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HAITI: ABSENCE OF DENGUE HEMORRHAGIC FEVER DESPITE HYPERENDEMIC DENGUE VIRUS TRANSMISSION

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Abstract. In 1994–1996, 185 strains of dengue (DEN) virus types 1, 2, and 4 were recovered from febrile United States and other United Nations military personnel in Haiti. We wondered whether risk factors for dengue hemorrhagic fever (DHF) existed and, if so, were DHF cases occurring among Haitian children. Dengue transmission rates were studied in 210 school children (6–13 years old) resident in Carrefour Borough, Port-au-Prince, Haiti. When sera were tested for plaque-reduction neutralizing antibodies to DEN 1–4 viruses, nearly 85% had antibodies to two or more DEN serotypes. The annual transmission rate was estimated at 30%, a rate observed in countries endemic for DHF. Haitian DEN 2 isolates were genotype I, which are repeatedly associated with DHF cases in Southeast Asia and American regions. Despite positive virologic pre-conditions, DHF cases were not recorded by experienced Port-au-Prince pediatricians. These observations, which are reminiscent of those in Africa, provide further evidence of a dengue resistance gene in black populations.

INTRODUCTION

In 1994, United States military personnel were assigned to supervise the transition from military to civilian government in the Republic of Haiti. The personnel assigned to duty in the city of Port-au-Prince contracted dengue viral infections almost immediately after arrival with the first cases reported seven days later.[1,2] During subsequent garrison duty, United States and other United Nations (UN) personnel continued to acquire dengue infections and large numbers of dengue (DEN) types 1, 2, and 4 viral strains were recovered.[2–6] Despite high attack rates of dengue fever among UN personnel, neither dengue fever nor dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) outbreaks were reported among Haitian civilians during that time. In fact, little dengue activity has been reported from Haiti. Only during 1969–1971 was the transmission of DEN-2 and -3 viruses reported[7] when viruses were isolated from dengue fever-like cases found largely among non-indigenous residents. Since then, neither sporadic cases nor outbreaks of DHF/DSS have been reported in Haiti.

Cases of DHF first occurred in the American region in 1981 when Southeast Asian genotype dengue (DEN) 2 virus strains were recovered from Cuban patients.[8] These or closely related DEN-2 strains spread widely throughout the Caribbean and most Central American countries, and the northern portions of South America.[9] In each of these sites, Southeast Asian genotype of DEN-2 viruses have been associated with sporadic cases or outbreaks of DHF/DSS.[10] During the 1981 epidemic in Cuba, blacks were hospitalized with DHF/DSS at lower rates than whites.[11] This observation was not an artifact of differential infection rates or preferential hospitalization, as was documented in a retrospective seroepidemiologic study in Havana.[12] Blacks and whites resident in the Cerro District were infected at identical rates with DEN-1 viruses during the 1977–1978 transmission period and again at similar rates with DEN-2 viruses during 1981. In the studied population, severe dengue disease was observed less frequently in dengue-infected black persons than whites.[12] Less hospitalization of blacks than whites was again observed during the epidemic of DHF/DSS in Santiago de Cuba in 1997.[13] From these observations, it was surmised that a human dengue resistance gene existed, which was more prevalent among blacks than whites. These observations led us to speculate that a dengue resistance gene might be prevalent among the children of Haiti, depressing the severity of dengue infections and explaining the apparent absence of the DHF/DSS syndrome. However, we did not know whether or not multiple DEN viruses were highly endemic in the indigenous population. Here we publish the first data documenting the hyperendemic transmission of multiple DEN serotypes, including DEN-2 of the Southeast Asian genotype in a population of Haitian children and the apparent absence of DHF/DSS in Haiti.

MATERIALS AND METHODS

Study site. The Republic of Haiti, with an area of 10,714 square miles and a population of 7,180,000, is located on the western part of the island of Hispaniola (Figure 1). It is sited 565 miles south of the United States mainland. About 95% of Haiti's population is black; mulattoes comprise most of the remainder and whites make up a very small percentage. Approximately 42% of the population is less than 16 years of age. The country has a high infant mortality rate with a life expectancy of 57 years. Haiti has a warm, humid tropical climate with average daily temperatures ranging from 24°C in January and February to 28°C in July and August. There are two rainy seasons, lasting from April to June and from August to October. The capital, Port-au-Prince, with a population of approximately two million, is located on the eastern end of the Gulf of Gonave. Most inhabitants live in high-density neighborhoods with homes lacking in screening. No systematic mosquito control programs are in place.

Collection of serum samples from Haitian children. In June 1996, a national dengue reference laboratory at Hospital Ste. Croix participated in a disease surveillance program. The serosurvey was performed on the population of

Carrefour borough of Port-au-Prince, Haiti. Finger-tip blood samples were collected into capillary tubes from all 210 children in grades 1–4 (6–13 years of age) attending the Lekol Basil Moreau School, Bizoton. The school is located in a densely populated area approximately 4 km southwest of downtown Port-au-Prince on a terrain sloping down to the Gulf that is less than 1 km away. Informed consent for this study was obtained from school administrators and parents to obtain age and sex data, and blood samples from the children. Except for informed consent, this study was exempted from a human use protocol because it was a public health survey involving serological testing and data analysis that were not linked to the children's names. Serum samples without any identifying information except age were sent to the Naval Medical Research Center Detachment (Lima, Peru) in August 1996 for serologic testing against all four DEN virus serotypes.

Virus recovery attempts from US and UN military personnel. The studies on US and UN personnel that resulted in the recovery of 161 DEN strains were described previously.[2,3] An additional 24 strains were recovered subsequently (Kanesa-Thasan N, unpublished data). Dengue viruses were isolated in C6/36 cells incubated for 14 days at 27°C.[14] Dengue virus serotypes were determined by an indirect fluorescent antibody (FA) test using serotype-specific monoclonal antibodies.[15] The following dengue viral strains, passaged 2–3 times in C6/36 cells, were sent to the Laboratory of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention (Fort Collins, CO) for partial sequencing and phylogenetic analysis: DEN-1 (H-059), DEN-2 (H-103, 120), and DEN-4 (H-119).

Dengue virus sequencing and genotyping. Nucleotide sequencing of the envelope (E) gene from position 639 to 1,233 was obtained for two DEN-2 isolates. This genomic region was amplified directly by reverse transcriptase-polymerase chain reaction from extracted viral RNA and sequenced by the dye-terminator method (Applied Biosystems, Foster City, CA).[16] The nucleotide sequence was resolved by a 377 automated DNA sequencer (Applied Biosystems). The sequences, combined with the E-gene database of other DEN viruses,[16,17] were phylogenetically analyzed by genetic distance of discrete character-state methods.[18]

Plaque-reduction neutralization tests. Dengue plaque-reduction neutralizing test (PRNT) antibodies were measured according to the method of Sangkawibha and others.[19] Briefly, plasmas were diluted to 1:30 in 24-well Falcon plastic trays (Apple Scientific Inc., Chesterland, OH), then mixed with equal volumes of dengue viruses to achieve a final plasma concentration of 1:60 and a plaque count of approximately 15. Plasma-virus mixtures in three replicate wells were incubated for 1 hr at 37° C. Next, BHK-21 clone 15 cells were added, incubated for 2 hr at 37°C, and carboxymethyl-cellulose (CMC) in maintenance medium was added as the first overlay. Plates were incubated at 37°C in CO_2 for five days (DEN-2) or seven days (DEN-1, -3, and -4 viruses). The CMC was removed, the cells were washed in tap water, stained with naphthol blue-black, rinsed again, and plaques were read. The following dengue strains were used: DEN-1 (Hawaii), DEN-2 (New Guinea C), DEN-3 (H-87), and DEN-4 (H-241). Seventy percent or more plaque reduction was scored as neutralization.

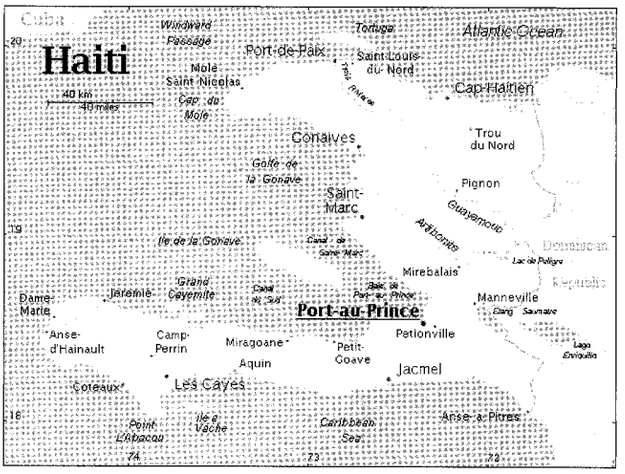

FIGURE 1. Map of Haiti, showing Port-au-Prince.

RESULTS

Between November 1994 and March 1996, acute-phase sera were received from 517 suspected dengue fever patients from the UN Mission in Haiti who were seen at the US Army's 86th Combat Support Hospital or the 131st Field Hospital. From these sera, 185 dengue viruses were recovered and typed by FA as DEN-1 = 65, DEN-2 = 73, and DEN-4 = 47.[2,3]

One strain each of DEN-1 and DEN-4 were genotyped. The DEN-1 strain belongs to genotype II, a group that also contains DEN-1 Jamaica 77 and viruses from Southeast Asia and Angola.[9,20] The DEN-4 strain belongs to genotype II, a group that contains Brazil 1982, Mexico 1984, Tahiti 1985, and Indonesia 1976 and 1977.[21] Both DEN-2 strains belong to genotype I, which is closely related to Brazil 1990 and Jamaica 1982.[16] This genotype also contains prototype DEN-2 New Guinea C and numerous Southeast Asian strains.[16]

Neutralization test data are summarized in Table 1 and

TABLE 1

Prevalence of dengue neutralizing antibodies in 210 school children, ages 6–13 years, resident in Port au Prince, Haiti, 1996

	Age (years)								
Dengue infection	6	7	8	9	10	11	12	13	Total
Negative	1		2		1				4
Dengue 1	1		2						4
Dengue 2		1	3	2	1	1			7
Dengue 3	2	1	1	1					5
Dengue 4	1	6	3	1					11
D1 + 2	1	3		3					7
D1 + 3	3			1	1				5
D1 + 4	1		1			1			3
D2 + 3	2		1	1					4
D2 + 4	1	9	3	3	5	1			22
D3 + 4	1	1		1					3
1 + 2 + 3	2	2	4		3	2			13
1 + 2 + 3	7	5	5	10	4	2			33
1 + 3 + 4	3			2	2				7
2 + 3 + 4	4	3	4	1	1				13
1 + 2 + 3 + 4	16	9	7	15	9	6	5	2	69
Total	46	40	36	41	27	13	5	2	210

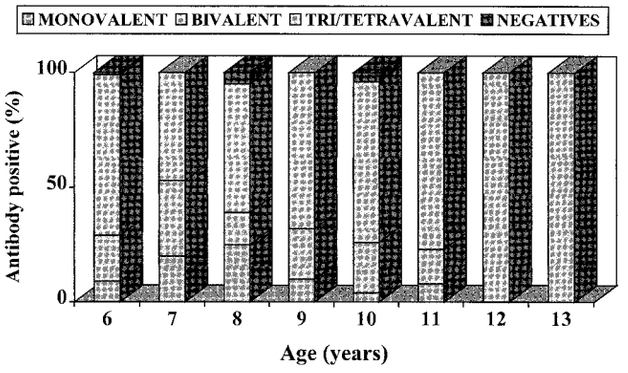

FIGURE 2. Age-specific prevalence of monovalent, bivalent, and tri/tetravalent neutralizing antibodies to dengue viruses in 210 Haitian children.

Figure 2. Dengue PRNT antibodies were highly prevalent. Only four (1.9%) of the sampled children had not experienced a dengue virus infection (Table 1 and Figure 2). During 6–13 years of residence in Port-au-Prince, only 27 (12.9%) of the children had experienced infection by a single dengue serotype (Figure 2). Despite absence of DEN-3 isolates among expatriates resident in Haiti in 1994–1996, five children had monotypic DEN-3 antibodies. The vast majority (85.2%) had experienced two or more dengue infections. Second, third, and fourth viral infections appeared to increase with age (Table 1 and Figure 2).

Personal communications from three pediatricians assigned to the University of Haiti General Hospital in Port-au-Prince with continuous experience of up to 16 years confirmed the absence of children hospitalized or dying with the clinical course, symptoms, or signs suggestive of DHF/DSS (Streit TG and Lafontant JG, unpublished data).

DISCUSSION

During 1969–1971, Ventura and Ehrenkranz[7] studied the distribution of DEN hemaglutination-inhibition (HI) antibodies in representative samples of the Haitian population. In the absence of outbreaks of dengue fever, DEN HI antibody prevalence in 603 sera increased from 43% in those 1–5-years old to 75% in those 21–30 years of age and older. Eleven DEN-2 viruses were recovered from febrile patients; also, a few sera demonstrated HI antibodies specific to DEN-3. Elsewhere in the Caribbean, DEN-3 and DEN-2 viruses were circulating.[22,23] With no DHF occurring in the American region at that time, the essentially silent transmission of one or more DEN viruses in Haiti was not surprising.

It is now established that during 1994–1996, at least three DEN serotypes were circulating in Haiti, as shown by virus isolations from garrisoned UN personnel. Phylogenetically, these viruses belonged to genotypes first found in Southeast or South Asia.[9,16,20,21] The Southeast Asian origin of the DEN-2 strains tested is particularly important because DEN viruses of these genotypes have been associated with DHF/DSS.[24] The DEN-2 strains identified in Haiti are members of a genotype that has been recovered from DHF/DSS cases in many countries in the American hemisphere during the past two decades.[10,24]

The age-stratified serologic study of children resident in Port-au-Prince provides evidence that dengue viruses of all four serotypes had been circulating in this population. Monotypic neutralizing antibodies were found to each DEN serotype. Despite the absence of any DEN-3 viral isolates in 1994–1996, five children in this study circulated monotypic DEN-3 antibodies and many more neutralized DEN-3 along with antibodies to one, two, or three other DEN types. It is not clear how many of these antibodies represent prior DEN-3 infection or cross-reactive responses to infections with other DEN viruses, particularly since some of the children sampled during the rainy season month of June may have had recent secondary dengue infections. It should be noted that DEN-3 viruses were introduced into the Caribbean in 1994.[25] All four dengue serotypes were circulating in nearby Puerto Rico in 1998.[26]

Using a simple mathematical model, it can be estimated that the average annual dengue infection rate in Port-au-Prince is approximately 30%. This is higher than the annual DEN infection rate reported for Yangoon, Myanmar, where DHF attack and death rates in children are high.[27] Although the precise sequence in which Haitian children experienced infections with first and second DEN viruses is unknown, the viral risk factors for DHF are clearly present. At the estimated total dengue infection rate, thousands of DHF/DSS cases and hundreds of deaths would be expected to occur annually in Haiti. Instead, neither DHF/DSS outbreaks nor sporadic cases have been reported. The situation in Haiti is similar to the less well-studied case of West Africa, where multiple DEN viruses have been recovered from indigenous and non-indigenous residents without reports of major dengue fever outbreaks or sporadic cases of DHF/DSS.[28–31]

This study points to the existence of a human gene that moderates the clinical expression accompanying dengue infection among individuals who are genetically Africans. There is a critical need for further studies on the natural history of dengue infections in blacks. Should a dengue resistance gene be identified, the information generated could lead to the development of powerful new modalities to assist physicians in reducing the morbidity and mortality burden imposed by dengue infections.

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