures are routinely made with Shannon traps and portable light traps. Opportunistic collections of ectoparasites and mosquito larvae are made. We started to use Disney traps in March and to operate a hamster sentinel program in conjunction with the use of these traps. A systematic scheme of collecting ectoparasites will be incorporated to the mammal trapping program. Other sampling methods to be included in this surveillance program are: (1) malaise traps; (2) black-light traps; (3) resting captures; (4) black-plates for mites; (5) dragging for ticks; and (6) house searches for triatomids and other house-dwelling insects.

These methods will be phased into the entomology program over the next few months. Additionally, to obtain more complete data on the population dynamics

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of disease vector species, the techniques of age grading and blood-meal identification will be employed when appropriate. All specimens collected in the surveillance program will be processed for pathogen isolation or identification, e.g., arboviruses, malaria, and others.

This entomologic activity is designed to permit detection of disease-vector interactions and has sufficient flexibility to focus on particular problems without interfering with the broad-based surveillance program.

Malaria, as a major cause of human illness along the Highway, has presented a problem that requires careful scrutiny. Two malarious areas, one near Marabá and the other near Altamira, have been selected for detailed study. Both sites are riverine environments. *Anopheles darlingi* has been found near Altamira but not near Marabá. The objective for studies in these riverine environments is to obtain basic entomological data for comparison with those malarious areas along the Highway where the circumstances for malaria transmission seem essentially different. The anopheline species found to date along the Highway are: *A. triannulatus; A. nunz-tovari; A. oswaldoi; A. fluminensis; A. intermedius; A. mattogrossensis*.

Additional species will be added to this list, probably *A. albitarsis*, as more collections are identified.

The ability to properly identify the insect specimens is fundamental to the success of this program. Thus, a reference collection of taxonomic specimens is being compiled. This collection is primarily for training purposes and to help in the routine identification of field-collected specimens. Presently the reference collection consists of approximately 1,500 pinned adults with accompanying larval and pupal skins. These specimens are from Marabá, Altamira, and Belém. Taxonomic studies will be undertaken as needed. In this regard, a special effort will be made to collect taxonomic series of *Anopheles nunz-tovari* in the Marabá area. The considerable morphologic variation of this species has already been the source of difficulty. It was noted by L. Deane, et al in 1946; but in the interest of future work we hope to document this finding in more detail.
Another fundamental component of this program is the collection, compilation, storage, and analysis of data. A computer program is being developed to cope with this aspect. The data are entered on field cards with the date, time, collector's name, locality, description of weather conditions, and detailed description of the habitat in which the collection has been made. Information is compiled in one card for each collection to facilitate input into the computer program. This system will alleviate the otherwise difficult task of reducing volumes of data to meaningful, summarized statistics, thus speeding the process of getting answers to questions relating to disease problems along the Trans-Amazon Highway.

C. Wildlife Ecology Surveillance*

Reported studies of wildlife ecology in the Amazon region have been mainly taxonomic, carried out in small areas, and conducted mostly in riverine ecology. Little work has been reported on the influence of seasonal changes in climate and vegetation on animal populations. Project Brazil 4311 has developed a Wildlife Ecology Surveillance Program to monitor changes in animal populations along the Trans-Amazon Highway, as man changes the habitat from forest to cropland and pasture.

It is well known that various wild and domestic animals are involved in the maintenance of many disease agents found in the tropics. Arboviruses and leishmaniasis have been reported in the Amazon area. Plague and rickettsial agents have been described elsewhere in Brazil and South America with both sylvatic and domestic rodents implicated in the transmission cycle. Information about the kinds of species present and their abundance in various habitats throughout the year is as important as the identification of these agents in various animals. This information, when compared with simultaneous epidemiologic and entomologic data from the same areas, should assist in understanding vector/reservoir interactions and the present and potential disease risk to humans from these zoonoses. Domestic animals must be included in these studies from the standpoint of disease hosts and reservoirs. With this framework in mind, the following procedures have been developed:

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1. Trapping

The trapping program compares the relative abundance of various animal species in different habitats over time. Mammals in the Trans-Amazon region are highly diversified and many species are present. Small changes in habitats can cause changes in the type of species and relative members of species found in each. A difference in both type and abundance of species could be expected between lowland and upland forest. These differences may be increased or reduced by seasonal climate and vegetation changes and by changes caused by man (for example, by clearing areas or creating areas of standing water). Because of the importance of different habitats, traps are dispersed in cropland, orchards, pastures, and in as many forest types as possible. The transition areas of secondary growth between forest and cleared areas is similarly covered. Traps are located in and around human dwellings and animal shelters. Trapping is carried out 5 days each week throughout the year at four sites (close to the entomology study sites) in the Marabá and Altamira study areas.

A wide variety of types and sizes of traps are employed because of variable species preference for entering certain traps. Baits including bananas, corn, cassava, sardines, peanut oil, rolled oats, local fruits, and combinations of these are used according to species preference. Baits are changed throughout the year as various forest or agricultural plants bear fruits making new natural food sources available.

Systematic placing of traps is necessary for comparing species and abundance from one area to another. Traps are placed along a grid in both forest and agricultural areas with a 30-m interval between traps. This interval and the baits employed will change as experience is gained. Traps are placed on lianas and in trees since some rodents and marsupials are essentially arboreal. Clearing of paths along the traplines is designed to disturb the natural vegetation as little as possible.

2. Bat Collecting

Bats have been implicated in the cycle of various viruses, most notably in rabies, which has been described in the Amazon. Collections are
made using 12 m x 2.6 m nylon nets placed across streams or trails in the forest, and parallel to the forest edge along clearings. Bats are also collected from day time roosts such as hollow trees, road culverts, house attics, or rolled up leaves of banana plants.

3. Hunting

Mammals not readily captured in traps (such as monkeys) are hunted. Schedules are arranged to include collections of both nocturnal and diurnal species.

4. Domestic Species

Domestic animals are periodically examined. Dogs, cats, pigs, horses, and cattle living in close proximity to man are sampled for evidence of present disease agents or previous infection.

5. Mammal Processing

Collected mammals are taken to the field laboratory where data cards are completed for each specimen. Ectoparasites, skin, and fur samples, blood, serum, internal organs, feces, and urine are removed from each specimen and preserved in either formalin or liquid nitrogen for shipment to the Evandro Chagas Institute in Belém. Mammals are tentatively identified in the field, and are preserved as skin and skull, skull only, or intact animal in formalin for later definitive identification.

6. Description of Study Areas

In order to extrapolate the information obtained by our investigations to other areas in the Amazon region, trapping sites must be described as to type of terrain, vegetation, soil, and other information. The description of each trapping site is therefore essential as is that of species and relative abundance of mammals collected. Each trapping area is identified with the number of the "lote" in which it is located for each trap site within the area is assigned a
trap number, and described in detail. The preferences of the various species may then be ascertained.

Photographic documentation is used to aid in the description of the areas. A series of photographic stations are located along the traplines, and monthly photographs are taken. Each photographic station contains a mount for the camera, and a target at which the camera is aimed. The photographs from each station may then be compared for monthly changes in vegetation. The photographic stations are located in agricultural areas as well as in the forest to document the influx of mammals as the crops produce fruit. The seasonal changes in the water level of swamps, and the inundation of lowland forests are also recorded in this manner. These photographs will be shown to botanists and tropical plant ecologists for identification of specific plants and vegetational types.

Small meteorological stations, consisting of a rain gauge and a maximum-minimum thermometer, are located at intervals along the Trans-Amazon Highway. Recording daily temperature fluctuations and rainfall from various points along the highway will enable us to compare local climatic conditions between areas. This information may also aid in determining why the forest differs from area to area.

The mammal surveillance program is being carried out in the same areas as the epidemiological and entomological programs. The role which mammals play, along with the insects and the human population, in the maintenance and/or introduction of diseases along the Trans-Amazon Highway may thus be studied. By monitoring the changes which occur in the mammal populations through trapping, netting, hunting, etc., and by determining the pathogens which are present, or which may be introduced in the future, health agencies can be alerted, and can plan accordingly. Information on the habitat preferences of certain species may be useful in controlling diseases through mammal control programs.