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Risk factors for the incidence of dengue virus infection in preschool children

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Abstract

OBJECTIVE To estimate the seroincidence of dengue in children living in Salvador, Bahia, Brazil and to evaluate the factors associated.

METHODS A prospective serological survey was carried out in a sample of children 0–3 years of age. A multilevel logistic model was used to identify the determinants of seroincidence.

RESULTS The seroprevalence of dengue was 26.6% in the 625 children evaluated. A second survey detected an incidence of 33.2%. Multilevel logistic regression showed a statistically significant association between the seroincidence of dengue and age and the premises index.

CONCLUSION In Salvador, the dengue virus is in active circulation during early childhood; consequently, children have heterotypic antibodies and run a high risk of developing dengue haemorrhagic fever, because the sequence and intensity of the three dengue virus serotypes currently circulating in this city are very similar to those that were circulating in Rio de Janeiro, Brazil, in 2008. Therefore, the authors strongly recommend that the health authorities in cities with a similar epidemiological scenario be aware of this risk and implement improvements in health care, particularly targeting the paediatric age groups. In addition, information should be provided to the population and actions should be implemented to combat this vector.

keywords dengue, seroincidence, children, environmental factors

Introduction

Each year around 50–100 million cases of dengue occur globally; 500 000 people with severe dengue require hospitalisation and around 2.5% of these die (WHO 2012). Since the beginning of this century, Brazil is the country that has reported most cases of dengue, having contributed approximately 60% of all cases reported by WHO between 2000 and 2008 (WHO 2012).

Until 2006, the highest rates of dengue fever (DF) and dengue haemorrhagic fever (DHF) in Brazil occurred in individuals over 15 years of age (Siqueira *et al.* 2005; Teixeira *et al.* 2005), the age pattern most commonly found in the majority of countries of the Americas (Halstead 2006). On the other hand, in South-East Asia, dengue, particularly DHF, is considered a childhood disease. This discrepancy has given rise to a debate on the possible factors that may contribute to the difference in the age distribution of the same disease in different geographical locations (Halstead 2006).

In Salvador, Bahia, a city located in the north-east of Brazil, epidemics of DF, associated with circulation of the DENV-2 and DENV-1 serotypes, occurred in 1995 and 1996, respectively. In both years, the highest incidences were reported in adults (Teixeira *et al.* 2001). From 2001 onwards, with the introduction of DENV-3 into Brazil, circulation of DENV-2 and DENV-1 decreased in various Brazilian cities, including Salvador in 2002 (Melo *et al.* 2007).

In 2007, a sudden and unexplained shift was observed in the age pattern of patients with dengue in Brazil. Initially, this shift was reported in cities in the north-east (Teixeira *et al.* 2008); however, in 2008, this inversion also began to be registered in Brazil's second largest city, Rio de Janeiro in the south-east (Rio de Janeiro 2008). For Brazil as a whole, from 2007 to 2010, the hospitalisation rates for DHF in individuals over 14 years of age were lower (ranging of 0.91–2.53 per 100 000 inhabitants) than for children under 15 years of age (ranging 2.74–7.5 per 100 000 inhabitants) (Ministério da Saúde 2012).

This study aimed to measure the incidence of dengue virus infections in preschool age children living in the city of Salvador, Bahia, Brazil between 1998 and 2000 and to evaluate possible factors associated with their occurrence.

We believe that this could help us understand the factors leading to the age shift observed in DHF occurrence.

Methods

A prospective study was carried out in Salvador, Bahia between 1998 and 2000 involving children 0–3 years of age at the time of enrolment to the study. Bahia at that time had a population of around 2.3 million. The sample population of this study consisted of a single child from each household selected from a random sample of households located in 24 sentinel areas within the metropolitan area of Salvador. These areas were defined according to whether or not they were connected to the basic sanitation network and according to income level, indicators considered representative of the life conditions of the resident populations. Further details of these procedures are available in previous publications (Teixeira *et al.* 2002b; Barreto *et al.* 2007).

After submission of the study protocol to the Internal Review Board of the Oswaldo Cruz Foundation (FIOCRUZ), Bahia and its approval, signed informed consent was obtained from the parents or guardians of the children. A structured questionnaire was applied between May and July 1998 to obtain data on the sex and age of the child and his/her history of yellow fever vaccination. Whether the household was connected to the mains water supply and whether there was a regular refuse collection service was also recorded.

Blood samples were taken from the children at the time the questionnaire was applied. Two years later another blood sample was taken from those children who had tested negative for antibodies against the dengue virus in the test performed in 1998. The techniques used consisted of ELISA for the detection of IgG antibodies and modified haemagglutination inhibition (HI) assay (Shope 1963), using antigens from four serotypes of the dengue virus and another four types of flavivirus: yellow fever (YF), Rocio (ROC), Ilhéus (ILH) and Saint Louis encephalitis (SLE), although these viruses are not in circulation in Salvador. These tests were performed at the Arbovirus Laboratory of the Evandro Chagas Institute.

In May 1999, 100% of the households in 24 sentinel areas were inspected to check for the presence of usable or unusable, uncovered, water-containing recipients (potential breeding grounds of *Aedes aegypti*) inside or around the household and whether they contained the larvae of this mosquito. Based on this information, the premises index (PI) was estimated by calculating the proportion of households in which recipients with immature forms of *Aedes aegypti* were found in relation to the total number of households inspected.

The mean seroprevalence and seroincidence rates for dengue were calculated for each sentinel area and for the sample as a whole. A multilevel logistic model (Hox 1995) was used (Figure 1) to evaluate the potential risk factors for seropositivity for the dengue virus. Level 1 deals with factors that are more proximal to the individual (sex and

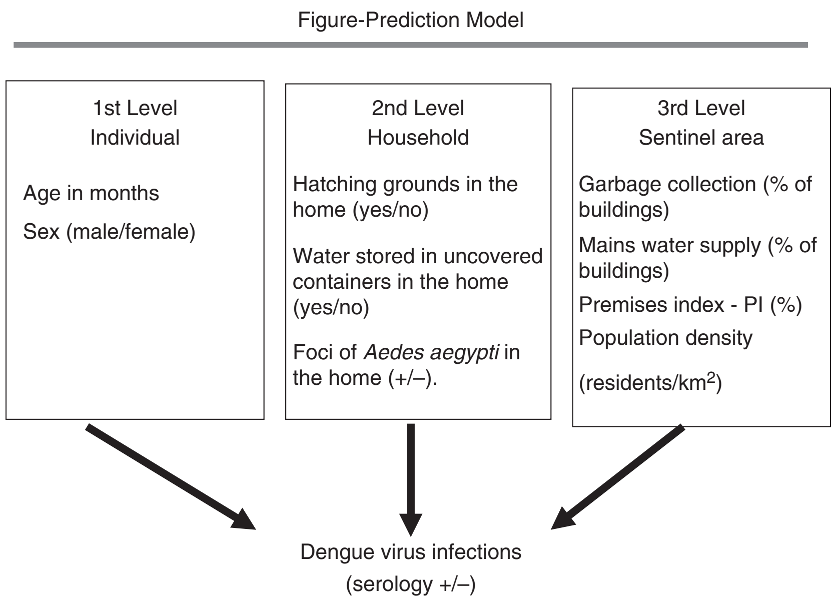

Figure 1 Prediction model.

age in months); the second level concerns factors related to the household (the presence of potential breeding grounds of *Aedes aegypti*, the presence of breeding grounds containing larvae of *Aedes aegypti* and uncovered containers used for water storage inside or around the household); level 3 was related to more distal factors concerning the sentinel area [mean (PI), mean population density, percentage of houses with garbage collection and the percentage of households connected to the mains water supply]. The selection of the best model was based mainly on theoretical reasons related to the construction of the question under investigation and according to the statistical criteria of the multilevel logistic model. The dependent variables were seroprevalence and seroincidence of the dengue virus. Odds ratios for the seroprevalence (1998) and seroincidence (1998–2000) of dengue virus infections and their respective 95% confidence intervals were calculated. The data were processed using the Epi-Info software program, version 6.0 (Dean *et al.* 1994) and analysed using the STATA software program, version 9.0 (StataCorp 2005).

Results

Around 54% of the children followed up in this study were boys; 23.9% were less than a year old, 36.1% were between one and 2 years of age and 40.0% were 2 years of age or older. None of the children had been vaccinated against yellow fever.

Of the 625 children who participated in the 1998 dengue seroprevalence survey, 26.6% tested positive. Mainly due to address changes in the families of children, there was a loss of 37% of 459 individuals who tested negative for antibodies against dengue virus in the first survey. Thus, 289 children participated in the seroincidence study conducted in 2000, when incidence of infection was 33.2% (Table 1).

Dengue virus infection seroprevalence in the sentinel areas varied from 3.2% to 53.3% with the exception of one area in which none of the children tested positive. With respect to the second survey (seroincidence), two sentinel areas were excluded because of the small number of negative children (≤2) followed up in these areas; in four sentinel areas, no negative child became positive, while in the others the seroincidence in the negatives varied from 21.4% to 63.5%. The minimum PI was 0.27% and the highest was 25.6%; however, PI was over 3% in 75% of the 24 studied areas.

Multilevel logistic regression analysis of all the variables investigated showed that for 1998 statistically significant associations with the seroprevalence of dengue were found only with respect to the variables of age (level 1 – individual) and the percentage of households connected to the mains water supply (level 3 – sentinel area). The non-conditional variance component calculated for the intercept of the simplest model (empty model) was 0.538; however, following inclusion of the second-level variables, the estimated variance, now conditional, decreased to 0.381 (Table 2). The variable concerning the presence of foci of *Aedes aegypti* in or around the home of the child failed to contribute towards explaining the seroprevalence of dengue in children.

As shown in Table 3, of the individual and ecological variables analysed only age and PI were found to be statistically associated with the seroincidence of dengue.

Table 1 Seroprevalence and seroincidence of dengue in preschoolers in Salvador, Bahia, Brazil, 1998–2000

	Seroprevalence (1998)		Seroincidence (2000)	
Serological result	*N*	%	*N*	%
Positive	166	26.56	96	33.22
Negative	459	73.44	193	66.78
Total	625	100.0	289	100.0

Table 2 Estimated odds ratios (OR) for the multilevel analysis of the association between seroprevalence of dengue in preschoolers and selected variables. Salvador, Bahia, Brazil, 1998

Variables	OR	*P*-value
Sex	1.09	0.659
Age	1.097	<0.001
% of households connected to the mains water supply	2.52	0.050
Premises index (PI)	0.98	0.583
Population density	1.00	0.184

Intercept = 0.538 (empty model); Intercept = 0.381 (conditional model); Explains 29% of the variance.

Table 3 Estimated odds ratios (OR) calculated by the multilevel analysis of the association between the seroincidence of dengue in preschoolers and selected variables. Salvador, Bahia, Brazil, 2000

Variables	OR	*P*-value
Sex	0.70	0.195
Age	1.06	0.000
% of households connected to the mains water supply	1.13	0.797
Premises index (PI)	1.08	0.007
Population density	1.00	0.976

Table 4 Odds ratios (OR) calculated by the multilevel analysis of the association between the seroincidence of dengue in preschoolers and selected variables (Final Model). Salvador, Bahia, Brazil, 2000

Variables	OR	*P*-value
Age	1.06	<0.001
Premises index (PI)	1.07	0.004

Proportion of variance: 64%.

When all other variables were removed from the model leaving only these two, the significance of the association did not change. The variables regarding the house were not found to be associated with the seroincidence and were, therefore, removed from the final model. The proportion of variance in the random component of the intercept, explained by the ecological level variables, principally the PI, was 64% with respect to seroincidence (Table 4).

Discussion

The high incidence of dengue virus infections found in this preschooler population (33%) indicates the intense circulation of this viral agent among in a period of 2 years (1998–2000). This period followed the introduction of DENV2 and then DENV1 serotypes, in the city of Salvador (Teixeira *et al.* 2001). However, circulation was less intense among preschoolers than in individuals aged 15 years or older in whom the seroincidence was almost 77% over a period of only 1 year (1998–1999) (Teixeira *et al.* 2002a,b).

Despite the fact that these surveys were performed by the end of the 1990s, the difference in the intensity of dengue virus transmission could give clues to the shift in the clinical and epidemiological patterns of dengue reported in Brazil since 2007 (Teixeira *et al.* 2008). Until 2001 in Salvador, practically no cases of DHF had been reported (Bahia 2002) despite the intense circulation of two serotypes of the dengue virus (DENV1 and DENV2). Only when DENV3 was introduced in 2002 in this city, the first cases of DHF were reported (Bahia 2002; Melo *et al.* 2007), with almost 80% of cases occurring in adults. Since that time, DENV3 has become the predominant serotype in circulation, and a simultaneous reduction occurring in the circulation of DENV2 and DENV1 was observed (Melo *et al.* 2007). Consequently, the number of individuals susceptible to these two serotypes is increasing, this population consisting of the infants born since that time and of the children who still had no antibodies against DENV2 or DENV1 at the end of the 1990s, which constitute approximately 50% of the preschool population.

Between 2003 and 2006, only DENV3 was circulating in Salvador, and the DHF hospitalisation rate was very low (ranging from 0 to 0.3 per 100 000 inhabitants). In 2007, DENV2 was again isolated in this city, and from 2008, this serotype was the predominant one. So, in 2009 and 2010, the DHF hospitalisation rates were very high in individuals under 15 years old (16.3 and 32.9 per 100 000, respectively) compared with adults (1.6 and 1.4 per 100 000, respectively) (Ministério da Saúde 2012). This situation made imperative that the health care service network, particularly the paediatric health care units, was prepared to provide emergency assistance in accordance with the standards defined for the clinical management of DHF in children to reduce the number of fatalities.

Of the environmental risk factors evaluated in this study, the PI was the only one associated with the incidence of dengue virus infections, confirming that even individuals who do not live in households in which foci of *Aedes aegypti* are found may be at risk of becoming infected if there is a high proportion of these foci in close proximity to their home. Several reports (Tun-Lin *et al.* 1995; Gomes 1998; Focks 2003) have indicated problems with the use of the PI as a predictor of the risk of dengue epidemics, because this larval indicator does not estimate the number of adult female *Aedes aegypti*, the form of the mosquito that is responsible for viral transmission. Nevertheless, it is understood that, when obtained in small spatial units such as those evaluated in the present study, the values of this indicator reflect possible situations of increased risk of dengue virus transmission. The PI has been the principal entomological indicator for programs focused on *Aedes aegypti* control, because its calculation is operationally practical and feasible on a large scale. Therefore, in view of our findings and those of others (Newton & Reiter 1992; Coelho *et al.* 2008), elevated PI should be considered a warning sign for the health authorities with respect to the risk of dengue virus transmission and should be used as an indicator of the need to intensify activities of vector control in each geographical space.

On the other hand, environmental education strategies aimed at reducing potential breeding grounds of *Aedes aegypti* must be innovative in the sense of developing mechanisms that appeal to and sensitise each and every family and community to adopt effective habits and practices to reduce the number of breeding grounds of this mosquito in each geographic space in the city. If the larvae of the *Aedes aegypti* remain in even a few households, this will be sufficient to produce the winged form of the insect that is capable of infesting neighbouring houses, putting the surrounding population at risk.

Other cities of Brazil and the Americas have similar epidemiological scenarios with respect to dengue, particularly as regards the possibility of rising infections in younger age groups. Thus, the monitoring and analysis of data generated by the surveillance system, together with data obtained in serological surveys, may be useful in predicting occurrences of severe forms related to this virus. In view of the severity of dengue in infancy, the absence of a vaccine, and new outbreaks of different serotypes of the virus in each social space in many regions of the world, it is crucial to intensify measures against *Aedes aegypti*.

Evidence seems to indicate that we will have a safe and effective vaccine against all four serotypes of the dengue virus in the near future. Serological surveys similar to the one carried out here can help define the priority populations to be vaccinated.

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References

Bahia (2002) Secretaria da Saúde. *Boletim Epidemiológico* **8**: 7.

Barreto ML, Barreto M, Genser B *et al.* (2007) Effect of city-wide sanitation programme on reduction in rate of childhood diarrhoea in northeast Brazil: assessment by two cohort studies. *Lancet* **9599**, 1622–1628.

Coelho GE, Burattini MN, Teixeira MG, Bezerra FA & Massad E (2008) Dynamics of the 2006/2007 dengue outbreak in Brazil. *Memorias do Instituto Oswaldo Cruz* **103**, 535–539.

Dean AG, Dean JA, Coulombier D *et al.* (1994) *Epi Info, Version 6: A Word Processing Database, and Statistics Program for Epidemiology on Microcomputers*. CDC, Atlanta, GA.

Focks DA (2003) *A Review of Entomological Sampling Methods and Indicators for Dengue Vectors*. WHO, Geneva.

Gomes AC (1998) Medidas dos níveis de infestação urbana para *Aedes(stegomya)aegypti* e *Aedes (stegomya) albopictus* em programa de vigilância entomológica. *Informe Epidemiol SUS* **7**, 49–57.

Halstead SB (2006) Dengue in the Americas and Southeast Asia: do they differ? *Revista Panamericana de Salud Publica* **6**, 407–415.

Hox JJ (1995) *Applied Multilevel Analysis*. TT-Publickaties, Amsterdan.

Melo PRS, Reis EAG, Ciuffo IA, Góes M, Blanton RE & Reis MG (2007) The dynamics of dengue virus serotype 3 introduction and dispersion in the state of Bahia, Brazil. *Memorias do Instituto Oswaldo Cruz* **102**, 905–912.

Ministério da Saúde (2012) Departamento de Informatica do SUS (DATASUS). http://www2.datasus.gov.br/DATASUS/index.php?area=0203. (Accessed 5 February 2012).

Newton EA & Reiter P (1995) A model of the transmission of dengue fever with evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. *American Journal of Tropical Medicine and Hygiene* **47**, 709–720.

Rio de Janeiro. Secretaria de Estado de Saúde e Defesa Civil (2008) Relatório de casos de dengue-2008. http://www.saude.rj.gov.br/Acoes/Dengue_estado.shtml. (Acessed 1º out. 2008).

Shope RE (1963) The use of micro-hemagglutination-inhibition test to follow antibody response after arthropod-borne virus infection in a community of forest animals. *Annals of Microbiology* **11**, 167–171.

Siqueira JB Jr, Martelli CT, Coelho GE, Simplício ACR & Hatch DL (2005) Dengue and dengue hemorrhagic fever, Brazil 1981–2002. *Emerging Infectious Diseases* **11**, 48–53.

StataCorp (2005) *Stata Statistical Software: Release 9*. StataCorp LP, College Station, TX.

Teixeira MG, Costa MCN, Barreto ML & Barreto FR (2001) Epidemiologia do dengue em Salvador-Bahia 1995–1999. *Revista da Sociedade Brasileira de Medicina Tropical* **34**, 269–274.

Teixeira MG, Barreto ML, Costa MCN, Ferreira LDA, Vasconcelos PFC & Cairncross S (2002a) Dynamics of dengue vírus circulation: a silent epidemic in a complex urban área. *Tropical Medicine and International Health* **7**, 757–762.

Teixeira MG, Barreto ML, Costa MCN, Strina AM, Martins D Jr & Prado M (2002b) Sentinel areas: a monitorin strategy in public health. *Cad Saúde Pública* **18**, 1189–1195.

Teixeira MG, Costa MCN, Barreto ML & Mota LE (2005) Dengue and dengue hemorrhagic fever epidemics in Brazil: what research needs are indicated by its trend, surveillance and control experiences? *Cad Saúde Pública* **21**, 1307–1315.

Teixeira MG, Costa MCN, Coelho G & Barreto ML (2008) Recent shift in age pattern of dengue hemorrhagic fever, Brazil. *Emerging Infectious Diseases* **14**, 1663–1664.

Tun-Lin W, Kay BH & Barnes A (1995) Understanding productivity, a key to *Aedes aegypti* surveillance. *American Journal of Tropical Medicine and Hygiene* **53**, 595–601.

WHO (2012) Dengue and dengue severe. Fact sheet No 117, January 2012 http://www.who.int/mediacentre/factsheets/fs117/en/index.html. (Accessed 4 April 2012).

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