Dengue Serologic Survey of Schoolchildren in Rio de Janeiro, Brazil, in 1986 and 1987[1]

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Two major epidemic waves of dengue 1 occurred in Rio de Janeiro, Brazil, in 1986 and 1987. This article reports the results of a survey of Rio schoolchildren who were tested serologically for antibodies to dengue 1 before and after the second major epidemic wave.

The highest percentages of positive subjects were found in districts with relatively poor socioeconomic conditions and mosquito control problems. It also appears likely that the estimated number of dengue cases occurring in 1986–1987 was substantially below the number that actually occurred. In addition, because of this exposure to dengue 1, Rio de Janeiro now runs the risk of dengue hemorrhagic fever/dengue shock syndrome occurring should another dengue serotype be introduced within the next few years.

Dengue and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) are considered the most important arboviral infections of man (*1*). Dengue viruses belong to the family Flaviviridae (*2*) and are classified into four serotypes. They cause a spectrum of acute human diseases ranging from mild undifferentiated febrile illness to severe DHF/DSS (*3*), the latter being an important cause of hospitalization and mortality among children and teenagers in South East Asia (*4*).

During the 1980s Brazil experienced several dengue epidemics. Dengue 1 and dengue 4 outbreaks occurred in the Federal Territory of Roraima to the north in 1982 (*5*). Another major dengue 1 epidemic began in the Rio de Janeiro metropolitan area, the nation's second largest urban area with a population of nine million inhabitants, in March of 1986 (*6*). About 80,000 clinical cases were reported in that area up to 1987 (*7*), while a large number of dengue-infected people, including children, presented no symptoms or mild, undifferentiated febrile illness (*8*).

The disease in Rio de Janeiro was characterized by fever, headache, retrobulbar pain, pains in the muscles and joints, weakness, and prostration. Cutaneous rash and pruritus were also found. Hemorrhagic phenomena (epistaxis, intestinal bleeding, and gum hemorrhages) were observed in a very few cases (*9*). Convalescence took about two weeks in some cases. The rate of lethality was low.

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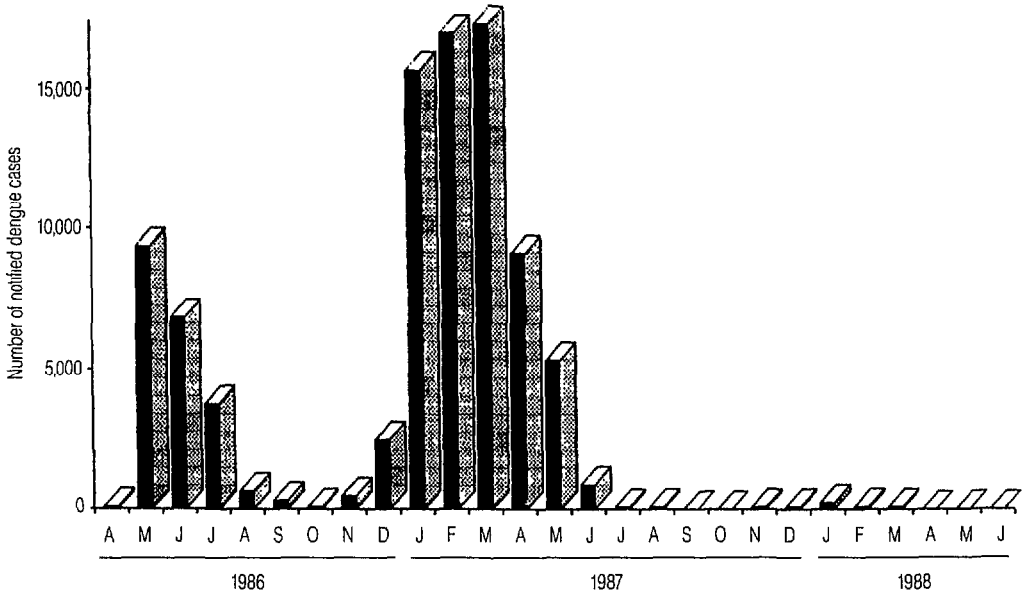
Figure 1. Notified dengue 1 cases in Rio de Janeiro, by month, from April 1986 through June 1988.



Overall, this dengue outbreak in Rio was characterized by two large epidemic waves, the first occurring between April and August of 1986 and the second between December 1986 and May 1987 (see Figure 1). The average weekly number of dengue cases notified during 1986 and 1987 was directly correlated with higher average temperatures and rainfall levels (M. Dias, personal communication). Such higher temperatures and rainfall levels favor proliferation and activity by the dengue vector *Aedes aegypti*, a circumstance that accounts for the occurrence of the largest wave of epidemic dengue in the summer (December–February) of 1986–1987.

This dengue 1 epidemic spread from the Rio de Janeiro area toward Brazil's northeast coast, reaching Alagoas State in June 1986 and Ceará State that September (*10*). A few dengue cases were still occurring in Rio de Janeiro and surrounding towns as late as 1988 (*11*). No other dengue virus serotypes were detected during this epidemic.

Studies done in Thailand have shown an initial infection with dengue virus serotypes 1, 3, or 4 followed by infection with the dengue 2 serotype to constitute an important risk factor for occurrence of DHF/DSS, the most potent combination in this regard being primary infection with dengue 1 followed by dengue 2 (*12*). The first DHF/DSS epidemic reported in the Americas, which occurred in Cuba in 1981, was associated with a dengue 1 epidemic in 1977 followed by a dengue 2 epidemic in 1981. The 1981 epidemic gave rise to over 10,000 DHF/DSS cases, and 158 deaths occurred among subjects of both sexes. Most of those fatally afflicted were children between the ages of four and six years; no deaths occurred among children less than two years old (*13*).

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The present article reports preliminary results of a dengue cohort serologic survey of pupils at Rio de Janeiro public ele-

mentary schools in 1986 and 1987. The survey sought information relating to the prevalence of dengue antibodies and the occurrence of seroconversion to dengue 1 in different areas of Rio de Janeiro; the proportion of clinical and subclinical dengue cases involved; and the public's knowledge of the disease.

MATERIALS AND METHODS

Serologic Survey Plan

The participating population was selected so as to maximize the survey's economy and ease of execution. Initially, elementary public schools were selected in five districts of the city of Rio de Janeiro—these districts being Copacabana, Lins de Vasconcelos, Penha, Rocinha, and Taquara. The locations of these districts are shown in Figure 2. Three schools were selected in the districts of Copacabana, Penha, and Lins de Vasconcelos, while four schools were chosen in Taquara and one in Rocinha. The survey in Rocinha was limited to 1987.

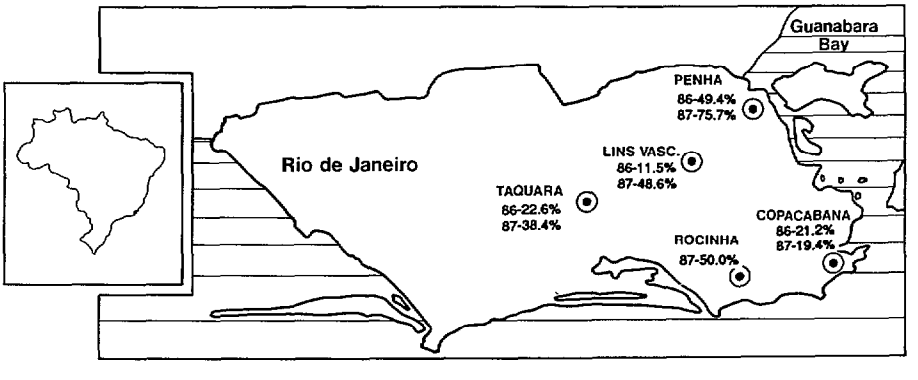
The total numbers of pupils surveyed at all of the schools were 402 in 1986 and 384 in 1987. These sample sizes are considered acceptable for a survey of this kind (*14*). Male and female children between four and 14 years of age were selected, and blood samples were collected from them at the beginning and end of a 12-month interval, in December 1986 and December 1987. Only 143 pupils were bled on both occasions, partly because many pupils moved to other schools or areas in the interim.

Serologic Survey Procedure

The aims of the study were explained to the selected pupils and their parents before blood sampling, and the parents' consent was obtained. The question "Did your child have dengue?" was answered by 271 of the participating children's parents during the 1987 survey. The results of the serologic tests conducted were reported to the parents.

Blood samples were obtained by fingertip puncture and collected on 13.0 mm diameter filter paper discs (Whatman, England). Fully saturated discs, containing approximately 100μl of blood, were dried and kept at $-18°C$ until use.

Figure 2. The locations of the five survey districts within the city of Rio de Janeiro, showing the percentages of December 1986 and December 1987 study children found to have dengue 1 antibodies in each district.



Serologic Test

Filter paper discs were eluted in 500μl of borate saline buffer (pH 9). Nonspecific hemagglutination inhibitors were removed by the kaolin method, and nonspecific agglutinins were adsorbed with goose red blood cells (*15*). Treated sera were tested with dengue 1 antigen by the hemagglutination-inhibition (HAI) technique (*15*). HAI antigens were prepared by the sucrose-acetone method, using brains of dengue 1 virus-infected baby mice (Mochizuki strain) (*15*).

RESULTS

The percentages of children, by sex and district, who yielded positive results for dengue 1 antibodies in the 1986 and 1987 serosurveys are shown in Tables 1 and 2 and in Figure 2. Overall, antibodies to dengue 1 virus were detected in 24.9% of the 402 pupils tested in 1986 and in 44.5% of the 384 pupils tested in 1987. The age range of these study subjects was too small to permit analysis by age distribution.

In 1987, the highest percentage of dengue 1 positive sera (75.7%) was found in the district of Penha and the lowest (19.4%) was found in the district of Copacabana. No homogeneity was observed in the percentages of children with dengue 1 antibodies in the whole set of districts ($\chi^2 = 56.203$; $p \leq 0.001$). Significantly different percentages were observed in Penha and Copacabana ($\chi^2 = 54.342$; $p \leq 0.001$), Penha and Taquara ($\chi^2 = 20.904$; $p \leq .001$), Penha and Lins de Vasconcelos ($\chi^2 = 13.329$; $p \leq 0.001$), and Rocinha and Copacabana ($\chi^2 = 11.547$; $p \leq 0.01$).

The percentages of the 143 children

Table 1. Percentages of children in the 1986 serosurvey with dengue 1 antibodies, by sex and district.

	Sex					
	Male		Female		Total	
District	No.[a]	%+[b]	No.	%+	No.	%+
Copacabana	44	25.0	69	18.8	113	21.2
Lins Vasc.	18	5.5	86	12.8	104	11.5
Penha	45	42.2	38	57.9	83	49.4
Taquara	52	21.2	50	24.0	102	22.6
Total	159	26.4	243	23.4	402	24.9

[a]No. = Number of participating children.
[b]%+ = Percent yielding positive results.

Table 2. Percentages of children in the 1987 serosurvey with dengue 1 antibodies, by sex and district.

	Sex					
	Male		Female		Total	
District	No.[a]	%+[b]	No.	%+	No.	%+
Copacabana	42	14.3	56	23.2	98	19.4
Lins Vasc.	18	72.2	89	43.8	107	48.6
Penha	39	74.3	35	77.1	74	75.7
Taquara	35	45.7	38	31.6	73	38.4
Rocinha	13	61.5	19	42.1	32	50.0
Total	147	49.0	237	42.4	384	44.5

[a]No. = Number of participating children.
[b]%+ = Percent yielding positive results.

Table 3. Percentages of the 143 study children tested in December 1986 and December 1987 who yielded positive results in those years, by sex and district. The attack rates calculated from these data show the percentages of children seronegative in 1986 who were seropositive in 1987.

District	No.[a]	% positive		Attack rate (%)
		1986	1987	
Copacabana	59	15.2	22.0	8.0
Lins Vasc.	15	6.7	26.7	21.4
Penha	17	41.2	76.5	60.0
Taquara	52	19.2	44.2	31.0
Total	143	18.9	37.0	22.4

[a]No. = Number of participating children.

tested in both 1986 and 1987 who yielded positive results are shown in Table 3 and Figure 3 by district, together with the observed attack rates among the study subjects in each district over the one-year interval between tests. The overall dengue 1 attack rate in these children for the year 1987 was found to be 22.4%.

Table 4 shows the changes in the dengue 1 serum titers observed in these 143 subjects. Eleven subjects whose sera tested positively for dengue 1 antibodies in 1986 provided sera yielding negative results in 1987.

Affirmative or negative answers to the question "Did your child have dengue?" were obtained from 263 parents of the 384 children participating in the 1987 survey. Table 5 compares these answers with the serologic results obtained for each child. As indicated, the predictive value of the affirmative answers was found to be 71.2% (52/73) and that of the negative answers was found to be 67.9% (129/190). Statistically significant associations were observed between the proportions of affirmative answers and positive HAI results, and negative answers and nega-

Figure 3. Results obtained from the 143 children tested in both 1986 and 1987, by district, showing the percentages positive in December 1986 and the higher percentages positive in December 1987.

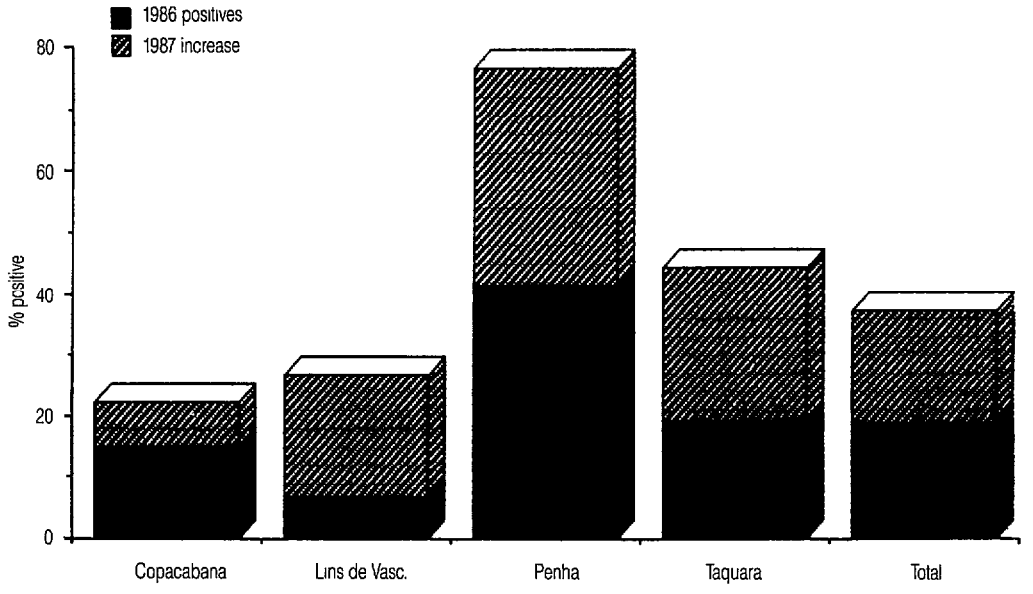


Table 4. Dengue 1 serum antibody titer changes found among the 143 children tested in 1986 and 1987.

1986		1987	
No.[a]	Titer[b]	No.	Titer
2	320	1	640
		1	80
5	160	2	40
		2	20
		1	N[c]
7	80	1	160
		2	80
		1	40
		1	20
		2	N
9	40	1	160
		2	80
		1	20
		5	N
4	20	1	40
		3	N
116	N	2	640
		2	160
		5	80
		19	40
		9	20
		79	N

[a]No. = Number of participating children.
[b]Titer = Reciprocal titer.
[c]N = Negative serologic test (titer < 20).

Table 5. A comparison of parental answers to the question "Did your child have dengue?" and HAI results obtained from the 263 children tested in 1987.

	HAI results		
Answers	+	−	Total
Yes	52	21	73
No	61	129	190
Total	113	150	263

tive HAI results ($\chi^2 = 32.953$; $p \leq 0.001$). Regarding specific districts, positive associations between parental answers and HAI results were found in the districts of Penha, Copacabana, Taquara, and Rocinha.

DISCUSSION

Interpretation of HAI flavivirus survey results can be complicated by serum cross-reactions that impair identification of the specific infecting agent (*16*). We reduced this source of error by studying children at relatively low risk of previous endemic flavivirus infection. A 1968 serosurvey of children in rural areas of the state of Rio de Janeiro detected St. Louis encephalitis virus neutralizing antibodies in no more than 3% of the test subjects (*17*). None of the children included in the present study had been vaccinated against yellow fever.

Technical and economic difficulties relating to Rio de Janeiro's size prevented us from conducting a serologic survey with randomized samples. Despite this limitation, we obtained good-quality indicators that should help to answer important questions about Rio's dengue 1 epidemic.

No significant difference was observed in the percentages of boys and girls yielding positive results, a finding consistent with occurrence of an epidemic in a large population fully susceptible to arthropod-borne infections.

With regard to particular districts, the highest percentage of subjects with dengue 1 antibodies (75.7%) was found in the district of Penha in 1987. This result is consistent with the large numbers of 1987 dengue cases reported from this northern Rio de Janeiro district and from the neighboring towns of Duque de Caxias, Nilopolis, Nova Iguaçu, and São João do Meriti. The northern area of Rio de Janeiro has relatively poor socioeconomic circumstances and offers conditions appropriate for *Aedes aegypti* breeding in houses and backyards.

In Copacabana, one of the most urbanized districts of Rio de Janeiro with relatively good sanitary conditions, only

19.4% of 98 children tested in 1987 were found to have dengue 1 antibodies. In contrast, 50% of the 32 children from Rocinha, a hill district near Copacabana with far worse sanitary conditions, had positive serum titers. This significant difference between the Rocinha and Copacabana data can be explained by the difficulties involved in controlling mosquitos in the Rocinha hill slum area.

Overall, the observed 22.4% dengue 1 attack rate suggests widespread circulation of the virus in Rio de Janeiro in 1987. As Figure 1 indicates, the interval between the December 1986 and December 1987 surveys includes nearly all of the epidemic wave that occurred in the summer of 1986–1987. Examination of the attack rates among children from different districts showed new dengue infections in all the survey districts, with the highest attack rates occurring in Penha and the lowest in Copacabana (Figure 3).

A significant difference was found in the rates of positive serology among children whose parents said they had had dengue as compared to those whose parents said they had not had it. Although the answers to retrospective questions of this sort have limited reliability, comparison of the parents' answers with the serologic test results provided information about the parents' (and general population's) awareness of the disease and its clinical picture.

The predictive values of the parents' affirmative and negative answers (71.2% and 67.9%, respectively) suggest the population has substantial knowledge of the disease and its clinical picture. Regarding the 33% of the seropositive children whose parents gave a negative answer, many of them could have experienced asymptomatic dengue cases or mild, undifferentiated febrile cases. Regarding the 29% of the seronegative children whose parents answered affirmatively, many could have experienced childhood diseases resembling dengue that were caused by other agents.

Theiler and Downs (*16*) have reported a tendency of HAI titers resulting from primary flavivirus infections to decline over time. Our analysis of dengue 1 serum titers in 143 children over a one-year period showed no significant change in most cases (see Table 4). In five cases there was a significant titer reduction (a reduction of at least three serum dilutions)—including three cases in which the test result became seronegative. The reversion to negative serum titers in eight other cases was not considered significant because the starting dilution in our HAI test was very low. Some of these sera could have contained dengue 1 antibodies while yielding a positive titer below the threshold. This idea has been corroborated by detection of IgG antibodies to dengue 1 in two of these sera by enzyme immunoassay (*18*).

CONCLUSIONS

The high percentage of test children with dengue 1 antibodies (44.5%) points up the great magnitude of the Rio de Janeiro 1986–1987 epidemic. The number of infected cases estimated on the basis of notifications was about one million (*7*). However, our data suggest that this number was an underestimate. All in all, it seems probable that a very large number of dengue cases were not notified because of the problems involved in gaining access to public health care facilities, public awareness of the fact that no specific treatment is available for dengue, and the occurrence of many asymptomatic or subclinical dengue cases—especially in children.

The barrier produced by a large population previously infected with dengue 1 virus, combined with *Aedes aegypti* con-

trol measures, effectively interrupted development of a new epidemic wave of dengue 1 in the summer of 1987–1988. However, Rio de Janeiro now runs the risk of DHF/DSS occurring in the event another dengue serotype is introduced within the next few years, as happened in Cuba in 1981 (*13*). Such a new serotype could easily be introduced into the Rio de Janeiro area via airplanes or ships coming from the Caribbean or other parts of the world (*15*).

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WHO Experts Make Recommendations on Vaccines

Experts in collaboration with the World Health Organization's program on influenza surveillance held their yearly meeting on 14–15 February to review the global influenza situation and to make recommendations for the composition of influenza vaccines to be used in the 1990–1991 season. Because of variability in influenza virus strains, vaccine composition is reviewed annually and changed in order to maintain effectiveness. Recommendations are based on data received from over 100 laboratories under WHO's global "influenza virus watch."

Two types of influenza viruses are commonly associated with outbreaks: influenza type A—of which two subtypes have circulated for many years, A(H1N1) and A(H3N2)—and influenza type B. Influenza A(H3N2) strains predominated this past season, but influenza B viruses have also been isolated in a number of countries. There have been few isolates of A(H1N1).

The 1989–1990 influenza season began in late 1989, with outbreaks of influenza A(H3N2) in North America, Europe, and Asia. Both the United Kingdom and United States reported excess mortality related to influenza. In the U. K. the epidemic was the most severe since 1976.

After reviewing the prevalence and antigenic character of various virus strains isolated during the current season, the WHO experts recommended that the vaccine for use in the 1990–1991 season be trivalent and contain the following antigens: an A/Guizhou/54/89 (H3N2)-like antigen, an A/Singapore/6/86 (H1N1)-like antigen, and a B/Yamagata/16/88-like antigen.

Vaccines against viruses causing influenza are very effective in protecting at-risk populations from illness and death during influenza outbreaks. Immunization is recommended for individuals over the age of 65, those who suffer from chronic disease of the heart, lung, or other conditions such as immunodeficiency and diabetes mellitus, and those who live or work under crowded conditions. The vaccine should be received in autumn (October in the Northern Hemisphere and May in the Southern Hemisphere) before the influenza outbreak season begins.

Source: World Health Organization, Press Release WHO/18. Geneva, 23 March 1990.