

HOUSEHOLD SURVEY OF DENGUE INFECTION IN CENTRAL BRAZIL: SPATIAL POINT PATTERN ANALYSIS AND RISK FACTORS ASSESSMENT

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Abstract. Urban dengue fever is now considered a major public health threat in most American countries. A household survey was conducted in the city of Goiânia in central Brazil in 2001 to assess prevalence of dengue infection and individual and area-based risk factors. Spatial point pattern analysis was performed using the dual Kernel method. A total of 1,610 households were surveyed; 1,585 individuals more than five years old had blood and data collected. Sera were tested for IgM/IgG antibodies by an enzyme-linked immunoassay. Area-based indicators derived from census data were linked to geocoded residential address. The seroprevalence of dengue was 29.5% and the estimate prevalence surface reached 50% in the outskirts areas. The risk of infection was significantly associated with older age ($P < 0.01$), low education (odds ratio [OR] = 3.45, 95% confidence interval [CI] = 1.82–6.55), and low income (OR = 1.32, 95% CI = 1.02–1.71) in multivariate analysis. This study highlighted the heterogeneity of dengue transmission within the city and can assist in spatial targeting control interventions.

INTRODUCTION

Dengue is a tropical and subtropical mosquito-borne flavivirus infection with four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). All four serotypes can cause dengue fever or its severe forms known as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Individuals infected with one serotype are considered predisposed to develop DHF when subsequently infected with a different serotype.¹ In the past four decades, the incidence of dengue fever and its more severe forms have increased dramatically in the Americas.^{2–5} Several highly urbanized cities have been affected by explosive dengue fever and DHF epidemics, which demonstrate the disease as public health priority.^{6–8}

Since 1986, Brazil has experienced several epidemics of dengue, starting with DENV-1, followed by DENV-2, and the recent introduction of DENV-3 in the southeastern region of this country.^{9,10} By 2001, 413,067 cases of dengue were reported in the country, mainly in the adult population, representing 63.3% of all cases reported in the Americas.¹¹ Confirmed DHF cases increased from 0 in 1992 to 679 cases with 29 deaths in 2001. In the mid 1990s, the widespread distribution of *Aedes aegypti*, the common vector of dengue, was demonstrated in Brazil, and large-scale epidemics of dengue were reported on the Atlantic coast and Amazon and frontier regions of this country.¹²

In central Brazil, there has been a nearly 20-fold increase in the number of reported cases of dengue since the introduction of the virus (from 1,660 in 1990 to 20,552 in 2000). Reinfestation of cities with *Ae. aegypti* the common vector of dengue and urban yellow fever, has resulted in public health concern about the possible risk of yellow fever in dengue-infested areas.^{13,14} A few cases of DHF were reported in this region from 1998 to 2000 with an upward trend in the past two years. Despite the increasing circulation of dengue virus, population-based prevalence data are not available in this region. We carried out a large-scale household survey to assess the extent of previous exposure to dengue virus in a highly urbanized city in central Brazil. This report presents the spatial point pattern analysis and the assessment of indi-

vidual and area-based risk factors for the occurrence of dengue infection.

MATERIAL AND METHODS

Study design. The current study was designed as a household survey to assess the prevalence of antibody to dengue virus and to collect information at individual level associated with viral infection. We also used the available data from the year 2000 population census to estimate socioeconomic indicators and other potential risk factors related to dengue transmission, herein described as area-based indicators. These data aggregated by census tract units can reflect possible contextual exposures at the area level, and address questions about the characteristics of the area where the participant lives as risk factors. This type of multilevel design has the potential to combine individual-level and area-based indicators.¹⁵

Study site and population. This study was conducted in one of the largest urban settings in central Brazil (Goiânia, population = 1.1 million inhabitants) in February 2001. The city is an important commercial route towards inland regions and the capital of the country.¹⁶ Ecologic tourism is common in the region and sylvatic yellow fever is enzootic. The urban area is divided into nine macro-regions defined by the Data Processing Company of the Municipality of Goiânia for administrative purpose. These macro-regions aggregate the census tracts ($n = 1,066$) and vary in population size from 59,123 to 279,806 inhabitants. In Brazil, a census tract has approximately 1,000 inhabitants, which is the smallest areal unit for which demographic and socioeconomic data are tabulated.

Sampling and sample size. Approximately 1,600 individuals more than five years old were selected in this serosurvey using a three-stage sampling approach. This multistage approach was planned to be representative of all regions of the city and used a prior vector investigation performed by the Health Secretariat (January, 2001) as the first stage. The methodology for vector assessment followed the standards established by the federal agency (National Health Foundation), described elsewhere.¹⁷ Briefly, the administrative neighborhoods of the city were divided into blocks in which each building was enumerated by a unique code number. Within each block a corner was chosen and moving leftward, one in every

four houses was systematically selected for vector inspection. Approximately 40,000 houses corresponding to 25% of the estimated 160 thousands building units were inspected. The Health Secretariat made available the maps displaying the buildings location. We used this sampling frame to randomly select 4% of the houses as the second stage sampling. During the household visit, an attempt was made to list all residents more than five years old and to randomly select one individual for blood collection and interview. This household survey covered all nine macro-regions of the city.

The sample size of approximately 1,600 participants was calculated under the assumption that 50% of the prevalence for dengue infection maximizes the sample size with a $\pm 5\%$ error. As a means for accounting for the multistage sampling, the sample size was weighted by a design effect of 3.0.¹⁸ For the risk factors assessment, this sample size had 90% power to detect an adjusted odds ratio (OR) greater than 1.5 with a 95% confidence interval (CI) assuming exposure levels of 30% among non-infected individuals and a refusal rate of 25%.¹⁹

Independent variables. Individual level variables were collected during the household visit that included data on sex, age, education, yellow fever vaccination status, and self-reported and family history of dengue. The following area-based indicators were estimated using the primary data, which were aggregated by census tract unit and available from the National Census Bureau.¹⁶ 1) For income evaluation, we used the Brazilian standard poverty line established as two minimum wages. The head-of-household income was calculated as the percent of individuals earning less than two minimum wages divided by the total number of households. We defined as low-income areas those census tracts where more than 50% of the heads of household earned less than two minimum wages. 2) population density was estimated considering the census tract population by its area. A high- or low-density area was defined using the median value of the population density (15,515.4 inhabitants/km²) as the cut-off point. 3) Housing density was expressed as the average number of persons per household in a census tract. The person per household indicator was used to distinguish the census tract with crowded households taken as a cut-off point of 3.5 persons per household. 4) Households without an indoor water supply were used to identify settings likely for water storage in the houses or vicinities and therefore potential vector breeding sources. This indicator was expressed as the percent of households with an indoor water supply in a census tract. Based on the dataset distribution, we considered areas with less than 80% of the households with indoor piped water as risk areas. A linked database was constructed relating individual records to their area-based indicators. A total of 99.2% of all participants' records were successfully matched to the corresponding area-based indicators.

Serologic tests. A blood sample (10 mL) was collected in a Vacutainer® tube (Becton Dickinson, Franklin Lakes, NJ) for all participants during the household visit, processed within 12 hours, and stored at -20°C . Serum samples were screened for IgM/IgG by an enzyme-linked immunoassay (EIA-PANBIO® Indx, Inc., Baltimore, MD). The cut-off point for seropositive samples in the EIA was established as ≥ 0.5 optical density units according to the manufacturer. Each batch of samples included positive and negative control sera on each plate for quality control and ascertainment of the expected cut-off values. Borderline and negative results (≤ 0.3

optical density units) were polled for data analysis and interpreted as noninfected individuals. This commercial kit identifies antibodies for all four dengue virus serotypes. The use of this semi-quantitative assay does not permit the discrimination of primary versus secondary infection or the serotypes circulating at the population level. All serologic tests were performed at the Institute of Tropical Pathology and Public Health which is the Reference Virology Laboratory of the Federal University of Goiás.

Statistical analysis. Prevalence was defined as the number of seropositive individuals detected per 100 participants. The overall seroprevalence rate with the respective 95% CI was standardized by age group and sex according to the structure of the population census data for the year 2000.¹⁶ Seroprevalence of dengue infection was also stratified by the nine macro-regions.

We used the Generalized Linear Model, which is considered an adequate statistical methodology for multilevel study, taking into account two levels of exposures (individual and area-based information).²⁰ The model building strategy involved two steps.²¹ First, all single explanatory variables at individual and area-based levels were checked for correlation with the outcome by Pearson's R correlation coefficient. In the second step, all statistically significant predictors ($P < 0.15$) of dengue infection in the univariate analysis were included in the multivariate model by backward stepwise to control for confounders. Another model with all independent variables included was also tested using backward selection procedure. Adjusted ORs and 95% CIs were computed using the Wald statistics for unconditional maximum likelihood estimation to assess the risk of dengue infection. Statistical significance was set at 5% level. We performed the statistical analysis using SPSS software for Windows version 10.0 (SPSS, Inc., Chicago, IL).

Spatial analysis. The residential address of each participant was manually geocoded into the available digital map of the city using ArcView GIS software version 3.2 (Environmental Systems Research Institute, Inc., Redlands, CA), which generated a point pattern layer. The spatial point distribution of the seropositive individuals was statistically evaluated to obtain a smooth estimate of prevalence values applying the dual Kernel method using CrimeStat software version 2.0 (Ned Levine and Associates, National Institute of Justice, Washington, DC). The Kernel density estimation is an interpolating and smoothing technique for generalizing point location to an entire area. It consists of placing a moving three-dimensional function (the kernel) of a given radius or bandwidth that visits each event in turn, and weights the area surrounding this point location according to its distance from the center of the sphere (reference point). The sum of these individual kernels is then calculated for the study region and generates a smoothed surface of disease risk.²² In our study, a dual kernel technique was applied to compare the density estimate of seropositive individuals to the density estimate of the total participants (population at risk). This ratio (seropositive/total) provides a spatial distribution of the estimate dengue prevalence for the study area. We used the normal method of interpolation and a fixed bandwidth of 1,500 meters and a cell size of 250 meters. The results were plotted using ArcView GIS software.

Ethics. This study was conducted in accordance with human subjects protocols approved by the Regional Ethical

TABLE 1
Seroprevalence of dengue virus infection

Characteristics	No. seropositive*/ total (%)	95% confidence interval
Sex		
Female	343/1,066 (32.2)	29.4–35.1
Male	163/519 (31.4)	27.4–35.5
Age groups† (years)		
5–14	14/63 (22.2)	12.7–34.5
15–49	329/1,084 (30.4)	27.6–33.2
≥ 50	163/438 (37.2)	32.7–41.9

* Seropositive refers to IgM/IgG-positive results.

† $\chi^2 = 9.7$, degrees of freedom = 2, $P < 0.01$.

Committee of the Federal University of Goiás. All adult participants and legal guardians of minors provided informed consent.

RESULTS

A total of 1,610 households were visited during a 14-day period including weekends, and 21 individuals (1.3%) refused to participate. A total of 1,589 individuals agreed to be interviewed and had a blood sample taken; 4 samples were excluded due to inadequate specimens. Data from 1,585 participants were available for seroprevalence analysis. The overall prevalence of dengue infection was 29.5% (95% CI = 27.3–31.8%) after standardization by age group and sex. Among the survey participants, children and young adults were under-represented and females comprised two-thirds of the survey participants. Seropositivity of dengue infection was found in those as young as seven years old with an increased tendency with age. There was no difference in prevalence rate according to sex (Table 1).

Spatial point pattern analysis. Prevalence rates by macro-regions ranged from 23.6% (95% CI = 16.8–31.5%) to 41.6% (95% CI = 34.2–49.2%), with higher rates detected in the

outskirts of the city. In contrast, statistically significant lower prevalence rates (23.6–28.8%) were observed in central areas. Figure 1 shows the distribution of seropositive and seronegative participants geocoded by residential address. The prevalence density surface, generated by the spatial point analysis (dual Kernel), showed marked variation from 20% to 50% within the city. It discriminated two hot spots with an estimate prevalence of up to 50% in the southeast and the northwest areas, in contrast with lower prevalence estimate in the central areas (Figure 2).

Risk factors assessment. At individual level, there was a significant positive correlation between seroprevalence and education and personal and family history of dengue. Likewise, a positive correlation coefficient between seroprevalence and the head-of-household income (area-based indicator) was found. Yellow fever vaccination was not statistically related with dengue seropositivity (Table 2). The multivariate analysis showed that individuals more than 50 years old had a two-fold risk of being infected compared with younger age groups. Illiterate individuals had an increased risk of past dengue infection (OR = 3.45) when compared with college-educated individuals. Self-reported dengue infection (OR = 3.13) and a family history of dengue (OR = 1.71) were also associated with a higher prevalence of dengue infection. When the contextual variables were considered, head-of-household income remained statistically associated with dengue infection, even when controlled by education measured at individual level (Table 3). The affirmative response to self-reported and family history of dengue to ascertain dengue seropositivity yielded 60.0% and 51.1% of positive predictive value, respectively (Table 3).

DISCUSSION

This household survey conducted in a highly urbanized region in central Brazil showed a 29.5% prevalence of previous

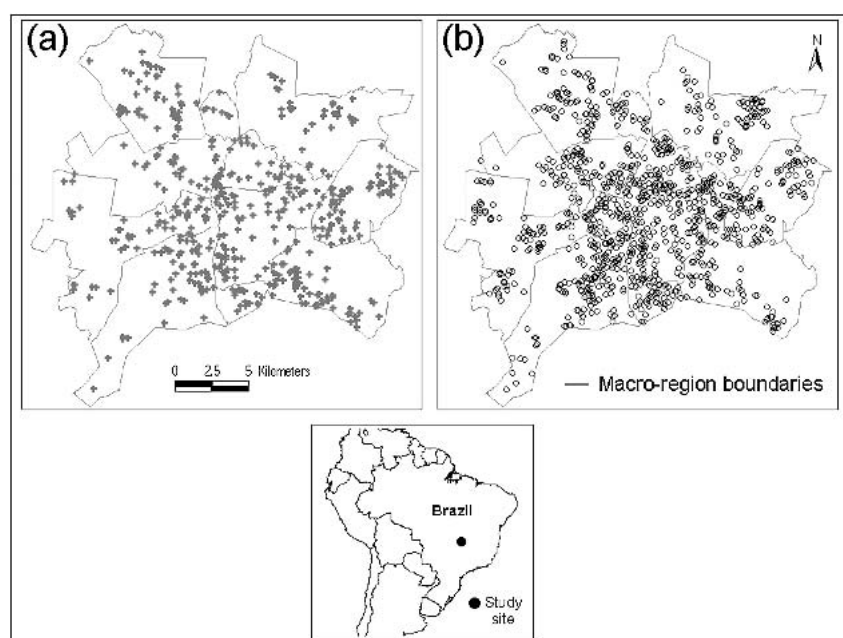


FIGURE 1. Distribution of (a) seropositive and (b) seronegative participants in the household survey of dengue infection in Goiânia, Brazil in 2001.

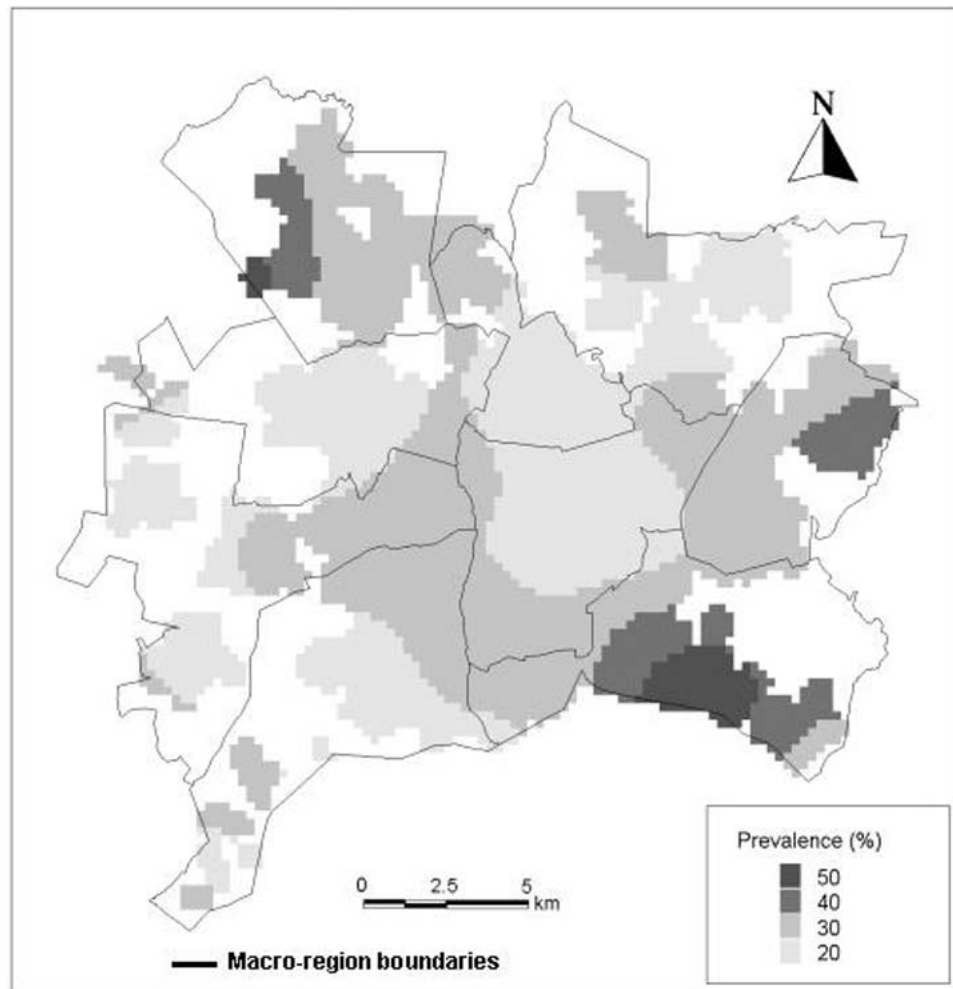


FIGURE 2. Kernel estimate of dengue seroprevalence in Goiânia, Brazil in 2001.

dengue virus exposure in the entire city. Using spatial point pattern analysis, the estimate prevalence surface peaked at 50%, which corresponded to possible clustering of infected individuals located in the outskirts of the city. The dual kernel method applied herein allows exploring the variation in the

past viral exposure within the city, taking into account both the density of the events (positive samples) and the population at risk (total samples). In the multilevel approach, the risk of acquiring dengue infection was significantly associated with lower socioeconomic status measured by education at individual-level and income at area-based level.

The hot spots identified by spatial analysis suggest heterogeneity in the transmission of dengue infection in this urban setting. The spatial clustering of infected individuals in high-risk and in non-contiguous areas discriminates areas for further investigations, as suggested by other investigators.²² Despite the association of low socioeconomic status with dengue infection, a correlation between dengue seroprevalence and crowding was not detected at the area-based level, which is different from results reported in northeastern Brazil.²³ This lack of correlation could be explained by wealthy residents of vertical buildings located in the central region of the city. In our setting, higher occurrences of other infectious diseases were previously described in areas where low socioeconomic status and lower population density overlap.²⁴ The absence of a relationship between areas with poor indoor water supply and prevalence of dengue infection is consistent with the findings of routine vector surveillance in the city, which identified disposable receptacles and not water storage containers as the

TABLE 2

Correlation coefficient according to individual and area-based potential risk factors

Variables	Correlation coefficient	P
Individual level*		
Sex	0.008	0.7
Age group (years)	0.078	0.002
Education	0.116	< 0.001
Self-reported dengue	0.186	< 0.001
History of dengue in the family	0.127	< 0.001
Yellow fever vaccine	0.004	0.9
Area-based indicators†		
Head-of-household income	0.092	< 0.001
Population density	-0.006	0.8
Housing density	0.032	0.2
Indoor piped water	0.011	0.6

* Serosurvey data.

† Indicators derived from census data.

TABLE 3

Multivariate logistic model for dengue virus infection using individual and area-based potential risk factors

Risk factor	No. seropositive*/total (%)	Adjusted odds ratio (95% CI)	P
Age group (years)			
≥ 50	163/438 (37.2)	2.11 (1.09–4.08)	0.03
15–49	329/1,084 (30.4)	1.75 (0.92–3.34)	0.09
5–14	14/63 (22.2)	1	
Education			
Illiterate	48/99 (48.5)	3.45 (1.82–6.55)	< 0.001
Elementary school	311/920 (33.8)	2.02 (1.24–3.28)	0.004
High school	117/423 (27.7)	1.62 (0.98–2.69)	0.06
College	29/133 (21.8)	1	
Self-reported dengue			
Yes	81/135 (60.0)	3.13 (2.11–4.65)	< 0.001
No	416/1,430 (29.1)	1	
History of dengue in the family			
Yes	67/131 (51.1)	1.71 (1.15–2.54)	0.008
No	417/1,386 (30.1)	1	
Head-of-household income			
< 2 minimum wages	172/448 (38.4)	1.32 (1.02–1.71)	0.03
≥ 2 minimum wages	325/1,124 (28.9)	1	

* Seropositive refers to IgM/IgG-positive results; percentages excluding missing data.

predominant breeding sites for *Ae. aegypti*. It is worthwhile mentioning that individual-level variables and census data were closely matched in time (2000–2001).

When the global seroprevalence as an indicator of the past infection was considered, approximately 29,500 infections per 100,000 persons occurred, which corresponded to approximately 340,000 residents previously infected with dengue virus in the city. This figure is markedly higher when compared with the 9,544 reported cases in the municipality, since the introduction of dengue in 1994. Data obtained in other Brazilian cities have also shown that seropositive individuals largely outnumbered the official numbers of dengue cases reported,^{25–28} even during the epidemics in Rio de Janeiro in the 1980s.^{29,30} In fact, inapparent and subclinical infection or mild disease caused by all dengue serotypes have been previously documented, which yielded undetected dengue transmission by routine surveillance.^{31–34} In our study, the higher seroprevalence among adults is in consonance with the age pattern of the reported cases from the surveillance data. Nevertheless, children had markers of dengue infection showing early virus exposure, suggesting a possible age shift in dengue occurrence, from adults to children, in the future.

In our study, women and older age groups were over-represented, which was probably due to their presence during daytime interview and perhaps their willingness to participate. It is well known that males and young age groups are frequently under-represented in serosurveys in Brazil²⁹ and in other countries³⁵ due to various cultural aspects. However, the prevalence of dengue infection was similar between males and females and increased with age, indicating cumulative virus exposure, similar to the official age distribution of reported dengue cases in Brazil.

The advantage in using a commercial EIA to perform a high volume of testing as required for dengue serologic screening is well documented.^{35,36} The EIA testing is expected to detect all serotypes (DEN-1–4) and to have similar sensitivity and specificity as the hemagglutination inhibition

test, which is considered the standard for dengue virus diagnosis.^{36,37} Routine laboratory surveillance indicated the circulation of DENV-1 as the predominant serotype and the introduction of DENV-2 in 1998, suggesting a short-term co-circulation of only two serotypes in the city. Therefore, most seropositive individuals detected by this community survey could be assumed to have primary dengue infection. In our region, high coverage for yellow fever vaccine has been maintained due to ecologic tourism and the risk of sylvatic yellow fever transmission. Although cross-reactivity in EIAs has been described in individuals who received yellow fever vaccine,³⁸ positive results in our study were not statistically different among vaccinated and unvaccinated participants, thus corroborating the described high specificity of the test.³⁹ To our knowledge, other flaviviruses with the potential to cause cross-reactivity in this immunoassay had never been locally detected.

In conclusion, our results identified target areas where behaviorally focused social mobilization and communication programs (Communication for Behavioral Impact–World Health Organization)⁴⁰ should be implemented to reduce mosquitoes breeding sites based on community involvement. Additionally, where resources allow, population-based serosurveys should be conducted as a component of a comprehensive dengue virus control surveillance.

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