The dynamics of dengue virus serotype 3 introduction and dispersion in the state of Bahia, Brazil

Paulo Roberto Santana de Melo/*, Eliana Almeida Gomes Reis, Isolina Allen Ciuffo**, Mônica Góes, Ronald Edward Blanton***, Mitermayer Galvão dos Reis/+

Centro de Pesquisa Gonçalo Moniz-Fiocruz, R. Valdemar Falcão 121, 40295-001 Salvador, BA, Brasil *Universidade Estadual de Santa Cruz, BA, Brasil **Laboratório Central do Estado da Bahia, Salvador, BA, Brasil ***Case University, Cleveland, OH, USA

By 2002, dengue virus serotype 1 (DENV-1) and DENV-2 had circulated for more than a decade in Brazil. In 2002, the introduction of DENV-3 in the state of Bahia produced a massive epidemic and the first cases of dengue hemorrhagic fever. Based on the standardized frequency, timing and location of viral isolations by the state's Central Laboratory, DENV-3 probably entered Bahia through its capital, Salvador, and then rapidly disseminated to other cities, following the main roads. A linear regression model that included traffic flow, distance from the capital and DENV-1 circulation ($r^2 = 0.24$, $p = 0.001$) supported this hypothesis. This pattern was not seen for serotypes already in circulation and was not seen for DENV-3 in the following year. Human population density was another important factor in the intensity of viral circulation. Neither DENV-1 nor DENV-2 fit this model for 2001 or 2003. Since the vector has limited flight range and vector densities fail to correlate with intensity of viral circulation, this distribution represents the movement of infected people and to some extent mosquitoes. This pattern may mimic person-to-person spread of a new infection.

Key words: dengue - emerging diseases - surveillance - *Aedes aegypti* - public health - modeling

Dengue viruses are mosquito-borne RNA viruses of the Flaviviridae family. The four closely related serotypes (DENV-1-4) are thought to represent at least three independent introductions into human populations from sylvatic primates, the most recent (DENV-1) occurring within the last century (Wang et al. 2000). Dengue is one of the most important re-emergent infectious diseases and one of the most serious health problems affecting tropical and subtropical regions of the Americas (McBride & Bielefeldt-Ohmann 2000, Guzman & Kouri 2002). All four serotypes cause dengue fever (DF), a febrile illness characterized by severe joint and muscle pains, and in some cases, the potentially fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The wide distribution of the mosquito vector *Aedes aegypti* in the tropics and subtropics results in the exposure of approximately 2.5 billion individuals to dengue infection yearly (Mackenzie et al. 2004).

Consistent with this, dengue viruses are the most common, important *Flavivirus* causing human disease in Brazil (Figueiredo 2000). In the last 14 years, Brazil has accounted for nearly 70% of the 3,141,850 reported cases of DF in the Americas in the last five years (PAHO 2003). Some viral serotypes and the mosquito vector are now distributed throughout the country, and dengue outbreaks occur in all regions, especially the most populated areas, such as in the state of Bahia (BA). By the

end of 2001, BA had only experienced epidemics with DENV-1 and DENV-2. Both of which were actively circulating when DENV-3 was introduced in 2002 and produced the first cases of DHF in BA. In 1987, DENV-1 was isolated for the first time in BA in the city of Ipupiara, located in the center of the state (the Chapada Diamantina region) (Vasconcelos et al. 2000). A second serotype, DENV-2, was introduced in 1995 in the extreme south of the state, although its exact point of introduction was not identified. During these initial epidemics in BA, the dynamics of introduction and subsequent spread of the infection could not be followed statewide, since the necessary public health protocols and the mechanism for viral isolation were not available. These were introduced in 1995 with development of a special unit at the state of Bahia's Central Laboratory (LACEN) for DENV isolation. In this study, we used the information from viral isolations at the LACEN from 2001 to 2003 to assess the dynamics of circulation of DENV serotypes in BA before, during and after the first introduction of DENV-3.

MATERIALS AND METHODS

Study site - BA is the fifth-largest Brazilian state in territorial extension and comprises 6.64% of the total area of the country. BA has 13,323,212 inhabitants with nearly 25% concentrated in the metropolitan area close to Salvador, the state's capital. Approximately 69% of the state is considered arid or semi-arid. By contrast, the 1,183 km long coastline is generally tropical and humid. Urbanization in BA is marked by concentration of the overwhelming part of the urban population in the capital, thus making Salvador the only city in the state whose population exceeds 500,000. Nevertheless, the state is also characterized by the dispersion of the population in hundreds of smaller urban centers. In the last two de-

cades, there has been explosive growth of medium-sized urban centers (cities with more than 100,000 inhabitants). This has strengthened interurban networks and facilitated individual movements for commerce and migration. Although the majority of the state's territory is agricultural, there is a good level of infrastructure with electricity and paved roads throughout most of its area.

Sampling - For administrative and epidemiologic purposes, the state is divided into municipalities, which are the basic reporting unit for the State Secretariat of Health (SSH). Dengue is a reportable disease in Brazil. The secretary of health in each municipality is responsible for collecting serum from each suspected case of dengue within five days of onset of symptoms, and sending this on to the LACEN for viral isolation and serology. All data were obtained directly from the LACEN or online public databases of the Brazilian government in 2004. In the analysis, the simplifying assumptions were made that the only difference between municipalities was the frequency with which serum was collected from suspected cases, and errors were not systematic, but random.

Viral isolation - Viral isolation was performed by inoculation of an *Aedes albopictus* C6/36 cell line with 25 µl of undiluted acute serum. The cell line was then incubated for seven days at 32°C in Leibovitz´s medium supplemented with 5% fetal calf serum. After fixation to slides in quadruplicate with cold buffered acetone, the cells were incubated with serotype-specific monoclonal antibodies for each of the four serotypes followed by fluorescein conjugated anti-mouse antibodies. The slides were then scored as positive or negative by fluorescent microscopy (Gubler et al. 1984).

Statistical analysis - Since municipalities differed in their efficiency of submitting samples, a correction factor was calculated for each community for each month. The efficiency with which each municipality would send samples was calculated as the number of serum samples submitted for viral isolation divided by the number of cases notified for the month. The median efficiency for all municipalities was used to correct the

total number of isolates per 100,000 populations. Serum samples were to be collected within the first five days of presentation and transported to Salvador within 24 h. This was clearly not always possible, and cases often were identified after more than five days of symptoms. Availability of roads is not an obstacle to transportation of serum samples. In order to compare the numbers of DENV isolates obtained from different municipalities, the efficiency of sample submission was calculated as in the following equation:

$$\text{efficiency} = \text{frequency/median}$$

where frequency is the ratio between number of sample submitted for viral isolation and the number of dengue cases registered by the SSH, and median is the median ratio for all municipalities submitting samples. The efficiency of sample collection was then used to standardize the number of isolates per 100,000 population as calculated in the following equation:

$$\text{standardized number of isolates} = \text{efficiency X recorded number of isolates X (population X } 10^{-5})$$

For this analysis, the standardized number of isolates/ 100,000 will be a proxy for the intensity of serotype-specific viral circulation. Log transformation was used to normalize the viral circulation intensity per municipality, the number of cases of dengue, number of samples arriving at the LACEN and the number of viral isolates. Distance from Salvador was normally distributed as assessed by probability-probability plots.

Viral circulation was plotted using the program Tabwin V. 3.0.1 (http://www.datasus.gov.br/tabwin/download.htm) from the Brazilian Ministry of Health. Data on the number of notified cases, municipal population sizes and *Ae. aegypti* infestation indices were provided by the BA-SSH through the Health Communication and Information Directorate (DICS). Demographic data for the state and municipalities was obtained from the National Census Bureau (IBGE, http://www.ibge.gov.br). Traffic flow data (mean number of vehicles passing per day), and distances from Salvador were obtained from the National Department of Infrastructure and Transportation. In BA, there are six electronic counting stations installed along the most important federal highways (Table I). The data from municipalities that were directly on these routes were analyzed separately from those that were located nearby. Since no qualitative differences were noted in the results, data on traffic flow for municipalities on and near (< 20 km) the major highways was used.

Multiple linear, forward stepwise regression analysis was conducted to identify predictor variables with an inclusion criterion of p < 0.15. Traffic flow, distance from Salvador (putative site of introduction), other viral serotypes, population size and vector infestation indices (Surveillance Epidemiology Division from SSH) were used as independent variables to explain the intensity of DENV-3 circulation. The plot of the standardized residuals against the standardized predicted values showed no patterns by visual inspection, and the probability-probability plots were essentially linear for all regression analyses.

TABLE I

Dengue virus serotype 3 (DENV-3) circulation[a] in 2002 was associated with distance from Salvador, traffic flow and DENV-1 circulation in the state of Bahia

Model	Adjusted r^2	(p)	ANOVA
Distance from Salvador	0.138	0.004	0.004
Distance from Salvador, traffic flow[b]	0.202	0.032	0.002
Distance from Salvador, traffic flow, DENV-1	0.235	0.087	0.001

a: linear multiple regression on log-normalized circulation intensity for DENV-3 (standardized isolates/100,000) per municipality for 2002. Distance from Salvador, traffic flow on main highway and log-normalized circulation intensity for DENV-1 were independent variables; *b*: for communities < 50 km away from a monitored highway provided by the National Department of Infrastructure and Transportation measured as mean daily number of passing vehicles.

RESULTS

Characteristics of reporting - In 2002, the LACEN received samples from 58 of 417 municipalities in BA that also reported cases of dengue (Table II). DENV-1 or DENV-3 was isolated from 8% of the 4,777 samples received. DENV-3 isolates comprised 69% of the total. The number of samples per municipality arriving at the LACEN did not correlate with distance from Salvador ($r^2 = 0.01$), but the number of samples did correlate with the number of cases ($r^2 = 0.56$), and the number of cases correlated with the number of viral isolates ($r^2 = 0.54$). This analysis was performed with the values for Salvador removed. The inclusion of Salvador produced higher correlations (data not shown).

Temporal analyses - There was very active DENV-1 and DENV-2 transmission reflected in the number of viral isolates in January through April of 2001. This fell from a high of 403 in March to 23 by June (Fig. 1). Uneven and sporadic isolations continued through the end of the year. In January of 2002, there was a small rise in the total number of samples submitted (193), and only DENV-1 and DENV-2 were isolated early in the month. Significantly, on January 18, the first recorded isolation of DENV-3 in BA was obtained from an individual living in a suburb of Salvador. Later in the month, there was a single isolation from the municipality of Valença, 100 km south along the coast from Salvador. Isolation of all circulating serotypes continued to increase during February and March, but isolation of DENV-3 clearly reached epidemic proportions relative to the other serotypes. Viral isolations as well as dengue cases declined steeply in April 2002.

In 2003, the initial DENV-3 isolates appeared in February instead of January. Prior to obtaining a positive sample from Salvador at the end of March 2003, the virus was isolated from eight small communities (mean population 29,000 ± 26,000) each on average > 500 km from Salvador.

Spatial analyses - Samples were received for isolation from 15% (60/417 and 65/417) of municipalities for both 2001 and 2002, while cases of dengue were reported from 84% (352/417) and 94% (391/417), respectively. In 2001, DENV-1 circulated as intensely in some distant municipalities as in Salvador (Fig. 2). DENV-2 circulated with much lower intensity and no

DENV-3 was isolated. There is no indication that either virus might be distributed from a common source. Following its appearance in BA, DENV-3 circulated in many of the same municipalities as DENV-1 (Fig. 2), but Salvador is located at the center of this subsequent diffusion with the intensity of circulation generally decreasing inversely with distance from Salvador.

Following the initial isolation of DENV-3 near Salvador in January 2002, all but one of the DENV-3 isolates for the month were from Salvador as well. By February, DENV-3 was isolated from some of the most distant municipalities from Salvador (Fig. 3). The capital remained one of the most important foci throughout the rest of this quarter and remained a focus of circulation even as the epidemic waned in April. The pattern of isolations for DENV-1 and DENV-2 did not conform to this pattern in any year studied. The pattern of appearance of DENV-3 did not appear as an enlarging concentric ring around the capital, nor did it appear random within the state. An overlay of the principal highways of the state shows that these roadways extend through the municipalities from which DENV-3 was most isolated in 2002 (Fig. 4).

DENV-1 and DENV-3 in 2003 continued to circulate in generally overlapping areas, except that DENV-3 continued to be more intensely associated with Salvador than DENV-1. DENV-2 did not circulate in 2003 (data not shown), and there was no single predominant region for intensity of DENV-1 or DENV-3 circulation.

Relationship of traffic volume and distance to DENV-3 circulation - Since *Ae. aegypti*, the vector of the dengue virus, has a very short flight range, human movement is the principal method for spread of the disease by either taking infected individuals to uninfected mosquitoes or humans carrying infected mosquitoes to where there are uninfected people (McDonald 1977, Trpis & Hausermann 1986, Reiter et al. 1995, Getis et al. 2003, Huber et al. 2004, Harrington et al. 2005). If Salvador represented the initial focus and point of dissemination, the intensity of circulation in the state should be a function of distance from Salvador modified by the degree of human movement (migration and commercial) in the area. Linear regression was used to test this hypothesis. The log normalized intensity DENV-3 circulation was modeled with traffic flow along the main highway, distance from Salvador, log-normalized DENV-1 circulation intensity and DENV-2 circulation (Table III). The stepwise analysis revealed that a model that included (in order of significance) traffic flow and distance from Salvador produced an adjusted $r^2 = 0.278$ when direct traffic flow was used (p = 0.031, Table I). The model worsened with the addition of any other variable, and the r^2 was also reduced when flow estimates were assigned to municipalities near but not directly on routes with counting stations. The coefficients for traffic flow were positive, while those for distance from Salvador were negative. The inverse square of distance was also tested, but did not improve the correlation. When DENV-1 or DENV-2 was used as the dependent variable, neither showed any significant correlations with distance or traffic volume in 2001 or 2002 (Table IV). In 2003, DENV-2 did not circulate, and isolation of DENV-1 or DENV-3 did not correlate with any of the tested variables.
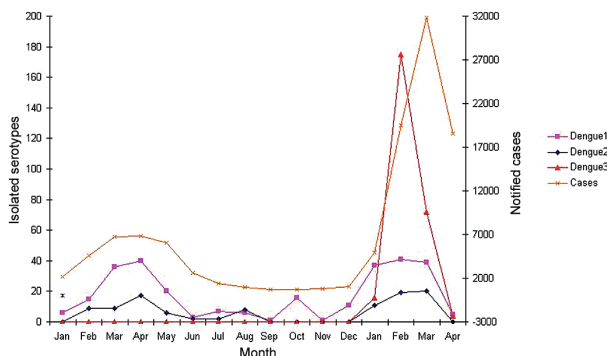

Fig. 1: monthly profile of dengue viral isolates and cases notified 2001 - 2002 in Bahia, Brazil.

TABLE II

Origin and characteristics of samples sent to Bahia's Central Laboratory (LACEN), 2002

Municipality	Population	Distance[a]	Cases[b]	Samples[c]	Isolates DENV1[d]	Isolates DENV-3[e]
Alagoinhas	132873	107	546	59	2	6
Anguera	9039	146	119	21	0	1
Antônio Cardoso	11681	139	11	3	1	0
Apuarema	7268	353	65	3	0	1
Barra do Choça	44179	537	128	38	17	0
Barreiras	120321	857	223	29	10	1
Brumado	62616	654	44	12	7	0
Cachoeira	30863	110	103	6	0	1
Caculé	20871	762	16	4	1	0
Cairu	10609	308	22	2	0	0
Camaçari	171845	41	718	15	0	2
Cândido Sales	29557	595	144	19	1	1
Capela do Alto Alegre	10795	208	3	3	1	0
Cícero Dantas	31719	302	4	1	1	0
Coaraci	27176	442	44	5	0	0
Conceição da Feira	17818	119	35	8	2	1
Coração de Maria	24214	104	748	169	1	9
Cruz das Almas	54562	146	80	36	0	3
Feira da Mata	6212	964	8	23	10	2
Feira de Santana	496625	109	3214	809	12	13
Gandu	27307	290	44	6	1	0
Ichu	4961	181	7	4	1	0
Ipiaú	43279	355	53	14	1	3
Ipirá	61551	202	69	7	1	1
Itaeté	13945	381	88	14	1	0
Itapetinga	58868	571	67	7	0	1
Ituaçu	17515	524	27	8	1	0
Ituberá	24886	308	58	24	1	0
Jaguarari	26627	398	179	1	0	1
Jequié	147713	358	591	16	1	1
Jitaúna	21103	387	15	9	0	1
Juazeiro	184204	500	148	49	1	4
Lauro de Freitas	122858	22	575	60	1	5
Macaúbas	43120	682	39	19	2	1
Miguel Calmon	28900	368	64	10	1	0
Monte Santo	55240	352	89	18	0	1
Morro do Chapéu	35068	386	43	8	1	0
Mundo Novo	19257	294	49	21	2	0
Mutuípe	20456	241	11	1	0	1
Piripá	17330	630	17	19	0	0
Poções	45555	444	5	1	0	0
Pojuca	26960	67	35	25	0	2
Prado	27311	787	37	2	0	0
Salvador	2520504	0	29309	2723	34	183
Salinas da Marg	13154	229	60	3	0	2
Santo Antônio de Jesus	80111	185	0	16	0	1
São Miguel das Matas	10169	224	11	5	0	1
São Sebastião do Passe	40620	58	247	39	2	2
Sátiro Dias	18003	205	12	5	0	0
Saúde	11509	353	7	1	0	1
Simoes Filho	98598	22	1061	55	0	3
Taperoá	15699	282	15	4	1	0
Uauá	26340	416	8	5	0	1
Ubaitaba	24441	369	28	3	1	0
Valença	79735	262	172	45	0	2
Vera Cruz	31352	289	143	20	0	3
Vitória da Conquista	270364	509	714	154	1	3
Xique-Xique	45632	577	99	91	0	0

a: distance from Salvador in km; b: cases of dengue officially reported to the State Secretariat of Health; c: samples is the number of samples sent to the LACEN for processing; d: DENV-1 isolates is the number of samples producing this serotype; e: DENV-3 isolates is the number of samples producing this serotype. There were no DENV-2 isolates in 2002.
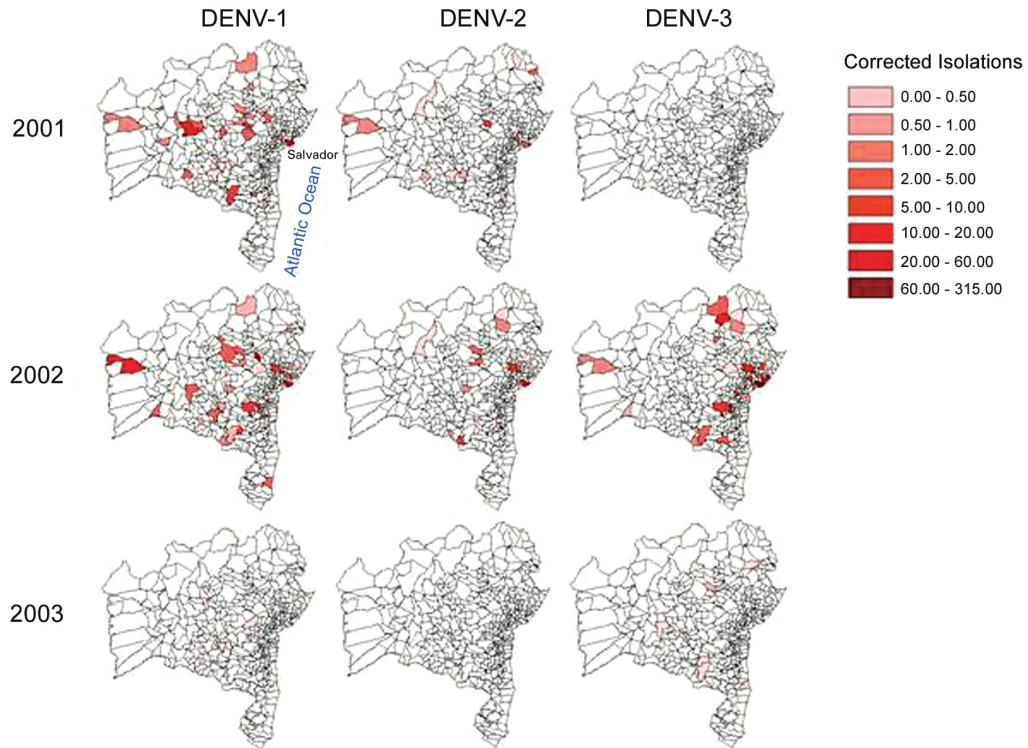
Fig. 2: comparison of yearly intensity of dengue virus serotypes circulation (DENV-1, 2 and 3) 2001 - 2003 in Bahia, Brazil. Maps of state of Bahia municipalities (www.datasus.gov.br/tabwin/tabwin.htm) by year and by serotype showing intensity of viral circulation (standardized number of viral isolations) indicated in legend.

Fig. 3: comparison of monthly intensity of dengue serotype 3 (DENV-3) circulation (standardized isolates/100,000), Jan-Apr 2002 in Bahia, Brazil.

Additional factors associated with intensity of DENV-3 circulation - Traffic volume and distance from Salvador explain approximately 27% of the variation in DENV-3 circulation intensities in 2002. To assess the contribution of other factors, both population size and municipal vector infestation indices (household and Breteau) were included in the model. The size of the population had the largest effect of any variable, and produced a model with $r^2 = 0.565$, p = 0.0003 (Table V). The variables traffic volume, distance from Salvador and log of the standardized DENV-1 circulation also entered into the model, but neither of the infestation indices contributed. The r^2 was reduced when traffic volume was removed ($r^2 = 0.431$) or the estimated traffic volume for municipalities not directly on monitored routes was used ($r^2 = 0.488$). Collinearity diagnostics showed that population and traffic volume were independent, and thus traffic volume was not merely a surrogate for population size. For this epidemic: DENV-3 circulation = 0.663 + (0.574) population size + (0.190) traffic volume + (-0.266) distance from Salvador + (0.158) $\log_{10}$ other viral circulation.

DISCUSSION

The dengue epidemic of 2002 was the most severe yet experienced BA. The first recorded cases of DHF occurred during this period. Although the epidemic spike was clearly due to the introduction of DENV-3, the stage was set by a previous decade of spreading *Ae. aegypti* infestation and DENV-1 and DENV-2 in-
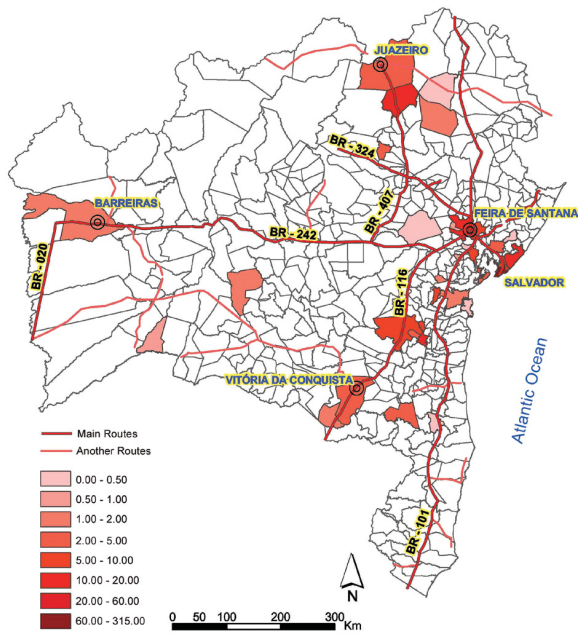
Fig. 4: main transportation routes and spatial distribution of dengue serotype 3 (DENV-3) intensity (standardized isolates/100,000) in Bahia, 2002.

fections. Thus, prior to the introduction of DENV-3, 69% of the population of Salvador was seropositive for at least one dengue virus (Teixeira et al. 2002). Viral isolation from clinical samples was instituted in the LACEN after DENV-1 and DENV-2 began to circulate, so that the 2002 epidemic was the first that could be tracked state-wide from its introduction. The institution of state-wide viral isolations was an essential tool in the epidemiology of this disease, since the registration of dengue cases alone does not allow for a reconstruction of what was occurring and how it occurred in the presence of multiple circulating serotypes.

Both time and space place the introduction of DENV-3 infection in Salvador in January 2002. For two weeks this serotype was only isolated from infected individuals in Salvador despite continued receipt of samples and isolation of other serotypes from all corners of BA. The next site for the dissemination of the virus was the transportation hub of Feira de Santana, 40 km west of the capital. In less than 30 days this virus was distributed to the rest of the state. Even after its introduction and dissemination, the capital remained the site of the most intense circulation of DENV-3, but not necessarily the other serotypes. The significant positive correlation with traf-

TABLE III

Distance and estimated traffic volume used for municipalities with DENV isolations, 2002

City	Traffic[a]	Distance[b]	City	Traffic	Distance
Alagoinhas	2357[c]	107	Jequié	6863	358
Anguera	9525	146	Jitaúna	4530	387
Antônio Cardoso	9525	139	Juazeiro	3712	500
Apuarema	7961	353	Lauro de Freitas	9525	22
Barra do Choça	6863	537	Macaúbas	3712	682
Barreiras	817	857	Miguel Calmo	-	368
Brumado	-[d]	654	Monte Santo	-	352
Cachoeira	2357	110	Morro do Chapeu	-	386
Caculé	-	762	Mundo Novo	3712	294
Cairu	2357	308	Mutuípe	2357	241
Camacan	2196	526	Piripá	-	630
Camaçari	9525	41	Poções	6863	444
Cândido Sale	6863	595	Pojuca	-	67
Capela do Alto Alegre	3712	208	Prado	-	787
Cícero Dantas	-	302	Salinas da Margarida	2357	229
Coaraci	2196	442	Salvador	9525	0
Conceição da Feira	2357	119	Santo Antônio DJ	2357	185
Coração de Maria	2357	104	São Miguel das Matas	2357	224
Cruz das Almas	2357	146	São Sebastiã	9525	58
Dom Basílio	817	709	Sátiro Dias	-	205
Feira da Mata	817	964	Saúde	3712	353
Feira de Santana	9525	109	Simoes Filho	9525	22
Gandu	2357	290	Taperoá	2357	282
Ichu	-	181	Uauá	-	416
Ipiaú	7961	355	Ubaitaba	2196	369
Ipirá	817	202	Valença	2357	262
Itaeté	-	381	Varzedo	2357	189
Itapetinga	-	571	Vera Cruz	9525	289
Ituaçu	-	524	Vitória da C	6863	509
Ituberá	2357	308	Xique-Xique	-	577
Jaguarari	3712	398			

a: mean daily volume as measured in 2001 by fixed electronic sensors at six stations (Table I); b: distance from Salvador; c: values in bold indicate municipalities directly on the monitored routes; d: municipalities too distant from monitored routes to estimate traffic flow.

TABLE IV

Distance from Salvador and traffic flow correlate only with dengue virus serotype 3 (DENV-3) circulation and only in 2002[a]

Year	DENV-1 Adjusted r^2 (p)	DENV-2 Adjusted r^2 (p)	DENV-3 Adjusted r^2 (p)
2001	0.001 (0.388)	0.038 (0.287)	(Not circulating)
2002	0.015 (0.262)	-0.038 (0.923)	0202 (0.002)
2003	-0.310 (0.949)	(Not circulating)	-0.165 (0.666)

a: linear multiple regression where the dependent variable is viral circulation (standardized isolates/100,000) per municipality and the independent variables are distance from Salvador (km) and traffic flow (mean daily number of passing vehicles on the major highway < 50 km away).

TABLE V

Linear multiple regression analysis of dengue virus serotype 3 (DENV-3) circulation in 2002

Model[a]	Adjusted r^2	P
Population[b]	0.399	$< 10^{-5}$
Population, traffic flow	0.490	$< 10^{-6}$
Population, traffic flow, distance	0.487	$< 10^{-5}$
Population, traffic flow, distance, logDENV-1[c]	0.488	$< 10^{-5}$

a: both of *Ae. aegypti* infestation indices (household and Breteau) failed to enter into the model; *b*: population size from the 2000 National Census, traffic flow (mean daily number of passing vehicles on the major highway < 50 km away) and distance from Salvador (km); *c*: log-normalized circulation intensity of DENV-1 and DENV-3 were used.

fic flow, the negative correlation with distance from Salvador, the absence of this pattern for the serotypes already present and for DENV-3 in the following year all support the hypothesis that Salvador was the center of distribution for DENV-3 during the 2002 epidemic.

While it may not be surprising that this virus would follow the major transportation routes out of the capital, this was not the pattern for the introduction and distribution of DENV-1 and DENV-2 (Vasconcelos et al. 2000). These two were introduced in smaller cities in the state and had a less clear pattern for subsequent distribution. One important factor in the more halting spread for those earlier introductions was that the distribution of *Ae. aegypti* was less extensive. From the time of first introduction of dengue in the state (1987) up to 2001, the number of municipalities reporting *Ae. aegypti* infestation in Brazil increased by 800% (Teixeira et al. 2001). Infected individuals traveling to a new area in the 1980s and 1990s were not as likely to encounter a vector as in 2002.

The low percent of municipalities sending samples for viral isolation may have produced a bias toward collections from population and transportation centers and this might have explained the pattern for isolations in 2002. However, no such pattern was observed for 2001 when there was a similar percentage of municipalities providing samples. In addition, those collecting samples had no idea of the serotype of the infecting virus, but only DENV-3 demonstrated a distribution associated with distance from Salvador and traffic flow.

Although a vector-borne disease, the pattern of spread of DENV-3 in BA in 2002 has some features in common with a point source introduction and subsequent distribution of a new pathogen with person-to-person spread. The mosquito vector, *Ae. aegypti*, has a short flight range, so that its own movements are unlikely to contribute much to the rapid distribution of this serotype of dengue (McDonald 1977, Trpis & Hausermann 1986, Reiter et al. 1995, Getis et al. 2003, Huber et al. 2004, Harrington et al. 2005). The heavy dependence of this infection on human movement and, indirectly, human contact may make the distribution of new dengue serotypes a model for the introduction of diseases that require person-to-person spread. While the movement of the human population is often cited a key to dengue transmission (Rodhain 1992, Pinheiro & Corber 1997, da Costa-Ribeiro et al. 2006), models of dengue transmission, however, often fail to include human mobility as an explanatory factor (Newton & Reiter 1992, Focks et al. 1995, 2000, Lifson 1996, Atkinson et al. 2007). Our findings further support the central role of the human population in viral dissemination.

The greatest weakness of these data is that they are secondary or relatively incomplete. Thus, the results must be viewed with caution. Nonetheless, lack of correlation between numbers of serum samples received and distance from the LACEN in Salvador as well as the correlation between number of samples received and number of cases of dengue indicate that the data are not random or a function of convenience. They likely represent the intensity of viral circulation. While distance was measured with good accuracy, traffic volume was sometimes assigned based on some distant point of measurement. Since misspecification lowers the power and significance of analyses, it is likely that more accurate measurements would produce even stronger correlations. Distance and traffic flow, however, only explained 28% of the variance. Clearly there were other factors that could influence this pattern. One of these was population size. The intensity of viral circulation was strongly dependent on density and availability of susceptible individuals. Vector density did not correlate well with intensity of viral circulation in this study.

One issue related to this being a "new" virus for this population was the active circulation of two other dengue serotypes. There is a brief period of two months following acute infection in which immunity develops to all dengue serotypes (Sabin 1952), so co-circulation of two serotypes might interfere with the spread of a third serotype. From the regression analysis, however, the co-circulation of other DENV did not lead to rela-

tive inhibition of DENV-3. DENV-1 circulation was in fact positively correlated with DENV-3 circulation. This might suggest that the factors that allowed DENV-1 to circulate successfully may have also promoted DENV-3 circulation to overcome whatever degree of cross-immunization that might have existed. On the other hand, no cases of dual infection were detected, which may suggest some interference, albeit the viral isolation method may be insensitive to dual infection.

Finally, the analysis of new introductions is very important to the study of the dynamics of viral diseases, since even one year after the introduction, understanding the pattern of dissemination becomes more complex. The level of immunity in the population must be accounted for. Thus, the opportunity to analyze this circumstance again any time soon in Brazil will only present itself with the introduction of DENV-4 or another new disease.

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