A MULTIDISCIPLINARY PROGRAM OF INFECTIOUS DISEASE SURVEILLANCE ALONG THE TRANSAMAZON HIGHWAY IN BRAZIL: EPIDEMIOLOGY OF ARBOVIRUS INFECTIONS[1,2]

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For two years a major epidemiologic surveillance program was conducted along Brazil's Transamazon Highway. This article describes the serologic results of that program in terms of human exposure to selected arboviruses.

Introduction

In the past, disease surveys in the Amazon Basin were generally confined to riverine areas, since human settlements were almost invariably established along the banks of rivers serving as the Amazon's major transportation network. The construction of the Transamazon Highway, running for the most part perpendicular to the major rivers along higher and drier land, is changing settlement patterns and quite possibly disease patterns as well. The human population in the area now includes colonists from every state of Brazil—colonists who will undoubtedly be encountering some disease agents for the first time and who may be carrying with them organisms foreign to this huge region.

Ecological change induced by this human influx may augment or diminish the potential for transmission of various disease agents; but in either case it affords an opportunity to study disease in a large group of people—of all ages and both sexes—who recently immigrated to a previously almost uninhabited portion of the Amazon Valley. This opportunity provided an especially worthwhile vehicle for learning about arbovirus diseases—since these are primarily zoonoses, and since the colonists' close contact with a variety of animal habitats provided an appropriate setting for studying the rural distribution of arbovirus diseases in man.

Previous serologic surveys for evidence of arbovirus infections in the area (Table 1) began with the 1958 work by Causey and Theiler *(1)* that reported the results of mouse protection tests using 14 viruses. The group of human subjects tested was comprised of 509 people from 15 different Amazon Valley localities. Although the sampling methods and features of the study population were not described in detail, the research involved an

[1]This article and three others relating to this program will appear in Portuguese in the *Boletín de la Oficina Sanitaria Panamericana.* The three other articles deal with the ecology of the study area, the area's mammal population, and entomologic surveillance.

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Table 1. Summary of selected past surveys for antibodies to arboviruses in the Amazon Valley.

Reporting authors and year	Population data: Population studied	Age and sex distribution	Subgroups studied	Percentage of study populations or subgroups possessing antibodies to the following arboviruses: Group A virus	Mayaro virus	Group B virus	Yellow fever virus	Bunyamwera group virus	Guaroa virus	Simbu group (Oropouche) virus	Group C virus
Causey and Theiler *(1)*, 1958	509 residents of 15 localities in the Amazon Valley	29 per cent less than 14 years old; sex ratio not stated		— [a]	13.9	—	44.7	4.7	—	—	—
Causey and Maroja *(2)*, 1957	551 residents of localities in the Amazon Valley	Adults only; sex ratio not stated	56 Marabá residents	—	0	—	—	—	—	—	—
			24 Altamira residents	—	4	—	—	—	—	—	—
			551 total residents	—	9.6	—	—	—	—	—	—
Bensabath and Andrade *(3)*, 1962	534 workers in the city of Belém	Adults only; avg. age 28.6 years; 64 per cent male		14.4	8.7	76.8	—	—	5.2	—	19.1
Neiderman et al. *(4)*, 1964	100 military recruits from the Amazon Valley	Avg. age 19 years; all male	50 recruits from Pará State	—	4	36	—	—	4	—	0
			50 recruits from Amazonas	—	12	50	—	—	0	—	0
Pinheiro et al. *(5)*, 1974	4,000 persons living along the Tranzamazon Highway	All ages, both sexes	Marabá residents	13.2	—	50.9	—	8.1	—	1.4	4.3
			Altamira residents	10.3	—	22.4	—	0	—	4.3	0.8
			Immigrants	1.2	—	25.0	—	0	—	0.3	0
Black et al. *(6)*, 1974	Isolated Indian tribes in the Amazon Valley	All ages, both sexes		44	42	30	11	—	—	—	—

[a] — = Not tested or no results reported.

enormous amount of field and laboratory time and provided the foundation for all later research. Its data, supplemented by data on 42 people from two other localities, is used by Causey and Maroja in their paper on Mayaro virus *(2)*.

Subsequent studies by Bensabath and Andrade *(3)*, Neiderman et al. *(4)*, Pinheiro et al. *(5)*, and Black et al. *(6)* reported data obtained from various subgroups of the Amazon Valley population, each population being characterized in terms of age, sex, occupation, locality, and length of residence. With the exception of the study by Black et al., which reported data collected from the population of an entire Indian tribe, there were no descriptions of how the study samples were selected.

All the studies used the relatively efficient hemagglutination-inhibition (HI) test, and all described the prevalence of arbovirus antibodies (that is, the proportion of the sample population that had antibodies at the time of the study and thus was presumably infected by the arbovirus tested, or a related arbovirus, at some time in the past). Only Pinheiro et al. *(5)* tested serial blood specimens from the same population in a nonepidemic situation to measure disease acquisition; but since the time interval between the first and second bleedings was not presented, a true incidence rate could not be calculated.

Bensabath and Andrade *(3)* showed that prevalence rates were higher in men than in women—and also higher in residents of Belém's periphery than in residents of central Belém. Pinheiro et al. *(5)* found that long-time residents living along the Transamazon Highway had higher prevalence rates than recent immigrants. Little other work has been done to relate environmental and behavioral variables to the acquisition of arbovirus antibodies.

The present study presents data on both the prevalence and incidence of arbovirus antibodies in a stratified random sample of colonists living along the Transamazon Highway. In addition, it seeks to define the relationship between these rates on the one hand and selected environmental and behavioral variables, clinical attack rates, and the temporal decline of antibody titers on the other.

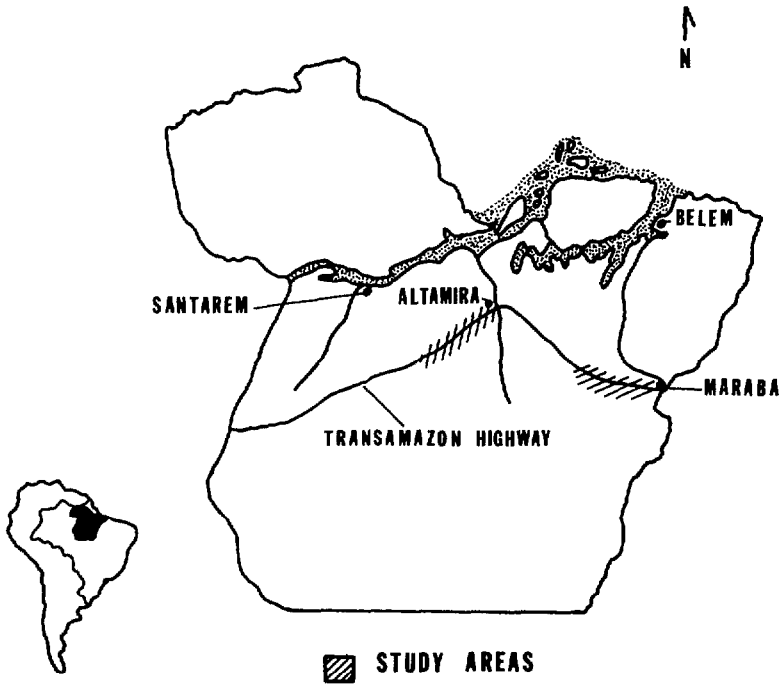
The Study Area

In 1970 the Brazilian Government began construction of the Transamazon Highway on an east-west axis between the Belém-Brasília Highway and the Peruvian border (Figure 1). A government-sponsored colonization program began in 1971. The heaviest and earliest colonization took place near Marabá and Altamira, the two easternmost towns along the highway; the sections of highway stretching west from each town for approximately 260 km (cross-hatched zones) were selected as study areas.

Common assumptions about the Amazon Basin are based on descriptions of riverine areas, where most of the population originally settled; but the opening of the Transamazon Highway has belied the Amazon stereotype of low flatland subject to annual flooding and has revealed a land of rolling, sometimes rather steep, hills with corresponding variations in flora and fauna. A more detailed description of the terrain and climate of the study area is provided by Peterson et al. *(7)*.

The two study areas differed in that Marabá was a fairly large and established town of about 10,000 inhabitants before creation of the highway, while Altamira was more of a boom town whose population rose rapidly from only a few thousand before the highway came to over 10,000 after it was built. The area west of Marabá was settled only along the edge of the road, one farm deep. In contrast, many farms west of Altamira are off the main highway, being served by side roads, and various small and medium-sized planned towns are scattered along the way. The farms in both areas had a size of 100 hectares, but the amount of cleared land per farm averaged 37 hectares in the Altamira area as compared to only 27 hectares in the area of Marabá.

Figure 1. A map of the state of Pará, Brazil; cross-hatching shows the two study areas.



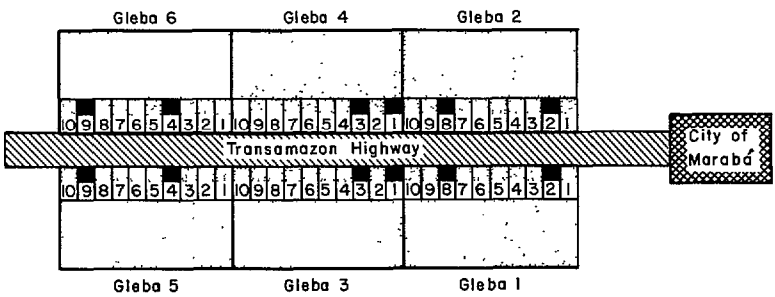
Collection of Specimens and Data

In addition to the base laboratory at the Evandro Chagas Institute in Belém, field laboratories were established in Marabá and Altamira for the initial processing of specimens and data. Each field laboratory was manned by a supervisor and two to four locally recruited field workers.

For ease of administration, the Government has divided the highway into segments called *glebas,* each of which contains 10 roadside lots and a variable number of internal lots. This study was restricted to the approximately 700 families, a population of just under 4,000 persons, who lived on roadside lots in the two study areas. The study sample was selected by using a table of random numbers to choose two lots in each odd-numbered *gleba.* The corresponding lots in the even-numbered *glebas* were then included to reduce travel time and increase the field teams' efficiency (Figure 2). All the people living on each lot selected (including not only members of the nuclear family but also other relatives and hired laborers) became part of the study samples.

On the first visit to a family, individual questionnaires and a family questionnaire were completed—on the basis of both interviews and observations made by the field worker. The data collected included demographic data (age, sex, location and length of residence, etc.); behavioral data (whether or not each person ever slept outside the house); and environmental data (the kind of house construction, the distance of the house from the forest, the distance of the house from the nearest standing or running water, etc.). An attempt was made to obtain blood for serologic studies and a malaria smear from each of the family members. Thereafter, at six-month intervals, environmental data were brought up-to-date and the specimen collection procedure was repeated.

Figure 2. A schematic diagram of land organization and sample selection along the Transamazon Highway, using as an example the first six *glebas* to the west of Marabá.



Each family was visited by a field team once every two weeks after the original survey. If any family member had experienced an episode of illness during the previous two weeks, the symptoms and course of the disease were noted on another questionnaire and blood was drawn for a malaria smear, serologic studies, and virus isolation. To carry out this work, once during each two-week period the field teams would travel the highway making visits until all lots in the sample had been visited. On the return trip the team would make a second attempt to interview those who had been away from home. The round-trip of 500 km usually took 4-6 days for the two-week visits and 8-14 days for the six-month visits.

Serologic Testing

After clot retraction, the serum was poured into 2cc plastic vials and shipped in liquid nitrogen to the Evandro Chagas Institute in Belém, where it was stored at -70°C. Serologic results were obtained by hemagglutination-inhibition testing for 20 arboviral agents found in the Amazon region (Table 2). Antigen for the study was obtained by making two acetone extractions of infected hamster serum. A 1:200 dilution of goose cells was used for hemagglutination. Nonspecific inhibitors in the sera were removed by acetone extraction according to the method of Clarke and Casals *(8)* and goose-cell agglutinins were removed by adsorption. The resulting 1:20 serum dilution was used for screening with four units of antigen in microtiter plates, as described by Sever *(9)*. End-points were determined for positive sera using microtiter loops. Virus isolation was attempted either at the Evandro Chagas Institute, using mice, or at the Walter Reed Army Institute of Research, using mice and Vero cells. The results of these various procedures for virus isolation will be reported in a separate publication.

Data from the field interviews and labora-

Table 2. Antigens used in the testing of Transamazonian serum specimens.

Alphavirus antigens	Flavivirus antigens	Other viral antigens	
EEE	Yellow fever	Caraparu	Belém
WEE	17-D	Catu	Araguari
Mayaro	Bussuquara	Guaroa	Tacaiuma
Mucambo	Ilheus	Oropouche	Triniti
	St. Louis	Utinga	AN 235467
		Itaporanga	

tory results were coded in either Belém or Washington, D.C., and put on cards. The analysis programs centered on two measures: antibody prevalence (the proportion of all subjects tested who showed positive results) and antibody incidence (the proportion of subjects whose test results changed from negative to positive over a given time period). Behavioral, environmental, and demographic variables measured at the time of the original interview were then analyzed to determine what effect they might have had on the incidence and prevalence of arboviral disease.

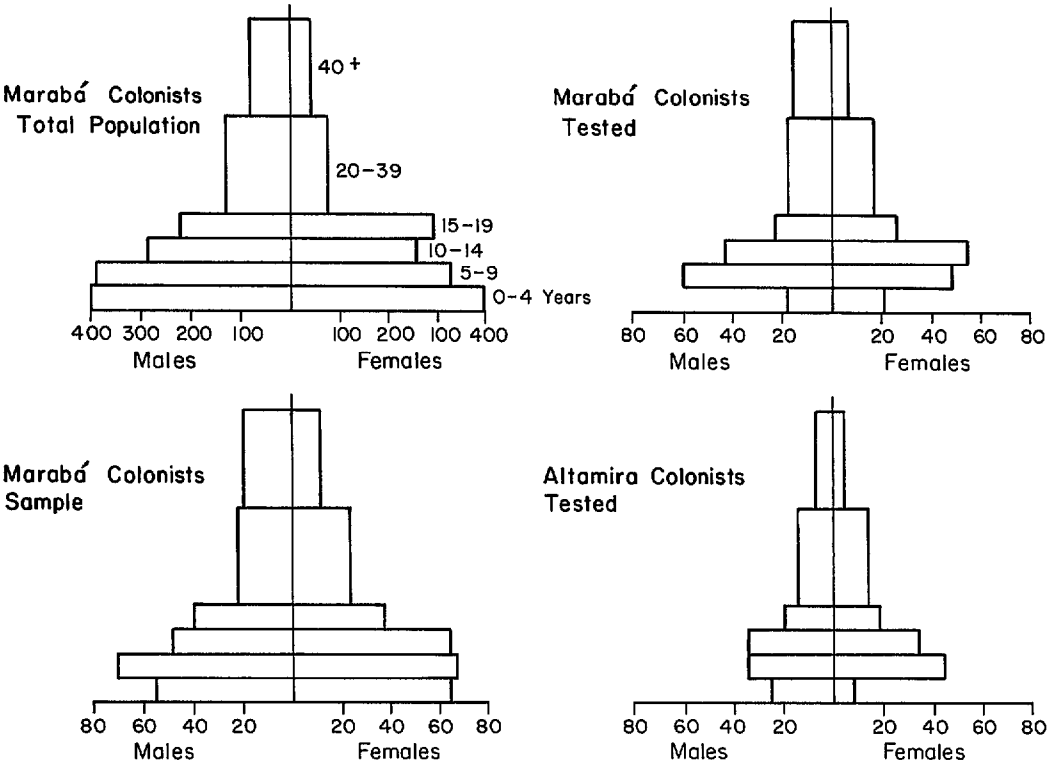
Results

During the first round of visits, 801 people were enrolled in the Marabá area and 802 in the Altamira area. As the study progressed some families left their homes, and their places were taken by new arrivals; but the total number of individuals enrolled varied by less than 2 per cent.

A constant 13-14 per cent of the Marabá sample was either absent at the time of the semiannual visits or refused to have blood drawn. In the Altamira area this figure ranged as high as 33 per cent. The principal reasons for being absent were work in the interior of the lot (adults) and being away at school (children). In cases where venipuncture was considered difficult, especially among children under the age of two years, the field workers would obtain fingertip capillary blood for a malaria smear and forego the collection of sera.

As shown in Figure 3, the age and sex distribution of the sample population in the

Figure 3. The age and sex distribution of selected population groups along the Transamazon Highway.



Marabá area was very similar to that of the entire colonist population from which the sample was derived (data obtained from INCRA[6]). Similar information on the entire colonist population living in the Altamira area was not available. Figure 3 also shows the age-sex distribution of those people in the sample who actually had blood drawn, tested for arbovirus antibodies, and used in the final analyses. These graphs differ from the graph of the total population mainly in the under-representation of the 0-4 year age group—a difference resulting from the difficulty of obtaining blood specimens by venipuncture.

Flaviviruses

The prevalence of flavivirus antibodies in the Marabá area declined from 37 per cent on the original visit to 27 per cent on the final visit. The levels in Altamira were lower, declining from 18 per cent to 11 per cent. The incidence rates in Marabá declined from 6 per cent in the first six-month period to 1 per cent in the final period, while in Altamira they fell from 1.2 per cent to 0 per cent (Figure 4). The fall in prevalence rates, a fall occurring while antibodies were still being acquired, can be at least partly accounted for by declining antibody titers in those persons who reacted to 17-D antigen (Figure 5). As these titers fell below the screening level of 1:20 they were no longer counted as positive results.

An examination of the age-specific prevalence rates found by the original visits in Marabá shows that the overall flavivirus prevalence rates increased sharply at age 20, from about 20 per cent to over 50 per cent. For people over 15 years of age, the rates were higher among men than among women (Table 3). The same general trends applied to the sample from the Altamira area, although the rate of positivity was higher among women in the 15-19 year group and the age-specific rates were consistently lower for all

Figure 4. The incidence and prevalence of flavivirus infections among colonists living along the Transamazon Highway.

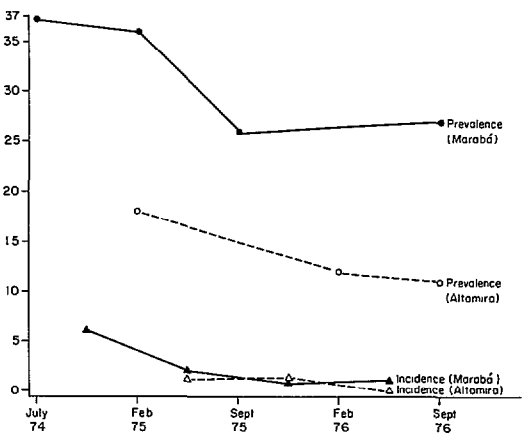
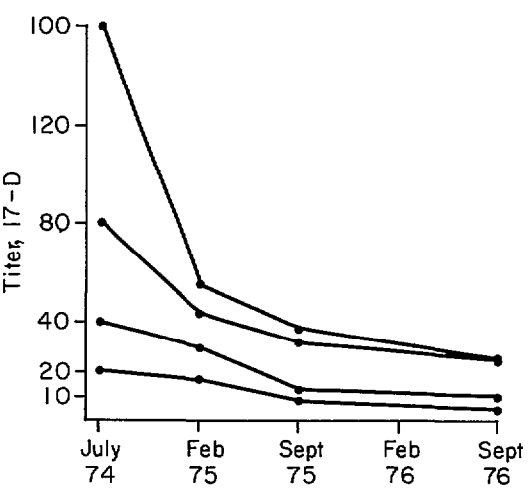


Figure 5. Changes observed in the mean titer of antibodies to 17-D virus over time, by the titer found in serum obtained during the initial (July 1974) visit.



age groups than the rates at Marabá. The 25 subjects from both areas who seroconverted to a flavivirus were evenly divided by sex (they included 13 males and 12 females), and 89 per cent were over 15 years of age.

Broad cross-reactions and specific reactions to the 17-D vaccine accounted for 78 per cent

[6]National Institute of Colonization and Agrarian Reform.

Table 3. Age and sex-specific prevalences of antibodies to flaviviruses in sera from colonists living along the Transamazon Highway.

		Age group (in years)							
		0-4	5-9	10-14	15-19	20-29	30-39	40-49	>49
Marabá area	Males	28%	22%	23%	27%	64%	58%	52%	74%
	Females	14%	24%	27%	15%	43%	42%	55%	43%
Altamira area	Males	4%	9%	11%	10%	36%	33%	47%	54%
	Females	0%	2%	12%	16%	15%	25%	30%	40%

of the observed flavivirus prevalence in Marabá and 77 per cent in Altamira. Rates specific for sex and age closely paralleled those found for all flavivirus antibodies. Nevertheless, specific reactions to Ilheus virus were quite common in Marabá, accounting for 19 per cent of all flavivirus infections; such reactions were rare in Altamira (Table 4), and this difference persisted throughout the study period. On the other hand, specific reactions to St. Louis encephalitis virus were more common in Altamira, although the magnitude of this difference declined as time passed. Only two subjects yielded results positive for Bussuquara virus; both of these people lived in the Marabá area. Of the 25 subjects who seroconverted, all had either broad cross-reactions or reactions to 17-D—with the exception of two seroconversions to Ilheus in Marabá, one to Ilheus in Altamira, and one to St. Louis encephalitis in Altamira.

Table 4. Flavivirus reactions, by specific virus, obtained with sera collected during the initial visit to Marabá and Altamira area residents.

Virus	Positive sera from:			
	Marabá area residents		Altamira area residents	
	No.	% of pos. sera	No.	% of pos. sera
17-D	69	36	35	51
Ilheus	36	19	2	3
St. Louis encephalitis	3	2	14	20
Bussuquara	2	1	0	0
Broad cross-reaction	81	42	18	26
Total (all flavivirus)	191	100	69	100

Alphaviruses

The prevalence of observed alphavirus antibodies varied between 12 and 15 per cent in Marabá during the study period and between 8 and 9 per cent in Altamira. In Marabá the incidence rate declined from 3.5 per cent in six months to 0.7 per cent in six months, but in Altamira it rose from 0.5 to 1.5 per cent (Figure 6). An examination of the age-specific prevalence rates obtained from the original visit shows that up to the age of 19 years the rates are low (below 10 per cent) and there is little difference between the two study areas. After age 20 the rate increases sharply and is consistently higher in the Marabá area (Table 5). The rates were found to be higher among males than among females of most ages in both areas.

Of the four alphaviruses tested, Mayaro accounted for the greatest number of positive reactions. In fact, of the 59 persons yielding results positive for alphavirus antibodies in Marabá at the time of the first visit, 35 reacted only to Mayaro virus. The age distribution of Mayaro antibodies in the Marabá area closely paralleled that of the total alphavirus antibodies. In Altamira the increase of Mayaro antibody prevalence with age was not as striking.

The other alphaviruses tested were Mucambo, eastern equine encephalitis (EEE), and western equine encephalitis (WEE). Not counting broadly cross-reactive sera, there were 17 people with positive titers (7 to Mucambo, 6 to WEE, and 4 to EEE). Nine of these 17 subjects were over 40 years old.

Figure 6. The incidence and prevalence of alphavirus infections among colonists living along the Transamazon Highway.

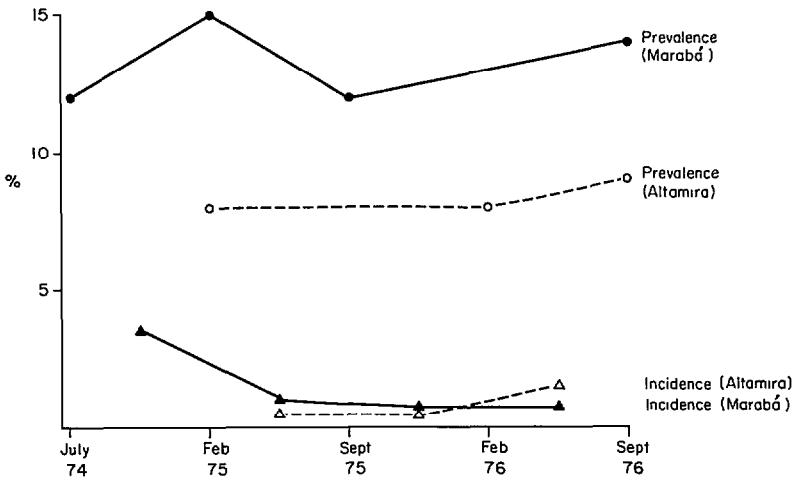


Table 5. Age and sex-specific prevalence of antibodies to alphaviruses in sera from colonists living along the Transamazon Highway.

		Age group (in years)							
		0-4	5-9	10-14	15-19	20-29	30-39	40-49	>49
Marabá area	Males	6%	2%	5%	9%	23%	22%	29%	33%
	Females	10%	0%	2%	0%	11%	11%	22%	36%
Altamira area	Males	0%	0%	8%	10%	11%	23%	12%	31%
	Females	0%	2%	0%	0%	7%	0%	0%	40%

There were 21 people (15 males and six females) who seroconverted to an alphavirus. All but four were over the age of 10 years. The most common specific reaction was to Mayaro (11); two subjects reacted to Mucambo, and two reacted to WEE. There were six converters who showed a broad cross-reaction to several alphaviruses.

Other Arboviruses

Guaroa. The prevalence of Guaroa antibodies was higher in Marabá (5.8 per cent at the original visit) than in Altamira (3 per cent at the original visit) and declined over the study period in both areas. Women and those under the age of 20 had much lower rates than adult males (Table 6). No colonist in our sample had a definite seroconversion to Guaroa.

Oropouche. The prevalence of antibodies to this virus in both study areas was about 1 per cent—with most (60 per cent) of the antibodies being found in adult females.

Itaporanga. On the original visit, 18 (3.4 per cent) of the Marabá area residents tested had antibodies to Itaporanga, while the corresponding figure for the Altamira area was 5 (1.3 per cent). Of the 23 people with antibodies to Itaporanga in the two areas, 17 were over the age of 20 and all but two were men.

Caraparu. In the Marabá area, 10 of the 524 sera tested (1.9 per cent) were positive for antibodies to Caraparu, while in the Altamira area three of 379 sera (0.8 per cent) were posi-

Table 6. Age and sex-specific prevalences of antibodies to Guaroa virus in sera from colonists living along the Transamazon Highway.

		Age group (in years)							
		0-4	5-9	10-14	15-19	20-29	30-39	40-49	>49
Marabá area	Males	5%	0%	0%	2%	14%	20%	13%	15%
	Females	5%	0%	0%	4%	0%	5%	6%	29%
Altamira area	Males	0%	0%	0%	0%	7%	7%	6%	31%
	Females	0%	0%	0%	0%	0%	0%	0%	0%

tive. All but one of the 13 positive subjects were over 15 years of age; males and females were about equally represented.

Catu. Nine of 254 sera tested from the Marabá area (1.7 per cent) showed antibodies to Catu, while only four of 379 sera from the Altamira area (1.1 per cent) yielded a positive response. All but one of the 13 positive subjects were over 20 years old, and nine of the 13 were males.

Others. The prevalences of antibodies to all other arboviruses tested, which were low in both areas, are shown in Table 7.

Relation of Antibody Prevalence to Environmental Variables

A search was made for differences within the Marabá and Altamira study areas by comparing differences in antibody prevalence between successive groups of 10 *glebas,* each group spanning 20 km along one side of the highway. With regard to all the viruses studied, only minor focal differences were found between the ten-*gleba* groups—except that six of seven persons with antibodies to Oropouche in the Marabá area lived in *glebas* 31-50.

Table 7. Prevalences of antibodies to selected arboviruses in sera from colonists living along the Transamazon Highway.

Arbovirus	Area	Prevalence
Araguari	Marabá	4/535
	Altamira	2/373
Belém	Marabá	2/535
	Altamira	2/372
Tacaiuma	Marabá	4/524
	Altamira	1/379
Utinga	Marabá	6/524
	Altamira	1/379
AN 235467	Marabá	1/524
	Altamira	1/373

Sleeping in the Forest

Many of the colonists, especially adult males, would sleep outside their homes (in the forest) when hunting or when clearing land far from home. The number of men over 15 years of age who reported sleeping outside the house was 129 out of 212 (43 per cent) in the Marabá area; people with antibodies to Mayaro, Guaroa, Itaporanga, Caraparu, and Catu viruses were more likely to have reported sleeping outside their homes than were members of the general population (Table 8). This was not true in the Altamira area, where the prevalences of these antibodies were lower and people were less likely to sleep outdoors. On the average, neither seroconverters to alphaviruses nor seroconverters to flaviviruses slept outside their homes more frequently than the general population.

The Distance between Home and the Forest

The interviewer estimated this variable for each house during the first visit to that house. It was not feasible to make exact measurements, and the estimates were grouped into the following distance categories: 0-50m, 51-150m, 151-250m, and over 250m. The data were then examined to see if increasing

Table 8. Relationships between sleeping outside the home and the presence of antibodies to selected arboviruses in sera collected during the initial visit from males at least 15 years old in the Marabá area.

	Positive reactors		
	No. sleeping outside	No. in total population sample	Percentage sleeping outside
Total population	129	212	61%
No. with sera showing antibodies to the following viruses:			
Mayaro	14	18	77%
Guaroa	14	17	82%
Itaporanga	9	12	75%
Caraparu	3	3	100%
Catu	7	7	100%

distance from the forest produced a consistent change in the prevalence of any arbovirus antibodies among the total population or among any age-sex subgroup. Changes in the incidence of alphavirus, flavivirus, or Guaroa antibodies were also sought. No significant differences were found.

Home-building Materials

In the Marabá area, people living in straw houses were more likely to have antibodies to certain arboviruses than were people living in more tightly constructed wood and mud houses (Table 9). This association was most striking in those who seroconverted to an alphavirus in the Marabá area, with 13 of the 14 seroconverters (93 per cent) living in straw houses. No difference was apparent in the Altamira area, where there were very few mud or straw houses.

Other Environmental Variables

Other variables that were studied, but that seemed to have little effect on the acquisition of antibodies, were distance from standing water, distance from running water, and the steepness of the terrain.

The Biweekly Morbidity Survey

The biweekly morbidity survey was more vulnerable than the semiannual survey to such factors as inclement weather, vehicle breakdown, fuel shortages, and unavailability of supervisory personnel. The data collected did not show any definite relation between reported disease, with or without fever, and the acquisition of arbovirus antibodies. Of 418 specimens of whole blood from recently febrile colonists that were injected into Vero cells and suckling mice, all were negative for viral pathogens. Several isolates were obtained from febrile patients admitted to the Marabá Hospital; these will be described in a later paper.

All persons found to be febrile were examined for malaria. Thirty-three blood samples obtained during the original visit (mostly from adult males and children) yielded positive results; but only three more cases were detected in the following six months and no more were found thereafter *(10)*.

Discussion

There was a clear-cut and consistent relationship between age and sex on the one hand and acquisition of arbovirus antibodies. In the

Table 9. Relationships between types of house construction and the proportion of Marabá area residents whose sera (collected on the initial visit) showed antibodies to selected arboviruses.

	Residents of homes made of:								
	Wood		Straw		Mud		Other materials		
	No. of residents	% of total population and positive reactors	No. of residents	% of total population and positive reactors	No. of residents	% of total population and positive reactors	No. of residents	% of total population and positive reactors	Total no. of residents
Total population	145	35%	220	53%	20	5%	30	7%	415
No. with sera showing antibodies to the following viruses:									
Mayaro	6	23%	18	69%	2	8%	0		26
Guaroa	6	23%	19	73%	1	4%	0		26
Itaporanga	4	25%	11	69%	1	6%	0		16
Catu	1	12%	7	88%	0		0		8

case of most arboviruses, adult men had the highest attack rates—and those who worked and slept in the forest were even more likely than others to acquire antibodies. One could propose various hypotheses to account for these observations, but when considering arthropod vectors the point that stands out is that the men were spending most of their time clearing forests and working in the fields, while the women and children were most often found at or near the home or in school. The finding that antibody rates were higher in Marabá than in Altamira for almost every virus tested reinforces this association—because on the average Marabá had less cleared land per lot and homes were much closer to the nearest forest border than was the case in the Altamira area. Another supporting factor is the decline in the observed incidence of arbovirus antibodies arising during the study period in the Marabá area. That is because a family's contact with the forest is greatest when it first settles on the lot, since the first job is to clear the land in preparation for planting. As time goes on, the family members spend less time clearing land and more time working on land already cleared.

The only virus that did not appear to subject adult males to greater risk than other people was Oropouche. Here the higher attack rates in adult females can be explained by the fact that most of the cases occurred during an epidemic in a nearby village, and we have shown that adult females tend to experience higher rates of attack during urban epidemics of Oropouche (*11*).

As reported by Peterson et al. (*12*) and by Roberts et al. (*13*), the forest through which the Transamazon Highway passes contains a large and diverse representation of both mammals that may act as reservoirs and potential insect vectors—with relatively little between-site variation in species composition. The arbovirus transmission cycles in such a system should be relatively stable, providing the disease is not lethal, and the degree to which man is affected will depend largely on how closely and for how long a time he has contact with the forest. In the case of the colonists, the original clearing of the land, future expansion of cleared areas, and hunting are the activities most likely to involve contact with the forest and most likely to result in arbovirus infection.

The cleared areas represent a much more complex situation. In the slash-and-burn method of agriculture, the flora undergo major cyclic changes as cropland is replaced by secondary growth that is later cut and burned to again make cropland. Many jungle animals do not adapt well to these conditions and are rarely found outside their jungle habitat. Others, often found only in low numbers in the undisturbed forest, seem to flourish in cropland areas (*12*). Domestic animals, being introduced to the area in increasing numbers, further complicate the potential disease reservoir picture.

The missing factor needed for a large epidemic of arboviral disease generally seems to be an efficient vector willing to cross the cleared area and infect man in or around his home. Roberts et al. (*13*) report that with the exception of a rare *Culex* or *Culicoides,* no endophilic or endophagic vector species were identified—and that cleared land posed an effective barrier to all but the anopheline mosquitoes. The occurrence of large-scale outbreaks of arboviral disease in the future will largely depend on the adaptation of forest vectors to a new environment or the importation of urban vectors into the area.

In the present study, the measured rate of disappearance of antibodies to 17-D vaccine virus was significant. Without this development, it would be hard to explain how the antibody prevalence rate could remain steady or even decline while new people were acquiring arbovirus antibody at a fairly high rate. We were unable to calculate decay rates for antibody to other arboviruses because of insufficient numbers of positive sera and observation times. However, infection with wild yellow fever strains may produce longer-lasting antibodies at a higher titer than does the 17-D vaccine strain.

A continuing field study such as this is expensive and requires a long-term commitment from all parties involved. There must be constant supervision of data and specimen collection as well as strong laboratory support. Access to a computer and data-analysis expertise is essential, and there must be constant communication between the investigators in epidemiology, entomology, wildlife ecology, and all the specific laboratory disciplines required. Above all, a sense of flexibility and optimism must be maintained in order to cope with the inevitable logistic problems that arise. The advantages of this type of study are that more than one disease can be studied, thereby lowering the unit cost per disease; all the factors contributing to a disease problem can be studied in their natural setting; and temporal changes in disease incidence can be measured. These types of data can then be used to generate new questions, which in turn can be answered by employing more specific research protocols. Additional benefit occurs in the event of an epidemic, in cases when the resources to mount an investigation are available and can be quickly mobilized.

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SUMMARY

A program of epidemiological surveillance was conducted for two years (1974-1976) along the Transamazon Highway in Brazil's Pará State. Approximately 300 randomly selected families (including about 1,600 persons) were incorporated into the study population. Visits to the study populations were conducted at six-month and two-week intervals. Blood and serum samples were collected and individual interviews were conducted during the six-monthly visits. The biweekly visits were performed to detect and document episodes of illness. The blood samples were processed for virus isolation attempts, and the sera were tested for antibodies to arboviruses.

This report presents serologic data that was obtained on flaviviruses, alphaviruses, and other viruses such as Guaroa and Oropouche. It also assesses the relationship of antibody prevalences to several environmental variables.

Among other results, a clearcut and consistent relationship was documented between age and sex on the one hand and acquisition of arbovirus antibodies on the other. Most arbovirus attack rates were higher for adult males than for other segments of the study population, and men who slept as well as worked in the forest were especially likely to be attacked.

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