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New entomological and virological data on the vectors of sylvatic yellow fever in Brazil

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The present report focuses on recent ecoepidemiological data on yellow fever, obtained recently in two very distinct ecoepidemiological contexts: the Barcarena (PA) area, situated in the dense Amazonian rain forest, and the Campo Grande (MS) region, situated in the cerrado with gallery forest in central Brazil. In the first region, one strain was isolated from a pool of 6 *Haemagogus janthinomys*. In the other region, 2,480 anthropophilous mosquitoes were collected, of which near 40% were potential YF vectors. These species, classified by decreasing relative abundance, were: *Aedes scapularis*, *Sabethes chloropterus*, *Hg. janthinomys*, *Hg. leucocelaenus*, *Hg. spegazzinii*, and *Sa. soperi*. Four strains of YF virus were isolated from *Hg. janthinomys*, one from *Sa. cheoropterus* (first report for South America) and one from *Sa. soperi* (first report). The minimal infection rates varied among the three localities of collection but were all high, compared with previous data. The mean daily survival rate was 0.9635 for the populations of *Hg. janthinomys*, allowing extrapolation of the value of the infection rate when people were infected most recently. It was deduced that the epizootics were intense and more or less concomitant in the three areas. The main problem that remained to be solved concerns the mode(s) of reintroduction or survival of YF virus in each of the two regions under study.

O presente trabalho enfatiza resultados recentes sobre eco-epidemiologia da febre amarela, obtidos em duas localidades ecologicamente muito diferentes: Barcarena (PA), situada na floresta amazônica densa, e a região de Campo Grande (MS), coberta de cerrado atravessado por florestas de galeria na região Centro-oeste. No primeiro local foi isolada uma amostra a partir de um lote de 6 Haemagogus janthinomys. Na região de Campo Grande, 2.480 mosquitos antropófilos foram coletados, dos quais cerca de 40% eram vetores potenciais da FA. Essas espécies, classificadas por ordem decrescente de abundância, eram: Ae. scapularis, Sa. chloropterus, Hg. janthinomys, Hg. leucocelaenus, Hg. spegazzinii e Sa. soperi.

Quatro amostras de FA foram isoladas a partir de Hg. janthinomys, uma de Sa. chloropterus (primeiro registro na América do Sul), e uma de Sa. soperi (primeiro registro). As taxas mínimas de infecção variaram segundo os locais de coleta, mas foram sempre altas em comparação com dados anteriores. A taxa média de sobrevivência diária do Hg. janthinomys é igual a 0,9635, permitindo estimar o seu valor na ocasião em que os casos humanos mais recentes foram provavelmente contaminados. Deduziu-se que as epizootias foram muito intensas e mais ou menos simultâneas nos dois locais considerados. O maior problema a ser resolvido é o modo de re-introdução do vírus, ou sua sobrevivência, em cada região sob estudo.

Despite the existence of a good vaccine, yellow fever (YF) virus continues to cause, almost every year many human deaths in its area of distribution (1). The reasons of the failure to control this disease are multiple. The main reason is related to the difficulty of accessing and vaccinating all people in the risk regions. Another reason is the lack of the ecological data which would be needed for establishing vaccination priorities. In other words, it would be necessary to better understand the mechanisms of *i*) the transmission of the virus and *ii*) emergence of epizootics, with or without human cases,

in order to define the places and times where and when the risk of transmission is highest.

Recent studies in Africa (2,3) and South America (4) have shown that diverse ecoepidemiological situations may exist, which are characterized by the nature of man-made changes of the environment and vector populations. Further, it became evident that the studies in Brazil must be related to the phytogeographical zones present in the country (1). The present report will focus on data, obtained recently in two very distinct ecoepidemiological contexts, with a discussion of implications for future studies.

The three main phytogeographical zones where YF virus is circulating in Brazil are characterized by dense Amazonian rain forest, open rain forest and gallery forests run-

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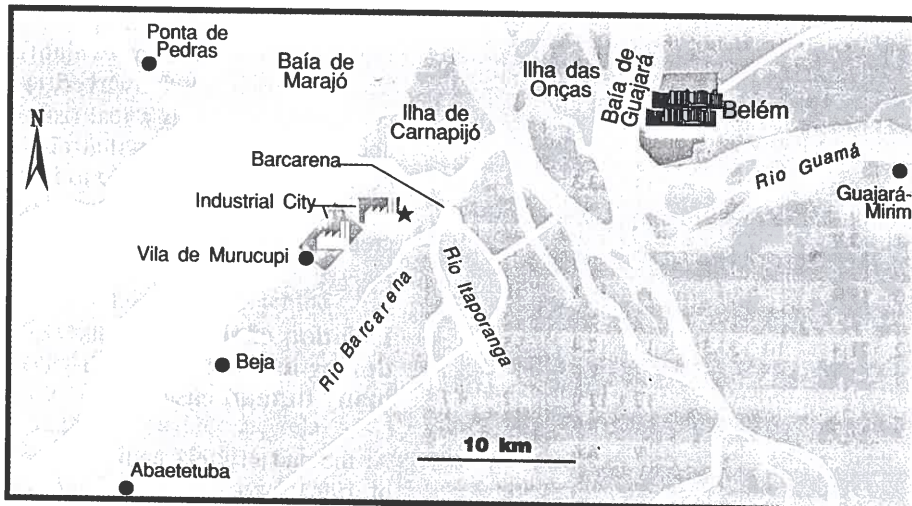


Figure 1. Map of the Barcarena region, Pará State, Brazil; the star shows the localization of mosquito collecting.

ning through arboreal savannahs. We recently have been able to show active circulation of YF virus in the former and latter, thus under very different conditions (5).

Isolation of YF virus in the absence of human cases

During routine mosquito collecting work, done in Barcarena, Pará (Figs. 1 to 4) (6), from June 12 to 26, 1991, one strain of YF virus was isolated from the six mosquitoes which constituted the only inoculated pool of *Ha. janthinomys*. Such a high infection rate (16.66%), associated

with a very low relative density (0.3 mosquitoes/man X hour), is probably the result of a high sampling error. The only other potential vectors which were also collected were *Ae. scapularis* (1 specimen), *Hg. leucocelaenus* (4 spec.) and *Sa. chloropterus* (23 spec.; 1 pool) (7).

Two studies, conducted in the same region, from June 21 to 28 and September 20 to 28, 1990, gave mainly the same entomological pattern (8). More recently, no strains were isolated on a collecting trip, made between December 3 and 21, 1991 (9).

In order to identify the possible incidence of YF in the human population (by sylvatic contacts or vaccination), four serological surveys were done, the positive results of which are reported in Table 1 (10).

Unexpectedly, antibody rates to YF virus were not high among the riverine populations in this region. This may be due either to a low rate of vaccination or to a lack of contacts between man and sylvatic YF virus, or both. On the other hand, high immunity rates against Mayaro virus, which is transmitted mainly by *Hg. janthinomys* (11), show that the contacts with the vectors actually exist or have existed. In fact, the active circulation of YF virus was detected in this region or the nearby Abaetetuba county only in 1968 (12) and 1988 (13). YF virus may have circulated



Figure 2. Collecting anthropophilous mosquitoes with sweep-net at ground level. Photo: N. Dégalier, 1992.

Figure 3. Climbing a tree to construct a platform in the canopy, for collecting YF vectors. Mr. Raimundo Benedito da Silva, in memoriam. Photo: Akemi Suzuki, 1991.

Figure 4. Collecting anthropophilous mosquitoes at canopy level. Photo: N. Dégalier, 1992.



Table 1 — HI positive human sera and rates for arboviruses in the region of Barcarena, Pará, according to year of survey.

Arbovirus	Year				Total ^a					
	1968	1980	1982	1991						
Yellow fever (sylvatic)	21	18.7		1	0.9	1	0.4			
Yellow fever (vaccinal)				14	13.3	14	5.5			
Ilheus	3	2.6	30	29.7	15	50	8	7.6	53	20.9
Saint Louis encephalitis	4	3.6	4	3.9					4	1.6
Western equine encephalitis					2	1.9	2	0.8		
Mayaro		16	15.8		19	18.1	35	13.8		
Mucambo		3	3	3	10	7	6.7	13	5.1	
Oropouche		72	71.3	10	33.3	13	12.4	95	37.4	
Caraparu		2	1.9			8	7.6	10	3.9	
Catu					12	11.4	12	4.7		
Guaroa		2	1.9			1	0.9	3	1.2	
Maguari					7	6.6	7	2.8		
Icoaraci		2	1.9	1	3.3			3	1.2	
Itaporanga				2	6.6			2	0.8	

^a Due to lack of homogeneity between the 1968 survey and the following surveys, the results of the former have not been included in these figures.

at a very low rate, and only among the monkey population which is probably diffuse. However, we consider that there is a real risk of sporadic human cases that could occur or even an epizootic to initiate if the simian population increases.

Isolation of the YF virus during an epizootic with many human cases

At the end of 1991 and beginning of 1992, a total of 14 human YF cases were confirmed in the region of Campo Grande (MS). This region is phytoclimatically very dif-

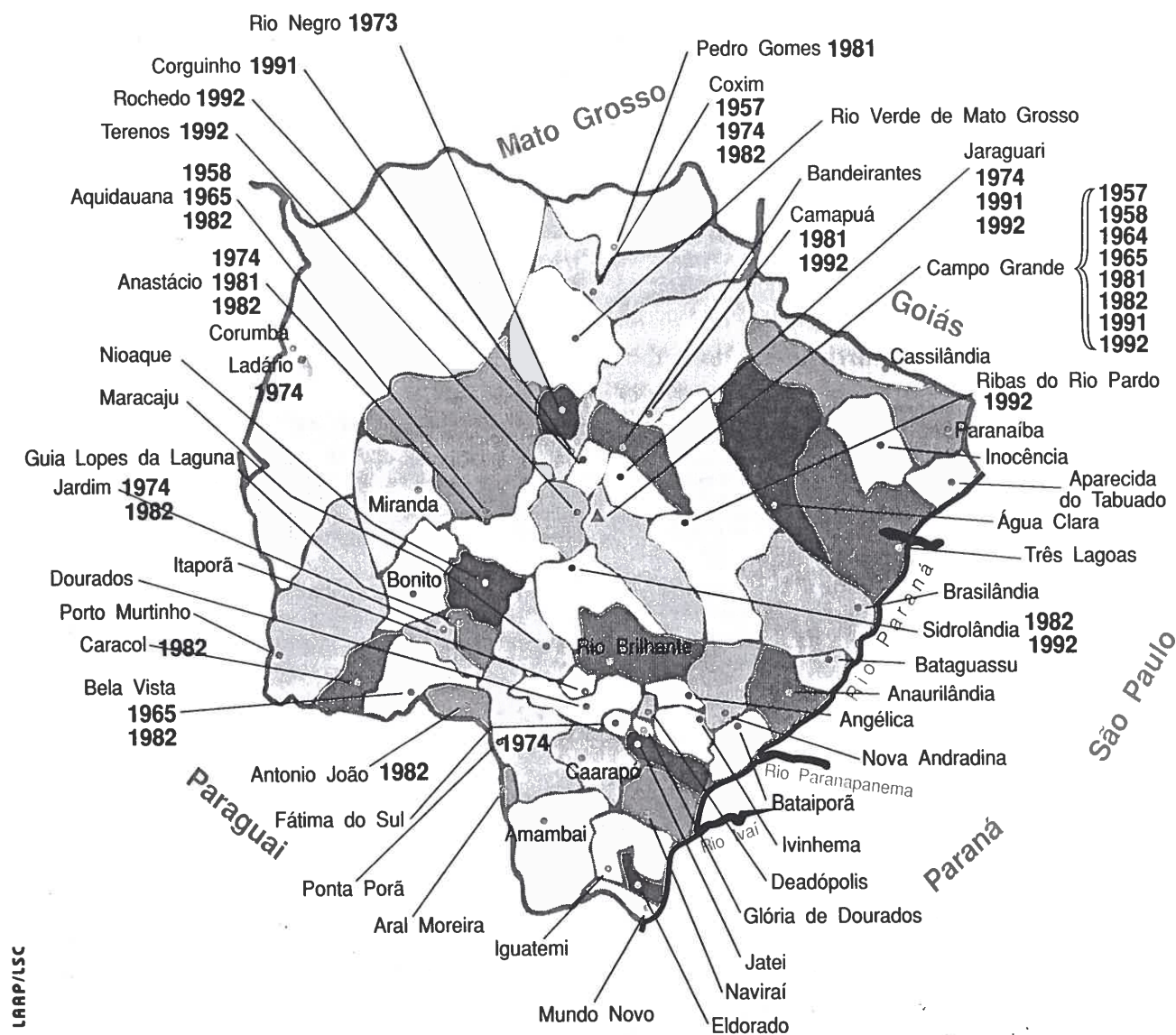


Figure 5. Map of Mato Grosso do Sul State with divisions of counties, localization and year of report of cases of YF during the last 39 years.

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2. Cordellier R 1991 L'épidémiologie de la fièvre jaune en Afrique de l'Ouest. *Bull Org Mond Santé* 69: 73-84
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 5. **Material and methods:** Anthropophilous mosquitoes were collected from human bait (all individuals were volunteers and with protecting antibodies), at canopy and ground levels, during the midday hours (the maximum biting activity of the potential vectors of YF in South America extends between 10:00 and 15:00 h). The mosquitoes were anesthetized with cold and immediately put in liquid nitrogen. In the laboratory, monospecific pools were done above a chilling table (-20°C). Thorax and heads of the potential vectors of YF were simultaneously inoculated into suckling mice and C6/36 *Aedes albopictus* cells, without refreezing and on the same day. Their abdomens were dissected for estimation of the mean parous rate of the population
 6. Barcarena (1° 31' S; 48° 40' W; alt. < 100 m) is a little town of 46.082 inhabitants (source IBGE, "Sinopse preliminar do Censo de 1991 p 44"), who are mostly employed by the Albras Company for extraction of bauxite and manufacture of aluminum. The mean annual rainfall is between 2,500 and 3,000 mm, with 180-240 rainy days. Drier and wetter seasons extend from September to November and February to April, respectively. The wetter months are also the coldest. The rains during the driest month are more than 60 mm
 7. 674 individuals of at least 20 different species of other mosquitoes were also collected from human bait during the same period
 8. In June, 1990, 17 YF potential vectors were collected, of which 15 were *Hg. janthinomys* (relative density rate = 0.36). The other anthropophilous mosquitoes were 634 in number, with at least 16 different species. In September of the same year, only 5 YF potential vectors were collected, 3 of which were *Hg. janthinomys* (relative density rate = 0.06); the other anthropophilous mosquitoes were 446 in number, with at least 15 different species
 9. 22 YF potential vectors were collected, of which 7 were *Hg. janthinomys* (relative density rate for canopy collecting only = 0.02). The other anthropophilous mosquitoes were 200 in number, with at least 12 different species
 10. The sera were tested for HI antibodies against the most prevalent pathogenic virus species. The sera were considered positive when reacting with only one virus, or when the titer of antibodies was at least two-fold higher than the higher titer of other cross-reacting ones. Rates (%) are indicated in boldface
 11. Travassos da Rosa APA, N Dégallier, JP Hervé, GC Sá Filho 1987 La recherche sur les arbovirus en Amazonie, p 223-247. In *Connaissance du milieu amazonien Actes du séminaire*, éd., Orstom, coll Colloques et Séminaires, 15 et 16 octobre, Paris
 12. One, two and four strains were isolated from mosquitoes, man and monkeys, respectively
 13. One human case was confirmed serologically
 14. Despite some dispersion of the cases around the Campo Grande county, the latter will be considered as representative of the general climate of the region. The following data concern the Campo Grande station (20° 27' S; 54° 37' W; alt. 556 m) for the period 1931-1960. Mean annual rainfall is 1,442 mm, with the drier and wetter seasons in June to August and December to February, respectively. The wetter months are also the hottest. The rains during each of the three drier months are less than 60 mm
 15. Field collecting of mosquitoes was done between January 24 and February 10; between January 26 and February 5; and from February 6 to 14 in the three ranches, Fazendas Cabeceira do Jaraguari, Rincão and Água Encanada, respectively
 16. Other authors are considering this species as only a "possible potential vector" and not a "proven potential vector", because it has never been encountered naturally infected by YF virus
 17. Dégallier N, JP Hervé, APA Travassos da Rosa, GC Sá Filho 1988 *Aedes aegypti* (L.): importance de sa bioécologie dans la transmission de la Dengue et des autres arbovirus. Première partie. *Bull Soc Pathol* 81: 97-110. *Idem* Deuxième partie: 111-124
 18. The mean daily survival rate may be estimated when the duration of the gonotrophic cycle and the parous rate of the population are known. The ovaries of the mosquitoes were dissected, left to dry at room temperature, and examined under the microscope. Their nulliparous/parous state is inferred from the presence/absence of coiled tracheoles
 19. *Hg. (Haemagogus) janthinomys*: Sidrolândia, 3 strains, Campo Grande, 1 strain; *Sa. (Sabethoides) chloropterus*: Sidrolândia, 1 strain; *Sa. (Sabethinus) soperi*, Jaraguari, 1 strain
 20. The very high infection rate is probably due to a sampling error as was the case for the strain isolated from Barcarena
 21. Karabatsos N 1985 *International catalogue of arboviruses including certain other viruses of vertebrates*, 3rd ed, American Society of Tropical Medicine and Hygiene, San Antonio, Texas
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 23. Hervé JP, APA Travassos da Rosa 1983 Ecologia da febre amarela no Brasil. *Rev Fund SESP* 28: 11-19
 24. Rawlins SC, B Hull, DD Chadee, R Martinez, A LeMaitre, F James, L Webb 1990 Sylvatic yellow fever activity in Trinidad, 1988-1989. *Trans R Soc Trop Med Hyg* 84: 142-143
 25. Strains of YF virus have been isolated by the IEC once from a pool of unidentified *Sabethes* mosquitoes and once from a mixed pool of *Aedes* sp. and *Sabethes* sp.
 26. Galindo P 1958 Bionomics of *Sabethes chloropterus* Humbolt, a vector of sylvan yellow fever in middle America. *Am J Trop Med Hyg* 7: 429-440
 27. Dégallier et al, unpublished data
 28. The mean relative density and infection rate are 3.1 mosq./man h and 0.21% (35/16,573), respectively
 29. We were able to see remains of dead Howler monkeys (*Alouatta caraya*) in the Jaraguari and Sidrolândia counties (see Fig. 9)
 30. A mean incubation period of five days is assumed (see note no. 4)
 31. In the Jaraguari county no strain was isolated from *Hg. janthinomys* 52 days after the time the last human patient was theoretically infected. As the sensibility of suckling mice is sufficient to allow isolation at very low rates (around 0.01%), it is reasonable to consider that the YF virus has vanished from the adult population of this species, at this time
 32. From the virus point of view!
 33. **Acknowledgments:** We are much indebted to the technical staff of the Evandro Chagas Institute who has collected material in the field (Francisco C. Castro, Hélio A.C. Saraiva, Jonas R. dos S. Moraes, Orlando V. da Silva, Raimundo B. da Silva, Roberto C.F. Brandão) and processed it in the laboratory (Armando de S. Pereira, Basilio S. Buna, Hamilton A. de O. Monteiro, Ivan C. Alves, Iveraldo F. da Silva, Luiz R.O. da Costa, Maria R.C. Freitas, Maxwell F. de Lima, Osvaldo V. da Silva, Raimundo F. do Nascimento) with much competence and responsibility. We sincerely acknowledge the Albras Company which gave us the opportunity to work in the Barcarena area. The studies conducted in the Campo Grande region would not have been possible without the logistic help of the Regional Direction of National Health Foundation in Mato Grosso do Sul (Director Dr. Aroldo Ferreira Galvão) and the special dedication of field entomology staff of this institution (under the responsibility of Dr. Ari Alves Ribeiro). All meteorological and a part of epidemiological data used in this report were provided by Dr. Carlos J. Mangabeira da Silva (Cenapi/Ministry of Health, Brasília, DF). This study was funded by the National Health Foundation, CNPq, Orstom (France) and the Albras Company, Pará State, Brazil.