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# Modifications of arbovirus transmission in relation to construction of dams in Brazilian Amazonia

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The Amazonian region of Brazil seems to be the world's richest reservoir of arboviruses. To date, 183 different types of arboviruses have been detected in the Amazonian region, accounting for more than one third of the 535 arboviruses reported in the world. Of these, 136 (74.3%) are endemic. The main objective of this paper was to evaluate what the effects of the construction and flooding of dams would be on the transmission and epidemiology of sylvatic arboviruses, in order surveys: *i*) inside the dam region, before, during and after the flooding period; *ii*) outside the dam region, in an area with a similar climate, landscape and vegetation. during the total time for studies. The two chosen areas to define the potential health hazards to which the human population would be subjected. Five studies. The two chosen areas, namely Altamira and Tucuruí, were studied since 1974, and since

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September, 1982, respectively. The former, and the latter before the outset of flooding (September 6. 1984) were considered as a control or reference for comparisons with data obtained in Tucuruí during flooding and thereafter. The surveys consisted of sampling as many wild vertebrates and haematophagous Diptera as possible. Human sera were collected from febrile cases and random serological surveys. Very few variations seem to have occurred among the three phases of the study, with a maximum of positive serologies during the flooding period, but the differences were not statistically significant. Similarly, in the reference region, the prevalence of antibodies against the four more important arbovirus groups did not vary significantly. The arboviruses were classified under two categories: i) those which showed enhanced transmission and ii) those which showed no apparent modifications in their transmission patterns (referring to the control area). Three new and three already known types in the Anopheles A group of *Bunyavirus* were favoured by the great proliferation of Anopheles nuneztovari and An. triannulatus which accompanied the flooding of the dam. An epizootic of the Gamboa virus (Bunyavirus, Gamboa serogroup), newly reported in Brazil, was observed one year after the outset of flooding, when the mosquito Aedeomyia squamipennis showed very large populations. Guaroa (Bunyavirus, California group), an endemic arbovirus, showed an epizootic probably as a consequence of the proliferation of anopheline mosquitoes. A temporary proliferation of *Culex* spp. mosquitoes and the presence of a rich avifauna in the dam area during flooding are probable causes of the enhanced circulation of Turlock (Bunyavirus, Turlock group) and Kwatta-like (Rhabdoviridae, Kwatta group) viruses. Oropouche (Bunyavirus, Simbu group), Saint Louis Encephalitis (Flavivirus, group B), Maguari/ Xingu (two jointly studied *Bunyavirus*, group Bunyamwera), and Triniti (ungrouped Togaviridae) arboviruses showed at least patterns of enhanced circulation but probably for diverse reasons, not all due to the flooding of the dam. The last two types showed a possible shift of mosquito vectors, from diurnal ones to the nocturnal above cited anopheline. Eight arboviruses showed no clear-cut differences between their transmission patterns in the reference region and the ecologically modified dam area. They are: Yellow Fever (*Flavivirus*, group B), Mayaro (*Alphavirus*, group A), Ilheus (Flavivirus, group B), Tucunduba (Bunyavirus, Bunyamwera group), Eastern (and Western) Equine Encephalitis (Alphavirus, Group A), Icoaraci (Phlebovirus, Phlebotomus group), and Itaporanga (Phlebovirus, Phlebotomus group) viruses. The first six are pathogenic to man. The usefulness of classifying ecologically the arboviruses is highlighted, when studying or forecasting possible impacts of dam impoundment on human health hazards due to these agents. The scarcity of such studies does not allow comparisons for future studies, but it would be most important (i) to define the ecological characteristics of the new equilibrium established in modified regions, and (ii) to adopt a more previsional attitude (hypothesis testing) during project planning.

O objetivo principal do estudo foi avaliar os impactos da construção e enchimento de barragens, sobre a transmissão dos arbovírus silvestres e os riscos de contaminação da população humana. Resultados signi-ficativos foram obtidos apenas durante a execução do projeto Tucuruí/ Altamira. Somente nesse caso foi possível realizar comparações entre coletas de dados virológicos e sorológicos: 1) na área da barragem, antes, durante e depois do enchimento do lago; e 2) numa outra região, com clima, vegetação, e paisagem similares, mas longe das possíveis influências da barragem: município de Altamira, cidade localizada às margens do crio Xingu e da rodovia Transamazônica. As coletas de mate-rial foram organizadas a fim de se obter o maior número possível de animais silvestres, dípteros hematófagos e soros humanos em ambas as regiões e durante cada um dos períodos. A evolução da sorologia humana é apresentada em relação ao tempo para Altamira e em relação às fases do enchimento, para Tucuruí. As variações do percentual de soros positivos para os principais arbovírus patogênicos para o homem não foram significativas, tanto na região de referência, como durante as fases do enchimento da barragem. O número mais elevado de sorologias positivas durante o enchimento não foi significativo. No que concerne à circulação silvestre dos arbovírus, numerosos dados já estão disponíveis, e mostram uma complexidade e variedade até então insuspeitáveis. Com relação aos efeitos das mudanças ambientais sobre os seus ciclos de transmissão, os arbovírus foram agrupados em duas categorias: 1) aqueles cujo nível de transmissão subiu, causando epizootias ou não; e 2) aqueles cuja transmissão aparentemente não foi modificada. Na primeira categoria encontram-se: a) três tipos novos e três tipos conhecidos no grupo Anopheles A, que foram ativamente transmitidos pelos Anopheles nuneztovari e An. triannulatus; b) o vírus Gamboa, novo para o Brasil,

que causou um epizootia em aves, evidenciada um ano após o início do enchimento, quando as populações do vetor Aedeomyia squamipennis eram muito numerosas; c) o arbovírus endêmico Guaroa, que foi também ativamente transmitidos pelos mosquitos do gênero Anopheles na área de Tucuruí; d) os arbovírus Turlock e Kwatta-like, cuja transmissão foi favorecida por uma proliferação temporária (durante o enchimento e alguns meses após) de mosquitos do gênero Culex e a presença de uma fauna muito rica de aves; e) os vírus Oropouche, Encefalite de São Luís, Maguari/Xingu e Triniti foram também evidenciados, mas os seus picos de transmissão não foram devidos somente ao enchimento do lago. Em particular, os dois últimos tipos mostraram uma possível mudança de vetores, passando de mosquitos diurnos (Sabetíneos) a noturnos (os anofelíneos já citados). Um fator ecológico, que teve uma possível ação sobre a amplificação da transmissão desses arbovírus, pode ter sido a concentração (natural ou através da operação de resgate de fauna) de animais silvestres estressados, nas ilhas ou nas margens do lago. Na segunda categoria encontram-se oito tipos de arbovírus, que não mostraram nenhuma diferença marcante nos padrões de transmissão entre a região de referência e a região de Tucuruí. Compreendem seis tipos patogênicos para o homem, tais como Febre Amarela, Mayaro, Ilheus, Tucunduba, Encefalites Equinas do Leste e do Oeste, e dois arbovírus, Icoaraci e Itaporanga, isolados de roedores e marsupiais, respectivamente. No que concerne aos resultados globais, alguns números permitem mostrar a grande riqueza e diversidade do meio amazônico. De um total de 74 tipos diferentes isolados, pelo menos 53 (71%) — ou 113 do total conhecido na Amazônia Brasileira — foram isolados do material coletado em Tucuruí; 37 (50%) tipos foram obtidos na região de Altamira, distante de Tucuruí apenas 300 km. Até agora, as

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duas regiões forneceram somente 16 (21,6%) tipos em comum. Esse estudo sobre os arbovírus e a ecologia de um lago artificial é o primeiro realizado na Bacia Amazônica. Outros estudos na região neotropical foram os de Bayano no Panamá e de Brokopondo no Surinam. Embora as regiões em questão sejam bastante diferentes, é possível comparar nossos resultados com os do projeto Bayano, que tratou detalhadamente de arbovírus: 1) Os efeitos das barragens parecem ter sido diferentes no que concerne os vírus do complexo da Encefalite Equina da Venezuela e do complexo Chagres, não evidenciados em Tucuruí; 2) os vírus SLE e Gamboa mostraram o mesmo padrão epizoótico, favorecido pelas novas condições ecológicas; 3) igualmente nas duas regiões, a transmissão dos vírus Mayaro e da Febre Amarela não parece ter sido modificada devido às barragens; 4) tanto no Panamá como em Tucuruí, numerosos tipos novos foram isolados, pertencentes em sua maioria aos grupos Changuinola e Phlebotomus. A escolha de uma zona de referência, além da zona da barragem antes do enchimento, foi de importância primordial para análise de fenômenos imprevisíveis, tais como a transmissão de arbovírus silvestres ou a infecção do homem, ligados a fatores climáticos e biológicos, ainda desconhecidos. As precedentes conclusões pareceram óbvias quando se consideram os resultados fragmentados e inespecíficos, obtidos durante os escassos estudos feitos nas outras barragens, Balbina, Samuel e Santo Antonio. Estudos futuros serão planejados para definição das características do novo equilíbrio ecológico. Além disso, projetos para estudos de campo em outros UHE, deverão ser fundamentados na experiência de Tucuruí, objetivando testar as hipóteses acima apresentadas.

S ioli (1), Marlier (2), Paiva (3) and Guillaumet (4) have summarized the main features of the Amazonian region as follows: the Brazilian part of the hydrographic basin of the Amazon river and tributaries covers 3,984,467 km<sup>2</sup>, or 63.1% of the total area (6,315,217 km<sup>2</sup>); maritime and land borders of the region represent 55.1% of the total borders of Brazil; approximately 3,373,000 km<sup>2</sup> (84%) of the Brazilian Amazonia are covered by dense equatorial forest.

Since the discovery of its numerous natural resources more than 100 years ago, this region has been developing rapidly, with an ever increasing need for energy, both for agriculture and the extraction of minerals (5).

On the other hand, this region is sparsely inhabited, with less than 10% of the total Brazilian population and, excluding the capital cities of the Amazonian States, it has one of the world's lowest population densities, less than 2 inhabitants/km<sup>2</sup>. With its equatorial or humid tropical climate, its enormous potential for employment and its natural resources, Amazonia is indeed very attractive especially for people from regions which suffer from severe droughts like the Brazilian Northeast.

The area has many large rivers and their energy has been made available, since the 50s, by construction of various dams.

Besides evaluating the ecological problems which may arise from cutting and flooding large forest areas (1,6), the appraisal of the health situation of the local autochtonous and allochtonous human populations was among the priorities of the country's development agencies.

Thus, agreements were made between the company Eletronorte (Eletrobrás) and the Evandro Chagas Institute (FNS-MS), with the objective of studying the possible impact of the construction of large dams on the epidemiology of arboviruses. Since much of the material collected during the 10 preceding years remains to be studied, the results presented here can only be considered as preliminary.

It seems that the Amazonian region of Brazil is the world's richest reservoir of certain infectious agents. Thus, the 183 different types of arboviruses so far detected in the Amazonian region (7-14), accounts for more than one third of the world's arboviruses (N=535) (15). Of these, 136 (83.4%) are endemic and 34 (18.5%) are pathogenic to man.

I he main objective of these studies was to evaluate the effects of the construction and flooding of a dam on the transmission and epidemiology of sylvatic arboviruses so as to determine the potential health hazards that human populations entering the area would be subjected to.

Whenever possible, comparisons were made between a number of surveys: 1) inside the dam region, before, during and after the flooding period; 2) outside the dam region, but in an area with a similar climate, landscape and vegetation, during the total period of the studies.

The reasons for using the latter as a control were: a) the much lower number of isolations obtained in the dam area prior to flooding, and b) the dam area used during the preflooding period, and the neighboring area could not be monitored because of floods or inaccessibility. A third reason was the annual variability of intertropical climatology (16) which probably greatly influenced the seasonal and/or annual pattern of arbovirus transmission.

The two chosen areas, namely Altamira and Tucuruí, were studied since 1974, and since September, 1982, respectively. The former, and the latter before the outset of flooding (September 6, 1984), were considered as a control or reference for comparisons with data obtained in Tucuruí during the flooding period and thereafter.

In the case previously described (Tucuruí/Altamira) the conditions of study were ideal. On the other hand, despite different local ecological conditions, it was not possible to do the same type of longitudinal studies in the three other regions studied: Balbina-AM, Samuel-RO and Santo Antonio (Almeirim-PA), and only fragmentary results will be reported.

#### **Description of study areas**

### The two areas of the Tucuruí/Altamira project

*Tucuruí*, or the flooded area:  $3^{\circ}45'$  S -  $49^{\circ}41'$  W, is located at the low portion of the Tocantins river basin, at an altitude of 40 m above sea level.

Technical and physical data about the dam and the artificial lake, and the neighboring human population have already been published (17,18,19).

During the preflooding period, a field station, located along the old (now flooded) BR 422 road, at km 67 between Tucuruí and Marabá, near a little river named "Pucuruizinho", was used for collection of vertebrates and insects.

During the six-month duration of the flooding period,

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Figure 1. Situation map of the dam impoundment area. Tucuruí. Pará. Brazilian Amazonia. The flooded area is striped and the locations of collecting are numbered 1 through 5. The postflooding collecting station is indicated with a star.

various small localities, situated in the area of the actual lake, were visited and their populations sampled. Of these, "Arumateus", "Vale do Caraipé", "Canoal", "Jacunda", "Jatobal" survive only as virus' names.

Of the five field stations used during the rescuing of the fauna, nos. 3 and 4 were also occasionally used and no. 4 was assigned for all postflooding studies (Fig. 1).

Altamira, or the reference area: 3°12' S - 52°12' W, is located at the lower portion of the Xingu river basin, at an altitude of 74 m. The Xingu basin, a tributary of the Amazon, is not as extensive as the Tocantins/Araguaia basin. Four field stations were used in the same region, along the Itaituba-Altamira-Marabá road (Transamazonian highway).

From 1974 to 1982, the surveys were done at various locations between km 10 and km 190 on the Altamira-Itaituba road section and between km 8 and km 90 on the Altamira-Marabá section. Since 1983, only one field station at km 18 at the latter section has been used.

Peterson et al (20) have already provided numerous ecological information about this region.

The results of the studies conducted during the 1974-76 period have already been published (21,22,23,24).

The reference and study areas, approximately 300 km from each other, are geologically and climatically similar, and have nearly the same vegetation (16,25,26,27,28).

Both are situated near the most northern limit of the precambrian Brazilian shield, which here, is covered with eroded late tertiary sedimentary deposits.

The climate, also very similar in the two regions, is classified as warm (mean t° > 22° C), of the tropical humid type, with three dry months (< 60 mm). The mean annual relative humidity is between 85% and 90%. The mean monthly rainfalls in the two localities are well correlated (Spearman correlation coefficient = 0.96 with p < 0.021%). However, there is a 1°C difference between the annual means of the two counties, which are situated on either sides of the 26° isotherm. Altamira has a mean maximum annual temperature 2°C lower than Tucuruí. Similarly, the mean annual rainfall is slightly lower in the Altamira region, being between 1,500 and 2,000 mm, while in Tucuruí it is between 2,000 and 2,500 mm. The mean number of rainy days is also a little lower for the Altamira region, 120-180 versus 180-240 for Tucuruí (Fig. 2).

During the period from 1972 to 1988, the monthly rainfalls of the two counties were highly correlated (Spearman correlation coefficient = 0.85 with p < 0.006%).

The forests covering the two areas are classified by Kuhlmann (27) within the same category of vegetation: hilean hygrophilous perennifolian Amazonian forest, or dryland ("terra firme") forest.

### The area of the Balbina project

The dam is crossing the Uatamã river (AM). The whole area is covered by dense tropical rain forest.

In the area of the Balbina project, only three collecting trips were made, from April 18 to 27, from May 23 to June 5, and from December 6 to 14, 1988.

### The area of the Samuel project

The dam is crossing the Jamari river (RO). The vegetation is mainly constituted of dense tropical rain forest. In the lower areas, the forest may be periodically inundated (29). Only one trip was made, from November 17 to December 12, 1988.

### The area of the Santo Antonio project

The dam is crossing the Jari river which separates the Amapá and Pará States. It is located near Monte Dourado town. The vegetation is constituted of dense tropical rain forest of the submontane type and cultivated areas on the left and right sides of the Jari river, respectively. Only one trip was made, from May 11 to June 9, 1987.

In the present study, the surveys were oriented to obtain as many samples of vertebrates, and hematophagous diptera as possible. Human sera were collected from febrile patients and random serological surveys. Changes in the profile of human serology and in the rate of virus isolations in people may be helpful indicators to assess the impact of ecological transformation on arbovirus transmission to man. Unfortu-



Figure 2. Mean monthly rainfall (in mm) in Tucuruí (black) and Altamira (white), Pará, Brazilian Amazonia, 1972-1988.

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Table 1 — Hematophagous Diptera, wild and sentinel ("s") vertebrates' viscera and sera, and human sera, collected and inoculated in the Altamira region, Pará, Brazilian Amazonia, with, in parentheses, the number of arbovirus isolations, 1974-1988.

					Human		
Year/Origin	r/Origin Dipte		Vertebrates		Collected	Inoculated	
1974	15,079	(6)	47 s	(4s)	3,667	442 (3)	
1975	4,746		54 s		5,852	471 (1)	
1976	3,167		69s, 66		1,914	220 (1)	
1977	12,920	(1)	70s, 40		1,244	325 (1)	
1978	9,347	(4)	64 s		594	257 (1)	
1979	24,329	(2)	69 s		666	484 (3)	
1980	24,237	(6)	72 s		454	393 (2)	
1981	26,733	(1)	34 s		322	301 (1)	
1982	14,107	(4)	17s, 14		245	232 (1)	
1983	70,556	(11)	214	(2)	213	141 (1)	
1984	8,389	(9)	30	(1)	467	168	
1985	28,210	(1)	450		750	87	
1986	47,942	(5)	216	(2)	437	25	
1987	-	-			352	30	
1988	10,361	(4)	154		13	11	
Totals	300,123	(54)	496s,1,18	4 (4s,5)	17,190	3,587 (15)	

nately, many data on historical and social aspects of the colonization are missing, for they are rarely collected at the same time as the ecological data. Despite these limitations, we collected data on age, sex, birthplace, residence (but not the date of entering the area) and occupation, thus allowing us only to infer about the infection inside or outside the region.

Wild vertebrates were collected with fruit-baited traps or selective hunting in the case of monkeys, and mist nets in the case of bats and birds. Blood and viscera were usually collected from each animal. Insects, mostly mosquitoes, phlebotomine sandflies and ceratopogonid midges, were sampled during human-baited canopy or floor diurnal collections, nightly "CDC miniature light-trap" canopy or floor runs, and active search in the understory vegetation. In the particular case of the terrestrial tortoises in the dam area, it was possible to do a marking-release-recapture experiment.

The collected materials were preserved in liquid nitrogen in the field, transferred in freezers to the

laboratory, before being identified and inoculated intracerebrally into 48 h-old Swiss suckling mice. Sera were tested against 19 antigens, most of them pathogenic to man, by hemagglutination-inhibition (HI). In some instances, neutralization tests (NT) were also performed, usually on mice.

### The Tucuruí/Altamira project

The numbers of hematophagous Diptera, wild and sentinel vertebrates' viscera and sera, and human sera, collected and inoculated at the Altamira and Tucuruí areas are reported in Tables 1 and 2, respectively.

#### Evolution of human serology — A total of

1,940 sera were obtained, sometimes from the same donor more than once. Of these, 132 (6,8%) were serologically converted for at least one arbovirus. However the great majority (99/132 or 75%) were either group responses to gr. B and gr. A viruses, or specific responses to YF virus. In these two cases, no conclusions can be drawn about the nature and time of human contaminations with sylvatic arboviruses. Thus, only serological conversions specific for one virus will be dealt with in the following account.

Results are shown in Figures 3 and 4 for the reference region and for the dam area, respectively. For the latter, the proportions are grouped according to the three periods: before, during and after flooding.

Among the group A arboviruses (Togaviridae), Mayaro virus gave by far the greatest proportion of positive reactions. The few other positive reactions in the same group were due to Mucambo (MUC) virus (member of Venezuelan Equine Encephalitis (VEE) complex), and to Eastern Equine Encephalitis (EEE) virus.

With respect to the prevalence of group B arbovirus (Flaviviridae) antibodies, two facts should be emphasized. First, most of positive results were probably due to yellow fever (YF) vaccination. Second, among the specific reactions, Ilheus (ILH) and Saint Louis Encephalitis (SLE) viruses were responsible for 355 and 56 positive results, respectively.

Antibodies to Oropouche (ORO) virus, a *Bunyavirus* of the Simbu group, were not very prevalent, despite the large impact in Amazonia during past epidemic episodes (10).

The antibody prevalence of the other groups was very low, below 2%.

Few variations seem to have occurred between the three phases of the study, with a maximum of positive serologies during the flooding period, but the differences were not statistically significant ( $X^2 = .818$ ; df = 6; p = .99).

Virus-specific serological conversions (except for YF: vide supra) have been shown for the arboviruses Oropouche (12 conv.), Ilheus (8 conv.), Mayaro (6 conv.), Guaroa (3 conv.), Mucambo (2 conv.), and Eastern Equine Encephalitis (1 conv.), demonstrating few contacts of this population

Table 2 — Haematophagous Diptera, wild vertebrates' viscera and sera, and human sera, collected and inoculated at the Tucuruí hydroelectric dam area, Pará, Brazilian Amazonia, with, in parentheses, the number of arbovirus isolations. The period of study, 1982-1988, is divided according to the phases of flooding of the lake. <sup>*a*</sup> indicates that the collected material is still under identification and/or inoculation.

		Diptera Vertebrates			Human		
Period	Date			Vertebrates		Collected	Inoculated
	1982	53,118	(2)	818	(1)	163	59
Preflooding	1983	30,672	(4)	507	(4)		
	Jan 1,1984 to	+					
x	Sept 6,1984	90,361	(12)	679	(10)	352	7
Sub-totals		174,151	(18)	2,004	(15)	515	66
During	Sept 7,1984 to						
flooding	Dec 31,1984	975,632	(43)	590	(1)	1,165	72 (2)
	1985	265,114	(94)	915		1,764	68
Post-	1986	170,681	(26)	695	(2)	1,678	71
flooding	1987	94,462	(12)	357		384	23 (1)
-	1988	2,708 <i>ª</i>		333		476	32 (2)
Sub-totals		532,965 (	(132)	2,300	(2)	4,302	194 (3)
Grand totals		1,682,748 (	(193)	4,894	(18)	5,982	332 (5)

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with these primarily sylvatic agents. The case of Oropouche virus is different, for it can cause large urban and rural epidemics.

*Ecoepidemiology of selected arboviruses* — In order to emphasize what may have happened in the transmission patterns of the arboviruses in relation to ecological modifications in the dam area, we will discuss the results under two somewhat arbitrary but convenient categories. These will concern arboviruses: *i*) which have shown an enhancement of their transmission levels, whether or not caused by the new ecological conditions, and *ii*) which have shown no apparent modification in their transmission patterns (referring to the control area).

New ecological and virological data obtained as byproducts of these studies were published in other places (30,31) or are yet to be published.

Epizootics of newly described, exogenous or endemic arboviruses

The transmission patterns of the following arboviruses were probably pertubated due to ecological modifications caused by the dam construction and flooding: six different types in the Anopheles A group, Gamboa, Guaroa, Turlock, Kwatta-



'igure 4. Percentage of positive HI antibodies against the four most prevalent groups of arboviruses, in human sera from the Tucuruí dam area, Pará, Brazilian Amazonia, grouped according to the three flooding periods, 1982-1988.



Figure 5. Minimum infection rate of Anopheles spp. mosquitoes, positive for six different Bunyaviridae (Bunyavirus) of the Anopheles A group, from the Tucuruí dam area, Pará, Brazilian Amazonia, grouped according to the three flooding periods, 1982-1988.

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Figure 3. Annual percentage of positive HI antibodies against the four most prevalent groups of arboviruses, in human sera from Altamira, Pará, Brazilian Amazonia, 1974-1987.

like, Oropouche, Saint Louis Encephalitis, Maguari/Xingu, and Triniti viruses. The following discussion will emphasize the ecoepidemiologic peculiarities of the cycle of each one.

Anopheles A group (Bunyaviridae: Bunyavirus). These arboviruses showed a very distinctive transmission pattern as a doubtless consequence of the dam impoundment. Until 1984, only three different types were known in Brazilian Amazonia: Tucaiuma, Lukuni and Trombetas, the first two being pathogenic to man (15). These arboviruses, and three new types appeared in the dam area during flooding or a little before, as a probable result of the proliferation of their mosquito hosts, mainly Anopheles (Nyssorhynchus) spp. (Fig. 5). An. nuneztovari and/or An. triannulatus furnished all the strains and thus, seem to be the normal hosts of these viruses. However, it is the first time that Tacaiuma virus was isolated from nocturnal anophelines. known only from the diurnal Haemagogus janthinomys, Ae. scapularis and An. (Kerteszia) sp. Similarly, Lukuni virus was known only from the latter mosquito and the diurnal-crepuscular An. nimbus.

Considering the group as a whole, the numbers of isolated strains were 3 for the Altamira region, and 2, 26, and 54, for the pre, during, and postflooding periods, respectively, in the dam area. This suggested a relationship between the flooding periods and intensity of transmission was confirmed when we considered the real infection rates of the mosquitoes for each arbovirus of that group ( $X^2 =$ 21.34; df = 10; p = .0188).

Serological data were routinely available only for the Tacaiuma virus, which is the only one of these six Anopheles A viruses, from which a hemagglutinin has thus far been obtained. Despite the two isolations obtained from man in the Altamira region, yearly antibody prevalence never exceeded 1.5%, and in the dam area the yearly prevalence was always less than 1.2%. The sera of five birds (5 different species), three monkeys (*Alouatta belzebul*) and two armadillos (*Dasypus novemcinctus*), collected either before or after flooding, had antibodies for this arbovirus. Some neutralization tests have shown that carnivorous and ungulate mammals are probably involved as vertebrate hosts of the Tucuruí virus.

Gamboa virus (GAM: Bunyaviridae, Bunyavirus). Although very largely distributed in Central and South America (Panama, Ecuador, Argentina (15,32), the Gamboa virus is new in Brazil. Epizootic-level transmissions of this virus (probably including various related types) occurred in Panama during the period from April, 1985 to July, 1987 (33).

It is noteworthy that our first two isolated strains were from the blood of birds (*Geotrygon montana*: Columbidae, and Xenops minutus: Furnariidae) collected on August 2 and 3, 1984, just one month before the outset of flooding. These two species are very common from tropical Central America to Argentina (34) and the former is known as a possible although not proven migrant (35). Seventy six strains were isolated from mosquitoes Aedeomyia squamipennis, one from Anopheles triannulatus, and two more from the birds G. montana and Glyphorhynchus spirurus (Dendrocolaptidae), all collected in the dam area during the postflooding period, or at least one year after the initial isolations (Fig. 6).

Two hypotheses may be formulated to explain what happened in this region: *a*) the virus was present in the area, but was never encountered despite the good sample size of vertebrates and mosquitoes that was examined; *b*) the virus was newly introduced in the area by a migrating bird from Central America, where the agent was circulating since its first isolation in 1962, and with greater intensity during the period from 1985 to 1987. Further, a delay of eight months was necessary for the virus to become detectable in the mosquito host. *Ad. squamipennis* is known to breed in waters invaded by floating plants, thus it is probable that such favorable conditions were not met until some months after completion of the flooding. The unique strain from *An. triannulatus*, although probably not a laboratory artifact, is not thought to be of ecological importance.

As it was already noted by other authors, the vertebrate hosts of this virus are birds living in the understory of humid tropical forest. Birds of the same families as those from Tucuruí were found with antibodies in Argentina (32).

Guaroa (GRO: Bunyaviridae: Bunyavirus, group California). Until the present studies, GRO virus had been isolated only from man in Brazil (7) and from anopheline mosquitoes in Colombia, Panama, and French Guiana (15,36). In Brazilian Amazonia, it seems to be restricted to the eastern part of Pará State and was isolated only once (August, 1976) in Altamira, its westernmost station. Antibody prevalence in human sera is generally low and was zero in the reference region in 1986 and 1987 (Fig. 3).

In the dam area, it was isolated twice from man and four and three times from *An. nuneztovari* and *An. triannulatus*, respectively. The virus was present probably during the flooding period (November, 1984) and an epizootic occurred one year later between October and December, 1985 (Fig. 7). Although three human seroconversions occurred between June, 1985 and November, 1988, and one strain was isolated in July, 1987, its serological prevalence in human sera remained negligible. The only wild vertebrates which showed antibodies were three monkeys (*Alouatta belzebul*), captured during the flooding period.

Available data about this arbovirus indicate clearly that it has been favored by new ecoepidemiological conditions due to the dam construction and flooding. It is also probable that a lot of human cases were dismissed because of the lack of active searching of febrile cases.

Turlock (TUR: Bunyaviridae: Bunyavirus, group Turlock). TUR virus is typically a Culex-bird transmitted agent in Brazilian Amazonia, it has been isolated sporadically from at least 11 different bird species (7) and from mosquitoes. In the dam area, pre and during flooding periods have each furnished two strains. Although the virus has not been isolated again from the area —as it is also the case for the Altamira region since 1974 — it is probable that the enormous proliferation of Culex spp. during flooding favored a raise of enzootic circulation, which was reflected in the positive serology of the sera of 6 birds, bled during the first half of 1986 (during the total period of study, 13 birds of 12 species were positive).

*Kwatta-like (Rhabdoviridae, group Kwatta)*. This virus is ecologically comparable to Turlock, with a *Culex*-bird cycle (7). It was present in the area before flooding (one strain) and showed an enhanced circulation during flooding, four strains being isolated from *Culex (Culex)* sp.

However, unlike TUR virus, it was also present in the Altamira area at the same time (three strains isolated in 1982-1983), indicating that favorable ecoclimatic conditions were possibly independent of changes caused by the dam.

**Oropouche (ORO: Bunyaviridae: Bunyavirus, group Sim**bu). This virus, not prevalent in the Tucuruí area's human serological surveys (cf. supra), nor the populations of its usual ceratopogonid midge vector at first did not appear to us to have been favored by the dam impoundment.

However, one strain was isolated recently (April, 1988: possibly an imported case, from Araguaina, Goiás, see below) and ten seroconversions occurred in the human population between the beginning of 1986 and the middle of 1988. Further, the continuous circulation of ORO virus among the wild vertebrates is attested by relatively high annual rates of positive animals, mainly birds and monkeys during the flooding period. The annual prevalence rates of positive birds (during the flooding phase, the bird sera were all negative) were between 2% and 7.1%, with two peaks in 1983 and 1986. The monkey sera collected during the flooding period showed a positivity of 8.5% (too few monkeys were tested during the other periods).

Besides the confirmation of the effective role of birds (22 different species), monkeys (5 species) and sloths (1 species) in the sylvatic cycle of this arbovirus (cycle hypothesized by Hervé et al, (7) and previous authors), these findings are compatible with the creation of ecological conditions that favored the transmission of ORO virus.

But recent studies (37) have brought some support for another interpretation. In fact, such an enhancement of transmission may also have been due to general favorable





Figure 6. Minimum monthly infection rates of the mosquitoes Anopheles triannulatus and Aedeomyia squamipennis for arboviruses of the Gamboa group (Bunyaviridae: Bunyavirus), from the Tucuruí dam area, Pará, Brazilian Amazonia, during the postflooding period, 1985-1988.

Figure 7. Monthly minimum infection rates of the mosquitoes Anopheles nuneziovari and An. triannulatus for the arbovirus Guaroa (Bunyaviridae: Bunyavirus, California group), from the Tucuruí dam area, Pará, Brazilian Amazonia, during the postflooding period, 1985.

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ecological conditions that developed simultaneously in a much wider area. The latter is supported by: a) a raise in antibody prevalence in human sera collected during 1987 both in the reference region and the dam area, and b) the demonstration of a large epidemic which occurred some 300 km southeast of Tucuruí from December, 1987 to March, 1988. Unfortunately, ceratopogonid midges collected from the dam area during 1988 have not yet been sorted.

Saint Louis Encephalitis (SLE: Flaviviridae: Flavivirus, group B). SLE virus is predominantly a Culex-bird circulation virus. However, it has been isolated also from monkeys and other canopy-dwelling mammals (sloths, marsupials and bats). Until recently, it was considered as a canopytransmitted agent, but isolations from a great variety of ground-living mosquitoes and from an armadillo (Dasypus novemcinctus) suggest the existence of other cycles at lower levels (7,38).

In the reference region, it has probably been circulating from 1974 to 1986, with two epizootic periods in July-No-

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vember, 1974 and February-August, 1983. It probably remained in the area at a low (enzootic) level of transmission, as was suggested by the isolation of two strains, in May, 1984 and April, 1986, respectively.

In the dam area, SLE virus was isolated in 1982, in 1984 before flooding and during a two-month period after outset of flooding, and once more one year after, in 1985. Human seroconversions were detected in November, 1984 (1), April and May, 1985 (30, see 46). A positive reaction against this arbovirus has been detected in the serum of one marsupial *Didelphis marsupialis*, captured in 1985, thus confirming the possibility of this very common, versatile mammal, being one of the amplification hosts at groundlevel. Annual rates of antibodies in birds (10 species) were low (0.4-0.8%) but attained 3.4% in 1984.

Although the flooding of the dam has favored a great proliferation of culicine vectors (especially Culex declarator), and six strains were isolated during this period (versus three from Altamira), the present data has not revealed a clear-cut difference between the transmission patterns in the two regions. One interesting finding was a very high prevalence of group B antibodies (35.7% of 255 sera, 1985) in two sympatric species of large terrestrial tortoises (Chelonoidis carbonaria and C. denticulata), the populations of which were concentrated on islands. Unpublished experimental infection of tortoises by SLE virus have shown that both specific antibodies and group reacting ones appear simultaneously (although the latter at lower rates), allowing us to suspect SLE virus as

the natural causative agent of these serological findings. However, the exact significance of this has not yet been elucidated, especially the intertortoise vector. An as yet unidentified diurnal *Culex (Melanoconion)* mosquito was observed to be avidly attracted by these reptiles, but this aspect deserves more studies.

Maguari/Xingu (two jointly studied Bunyaviridae: Bunyavirus, group Bunyamwera). Strains, possibly of one or both of these two arbovirus types (39), have shown a very interesting fact: a possible shift of mosquito hosts.

With reference to other areas where these arboviruses were encountered, the natural hosts are diurnal mosquito species such as *Aedes scapularis*, *Ae. Serratus*, *Ae. fulvus*, *Psorophora ferox*, various Sabethini, etc.

In the Tucuruí dam area, there were four isolates between 1986-87 (near two years after flooding completion), from the crepuscular-nocturnal *Anopheles nuneztovari* and *An. triannulatus*. However, the hypothesis of host shift needs to be tested by a precise identification of the strains — for example, a third distinct type may have appeared —

and by looking for the presence of specific antibodies in wild vertebrates.

*Triniti (TNT, ungrouped Togaviridae).* The sylvatic cycle of TNT virus was not characterized until recently. It seems to be transmitted between diurnal sabethine (genera *Sabethes, Wyeomyia, Trichoprosopon*) and/or diurnal anopheline (*An. nimbus*) mosquitoes and diurnal rodents (7).

It was isolated from these vectors in 1983, in the Altamira region (two strains) and in Tucuruí during the preflooding period (three strains). One year after the flooding of the dam area (November-December, 1985), four strains were unexpectedly isolated from the crepuscularnocturnal species *Anopheles triannulatus*.

A shift — and delay — similar to those already noted for the Maguari/Xingu viruses may also well have occurred with TNT virus. Nevertheless, it is not clear if such a shift in vector species was accompanied by a shift in vertebrate hosts.

Arboviruses with transmission patterns apparently not modified

Eight arboviruses did not show any clear-cut difference between their transmission patterns in the reference region and the ecologically modified dam area.

Yellow Fever (YF: Flaviviridae: Flavivirus, group B) and Mayaro (MAY: Togaviridae: Alphavirus, group A). The YF virus is the most important cause of human deaths by arbovirus in Brazil, despite the well-proven efficaciousness of the vaccine. In fact, it appears logistically impossible to vaccinate a sufficient proportion of the people living far from cities, to prevent the few man-to-man rural and/or sylvatic outbreaks which seem associated with fatal cases (10).

In the Altamira region, human cases were confirmed, either by virology, histopathology, and/or serological conversions, nearly each every from 1977 to 1984, and in 1987-88. In the Tucuruí dam area, one strain was obtained from a monkey (Chiropotes satanas) a little before the outset of flooding and two human cases (one fatal) were confirmed in 1987. With regard to human serology, the routine tests have not allowed separation between natural contaminations and antibodies acquired from vaccination. However its actual sylvatic circulation was indicated by a 9.1% rate of antibodies in the monkey sampling done in 1984, and one positive reaction in the sera of a Cebus apella monkey, bled in 1985. It is probable that the Haemagogus-monkey cycle (with sporadic contacts with man) of this virus was not modified by dam construction and thus, vaccination of all people entering such an area must be maintained.

The same can be said for MAY virus, which is ecologically extremely similar. Isolated from man in Tucuruí in November, 1984 and during the seventies in Altamira, it has shown a high and fairly constant prevalence of antibodies in human sera collected in the two areas (40). In Tucuruí, during 1984, 48.2% of the sampled monkeys had antibodies against it. Thereafter, four monkeys and two birds (Arremon taciturnus and Tyrannus melancholicus) were positive in 1985. Two other birds, Formicivora grisea and Cercomacra tyrannina were positive in 1983 and 1987, respectively.

This arbovirus, whose sylvatic cycle seems to be identical to that of YF (7), would be a good "indicator" of the ecological potential of any area for the sylvatic circulation of YF virus in the absence of human cases or when vaccine-acquired antibodies against YF are obscuring the sylvatic pattern.

Itaporanga (ITP: Bunyaviridae: Phlebovirus, group Phlebotomus). This virus was isolated from Culex sp. mosquitoes, collected in Altamira and Tucuruí, in May and June, 1985, respectively. From Tucuruí, one monkey (Saguinus midas: 1982), three marsupials (Didelphis marsupialis: 1984, 1985) three rodents (Proechimys sp., Dasyprocta primnolopha: 1982; Agouti paca: 1984), one bat (1986) and three birds (Micrastur gilvicollis: 1986; Thamnomanes caesius: 1986; 1988) had antibodies against this arbovirus. Thus, its enzootic circulation may not have been modified by the construction of the dam.

Ilheus (ILH: Flaviviridae: Favivirus, group B). ILH, a widely distributed and pathogenic arbovirus (8), does not seem to have been influenced by the dam. Although it has not been isolated in the two regions of study since May, 1984, and March, 1984, respectively, human seroconversions, detected in September, 1985 (1), February, 1986 (1), April, 1986 (1), February, 1987 (5), and August, 1987 (1), attested its continuous presence and enzootic circulation in the dam area. In Altamira, specific antibodies were detected in the sera of bats (one in 1985 and three in 1986). In Tucuruí, various birds (1982; 1988), one monkey (Alouatta belzebul: 1984), two rodents (Dasyprocta prymnolopha: 1982; Myoprocta sp.: 1984) and one marsupial (Marmosa cinerea: 1987) were positive.

Icoaraci (ICO: Bunyaviridae: Phlebovirus, group Phlebotomus). ICO virus has not been isolated from either vertebrate or from arthropods, after August, 1983, and November, 1983 in Altamira and Tucuruí, respectively. However, its continuous enzootic circulation in the dam region was demonstrated by the detection of specific antibodies in the sera of two monkeys (Alouatta nigerrima and Saimiri sciureus: 1983), one marsupial (Marmosa cinerea: 1983) and seven rodents (Proechimys sp.: 1982-83, 1985). In the reference region, rodents of the same genus were found positive in 1983, 1984 and 1986.

*Tucunduba (Bunyaviridae: Bunyavirus, group Bunyam-wera).* This arbovirus is transmitted mostly by sabethine mosquitoes and some diurnal species of other tribes. It was active in the Altamira region from 1978 to 1981 (8 strains), and in the dam area before (1 strain, 1983) and after (1 strain, 1987) the flooding period. It is noteworthy that the latter strain was not isolated from the known mosquito hosts but from *An. triannulatus*.

As there has been apparently no epizootic episode, a dead-end infection seems a more acceptable hypothesis than any vector shift to explain this aberrant isolation. Whatever the case, the dam does not seem to have affected the transmission of this arbovirus.

Eastern Equine Encephalitis (EEE) and Western Equine Encephalitis (WEE) (Togaviridae: Alphavirus, group A). EEE virus, which is largely distributed in the Amazonian region (8) and with a complex sylvatic cycle (7), has not been isolated from the Altamira region since October, 1974, and from the dam area since July, 1983, but it is probably present in the latter area as was suggested by a seroconversion detected in November, 1985 in a human serum and positive serological reactions detected in the sera of a monkey (Saimiri sciureus: 1984) and a bird (Thamnophilus aethiops: 1985).

The presence of the WEE virus in the dam area was suggested by the detection of specific antibodies in the sera of numerous birds (at least 14 species), collected each year from 1982 to 1988.

However, the dam impoundment does not seem to have affected the transmission patterns of these two arboviruses.

### The Balbina project

Sylvatic hosts — 11,931 haematophagous Diptera have been inoculated in 243 pools. They represented at least 39 different species.

Two strains, which are considered new types in the Changuinola serogroup of *Orbivirus* (Reoviridae) (41), have been isolated from phlebotomine sandflies.

Human serology — 284 human sera were tested for antibodies, of which five were also inoculated to attempt isolation. Among the most prevalent antibodies in these sera, and excluding the numerous multiple positivities due to YF vaccination and/or serogroup cross-reactions, ILH, MAY, MUC, ORO and CAR were notable. The antibody rates did not seem to be different from those of other areas in Amazonia.

The lack of data collected during the preflooding period or in an adjacent nonmodified area unables us to draw any conclusion about the effects of the dam impoundment on arbovirus transmission.

### The Samuel project

Arthropod sylvatic hosts — 10,267 haematophagous Diptera, representing at least 30 different species, were collected and inoculated in 184 pools.

One strain of SLE virus was isolated from one pool of *Culex (Culex)* sp. mosquitoes.

Vertebrate sylvatic hosts — A total of 101 wild vertebrates, representing 18 different species, were tested for viruses and/or antibodies.

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The only interesting serological results were: *i*) two sera from the rodent *Dasyprocta fuliginosa*, reacting with ILH virus and *ii*) one monkey (*Callicebus brunneus*), reacting against MAY and TCM viruses.

As in the precedent case, many data are lacking in relation with environmental modifications due to the dam.

#### The Santo Antonio project

The results of this project were even poorer than the two preceding ones. Only vertebrates were collected: 573 specimens, representing at least 21 different species.

No viral strains have been isolated. However, some interesting serological data deserve being reported here. Seven monkey sera among the 14 examined were positive for YF and MAY viruses, of which five for the two agents (42). This finding confirms the great similarity which had already been noted between the cycles of both viruses. One "guariba" (*Alouatta seniculus*) monkey was also positive for Tacaiuma virus, confirming the similar data obtained in the Tucuruí area (*cf. supra*).

In this first attempt to classify the different arboviruses according to their various ecological reactions to the construction of a big dam, we encountered one main difficulty: the possible grouping of the same virus in more than one category. This is the consequence of a great variety and/or complexity of the cycles of these arboviruses, combined with a lack of ecological information.

Some numbers will express the richness of the region: of a total of 74 different types, at least 53 (71%) of them were isolated from the Tucuruí area — one third of the total known types in Brazilian Amazonia — versus 37 (50%) from Altamira, only 300 km distant from the former. To date and with comparable sampling efforts, the two areas have furnished only 16 (21.6%) viral types in common. These results merit the following remarks: *i*) the diversity of arboviruses in the studied regions is high, *ii*) the endemicity rate of the arboviruses is high, and *iii*) the initial hypotheses made about the ecological similarity of the two studied regions may need to be revised.

Amazonian arboviruses were classified by Hervé et al (7) in to various types of cycles, based on the usual hosts (vertebrates and arthropods), and eventually incorporating human contaminations. The presently reported studies do not modify these schemes, but on the contrary, they show that they may be useful for the forecasting of possible impacts.

For example, a great proliferation of mosquitoes such as *Anopheles (Nyssorhynchus*) spp. (entomological data analyzed in papers to be published), has favored the circulation of the arbovirus Guaroa and six types pertaining to the Anopheles A group. Temporary conditions, which prevailed during the flooding period, were favorable to a great increase in *Culex (Culex)* spp. density, thus enhancing the enzootic circulation of various arboviruses like Turlock, Kwatta-like, and SLE. *Aedeomyia squamipennis*, multiplying with a certain delay after the flooding was completed,

was supportive for an outbreak of the bird-associated Gamboa virus. This agent, formerly not isolated in Brazilian Amazonia, was possibly introduced by a migrating bird.

Another ecological factor, which possibly played a role in the temporary amplification of arbovirus circulation, and in the appearance of new types so far undetected, may be the concentration (natural or through the saving operation) of stressed wild animals on the isles and the banks of the lake. New types of biotopes (swamps, etc.) may also have attracted some species new to the region.

Arbovirus studies in relation to the construction of hydroelectric dams are scarce, especially in the neotropical region. Two projects have implicated arbovirological studies: the Bayano dam in Panama (43) and the Brokopondo dam in Surinam (Dutch Guiana) (44). In the Amazonian river basin, the present studies are the first of this kind, therefore it may be ecologically unreliable to compare them with other studies done in fairly distinct geographical and/ or climatic environments.

The only detailed information of a similar nature is that of the Bayano Project and the following discussion emphasizes the most salient differences and similarities.

The effects of flooding seem to have been very different in some cases:

• neither the Venezuelan Equine Encephalitis (VEE) virus or its closest Amazonian relative Mucambo virus have been isolated in Tucuruí (versus numerous strains in Panama); the only indication of the presence of the latter virus in Tucuruí was a human seroconversion detected in May, 1985;

• no virus of the Chagres (Bunyaviridae: *Phlebovirus*: Phlebotomus group) complex has been isolated in Tucuruí (versus numerous strains in Panama);

• the EEE virus was possibly favored by the Bayano dam impoundment, but it nevertheless circulated at a low level. As in the Tucuruí area, its real impact remains to be appraised.

On the other hand, the effects of dam impoundment were comparable in the following instances:

• SLE virus showed an epizootic-like episode during the flooding periods in both projects;

• the epizootic of Gamboa group viruses followed a similar pattern in both places, characterized by an eightmonth delay between the outset of flooding and the first isolation from the mosquito host; however, the duration of the epizootic (not including the two preflooding strains isolated from birds) was significantly shorter in Tucuruí than in the Bayano lake region (14 versus probably more than 18 months); another difference between the two series of results is that no strain has been isolated from male Ad. squamipennis mosquitoes collected in Tucuruí, thus making the possibility of transovarial transmission a very improbable one in the latter area;

• the enzootic transmission of YF and MAY viruses does not seem to have been modified in any respect, in either of the two regions;

• after an initial outbreak, some arboviruses, like the Utinga/Utive/Pintupo complex (Bunyaviridae: Simbu group) (45), seem to have "disappeared" from the Bayano

area, but doubts remain about the significance of such a lack of isolation;

• a fair number of new (or unclassified) types were isolated in the groups Changuinola, Phlebotomus and others, in both cases.

### Conclusions

The findings reported in this paper are of a preliminary nature, especially due to the lack of analysis of entomological data, but it is evident that — as suggested by the poor results obtained from the other three regions (Balbina, Samuel and Santo Antonio) — longitudinal and long-term studies are necessary for a better understanding of the extremely diverse and complex cycles that characterize the Amazonian arboviruses.

An important factor that emerged from the studies is the necessity of a reference or control area for comparative purposes and that it cannot be just the dam area before flooding (for the present study in the Tucuruí/Altamira project, the reference area was chosen *a posteriori*). Moreover, a nearer location to the dam as in the present case ought to be chosen.

For future studies, it would be interesting: i) to define the ecological characteristics of the new equilibrium which, we think may now have been established in the region of the Tucuruí dam, and ii) to adopt a more previsional attitude (hypothesis testing) during field work in regions of planned dam impoundments (46).

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- 41. They have received the provisional names Uatumã and Balbina, respectively
- 42. These "positive" species (No.) were: Pithecia pithecia (1), Alouatta seniculus (2), Saimiri sciureus (1), Saguinus m. midas (1), and Ateles paniscus (2); these results show that YF may not be so lethal to New World monkeys as is frequently said; however, it may be possible that the susceptibility varies according to the location of monkey populations inside or outside the enzootic permanent focus.
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- 46. Acknowledgments: The results reported in the present study are based in part on those presented by Dégallier et al (30). We wish to thank all staff members from the Evandro Chagas Institute and other institutions for their past and present participation in the field and laboratory work which have made this paper possible. The present work was supported by the invaluable financial and/or logistic help from Eletronorte (Eletrobrás), SUDAM (Polos Agropecuários da Amazônia), CNPq, ORSTOM and SESP Foundation. We are especially grateful to Dr. Evaldo G.M. César (SUDAM-M.I.) who kindly provided us data on Amazonian meteorology. Dr. Jeffrey J. Shaw (Wellcome Parasitological Unit, Belém) and Mrs. Michelle Abriol (Belém) very kindly accepted to revise our paper in English.

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