Dengue Serologic Survey in Ribeirão Preto, São Paulo, Brazil[1]

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The city of Ribeirão Preto suffered a dengue 1 epidemic that began in November 1990 and ended in March 1991. A serologic survey designed to detect IgG antibodies to the four dengue serotypes and other flaviviruses was carried out in Ribeirão Preto during September and October of 1992. Dengue 1 antibodies were detected in 5.4% of the survey participants. Significantly higher seropositivity (9.3%) was found among subjects residing in the Northwest Sector of Ribeirão Preto than among those living in the city's other three sectors. The Northwest Sector also exhibited relatively high levels of breeding sites used by the Aedes aegypti *vector, the highest number of reported dengue cases of any sector, and relatively poor socioeconomic conditions. The fact that the epidemic was limited mainly to the Northwest Sector probably resulted mainly from intense vector control and educational measures undertaken in response to the outbreak. As of the 1992 survey, most of the city's population remained vulnerable to dengue 1 infection; however, an estimated 23 000 with dengue 1 antibodies appeared to be at relatively high risk of developing dengue hemorrhagic fever/dengue shock syndrome in the event of infection with dengue 2. Both of these considerations indicate an ongoing need to maintain dengue education and vector control measures.*

Because of the degree of morbidity and mortality involved, dengue is considered the most important arboviral disease of man (*1*). The four dengue virus serotypes, designated 1, 2, 3, and 4, are RNA viruses belonging to the genus *Flavivirus* of the family Flaviviridae (*2, 3*). According to strict epidemiologic criteria, dengue viruses are arboviruses (arthropod-borne viruses) transmitted by the mosquito vectors *Aedes aegypti* and *Aedes albopictus* (*1, 4*).

Since the mid-1980s, dengue epidemics have occurred in all of Brazil's geographic areas except the southern states, producing millions of infections. Dengue types 1 and 2 were isolated during these epidemics (*5–7*). The vast majority of the disease cases involved were not life-threatening. However, dengue hemor-

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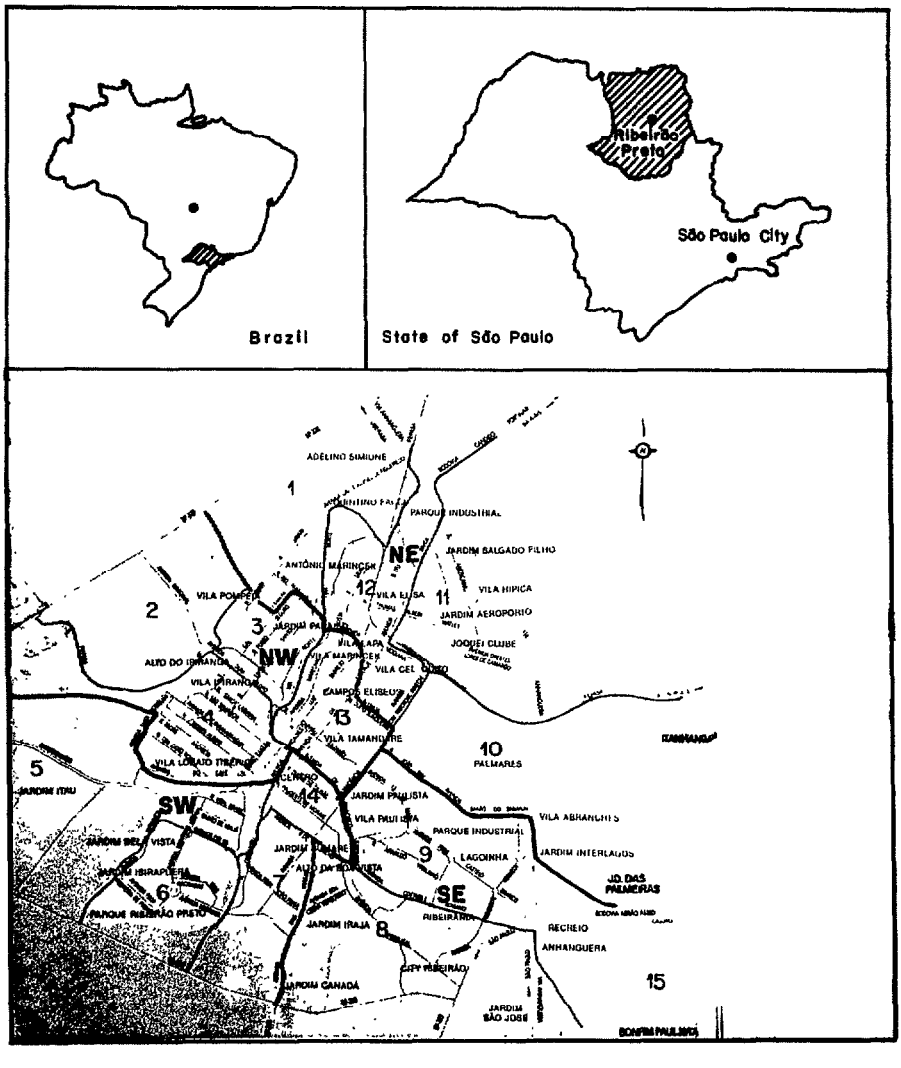
rhagic fever (DHF) and dengue shock syndrome (DSS) have also occurred, mostly in Rio de Janeiro, causing hundreds of DHF/DSS cases and many deaths (*8*).

This article deals with a dengue 1 outbreak in Ribeirão Preto, a city of approximately 430 000 inhabitants in São Paulo State that serves as the cultural and economic center for a region of roughly 3.5 million people and is located 329 km north of the city of São Paulo (Figure 1) (*9*). A dengue 1 outbreak that began elsewhere in Brazil reached Ribeirão Preto in November 1990. This was the first dengue outbreak to strike the region. Reported dengue cases increased from 174 in No-

Figure 1. A map of the city of Ribeirão Preto showing the locations of the 15 zones and of the Northeast (NE), Northwest (NW), Southeast (SE), and Southwest (SW) sectors. The NE Sector includes zones 1, 10, 11, and 12; the NW Sector includes zones 2, 3, 4, and 13; the SE Sector includes zones 8, 9, and 15; and the SW Sector includes zones 5, 6, 7, and 14.



vember to 7 325 in December, thereafter declining to 1 122 in January 1991, 277 in February, and 65 in March when the outbreak ceased. Dengue patients presented either an undifferentiated fever (viral syndrome) or classical dengue fever. Dengue 1 was the only virus isolated. Following this epidemic, the virus spread to neighboring towns and other parts of São Paulo State (*7, 10*). However, fewer than 10 autochthonous dengue cases were reported in Ribeirão Preto from the beginning of April 1991 to October 1992.

In response to the epidemic, vector control measures were begun in the city during November 1990. These included a massive cleaning program of houses and back yards, combined with application of insecticides and larvicides. Educational activities encouraging appropriate control measures were also conducted at that time.

It was difficult to determine the true magnitude of the Ribeirão Preto dengue outbreak, mostly because many of the reported cases were incorrectly diagnosed and also because there appeared to have been a high incidence of asymptomatic pediatric cases. Therefore, a randomized serologic survey was performed to detect dengue 1 antibodies. Besides seeking to gauge the epidemic's magnitude, the survey also sought to determine infection rates in different parts of the city and to correlate these findings with levels of *Aedes aegypti* infestation, numbers of reported cases, and prevailing socioeconomic levels in different sectors.

MATERIALS AND METHODS

Survey Plan

The survey adapted the methodology used to determine the Breteau index (the number of vector breeding sites per 100 residences visited) to a population study, employing a stratified and probabilistic approach to select the study sample. Individual Ribeirão Preto residences to be included in the survey were selected in a randomized manner based on the desired sample size and the number of residences in the study area (*11*). The survey participants were chosen from among residents 5–15 years of age. Although no specific method was used to select the children studied within this age range at any given residence, no more than three children from any one residence were included in the survey.

A desired sample size of 648 participants was determined by applying the formula

$$n = \frac{z_\alpha^2 \, pq}{d^2}$$

where: n = sample size; α = significance level of 5%; $z_\alpha = 1.96$ (critical value); $p = 2.083\%$ (proportion of dengue infections indicated by 8 963 reported cases in a population of 430 312 inhabitants); $q = 97.917\%$ $(1 - p)$; and $d = 1.1\%$ (acceptable variation for the sample size) (*11*). For purposes of statistical analysis the participants were arranged into 5–6 year, 7–10 year, 11–14 year, and 15 year age groups.

Ribeirão Preto was divided into 15 zones which in turn were grouped in four sectors, these sectors being the Northeast (NE), Northwest (NW), Southeast (SE), and Southwest (SW) sectors shown in Figure 1. This zone division followed criteria designed to group the study population into geographic subgroups with similar socioeconomic levels and *Aedes aegypti* densities.

The serologic survey plans employed a randomized model to determine the Breteau index (*12*). The study participants were selected in accordance with the relative numbers of residences in each zone, assuming a generally homogeneous house-by-house distribution of the

study population. City blocks and houses were selected so as to choose no more than three participants from any given block.

Survey Procedure

The survey was carried out in September and October of 1992. The selected houses were visited, and the parents of the study subjects were informed about the study and its aims. Enrollment of the subjects was only permitted with parental consent. Blood samples were obtained by fingertip puncture, using sterile disposable lancets; and the blood was collected by absorption on paper filter strips.

Samples were obtained from 662 participants. The material was dried, labeled, and kept at −18 °C until use. The serologic tests employed blood halos with a diameter of 18 mm, containing about 50 µl of serum (100 µl of blood). These were punched out and eluted in 450 µl of an aqueous solution at pH 9 made up with 1 000 ml H_2O, 1.5M NaCl, 0.5M H_3BO_3, and 1N NaOH (*13*). After elution the sera were at a dilution of 1:10 (*14*).

Enzyme Immunoassay on Infected Cultured Cells (EI-ICC)

An EI-ICC designed to detect anti-flavivirus IgG antibody was used to test the sera (*15, 16*). C6/36 *Aedes albopictus* cells were grown in Leibowitz L15 medium (GIBCO, U.S.A.) containing 10% heat-inactivated fetal bovine serum, 10% tryptose phosphate broth, nonessential amino acids, 100 U/ml penicillin, and 100 mg/ml streptomycin. The cultures were incubated at 28 °C in a humidified atmosphere (*17*). The C6/36 cells were placed in 96-well plastic plates (Falcon, U.S.A.) containing 150 µl of growth medium at a density of approximately 5×10^4 cells per well.

After 24 hours the cells were infected with one of the following flaviviruses: dengue 1 strain RibH830 (isolated in Ribeirão Preto); dengue 2 strain NgC (supplied by Dr. R. Shope, Yale Arbovirus Research Unit, U.S.A.); dengue 3 strain H87 (supplied by Dr. D. Gubler, Centers for Disease Control and Prevention, U.S.A.); dengue 4 strain H241 (also supplied by Dr. Gubler); yellow fever strain 17D (vaccine strain, supplied by Dr. H. Schatzmayr, FIOCRUZ, Brazilian Ministry of Health); Ilheus strain HBe7445 (supplied by Dr. A. Travassos da Rosa, Evandro Chagas Institute, Brazilian Ministry of Health); and St. Louis encephalitis strain SPAn1916 and Rocio strain SPH 34675 (both supplied by Dr. L. T. M. de Souza, Adolpho Lutz Institute, São Paulo State Ministry of Health).

Alternate columns of the microplate wells contained uninfected cells. At 6 days postinfection, all the wells received 100 µl of a neutral (pH 7) buffered formalin solution made up with 100 ml of 37–40% formaldehyde, 6.5 g of anhydrous dibasic sodium phosphate, 4.0 g of monobasic sodium phosphate, and 900 ml of distilled water. The wells were then held overnight at 4 °C.

Within 18–24 hours of fixation, the cells were washed twice with phosphate-buffered saline (PBS). Some microplates were used at once in the EI-ICC test; others were dried, put in plastic bags, and stored at −70 °C (*15, 16*).

The infected and uninfected cells to be used in the EI-ICC test were blocked for 2 hours with 200 µl per well of 5% gelatin in PBS. The microplates were then washed three times with 0.05% Tween 20 in PBS before being inoculated with the study subjects' sera. One hundred microliters of serial dilutions of each subjects' sera (the dilutions ranging from 1:200 to 1:32 000 in bovine serum albumin-PBS) were added to both infected and uninfected wells. The inoculated microplates

were then incubated for 1 hour at 37 °C and washed three times.

The wells next received 100 μl of goat anti-human IgG serum conjugated with horseradish peroxidase diluted 1:2 000 in bovine serum albumin-PBS (SIGMA, U.S.A), and the microplates were incubated for 1 hour at 37 °C. After washing the microplates five more times in PBS-Tween, a 100 μl portion of o-phenylenediamine dihydrochloride substrate (OPD) (SIGMA, U.S.A.) consisting of OPD (10 mg), 0.1M citric acid (6 ml), 0.2M dibasic phosphate (6.5 ml), water (12.5 ml), and hydrogen peroxide (40 μl) was added to each well. The test results were read visually by comparing the yellow color of virus-infected wells with the colorless appearance of uninfected wells 5 to 10 minutes after addition of the substrate (*15, 16*).

RESULTS

Table 1 lists the reciprocal titers of sera positive for dengue 1 antibodies, shows the responses of those sera to other dengue virus serotypes, and provides basic information about the study subjects involved. As may be seen, IgG antibodies to dengue 1 were found in 36 (5.4%) of the 662 sera tested, yielding positive reciprocal titers in the range of 200 to 8 000. When these positive sera were tested with dengue serotypes 2, 3, and 4, six of them were found to cross-react with dengue 3, yielding reciprocal titers of 500 or 1 000, and two of these six also responded positively to dengue 4, yielding reciprocal titers of 500. One serum positive for dengue 1 but not dengue 3 was also positive for dengue 4 at a titer of 500. None of the sera with dengue 1 antibodies were positive for dengue 2.

The positivity rate of 5.4% suggests that roughly 23 000 people were infected by dengue 1 during the 1990–1991 outbreak in Ribeirão Preto. (This extrapolation assumes that the rate of infection among the general population was the same as the rate of infection among the 5- to 15-year-old study subjects.)

Regarding sex, 5.3% of the males tested and 5.5% of the females were seropositive. Regarding age, levels of seropositivity ranged from 1.4% among the study children 11 years old to 8.6% in those 13 years old. However, no significant differences were found between the levels of seropositivity to dengue 1 in the four age groups cited previously (5–6, 7–10, 11–14, and 15 years); $X^2 = 3.63$ (3 degrees of freedom, $p > 0.05$).

To help relate seropositivity to socioeconomic levels and *A. aegypti* densities, seropositivity rates were determined for subjects residing in the 15 study zones. These rates ranged from 0% in five zones to 22% in zone 3, the next highest rate being 7.7% (in zone 14) and the median for the 15 zones being 2.5% (Table 2). Zones with no seropositive subjects (these being zones 7, 8, 9, 11, and 13) were deemed at low risk with regard to dengue infection, while the zones with seropositivity rates exceeding 6% (zones 3, 5, 10, 14, and 15) were considered high-risk areas.

The seropositivity rates indicated by the survey for the four sectors of Ribeirão Preto are shown in Table 3. The Northwest (NW) Sector study subjects yielded the highest positivity rate (9.3%), the next highest (4.9%) being found for subjects from the Southwest (SW) Sector. Overall, a significant difference was found between the dengue 1 infection rates in the four sectors of Ribeirão Preto ($X^2 = 13.58$, 3 degrees of freedom, $p < 0.01$). A similar result was observed after grouping the NE, SE, and SW sectors and comparing their combined seropositivity with that of the NW sector ($X^2 = 11.58$, 1 degree of freedom, $p < 0.001$).

The dengue 1 seropositivity rates of study subjects in the four sectors of Ri-

Table 1. Data relating to the 36 sera positive for dengue 1 antibodies, showing the donors' initials, zone of residence, sex, and age, together with the reciprocal IgG serum titers obtained through EI-ICC testing with dengue virus serotypes 1, 2, 3, and 4. (N = negative test.)

				Reciprocal titer obtained with:			
Identification	Zone	Sex	Age (years)	Dengue 1	Dengue 2	Dengue 3	Dengue 4
B.C.G.	1	M	5	500	N	N	N
R.B.	2	F	9	500	N	N	N
E.L.S.	3	M	9	200	N	N	N
W.M.F.O.	3	M	13	200	N	N	N
M.M.S.	3	M	13	200	N	N	N
F.M.A.	3	F	6	2 000	N	N	N
P.J.S.	3	F	5	500	N	500	N
A.J.S.	3	M	6	500	N	N	N
S.S.G.	3	F	15	500	N	500	N
S.M.B.	3	F	10	8 000	N	500	N
D.M.S.	3	M	10	4 000	N	N	500
S.V.C.	3	F	5	2 000	N	N	N
F.B.O.	3	F	12	2 000	N	N	N
J.C.V.	3	M	15	2 000	N	N	N
F.V.	3	M	7	4 000	N	N	N
A.G.S.	3	M	11	4 000	N	N	N
C.M.F.	3	F	9	8 000	N	N	N
C.G.M.	3	M	12	500	N	N	N
P.M.G.	3	F	8	2 000	N	N	N
D.C.B.	3	F	12	8 000	N	N	N
G.B.	4	M	14	4 000	N	1 000	N
L.C.N.	4	M	13	500	N	500	500
L.R.S.	4	M	7	2 000	N	N	N
G.C.C.	4	M	6	200	N	N	N
P.S.	5	F	13	500	N	N	N
G.L.	5	F	13	500	N	N	N
C.A.P.	5	F	15	500	N	N	N
J.S.	5	M	9	500	N	N	N
P.L.A.	5	F	10	200	N	N	N
A.F.	6	F	10	200	N	N	N
E.C.F.	10	F	15	200	N	N	N
T.E.F.	10	F	5	200	N	N	N
L.M.S.	12	M	13	500	N	N	N
J.S.F.	14	F	6	500	N	500	500
E.A.P.G.	14	M	14	500	N	N	N
C.B.	15	M	12	500	N	N	N

Table 2. Rates of dengue 1 seropositivity indicated by the survey findings for each of the 15 zones of Ribeirão Preto, in order of increasing positivity.

	Zone														
	7	8	9	11	13	1	6	2	12	4	5	10	15	14	3
% positive	0	0	0	0	0	1.6	2.3	2.5	2.7	3.5	6.1	6.1	6.7	7.7	22.2

Table 3. Dengue-related data for each of Ribeirão Preto's four sectors and the zones included in each sector. The data shown include the dengue 1 seropositivity found for survey subjects residing in each sector, the sector's percentage share of all reported dengue cases during the 1990–1991 outbreak, and the sector's Breteau index as of November 1990.

Sector	Zones in the sector	% seropositive (No. positive/total)	% of all reported cases	Breteau index
NE	1, 10, 11, 12	2.9% (4/139)	19.4%	13.1
SE	8, 9, 15	0.9% (1/112)	7.5%	3.1
SW	5, 6, 7, 14	4.9% (8/164)	16.5%	7.5
NW	2, 3, 4, 13	9.3% (23/247)	56.7%	13.1

beirão Preto were compared with the numbers of reported dengue cases in each sector during the epidemic and also with the Breteau *A. aegypti* breeding site indexes found in November 1990 (at the beginning of the outbreak) in each sector. These data are also shown in Table 3. Positive correlations were found between study subject seropositivity and reported dengue cases in the four sectors ($r = 0.889$) and also between study subject seropositivity and the Breteau indexes ($r = 0.417$).

The socioeconomic levels prevailing in some but not all of the zones were estimated by R. J. S. Pontes (*18*) on the basis of rent and purchase prices of residences and other properties, and each zone evaluated in this manner was assigned a letter code—"A" indicating a high level, "B" an intermediate level, "C" a low level, and "D" a very low level (*18*). Using these codes, a negative correlation was found between the study subject seropositivity rates and socioeconomic levels in six zones (Table 4). An exception to this general pattern was found in Zone 14 (downtown), where the socioeconomic level was high (code A) and the level of study subject seropositivity was also high (7.7%).

Table 4. Relationships observed between socioeconomic levels prevailing in various zones of Ribeirão Preto (A = high, B = intermediate, C = low, D = very low) and the dengue 1 seropositivity rates found for subjects residing in those zones. Note that Zone 14 constitutes an exception to the prevailing inverse relationship observed between socioeconomic level and seropositivity.

Zone	Assigned socioeconomic code	Dengue 1 seropositivity (%)
14	A	7.7
8	A	0
9	B	0
12	C	2.7
13	C	0
3	D	22.2
4	D	3.5

DISCUSSION AND CONCLUSIONS

Over the past 20 years many dengue serologic surveys have been done in the Americas (*14, 19, 20*). However, most of them have been limited by methodology problems relating to randomness and sample size determination. Such limitations typically arise because of the difficulties involved with working in large urban areas with chaotic development patterns, in the absence of precise demographic information about the population, the study area, and the numbers of blocks and residences involved. The survey reported here was designed in a manner calculated to avoid most of these problems.

The study population's age range of 5 to 15 years was chosen to limit the pos-

sibilities for cross-reactions involving non-dengue flaviviruses. So far as was known, Ribeirão Preto had never had a dengue outbreak before; and so the whole population, independent of age, should have been susceptible to the virus. However, it was felt that those 5 to 15 years old were less likely than older subjects to have been infected by other flaviviruses endemic to Brazil, and hence selecting them would reduce potential cross-reaction problems. This idea was supported by a serologic survey done in the Ribeirão Preto region in 1984, which found evidence of flavivirus infections in only 2% of the population under 20 years old (*21*). It is also true, however, that Ribeirão Preto underwent massive vaccination against yellow fever in 1992, and as a result many of the dengue survey participants were apparently exposed to the 17D vaccine strain of that virus.

The serum samples were collected by fingertip puncture rather than venipuncture because the former procedure has been found to enhance voluntary participation (*14, 19*). Absorbing the blood samples on filter paper and storing them at −20 °C after drying afforded a quick and inexpensive way of avoiding microbial contamination. The EI-ICC used to detect IgG antibodies against specific dengue viruses has greater sensitivity than the hemagglutination inhibition test (*13, 16*). The former's simplicity, together with the subsequent reproducibility of the positive results (all the sera were tested at least twice) and the relatively high positive titers obtained (≥2 000 in 36% of the sera positive for dengue 1 antibodies), show that the EI-ICC is a suitable technique for this kind of study.

As Table 1 indicates, only 7 of the 36 sera that responded positively to dengue 1 (19.4%) exhibited apparent cross-reactions by responding positively to dengue 3 and/or dengue 4. None of the 36 sera responded positively to the test strains of Ilheus, St. Louis encephalitis, Rocio, or yellow fever virus. Nor was any positive response found to dengue 2, suggesting that this virus did not participate in the Ribeirão Preto outbreak. It also appears that all 36 of the seropositive study subjects had experienced a primary flavivirus infection.

It is difficult to explain the absence of positive reactions to 17D yellow fever virus, since many of the participants indicated they had been vaccinated. However, the efficacy of the yellow fever vaccination campaign of 1992 has been questioned, and another study has been initiated in order to evaluate this matter.

Analysis by sex and age of the participants showed no significant differences in terms of dengue 1 positivity. It thus appears that the Ribeirão Preto dengue 1 epidemic attacked a completely susceptible population and that, at least so far as the study participants were concerned, the mosquito-mediated disease transmission process did not discriminate with respect to sex or age.

The size of Ribeirão Preto's population (430 000) and the 5.4% seropositivity rate suggest that roughly 23 000 people were infected by the dengue 1 virus during this outbreak. However, this number is based on data obtained from a limited age group. It thus supposes a dengue infection risk independent of age. While this assumption seems reasonable for a completely susceptible population, it is noteworthy that a serologic survey done after the Cuban dengue 1 epidemic showed a higher infection rate among people over 35 years old (*19*). If the same phenomenon occurred in Ribeirão Preto, the number of people infected could well have exceeded 23 000.

The seropositivity rate of 5.4%, and even the 22.2% positivity found in zone 3, cannot be considered high values compared to those found in other places. A prospective dengue 1 serologic survey of Rio

de Janeiro schoolchildren found positivity rates of 18.9% in 1986 and 37% in 1987, with a positivity rate of 75% being found for the District of Penha in Rio de Janeiro (*14*). In this same vein, in 1982 a national serologic survey of dengue 1 antibodies in Cuba found a positivity rate of 42% (*19*).

Of the nearly 9 000 dengue cases reported in Ribeirão Preto during the outbreak, some 40% (roughly 3 600) were confirmed by laboratory tests (serology and/or virus isolation). The remaining 60% presented an undifferentiated fever probably caused by another virus such as rubella or influenza. (A study carried out during the dengue 1 epidemic in Rio de Janeiro found that diagnosis made on the basis of the most accurate clinical picture model was confirmed by laboratory tests in only 62% of the cases—*20*). Considering these data, and supposing that 23 000 people were actually infected by dengue 1 during the Ribeirão Preto outbreak, it appears that only 3 500 real dengue cases (15% of the infections) were reported and confirmed. Most of the remaining 85% were not reported, probably because they were not symptomatic or because those afflicted knew from relatives or friends about the lack of any specific medical treatment for uncomplicated dengue fever.

Three of the five zones (7, 8, and 9) where the risk of dengue infection was found to be low were mostly inhabited by middle class people living in adequately built homes with suitable basic sanitation and without accumulations of trash in the back yards. The Breteau index found in November 1990 in these zones was equal to or less than 3.1. Another low-risk area, Zone 11, containing an airport and industrial area with a small and scattered population, had a Breteau index of 2.2. Three zones considered high-risk areas (3, 10, and 14) were mostly inhabited by residents with a low socioeconomic level living in small houses with poor sanitary conditions, inadequate garbage disposal, and Breteau indexes ranging from 3.1 to 13.1. Another area considered high-risk (Zone 5 on the outskirts of the city) had a scattered population living mainly at a low socioeconomic level. Zone 13 (considered low-risk) and 15 (considered high-risk) exhibited mixtures of socioeconomic conditions; the reasons for the apparently differing levels of dengue infection risk in these zones is unclear.

The survey found dengue 1 seropositivity to be significantly higher among study subjects residing in the Northwest Sector of Ribeirão Preto than among those residing in other sectors, while relatively low seropositivity was found among Southeast Sector subjects. This indicates that during the outbreak the virus attacked different parts of the city to differing degrees.

The absence of a good indicator of adult *A. aegypti* vector density makes the Breteau larval index the most suitable vector parameter to compare with the serologic survey data. The positive correlations found between the seropositivities, numbers of reported cases, and November 1990 Breteau indexes in the four sectors reveals a logical pattern that aids in understanding more about dissemination of the virus and the magnitude of the outbreak. All of these indicators attained their highest values in the Northwest Sector. It thus appears that there was more vector activity and greater dengue virus transmission in that sector, resulting in higher numbers of infections and disease cases.

Also, a negative correlation was found between the dengue 1 seropositivity in particular zones and the socioeconomic status of the inhabitants. For the most part, the lower the socioeconomic level, the higher the seropositivity. Districts where the socioeconomic level was low

tended to have high concentrations of people living in small homes—circumstances enhancing vector transmission of dengue virus. Such districts also tended to have inadequate basic sanitation, deficient solid waste disposal, and sites littered with many tires and cans making good *A. aegypti* breeding places. These districts also tended to have numerous places where scrap iron and used tires were traded—places that typically serve as fine *aegypti* breeding grounds. Most of the people living in these districts are aware of neither the dengue transmission mechanism nor the importance of keeping their homesites free of small water-bearing containers (*18*).

Downtown Ribeirão Preto (Zone 14) constituted an exception to the general rule, in that high real estate rental and purchase prices (establishing socioeconomic level A) were found together with high dengue 1 seropositivity (7.7%). This circumstance can be explained by the zone's role as a commercial district. On the one hand, the infection could be brought in readily by people working there who lived in other zones; and it could be disseminated readily because the Zone 14 population included a substantial number of construction workers living at low socioeconomic levels, many of them migrants from other parts of Brazil, who were occupying small crowded residences close to their jobs.

Overall, dengue 1 dissemination during the outbreak was clearly related to biologic and economic circumstances. The virus was probably introduced into Ribeirão Preto—the cultural, trading, and economic hub for a region of 3.5 million people—as a result of extensive transit by people from other parts of Brazil. The virus spread in the Northwest Sector, which had suitable vector infestation and human population levels to sustain the dengue transmission cycle. The fact that the epidemic was limited mostly to the Northwest and declined steadily from January through March can probably be attributed to public health activities, most notably the vector control and education measures already mentioned. Presumably as a result of these activities, the Breteau index in Ribeirão Preto declined from 6.9 in November 1990 (prior to any control measures being taken) to 2.1 in January 1991 and 0.3 in February before increasing slightly to 1.6 in March (*18*).

Looking beyond the epidemic, two conclusions derived from the survey data seem especially worth noting. First, the data suggest that most of the Ribeirão Preto population remains susceptible to dengue 1. Second, should an expansion of the Brazilian dengue 2 outbreak strike Ribeirão Preto, the estimated 23 000 inhabitants previously infected by dengue 1 will be at relatively high risk of developing life-threatening DHF/DSS (*22*). These circumstances provide considerable justification for having ongoing vector control work and dengue education programs in Ribeirão Preto directed at preventing new outbreaks.

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