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. spegazinii,* 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. spegazinii*** *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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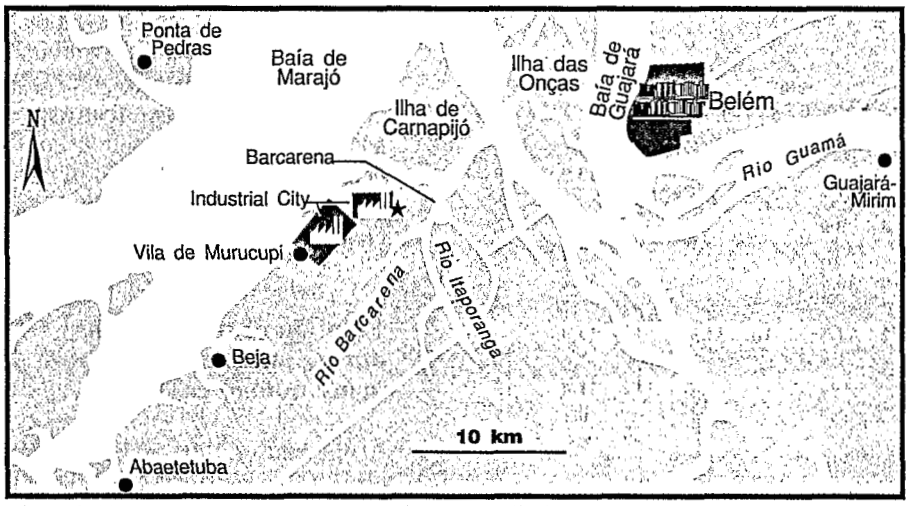


Figure 1. Map of the Barcarena region, Pará State, Brazil; the star shows the localization of mosquito collecting.

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

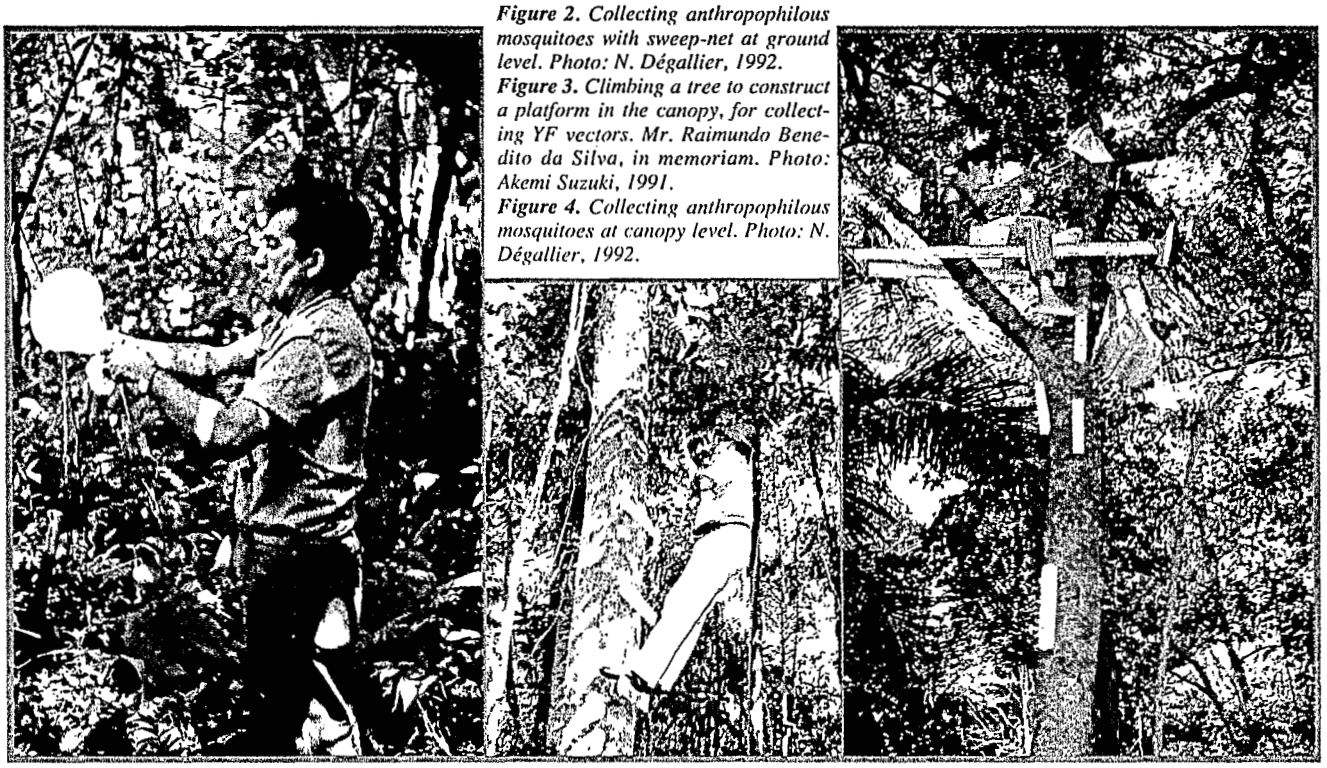


Figure 2. *Collecting anthropophilous mosquitoes with sweep-net at ground level. Photo: N. Dégallier, 1992.*
Figure 3. *Climbing a tree to construct a platform in the canopy, for collecting YF vectors. Mr. Raimundo Benedito da Silva, in memoriam. Photo: Akemi Suzuki, 1991.*
Figure 4. *Collecting anthropophilous mosquitoes at canopy level. Photo: N. Dégallier, 1992.*

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

	Year									
Arbovirus	*1968*		*1980*		*1982*		*1991*		*Total* [a]	
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-

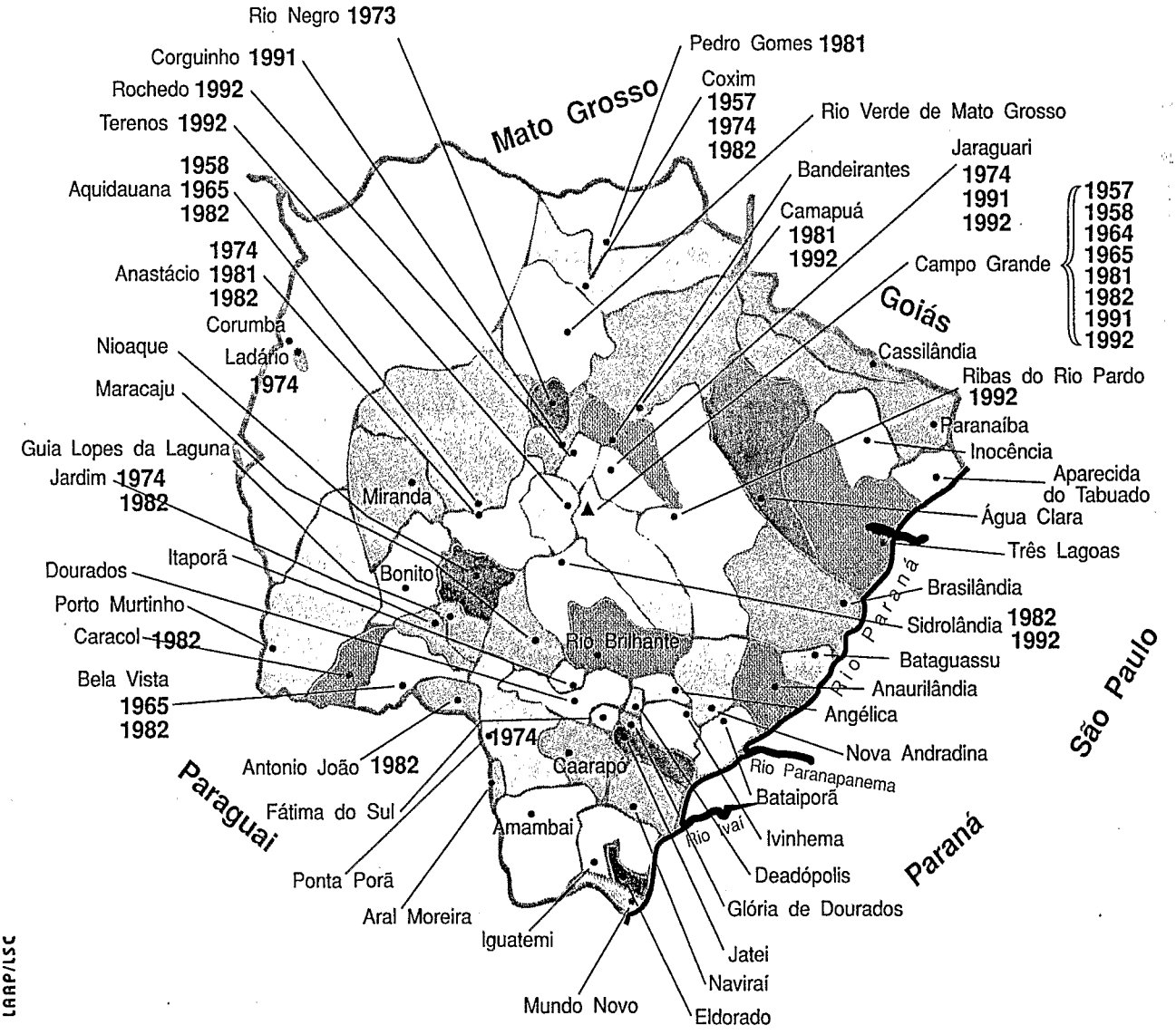


Figure 5. Map of Mato Grosso do Sul State with divisions of counties, localization and year of report of cases of YF during the last 39 years.

ferent from the Barcarena region (14). Previously described collecting and processing methods were used (5). Three counties, Jaraguari, Campo Grande and Sidrolândia, with fatal human cases, were investigated (Fig. 5) (15).

A total of 986 potential vectors of YF were collected, making up near 40% of all mosquitoes (Table 2).

If we consider the relative densities of all species (Table 3), *Ae. scapularis* (Fig. 6) (16) was globally the most abundant of the potential YF vectors, followed by *Sa. chloropterus* (Fig. 7), *Hg. janthinomys* (Fig. 8), *Hg. leucocelaenus, Sa. soperi* and *Hg. spegazinii.*

All the above species, except for *Hg. leucocelaenus* and *Sa. soperi*, were less and more abundant in the gallery forests of Jaraguari and Campo Grande counties, respectively. Nevertheless, it was in Sidrolândia county that the global relative density of anthropophilous mosquitoes was the highest. The number of different species was lower in the ranch Fazenda Rincão (Campo Grande county) than in the other counties (Table 2).

These differences in species richness may be re-

Table 2 — Anthropophilous mosquitoes collected in the region of Campo Grande (MS), between January 24 and February 14, 1992, according to species, place and level, with the potential YF vectors in boldface.

County	*Jaraguari*		*Sidrolândia*		*Campo Grande*		
(Fazenda name)	*(Cab. do Jaraguari)*		*(Água Encanada)*		*(Faz. Rincão)*		*Total*
Species/Level	*Canopy*	*Ground*	*Canopy*	*Ground*	*Canopy*	*Ground*	
Anophelinae							
Anopheles (Ano.[a]) mediopunctatus			1	23			24
An.[a] (Nys.) triannulatus			1	14			15
An. (Sth.) nimbus				31			31
Chagasia bonneae	2	10				3	15
Culicinae							
Aedini							
Aedes (How.) sp.		2		2			4
Ae. (How.) septemstriatus		2				8	10
Ae. (Och.) scapularis	***2***	***85***	***16***	***212***	***4***	***88***	**407**
Ae. (Och.) serratus		9		9			18
Ae. (Pro.) argyrothorax				12			12
Haemagogus (Hag.) janthinomys	***8***	***29***	***35***	***33***	***11***	***68***	**184**
Hg. (Hag.) spegazinii		***6***					**6**
Hg. (Con.) leucocelaenus	***15***	***70***	***11***	***46***	***2***	***17***	**161**
Psorophora (Jan.) albipes	13	195	88	271	4	183	754
Pa. (Jan.) ferox	1	34	2	31		1	69
Culicini							
Culex sp.		4	1	21			26
Cx. (Cux.) coronator		1		2			3
Cx. (Cux.) declarator		5		5			10
Cx. (Mel.) sp.		1		2			3
Mansoniini							
Coquillettidia (Rhv.) albicosta				8			8
Cq. (Rhv.) venezuelensis				1			1
Sabethini							
Limatus sp.		2				5	7
Li. durhamii				11			11
Sabethes (Sab.) albiprivus	3	51	5	9	16	67	151
Sa. (Sab.) belisarioi	10	1	10		2	8	31
Sa. (Sab.) forattinii					2		2
Sa. (Sab.) quasicyaneus		8		2			10
Sa. (Sab.) tarsopus					2		2
Sa. (Sbn.) intermedius		17					17
Sa. (Sbn.) soperi		19				1	**20**
Sa. (Sbo.) chloropterus	20	33	46	14	42	53	**208**
Sa. (Sbo.) glaucodaemon	45	60	55	37	5	8	210
Trichoprosopon obscurum		7					7
Wyeomyia sp.	1	17		13	1	11	43
Total[b]	120	668	271	809	91	521	2480
No. of species	11	24	12	23	11	14	33

[a] The abbreviations follow the paper of Reinert (1975 *Mosq Syst* 7: 105-110).
[b] A total of 144 pools were inoculated, with 50, 57 and 37, respectively from each place of collection; among these the potential YF vectors represent 16, 17 and 14 pools, respectively.

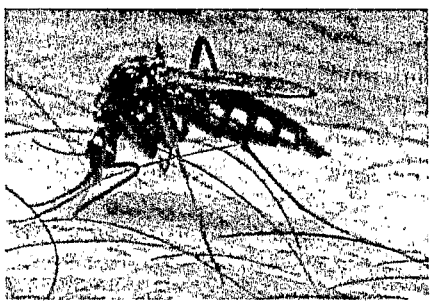
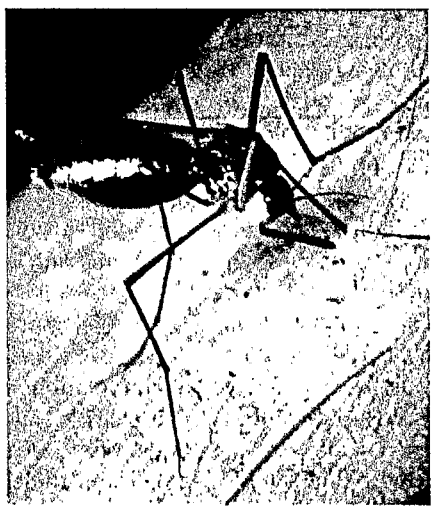
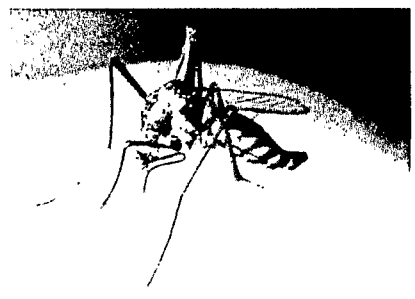


***Figure 6. Aedes scapularis**, potential vector of YF, collected mainly at ground level in the Campo Grande region, MS. Photo: N. Dégallier, 1992.*



(↑) ***Figure 8. Haemagogus janthinomys**, main vector of YF virus in Brazil. Photo: N. Dégallier, 1992.*



(←) ***Figure 7. Sabethes chloropterus**, proven secondary vector of YF virus in the Campo Grande region, MS. Photo: N. Dégallier, 1992.*

Table 3 — Relative densities (No./man x hour) of anthropophilous mosquitoes collected in the region of Campo Grande (MS), between January 24 and February 14, 1992, according to species and county, with the potential YF vectors in boldface.

Species/Places (Men x Hours)	*Jaraguari (181.5)*	*Sidrolândia (183)*	*Campo Grande (127.5)*	*Total (492)*
Anophelinae				
Anopheles (Ano.[a]) mediopunctatus		0.13		0.049
An.[a] (Nys.) triannulatus		0.08		0.03
An. (Sth) nimbus		0.17		0.063
Chagasia bonneae	0.07		0.02	0.03
Culicinae				
Aedini				
Aedes (How.) sp.	0.01	0.01		0.008
Ae. (How.) septemstriatus	0.01		0.06	0.02
Ae. (Och.) scapularis	***0.48***	***1.26***	***0.72***	**0.827**
Ae. (Och.) serratus	0.05	0.05		0.037
Ae. (Pro.) argyrothorax		0.07		0.024
Haemagogus (Hag.) janthinomys	***0.20***	***0.37***	***0.62***	**0.374**
Hg. (Hag.) spegazinii	***0.03***			**0.012**
Hg. (Con.) leucocelaenus	***0.47***	***0.31***	***0.15***	**0.327**
Psorophora (Jan.) albipes	1.15	1.96	1.47	1.533
Ps. (Jan.) ferox	0.19	0.18	0.01	0.14
Culicini				
Culex sp.	0.02	0.12		0.053
Cx. (Cux.) coronator	0.01	0.01		0.006
Cx. (Cux.) declarator	0.03	0.03		0.02
Cx. (Mel) sp.	0.01	0.01		0.006
Mansoniini				
Coquillettidia (Rhv.) albicosta		0.04		0.016
Cq. (Rhv.) venezuelensis		0.01		0.002
Sabethini				
Limatus sp.	0.01		0.04	0.014
Li. durhamii		0.06		0.022
Sabethes (Sab.) albiprivus	0.30	0.08	0.65	0.307
Sa. (Sab.) belisarioi	0.06	0.05	0.08	0.063
Sa. (Sab.) forattinii			0.02	0.004
Sa. (Sab.) quasicyaneus	0.04	0.01		0.02
Sa. (Sab.) tarsopus			0.02	0.004
Sa. (Sbn.) intermedius	0.09			0.035
Sa. (Sbn.) soperi	***0.10***		***0.01***	***0.041***
Sa. (Sbo.) chloropterus	***0.29***	***0.33***	***0.75***	***0.423***
Sa. (Sbo.) glaucodaemon	0.58	0.50	0.10	0.427
Trichoprosopon obscurum	0.04			0.014
Wyeomyia sp.	0.10	0.07	0.09	0.087
Total	4.34	5.90	4.80	5.041

[a] The abbreviations follow the paper of Reinert (1975 *Mosq Syst* 7: 105-110).

Table 4 — Minimal infection rates (%) of each positive species for YF virus, according to the place of collection in the region of Campo Grande, MS; the dates of the most recent human cases in each locality give an indication of the end of the epizootics.

Species/Place Most recent human case	*Jaraguari Dec. 20,'91*	*Campo Grande Dec. 31,'91*	*Sidrolândia Feb. 3,'92*	*Total*
Haemagogus (Hag.) janthinomys		***1.27***	***4.41***	***2.17***
Sabethes (Sbo.) chloropterus			***1.67***	***0.48***
Sabethes (Sbn.) soperi	***5.26***			***5.00***

Figure 9. *Remains of a dead Howler monkey (**Alouatta caraya**), found in the gallery forest after the 1992 epizootic in the Campo Grande region. Photo: Sr. José Mendes, 1992.*

lated to ecological (man-made?) disturbance of the environment.

The relative density is not the only important factor related to vector competence of mosquito populations. Of prime importance is also the value of the parous rate (17, 18), which allows us to estimate the mean daily survival rate. A global proportion of 57.31% of the 164 dissected *Hg. janthinomys* were determined as parous. With a 15-day long gonotrophic cycle (4), the global survival rate is thus 0.9635 (Table 5).

Three species were found to be infected by the YF virus (19) and this is the first time that YF virus was isolated from sylvatic vectors in Mato Grosso do Sul State, despite attempts made during previous surveys.

It is noteworthy that the infection rates of the main vector, *Hg. janthinomys* (Table 4), are higher in the localities with more recent human cases, perhaps indicating the progressive decrease of the virus in the mosquito population, because of the immunization of susceptible monkeys. This hypothesis will be discussed in the next section.

The species *Sa. soperi* was encountered naturally infected for the first time but its actual role in the transmission of YF needs to be further studied (20). It may be more ornithophilous than primatophilous, because the arbovirus Macaua, for which it is probably a natural host, has been isolated from birds (21). To date, the *Sa. chloropterus* has been found naturally infected by YF virus only in Central America (22,23) and recently in Trinidad island (24). Thus, here is the first time that it was found naturally infected in continental South America (25). Contrary to the *Haemagogus* spp., these mosquitoes are known to survive during the dry season (26,27), therefore being good candidates for supporting a short-term maintenance of YF virus during the unfavorable season.

The role of *Hg. janthinomys* as the principal vector of sylvatic YF in Brazil and responsible for most human contaminations has been confirmed once more. The relative densities and infection rates have been compared with those estimated from previous studies in Brazil (Table 5; ref. 28). The high densities and infection rates, together with the presence of infected *Sabethes*, may suggest that our field work

Table 5 — Relative densities (Nr./man x h) and minimum infection rates (Nr. of strains/Nr. of mosquitoes) for YF (%) of potential vectors of sylvatic YF in Brazil, 1954-1992.

Species	*Place (State)*	*Density*	*Infection rate*
Hg. spegazzinii	Ilhéus (BA)	1.6	0.01 (1/9119)
Hg. species	Belém-Brasília km 87 (PA)	-	1.00 (1/222)
Hg. species	Belém-Brasília km 94 (PA)	-	5.00 (1/20)
Ae. fulvus	Serrado Navio (AP)	-	0.60 (1/165)
Hg. species	Barcarena (PA)	-	0.29 (1/335)
Hg. species	Rio Jaburu (Il. Gurupa) (PA)	-	0.75 (1/132)
Hg. species	Alenquer (PA)	-	1.19 (3/252)
Hg. capricornii[a]	Belterra (PA)	-	0.27 (2/732)
Hg. species	Uruaçu-Niquelândia (GO)	3.2	0.86 (4/461)
Hg. janthinomys ?	Formosa (GO)	2.3	0.57 (2/347)
Hg. janthinomys ?	Bela Vista (GO)	2.7	0.78 (7/891)
Hg. janthinomys	Faro (PA)	0.8	0.22 (1/448)
Hg. albomaculatus	Monte Alegre (PA)	3.5	0.11 (2/1798)
Hg. jant. + Hg. albom.	Monte Alegre (PA)		0.22 (1/436)
Hg. janthinomys	São Domingos do Capim (PA)	15.0	0.34 (3/858)
Hg. janthinomys	Sinop (MT)	1.0	0.47 (1/209)
Hg. janthinomys	Ilha Japichaua (PA)	0.6	1.40 (2/142)
Hg. janthinomys	Barcarena (PA)	0.3	16.66 (1/6)
Hg. janthinomys	Campo Grande (MS)	0.6	1.27 (1/79)
Hg. janthinomys	Sidrolândia (MS)	0.37	4.41 (3/68)
Sa. chloropterus	Sidrolândia (MS)	0.33	1.67 (1/60)
Sa. soperi	Jaraguari (MS)	0.1	5.26 (1/19)

[a] As this species is not known from Amazonia, and its females are undistinguishable from those of *Hg. janthinomys*, it was probably the latter which has been collected (see Arnell 1973 *Contr Amer Ent Inst* 10: 174 p)

Table 6 — Summary of ecoepidemiological parameters for the *Haemagogus janthinomys* mosquito population, collected in the region of Campo Grande, MS, by county, from January 24 to February 14, 1992.

	Jaraguari	*Campo Grande*	*Sidrolândia*	*Estimated*[a]
No. of days since the last human case	52	41	17	0
MIR (%)	0	1.27	4.41	6.54 (r2=0.99)
Relative density	0.20	0.37	0.62	0.83 (r2=0.99)
Parity rate (%)	57.5	53.62	61.81	63.45(r2=0.48)
Daily survival rate (%)[b]	96.37	95.93	96.84	
Surviving infected mosquitoes (%)[c]	14.62	18.20	57.93	
MIR at time of epizootics	?	6.97	7.61	

[a] Values at origin estimated by linear regression of the known values; the values of the coefficients of regression are indicated between parenthesis.
[b] Assuming a 15-day long gonotrophic cycle.
[c] This value represents the proportion of infected mosquitoes surviving at the end of the period = (daily survival rate) (No. of days since the last human cases).

in Mato Grosso do Sul was done only a short time after a YF epizootic (29). In the case of the Barcarena isolation, it is not known if there was a previous epizootic during the first half of 1991.

An interesting exercise would be to compare some ecoepidemiological parameters of the *Hg. janthinomys* populations (infected or not) from the three places of study in the Campo Grande region. As shown in Table 6, the time lags between the last human contaminations and our collecting of infected mosquitoes (30) would be 41 and 17 days for Fazenda Rincão and Sidrolândia, respectively (31). With survival rates equal to 0.9593 and 0.9684, the infected mosquitoes would represent 18.20% and 57.93% of those infected during the epizootics in the respective areas. Thus, the infection rates at the respective times of human contaminations would be estimated to be at least equal to 6.97% and 7.61% (Table 6). Therefore, these are of the same order. We have obtained a similar value for the initial MIR (6.54%) when fitting a line with the values estimated at 17,41 and 52 days after the last human contamination. As for the MIR, the relative density index seems to decrease linearly with time (Table 6). On the other hand, the parity rate does not seem to vary linearly with time. No hypothesis is yet available to explain these phenomena.

Various other ecoepidemiological aspects remain to be studied in relation to the maintenance of the YF virus in its area of endemicity. In the southern part of this area, it is not known if the virus is periodically reintroduced from the north (the permanent endemic focus of the French authors (2)) or if it is able to persist during the dry and cold season, either in the drought-resistant eggs of the *Haemagogus* species, or in the more resistant *Sabethes* adults, or in both. Whatever the case, the "purpose" (32) may not be the same. The long-term survival of the virus in the eggs would necessarily have to wait for the reconstitution of the local population of susceptible monkeys (Fig. 9). On the other hand, a shorter-term survival in adult mosquitoes may be a possibility of initiating another epizootic in the case of new vertebrate hosts entering the area.

The data obtained in the Barcarena area were not sufficient to conclude if there was an epizootic or not, before the recent isolation of YF virus from mosquitoes. However, the low rate of HI antibodies in the human population and the absence of recent human cases, despite a close contact of these people with the forest, suggest a nonepizootic mode of transmission.

Conclusions

Further studies are needed in both types of phytoclimatical zones, in order to determine what are the means of *reintroduction* or *survival* of the YF virus between the successive epizootics. Eggs of *Haemagogus* would be collected with ovitraps, and sentinel monkeys would be maintained to detect any early seasonal circulation of YF virus. The seasonal variations of the relative densities and survival rate of the vectors, and the reconstitution of the monkey populations would be the subjects of other very interesting studies. Finally, the survey of susceptible (nonvaccinated) people would be of prime importance in relation to the risks of sporadic cases emergences. When all these aspects are better known with fresh data, it would be possible to attempt some modeling work (33). ■

References and notes

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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
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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
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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
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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.
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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!
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