Exposure to the risk of dengue virus infection in an urban setting: ecological *versus* individual heterogeneity

Maria Glória Teixeira[a✉]***, Maurício Lima Barreto***[a]***, Maria da Conceição N. Costa***[a]***, Leila Denise Alves Ferreira***[b]***, Vanessa Morato***[a]***, Pedro Fernando Vasconcelos***[c] **and *Sandy Cairncross***[d]

[a]*Instituto de Saúde Coletiva/UFBA – Rua Basílio da Gama, s/n Campus Universitário, Canela, Salvador –Bahia CEP:41.110-170*

[b]*Instituto de Matemática da Universidade Federal da Bahia*

[c]*Instituto Evandro Chagas – SVS/Ministério da Saúde do Brasil*

[d]*London School of Hygiene and Tropical Medicine*

Abstract

The dynamics of dengue virus circulation in the intra-urban spaces of large cities and the risk factors for the occurrence of such infections are still not well known. Although it has been established that poverty is one of the determinants of the majority of infectious and parasitic diseases, in the case of dengue this is still a matter of some controversy. This study had the objective of describing the distribution of dengue seroprevalence and seroincidence in different intra-urban spaces within a large and complex city in north-eastern Brazil. The study investigated whether there is any relationship between the intensity of virus circulation and the population's living conditions or between group immunity and *Aedes aegypti* infestation rates. The variability in the risk of such infections was also examined. A prospective study was conducted by means of serological investigations among a sample of people living in 30 different spaces ("sentinel areas") in the city of Salvador, which was selected according to extreme differences in living conditions. High rates of seroprevalence (67.7%) and seroincidence (70.6%) were found for the circulating serotypes (DENV-1 and DENV-2). Similar to what has been occurring in south-east Asia, the seroincidence was high (55%) even when the group immunity had already been partially established (42%) and the *Ae. aegypti* infestation rates were relatively low (<3%). Contrary to the ecological analysis, at the individual level, substantial heterogeneity in dengue exposure was observed. This paper discusses this apparent contradiction, highlighting its implications for the effectiveness of vector control strategies.

Keywords: Dengue; Prospective study; Herd immunity; Seroprevalence; Seroincidence; Spatial distribution; *Aedes aegypti*; Epidemiology.

Introduction

The dynamics and determinants of dengue virus circulation in urban areas, particularly in large cities, are not well-established. Epidemiological studies of dengue infections have been neglected,[1] even though they are important for developing dengue prevention and control strategies, as has recently been recognized in the dengue research agenda of WHO.[2]

✉*E-mail: magloria@ufba.br; Tel (71) 32837412; Fax 32837460*

The re-emergence of this disease in several continents and its potential virulence is of great concern to public health worldwide. Moreover, the available control strategies are based on vector control, and these have not always been effectively implemented to prevent transmission of the disease.[3,4] Unlike yellow fever, restrictions on human movement through quarantine are not applicable for the control of dengue for lack of vaccines.

In the large urban centres that are infested with *Aedes aegypti*, large numbers of susceptible individuals and high population density[3] probably facilitate more extensive transmission of dengue virus as mosquito vectors, because of multiple probing/feeding behaviours are likely to infect multiple individuals in different households. However, the risk of exposure to dengue virus infection in relation to the range of social and economic conditions in big cities remains unclear. These risk factors are related both to low income areas and areas with more favourable conditions.[5,6]

In Salvador, a large city in north-eastern Brazil, the dengue virus was first detected in January 1995, and, by the end of 1999, there had been two recorded epidemics. The mean annual incidence over that period reached 691 reported cases per 100 000 inhabitants.[7] We carried out a serological study of the prevalence and incidence of dengue virus infection in this city,[8] at a time when only DENV-1 and DENV-2 were circulating.[7] The present study attempts to examine the relationship between the spatial distribution of dengue virus circulation and the population's living conditions, group immunity and *Ae. aegypti* infestation rates, and also to find out any variability in the risk of such infections.

Materials and methods

This prospective study on dengue seroprevalence and seroincidence was carried out in Salvador, Bahia state, Brazil. This city had more than 2.3 million inhabitants in 1998 and presented marked differences between specific areas with regard to socioeconomic conditions and environmental sanitation. The present study considered 30 spatial units of analysis that were named "sentinel areas". These were selected by stratified sampling using data obtained from the Brazilian Institute for Geography and Statistics[9] regarding sanitation system coverage and income levels, which were taken to be estimators of living conditions, using the following strata:

(1) High: more than 80% of the homes were connected to the sanitation system and more than 50% of the families had an income of more than five minimum salaries (80 US dollars at the time of the study) (6 areas).

(2) Medium: 50% to 80% of the homes were connected to the sanitation system and more than 50% of the families had an income of between one and four minimum salaries (19 areas).

(3) Low: less than 50% of the homes were connected to the sanitation system and more than 50% of the families had an income of less than one minimum salary (5 areas).

This selection strategy has been described in more detail elsewhere.[10]

To determine the number of individuals for the serological surveys, a seroprevalence of 50% was assumed because this was the mean observed in previous surveys in Brazilian state capitals.[6,11] With this, and assuming precision of less than or equal to 3% and a confidence level of 5%, the sample size was estimated to be 1503 individuals. After adding 30% to compensate for possible losses, the result was 2149 individuals. Using the database of a demographic census carried out in 30 sentinel areas in 1997, when 68 749 individuals were counted, a random draw for the participants was made (without replacements). These individuals were then grouped in areas by taking the home address into consideration.[12]

Two surveys were carried out. The first, in 1998, is referred to here as “the seroprevalence survey”. The second, one year later in 1999, is termed “the seroincidence survey.”

After the approval of the study protocol by the Ethics Committee for Scientific Research of the Gonçalo Moniz Research Centre (Oswaldo Cruz Foundation, Bahia), a structured questionnaire was formulated during May through July 1998. The data sought in the questionnaire included: name, address, sex, age, educational level and history of vaccination against yellow fever. Just after the interview, these individuals were provided with clarifications regarding the nature of the study and they were asked to sign an informed-consent form. Following this, the first blood sample was collected. Three individuals previously vaccinated against yellow fever were excluded in order to avoid false positive serological test results due to cross-reactions. A second blood sample was collected a year later, from the individuals who were negative during the first serological survey, or had positive reactions to only one serotype of the dengue virus.

Blood samples were collected in 10 ml sterilized vacuum tubes, and the serum was separated by centrifugation and stored at –20 °C. These samples were sent in thermal boxes containing ice to the arbovirus laboratory of the Evandro Chagas Institute. There, the haemagglutination inhibition (HI) test,[13] as modified by Shope,[14] was carried out using antigens for the four serotypes of the dengue virus and four other flaviviruses (YF, Rocio, Ilhéus and St. Louis encephalitis), although these do not circulate in Salvador. There is a controversy regarding the interpretation of the serological response to flaviviruses, which differs between the first (primary) infection and any subsequent (secondary) infection with another flavivirus or serotype (flaviviruses show an increasingly strong response on subsequent infection and serological cross-reactions are frequently observed).

Thus, for the interpretation of serological response, the WHO[15] criteria was followed, i.e. HI titres of 1:20 or higher, exclusively for a specific dengue serotype, or titres four times higher for one serotype than for another (DENV-1 or DENV-2), were considered positive and specific for that serotype (primary response). Titres indicative of secondary response also followed the WHO criteria.[15] These were also confirmed by IgG enzyme-linked immunosorbent assay[16] and were considered positive to both serotypes, meaning that infection with both DENV-1 and DENV-2 had occurred.

For each sentinel area, the seroprevalence and seroincidence rates of dengue infection were estimated both unadjusted (crude) and with standardization by age using the indirect method (Rothman),[17] and the total composition of the study sample as the reference population. Since the interval between the two surveys was one year, the seroincidence was expressed as an annual rate per cent. The prevalence ratio (PR) and relative risk (RR) of dengue virus infection with 95% confidence intervals (CI) were estimated by taking as the reference standard the sentinel areas with the lowest seroprevalence (area 427) and seroincidence (area 7), respectively, among the areas in the highest socio-sanitary stratum. For the three strata according to living conditions, the respective seroprevalence and infection incidence were calculated after Rothman,[17] and the chi-squared test for trend was applied.

From the information collected using the questionnaire, the frequency indicators were estimated for each area by taking into consideration the proportions of individuals according to sex, age greater than or equal to 15 years, schooling (assuming that individuals were at risk if they were 15 or more years old and had not completed elementary schooling) and mean family income less than or equal to two minimum monthly salaries. The mean population density was obtained from the 1996 census.[9] Calculations of Pearson's correlation coefficient were used to investigate the existence of associations between the variables of interest.

In April 1999, health workers trained and supervised by the research team visited and inspected all the houses in the 30 sentinel areas. The existence of foci of *Ae. aegypti* was checked and records were made of these visits. The unit of analysis for these data was the sentinel area. Every building with one or more breeding sites containing the larvae of this mosquito was considered to be positive, and the Premises Index (PI) was estimated as the percentage of positive buildings. The infection incidence was calculated for different PI bands (less than or equal to 3%; 3.1% to 5%; 5.1% to 10%; and over 10%) using covariance analysis,[18] with adjustments for age and mean seroprevalence (herd immunity indicator). The preventable fraction was estimated by considering individuals who lived in areas with PI less than or equal to 3% to be "non-exposed".

The data were entered using Epi-Info 6.0 and analysed using SAS and STATA.

Results

Among the 1515 individuals who took part in the seroprevalence survey, 58% were female and 71% were aged 15 years or older, mainly in the age groups 15 to 29 (33%) and 30 to 49 (29%). The majority (68%) had had schooling for eight years or less. Around 25% reported that their family income was less than two minimum salaries and 50% earned from two to less than five minimum salaries. There were 595 individuals in the seroincidence survey, out of 860 who were eligible according to the criteria established, which represented a loss of 31%. The great majority of these losses were due to changes of address, and it was not appropriate to locate these losses since this was an ecological study of sentinel areas. Nonetheless, the social and demographic structure of the sample remained similar to what was found in the first survey. Among the sentinel areas, the population density varied widely, from a maximum of 49 980 to a minimum of 1834 inhabitants per km^2.

The mean seroprevalence was 69% (ranging from 16% to 98% among the sentinel areas), and this distribution was little changed after standardization for age. The prevalence ratio (PR) indicated a risk of positive findings that ranged from 0.36 in sentinel area 1011 to 2.20 in area 1054, which were both in the medium stratum of living conditions (Table 1).

As shown in Table 2, the stratum of lowest living conditions had the highest mean seroprevalence (74.0%), and this trend was statistically significant ($\chi^2 = 8.386$; $p = 0.004$). It can be seen from Table 3 that the seroprevalence presented a positive correlation ($r = 0.4914$; $p = 0.006$) with population density and a weak negative correlation ($r = -0.2778$; $p = 0.137$) with the proportion of individuals aged 15 years or over who had had less than eight years of schooling. No statistically significant association was found with mean income ($r = 0.0571$; $p = 0.764$).

The mean seroincidence was 71.0% per year. Three areas in which the sample size was less than four were not taken into consideration; in the others, the seroincidence ranged from 50% (area 323) to 90% (area 678) (Table 1). In the areas in which the seroprevalence was

Table 1: *Seroprevalence and seroincidence (crude and standardized) for dengue, study population, population density and premises index (PI) according to sentinel area and strata of living conditions in Salvador – Bahia, Brazil, 1998-1999*

Sentinel area	Stratum	Population density/km²	Seroprevalence*				Seroincidence**				PI
			Sampled population	No. positive	Crude (%)	Standardized (%)	Sampled population	No. positive	Crude (%)	Standardized (%)	
7	H	13 200	70	40	57.1	54.4	22	13	59.1	57.5	5.40
444	H	7597	50	35	70.0	66.6	11	8	72.7	73.0	1.46
427	H	27 093	27	12	44.4	42.2	11	9	81.8	80.4	5.14
571	H	3074	21	9	42.9	42.3	8	3	37.5	38.2	0.36
575	H	49 745	11	9	81.8	80.9	2	–	–	–	0.27
595	H	38 879	114	85	74.6	73.9	25	20	80.0	82.8	5.53
243	M	21 247	33	30	90.9	90.5	3	2	66.0	65.9	5.25
309	M	17 638	31	15	48.4	49.2	11	7	63.6	67.1	6.95
315	M	25 369	64	59	92.2	88.9	3	3	100.0	100.0	3.46
204	M	37 591	63	50	79.4	80.2	8	7	87.5	91.9	8.06
263	M	48 578	51	47	92.2	92.1	6	4	66.7	64.9	2.54
323	M	26 207	46	33	71.7	72.8	14	7	50.0	49.6	2.48
327	L	36 911	42	32	76.2	77.1	10	8	80.0	83.9	4.04
322	L	33 376	44	27	61.4	61.3	21	13	61.9	62.3	4.14
330	L	49 979	40	26	65.0	64.3	13	8	61.5	61.5	2.90
1.054	M	23 770	42	41	97.6	99.0	31	26	83.9	85.0	15.83
1.057	M	14 810	65	39	60.0	58.8	32	18	56.3	54.3	12.90
1.072	L	28 459	49	42	85.7	86.4	14	11	78.6	78.0	14.81
1.026	M	16 321	117	45	38.5	38.1	82	54	65.9	64.5	16.73
1.025	M	6526	63	37	58.7	61.2	42	32	76.2	76.5	4.75
1.011	M	11 953	37	6	16.2	16.4	29	18	62.1	61.8	16.06
191	M	28 734	30	25	83.3	84.2	23	18	78.3	75.8	14.40
961	M	17 749	53	41	77.4	75.9	27	19	70.4	68.8	25.63
962	M	5698	55	39	70.9	69.4	23	14	60.9	59.8	5.57
672	M	7363	56	28	50.0	53.0	31	26	83.9	86.4	4.22
677	M	26 419	55	51	92.7	91.8	11	9	81.8	83.2	4.72
678	M	1834	32	19	59.4	62.8	10	9	90.0	91.1	10.02
118	L	38 063	33	27	81.8	87.3	13	7	53.8	56.8	4.49
205	M	26 462	44	35	79.6	78.8	12	9	75.0	78.4	10.15
208	M	26 558	77	57	74.0	77.7	47	38	80.9	81.3	3.00

*: 1998; **: 1999; H: High living conditions; M: Middle living conditions; L: Low living conditions

lower, the incidence of infection indicated by a change in serological status was high, except in area 571. There was only one area (575) in which no individuals seroconverted in the second survey, although the number of initially negative individuals was only two and this area had the lowest PI (0.27%) (Table 1). The relative risk of seroincidence among the areas in the second survey ranged from 0.64 to 1.52, excluding area 575, in which there were no new cases. In addition, there was a statistically significant negative correlation between the incidence of infection and the proportion of individuals aged 15 years or over, who had not completed elementary schooling. For mean income and population density, a negative correlation was also found, but without statistical significance (Table 4).

Differing from the seroprevalence survey, the highest unadjusted or standardized seroincidence rates were in the stratum of highest living conditions (Table 2), but the chi-squared test for trend did not show statistical significance ($\chi^2 = 1.332$; $p = 0.248$).

A comparison between the incidence adjusted for age and the mean seroprevalence in the sentinel areas (herd-immunity indicator), when grouped according to the PI ranges considered (Figure), revealed that the lowest seroincidence (55%) was in the group with PI less than or equal to 3% and the highest (77%) was in the group from 3% to 5%. The differences were statistically significant at the 5% level only between the first and second PI groups ($p < 0.01$) and between the first and fourth groups ($p = 0.02$).

***Table 2:** Seroprevalence (%) and seroincidence (%) of dengue, prevalence ratio (PR), relative risk (RR) and confidence interval (CI: 95%) according to living condition strata in 30 sentinel areas of Salvador – Bahia, Brazil, 1998-1999*

Strata of living conditions	Crude (%)	Seroprevalence* (1998)			Crude (%)	Seroincidence** (1999)		
		Standardized (%)	PR	CI: 95%		Standardized (%)	RR	CI: 95%
High	64.8	68.8	1.0	—	75.0	76.6	1.0	—
Middle	68.7	69.2	1.06	0.96; 1.16	70.6	70.3	0.94	0.81; 1.09
Low	74.0	78.4	1.19	1.07; 1.33	66.2	66.7	0.88	0.71; 1.09

Test for trend: *χ^2 = 8.386, p= 0.004; ** χ^2 = 1.332, p= 0.2484

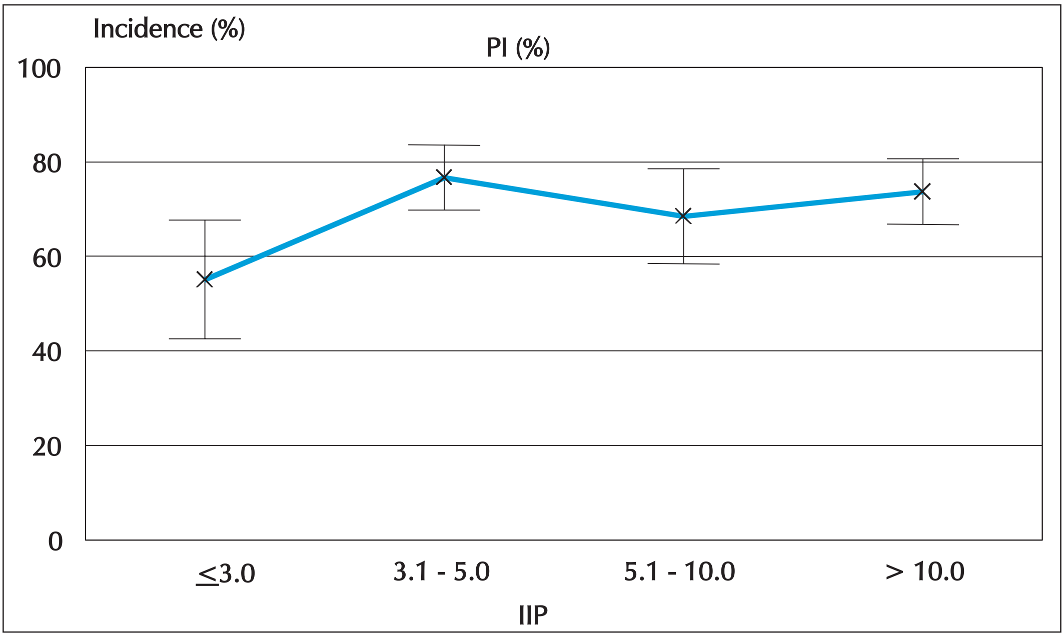
***Table 3:** Seroprevalence and seroincidence for two serotypes of dengue virus in Salvador, Brazil, 1998-1999*

Serology	No. examined	1 serotype		2 serotypes	
		No.	%	No.	%
Seroprevalence	1515	386	25.5	655	43.2
Seroincidence prior immune status					
Negative	331	77	23.3	124	37.5
Positive for one serotype	264	219	83.0	—	—
Total	595	296	49.8	124	20.8

***Table 4:** Spearman correlation coefficient (r) for association between crude and standardized seroprevalence and seroincidence for dengue virus and selected variables for residents of 30 sentinel areas in Salvador – Bahia, Brazil, 1998-1999*

Variable	Seroroprevalence (1998)				Seroincidence (1999)			
	Crude		Standardized		Crude		Standardized	
	r	p	r	p	r	p	r	p
Education (proportion ≥ 15 years old with less than middle school)	-0.26	0.17	-0.28	0.14	-0.50	0.01	-0.50	0.01
Average income	-0.04	0.85	-0.06	0.76	-0.09	0.63	-0.12	0.52
Population density (inhab/km^2)	-0.49	0.01	-0.49	0.01	-0.17	0.36	-0.15	0.43
Premises Index (PI)					0.21	0.27	0.17	0.36

Figure: ***Incidence of dengue (and 95% CI) adjusted for initial seroprevalence and mean age and compared with Premises Index (PI) for Aedes aegypti, in 30 areas of Salvador, Brazil***



In the individual analysis, it was observed that, among the individuals who in the seroprevalence survey had been negative, 38% presented a risk of being infected by the two serotypes within a period of approximately one year. Among those who had been positive for one of the serotypes in the first examination, 83% had a second infection within this period (Table 4).

Discussion

The high seroprevalence level for dengue virus infection found in this study (69%), and the subsequent high incidence level (71%), were surprising. This was particularly so given the short period of time (around four years) that had elapsed since the virus was introduced into Salvador. These findings demonstrate the force of the transmission of this virus in Salvador, given that surveys carried out in other Brazilian state capitals after similar periods of time of virus circulation revealed lower mean seroprevalence (41% to 44%).[6,19] One plausible explanation for the rates that were so high in Salvador may relate to the almost complete lack of vector control measures at the outset of virus transmission in this city. Such measures were only[7] had waned. On the other hand, in other cities[6,19] such measures had already been implemented routinely even before the introduction of the dengue virus.

It is worth emphasizing that the results from the present investigation have made it possible to estimate that, between 1995 and 1999, around two million individuals in Salvador were infected by the dengue virus. This means that the population was at a higher risk for haemorrhagic dengue, given that DENV-3 started circulating in 2002.

The association found in 1998 between dengue seroprevalence and precarious living conditions (Table 2) disappeared in the seroincidence for the subsequent year. In other words, the risk of being infected by the dengue virus became practically the same between the different economic strata of the population as viral transmission became established in Salvador. This distribution, which was relatively homogeneous between the strata, has also been observed in Fortaleza and São Luís do Maranhão. The risk in Fortaleza was even found to be slightly higher in the districts with better socioeconomic indices.[6,19] These facts strongly suggest that dengue in Brazil is a disease that affects all social classes.

Despite the statistically significant associations found between dengue seroprevalence and population density and also between seroincidence and degree of PI and schooling, it can be seen that the risk of infection was high in almost all the areas, including in the areas with good living conditions. It is likely that this dynamics is at least partially due to the fact that in Salvador high population density and PI are found both in areas with precarious living conditions and in those where economically more favoured populations live, even if some people may have been infected away from home, for instance at school or at work.

This possible similarity of exposure to the risk of being infected by the dengue virus, in different intra-urban environments, differentiates this agent from those of the great majority of infectious and parasitic diseases. In particular, it differentiates dengue from the microorganisms whose transmission routes are connected with the environment and which predominantly affect poor populations.

In contrast to the uniformity of exposure suggested by the ecological comparison described above, our results indicate substantial heterogeneity in exposure to dengue at the individual level. Since there is no evidence of variation in the susceptibility of naive individuals to dengue infection, our finding of a higher incidence of seropositivity (83%) among individuals who were initially seropositive to one serotype, is strongly indicative of heterogeneity in the degree of exposure among the individuals of the population of this city. The lack of variation in exposure between neighbourhoods, as described above, suggests that the main differences are between individual households or people.

These results suggest puzzling and apparently contradictory conclusions regarding the degree to which exposure to dengue infection varies across the population, unrelated to the observed *Ae. aegypti* density in each area. The lack of association between mosquito density and dengue seroincidence is particularly remarkable in view of the way in which differentials existing between individuals can be expected to be amplified in ecological comparisons.[20] It is hard to see how people's movement about the city could explain this phenomenon; by bringing each individual into contact with various local environments, one would expect mobility to render their exposure more homogeneous rather than the contrary.

The larval survey was carried out by the research team in 100% of the households, which is grounds for confidence in this indicator, but the use of only one measure of the vector population constitutes one of the limitations of this study. Perhaps an option to refine our results might have been to count viable pupae, or adult females, per person.[21,22] However, this was not possible, for reasons of cost and operational complexity. For these same reasons, most vector control programmes use only larval indicators in their routine monitoring. Though the larval densities vary widely during the course of a year,[23] the geographical patterns remain consistent through time.[24] So, our results have disturbing implications for the effectiveness of vector control strategies.

An approach that takes into account the lifestyles that potentially favour greater exposure to the risk of being infected by the dengue virus ought to form one of the lines of research for elucidating this question. This is because the differences may be related both to the public and the private domain, since the environment of the home and its surroundings have a decisive influence on the transmission of dengue virus.

Another important finding from this study relates to the high incidence of dengue virus infection even when the infestation indices were relatively low and group immunity had already been partially established (42%), which also expresses the transmission strength of this agent. It was found that seroincidence did not vary with PI when this was greater than 3%. This finding has strong implications for *Ae. aegypti* control programmes, since it suggests that it is necessary to intensify vector control and bring *Aedes* densities under 3%, before an observable impact can be registered. This finding is in agreement with the observation in Singapore that from the early 1990s the incidence of dengue rose considerably, even when the PI was less than 2%.[2] The lack of a safe and effective vaccine against the virus increases the need for improved strategies and technologies for vector control, and for epidemiological studies to identify changes in infection patterns, such as the locations of transmission foci, the age group of peak incidence, and falling herd immunity. The last of these was one of the most important factors in the re-establishment in recent years of intense dengue virus circulation in Singapore.[25]

It can be understood, therefore, that the results from this investigation should be considered at the time of defining dengue control policies and improving vector control measures. On the one hand, observation that the dengue virus in our environment does not respect social spaces strengthens the principle that vector control measures must always be universally applied in each territory. On the other hand, the identification of specific risk factors in the domestic domain may indicate a need for other evidence-based interventions which can help to eliminate the disease from cities such as Salvador.

Acknowledgements

The authors acknowledge the financial and technical support of the National Centre of Epidemiology, Ministry of Health, Brazil, especially its Director, Dr Jarbas Barbosa da Silva Junior.

References

[1] Kuno G. Review of the factors modulating dengue transmission. *Epidemiol Rev*. 1995; 17(2): 321-35.

[2] Farrar J, Focks D, Gubler D, Barrera R, Guzman MG, Simmons C, Kalayanarooj S, Lum L, McCall PJ, Lloyd L, Horstick O, Dayal-Drager R, Nathan MB, Kroeger A. WHO/TDR Dengue Scientific Working Group. Towards a global dengue research agenda. *Trop Med Int Health*. 2007 Jun; 12(6): 695-9.

[3] Newton EA, Reiter P. A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. *Am J Trop Med Hyg.* 1992 Dec; 47(6): 709-20.

[4] Gubler DJ. Dengue and dengue hemorragic fever: its history and resurgence as a global health problem. In: Gubler DJ, Kuno G., editors. *Dengue and dengue hemorragic fever*. New York: CAB International, 1997. p. 1-22.

[5] Rodhain F, Rosen L. Mosquito vectors and dengue virus-vector relationships. In: Gubler DJ, Kuno G. Editors. *Dengue and dengue hemorragic fever*. New York: CAB International, 1997. p. 45- 60.

[6] Vasconcelos PF, Lima JW, da Rosa AP, Timbó MJ, da Rosa ES, Lima HR, Rodrigues SG, da Rosa JF. [Dengue epidemic in Fortaleza, Ceará: randomized seroepidemiologic survey] *Rev Saude Publica*. 1998 Oct; 32(5): 447-54. Portuguese.

[7] Teixeira MG, Costa MC, Barreto ML, Barreto FR. [Epidemiology of dengue in Salvador-Bahia, 1995-1999] *Rev Soc Bras Med Trop*. 2001 May-Jun; 34(3): 269-74. Portuguese.

[8] Teixeira Mda G, Barreto ML, Costa Mda C, Ferreira LD, Vasconcelos PF, Cairncross S. Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. *Trop Med Int Health*. 2002 Sep; 7(9): 757-62.

[9] Censo Demográfico. Fundação IBGE. Rio de Janeiro, Brasil: Censo demográfico, 1991.

[10] Teixeira Md Mda G, Barreto ML, Costa Mda C, Strina A, Martins D Jr, Prado M. Sentinel areas: a monitoring strategy in public health. *Cad Saude Publica*. 2002 Sep-Oct; 18(5): 1189-95.

[11] Figueiredo LT, Cavalcante SM, Simões MC. Dengue serologic survey of schoolchildren in Rio de Janeiro, Brazil, in 1986 and 1987. *Bull Pan Am Health Organ*. 1990; 24(2): 217-25.

[12] Cochran WG, *Sampling techniques*. 3rd edition, New York: Wiley & Sons, 1977.

[13] Clarke DH, Casals J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am J Trop Med Hyg*. 1958 Sep; 7(5): 561-73.

[14] Shope RE. The use of a micro hemagglutination-inhibition test to follow antibody response after arthropod-borne virus infection in a community of forest animals. *Anais de microbiologia* (Rio de Janeiro) v. 11(parte A) 1963 Sep: 167-171.

[15] World Health Organization. *Dengue haemorrhagic fever: diagnosis, treatment, prevention and control*. 2nd edition, Geneva: WHO, 1997. p.84.

[16] Chungue E, Marché G, Plichart R, Boutin JP, Roux J. Comparison of immunoglobulin G enzyme-linked immunosorbent assay (IgG-ELISA) and haemagglutination inhibition (HI) test for the detection of dengue antibodies. Prevalence of dengue IgG-ELISA antibodies in Tahiti. *Trans R Soc Trop Med Hyg*. 1989 Sep-Oct; 83(5): 708-11.

[17] Rothman KJ. Standardization of rates. In: *Modern epidemiology*. Ed. Little Brown and Company, Boston-Toronto: Little Brown & Co,, 1986. p. 41-49.

[18] Montgomery DC. *Design and analysis of experiments*. 3rd edition. New York: Wiley, 1991.

[19] Vasconcelos PF, Lima JW, Raposo ML, Rodrigues SG, da Rosa JF, Amorim SM, da Rosa

ES, Moura CM, Fonseca N, da Rosa AP. [A seroepidemiological survey on the island of São Luis during a dengue epidemic in Maranhão] *Rev Soc Bras Med Trop*. 1999 Mar-Apr; 32(2): 171-9. Portuguese.

[20] Koopman JS, Longini IM Jr. The ecological effects of individual exposures and nonlinear disease dynamics in populations. *Am J Public Health*. 1994 May; 84(5): 836-42.

[21] Focks DA, Brenner RJ, Hayes J, Daniels E. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. *Am J Trop Med Hyg*. 2000 Jan; 62(1): 11-8.

[22] Strickman D, Kittayapong P. Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. *Am J Trop Med Hyg*. 2003 Feb; 68(2): 209-17.

[23] Secretaria Municipal de Saúde. Relatório do Plano de Erradicação do *Aedes aegypti* no Município de Salvador. Prefeitura Municipal de Salvador. Centro de Controle de Zoonozes, 1999.

[24] Morrison AC, Gray K, Getis A, Astete H, Sihuincha M, Focks D, Watts D, Stancil JD, Olson JG, Blair P, Scott TW. Temporal and geographic patterns of *Aedes aegypti* (Diptera: Culicidae) production in Iquitos, Peru. *J Med Entomol*. 2004 Nov; 41(6): 1123-42.

[25] Ooi EE, Goh KT, Gubler DJ. Dengue prevention and 35 years of vector control in Singapore. *Emerg Infect Dis*. 2006 Jun; 12(6): 887-93.