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SURVEILLANCE AND RESEARCH ON INFECTIOUS DISEASES

ALONG THE TRANS-AMAZON HIGHWAY

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ALONG THE TRANS-AMAZON HIGHWAY*

I. INTRODUCTION

The Trans-Amazon highway, the construction of which began in September 1970, branches off the east side of the existing Belém-Brasília highway and more or less parallels the Amazon river for about 3,000 km westerly to a point close to the Peruvian border.

The road crosses vast areas of forest, most of which has never been touched by man, and certainly passes through a wide variety of ecological environments.

The colonization of the highway area by immigrants from other parts of the country began in the middle of 1971. About 22,000 immigrants settled in three different sections along the road which were already built (Figure 1).

It was logical to assume that these people would come into contact with certain zoonotic agents such as arboviruses and Leishmania, and that other infectious diseases like schistosomiasis and Chagas' disease and their vectors, might be introduced into the Trans-Amazon area, where they were previously unknown. This situation offers, therefore, a unique opportunity for a long-term study of certain infectious diseases among the new settlers, and for ecological investigations of possible zoonosis foci and their threat to man.

Epidemiologic studies of yellow fever and other arboviruses, leptospirosis, leishmaniasis, and schistosomiasis have been under way

*Prepared by Drs. F. P. Pinheiro, G. Bensabath, A. H. P. Andrade, Zea C. Lins, H. Fraiha, A. T. Tang, R. Lainson, and J. J. Shaw, Instituto Evandro Chagas, Belém, Pará, Brazil.

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since 1971. Instances, in 1972 and 1973, of a hemorrhagic disease associated with some fatalities were also investigated. In the near future studies will be extended to cover the Labrea black fever, Chagas' disease, and malaria.

A multidisciplinary field team made six trips to different sections of the road, each one lasting between 40 to 70 days (Figure 2). Several shorter trips were also made. The team collected blood and other clinical specimens from both normal and sick persons. Specific serological surveys were made or are planned. A leishmanin skin-test survey of 157 persons, including adults and children, showed 47 percent of positive reactions. Very few active cases of leishmaniasis have been encountered along the Trans-Amazon highway up to now, however, and the positive skin tests were found in immune persons with past infections contracted elsewhere. Leishmaniasis along the highway will doubtless become more important in future generations.

In addition, reservoir hosts among wild animals and the role of certain arthropod vectors were investigated. Search for foci of planorbid snails was undertaken in ponds and streams located along almost 300 kilometers of the road.

In November, 1972, a permanent surveillance program was started in the Altamira area, aiming at the detection and study of cases of arboviral infections, viral hepatitis, and the hemorrhagic syndrome of Altamira (HSA). Certain parasitic and bacterial diseases will also be considered in this program.

II. STUDIES ON ARBOVIRUSES

A. In humans

The results of blood studies for attempted virus isolation in 207 febrile persons were normal. The majority of the patients, however, were bled after the 5th day of illness, when viremia is usually difficult to detect. But malaria parasites, most of them Plasmodium falciparum, were found in 18 out of 107 of these patients.

We were able to examine necropsy material from five patients who died along the highway. Two of them had malarial pigment in the liver; in two, histopathologic findings indicated viral hepatitis, and in one, the cause of death remained unknown.

Almost 4,000 blood specimens were taken from immigrants, both before and after they entered the Trans-Amazon area, residents, and road workers at various sites of the highway.

These sera were tested against 18 arbovirus antigens, most of them of medical interest. All were tested by the hemagglutination inhibition (HI) technique. In some instances, neutralization test (NT), usually in mice, and tests done with Piry virus, were also performed. The results of tests done on 2,823 serum specimens are expressed in Table 1, and demonstrate clearly that the prevalence of antibodies to arboviruses was lower among settlers than among residents of all four areas surveyed. For instance, the incidence of antibodies to group A arboviruses was 6 to 24 times higher in the residents than in the colonists. Reactions to the Mayaro virus were by far the most common in group A arboviruses, although a few positive reactions to the Mucambo virus, a member of the Venezuelan Equine Encephalitis (VEE) complex were found. In rare instances, positive reactions to the Western Equine Encephalitis (WEE) and the Eastern Equine Encephalitis (EEE) viruses were also detected. The difference in the incidence of antibodies to group B arboviruses among residents and settlers was less conspicuous than group A. Two points, however, should be emphasized with respect to antibodies against the group B arboviruses: (1) Most of them are probably due to vaccination against the yellow fever (YF) virus and (2) Specific antibodies to the St. Louis Encephalitis (SLE) virus were found by the HI technique, with confirmation by NT, in a small number of persons.

The incidence of antibodies to the arbovirus groups varied according to the area, but it was usually below 10 percent and sometimes 0. Low levels of antibodies to the Oropouche virus, an agent previously implicated in four major epidemics in localities of the Amazon area, were found in all areas.

Infections caused by the Piry virus, a Rhabdovirus, or by an agent antigenically related to it seem to be common in both colonists and residents, as judged by the rates of neutralizing antibodies found. These rates are especially high among the immigrants from Rio Grande do Sul, the southern state of Brazil. Table 2 shows that the rate of antibodies increases with age, reaching a 90 percent positivity in the group above 50 years of age. No marked sex difference was noticed.

Paired sera from 308 immigrants, obtained before and after going into the Altamira area, were tested. Results revealed 33 serological conversions to arbovirus (Table 3), 27 of them being to group B, possibly as a result of YF vaccination, although two reactions were specific to the Ilhéus and one to the SLE viruses. Of the remaining sera, two showed conversions to the Mayaro, two to the Catu, one to the Guaroa, and one to the Itaporanga viruses.

B. Studies on reservoir hosts and vectors

These were undertaken simultaneously with studies in humans, to determine the presence of known or of new arbovirus types, especially those of medical interest, and to gather information about their natural cycle.

Wild animals are captured by various methods such as trapping, shooting, or by the use of "mist nets". So far 4,465 animals have been captured:

Birds	3,218
Marsupials	220
Rodents	683
Primates	131
Edentates	21
Chiroptera	145
Miscellaneous	47

The blood or viscera of these animals yielded 11 strains of arbovirus. As may be seen below, the SLE virus, an agent well recognized as pathogenic to man, was isolated in four occasions:

Arbovirus group	Virus	Source	No. Isolates	Areas and Year
B	SLE	Bird	4	Marabá, March 71 (1) Altamira, August 71 (1) Itaituba, November 71 (2)
C	Murutucu	Bird	1	Altamira, August 71
Turlock	Turlock	Bird	3	Altamira, September 71 (1) Itaituba, November 71 (1) Itaituba, July 72 (1)
Phlebotomus	Candiru complex	Marsupial	1	Itaituba, December 71
Phlebotomus	Icoaracy	Rodent	1	Itaituba, July 72
Ungrouped	An 141106	Bird	1	Maraba, March 71

In addition, a strain of the Wyeomyia complex was isolated from a pool of mosquitoes (Wyeomyia) captured 25 Km from Itaituba City, in November 1971.

The distribution of HI antibodies to arboviruses among the wild animals is shown in Table 4. Birds showed by far the highest rates of immunity in comparison with the other animals tested, and they had antibodies to all groups of arboviruses for which they were tested. It should be noted that most reactions in group A were caused by the WEE virus, and in group B by the SLE virus. Birds of the family Formicariidae showed a greater antibody incidence than those of other families.

A few rodents had HI antibodies to Pixuna virus, a member of the VEE complex, with confirmation by NT. High antibody incidence was found to viruses of the "Phlebotomus" group, and low levels or negative results with the other groups.

Primates usually had a high immunity to the YF and Mayaro viruses in all surveyed areas, and smaller rates of antibodies to the Simbu, "Phlebotomus" and Anopheles A groups. The occurrence of YF antibodies in monkeys, indicating the circulation of the virus in the forest, constitutes a strong reason for continuous vaccination of the colonists against this agent.

The marsupials, bats, edentates, and other animals had low antibody levels or did not react to the viruses tested, but they all had antibodies to the "Phlebotomus" group viruses, although not as high as in the case of the rodents.

Arthropods were captured using human bait, mechanical suction, light trap, and the Trinidad No. 17 trap. A total of 17,583 hematophagous arthropods, 13,555 of them mosquitoes, were captured, and inoculated into mice for attempted virus isolations. As previously mentioned, only one isolation was obtained. This was a strain of the Wyeomyia complex.

The genus Culex was predominant and comprised 46.9 percent of the total mosquito catches. They were the only mosquitoes present in reasonable numbers in all types of collection.

There was a marked variation in the composition of catches, according to methods used (Table 5). Variations were also observed according to the areas, but the data are not strictly comparable, because in these areas captures were made at different seasons of the year.

III. INVESTIGATION ON THE HEMORRHAGIC SYNDROME OF ALTAMIRA (HSA)

In 1972, 22 settlers of the Altamira region, mostly young persons, suffered a hemorrhagic disease, diagnosed as thrombocytopenic purpura (TP). One of the patients died and his disease was confirmed by histopathology. Three other persons died with hemorrhagic manifestations which were not studied in two, and unconfirmed by histopathology in the other. From January to March 1973, 28 additional patients had the disease and two died, but no autopsy was done. One of them, however, had a very low number of platelets. Family grouping of cases were observed, as follows:

<u>Year</u>	<u>No. of Cases</u>	<u>Families with 2 or more cases</u>	<u>No. of cases in these families</u>	<u>Families with single cases</u>
1972	22	4	18	4
1973 (Jan-Mar)	28	4	10	18

In spite of an increase in bone marrow megakaryocytes the number of platelets was exceedingly low in some patients, occasionally dropping to about 10,000 cu m. The retraction of blood clot was poor or absent and the bleeding-time markedly prolonged. The patients had ecchymoses and petechiae throughout their bodies and some of them experienced bleeding of the gums and nose, and melena. Petechiae also covered the mucosa of the mouth of surviving patients and the pericardial membrane, lungs, brain, and intestine of one who died. Most patients were anemic, but this condition may have been caused by hookworm infections, iron deficiency, or other factors. The coagulation time and levels of prothrombin and fibrinogen of the blood were normal, as were the levels of SGOT, SGPT, and urea. Fever was of low grade, or absent.

Old residents link the illness to the bites of Simuliidae that are particularly abundant in the rainy season. They also say that the illness has been seen for many years, but in immigrants only. Sporadic cases occur, but not every year. Whether their opinion is correct or not, is not clear. The fact remains that no case of hemorrhagic syndrome was reported last year when the population of Simuliidae apparently decreased, nor was there a single case during the dry season, a time during which the black flies were absent. New cases reoccurred early this year, after the beginning of the rainy season and the reappearance of Simuliidae. Figure 3 shows the distribution of cases on a monthly basis.

Persons living in the Agrovilas, and constantly exposed to the bites of Simulium, had a high incidence of precipitin antibodies to macerated black flies. This indicates the possibility of either a hypersensitivity phenomenon or a toxin effect. Altamira city residents, and others who have little or no exposure to the bites of Simulium, have

a much lower incidence of precipitin antibodies to the antigen prepared from the flies, as shown below:

<u>INDIVIDUALS</u>	<u>No. POS/TESTED</u>	<u>%</u>
Natives of Altamira	6/123	4.8
Residents for about 1 month in the Agropolis of Km 46	0/8	0
Natives of Belém	0/38	0
Patients with HSA	17/18	94
Familiar contacts of cases of HSA	5/9	55

Improvement or complete remission of the symptoms is obtained with blood transfusions, vitamin therapy, and bed rest. Moderate doses of steroids bring a fast recovery.

Attempts (in mice, hamsters, and VERO and HEP - 2 cells) to isolate a virus from the blood and other specimens taken from the patients have been unsuccessful. One guinea pig inoculated with blood collected from a patient in the acute phase of the disease died 18 days after receiving the inoculation. The inoculation of organ tissues or blood from this animal killed guinea pigs in three subsequent passages at intervals varying from 23 to 69 days. The histopathologic findings on these animals revealed congestion of the lungs sometimes associated with pulmonary hemorrhages, but no lesions characteristic of thrombocytopenic purpura. No pathogenic bacteria or parasites could be identified in association with the syndrome. Paired sera from two patients did not react with a number of rickettsial (spotted fever and typhus groups, scrub typhus, and Q fever), leptospiral (microagglutination tests for 18 antigens) and arbovirus antigens (two group A and two group B).

IV. BACTERIOLOGY

A. Enterobacterias

Attempts are made to isolate pathogenic enterobacterias from humans, and Salmonella from wild animals. From March 1971 to November 1972, 418 coprocultures were made from road workers, colonists, and long-term residents along various sectors of the Trans-Amazon and Cuiabá-Santarém roads. During the same period attempts were made to isolate Salmonella from the large and small intestine of 1,432 wild animals caught in forest along the two roads mentioned above.

Three strains of Salmonella and two of Shigella dysenteriae were isolated from humans. The Salmonella strains belonged to sero-group B and C2(1).

Four isolations of Salmonella, two of sero-group D, and one each of sero-group C2 and subgenus IV (Kauffmann-White scheme), were made from wild animals. In addition, one strain of Arizona was cultured from a specimen of Ameiva.

The history of these isolates is given below:

<u>SOURCE</u>	<u>AGE</u>	<u>PLACE</u>	<u>DATE</u>	<u>ENTEROBACTERIA</u>
Human	3 years	Marabá Km 68	March 71	<u>Salmonella</u> group C2
"	8 months	Mirituba (Itai (Itaituba)	November 71	<u>Salmonella</u> group B
"	?	Km 10, Itaituba-Jacareacanga road.	July 72	<u>Shigella dysenteriae</u>
"	?	" "	" "	" "
"	20 years	Km 73, Santarém-Cuiabá road.		<u>Salmonella</u> sero-group B
<u>D. marsupialis</u>	-	Km 12, Marabá-Altamira road.	March 71	<u>Salmonella</u> sero-group C2
<u>Metachirus</u>	-	Km 19, Itaituba-Jacareacanga road	July-Sept 72	<u>Salmonella</u> sero-group D
<u>Proechimys</u>	-	" "	" "	" "
<u>Oryzomys</u>	-	Km 84, Santarém-Cuiabá road	Nov-Dec 72	" sub-genus IV (K-W scheme)
<u>Ameiva</u>	-	" "	" "	Arizona

B. Leptospira

Serologic studies on humans and wild animals, and attempts to isolate this microorganism from wild animals, revealed the presence of a number of infections caused by several serotypes of Leptospira.

For instance, 15 of 222 sera from rodents and marsupials captured in different road areas had antibodies against L. grippotyphosa, L. wolffi, L. bataviae, and L. butenko, with titers ranging from 1:200 to 1:400. Provisionally it may be recorded that the serotype most commonly found in marsupials appears to be L. wolffi, and in rodents L. grippotyphosa. In addition, five isolations were obtained as result of the inoculation of 90 urine samples and 157 pieces of renal cortex derived from rodents and marsupials. The cultures were made in Fletcher's medium and all five strains, which are not yet typed, were derived from the renal cortex.

So far, the presence of antibodies in humans has been looked for only in 131 sera of colonists from Rio Grande do Sul and Santa Catarina States, in southern Brazil, during the transit of these people through Belém, en route for one of the Trans-Amazon "Agrovilas". Two sera were found positive for L. bataviae and L. panama, both in titers of 1:200. It is intended to reexamine these persons after variable lengths of stay in their new environment on the highway.

Of 190 sera collected from other persons living along the road, 35 gave significant titers for L. panama (16), L. tarusovi (6), L. grippotyphosa (4), L. australis (4), L. bataviae (3), L. pyrogenes (1), and L. wolffi (1).

V. PARASITOLOGY

Details of the epidemiology of leishmaniasis are to be discussed by the next speaker, Dr. Lainson. It may be mentioned, however, that Leishmania has been isolated from 35 wild animals.

In addition, a variety of different parasites have been encountered in these same wild animals, either by direct examination of blood films, culture in NNN medium, or the inoculation of laboratory animals with various tissues.

Parasites of medical interest include Histoplasma, found in the viscera of three specimens of Proechimys guyannensis from the Itaituba-Jacareacanga sector, and Toxoplasma found in the viscera of a single opossum, Didelphis marsupialis from the Santarém-Cuiabá sector.

Hemogregarines, Babesia and Grahamella-like parasites have been isolated in a variety of animals, and Trypanosoma cruzi-like parasites continue to occur, principally in Didelphis, but also occasionally in rodents. These trypanosomes are morphologically and pathologically (in mice) indistinguishable from T. cruzi of man.

Schistosomiasis

A search for human cases was carried out in March 1971, by stool examination, using the method of Hoffman, Pena, and Jener. Eggs of S. mansoni were found in 3 of the 73 fecal samples examined, but all patients belonged to the imported labor force. The task of looking for human schistosomiasis has been taken over by another agency of the Brazilian Ministry of Health, and it is known now that the number of human cases is much higher. Many of these patients have been treated at the Altamira City hospital.

We plan to determine if antibodies against Schistosoma are present in the sera of settlers and residents of different sections of the highway, as well as in the serum samples collected in the future.

Foci of planorbid snails identified as Biomphalaria straminea (an important species in the epidemiology of schistosomiasis) were encountered in two streams. One of these is located approximately 9 Km from Altamira City, whereas the second one is in a suburban area of the same city. On the other hand, no planorbid species were found in 169 other sites examined along almost 300 Km of the road sections, from Altamira-Itaituba, Altamira-Vitoria and Altamira-Marabá, during 1971.

TABLE 1

Percentage of HI Antibodies to Arboviruses Among Residents Having Spent More than Six Months in Different Areas of the Trans-Amazon Highway, and Among Immigrants from Other Parts of the Country

Group of Arbovirus	R E S I D E N T S:					I M M I G R A N T S	
	Marabá	Altamira	Itaituba ^{1/}	Itaituba ^{2/}	Santarém	Tacajós ^{3/}	Altamira ^{4/}
A	13.9	10.3	26.4	21.2	14.9	2.2	1.2
B	50.9	22.4	35.0	41.8	30.8	9.9	25.0
C	4.3	0.8	2.8	4.2	0	1.0	0
Guamá	2.8	1.7	3.4	1.3	0	1.1	0
Bunyamvera	8.1	0	12.6	8.1	0.9	1.0	0
Simbu	1.4	4.3	0	1.9	2.2	0.2	0.3
Phlebotomus	4.8	2.5	6.8	6.5	2.9	0.3	0
Anopheles A	1.9	0.8	0.5	0.3	0.2	0.15	0
Piry ^{5/}	17.4	4.3	6.3	15.5	Not done	20.3	Not done

1/ Blood samples collected in 1971.

2/ Blood samples collected in 1972.

3/ Immigrants bled before entering the Trans-Amazon area.

4/ Immigrants with less than a month in the Trans-Amazon area.

5/ This agent is now considered to be a Rhabdovirus, and the determination of antibodies was by the neutralization test.

T A B L E 2
NEUTRALIZING ANTIBODIES TO PIKY VIRUS AMONG
IMMIGRANTS FROM RIO GRANDE DO SUL STATE

COUNTIES	SEX	A G E G R O U P S					
		0-9	10-19	20-29	30-39	40-49	50 +
TTE. PORTELLA	F	2/25 ϕ	11/33	7/24	3/7	10/15	2/3
	M	3/32	11/37	12/24	9/13	12/16	5/5
I R A I	F	1/1	3/5	1/2	3/3		1/1
	M	2/3	2/5	1/3	3/3		1/1
TOTALS	BOTH	8/61	27/80	21/53	18/26	22/31	9/10
PERCENTAGE	"	13.1	33.7	39.6	69.2	70.9	90

ϕ Positives / Tested.

T A B L E 3
SEROLOGICAL CONVERSIONS BY HI TEST TO ARBOVIRUSES IN
308 SETTLERS WITH PAIRED SERUM

Group	Viruses	Number of Conversions
A	Mayaro	2
B	Yellow Fever	6
	Ilhéus	2
	SLE	1
	Gr. B	18 ϕ
Bunyamwera	Guaroa	1
Guamá	Catu	2
Phlebotomus	Itaporanga	1
TOTAL		33

ϕ 5 Samples cross reacted with SLE and Ilhéus viruses, but not with YF virus.

TABLE 4

MINIMAL AND MAXIMAL RATES OF HI ANTIBODIES TO ARBOVIRUSES IN
WILD ANIMALS CAPTURED AT DIFFERENT AREAS OF THE TRANSAMAZON
HIGHWAY

Groups of Arboviruses	Birds	Marsupials	Rodents	Primates	Edentates	Chiroptera	Others
A	0.7-9.9+	0	0-2.08	20-67	0	0	0
B	6.9-18.6	0	0-0.6	0-45.4	0	0-12.5	5.4
C	0-3.3	0	0-4.7	0	0	0	0
Guamá	0-2.3	0-5.2	0-2.8	0	0	0	0
Capim	NT	0	0.9	NT	NT	NT	NT
Bunyanwera	0- 0.7	0	0	0	0	0	0
Simbu	1.0-2.8	0	0	0-8.0	0	0	0
Phlebotomus	0- 2.8	0-10.5	0-33.3	0-16.0	37.6	0- 2.0	2.7
Turlock	0.3-9.6	0	0	0	0	0	NT
Anopheles A	1.0-5.2	0	0	0- 6.0	0	0- 6.7	0

+ Percentage

NT - Not tested

TABLE 5

THE PERCENTAGE OF MOSQUITOES CAPTURED BY DIFFERENT METHODS IN AREAS OF THE TRANSAMAZON HIGHWAY

AREA / DATE	METHODS OF COLLECTION			
	HUMAN BAIT	TRINIDAD Nº 17 BAITED WITH MICE	CDC LIGHT TRAP	MECHANICAL SUCTION
Itaituba - Altamira, Km 25 March - April 72	<u>Psorophora</u> 57.4% <u>Culex</u> 15.5% <u>Aedes</u> 13.5% <u>Anopheles</u> 8.7%	<u>Culex</u> 56.9% <u>Psorophora</u> 40.7% <u>Aedes</u> 1.0%	+	No collection
Itaituba - Jacareacanga July - Aug. 72	<u>Culex</u> 87.4% <u>Anopheles</u> 8.4%	<u>Culex</u> 97.3%	<u>Uranotaenia</u> 75.8% <u>Culex</u> 22.5%	<u>Culex</u> 94.7% <u>Anopheles</u> 2.3%
Santarém - Cuiabá Km 84 Nov. - Dec. 72	<u>Eichoproson</u> 83.2% <u>Wyeomyia</u> 7.1% <u>Anopheles</u> 4.4%	++	<u>Uranotaenia</u> 79.3% <u>Culex</u>	<u>Culex</u> 81.2% <u>Anopheles</u> 9.7% <u>Uranotaenia</u> 8.5%

+ 16 mosquitoes captured but not included

++ 39 mosquitoes captured but not included

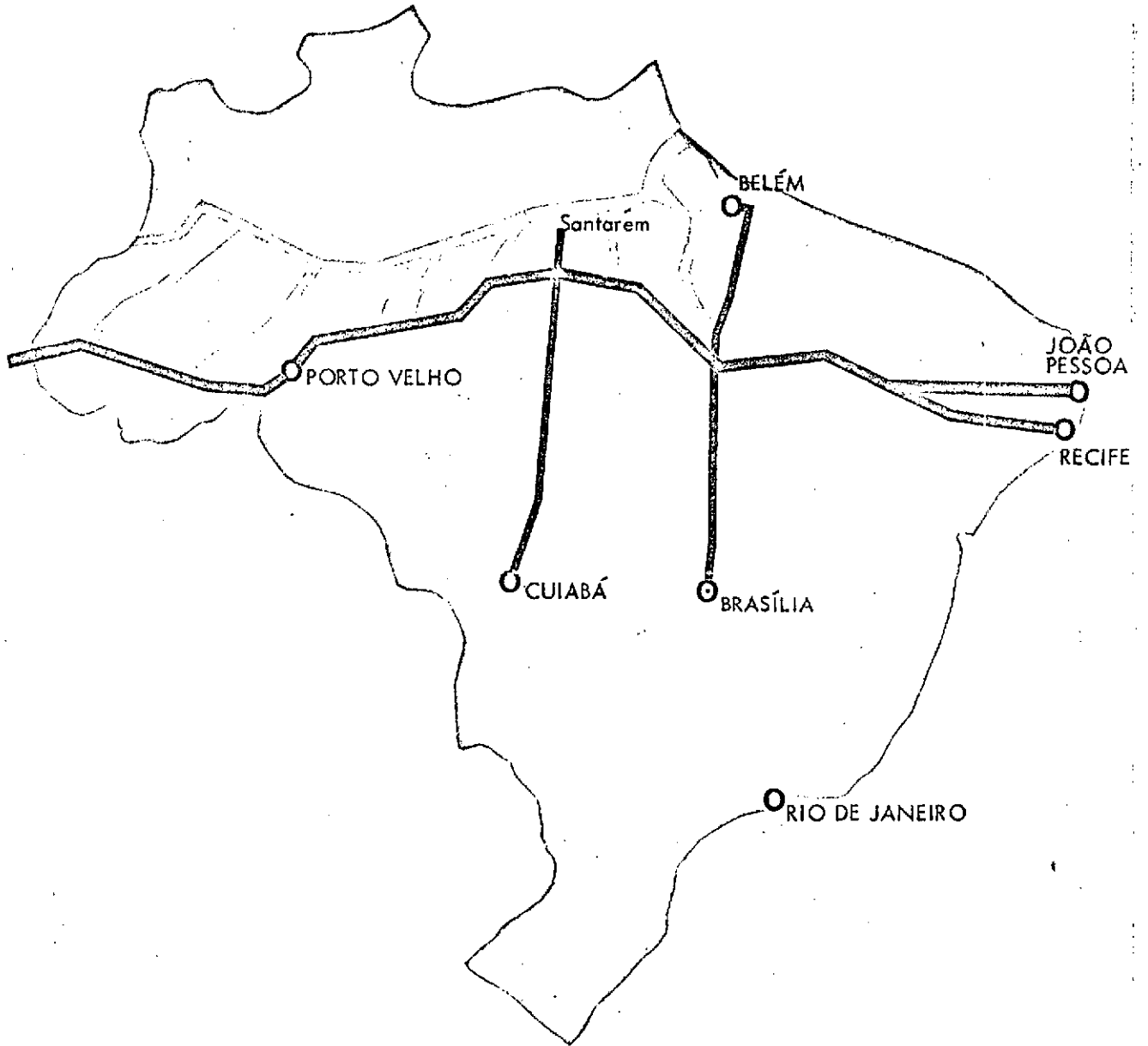
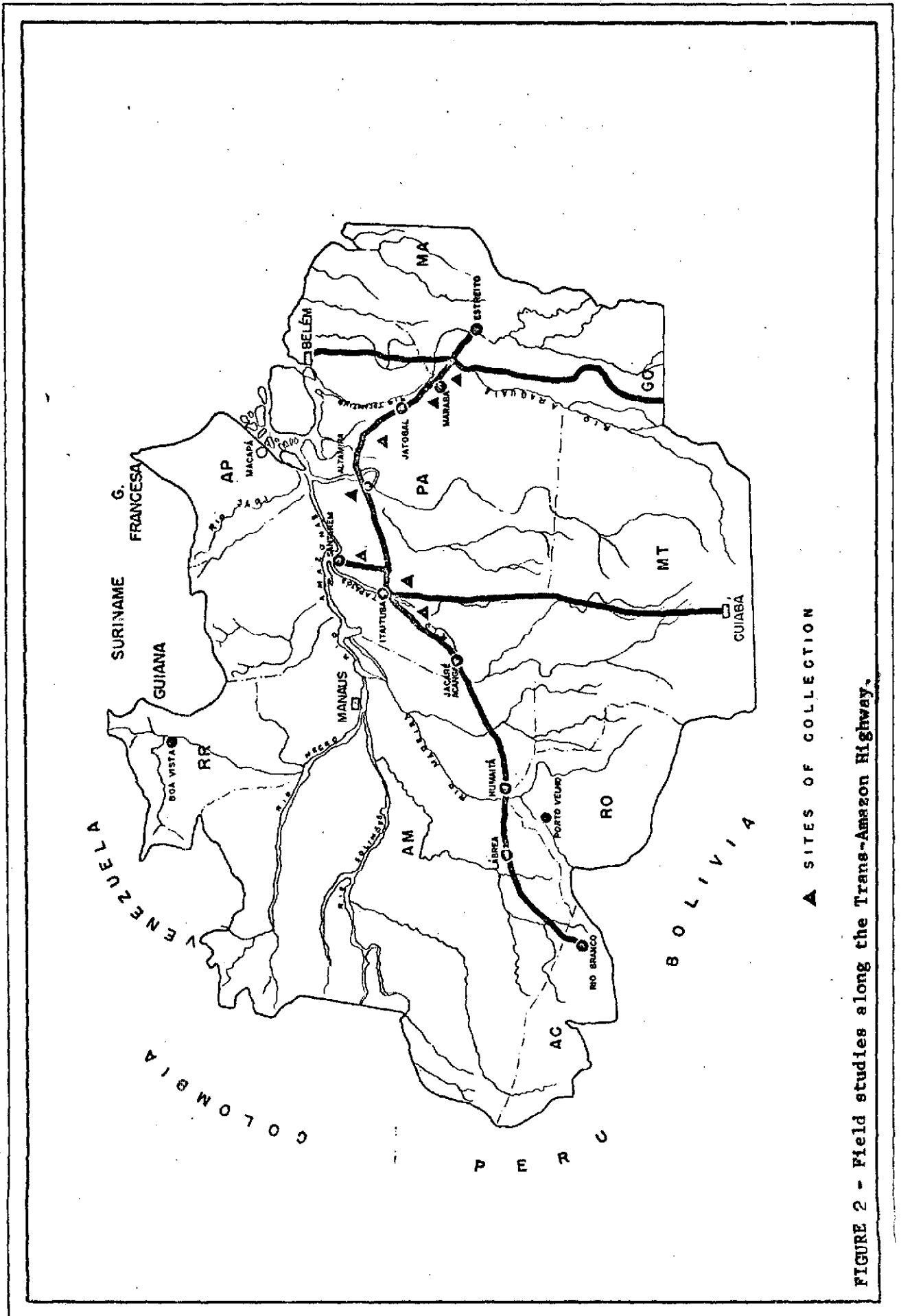


FIGURE 1

COLONIZED AREAS ALONG THE TRANS-AMAZON HIGHWAY



▲ SITES OF COLLECTION

FIGURE 2 - Field studies along the Trans-Amazon Highway.

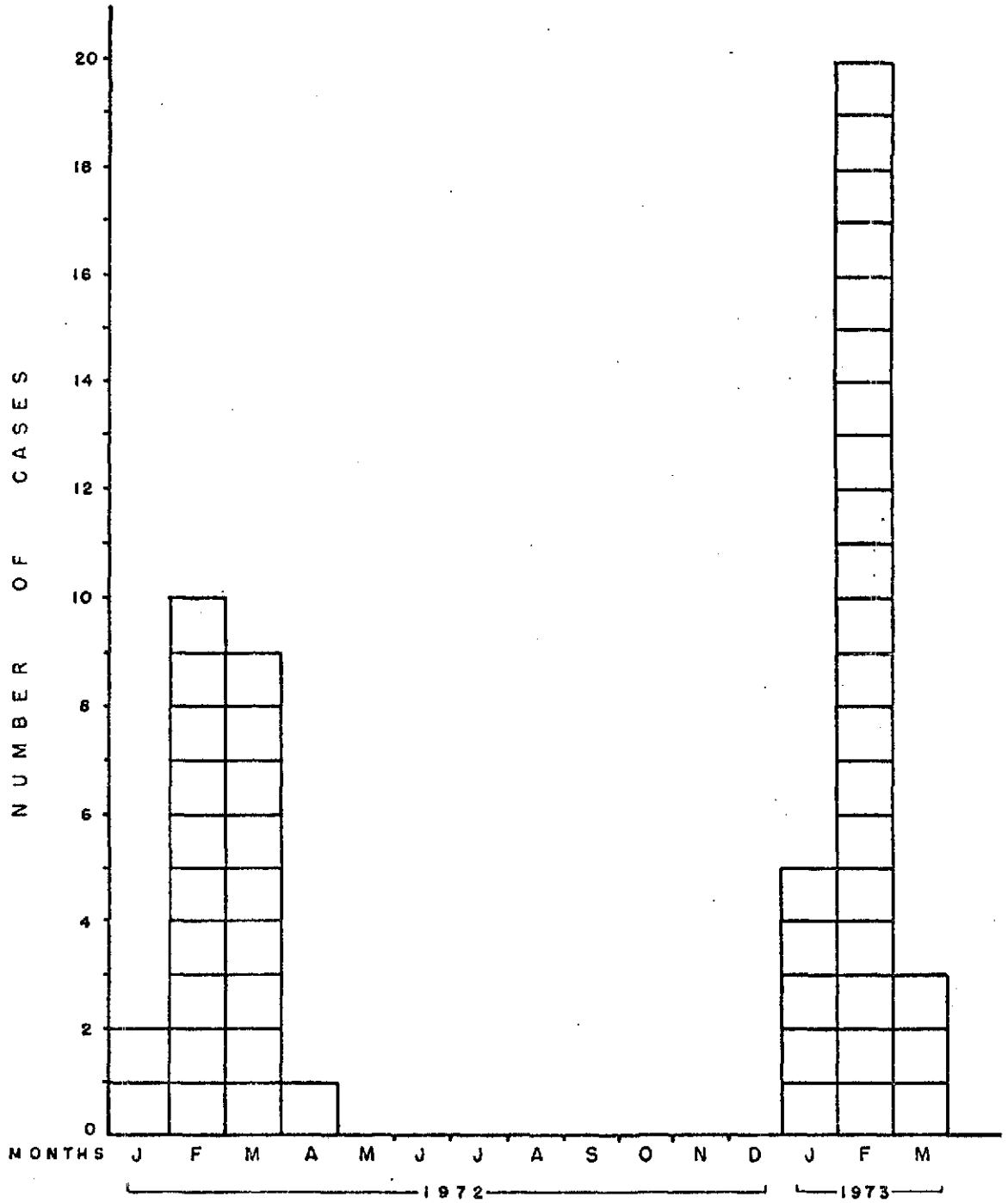


FIGURE 3 - Cases of hemorrhagic syndrome of Altamira, by month, in 1972 and in January-March 1973.