Original Report

Fatal Dengue Hemorrhagic Fever in Cuba, 1997

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ABSTRACT

Objectives: After more than 15 years without dengue activity, a dengue II epidemic was reported in Cuba in 1997. Three thousand and twelve serologically confirmed cases were reported, with 205 dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) cases and 12 fatalities. This report presents the clinical, serologic, and virologic findings in the 12 fatal DHF/DSS cases.

Methods: Serum and necropsy samples were studied by viral isolation in C636 cell line and polymerase chain reaction. Serum samples were tested by IgM capture enzyme-linked immunoassay (ELISA) and ELISA inhibition method (EIM).

Results: All 12 cases were classified as DHF/DSS according to the Pan American Health Organization Guidelines for Control and Prevention of Dengue and Dengue Hemorrhagic Fever in the Americas. All patients were older than 15 years. Women were more frequently affected. The symptoms and signs presented by these patients were similar to those previously described in DHF/DSS cases. Clinical deterioration occurred on average at day 3.75. Abdominal pain and persistent vomiting were the earliest and most frequent warning signs. Dengue infection was confirmed in all cases. IgM antibodies were detected in 11 of 12 cases, all of them with a secondary infection. Dengue II virus was detected by viral isolation in 12 samples and by polymerase chain reaction in 17. Virus or RNA was detected in various tissues, including kidney, heart, lung, and brain.

Conclusion: The clinical, pathologic, and laboratory features of 12 cases of fatal dengue hemorrhagic fever were reviewed. The results obtained demonstrate that adults with a primary dengue infection are at risk of developing the severe disease (DHF) if they are infected with a different serotype.

Key Words: *dengue, dengue hemorrhagic fever, fatal dengue hemorrhagic fever, PCR*

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After more than 15 years without dengue activity, a dengue II epidemic was reported in Cuba in 1997.[1] In 1977, a dengue fever (DF) outbreak caused by dengue I virus affected the whole country, with more than 500,000 mild cases.[2] A national serologic survey carried out in 1981 showed that 44.46% of the population had antibodies to dengue viruses compared to the 2.6% seropositivity rate before the epidemic.[3] In 1981, a second dengue epidemic was reported, with 344,203 cases; of these, 10,312 were clinically classified as dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). There were 158 fatalities (101 children).[4] Dengue virus II was the etiologic agent.

From 1982 to 1996 Cuba was free of dengue, owing to the effective campaign to control and eradicate *Aedes aegypti*. Most of the 169 Cuban municipalities have been free of the vector.

The high level of vector infestation, the increasing migration from dengue endemic areas, and the breakdown of eradication measures caused by the critical Cuban economic situation resulted in the re-introduction of dengue in the municipality of Santiago de Cuba, in the eastern part of the country. This outbreak, detected in January 1997, was controlled by August and 3012 serologically confirmed cases were reported, with 205 DHF/DSS cases (all in adults) and 12 fatalities. Secondary infection was detected in 98% of DHF/DSS cases. Dengue II virus (Jamaica genotype) was the etiologic agent.[1]

Secondary infections resulting in DHF/DSS (including fatalities) were observed for at least 16 years after the first dengue infection of this epidemic. This report presents the clinical, serologic and virologic findings observed in the 12 fatal DHF/DSS cases of the 1997 dengue II outbreak.

MATERIALS AND METHODS

Clinical Case Definition

All cases were classified according to the Guidelines for Control and Prevention of Dengue and Dengue Hemorrhagic Fever in the Americas.[5]

Specimens

Serum specimens were obtained during the acute phase of the disease. Blood samples were drawn by venipuncture, and after centrifugation, serum was removed. Samples were stored at -70°C. Necropsy samples were delivered to the reference laboratory at 4°C. A 10% suspension of the tissue specimens was prepared in minimum essential medium (MEM) containing 10% calf serum, 500 units of penicillin, and 500 μg streptomycin per milliliter.

Viral Isolation

Aedes albopictus cell line (C636/HT) was grown in MEM supplemented with 10% heated fetal bovine serum (56°C for 30 min), 1% nonessential amino acids, and 1% glutamine solution 200 mM. Sera (diluted 1 : 30) and tissue supernatants were inoculated (100 μL) onto monolayers of cells grown in 24 plastic plaque wells. Following centrifugation for 1 hour at 2500 rpm at 33°C, supernatants were discarded and 1 mL of culture medium with 2% fetal bovine serum was added. Cells were kept at 33°C and observed daily for viral cytopathic effect (CPE). Monolayers that showed no CPE were tested by indirect immunofluorescent assay (IFA), using hyperimmune ascitic fluid to dengue II. Negative cultures were passed on the same cell line twice. Virus isolates were identified by IFA, using specific monoclonal antibodies against the four dengue serotypes. (Antibodies were donated by Dr. D. Gubler from Centers for Diseases Control and Prevention, Atlanta, Georgia.)

Serologic Studies

Serum samples were tested for dengue-specific IgM antibody by IgM capture enzyme-linked immunoassay (ELISA).[6] The presence of IgM antibodies is usually considered presumptive evidence for a current dengue infection, but was classified as confirmed for this study. Serum samples also were tested serologically by ELISA inhibition method (EIM), using the method of Fernández and Vázquez.[7] Classification as primary and secondary infection followed previously established criteria.[8]

Molecular Studies

RNA was extracted from supernatants of necropsy tissue homogenates, using the previously described acid-guanidine isothiocyanate procedure, with minor modifications.[9,10] DNA amplification was done according to Lanciotti et al.[9]

RESULTS

Twelve cases of DHF/DSS were fatal, according to clinical and anatomopathologic criteria.[5] All of these patients were older than 15 years (mean = 40 y; range = 17-57 y). There were five (41.6%) males and seven (58.3%) females. Four (33.3%) were white, six (50%) were of mixed race, and two (16.6%) were black. Six of the patients were reported to have had an underlying disease (i.e., diabetes, Crohn's disease, gastritis, asthma, sicklemia, and duodenal ulcer). The disease was acute; on average, hospitalization occurred at 2.9 days of onset of fever, and worsening of the clinical picture and death occurred at 3.75 and 7.5 days, respectively.

All 12 patients developed fever, headache, persistent vomiting, malaise, bleeding manifestations, and shock. Hemoconcentration was present in 11 (91.6%) patients, and thrombocytopenia in 10 (83.3%). Ascites was observed in 11 (91.6%) cases and abdominal pain in 10 (83.3%). Hepatomegaly was detected in 8 (66.6%) patients and pleural effusion in 7 (58.3%). Coma and splenomegaly were rarely observed (2 and 3 cases, respectively).

The occurrence of hemorrhagic manifestations is characterized in Table 1. Hematemesis was observed in

Table 1. Main Hemorrhagic Manifestations in the Twelve Fatal Cases of Dengue Hemorrhagic Fever, Cuba 1997

	Patient Number											
Hemorrhagic Manifestations	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Petechiae (n = 5)		X				X	X		X			X
Hematemesis (n = 7)		X	X	X			X	X		X		X
Gum bleeding (n = 4)						X	X	X				X
Nasal bleeding (n = 4)						X		X	X	X		
Venipuncture bleeding (n = 2)					X		X					
Pulmonary bleeding (n = 4)								X	X		X*	X
Melena (n = 3)		X				X						X
Vaginal bleeding (n = 3)							X			X		X
Purpuric manifestations (n = 1)							X					
Oral bleeding (n = 3)						X		X*		X		
Ecchymosis (n = 3)				X							X	X
Hemoperitoneal bleeding (n = 2)				X							X*	
Conjunctival bleeding (n = 1)												X
Gastrointestinal bleeding (n = 1)	X*											

*Findings at necropsy.

Table 2. Clinical Manifestations of Dengue Hemorrhagic Fever/Dengue Shock Syndrome Observed in Various Epidemics

	Percentage of Clinical Manifestation in Cases of DHF/DSS				
	Cuban Epidemic			*Puerto Rico Epidemic*	
Clinical Manifestation	*1997 (%)*	*1981*[*11] (%)*	*1981 Fatal DHF*[†11] *(%)*	*1986*[5] *(%)*	*1990*[12] *(%)*
Fever	100.0	97.0	88.0	100	100.0
Vomiting	100.0	71.0	81.0	62	–
Hepatomegaly	66.6	11.0	35.0	45	10.5
Abdominal pain	83.3	23.0	58.0	–	–
Ascites	91.6	–	8.0	–	5.3
Pleural effusion	58.3	2.8	8.0	–	–
Hemoconcentration	91.6	34.0	92.0	100	100.0
Thrombocytopenia	83.3	80.3	71.8	100	100.0
Shock	100.0	0	100.0	–	14.0
Bleeding	100.0	90.0	65.0	–	100.0
Petechiae	41.6	55.0	38.0	48	45.6
Hematemesis	58.3	14.0	35.0	38	–
Melena	25.0	5.0	4.0	17	–
Vaginal bleeding	42.8	44.0	25.0	–	3.5

*Cuban adults with DHF; †Fatal DHF in Cuban adults.

seven (58.3%) cases; petechiae in five (41.6%); vaginal bleeding in three of seven females (42.8%); restlessness and hypotension in three (25%) cases; and sudden reduction in temperature, profuse perspiration, tachycardia, and faintness in two (16.6%). In 58.3% of patients, warning signs, of which abdominal pain and persistent vomiting were the earliest and most frequent, were observed on the second or third day of onset.

Table 2 shows a comparison of the main clinical manifestations observed in DHF/DSS cases during various epidemics.[5,11,12]

Dengue infection was confirmed in all cases by serologic, virologic, or molecular methods. IgM antibodies were detected in 11 (91.6%) of 12 cases, all of them with a secondary infection. The case with no IgM detected at day 4 had a titer of total immunoglobulin to dengue virus of 1:320. Table 3 shows the main virologic and molecular results. Dengue II virus was detected by viral isolation in 12 samples and by PCR in 17. Dengue II infection was confirmed in 10 cases. Virus or RNA was detected in the different tissues studied, including kidney, heart, lung, and brain. Dengue II was detected by both viral isolation and PCR in a liver sample of the patient with no detectable IgM.

Table 3. Virologic and Molecular Results on Samples from Fatal Cases of Dengue Hemorrhagic Fever

Specimens	*Viral Isolation Positive/Total (%)*	*PCR Positive/Total (%)*
Liver	5/10 (50.0)	9/10 (90.0)
Spleen	3/9 (33.3)	6/7 (85.7)
Kidney	1/3 (33.3)	2/2 (100.0)
Brain	1/1 (100.0)	–
Heart	1/3 (33.3)	0/1 –
Lung	1/2 (50.0)	0/1 –
Total	12/28 (42.8)	17/21 (80.9)

PCR = polymerase chain reaction.

DISCUSSION

Dengue is the most important arthropod-borne viral infection in man, and an estimated 50 to 100 million infections occur annually, with over 60,000 fatalities to date from DHF.[13-15] Currently, DF and DHF are emerging as important health problems in the tropics and especially in the American region, where the severe form of the disease has been recognized since 1981, when Cuba reported an extensive DHF epidemic.[2]

Excellent descriptions of the clinical spectrum of dengue virus infection and specifically DHF have been reported; however, most of these reports refer to clinical observations in children, because DHF has been observed mainly in this age group in southeast Asia.[16,17]

Dengue hemorrhagic fever in adults in epidemic form was observed for the first time during the Cuban DHF epidemic of 1981.[11] Reports of DHF in individuals older than 15 years have progressively increased, mainly in the American region; however, few detailed reports describing the clinical, laboratory, and anatomicopathologic features have been published.

During the 1997 Cuban DHF epidemic, 205 DHF cases were observed, all with serologic or virologic confirmation.[1] Of these, 12 patients died. The 12 fatal cases were classified as DHF according to the Guidelines for the Prevention and Control of Dengue and Dengue Hemorrhagic Fever in the American Region.[5] Hemoconcentration was not detected in one case and thrombocytopenia was not detected in two cases, because the rapid and fatal outcomes precluded hematology studies.

For years, DHF has been considered a childhood disease; however, in this study, the average age of patients (40 y) was different from that in previous reports.[18,19] Reports of 1975 to 1978 from areas where DHF is endemic revealed only 8 of 629 patients in Indonesia and 18 of 694 in Thailand to be older than 15 years.[18,19] By contrast, in 1988, Díaz et al described the clinical picture of the disease in Cuban adults. More recently Zagne et al reported the presence of DHF in 56 adults in Brazil.[20] Currently, DHF is observed in both children and adults in Cuba. In the particular case of Cuba, only individuals who were born before 1981 are susceptible to a secondary infection (currently documented as the most important risk factor for DHF). Frequently, DHF has been reported among young girls over 4 years of age. In this study, females were affected more frequently than males.

The symptoms and signs presented for these patients were similar to those previously described in DHF cases. In keeping with the observations made during the 1981 Cuban epidemic,[5,11,21,22] hepatomegaly, abdominal pain, ascites, hematemesis, and shock were relevant signs; however, the high incidence of ascites in this epidemic could be attributable to the introduction of abdominal ultrasound, which detects small amounts of fluid.

Clinical deterioration occurred on average on day 3.75, with a total disease course of 7 days. These data are similar to those described in some but not all reports.[11] It is important to point out that warning signs were observed in most cases at days 2 and 3; of these, abdominal pain and vomiting were the most frequently observed signs. The spectrum of these warning signs during the first 2 to 3 days of a febrile disease during a dengue epidemic should alert clinicans to the possibility of DHF so that early and adequate life-saving treatment can be applied.

Several risk factors for DHF related to the host, the vector, and the virus within an ecologic situation have been proposed; of these, sequential dengue infection is the most important.[23,24] A widely held explanatory hypothesis is that antibodies to any of the four dengue serotypes at subneutralizing concentrations, augment dengue virus infections of Fc receptor (FcR)-positive cells, such as monocytes. Dengue virions and immunoglobulin G (IgG) form virus-antibody complexes. The binding of immune complexes to FcR via the Fc portion of IgG results in augmentation of dengue virus infection. It is now known that primary dengue infections induce serotype-specific and serotype-cross-reactive CD4+ and CD8+ memory cytotoxic T lymphocytes (CTL). During secondary infections, the presence of cross-reactive nonneutralizing antibodies is thought to result in an increased number of monocytes that have been infected via dengue virus-antibody complexes. These cells activate serotype-cross-reactive CD4+ and CD8+ memory CTL, resulting in the rapid release of cytokines and chemical mediators that contribute to the plasma leakage and hemorrhage observed in DHF.[25,26] Secondary infection, the virus serotype sequence of infections, and the characteristics of the strains also have been considered as possible risk factors for DHF.

In all but one of the fatalities during the 1997 Cuban epidemic, a secondary infection was demonstrated. This finding is in agreement with previous reports from Cuba and southeast Asia where secondary infection has been observed as the main risk factor for the severe disease.[11,21,23,24,27-29] In the current study, the sequence of infection was dengue I (in the 1977 dengue I epidemic) and dengue II (in the 1997 dengue II epidemic). This sequence of infection (dengue I/dengue II) is probably one of those most frequently associated with cases and epidemics of DHF.[23] Finally, both dengue II Cuban strains, one isolated during the 1981 DHF epidemic and the other isolated in the Santiago de Cuba outbreak (1997) have an Asian origin, demonstrated by genomic sequences. The first one (1981) is close to old dengue II strains, such as New Guinea C and 16681 strains, and the second (1997) is close to more recent strains, such as Jamaica 1982 and some related Thailand strains.[30-32]

It was surprising how, after more than 17 years from the first infection, enhancing antibodies could still produce the immunoenhancement phenomenon. This may be a unique and not previously reported observation.

Bravo et al have suggested that the attack rate of DHF may be higher among whites than among other races.[24] They also reported that some chronic diseases, such as asthma, diabetes, and sicklemia, were risk factors for DHF. Similar to the findings observed during the 1981 Cuban DHF epidemic, DHF again appeared to occur at a higher rate among whites. The ethnic population distribution of Santiago de Cuba is 30.1% whites, 26.8% mixed race, 42.8% blacks, and 0.27% Asian; 33.3% of the fatal cases were among whites and only 16.6% among blacks, one of these was sicklemic (another DHF risk factor).

Despite the fact that death occurred after defervescence and, thus, during the convalescent phase, dengue II virus was detected by PCR and viral isolation in 10 cases. In the literature, rates of isolation of dengue virus from tissues of fatal cases are low, possibly owing to the presence of virus-neutralizing activity and the insensitivity of the viral isolation systems.[33] The use of the C636 HT cell line and PCR may have contributed to the high isolation rate obtained in the present study. Liver is the tissue that most often yields virus isolation, although a few isolations have been obtained from other tissues.[33] In the present study, viral antigen was detected in liver, spleen, brain, heart muscle, kidneys, and lung. In several cases, dengue II virus was recovered from tissues, suggesting that viral replication was occurring.

It has been suggested that the central factor that determines disease severity is the number of infected cells. The larger the number of infected cells, the more severe the illness. More specifically, DHF differs from DF because

there are more dengue virus-infected cells. Although the number of infected cells in the tissues of these patients is unknown, the presence of dengue II virus in different tissues in the same patient suggests widespread viral replication with consequent tissue injury.

Similar to other reports, the most frequent gross anatomic findings at autopsy were petechial hemorrhages and an increase in extravascular fluid represented by effusions in serous cavities. Microscopically, generalized vascular damage was the most important histopathologic lesion (data not shown).

Currently, DHF is emerging as an important health problem in the world and specifically in the American region. Annually, a high number of cases is reported even in countries without any antecedent reports of the disease. The complete study of the Cuban DHF epidemics will allow researchers to learn about the epidemiology, clinical manifestations, and etiopathogenesis of this severe disease.

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REFERENCES

1. Kourí G, Guzmán MG, Valdés L, et al. Reemergence of dengue in Cuba: a 1997 epidemic in Santiago de Cuba. Emerg Infect Dis 1998; 4:89-92.
2. Kourí G, Guzmán MG, Bravo J, Triana C. Dengue haemorrhagic fever/dengue shock syndrome: lessons from the Cuban epidemic, 1981. Bull WHO 1989; 67:375-380.
3. Cantelar N, Fernández A, Albert L, Pérez E. Circulación de dengue en Cuba 1978-1979. Rev Cubana Med Trop 1981; 33:72-78.
4. Kourí G, Más P, Guzmán MG, Soler M, Goyenechea A, Morier L. Dengue hemorrhagic fever in Cuba, 1981: rapid diagnosis of the etiologic agent. Bull Pan Am Health Organ 1983; 17:126-132.
5. Pan American Health Organization. Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control. Washington, DC: PAHO, 1994. (Scientific Publication 548).
6. Vázquez S, Saenz E, Huelva G, González A, Kourí G, Guzmán MG. Detección de IgM contra el virus del dengue en sangre entera absorbida en papel de filtro. Rev Panam Salud Publica 1998; 3:174-178.
7. Fernández R, Vázquez S. Serological diagnosis of dengue by an ELISA inhibition method (EIM). Mem Inst Oswaldo Cruz 1990; 85:345-351.
8. Vázquez S, Bravo J, Pérez AB, Guzmán MG. ELISA de inhibición. Una alternativa en el estudio serológico de los casos de dengue. Bol Epidem OPS 1997; 18:7-8.
9. Lanciotti RS, Calisher CH, Gubler DG, Chang G, Vordam V. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol 1992; 30:545-551.
10. Rosario D, Alvarez M, Díaz J, Contreras R, Rodriguez R, Vázquez S, Guzmán MG. Reacción en cadena de la polimerasa para la rápida detección y determinación del serotipo de virus de dengue en muestras clínicas. Rev Panam Salud Publica 1998; 4:1-5.
11. Díaz, A, Kourí G, Guzmán MG, et al. Description of the clinical picture of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) in adults. Bull Pan Am Health Organ 1988; 22:133-144.
12. Rigau JG, Puerto Rico Association of Epidemiologists. Clinical manifestations of dengue hemorrhagic fever in Puerto Rico, 1990-1991. Rev Panam Salud Publica 1997; 1:381-388.
13. Monath TP. Yellow fever and dengue: the interactions of virus, vector and host in the re-emergence of epidemic disease. Semin Virology 1994; 5:133-145.
14. Gubler D, Clark G. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerg Infect Dis 1995; 1:55-57.
15. Gubler DJ. Population growth urbanization, automobiles, and airplanes: the dengue connection. In: Greenwood B, deCock K, eds. New and resurgent infections: prediction, detection, and management of tomorrow's epidemics. New York: Wiley & Sons, 1998.
16. Nimmannitya S. Management of dengue and dengue haemorrhagic fever. Monograph on dengue/dengue haemorrhagic fever. Regional publication SEARO No. 22. New Delhi: WHO Regional Office for South-East Asia, 1993:48-54.
17. Jatanasen S, Thongcharoen P. Dengue haemorrhagic fever in south-east Asian countries. Monograph on dengue/dengue haemorrhagic fever. Regional publication SEARO No. 22. New Delhi: WHO Regional Office for South-East Asia, 1993: 23-30.
18. Sumarmo, Wulur H, Jahja E, Gubler DJ, Suharyono W, Sorensen K. Clinical observations on virologically confirmed fatal dengue infections in Jakarta, Indonesia. Bull WHO 1983; 61:693-701.
19. Nimmannitya S. Clinical spectrum and management of dengue haemorrhagic fever. Southeast Asian J Trop Med Public Health 1987; 18:392-397.
20. Zagne SMO, Alves VGF, Nogueira RMR, Miagostovich MP, Lampe E, Tavares W. Dengue haemorrhagic fever in the state of Rio de Janeiro, Brazil: a study of 56 confirmed cases. Trans R Soc Trop Med Hyg 1994; 88:677-679.
21. Guzmán MG, Kourí G, Martínez E, et al. Clinical and serologic study of Cuban children with dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Bull Pan Am Health Organ 1987; 21:270-279.
22. Guzmán MG, Kourí G, Morier L, Soler M, Fernandez A. A study of fatal hemorrhagic dengue cases in Cuba, 1981. Bull Pan Am Health Organ 1984; 18:213-220.
23. Halstead SB. Pathogenesis of dengue. Challenges to molecular biology. Science 1988; 239:475-481.
24. Bravo J, Guzmán MG, Kourí G. Why dengue haemorrhagic fever in Cuba? I. Individual risk factors for dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) in adults. Trans R Soc Trop Med Hyg 1987; 81:816-820.
25. Kurane I, Ennis FA. Immunity and immunopathology in dengue virus infections. Semin Immunol 1992; 2:121-127.
26. Kurane I, Ennis FA. Cytokines in dengue virus infections: role of cytokines in the pathogenesis of dengue hemorrhagic fever. Semin Virology 1994; 5:443-448.
27. Halstead SB. Dengue viruses. In: Gorbach SL, Bartlett JG, Blacklow NR, eds. Infectious diseases. Philadelphia: WB Saunders, 1992:1830-1835.
28. Guzmán MG, Kourí G, Bravo J, Soler M, Martínez E. Sequential infection as risk factor for dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) during the 1981 dengue hemorrhagic Cuban epidemic. Mem Inst Oswaldo Cruz 1991; 86:367.

29. Guzmán MG, Kourí G, Bravo J, Soler M, Vazquez S, Morier L. Dengue hemorrhagic fever in Cuba, 1981: a retrospective seroepidemiologic study. Am J Trop Med Hyg 1990; 42:179–184.
30. Chungue E, Cassar O, Drouet MT, et al. Molecular epidemiology of dengue 1 and dengue 4 viruses. J Gen Virol 1995; 76:1877–1884.
31. Guzmán MG, Deubel V, Pelegrino JL, et al. Partial nucleotide and amino acid sequences of the envelope and the envelope/nonstructural protein-1 gene junction of four dengue-2 virus strains isolated during the 1981 Cuban epidemic. Am J Trop Med Hyg 1995; 52:241–246.
32. Rico-Hesse R, Harrison LM, Salas RA, et al. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology 1997; 230:244–251.
33. Innis BL. Dengue and dengue hemorrhagic fever. Exotic viral infections. In: Porterfield JS, ed. London: Chapman & Hall, 1995:103–140.