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Epidemiologic analysis of the 1999 dengue fever epidemic in Nicaragua

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Epidemiologic analysis of the 1999 dengue fever epidemic in Nicaragua

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Study Objectives

The present study analyzes cross-sectional patient information from the 1999 dengue fever epidemic in Nicaragua. It attempts to achieve the following eight goals:

1. Determine if demographic factors like age, sex, race, occupation, and distance from the hospital are associated with dengue disease severity, hospitalization, and length of hospital stay. 2. Determine if clinical history and presentation (dehydration, history of chronic disease, or superimposed acute infection) are risk factors for severe disease, hospitalization, and length of hospital stay. 3. Determine if secondary infection or viral serotype is a risk factor for severe disease and hospitalization. 4. Determine if behavioral factors (use of aspirin, vitamins, and "traditional" medications, 24 hour fluid intake, and ability to take time off from work and school) are associated with severe disease and hospitalization. 5. Compare the aforementioned clinical, demographic, and behavioral risk factors with results from the 1998 Nicaragua dengue epidemic. 6. Compare secondary infection as a risk factor for severe disease during the 1999 Nicaragua dengue epidemic with past dengue epidemics in Nicaragua, Cuba, and Southeast Asia. 7. Analyze the way in which changes in baseline hematocrit assumptions and definition of shock affect the final distribution of severe disease. 8. Determine the effects of changing the definition of secondary infection on the relationship between secondary infection and severe disease.

Introduction

Dengue fever has long plagued mankind. From its origins in the jungles of Africa and Asia to the urban centers of Latin America and Southeast Asia, dengue has been a source of considerable suffering. Dengue is transmitted by the mosquitoes, *Aedes aegypti*, *Aedes albopictus*, and a number of other species in the *Stegomyia* subgenus. Dengue viruses are single-stranded, enveloped positive-polarity RNA flaviviruses, grouped into four serotypes (DEN-1, DEN-2, DEN-3, DEN-4), that cause a spectrum of disease ranging from mild dengue fever (DF) to severe and life-threatening dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). The pathogenesis of severe disease is not clearly known and is hampered by the lack of an animal model. Because of this deficit, epidemiologic studies have proven to be one of the best ways to identify potential etiologies.

In the late 20th century, increasing movement of people and merchandise via ship and airplane from Asia, where dengue had been hyperendemic since WWII, led to the introduction of various dengue virus strains into the Americas. They have produced epidemics of DF, DHF, and DSS in the Caribbean, northern South America, and Central America. Since the first reported cases in 1985, over 75,000 cases of DF have been documented in Nicaragua (Kouri et al. 1991; OPS/OMS 1999). The occurrence of several thousand cases each year in 1998 and 1999 stressed the financial resources of the country (Harris et al. 2000). Given the increasing use of airplanes for travel and movement of merchandise, dengue could pose a major threat to the United States in areas infested by *Ae. aegypti* and *Ae. albopictus*.

History of dengue

The origin of DF, and the mosquitoes that transmit the disease, is shrouded in mystery. Although the first incident of a dengue-like disease is not known, a description matching that of DF was published in a Chinese encyclopedia of disease symptoms and remedies in 992 A.D (Gubler 1997). The description of this disease included: rash, fever, eye pain, arthralgias, myalgias, and hemorrhagic manifestations and was thought to be somehow connected to flying insects associated with water. This relationship earned it the Chinese name, "Water Poison." From the end of the 10th century to the 18th century, little mention of the disease was made in published reports, although it was undoubtedly present.

Mention of the disease we now call dengue began with sporadic epidemics throughout the 1600's and was described extensively beginning in the late 1700's. Benjamin Rush described a disease characterized by intense myalgias, arthralgias, and retroocular headaches during a 1780 epidemic in Philadelphia. He was the first to use the English title, "Breakbone Fever" to describe these symptoms (Vaughn and Green 2000). The first use of the Spanish term, "Quebranta Huesos" or literally "breaker of the bones," was employed by a physician in 1771 in Puerto Rico to describe a febrile illness with characteristic dengue symptoms (Rigau-Perez 1998). Early epidemics of dengue-like illnesses that occurred in Indonesia and Egypt in 1779 were called, "Knockelkoorts," (Bone Fever) and "Mal de Genoux" (knee trouble); however, based upon the symptomatology, it is more likely that these epidemics were caused by Chikungunya

virus, another febrile illness that can cause a rash, myalgias, and arthralgias (Gubler 1997).

The currently accepted origin of the term "dengue" comes from the Swahili, "Ki Dinga Po," used in Zanzibar, East Africa in 1823, which meant, "a disease characterized by a sudden cramp-like seizure, caused by an evil spirit." Subsequently, the terms, "Dandy fever," or "The Dandy," were used in the Virgin Islands in 1827, perhaps as an adaptation of the Swahili term learned from slaves transported from East Africa. Although the first time the term, "dengue fever" was employed is not clear, it has been used continuously to the present since an 1828 epidemic in Cuba (Gubler 1997). It is possible, however, that the term was in general use for at least thirty years before 1828. In 1801, n the Spanish royal archives and letters, the Queen of Spain, Maria Luisa, referred to an illness that she had by stating, "I was sick with a disease called dengue and since yesterday had bleeding." (Gubler 1997; Rigau-Perez 1998)

Epidemiology

An estimated 2.5 billion people in tropical and sub-tropical countries around the world are at risk for dengue virus infection. Annually, more than a hundred million people develop DF, with approximately 250,000 progressing to DHF and DSS (Monath 1994). From 1956-1995, over 3.5 million cases of DHF and nearly 60,000 deaths were reported to the World Health Organization (WHO) (Halstead 1997). Furthermore, if global warming continues over the next several decades as many environmentalists fear, temperature increases could lead to an increased distribution of the *Aedes aegypti*

population with a concomitant increase in epidemic potential (Jetten and Focks 1997; Patz et al. 1998).

Dengue virus infection is a serious risk to United States citizens that travel to endemic regions. Endogenous transmission of dengue in the United States may also increase if *Ae. aegypti* and *Ae. albopictus* continue to spread into the southern states. The number of laboratory-confirmed dengue virus infections reported to the CDC increased in 1998 relative to 1997 (CDC 2000). While travel histories were not available for all cases, it appeared that most if not all positive cases occurred in travelers to dengue endemic regions. In 1995, seven cases of locally acquired dengue infection were reported in Texas (Rawlings et al. 1998). Despite this, the actual threat of dengue infection in this country still remains low. In fact, the increased number of cases reported to the CDC could easily be explained by the creation of multiple dengue surveillance sites around the country.

DHF and DSS have not always been associated with dengue virus infections. Although the origin of DHF is not entirely clear, one hypothesis has been proposed that provides a logical explanation for its emergence (Gubler 1997). World War II was a major turning point in the epidemiology of DF as troop movements in Southeast Asia and the South Pacific increased the distribution of *Ae. aegypti*. The armies left massive amounts of used equipment that provided fertile breeding grounds for *Ae. aegypti*. Furthermore, it is likely that the majority of these troops had not been exposed to dengue previously and therefore were susceptible to infection by any dengue virus strain. This mass exposure of millions of susceptible individuals resulted in many cases of DF from 1942-1945. Large scale troop movements also could have spread multiple viral serotypes

throughout the region. This dissemination and intermingling of multiple dengue serotypes may have played a role in the subsequent development of the more serious forms of dengue fever. In fact, hemorrhagic manifestations were not seen until an epidemic of DHF/DSS occurred in 1953-1954 in Manila, Philippines and in Bangkok, Thailand in 1958 (Hammon 1960). This was also the first time that DEN-3 and DEN-4 viruses were isolated in Asia (Hammon et al. 1960).

Epidemic DHF was seen throughout Southeast Asia in the 1970's. It seems logical that this was a result of the urbanization and economic expansion following the war that resulted in a major shift of people from rural to urban locations with a resultant deterioration of housing, water, and waste management infrastructures. The increased density of hosts coupled with numerous breeding sites for *Ae. aegypti* may have been the cause for the epidemic DHF. In the 1980's and early 1990's, DHF/DSS spread north to southern China and Hainan Island, and west to India, Pakistan, Sri Lanka, the Maldive Islands, and Singapore (Gubler 1997).

The situation in the Americas was somewhat different due to mosquito control programs enacted in the 1940's and 1950's. The *Ae. aegypti* eradication effort, initiated by the Rockefeller foundation in 1943 and continued by the Pan American Health Organization (PAHO) in 1947, was an effort to rid the continent of yellow fever, caused by a virus also transmitted by *Ae. aegypti*, and was successful in the vast majority of Central and South American countries, with the exception of Suriname, Guyana, French Guiana, Venezuela, the Caribbean Islands and the southern United States. Despite or perhaps because of its success, this program was discontinued in the early 1970's. As the mosquito eradication program was perceived to have eradicated *Ae. aegypti*, funding was

no longer necessary for it. By the end of the 1970's nearly all of the countries in which *Ae. aegypti* had previously been eliminated were reinfested. In 1997, the distribution of *Ae. aegypti* was the same as that in 1940, considerably increased relative to 1970 (See Figure 1).

The eradication efforts did have the positive effect of dramatically decreasing the number of dengue epidemics in the Americas throughout the 1940's and 1950's. Following mild epidemics in Panama, DEN-2 and DEN-3 were identified for the first time in Latin America in 1954 (Rosen 1958; Rosen 1974). Signs of change in the infrequent, mild epidemic pattern could be seen when DEN-3 caused outbreaks in Jamaica and Puerto Rico in 1963. Until 1977, only DEN-2 and DEN-3 were documented in the Americas. At this point, only one serotype was present in any given location.

This situation did not last for long, as DEN-1 outbreaks occurred in Jamaica in 1977 and in Puerto Rico and Venezuela in 1978 (PAHO 1979). Over the next four years, this serotype spread throughout Central America and northern South America. In 1978-1979, an epidemic in Cuba of DEN-1 caused more than 500,000 cases of relatively mild DF with no reported cases of DHF (Bravo et al. 1987). At this time, DHF/DSS was unknown in the Caribbean.

In 1981, upon a background of DEN-1 immunity in Cuba, a newly introduced DEN-2 strain from Southeast Asia caused a huge epidemic with 344,203 reported cases of DF, 10,312 cases of DHF (WHO grades II-IV), and 158 deaths (Bravo et al. 1987; PAHO 1995). Many more deaths would have undoubtedly occurred if it were not for the rapid hospitalization of patients with DHF/DSS. To date, this has been the most important dengue epidemic in terms of numbers of people affected with severe disease

and with respect to the careful classification of cases. Subsequently in 1989-1990, DEN-1, DEN-2, and DEN-4 were isolated in Venezuela during a six month epidemic that caused 5,990 DHF cases and 70 deaths. Since these two epidemics, many other outbreaks of DHF have occurred in the Americas (See Figure 2).

While all four serotypes have been found in Nicaragua, disease is usually of the endemic-epidemic pattern in which no more than two viruses are circulating simultaneously, with one predominating throughout the epidemic. Because not all serotypes are found concurrently, many adults still remain susceptible to later outbreaks. The first recorded epidemic of dengue in Nicaragua, caused by DEN-1 and DEN-2, occurred in 1985 with 17,000 cases and 7 deaths (Kouri et al. 1991). Sporadic cases were observed until 1990 when 4,137 DEN-4 cases were reported. From the end of 1994 through the rainy season of 1995, DEN-3 of the "Sri Lanka" strain caused more than 20,000 cases. The next epidemic didn't occur until 1998 when DEN-2 ("Jamaica") and DEN-3 ("Sri Lanka") caused more than 2500 cases (OPS/OMS 1999). The cases of DHF/DSS in Nicaragua were not associated with sequential infection (Harris et al. 2000).

Economic impact of dengue fever

Fortunately, even during epidemics, dengue has a relatively low mortality rate. The 1989-1990 Venezuelan epidemic had a mortality rate of approximately 1%. During the 1981 Cuban epidemic, the mortality rate was 4.5 per 10,000 cases of dengue fever (.04%). On the other hand, a recent study conducted in Puerto Rico, using the disability-adjusted life year (DALY), measured the amount of lost productivity due to dengue illness in order to assess its economic impact. This study found that DALY losses were

on the order of those due to malaria, tuberculosis, hepatitis, meningitis, the childhood cluster (polio, measles, pertussis, diptheria, tetanus), the tropical cluster (Chagas' disease, leishmaniasis), sexually transmitted diseases (excluding HIV), or intestinal helminths (Meltzer et al. 1998).

An earlier study of the 1977 dengue epidemic in Puerto Rico estimated that costs of medical care and loss of work were between US $6 million and US $16 million (Von Allmen et al. 1979). The total cost for dengue epidemics in Puerto Rico from 1977 to 1997 was somewhere between US $150 and US $200 million (PAHO 1995). The cost of the 1981 Cuban epidemic was estimated at around US $103 million (PAHO 1995).

One report estimated the cost of the 1994 epidemic in Nicaragua, including medications, hospitalization, and vector control efforts, at greater than US $2 million (Ferrando 1994). Furthermore, a PAHO study revealed that it cost US $130 per day for a hospital bed and estimated US $3,000 for a case of dengue with complications. This is very significant in a country where the per capita gross national product is only US $ 449 (Nicaragua 1996).

Virology

Dengue viruses (See Figure 3) are members of the Flavivirus genus that is antigenically related to the yellow fever, West Nile, Murray Valley, Japanese encephalitis, St. Louis encephalitis, and tick-borne encephalitis viruses. The *Flaviviridae* family was named for the prototype virus in this group, yellow fever (flavus is yellow in Latin) and encompasses the Hepatitis C group, Flavivirus genus, and the Pestivirus group (e.g. BVDV-bovine viral diarrhea virus). All of the viruses in this

family share a virion diameter in the range of 40-60nm, are composed of single-stranded positive polarity RNA, and possess a very similar gene structure and order.

Four dengue virus serotypes, DEN-1, DEN-2, DEN-3, and DEN-4, cause dengue fever. Dengue viruses share significant amino acid homology, with DEN-1 and DEN-3 being the most similar to each other (77.4% homology), followed by DEN-1 and DEN-2 (68.3% homology), DEN-2 and DEN-3 (67.3% homology). DEN-4 has between 62% and 63% homology with the other three serotypes.

Structurally, each virion possesses an envelope (E) protein, an isometric nucleocapsid (C) protein, and a membrane protein (M), in addition to various non-structural (NS) proteins that appear to be involved in viral replication. E protein is the major surface protein to which most neutralizing antibodies are directed. It is thought to be involved in receptor binding, erythrocyte hemagglutination, viral assembly, and membrane fusion in acid pH endosomes (Chang 1997). The gene order in the Flavivirus genus is 5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3' (Westaway and Blok 1997). The dengue virus genome encodes an uninterrupted open reading frame, and is translated as a single polyprotein before being post-translationally cleaved into functional proteins. The four serotypes (DEN-1, DEN-2, DEN-3, DEN-4) are 10,188; 10,173; 10,170; and 10,158 nucleotides in length, respectively (Westaway and Blok 1997).

Vector

Dengue was known to be spread by mosquitoes of the *Aedes* genus as early as 1903; however, the virus was not cultured until the 1940's. Before the advent of virus culturing and identification techniques, human volunteers were used to determine which

mosquito species were capable of spreading dengue. Although many species of *Aedes*, including *Ae. albopictus* and *Ae. polynesiensis*, can transmit dengue virus, *Ae. aegypti* (See Figure 4) is the species responsible for the most serious epidemics of DF and DHF (Rodhain and Rosen 1997).

Aedes albopictus originated in Asia and was localized in Southeast Asia, China, Japan, Indonesia, and islands in the Indian Ocean until large-scale commercial shipping spread it to the United States, Brazil, Mexico, Guatemala, El Salvador, Colombia, Bolivia, Dominican Republic, southern Europe (Albania, Italy), and various regions in Africa and the South Pacific (Gubler et al. 1978; Gubler 1997). The influx of *Ae. albopictus* to the United States may set the stage for the reestablishment of indigenous dengue infections. *Aedes aegypti* has been detected in the southern U.S. in Texas, Florida, and Louisiana (CDC 1996; CDC 2000; Rawlings et al. 1998).

Why has *Ae. aegypti* been the mosquito associated with the most severe epidemics? It has been observed that *Ae. aegypti* is infected by the oral route more poorly than *Ae. albopictus* or other *Aedes* species (Rosen et al. 1983). In addition, the threshold viremia for successful infection of *Ae. aegypti* is quite high. While this makes *Ae. aegypti* a less efficient vector for dengue virus infections, it does not necessarily make it a poorer vector for the spread of dengue virus. It is possible, given the strong association between *Ae. aegypti* and severe epidemics, that the decreased transmission efficiency establishes a selection process by which it transmits only the most virulent strains.

Aedes aegypti is a domestic daytime-biting mosquito that prefers to live in quiet dark areas of homes, under beds and in closets, or near houses in water storage

containers, flower pots, used tires, and bottle caps (Reiter and Gubler 1997). *Aedes albopictus*, on the other hand, is a peridomestic insect that is found most often in nearby forests in rainwater collections, tree knotholes, cut bamboo shoots, or closer to homes in used tires or discarded bottles. The average lifespan for *Ae. aegypti* females ranges from 8-15 days, while males live approximately 3-6 days. Their lifecycle consists of three different major stages: the aquatic larval and pupal stages lasting 7-9 days and 2-3 days, respectively at 25°C; and the adult phase, lasting 8-15 days for females and 3-6 days for males. After an infective blood meal, *Ae. aegypti* can transmit the virus after an 7-12 day extrinsic incubation period depending upon the ambient temperature. At 32 or 35°C, transmission occurred as early as 7 days, while at 30°C, transmission was not observed until 12 days after infection (Watts et al. 1987). Therefore, in order for the mosquito to spread dengue, the adult female must live for at least 7-12 days.

It is very likely that *Ae. aegypti* spread to the Americas via trade and slave ships in the 1600's. Continuing movement of merchant ships worldwide spread the mosquito to tropical Asia in the 19th century and to the Pacific islands in the late 1800's and early 1900's. By the mid-twentieth century, *Ae. aegypti* had spread to nearly every country in the Americas. In 1997, *Ae. aegypti* is widespread in the southeastern United States (See Figure 5).

Risk factors for progression to DHF/DSS

In the absence of an animal model that mimics human dengue infections, epidemiological studies of dengue epidemics have been the best approach in the search for a link between risk factors and severe disease. In each epidemic several

epidemiological features have turned out to be important. The number of dengue viruses circulating in a given region at a particular time, the serotype and genotype causing the epidemic, and the number of previous dengue infections with which individuals had been infected, may contribute to the severity of the epidemic. In the present study, gender, age, race, immune status, and history of chronic disease were compared between patients that had a disease other than dengue (negative laboratory test for dengue), mild dengue (DF, DFHem), and severe dengue (DHF, DSAS, DSS) in order to test some of these claims.

The progression to DHF/DSS is an area of very active research and various studies have reported risk factors. In Southeast Asia, DHF/DSS is almost always found in children. In Latin America, children comprise the majority of severe dengue infections but significant numbers of adults with DHF/DSS have been reported (Harris et al. 2000; Zagne et al. 1994). Although epidemics in Southeast Asia demonstrated a higher incidence of DHF/DSS in females, studies in Nicaragua and Puerto Rico have not shown this relationship (Harris et al. 2000; Rigau-Perez 1997; Rigau-Perez 1999). It has been suggested that the increased incidence of severe disease among female patients is due to a more competent immune response (Halstead 1997). Although this is possible, insufficient evidence exists to support this claim. The present study will attempt to evaluate the risk of severe disease within gender and age groups.

Black race was suggested as a protective factors against DHF/DSS relative to Caucasians and Asians by data from the 1981 dengue-2 Cuban epidemic (Bravo et al. 1987). Although later studies have not adequately studied race as a protective factor, if this association turns out to be true, it would suggest that a gene or group of genes may

exist that confers resistance to people that express it. Race will be examined in the present study to see if racial background is associated with a lower risk of severe disease.

Interestingly, moderate to severe protein-calorie malnutrition was shown in Bangkok, Thailand to decrease risk for DHF/DSS (Thisyakorn and Nimmannitya 1993). This effect could be due to a decreased production of immunoglobulins that could potentially decrease antibody-dependent enhancement of infection. Chronic illnesses like asthma and diabetes may increase the risk of severe disease (Bravo et al. 1987). Firm evidence does not exist to support either of these claims. Perhaps the underlying inflammatory processes in asthma lead to increased antibody production that would contribute to increased antibody-dependent enhancement. Any hypothesis for a relationship to diabetes would be premature given the extremely limited nature of evidence to link it with severe disease. Patient health conditions like peptic ulcer and menstruation may play a role in risk of DHF/DSS (Bravo et al. 1987; Halstead 1997) through a mechanism of increased bleeding. However, these risk factors have not been clearly defined. The present study will test the hypothesis that chronic illness and superimposed acute infections are associated with severe disease.

Several studies in Southeast Asia and in Cuba have shown that DHF/DSS is associated with secondary-type dengue infections in patients older than one years-old. In a five year prospective study in Myanmar, Burma, and Rayong, Thailand, a strong association was discovered between secondary infection and DHF/DSS (Sangkawibha et al. 1984; Thein et al. 1997). The present study will test the hypothesis that rates of secondary infection with a different dengue serotype are higher among patients with severe disease relative to those with mild disease.

A curious epidemiologic feature observed in the 1981 Cuban epidemic was that some infants less than one year-old were found to have DHF whereas no children in the one to three years-old age group were diagnosed with DHF. In Thailand, six to nine month-old infants with primary dengue infections born to dengue-immune mothers developed DHF or DSS (Halstead 1997; Kliks et al. 1988). Normal term infants are born with maternal IgG antibodies. These IgG antibodies have a half-life of 25 days, resulting in steadily decreasing concentrations and a concomitant decrease in protection from infection, reaching a nadir at three months (Rudolph and Kamei 1994). As the IgG neutralizing antibody titers fall, their potential to become "enhancing" increases (See Figure 7). Enhancing antibodies may increase the risk of DHF/DSS while neutralizing antibodies may decrease this risk (Kliks et al. 1989). The present study will attempt to determine if infants with severe disease are more likely to have a primary infection relative to older children.

Following the 1981 Cuban epidemic, after careful classification of DHF/DSS cases and their immune status, the rates of secondary infection were higher in DHF and DSS patients relative to the general population and in patients with DF (Bravo et al. 1987). Although this relationship had been suggested as early as 1970 (Halstead 1970), results from this epidemic provided the best evidence to-date in support of the hypothesis that secondary infection was causally linked to severe disease. If secondary infections were not associated with an increase in the risk of DHF/DSS, the rates of secondary infection in this group should have been the same as that in the general population. The rate of secondary infection was 44.5% in the general population whereas this increased to between 95 and 98.5% in the DHF/DSS groups. Along with similar observations from

the analysis of epidemics in Thailand, these have suggested a relationship between sequential infection and severe disease (See Pathogenesis & pathophysiology section).

Opponents to the sequential infection model of pathogenesis point to epidemics of DHF in the early 1970's in Oceania that do not correlate with secondary infection, but rather with viremia of the patient (Barnes and Rosen 1974; Gubler 1997; Moreau et al. 1973). On each of the islands affected, dengue viruses had not been seen for many years and as a result, all individuals were infected for the first time. The sequential infection model does not explain why these individuals with primary infections would suffer from DHF or DSS. Outbreaks of DHF in Tahiti and New Caledonia were associated with a high viral isolation rate suggesting a higher viremia in patients. At the other extreme, an epidemic of dengue fever in Tonga was so mild that the outbreak was not detected for over a year. In this outbreak, viral isolation rates were low, suggesting a low viremia (Gubler et al. 1978). In addition to these observations, a severe outbreak of dengue broke out in Tonga in 1975 that could not be explained by differences in immune status, density of mosquito vector, or increased susceptibility to infection by the mosquito (Gubler et al. 1978).

Given the observations from the Pacific Islands, Cuba, and Southeast Asia, viral strain in addition to serotype may play a role in progression to severe disease. The evidence for differences in viral virulence is contradictory, however, as several authors have shown that viruses that cause mild and severe illness appear to have identical genomes (Halstead 1997). Furthermore, after analyzing data obtained from a prospective five year study in Peru, researchers did not find a significant relationship between secondary infection and severe disease after an outbreak of DEN-2 of American origin

(Watts et al. 1999). It has been shown, however, that dengue genotypes of Asian origin are associated with DHF (Rico-Hesse et al. 1998; Rico-Hesse et al. 1997). The present study will attempt to determine if viral serotype is associated with severe disease or increased risk of hospitalization.

Pathogenesis & pathophysiology

Although risk factors for severe disease have been suggested by epidemiologic studies, the connection between risk factors and pathophysiologic mechanisms has been elusive, particularly in light of the fact that an appropriate animal model has not been found to reproduce human disease. It is clear that two major pathophysiologic changes occur in DHF and DSS. The first is an increase in vascular permeability that leads to hemoconcentration, hypoalbuminemia, decreased pulse pressure, and shock. The second involves changes in hemostasis resulting from a combination of thrombocytopenia and various coagulation defects.

The increase in vascular permeability is likely due to a combination of factors including circulating cytokines, complement factors, and other non-cytokine factors. Studies have not shown that elevations in cytokines can be correlated with severity of disease. It is known, however, that tumor necrosis factor-α, interleukin-1 (IL-1), IL-2, and IL-6 can induce plasma leakage like that seen in DHF. Elevations in IL-8, C3a and decreased C3, C4, C5, and factor B have been shown to be correlated with severity of disease (Avirutnan et al. 1998; Rothman 1997). Platelet activating factor and histamine may also play a role although this also has not been demonstrated experimentally. It is unlikely that the vascular permeability is caused by direct cytopathic damage as most

pathologic samples have shown little or no endothelial damage. Furthermore, the rapid recovery in patients who do survive the illness suggests an inflammatory-mediated process rather than a traumatic one.

The search for an explanation to this phenomenon of increased vascular permeability has been guided by the epidemiologic observation that the majority of patients with DHF are suffering their second dengue infection. This has led to the hypothesis that non-neutralizing antibodies or "enhancing" antibodies lead to an exaggerated immune response (Halstead 1970; Halstead 1988). While this hypothesis suggests a mechanism by which dengue infections cause shock, it does not explain all aspects of the epidemiologic and laboratory data (Rosen 1977; Rosen 1986; Rosen 1989).

In the normal immune response to viral infection, protection from the virus is generated either by the production of virus-specific neutralizing antibodies, or by the sensitization of cytotoxic CD8+ T-lymphocytes to intracellular pathogens. A protective antibody response is one in which antibodies bind to viral proteins and prevent viral entry into cells, increase complement-mediated cytolysis, or increase virus uptake and degradation by macrophages (Kurane and Ennis 1997). In the case of dengue virus, these antibodies are typically directed against the envelope protein. Long-term immunity in the form of neutralizing antibody and CD8+ T-cells is usually generated against each serotype with which a person has been infected. This does not, however, confer immunity to other dengue serotypes.

Various *in vitro* systems have demonstrated that serum from patients with preexisting anti-dengue antibodies can augment the infection in peripheral blood monocytes (See Figure 7). In some instances, these "enhancing" antibodies have been

shown to correlate with the presence of severe disease (Kliks et al. 1988; Kliks et al. 1989). In some *in vitro* and *in vivo* studies, cells of the monocyte/macrophage lineage have been the principal cells infected by dengue viruses (Kurane and Ennis 1997). Because macrophages play a critical role as antigen-presenting cells and in mobilizing other cells of the immune system, they have been implicated as an intermediary in the pathogenesis of DHF. It is known that following viral infection of a macrophage, it secretes various cytokines like interferon-α, tumor necrosis factor-α, and IL-1. These cytokines can in turn increase the concentrations of other cytokines like platelet activating factor and various interleukins, which play a role in vascular permeability and coagulation.

The basic model of antibody-dependent enhancement (ADE) is that non-neutralizing antibodies generated during an earlier dengue infection bind to epitopes on the surface of the dengue virion without stimulating complement-mediated destruction or opsonization. Instead, these antibody complexes are more actively taken up by macrophages through a Fc receptor-mediated process. As the macrophage phagocytoses more virus, the macrophage upregulates its Fc receptor, releases more cytokines, and eventually activates other virus specific T-cells.

The second arm of the immune response to dengue infections, that mediated by cytotoxic CD8+ T-cells or CD4+ helper T-cells, may also participate in the pathogenesis of DHF. T-cells from an earlier dengue infection have been shown to be cross-reactive against other serotypes, although most of the CD8+ T-cells function in a serotype-specific fashion (Kurane and Ennis 1992; Kurane et al. 1994). T-cells secrete a wide variety of cytokines that can increase vascular permeability.

An alternative to the ADE or cross-reactive T-cell models of pathogenesis is that different viruses possess varying levels of virulence. This hypothesis assumes that RNA viruses with their low-fidelity reverse transcriptase enzymes mutate and change in response to environmental pressures. This model posits that some viruses are better able to replicate, cause a higher viremia and greater epidemic potential (Gubler 1998; Rosen 1977). Some evidence of this has been demonstrated with DEN-2 of Asian origin (Rico-Hesse et al. 1998; Rico-Hesse et al. 1997). All three hypotheses are not mutually exclusive as enhancing antibodies, cross-reactive T-cells, and viral virulence could all contribute to the pathogenesis of DHF and DSS.

Clinical features

Signs & symptoms of dengue fever

DF ranges in its clinical presentation (See Figure 8) from a self-limiting febrile illness to a severe shock syndrome (George and Lum 1997; PAHO 1995). It is commonly classified as occurring in three different forms, classic DF, DHF, and DSS. Although this presupposes that these entities are discrete illnesses, clinically DF, DHF, and DSS fall along a spectrum of increasing disease severity.

At the least severe end of the spectrum, DF (See Table 1) causes a characteristic syndrome that frequently varies by age group. Infants and young children often present with a higher proportion of undifferentiated fevers and maculopapular rashes. Older children and adults tend to share the classical features of abrupt onset fever, severe retroorbital pain, throbbing headache, maculopapular rash, myalgias, and arthralgias (George and Lum 1997). DF lasts anywhere from 3 to 14 days with an average duration

of 5-7 days. In addition to the above symptoms, sudden onset extreme fatigue, anorexia, chills, nausea, vomiting, photophobia, profuse diaphoresis, dysuria, skin hyperesthesias, and lymphadenopathy are seen. Early studies described severe depression and suicidality during convalescence (Hammon 1969); however, this has not been documented in later studies.

Often the initiation of fever coincides with the onset of a severe headache with retro-orbital pain. DF characteristically presents with osteoarthralgia (bone pain) and severe muscle pain and weakness. Another classic feature is the so-called "saddle-back" fever profile. Following the initial fever spike to as high as 40.5°C in the first several days of illness, the temperature can drop to nearly normal. After approximately one day, it often rises again to between 39°C and 40°C (George and Lum 1997).

DHF is characterized by thrombocytopenia and hemoconcentration, various hemorrhagic phenomena, and hepatomegaly in addition to the signs and symptoms described for DF (See Table 1). Elevated liver transaminases, activation of the complement system, increased prothrombin time, and consumption of fibrinogen are also seen (Bokisch 1973; Cohen 1966; WHO 1973). Normal AST and ALT values may be negative predictors for progression to DHF (Kalayanarooj et al. 1997). DHF is further sub-classified into grades I and II (DHF without shock) and grades III and IV (DSS or DHF with shock). Hemorrhagic signs and symptoms can include easy bruising and bleeding at venipuncture sites, epistaxis, gum bleeding, and mild gastrointestinal hemorrhage. A positive tourniquet test is often used to confirm hemorrhagic manifestations. A positive test is defined as the appearance of 20 or more petechial hemorrhages following the inflation of a blood pressure cuff to the midpoint between

systolic and diastolic blood pressure for 5 minutes. Petechial rashes can also be found in the absence of a positive tourniquet test over the extremities, axillae, face, and soft palate (George and Lum 1997).

It is fortunate that DSS is an infrequent consequence of dengue virus infections. Unfortunately, the increasing numbers of people that contract dengue virus infections each year make DSS a serious risk of which physicians, particularly in tropical nations, must be aware. The clinical presentation of DSS (See Table 1) is similar to DHF and DF until defervescence, 3-7 days after the onset of symptoms, at which point the patient suddenly develops signs of shock. Pallor, cold extremities, cyanosis, a weak and rapid pulse, lethargy, and acute abdominal pains are all frequently found in various stages of shock. In this case, shock is defined as a narrow pulse pressure (less than or equal to 20mm Hg) or hypotension for age. Patients can die within 12-24 hours if they are not treated rapidly with intravenous fluids. If treatment is given rapidly, patients usually recover rapidly with a short and uneventful convalescence.

Differential diagnosis of fever, rash, myalgia and arthralgia

The differential diagnosis of DF depends strongly on the geographic location and age of the patient. Children under 16 in dengue-endemic regions are at the highest risk (Nimmanitya 1998). Furthermore, the time of year and presence of outbreaks in nearby regions also contributes to the diagnosis. DF is rarely seen in the United States, but its high and increasing frequency in Latin America, India, Southeast Asia, and sub-tropical Africa make it a likely diagnosis in anyone presenting with a fever, rash, myalgia, arthralgia, and headache in these countries. Unfortunately, the specificity of any of these

symptoms is very low. Influenza, malaria, typhoid fever, various rickettsial diseases, hepatitis A, meningococcal infections, measles, rubella, Mayaro, leptospirosis, and chikungunya virus can present similarly.

A diagnosis of DF can not be definitively established on the basis of clinical presentation alone; however, the presence of hemoconcentration along with thrombocytopenia, and leukopenia often distinguishes dengue from the rest of the diseases in the differential diagnosis. Liver function tests, increased prothrombin and partial thromboplastin times can also strongly suggest DHF. Positive dengue antibodies by ELISA, HI, or PRNT, direct virus isolation, or RT-PCR is required to definitively establish the presence of a dengue virus infection. Clinical signs and symptoms are used to classify each case after laboratory tests have confirmed the diagnosis.

Dengue fever treatment

In general, cases of DF and mild DHF do not need to be hospitalized. According to authors who have experience with dengue in Southeast Asia, only those patients that present with signs and symptoms of shock truly need to be hospitalized (Nimmanitya 1998). However, high hospitalization rates, ranging from 35% for classic DF, to 60% for DFHem, have been reported among dengue patients in Nicaragua (Harris et al. 2000). Unfortunately, there are no specific anti-dengue medications and treatment is always symptomatic and supportive. In most cases of DF, an antipyretic like acetaminophen is sufficient. Aspirin should not be used because of its inhibitory effects on platelets and to avoid the possibility of Reye's syndrome. Oral rehydration with glucose and electrolyte solutions or fruit juice should be used to correct dehydration caused by vomiting and

diarrhea. If these patients become severely dehydrated or if they maintain a hematocrit that increases by 20% over their baseline they should be given IV fluids.

For the more seriously ill patients who present with hemoconcentration, thrombocytopenia, or with any spontaneous hemorrhage other than petechiae, hospitalization should be strongly considered. If a patient presents with restlessness or lethargy, cold extremities, oliguria, rapid and weak pulse, narrowing of the pulse pressure(<= 20 mmHg), or a sudden rise in hematocrit despite the administration of IV fluids, they should be hospitalized immediately. A recent double-blind study performed on 50 children with DSS in Vietnam showed that treatment with colloids (dextran 70) restored hematocrit and normalized blood pressure faster than three other IV fluid regimens (Dung et al. 1999).

Problems with classification schemes

Despite the difficulties inherent in using the WHO diagnostic criteria for DF and DHF, they are an important and necessary classification scheme. Analyses that look for relationships between risk factors and severity of illness depend greatly upon an accurate diagnosis of disease status. In order to compare results from different epidemiologic populations, diagnostic criteria must be standardized. Most researchers believe that capillary leakage is the most characteristic feature of DHF/DSS and that hemoconcentration can be used as an indicator of this process. The WHO criteria requires a >= 20% increase in hematocrit relative to the convalescent hematocrit for the patient or relative to the baseline hematocrit for his or her age and gender group in order to support a DHF diagnosis.

Although hematocrit is technically simple to measure, requiring only a sample of blood and a centrifuge, the quality of the result must be judged carefully. False negatives can occur in a population who have chronic anemia, a common situation in developing nations (WHO 1994). Unless hematocrit levels are very high, they can fall within the normal range for age and sex. On top of that, hemorrhagic manifestations and intravenous fluids can blunt the rise in hematocrit and cause normal hematocrit readings. Establishing each patient's baseline is often challenging because convalescent hematocrit measurements are often difficult to obtain.

Most importantly, the assumptions that a researcher makes about the variables that comprise each disease severity category may influence the final distribution of cases. The way in which signs of shock and signs of plasma leakage are defined may markedly change the number of cases in each severity group. The present study will evaluate the effects of two different assumptions for baseline hematocrit and two different definitions of shock on the distribution of severe disease.

Thrombocytopenia is also a key diagnostic criterion for both DHF and DSS. Thrombocytopenia is defined as <= 100,000 platelets per mm^3, or alternatively, 2-3 platelets per 100X oil immersion field on a peripheral blood smear is considered low. Normal peripheral blood smears contain between four and ten platelets per 100X field. Quality control is difficult to monitor and consistency between laboratories can be highly variable.

Diagnostic & analytic techniques

The classic gold standard for diagnosis of dengue virus infection depends upon viral isolation or the detection of dengue virus IgM or IgG antibodies. Many laboratories within the United States and around the world are beginning to employ polymerase chain reaction (RT-PCR) techniques in addition to viral isolation and serologies because of its increased speed, sensitivity, and specificity. One barrier to the use of PCR has been its high cost. However, when researchers and laboratories implement cost-cutting steps, the actual cost of RT-PCR can be equal to or less than that of serology or viral isolation (Harris 1998).

Viral isolation techniques isolate viruses in tissue or mosquito cell cultures that are then identified using fluorescently-labeled antibodies directed at viral proteins, often the E protein. While viral isolation allows researchers to reliably determine the virus type and obtain the virus for future studies, it requires a fully functioning tissue culture facility, highly trained personnel, and good storage conditions. This technique requires 7-14 days to receive a positive or negative result and can be inhibited by high serum titers of neutralizing antibodies (Vorndam and Kuno 1997).

ELISA assays measure the levels of two major immunoglobulins classes: IgM and IgG. Following a first exposure to the dengue virus (primary infection), IgM antibodies are usually generated within 4 days (See Figure 9). IgG antibody levels are not detectable for about one week following antigen exposure. In a secondary infection, IgG antibodies are already present in the patient's serum and will rise quickly following

exposure to dengue virus. IgM antibodies in a secondary infection may not rise as quickly, remain at low titers or in rare cases are undetectable (Innis 1998).

Serologic analysis provides strong evidence for dengue infection and can be very sensitive and specific (Harris et al. 2000), but these techniques have limitations. False positives can occur in the presence of cross-reactive antibodies to other group-B arboviruses like yellow fever, Japanese-B encephalitis, and West Nile virus. Because IgM antibodies take at least four days to develop following infection, negative ELISA results can occur if serum samples are taken in this time period. Serological assays do not identify dengue serotype as reliably as other techniques like the plaque reduction neutralization test, viral isolation, and RT-PCR.

IgG antibodies are used to determine whether a person is having a primary or secondary immune response. This is determined by measuring the IgG titers in acute-phase serum samples. Patients with a primary infection will rarely have detectable levels of IgG in the first week, but in general a patient can be diagnosed as having a secondary antibody response if they possess hemagglutination inhibition titers > 1:10 in the acute serum sample. The equivalent inhibition IgG titer is >1:20. Dengue IgG antibodies are highly cross-reactive to other flaviviruses. In patients previously exposed to another flavivirus, like the yellow fever vaccine, a primary response to dengue virus can induce titers above 1:20.

PCR-based tests, in particular, RT-PCR, provide results quickly, can be relatively inexpensive, and are serotype specific. Because this technique measures viral RNA, it can be highly sensitive and specific for dengue virus. Unfortunately, because of the exquisite sensitivity of the technique, there is significant opportunity for cross

contamination with research products or residues of previous samples. RT-PCR can be technically challenging and require extensive trouble shooting to perform correctly. Furthermore, detection of the RNA depends upon the presence of virus in the bloodstream, leaving only a small window of opportunity for measuring viremia in dengue patients (See Figure 9). Typically patients have detectable levels of virus between zero and six days from the beginning of their symptoms and may have detectable titers 6-18 hours before symptom onset (Kuno 1997). After six or seven days, detection of antibodies must be used to identify the presence of virus (See Figure 9). In general, when performed in the hands of trained technicians or researchers, RT-PCR is an excellent technique that provides valuable information rapidly. Because of its limitations, however, RT-PCR is not a technique that is used for general diagnostic use

Methods

Study population

The Pacific region of Nicaragua (See Figure 10) has a population of approximately 4.4 million (Anon 1998), of which two million people live in the two urban centers where this study was conducted. The age distribution of the general population according to the 1995 Nicaragua census showed 45.4% of the population was in the 0-14 year age group, 51.8% in the group aged 15–64, and 2.8% were older than 65 (Nicaragua 1995). Participating hospitals in this study included the 336-bed Hospital Escuela Oscar Danilo Rosales (HEODRA) teaching hospital in León, and the 221-bed Hospital Infantil Manuel de Jesus Rivera (La Mascota), a pediatric reference hospital in Managua. HEODRA serves a population of approximately 375,000 while La Mascota serves over 1.2 million. The participating health centers, run by the Ministry of Health, included Centro de Salud (C/S) Morazan (Managua) serving 67,949 patients annually, C/S Francisco Buitrago (Managua) serves 132,268, C/S Silvia Ferrufino (Managua) serves 101,149, C/S Mateare (Managua), and C/S Villa Venezuela (Managua). Although specific data for C/S Mateare and C/S Villa Venezuela are not available, the combined population served by all of the health centers is approximately 500,000 people.

Study design

A cross-sectional study was initiated on January 1 and continued through December 31, 1999. To be eligible for the study, subjects had to present with an acute febrile illness and two or more of the following symptoms and signs: headache, retro-

orbital pain, myalgia, arthralgia, rash or hemorrhagic manifestations. Patients with a confirmed diagnosis of another disease were also included if strong clinical evidence existed to support a diagnosis of a concurrent dengue infection. Patients presenting to the participating hospitals and health centers who met the above criteria had to give informed consent to be invited to participate in the study. The consent forms in Spanish and English can be found in Appendix 1 and Appendix 2. The study was approved by the UC Berkeley Committee for the Protection of Human Subjects (#99-4-38) and the Institutional Review Committee of the Centro Nacional de Diagnóstico y Referencia (CNDR) of the Ministry of Health (#99-04). Subjects included both sexes, all ages, and ethnicities as reflected in the local population.

A standardized questionnaire (See Appendix 3 and Appendix 4) was administered to collect demographic data (age, gender, race, residence, occupation) and identify aspects of a patient's health seeking behavior and health practices. Clinical information (signs, symptoms, medical history), factors that could contribute to hospitalization (additional acute illness, pregnancy, onset of symptoms, and date of presentation to the hospital), and the clinical course of hospitalized patients was documented by chart review using a standardized data-entry form. Venous blood was drawn and a convalescent serum specimen was obtained when possible. Platelet count and hematocrit data were obtained from the associated clinical laboratories.

Definitions

The World Health Organization grading system (WHO 1997) was used to classify patients infected with dengue virus. The WHO defines classic dengue fever by the

presence of fever, a laboratory-confirmed dengue virus infection, and several constitutional signs and symptoms (See Table 1). Minor hemorrhagic signs are considered classic dengue fever in the absence of thrombocytopenia, hemoconcentration, or signs of shock. In the present study, classic dengue fever was divided into DF and dengue fever with hemorrhagic manifestations (DFHem). This separation was made in order to better characterize the features of patients who present with hemorrhagic signs in the absence of the diagnostic features of DHF and DSS.

DHF (See Table 1) was defined as fever with hemorrhagic manifestations, thrombocytopenia, and signs of plasma leakage, i.e. hemoconcentration (equivalent to WHO classification DHF grades I and II).

DSS was defined (See Table 1) using DHF criteria plus hypotension for age (systolic pressure <80 mmHg for <5 years of age and <90 mmHg for >= 5 years of age) and clinical signs of shock (Andreoli et al. 1997), e.g., poor capillary filling, cold clammy skin (equivalent to DHF grades III and IV) or narrow pulse pressure and tachycardia (>100 beats/minute). Pulse pressure is the difference between systolic and diastolic blood pressures. A narrow pulse pressure was defined as a <20 mmHg difference between the lowest systolic and diastolic blood pressure measured in the hospital.

An additional classification was designated "dengue with signs associated with shock," (DSAS) for patients that did not meet all of the WHO criteria for DSS. A diagnosis of DSAS required the presence of signs of shock (hypotension for age, narrow pulse pressure, poor capillary filling, rapid heart rate) without both thrombocytopenia and hemoconcentration (See Table 1). For the sake of analysis, severe dengue was defined as DHF, DSS, or DSAS.

Platelet counts were obtained by chart review. Hemoconcentration was defined by either of two methods: 1. A 20% increase in the highest hematocrit measured relative to the hematocrit measured upon leaving the hospital or 2. a hematocrit 20% above normal (Andreoli et al. 1997; Rudolph and Kamei 1994) for each age and gender group (See below for relaxed and conservative assumptions of elevated hematocrit). This second category was designed to capture patients who lacked hematocrit elevations relative to those measured at time of release from the hospital, but who possessed increased hematocrit relative to standard reference values for individuals in the United States.

To partially deal with the problem of measuring an individual's baseline hematocrit, the baseline hematocrit was taken as the value obtained upon release from the hospital and was compared with the highest measurement taken during the hospital stay. It is still possible that patients with true hemoconcentration were not observed because of underlying anemia, DHF-induced hemorrhage, or the hematocrit measured on the day of release from the hospital may not have completely returned to its baseline value. Because of the difficulties inherent in this technique, other measurements like increased liver transaminases, observation of severe hemorrhage, and prolonged prothrombin times may be more accurate in diagnosing and classifying DHF (Kalayanarooj et al. 1997; Murgue et al. 1999).

Cases were considered laboratory-confirmed if any of the following were true: 1) dengue virus was isolated; 2) viral RNA was demonstrated by RT-PCR; 3) IgM-ELISA was positive (absorbance twice the mean of the negative controls); 4) a 4-fold increase in antibody titer was demonstrated between paired acute and convalescent sera; or 5)

antibody titer by inhibition ELISA was >=2560 (equivalent to a hemeagglutination inhibition (HI) antibody titer of >=1280). Primary infection was defined by an antibody titer (by inhibition ELISA) of <20 in acute samples (equivalent to an HI titer of <10) in the first four days after symptom onset; titer <= 20 from five to seven days after symptom onset, or a titer <2560 in samples obtained after eight days following symptom onset (equivalent to a HI titer of <1280). Secondary infection was defined by an antibody titer by inhibition ELISA of >=20 in acute samples taken in the first four days following symptoms onset (equivalent to an HI titer of > 10), titer >20 after five to seven days, or titer >=2560 in samples obtained after 8 days (equivalent to an HI titer of >=1280) (Nogueira et al. 1993). These criteria were equivalent to the conservative assumptions described below for the definition of secondary infections.

Laboratory methods

IgM antibodies were measured using an antibody capture ELISA. The standard MAC-ELISA (Kuno et al. 1991) was modified to decrease the time required for the assay by reducing fixation and incubation times through increasing the temperature and by modestly decreasing the number of washes. The modified ELISA was validated against the standard MAC-ELISA, resulting in a sensitivity of 98.5% and a specificity of 97.6% (Balmaseda et al. unpublished results). Total antibody levels were measured using an inhibition ELISA (Fernandez and Vazquez 1990) that was validated against the HI assay (Clark and Casals 1958), resulting in values that were approximately one dilution higher than HI titers (Balmaseda A, Téllez L, personal communication). Viral isolation and reverse transcriptase-polymerase chain reaction (RT-PCR) detection of viral RNA were

performed with sera collected within five days of the onset of symptoms. Viral isolation in C6/36 cells and subsequent immunofluorescent detection of viral antigens were performed as described previously (Balmaseda A 1999). RNA was extracted, reverse transcribed, and amplified using serotype-specific primers directed to the capsid region (Harris 1998) or NS3 gene (Seah et al. 1995) with minor modifications.

Discussion of study design

Two additional sub-classifications of disease severity were used in the present study that are not used in the standard WHO guidelines. DFHem categorized patients who met the criteria for classic DF but possessed additional signs of hemorrhage in the absence of thrombocytopenia or elevated hematocrit. Although the WHO classification system would classify these patients as having DF, it is felt that this sub-classification more accurately describes the continuum of dengue illness.

DSAS was defined as DSS in without both hemoconcentration and thrombocytopenia (Harris et al. 2000). DSAS characterized patients that presented with signs of shock like cool skin, cyanosis, or restless, yet did not meet all of the WHO guidelines for DSS. In Nicaragua, it has been observed that some patients would likely have been classified as DSS were it not for IV fluid interventions that decreased their rising hematocrits (Harris et al. 2000). This new category thus captured a group of patients with severe disease that would not be diagnosed using standard WHO guidelines.

Difficulties existed in defining disease status. The definition of DHF requires hemoconcentration which is estimated by a hematocrit >=20% above baseline values for an individual. Unfortunately, baseline values for the general Nicaraguan population or

for each patient were not available. Because of this deficit, hemoconcentration was determined in one of two ways: 1. Comparison between the highest hematocrit observed for a patient with the hematocrit measured upon release from the hospital. This makes the assumption that upon release from the hospital, each patient's hematocrit will have returned to its normal level. 2. Determination of elevated hematocrit relative to age and sex of the patient. In this case, an elevated hematocrit was defined as a 20% increase in hematocrit relative to the 50^{th} percentile hematocrit for each age and gender group in the United States (equivalent to the conservative assumption described below). Standard values by age and sex were found in reference texts for patients in the United States (Andreoli et al. 1997; Rudolph and Kamei 1994).

The above assumptions regarding baseline hematocrit levels will influence the distribution of disease severity and may not accurately represent individuals in Nicaragua; however, given the limitations of the data collected, they are the most reasonable ones to make. One way to avoid these assumptions would be to measure the patient's baseline hematocrit several weeks after the acute illness. This additional measurement requires two tubes of blood drawn during convalescence, one for convalescent serologies and the other for hematocrit. The cost of getting the additional sample in terms of labor and material costs limited its use in the present study.

Obtaining convalescent sera to measure IgG levels was also a source of difficulty. Tracking down several thousand patients to obtain second serum samples frequently proved impossible due to staff time constraints and limited funding to support salaries for such work. In order to evaluate a patient's immune status, acute serum IgG levels were used. This is not as accurate as alternate systems such as plaque-reduction neutralization

tests and will miss any patients with secondary infections who possess undetectable levels of IgG.

The present study does not attempt to accurately measure the underlying incidence of DF or DHF in the region. While the majority of severe cases of DHF were likely observed, the study was biased toward enrolling the sickest patients or at least those sick enough to go to a local health center. Furthermore, the study depended strongly upon the willingness and desire of physicians to enroll patients. All physicians participating in the study were willing and enthusiastic partners, however, it is possible that not every patient in their care with potential DF was enrolled. Additionally, not all physicians in each hospital enrolled their dengue patients in the present study.

Although the hypothesis that geographic location may play a role in severity of illness by exposing some patients to increased vector density or a more virulent strain of dengue, this hypothesis could not be tested. The major problem with testing this was that no city-wide or national database of addresses exists to correlate patient addresses with map coordinates. Additionally, the patients were not asked in which neighborhood they lived, which might have provided a method by which dengue infection and severity could be correlated with geographical location.

Alternative assumptions for classification of disease severity

As is stated above, DHF was defined by the following: fever, positive laboratory result, at least one hemorrhagic sign, thrombocytopenia, and hemoconcentration, elevated hematocrit, ascites, or pleural effusion. DSS was defined as having all of the criteria for DHF in addition to hypotension and tachycardia or some sign of shock (cold skin,

restlessness, cyanosis) or narrow pulse pressure and tachycardia or signs of shock. DSAS was defined as having all of the features of DSS without both thrombocytopenia and hemoconcentration. The definitions for DF and DFHem were unchanged from the criteria outlined in the definitions section above.

Two assumptions about baseline population hematocrit levels were analyzed in the present study in order to observe changes in disease classification and possible effects on relationships between predictor variables and disease severity. Population hematocrit levels were defined by either relaxed or conservative criteria.

Two variations in the definition of shock were analyzed. In the first, shock was defined by narrow pulse pressure or hypotension. In the second, narrow pulse pressure and hypotension were considered major diagnostic criteria, but not sufficient to diagnose shock. In this case, shock was defined by narrow pulse pressure and tachycardia or signs of shock (pallor, restlessness, cyanosis); or hypotension and tachycardia or signs of shock.

The effect of two different definitions of secondary infection on the final distribution of primary and secondary cases will be described. See below for the definition of these two definitions.

Hematocrit Assumptions:

1. The first assumption for elevated hematocrit was named the **relaxed** case. In this definition, an elevated hematocrit was defined by a hematocrit equal to or greater than the 50th percentile of normal for U.S. children and women (Soldin 1997). Men and boys older than 12 were defined as having elevated hematocrits if their measured hematocrit was equal to or greater than 20% more than the 50th percentile of males in the

U.S for each age group (Soldin 1997). In children under two, elevated hematocrit was defined as greater than or equal to 34. For children aged 2-12, hematocrit values greater than 36 were considered elevated. In women and girls, hematocrits were elevated if they were greater than or equal to 38. In 12-18 year-old boys, a hematocrit of 46 was considered elevated, equivalent to greater than or equal to 20% above the 50^{th} percentile for this age group (1.2 * 38). Men older than 18 with hematocrits greater than or equal to 50 (1.2 * 42) were considered elevated. The increased values for men assumed a lower proportion of anemia in this population relative to younger children and women.

2. The second assumption of elevated hematocrit was named the **conservative** case. In this definition, children under 2 with hematocrit greater than or equal to 40, 2-12 year-old children with hematocrits greater than or equal to 43, and women and girls older than 12 with hematocrit values equal to or greater than 45 were considered elevated. These assumed a 20% increase over the 50^{th} percentile for age and gender. The elevated cut-off points for men and boys remained as described for the relaxed case above (46 in boys 12-18 years-old and 50 for men over 18 years-old).

Secondary Infection Assumptions:

Two different definitions of primary and secondary infection were tested to evaluate their effects on the relationship between severe disease and immune status. The conservative assumption captured fewer primary infections than the more stringent requirements, whereas the relaxed assumption captured more primary infections. The present study attempts to characterize the relationship between secondary infection and severe disease. While this relationship is important, if any cases of severe disease are

found to be caused by primary infections, one needs to be very certain that they truly are primary infections.

Using the **relaxed** criteria, secondary infections were characterized by: 1. An IgG titer > 20 in the first four days following onset of symptoms, or 2. An IgG titer >40 five to seven days after onset of symptoms, or 3. An IgG titer >2560 eight days after onset of symptoms.

The **conservative** criteria characterized secondary infections by: 1. IgG titers >= 20 in the first four days after symptom onset, or 2. IgG titers >20 five to seven days after onset of symptoms, or 3. IgG titers >=2560 eight days after onset of symptoms.

Methods of data analysis

Statistical analysis. Data were entered and analyzed using Epi-Info (Centers for Disease Control and Prevention, Atlanta, GA). Crude odds ratios, their Cornfield 95% confidence intervals, and their corrected Yates p-values were calculated.

Epi Info program

See Appendix 5. Epi Info program

Explanatory variables

Secondary infection, gender, weight, age, race, hospital (HEODRA or La Mascota), additional severe infection (malnutrition, malaria, pneumonia, diarrhea, or urinary tract infection), history of chronic illness (asthma, diabetes), occupation, pregnancy, distance from hospital, dehydration, "traditional" practitioners or medications, dehydration, amount of bed rest, remained at home/bed when sick, stopped work or school, use of vitamin C, aspirin, ibuprofen, multivitamins, or diclofenac.

Outcome variables

Length of hospital stay, hospitalization, severity of disease (DF, DFHem, DHF, DSS, death), stopped work or school due to illness.

Results

Description of study population

Unless otherwise specified, all reported results were determined using conservative elevated hematocrit and conservative shock assumptions. 2,808 patients were included in the current study of which 1,291 (45.7%) were laboratory-confirmed dengue infections, while 1,517 (53.8%) had a negative laboratory result (See Table 2). 96.2% had DF or DFHem, 3.8% had DHF, DSAS, or DSS, and 4.6% remained unclassified (See Table 2). The rates of hospitalization increased with increasing severity of disease (See Table 3). 54% of all laboratory-confirmed dengue cases came from outpatient clinical sites, whereas 46% were enrolled at La Mascota or HEODRA (See Table 4). 77% of all hospitalized patients were admitted to La Mascota, with the remainder admitted to HEODRA. Most cases of dengue were diagnosed between May and December of 1999, with the most severe cases occurring between August and October (See Table 5).

Among all laboratory-confirmed individuals, the mean age was 15.3 years (median = 11), 57.2% were female, 2% were black, 14% were white, and 84% were Mestizo (See Table 6). 64% of laboratory-confirmed dengue infections were in patients younger than 15 years-old, while 57% were under 15 in the patients with a negative dengue result. Nationally, the mean age of dengue patients was 19.8 (median = 15), with female patients outnumbering males (58% female to 42% male). 46 of 47 severe dengue cases (DHF, DSAS, or DSS) came from La Mascota, with one coming from HEODRA

hospital. The signs and symptoms of all laboratory-confirmed patients can be seen in Table 7.

The most common dengue serotype was DEN-2, followed by DEN-4, and DEN-3 (See Table 8 & Table 9). This result should be interpreted cautiously, however, because only a very small number of RT-PCR and viral isolation studies have been completed at the time of this analysis.

Description of unclassified laboratory-positive dengue cases using WHO standards

Using conservative estimates for elevated hematocrit and conservative shock assumptions, 59 positive cases by IgM or IgG serologies remained unclassified into a dengue subtype (See Table 10). Changing from conservative to relaxed assumptions of elevated hematocrit and shock did not result in any changes in this unclassified group. Ten of these patients, all without hemorrhagic or shock signs were hospitalized with an average six day hospital stay (n=6, no information for four). Of these hospitalized patients, eight did not have fever, headache, retroorbital pain, myalgias, arthralgias, rash, or leukopenia (For two patients, this information was unknown). Three patients had both thrombocytopenia and hemoconcentration, while two patients had either thrombocytopenia or hemoconcentration (Four patients lacked information for these variables). Several possibilities seem likely for this group of patients: 1. They were sick and truly did not have any hemorrhagic or general signs of dengue illness. 2. They were sick before they presented to the hospital and lacked symptoms at this time. 3. Relevant

information was not added to the patient chart or to the study database. If possible, information on these patients will be verified from the original patient charts.

Of the 49 remaining unclassified patients, 40 were not hospitalized. The other nine lack information about their hospitalization status. Of the 40 non-hospitalized patients, all patients did not have any hemorrhagic signs, cool skin, restlessness, agitation, thrombocytopenia, or hemoconcentration. Thirty-eight patients did not have fever, headache, retroorbital pain, myalgias, arthralgias, rash, or leukopenia. Two patients lacked information for this variable. Since all of these patients did not have hemorrhagic signs or signs of shock, and most did not have any general symptoms consistent with dengue, the question remains as to why these patients were included in the study at time of enrollment. It appears that they were having an asymptomatic infection at the time of presentation and perhaps had more severe symptoms earlier that prompted them to visit their health center or hospital. Interestingly, 29 of 40 went to C/S Francisco Buitrago, suggesting that this site did not completely record all patient data. The remainder of cases were spread fairly evenly among La Mascota (3/40), C/S Morazan (3/40), C/S Silvia Ferrufino (3/40), HEODRA (1/40), and C/S Villa Venezuela (1/40).

Risk factor analysis

All of these analyses were performed using the "419con.pgm" Epi-Info program that utilized conservative elevated hematocrit assumptions, conservative secondary infection assumptions, and shock defined by hypotension and tachycardia or signs of shock; or narrow pulse pressure and tachycardia or signs of shock.

It was discovered that children under 15 years-old were significantly more likely to have severe disease and to have been hospitalized more frequently relative to those 15 or older (See Table 11). Even after controlling for severity of disease, children with mild disease were still much more likely to have been hospitalized than adults. This relationship may have been the result of a selection bias for severe pediatric cases at La Mascota. Of all hospitalized patients under 15 years-old in the study, 89% were hospitalized at La Mascota and 11% at HEODRA. Relative to HEODRA, admission to La Mascota was an independent risk factor for severe disease (OR 4.22 95% CI 1.55-12.48). This association suggests that either more patients with severe disease present at this hospital or that some factor about the hospital itself actually promoted severe disease.

The rates of secondary infection for DF, DFHem, and for patients with severe disease were 87%, 88%, and 87%, respectively (See Table 6). The present study did not attempt to measure the rate of secondary infection among the general population of Nicaragua. Secondary infection was not associated with severe disease even after stratifying by age (See Table 12). When secondary infection was plotted against the percentage of severe disease in each age group, it appeared that among patients younger than 8, severe disease increased as percentage of secondary infection increased (See Figure 11). Among all laboratory-confirmed dengue cases that were hospitalized, 13% were having their first dengue infection. Six of the 47 severe dengue cases were caused by primary infections (four classified as DHF and two as DSAS).

Only one virus isolate was available from a patient with severe disease, although serologic results from RT-PCR or viral isolation were performed on 82 other patients. Because RT-PCR and viral isolation was performed on only a few severe cases, no

analysis could be performed for the relationship between viral serotype and disease severity.

Dehydration was a significant risk factor for severe disease (OR 6.05 95% CI 2.10-16.57) and hospitalization (OR 5.84 95% CI 2.41-14.60) among laboratory-confirmed cases. In patients with a negative laboratory result dehydration was a risk factor for hospitalization (OR 6.87 95% CI 2.69-17.78) (See Table 13). For the present study dehydration was defined by the presence of any one of the following clinical signs of dehydration: decreased urine output, dry mucous membranes, poor skin turgor, lack of tears when crying, sunken eyes, or sunken fontanelles in infants. In children, dehydration was associated with severity of illness (OR 4.08 95% CI 1.41-11.27) and increased hospitalization rates (OR 4.07 95% CI 1.60-10.76). In adults the relationship was inconclusive due to a low number of adults with dehydration and severe disease. Drinking less than eight cups of fluid in the 24 hours prior to presentation at the hospital or health center was a risk factor for severe disease (OR 8.51 95%CI 1.24-170). Interestingly, drinking less than 10 cups of fluid was a risk factor for hospitalization in laboratory-confirmed dengue patients (OR 40.70 95% CI 6.03-804.83), while drinking less than 12 cups of liquid was a risk factor for hospitalization (OR 4.41 95% CI 1.02-26.95) in patients with a negative laboratory result (See Table 13).

Proximity to health centers and hospitals played a significant role in severity of disease and rates of hospitalization. It was found that living greater than ten kilometers from the health center or hospital was a significant risk factor for severe disease (OR 3.76 95% CI 1.82-7.68) and hospitalization (OR 9.54 95% CI 5.65-16.23) (See Table 14). Furthermore, patients with mild disease who lived far from the health centers or hospitals

were hospitalized at a higher rate than those living near the health centers or hospitals (OR 9.10 95% CI 5.25-15.89). The relationship between distance and hospitalization rates was also observed among patients with a negative dengue laboratory result (OR 5.14 95% CI 3.18-8.32), implying that a higher rate of hospitalization among patients living far from the hospital was not specific to dengue patients.

An interesting association was observed between a patient's ability to take time off from work or school and rates of hospitalization. It was found that patients who were able to stay home when sick were less likely to be hospitalized (OR .43 95% CI .28-.67) (See Table 15). This decreased odds of being hospitalized was observed only in those who lived near the hospital. Working while sick was also associated with decreased rates of hospitalization (OR .16 95% CI .04-.54). Taking time off from work or school, or working while sick were not associated with severe disease.

Severe disease or hospitalization were not associated with the use of aspirin, ibuprofen, diclofenac, vitamins, "traditional" medicine or practitioners, nor with occupation, race, gender, chronic disease, superimposed acute infections, or pregnancy.

Alteration in distribution of disease by changing the definition of elevated hematocrit

Changing the criteria for elevated hematocrit resulted in nearly a two-fold increase in the number of severe cases of disease (See Table 16). Using the relaxed criteria, 85 cases of severe illness were observed, whereas using the conservative criteria, only 47 cases were defined as severe. This change was not due to newly classifying previously unclassified cases, but rather was the result of shifting cases from mild disease

to severe disease. Specifically, these cases moved from the DFHem category to either DHF, DSAS, or DSS.

Distribution of cases after changing shock criteria

It was observed that changing the definition of shock from the standard used in the present study (hypotension & tachycardia or signs of shock; or narrow pulse pressure & tachycardia or signs of shock) to a more inclusive, yet less specific definition (hypotension or narrow pulse pressure only), four additional severe cases of disease were classified (See Table 17). Not only were four cases shifted from the DFHem category to the severe group, but also five cases of DHF shifted to DSS and DSAS.

Distribution of secondary cases using relaxed and conservative criteria

The change from conservative to relaxed criteria for secondary infection resulted in an increase of 37 secondary infections among mild cases with no increases among severe disease (See Table 18). With the conservative definition, secondary infections were defined by IgG titers >=20 in the first four days after symptom onset. The relaxed definition defined a secondary infection by IgG titers >20 in the first four days after the onset of symptoms (See methods section for further details). Using the relaxed definition, 84.3% of mild cases had a secondary infection compared to 87.7% with the conservative definition. Independent of which assumption was used, six severe infections were classified as primary infections. Four of these patients had DHF, while two had DSAS. One DHF patient was four months-old, while the remaining five patients were between seven and ten years-old.

Comparison of 1999 results with the 1998 Nicaragua dengue epidemic

Despite having two fewer health centers in the 1999 study than in the 1998 study, over twice as many laboratory-confirmed cases were observed in 1999 (See Table 19). In 1999, 61% were classified as DF and 31% as DFHem. The 1998 study classified 43% as DF and 43% as DFHem. Overall, just under 4% of laboratory-confirmed cases were classified as severe in 1999. In contrast, 13% of cases were classified as severe in 1998.

In 1999, a greater proportion of cases in females had severe disease (55%) relative to 1998 (46%). The mean age of mild disease remained the same from 1998 to 1999 (15.7); however, the mean age of severe disease dropped from 11 to 7.4 in this time period (See Table 20). See Table 21 for the proportion of dengue signs and symptoms in 1999 and 1998. Among patients with DF or DFHem, the average length of hospital stay for all hospitals increased from 5.4 days in 1998 to 5.9 days in 1999. The mean hospital stay for patients with severe disease remained approximately constant. DEN-2 was the predominant serotype isolated in 1999, while DEN-3 was the major isolate in 1998 (See Table 22). In 1999, virus serotype was determined for 83 patients by RT-PCR or viral isolation. Only one of these patients had severe disease.

Discussion

Discussion of risk factor analysis

It was observed that patients under 15 years-old were more likely than patients older than 15 to have severe disease and to have been hospitalized. In Southeast Asia, children have been found to be at higher risk for hospitalization and severe disease, but most of these studies have not included adults in their study population. In the present study, adults were included from HEODRA hospital, but the majority of study participants with a confirmed dengue infection were in patients younger than 15 years-old (64%). In the group of patients with a negative dengue laboratory test, 57% were under 15 years-old. In the general Nicaraguan population, only about 45-50% are under 15. This suggests that children tend to be hospitalized slightly more in general independent of disease. When the dengue illness was mild, children were still at a higher risk of being hospitalized, suggesting that independent of severity, children were still more likely to be hospitalized. This observation may mean that something particular to children makes them more likely to get severe disease, and to be hospitalized even without severe disease.

The main enrollment hospital was La Mascota, a site where only the sickest children were sent, a selection bias toward severe disease could certainly have occurred. In support of this bias, relative to HEODRA, hospitalization at La Mascota was a risk factor for severe disease. This selection bias might explain the relationship between disease severity and young age. In fact, in the general hospital in Leon, where both children and adults were admitted, no significant relationship was found, supporting the

notion that children were not necessarily at higher risk of severe disease. While it is possible that children may be at higher risk for severe disease than adults, results from the present study does not provide conclusive evidence for this relationship.

Patients with DF or DFHem were hospitalized an average of 5.5 and 6.1 days, respectively. Patients with severe disease were hospitalized an average of 6.3 days. With such a narrow difference between these groups, it would appear that hospital length of stay for dengue infections is approximately equal independent of disease severity.

Although these hospital stays appear similar between the DF/DFHem patients and patients with severe disease, these numbers should be interpreted critically. Among the DF and DFHem cases, less than half (148/315) of the hospital data was collected or reported for such cases, while hospital length of stay was reported for all 47 severe cases. The remaining 160 DF and DFHem patients may have been hospitalized only briefly which would decrease the average length of hospitalization significantly. However, it is possible that these patients may have actually been hospitalized for longer than reported in the first 148 cases and would contribute to an increase in the average length of hospital stay.

At any rate, the similar lengths of hospital stay are a troubling result from an economic standpoint. In general, as described above in the clinical features section of the introduction, only patients with severe DHF or DSS need to be hospitalized for their dengue illness. With this in mind, it appears that physicians in Nicaragua are over-hospitalizing DF and DFHem patients. At US $130 per day for a hospital bed and given the large numbers of DF and DFHem patients, this increased hospitalization represents a large cost to the Nicaraguan health infrastructure.

Dehydration was also found to contribute to hospitalization and severe disease. In the present study, dehydration was defined by the presence of at least one clinical sign of dehydration (See methods section for further details). After stratifying by age, dehydrated children were more likely to be hospitalized (OR 4.07 95% CI 1.60-10.76) and to have severe disease (OR 4.08 95% CI 1.41-11.27) (See Table 13). In adults, only one patient with severe disease was dehydrated, making the relationship impossible to analyze. These relationships were not confined to dengue patients alone, as patients with negative laboratory results were also more likely to be hospitalized (OR 6.87 95%CI 2.69-17.78). Severity was not analyzed as an outcome variable because the definition of DHF, DSAS, and DSS requires a positive laboratory result.

A major limitation of the definition of dehydration used in the present study is its accuracy and diagnostic predictive value. While the clinical signs of dehydration used in the study may be accurate, measuring a patient's orthostatic blood pressure would increase the likelihood that a patient was truly dehydrated. Orthostatic blood pressure measurements were not performed because the physicians in Nicaragua were not familiar with the technique. Teaching and implementing this technique in the future would be very helpful to define dehydration, but would be a very difficult and time-consuming task.

Given that fluid loss from blood vessels is a major component of the pathophysiology in DHF and DSS, it would not be surprising to discover a relationship between dehydration and severe disease. If dehydration truly is an important predictor of hospitalization, what can be done to decrease or eliminate this problem? Educational programs and media campaigns could help by teaching parents about the dangers of

dehydration in young children. The success of these programs would be highly dependent upon successfully targeting at risk populations and upon the availability of clean drinking water.

Secondary dengue infections have been shown to be a risk factor for severe disease in several studies. Following the 1981 Cuban dengue epidemic (Bravo et al. 1987), the rate of secondary infection was 44.5% in the general population and between 95 and 98.5% in the DHF/DSS groups. In Rayong, Thailand (Sangkawibha et al. 1984), 100% of DSS cases and 81% of DHF cases had a secondary infection compared with only 34% for the general population. In another study from Burma, secondary infections were found in 98% of DSS patients, while only 6-18% had secondary infections in the general population (Thein et al. 1997).

In the present study, secondary infection was not found to be associated with severe disease. Changing the definition of secondary infection did not cause a relationship with severe disease to become apparent. Although secondary infection was shown to be associated with severe disease in Cuba, Thailand, and Burma, these observations have not been supported in Nicaragua in 1998 or 1999. This suggests that other factors may have been the primary factor causing severe disease in Nicaragua. The importance of viral serotype as a risk factor for severe disease could not be analyzed because at the time of this writing, too few samples had been analyzed by RT-PCR or viral isolation.

Of the six patients with a severe dengue illness caused by a primary infection, one patient was four months-old, while the rest ranged in age from seven to ten years-old. In terms of the probable pathogenesis of DHF, the antibody-dependent enhancement model

does not explain why the five children older than one with a primary infection were having a severe infection. For the four month-old, it is possible that the child possessed maternal "enhancing" antibodies that promoted severe disease; however, in studies from Southeast Asia, primary infections causing severe disease were only found in children six to nine months of age. It is possible given an approximate three month half-life of IgG antibodies, maternal antibodies may have decreased to enhancing levels by four months of age. This is purely conjecture, however, and given the cross-sectional nature of the present study, it was impossible to determine if this infant's mother had been exposed to dengue or if the child possessed maternal antibodies to dengue.

Living more than 10 kilometers from the hospital or health center was a risk factor for severe disease and hospitalization in Nicaragua. Even patients with mild disease who lived greater than 10 kilometers from the health centers were at greater risk for hospitalization. Why would these patients with mild disease be hospitalized more frequently when they lived far away? With mild disease they should be hospitalized less frequently. Perhaps this relationship was not unique to patients with dengue disease. Instead, independent of dengue infection, simply living far from the health centers might be the underlying risk factor for hospitalization. This effect was observed to some extent in this study, as patients who did not have laboratory-confirmed dengue infections were more likely to be hospitalized if they lived far from the health centers (OR 5.14 95% CI 3.18-8.32). Even given this observed relationship, something particular to dengue patients was observed as the effect among patients with laboratory-confirmed dengue infections and mild disease was larger (OR 9.10 95% CI 5.25-15.89).

One explanation for this relationship could have been that doctors and health staff were less willing to let patients leave the hospital if they lived far away. If a patient left the hospital and lived far away, the patient might be less willing to return even if subsequently he became more ill. When patients presented with mild disease, doctors could not be certain that he or she would not later progress to severe illness. This possibility appeared particularly relevant in DFHem patients (OR 10.04 95%CI 3.28-34.47 $p < .005$). If a patient presented with any hemorrhagic sign, he was more likely to have been hospitalized, perhaps because physicians were more suspicious that this patient would progress to severe disease.

Doctors need a way to determine if patients with mild disease are likely to progress to severe disease. In one study from Thailand, the liver enzymes, AST and ALT, were elevated in patients who later progressed to DHF or DSS (Kalayanarooj et al. 1997). Normal AST and ALT levels were predictive of eventual mild disease. Alternatively, doctors need to increase their certainty that a patient will return to the hospital if their condition worsens. Given the fact that the cost of one day in the hospital is approximately three months salary for the average Nicaraguan (Nicaragua 1996), alternative protocols should be considered in order to decrease hospital stays for those with DF or DFHem.

Interestingly, it was discovered that a patient's ability to stop work or school was a protective factor against hospitalization. In patients with a negative dengue laboratory finding, stopping work, but not school, was found to be protective against hospitalization. This demonstrates that the protective factor was not specifically related to dengue infections. Dengue patients who lived near the hospital were hospitalized less often if

they were able to take off work or school. This was not observed for patients who lived far away. The relationship between stopping work or school and hospitalization may be confounded by proximity to the hospital. While stopping work or school when sick may decrease hospitalization, proximity to the hospital may actually be the reason for their decreased hospitalization. Doctors may have felt confident that nearby patients could rest at home and return to the hospital if needed.

Although no relationship was observed between the use of various medications, vitamins, or traditional medications and severe disease or hospitalization, the sample size might not have been large enough to detect a subtle relationship. The use of aspirin, in particular, could possibly increase the severity of disease through its inhibitory effects on platelets. Future studies should examine this relationship more closely by increasing the sample size and by eliminating the large number of unknown responses entered into the patient database.

The failure of the data analysis to show a relationship between chronic disease or superimposed infection with severe disease or hospitalization was surprising but not altogether unexpected given the high "unknown" response in the database. If more information can be culled from patient charts in the future, chronic infection and superimposed acute infection may still be important risk factors for severe disease.

While race and gender may play a role in severe disease or hospitalization, an association was not demonstrated in the present study. With respect to black race as a risk factor, no black patients were diagnosed with severe disease. This observation could have merely been a result of their lower proportion in the study population, rather than representing any protective factor. More data from larger epidemics would be necessary

to examine this relationship more clearly. If gender were truly a risk factor , perhaps environmental factors caused increased exposure to mosquitoes. This might explain increased dengue infection in one gender group, but not necessarily severe disease. If severe disease were more common, some other explanation would have to be found.

Analysis of assumptions

In the present study, for all reported relationships, the most conservative estimates of elevated hematocrit and shock were used. This was done in an attempt to not inadvertently classify cases of DF or DFHem as DHF, DSAS, or DSS. All the same, the conservative assumptions for baseline hematocrit may not accurately represent the "true" population baseline. Until thorough and well-controlled studies are done to standardize hematocrit values for each age and gender group, conservative estimates are the safest and most reasonable. If conservative estimates are not used for either elevated hematocrit or definitions of shock, a misclassification bias could certainly occur. In the present study, it was demonstrated that changing the definition of shock and hemoconcentration skewed the distribution of cases toward the more severe disease categories. Whether these changes actually resulted in alterations in the relationships between risk factors and severe disease must be determined in future studies.

The definition of shock used for DSS by WHO standards is hypotension plus tachycardia or signs of shock; or narrow pulse pressure and tachycardia or narrow pulse pressure. A more accurate definition of shock is critical to determine the severity of dengue illness. Clinically, shock is not necessarily defined by the above variables. Altered mental status, decreased urine output, and multiple low blood pressure readings

could all be used to more accurately classify true shock. Unfortunately, in Nicaragua, obtaining reliable information about a patient's urine output is difficult. Recording multiple blood pressure readings are possible and would require that this information be requested in the hospital portion of the study questionnaire. Altered mental status in general is a difficult concept to define accurately which makes it less than ideal as a measure of disease severity.

Defining elevated hematocrits by comparing published standards in the United States with values obtained in a developing country like Nicaragua is bound to be subject to error. If the population were anemic, as has been documented by the World Health Organization for Latin America, much lower baseline hematocrit values than those observed in the United States would be considered "normal." Although 20% greater than the 50^{th} percentile for age and sex was used in the present study to define elevated hematocrit, high hematocrit values in Nicaragua may actually be significantly lower. To reduce this problem, either population standards need to be carefully defined in Nicaragua or convalescent hematocrit values must be obtained for each patient enrolled in the study. As has been done in other studies (Graham et al. 1999; Sangkawibha et al. 1984), two elevated hematocrit values, two low platelet values, and at least two low blood pressure readings should be used to increase the accuracy of these measurements.

Discussion of the differences between the 1999 and 1998 dengue epidemics in Nicaragua

The most obvious difference between the 1999 and 1998 epidemics was the percentage of cases diagnosed as severe. In 1998, 13% of all laboratory-confirmed cases

were diagnosed as either DHF, DSS, or DSAS, relative to about 4% in 1999. Several explanations could explain this difference. First, the predominant DEN-3 virus responsible for the 1998 epidemic was more virulent than the strains found in 1999. While this is possible, limited data exists to test this hypothesis. The difference could be due to a selection bias for more severe cases in 1998. Although the same two major hospitals were included in the present study, 75% of patients came from either La Mascota or HEODRA in 1998, relative to 46% in 1999. This difference in study population may be the reason that more cases of severe disease were observed in 1998. A final explanation for the difference in disease proportion could be due to the underlying assumptions used in 1998 relative to 1999. As was shown in the present study, changing the definition of shock and elevated hematocrit could change the distribution of severe disease. It seems probable that the increased proportion of severe disease classified in 1998 could be explained by variations in classification algorithms and differences in the study populations.

Limitations of the present study

1. Limitations of laboratory tests

The majority of the patients in the study were diagnosed by IgM or IgG serologies. While these tests are highly sensitive for dengue virus infections, they depend upon the presence of antibodies to dengue virus and will thus be negative during the first 5-7 days of a dengue infection. At the time of this analysis, only one serum sample had been collected for all patients in the present study. Attempts to obtain second serum samples for each enrolled patient are ongoing; however, previous work in Nicaragua

suggests that obtaining these second samples will be difficult. After analyzing the number of cases who were found negative by IgM or IgG, nearly 1100 patients presented to the health center within the first six days after they reported onset of symptoms. This suggests that, potentially, some patients went undiagnosed for dengue infections. These patients could be confirmed if a second blood sample were taken after the first 10 days or so following onset of symptoms.

Although the solution to this dilemma appears straightforward, taking a later blood sample is not a simple task. In the 1998 Nicaraguan study (Harris et al. 2000), a 25% rate of obtaining convalescent serum samples was considered quite good. The reasons for this are multiple and begin with economics; it costs money to hire people to track patients down and obtain blood samples. If more funding was available to hire more nurses or other staff, more convalescent samples could be obtained. An alternative to hiring additional staff would be to provide some type of incentive to patients to encourage them to return to the hospital to give a second sample. Unfortunately this request often goes unheeded, because once patients are healthy, most need to return to work and can not afford the luxury of returning to the hospital. Another solution would be to perform RT-PCR on all patients that present to the study within six days of symptom onset. Using the combination of RT-PCR and serologic assay, many more dengue virus infections would be diagnosed.

2. Quality of data collection and accurate input of data

Two major issues exist here, the actual collection of relevant data to the diagnosis or categorization of dengue illness, and the quality of data entry systems. With respect to collecting relevant data, clinicians and epidemiologists may not complete questionnaires

completely because of limited time. They may be prioritizing certain aspects of the questionnaire more than others given their time shortage. Future questionnaires should include the most important variables at the top of the form in a very concise format. Given that classification of severe disease is the first objective of any study of risk factors for severe disease, those variables that define severe disease are the most important. Age, fever, laboratory result, clinical signs of shock, pulse, blood pressure, acute and convalescent hematocrit, and platelet count are the most variables that need to be collected without fail on every patient.

Accurate data entry is a difficult activity to implement and monitor. Those responsible for entering data into the computer database typically work alone and their work is not double-checked. Errors are inevitable, but can not be identified in the current system. One way to improve the system would be to implement a dual-entry system for critical data. This has the obvious downside of being more time-intensive with up to double the amount of work. A streamlined process in which only the most critical data were verified may be cost-effective. The collection of data by chart review could be improved by creating a standardized form with pre-printed names and ID numbers.

3. Lack of funds and personnel for convalescent serum samples

Convalescent serum samples are very important to any epidemiologic study of dengue fever in order to verify negative serological results and to obtain baseline hematocrit values. Funding for the study was not sufficient to hire staff to track down patients and obtain convalescent blood samples. For future studies, either more staff members need to be hired or patients need to be encouraged in some other way to return

to the hospital. Additional funding might be possible to pay staff wages or perhaps a monetary incentive could be offered to patients.

4. Sample size and interpretation of results

As mentioned above, for many variables small sample size hindered the analysis. Larger sample size and fewer "unknown" data entries would clarify relationships between potential risk factors and outcome variables.

5. Lack of unique identifiers

Two databases were used in the present study to manage patient data. In one, initial patient contact information, laboratory results, demographic characteristics and initial clinical presentation were entered. In the second, hospital course and clinical progression were recorded. These two databases were correlated by a unique laboratory ID number. Unfortunately, many patients that were hospitalized did not have a recorded ID number. This made the correlation between initial clinical presentation and laboratory result with data obtained in the hospital like platelet count and hematocrit impossible. Future studies must meticulously record ID numbers for each and every patient or one database should be used.

A related issue arose when chart review of patients was attempted. In order to find patient records efficiently, a chart number is almost essential. Many of the patients in the present study lacked chart numbers. Although patients could be identified by name, frequent spelling errors and discrepancies between records made this a difficult process. Future studies must record chart numbers for each patient.

6. Slow analysis with relate command and depletion of memory; need to merge databases and use more rapid statistical software

Using EPI-Info, two factors were found to greatly decrease the speed of the analysis: increasing size of the dengue database and increasing the size of the program that analyzes the database. Future studies could decrease the size of the analyzing program. Another alternative would be to utilize other statistical software packages like STATA or SPSS, both of which possess many powerful statistical features and process data much more quickly.

Collaboration between clinics, laboratories, and epidemiologic surveillance

In the past three years, the connections between health care centers, laboratories, and epidemiologic analyses in Nicaragua have developed significantly. These connections have allowed researchers and health care workers to better understand the shape of the epidemics occurring in Nicaragua. Prior to 1997, neither funding nor scientific support were available to create and maintain these connections. As the epidemiologic, clinical, and laboratory infrastructure grows more sophisticated and more accurate analyses will be possible.

One objective of the present study was to support these growing connections by developing EPI-Info programs that could be used in the future. These programs will allow later epidemiologists to more easily and quickly understand the shape of an ongoing epidemic. Once these programs are developed and tested, utilizing them to answer questions about relationships between variables is an easy task.

Prevention & public health

Health care & prevention in Nicaragua

The control of epidemics in Nicaragua has been a difficult task. It is one of the poorest countries in Latin America and has been hard hit by a series of national disasters and war. From the Somoza government to the Sandinista reign and to the sweeping changes that began in 1990 and continue with Arnoldo Aleman today, health care has either been ignored or treated as the highest priority. The greatest concern for public health came after the Sandinista revolution in 1979. From that time until 1983, "health care for all," was one of the primary slogans and goals of the government. In 1983, the war with the Contras began to shift resources away from health care toward the military (Garfield 1989). The short period between 1979 and 1983 showed dramatic declines in infant mortality and maternal illiteracy rates. These were associated most strongly with improved access to health care (Sandiford 1991). In the aftermath of the war, efforts have been made to preserve access to health care, vaccinations, and improve literacy, but these have not met with tremendous success.

Vaccination efforts

Efforts to develop a vaccine for dengue have been ongoing since World War II. Unfortunately numerous technical obstacles have hindered the development of a safe vaccine. Because the presence of non-neutralizing (heterotypic) antibodies may increase the risk of severe disease, a tetravalent vaccine will likely have to be developed (Bhamarapravati and Yoksan 1997). A tetravalent vaccine is one that would offer

simultaneous protection against all four dengue virus serotypes. Recent work has been promising and has utilized whole virion peptides, synthetic peptides, infectious cDNA clone-derived vaccines, and naked DNA (Gubler 1998).

Vector Control

The elimination of *Ae. aegypti* has been a complicated, laborious, and often unfruitful task. The first successful attempt to eliminate *Ae. aegypti* came when the United States Army used a method appropriately named, "Source Reduction." In 1901, they reduced larval breeding grounds in Havana, Cuba, utilized an effective yellow fever vaccine, and instituted a strict quarantine (Reiter and Gubler 1997). These had the effect of eliminating yellow fever from Cuba. Source reduction was utilized many times in cities throughout the Americas and eventually eliminated yellow fever from the hemisphere. Efforts to eliminate dengue were not nearly so successful despite the fact that it shares the same vector. Decreasing strength of central governments, the concomitant decrease in resources for large scale eradication programs, the lack of an effective vaccine, and rising resistance to insecticides have all contributed to dengue's stubborn refusal to vanish.

Although *Ae. aegypti* source reduction is ultimately the only way dengue transmission can be reduced or eliminated, its implementation is exceedingly difficult. Even if governments possessed the resources and authority to hire inspectors to find and destroy larval hatching sites, this "top-down" approach is, by its very nature, politically unstable (Gubler 1989). Although it may appear counterintuitive, increasing success of a program can lead to decreasing financial support. Political and budgetary competition for

resources can shift funding away from a program that is perceived to have already accomplished its goals. This short-sighted reallocation leaves the program under-funded and ill-prepared to accomplish its objectives.

Because of these problems, attempts to develop a community-based, "bottom-up" approach has grown increasingly more popular. This type of program is less expensive and can potentially eliminate a larger number of larval breeding grounds, yet requires that citizens take responsibility for their own homes and neighborhoods. Unfortunately, there are many problems associated with this method. Often the work is laborious and boring as individuals search for and eliminate larval sites in used tires, bottles, tree knotholes, and rain gutters. Furthermore, even with sufficient wherewithal to find and eliminate the larvae, few of the 2.5 billion people living in *Ae. aegypti* regions understand biological life cycles. As a result, many do not understand that the winged-mosquito is in any way related to the aquatic larvae. Lastly, government officials and public health workers do a poor job educating people about the elimination of the mosquitoes. They use slogans like, "Clean up your backyard," without stating exactly what steps must be taken to achieve this goal.

Originally oils like kerosene were used to kill larvae and pupae, but they fell out of favor as organochloride insecticides were discovered. By the late 1940's, *Ae. aegypti* began to grow resistant to DDT and other organochlorides prompting the switch to organophosphorus insecticides like malathion and fenthion. Resistance has been slow to develop to these insecticides. Novel biological approaches have included the use of larvivorous fish or crustaceans living in large water supplies and the introduction of competing mosquitoes (*Toxorhynchites* genus) that prey upon *Ae. aegypti* larva (Lardeux

et al. 1992; Reiter and Gubler 1997; Riviere et al. 1987; Vu et al. 1998). These solutions have either been unpalatable to citizens or too expensive. Relatively simple interventions like covering water storage containers have been somewhat effective; however, government interventions must target the groups responsible for household water. In Puerto Rico, intensive efforts were made to educate women about the need to cover water containers. This was very successful, but unfortunately, in Puerto Rico, men were in charge of maintaining the large household water supplies (Waterman et al. 1985). As a result, a large source of mosquito breeding grounds was maintained. In Honduras, a bottom-up, community-based program was initiated and has shown some success in reducing the numbers of mosquitoes in uncovered water supplies and in standing water left in wash basins near the home, yet they face the same difficulties described above (Fernandez et al. 1998).

Over the years, many methods have been utilized to eliminate *Ae. aegypti*. These have met with varying levels of success, but ultimately the elimination of mosquitoes will depend upon a method that is sustainable over the long term. Efforts to eliminate *Ae. aegypti* became a goal of PAHO (PAHO 1985), but unfortunately *Ae. albopictus* was not included in this objective. If steps are not taken to decrease this vector in addition to *Ae. aegypti*, *Ae. albopictus* might prove to be an important vector for dengue in the Americas. Furthermore, in the long run, decreasing the threat of DF and DHF will rely upon a combination of vector control efforts as well as the creation of an effective vaccine.

Public health interventions

Screens

One seemingly straightforward intervention would be to screen all homes in Nicaragua. Because *Aedes aegypti* prefers to live indoors or very near homes, screens followed by insecticide use or intensive cleaning would be effective in decreasing exposure. Various studies have shown that house screens offer a decrease in the risk of contracting dengue virus infections (Ko 1992; McBride et al. 1998). Future studies should examine why people in Nicaragua do not use screens. Are there financial, social, or cultural barriers to their use?

Decrease hospitalization for less severe cases

In order to decrease hospitalization for less severe cases, the reasons for the increased hospitalization must be considered. Given the results of the present study and from personal conversations with physicians in HEODRA, it is likely that patients living far from the hospital are more likely to have been hospitalized because of the fear that they would not return or receive adequate care. If this is the main reason that patients are being hospitalized for longer periods of time, rather than for some uncharacterized factor that makes these patients more ill without being diagnosed with severe dengue disease, some alternative should be found that would allow patients to go home with mild disease and be able to return if they become more ill. Why would patients be less willing to return to the hospital? Perhaps financial considerations play a role? Do patients have to care for others at their home? Given that the majority of patients were children, a likely

scenario might be that parents of a sick child would not be able to leave work a second time. Alternatively, perhaps the cost of bus or taxi fare limits patients from returning to the hospital. The creation of day care services for children remaining at home might help with the first problem. Additionally, allocating funds for taxi or bus vouchers could help patients return to the hospital if they become more sick. Paying nurses to visit patients at home might be a cost-effective way of providing adequate follow-up care.

Decreasing the incidence of dengue infections is a major public health goal not only in Nicaragua, but also in many other countries around the world where *Ae. aegypti* and *Ae. albopictus* thrive. The sheer magnitude of the population exposed to dengue virus each year makes the appearance of severe disease an inevitable fact. The economic burden to the individual and society, due to lost wages, decreased productivity, and expensive health care make dengue a very major problem. While some solutions like the eradication of *Ae. aegypti*, or development of a vaccine, are difficult and fraught with many problems, they represent the best way to eliminate the threat of dengue fever in the long run. The development of surveillance systems through collaboration between clinical facilities, laboratories, and epidemiologists can provide warning for impending major epidemics, analyze risk factors unique to a given country, and target particularly vulnerable risk groups.

In the short run, specific actions could be taken to decrease the exposure to dengue-infected mosquitoes, minimize the severity of disease, and lower the cost of unnecessary hospitalization. Using screens in every home on all windows and doors, followed by household eradication of *Ae. aegypti* would decrease a large source of exposure to infected mosquitoes. This seems like a simple solution, but future studies

must examine why people in Nicaragua do not use screens already. Are there economic or social barriers to their use? The general public should be educated about the potential increased risk of severe disease from dehydration. The cost of this education program and of making water or oral rehydration therapy available to people will almost certainly be less than the cost of hospitalizing patients with severe disease. Finally, unnecessary hospitalization of mildly ill dengue patients should be decreased. In order to accomplish this, doctors need to be assured that mildly ill patients will return to the hospital if they become more sick. The creation of a system to provide money for taxi or bus vouchers, drivers to pick up patients, a day care service, or even paying workers for lost wages might be ways of encouraging patients to return to the hospital.

Conclusion

The present study attempted to achieve eight goals with respect to the analysis of the 1999 dengue epidemic in Nicaragua. To address the first of these goals, it was shown in the present study that gender, race, and occupation were not associated with severe disease or hospitalization. Following the 1981 epidemic of dengue in Cuba, black race was thought to be a protective factor against severe disease. The present study lacked a sufficiently large sample to evaluate this hypothesis conclusively. Some researchers have suggested that female gender may increase the severity of illness, however, this relationship was not observed in the current analysis. Young age was found to be a risk factor, as those under fifteen years-old were more likely to be hospitalized and to have severe disease than those older than fifteen. While this finding is concordant with findings from Southeast Asia and Cuba, it should be interpreted carefully, as 46 of 47 severe cases (98%) and 89% of hospitalized children came from La Mascota, the main pediatric referral center for Nicaragua.

Living more than ten kilometers from the hospital was a risk factor for severe disease and hospitalization. This is probably due to a combination of dengue specific and non-specific effects. It is possible that sick individuals that live far from health centers are more likely to wait until they are very ill before coming to the hospital, while sick patients that live near the hospital might go to the hospital with any severity of disease. This could contribute to a selection bias toward increasing severity of any disease in those living far from the hospital. With respect to dengue patients, physicians and health providers are aware that apparently mild disease can progress to severe disease over the

course of several days. If a patient lives far from the hospital, doctors may be more inclined to hospitalize all dengue patients for observation.

While researchers suggested that chronic diseases like asthma or diabetes may be a risk factor for severe disease following the 1981 Cuban epidemic, no relationship was found in the present study to support these observations. Superimposed acute infections like severe diarrhea or malaria did not contribute to an overall increased risk of severe disease or hospitalization, but these infections may have contributed to the length of hospital stay in some patients.

Dehydration was found to be associated with severe disease and hospitalization, particularly in children. In general, dehydration was a risk factor for hospitalization independent of dengue infection. Among all age groups, drinking less than seven cups of fluid in the preceding 24 hours before presentation to the health center was a risk factor for severe disease. Given these findings, it seems that an educational program to inform the general public about the benefits of drinking fluids when ill and the dangers of dehydration would be a prudent course of action.

The major difference between the 1998 and 1999 dengue epidemics was in the proportion of severe disease observed. In 1998, approximately 13% of all dengue cases were classified as either DHF, DSAS, or DSS, whereas only 4% was described in 1999. This could be attributed to changes in virus strain virulence from one year to the next, but little information was available to test this hypothesis. Although the decreased proportion of severe cases in 1999 could be due to increased diagnostic stringency used in 1999, the most likely possibility is that more patients were enrolled from a hospital in

1998 rather than from outpatient clinics. In 1998, 75% of enrolled patients came from hospitals compared with 46% in 1999.

In the present study it was observed that changes in the assumption of baseline population hematocrit could markedly increase the number of DHF cases diagnosed. For future studies, truly representative baseline hematocrit values should be developed for all age and gender groups in Nicaragua. Because these standard hematocrit values did not exist at the time of the current analysis, the combination of reasonable assumptions coupled with the clinical judgment of experienced physicians in Nicaragua suggest that defining elevated hematocrit in terms of United States standards may be the most appropriate. Specifically, hematocrit values measured in Nicaraguan patients 20% greater than the 50^{th} percentile for each age and gender group in the United States should be defined as elevated.

Changing the definition of shock from the relaxed (hypotension or narrow pulse pressure) to the conservative (hypotension and tachycardia or signs of shock; or narrow pulse pressure and tachycardia or signs of shock) definition, resulted in a modest decrease in the number of severe cases diagnosed (51 and 47, respectively). While this might mean that using the less stringent requirements are satisfactory for future studies, this would not be a wise decision. Shock is a difficult clinical state to diagnose accurately, particularly without repeated blood pressure readings, and reliable indicators of mental status changes, decreasing kidney function, and signs of shock. Utilizing the more stringent criteria, researchers can feel more certain that patients were accurately classified as having shock.

Unlike studies in Southeast Asia and Cuba, secondary infection was not a risk factor for severe disease or hospitalization. Additionally, six of forty-seven (13%) patients with severe disease had a primary infection. Four patients were diagnosed with DHF while two had DSAS. Of these six patients, five were older than one year-old. The antibody dependent enhancement model of secondary infection has no explanation for these patients. The sixth patient was four-months old and might have had maternal antibodies that promoted severe disease, although this is unlikely given results from previous studies. While the rate of secondary infection was not known for the general population, 88% of DF patients and 86% of DFHem patients had secondary infections compared to 88% for patients with severe disease. These findings suggest that having a secondary infection was not the characteristic responsible for severe disease. The pathogenicity of viral strain may have been a contributing factor to severe disease; however, too few samples were analyzed at the time of this analysis to evaluate their contribution to disease severity.

The results from the present study have demonstrated several important risk factors for severe dengue disease that should be further examined in future studies. The current analysis suggest that factors other than secondary infection were responsible for severe disease. Whether these factors are viral in nature, or due to other host factors remains to be solved by future analyses.

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Tables

Table 1. Definitions of disease severity

DF ***WHO 1997**	**Laboratory confirmed dengue infection:** Isolation of dengue virus from serum Demonstration of four-fold or greater increase in IgG IgM antibody titers to one or more dengue virus antigens Detection of dengue virus RNA sequences using reverse transcriptase polymerase chain reaction (RT-PCR) **Must also have 2 or more of the following signs and symptoms:** Acute onset fever Headache Retroorbital pain Myalgia Arthralgia Rash Leukopenia (WBC <4,000)
DFHem	**All of above for DF with some hemorrhagic manifestations:** Epistaxis, petechiae, melena, hematemesis, vaginal bleeding, hematuria, gingival bleeding, or positive tourniquet test
DHF ***WHO 1997**	**Must have:** Laboratory confirmed dengue infection Acute onset fever lasting 2-7 days Any of the following hemorrhagic signs: Positive tourniquet test, petechiae, bleeding from mucosa, GI bleed, injection sites, or any other location Thrombocytopenia (defined as • 100,000 platelets/mm^3) Signs of plasma leakage: Hemoconcentration Pleural effusion Ascites Hypoproteinemia
DSS ***WHO 1997**	**Must Have:** All of the above criteria for DHF in addition to either or both: Narrow pulse pressure (<20 mmHg) and rapid, weak pulse or cold, clammy skin, and restlessness Hypotension for age and rapid, weak pulse, or cold, clammy skin, and restlessness
DSAS	All signs and symptoms of DSS without both thrombocytopenia and hemoconcentration

Table 2. Distribution of dengue cases

	# Cases	%
DF	788	61
DFHem	397	30.8
DHF	32	2.5
DSAS	12	0.9
DSS	3	0.2
No diagnosis with positive dengue result	*59*	*4.6*
Total	1291	45.7
Laboratory negative	1517	53.8
Total	2808	100

Table 3. Hospitalization rates by disease severity

	Hospitalized (%)	Total Cases / Category	Mean Hospital Stay (days)
DF	117 (16)	732	5.54 (n=50)
DFHem	198 (60)	332	6.12 (n=98)
DHF	32 (100)	32	6.18 (n=32)
DSAS	12 (100)	12	6.17 (n=12)
DSS	3 (100)	3	7.7 (n=3)
Severe	47 (100)	47	6.3 (n=47)

Table 4. Clinical site for all enrolled and hospitalized patients

	Source of all laboratory-confirmed patients		Location of hospitalized patients	
	# Patients	%	# Patients	%
Outpatient clinics	700	54	NA	NA
La Mascota	409	32	282	77
HEODRA	181	14	83	23
Total	1290	100	371	100

Table 5. Presentation of cases by month

	DF	DFHem	Severe	Hospitalized
January	3	5	0	5
February	6	2	0	1
March	4	2	1	4
April	6	4	1	5
May	26	4	0	0
June	67	15	0	6
July	136	34	3	18
August	104	53	9	40
September	160	84	12	71
October	205	99	14	105
November	53	72	2	79
December	15	23	4	37
Total	785	397	46	371

Table 6. Age, gender, race, and immune status of study participants

	Negative lab result # (%)	Lab-Confirmed # (%)	DF # (%)	DFHem # (%)	DHF # (%)	DSAS # (%)	DSS # (%)
Age							
0-1	57 (4)	56 (4)	27 (4)	24 (6)	1 (3)	0 (0)	0 (0)
1-4	196 (13)	162 (13)	84 (11)	59 (15)	8 (25)	0 (0)	0 (0)
5-9	349 (23)	374 (29)	182 (23)	149 (38)	14 (44)	12 (100)	3 (100)
10-14	252 (17)	232 (18)	143 (18)	75 (19)	7 (22)	0 (0)	0 (0)
>=15	654 (43)	457 (36)	347 (44)	88 (22)	2 (6)	0 (0)	0 (0)
Mean, Median	16.7, 12	15.3, 11	17.7, 13	11.6, 8	7.8, 6.5	6.4, 6.5	6.7, 6
Range	0-81	0-85	0-85	0-69	0-30	5-8	5-9
Sex							
F	821 (54)	738 (57)	446 (57)	228 (58)	15 (47)	10 (83)	1 (33)
M	695 (46)	552 (43)	342 (43)	168 (42)	17 (53)	2 (17)	2 (67)
Race							
Black	28 (2)	26 (2)	19 (3)	6 (2)	0 (0)	0 (0)	0 (0)
White	232 (17)	168 (14)	110 (16)	49 (13)	4 (13)	2 (17)	0 (0)
Mestizo	1131 (81)	980 (84)	574 (81)	320 (85)	26 (87)	10 (83)	3 (100)
Primary Infection	NA	153 (13)	85 (12)	51 (14)	4 (13)	2 (17)	0 (0)
Secondary Infection	NA	1056 (87)	644 (88)	325 (86)	27 (87)	10 (83)	3 (100)

Table 7. Signs and symptoms by age group of laboratory positive patients

Signs & Symptoms	Patients under 15 # (%)	Patients 15 or older # (%)
Fever	776 (95)	431 (94)
Headache	337 (71)	93 (72)
Arthralgias	480 (61)	344 (79)
Myalgias	276 (59)	88 (68)
Retro-orbital pain	456 (59)	337 (77)
Abdominal pain	440 (56)	213 (49)
Anorexia	431 (54)	198 (45)
Rash	355 (46)	174 (41)
At least one hemorrhagic sign	352 (43)	90 (20)
Petechiae	187 (25)	48 (12)
Tourniquet test	180 (25)	41 (11)
Diarrhea	128 (16)	73 (17)
Epistaxis	107 (14)	19 (5)
Hepatomegaly	36 (5)	1 (.3)
Melena	19 (3)	4 (1)

Table 8. Serotype by dengue severity of laboratory-confirmed patients diagnosed by IgM or IgG

Serotype	DF	DFHem	Severe
DEN-2	50	11	1
DEN-3	4	1	0
DEN-4	9	7	0
Total	63	19	1

Table 9. Serotype collection by month

	DEN-2	DEN-3	DEN-4
January	0	0	0
February	0	2	0
March	3	0	0
April	4	0	2
May	17	3	1
June	8	0	7
July	0	0	1
August	0	0	0
September	1	0	0
October	25	0	4
November	4	0	1
December	0	0	0
Total	62	5	16

Table 10. Description of disease symptoms in unclassified laboratory positive cases

All cases were laboratory confirmed and unclassified by WHO or present study criteria	**Hospitalized** (n=10)			**Not Hospitalized** (n=40)		
	Y	N	Unknown	Y	N	Unknown
General symptoms (myalgias, arthralgias, fever, rash, etc.)	0	8	2	0	38	2
Hemorrhagic signs	0	10	0	0	40	0
Signs of shock (cool skin, cyanosis, agitation)	0	6	4	0	0	40
Both hemoconcentration and thrombocytopenia	3	2	4	0	0	40
Either hemoconcentration or thrombocytopenia (not both)	2	3	4	0	0	40
Mean length of hospital stay (n=6, with 4 unknown)	6 days			NA	NA	NA

Table 11. Relationship between age and hospitalization or severity of disease

Exposure	Outcome	Odds ratio (95% CI)
Under 15	Severe disease	13.17 (3.08-79.91)
Under 15	Hospitalization	5.55 (3.96-7.80)
Under 15 with mild disease	Hospitalization	4.78 (3.37-6.78)
Under 15 at HEODRA	Severe disease	1.74 (.23-15.53)
Under 15 at HEODRA	Hospitalization	.72 (.35-1.46)

Table 12. Primary vs. secondary infection by age with odds ratios for severe disease (conservative criteria: IgG >=20 in first four days)

Age	Primary Infection n (%)	Secondary Infection n (%)	% Severe Disease	Odds Ratio Secondary Infection vs. Severity
<2	37 (46)	44 (54)	1.3	0 (0-14.83)
2-4	15 (20)	60 (80)	5.6	?
4-6	16 (12)	117 (88)	9.1	?
6-8	17 (10)	152 (90)	10.8	.49 (.11-2.45)
8-10	8 (8)	96 (92)	3	.16 (.01-5.08)
10-12	4 (5)	78 (95)	3.7	.05 (0-2.22)
12-14	10 (10)	86 (90)	2.1	?
>=15	44 (10)	386 (90)	.5	?
All ages	191 (16)	1018 (84)	3.9	1.24 (.49-3.33)

Table 13. Dehydration and hospitalization or severity of disease

Exposure	Outcome	Odds ratio (95% CI)
Dehydrated and negative laboratory result	Hospitalization	6.87 (2.69-17.78)
Dehydrated	Severe disease	6.05 (2.10-16.57)
Dehydrated	Hospitalization	5.84 (2.41-14.60)
Dehydrated and under 15	Severe disease	4.08 (1.41-11.27)
Dehydrated and under 15	Hospitalization	4.07 (1.60-10.76)
Drinking less than 8 cups of liquid in preceding 24 hours	Severe Disease	8.51 (1.24-170)
Drinking less than 10 cups of liquid in preceding 24 hours	Hospitalization	40.70 (6.03-804.83)
Drinking less than 12 cups of liquid & a negative dengue test	Hospitalization	4.41 (1.02-26.95)

Table 14. Proximity to health center and hospitalization or severe disease

Exposure	Outcome	Odds ratio (95% CI)
Home >10km from hospital or health center	Severe disease	3.76 (1.82-7.68)
Home >10km from hospital or health center	Hospitalization	9.54 (5.65-16.23)
Home >10km from hospital or health center and mild disease	Hospitalization	9.10 (5.25-15.89)
Home <= 10km from hospital or health center with a negative lab result	Hospitalization	5.14 (3.18-8.32)

Table 15. Association between working while sick, staying home and hospitalization

Exposure	Outcome	Odds ratio (95% CI)
Worked while sick	Hospitalization	.16 (.04-.54)
Stopped work while sick	Hospitalization	.43 (.28-.67)
Stopped school when sick	Hospitalization	.66 (.48-.90)
Stopped school and lived >10km from hospital	Hospitalization	.91 (.25-3.28)
Stopped school and lived <= 10km from hospital	Hospitalization	.55 (.37-.81)
Remained at home while sick	Hospitalization	.68 (.48-.98)
Remained in bed while sick	Hospitalization	.70 (.52-.95)

Table 16. Relaxed vs. conservative assumptions for population hematocrit levels

	Relaxed * 50th percentile of U.S. standards for women and children; 20% > 50th percentile for adult men	**Conservative** *20% > 50th percentile of U.S. standards for all age and gender groups
DF	788	788
DFHem	359	397
Mild	1147	1185
DHF	69	32
DSAS	4	12
DSS	12	3
Severe	85	47
Total	1232	1232

Table 17. Distribution of disease following change in shock definition

	Shock defined by **hypotension** & tachycardia or signs of shock; or **narrow pulse pressure** & tachycardia or signs of shock	Shock defined by **hypotension** or **narrow pulse pressure** only
DF	788	788
DFHem	397	393
Mild	1185	1181
DHF	32	27
DSAS	12	16
DSS	3	8
Severe	47	51
Total	1232	1232

Table 18. Number of secondary infections with conservative and relaxed criteria

	Relaxed *IgG titer >20 in first 4 days of symptoms		**Conservative** *IgG titer >=20 in first 4 days of symptoms	
	# Secondary infections	**% of total infections**	**# Secondary Infections**	**% of total infections**
DF	615	84.3	644	88.3
DFHem	317	84.3	325	86.4
DHF	27	87.0	27	87.0
DSAS	10	83.3	10	83.3
DSS	3	100	3	100
Severe	40	86.9	40	86.9
Total	978		1009	

Table 19. Comparison of case distribution 1999 vs. 1998*

*Harris et al, 2000

	1999		1998	
	# Cases	%	# Cases	%
Laboratory Positive	1291	46	612	60
DF	788	61	260	43
DFHem	397	31	265	43
DHF	32	2.5	46	8
DSAS	12	1	18	3
DSS	3	0.2	20	3
No Diagnosis	*59*	*4.6*	*NA*	*NA*
Total	1291	100	612	100

Table 20. Comparison of age, gender, and hospital stay 1999 vs. 1998*

*Harris et al, 2000

	1999		1998	
	Mild Disease	Severe Disease	Mild Disease	Severe Disease
Gender M (%)	43	45	47	54
F (%)	57	55	53	46
Mean age (years)	15.7	7.4	15.7	11.0
Mean length of hospital stay (days)	5.9	6.3	5.4	6.1

Table 21. Signs and symptoms 1999 vs. 1998*

*Harris et al, 2000

Signs & Symptoms	1999		1998	
	Children <15 Years (%)	Adults >= 15 years (%)	Children <15 Years (%)	Adults >= 15 years (%)
Fever	95	94	92	99
Headache	71	72	77	91
Arthralgias	61	79	52	82
Myalgias	59	68	55	83
Retro-orbital pain	59	77	55	83
Vomiting	NA	NA	55	44
Abdominal Pain	56	49	46	48
Rash	46	41	62	56
Tourniquet test	25	11	37	33
Petechiae	25	12	38	35
Diarrhea	16	17	17	15
Epistaxis	14	5	17	11
Hepatomegaly	5	0.3	3	7
Melena	3	1	4	4

Table 22. Viral serotype by severity 1999 vs. 1998*

*Harris et al, 2000

Serotype	1999			1998		
	Mild Disease	Severe Disease	Total	Mild Disease	Severe Disease	Total
DEN-2	61	1	62	9	3	12
DEN-3	5	0	5	97	13	110
DEN-4	16	0	16	NA	NA	NA
Total	82	1	83	106	16	122

Figures

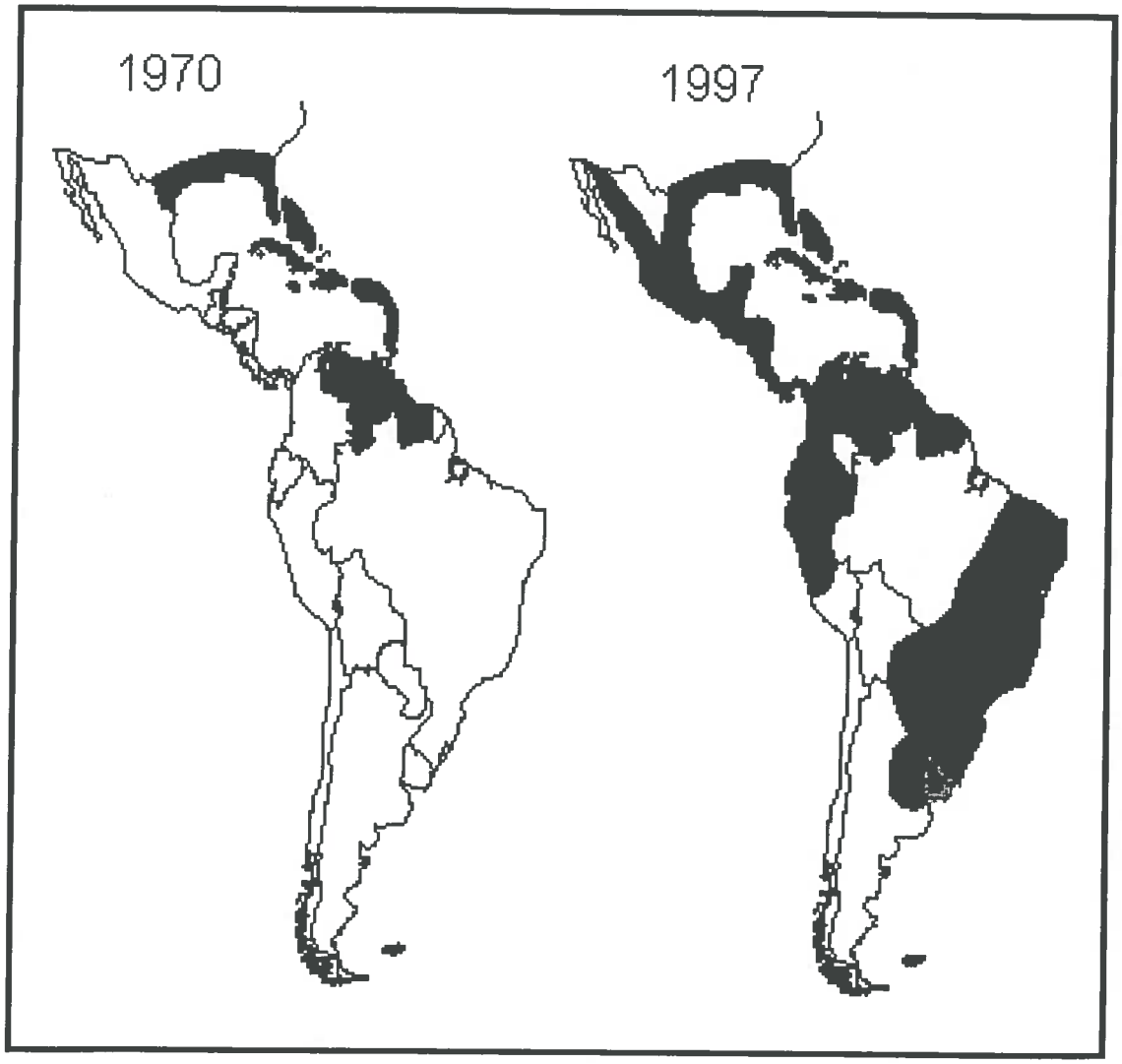

Figure 1. Distribution of Aedes aegypti in the Americas in 1970 and 1997

(http://www.cdc.gov/ncidod/dvbid/dhspot98.htm)
Centers for Disease Control and Prevention, 2000

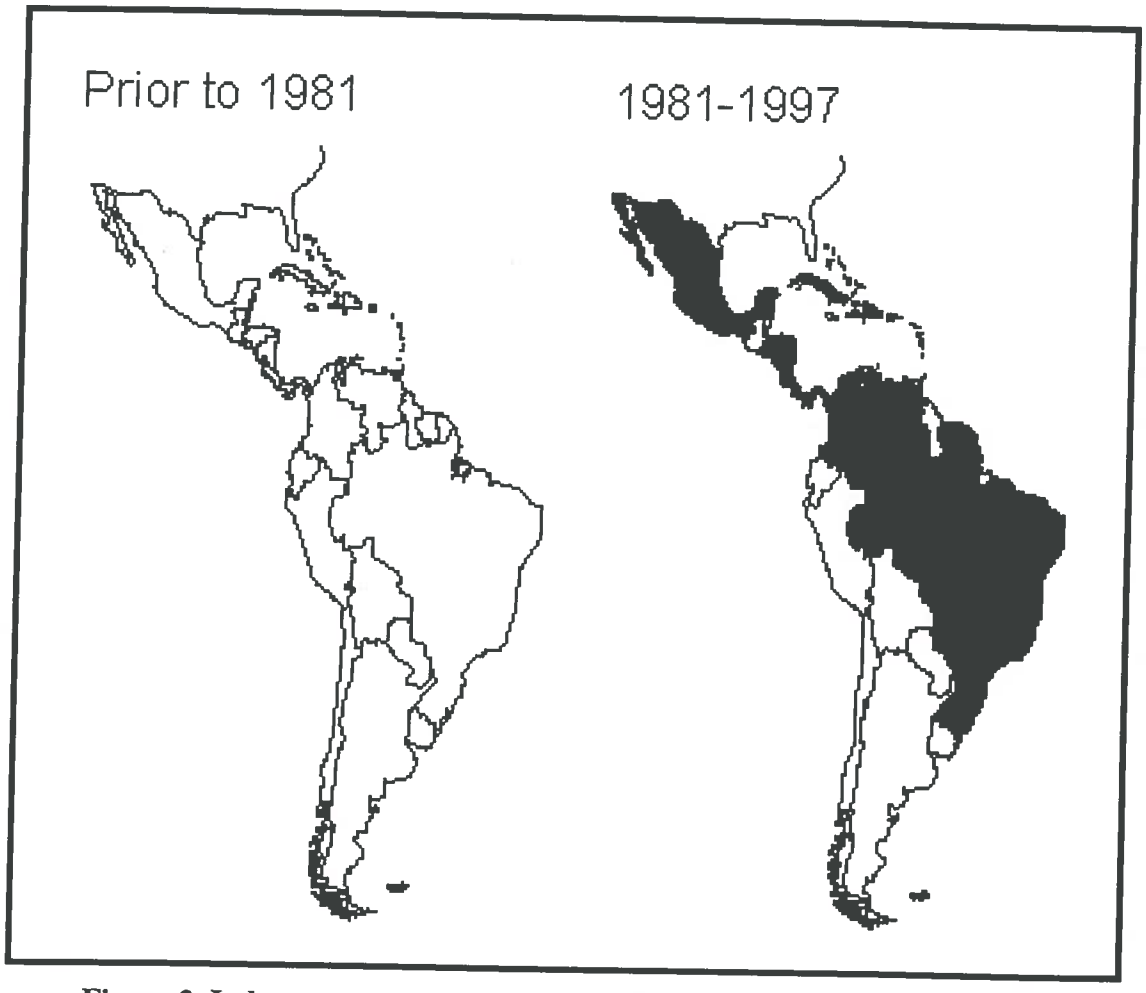

Figure 2. Laboratory-confirmed DHF before 1981 and from 1981 to 1997

(from http://www.cdc.gov/ncidod/dvbid/dhspot98.htm)
Centers for Disease Control and Prevention, 2000

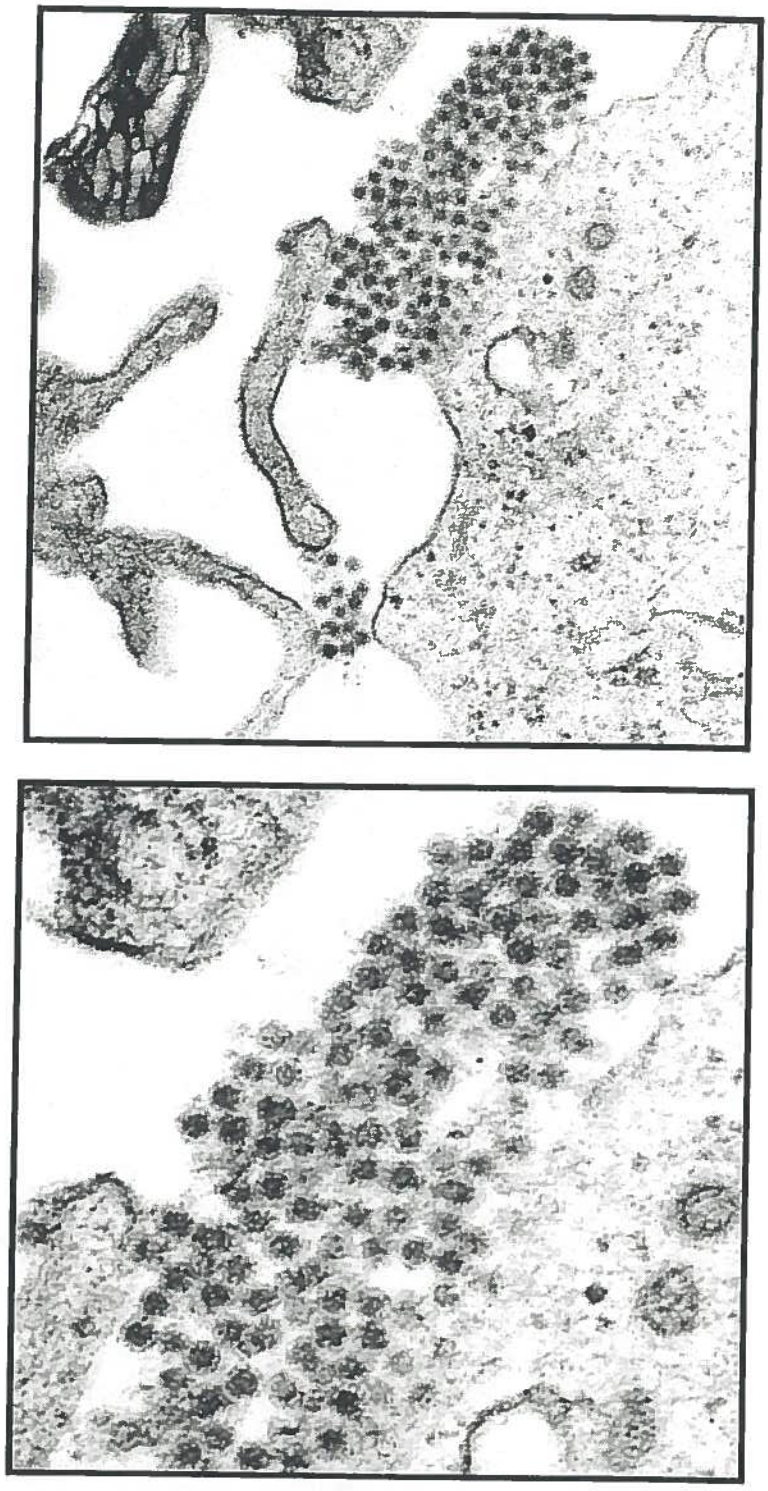

Figure 3. Budding DEN-2 magnified 123,000 times

(http://www.cdc.gov/ncidod/dvbid/dhspot98.htm)
Centers for Disease Control and Prevention, 2000

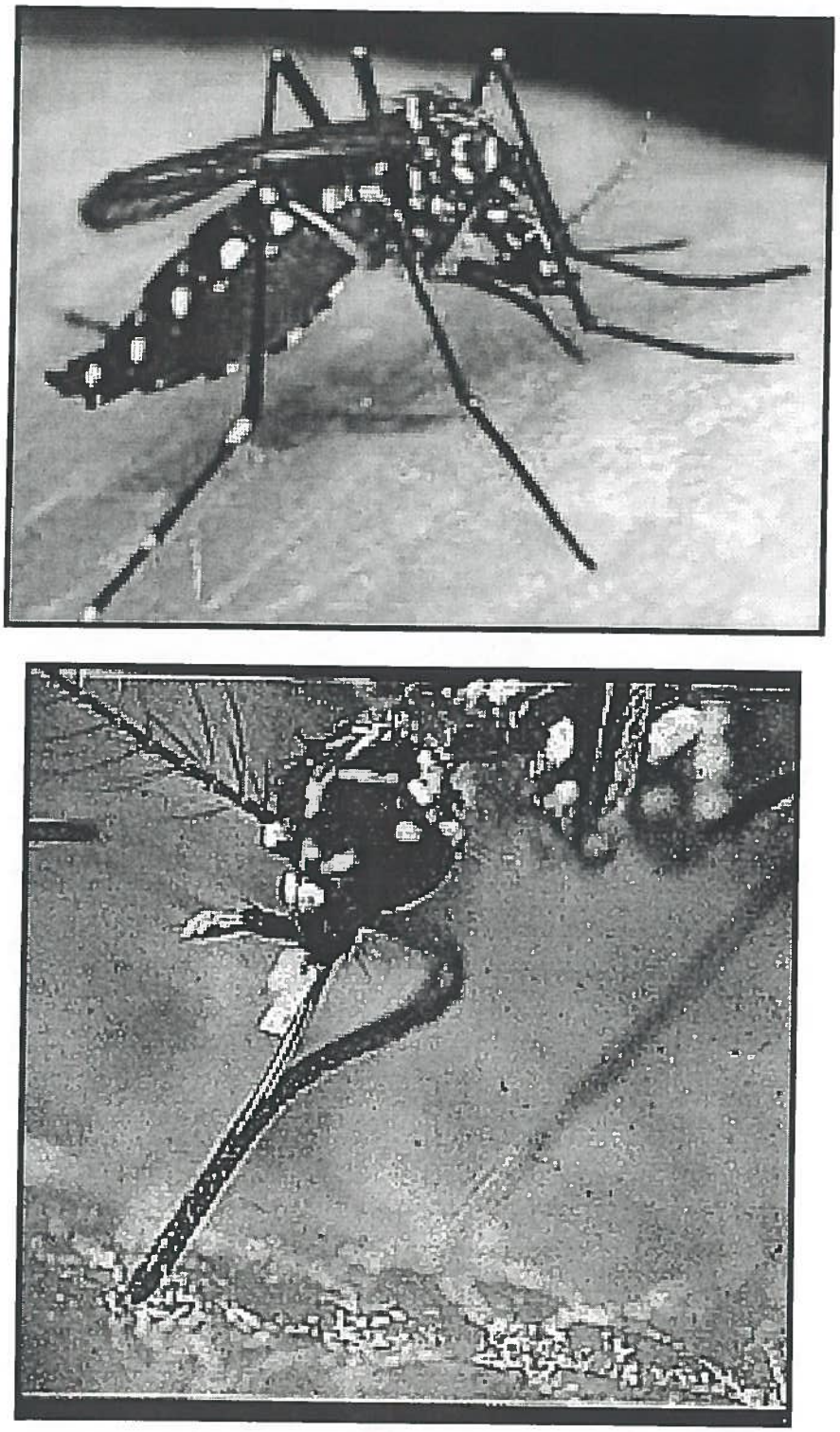

Figure 4. Aedes aegypti

(http://www.cdc.gov/ncidod/dvbid/dhspot98.htm)
Centers for Disease Control and Prevention, 2000

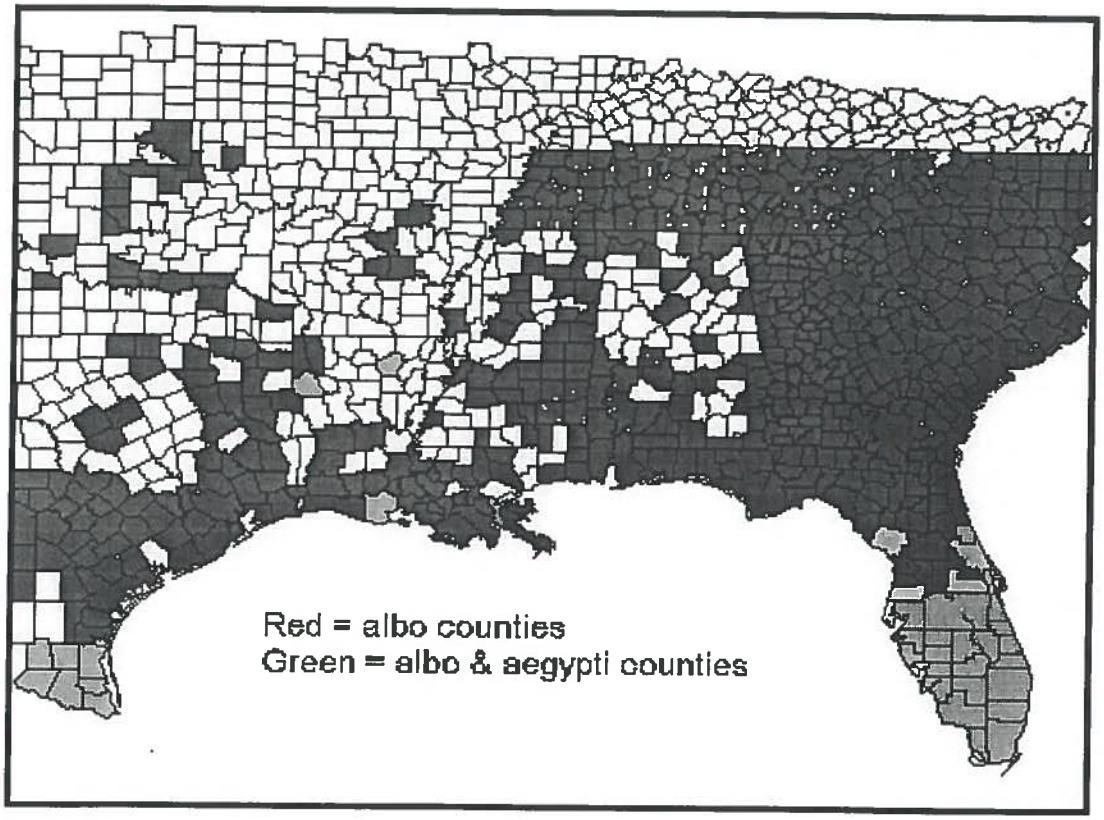

Figure 5. Distribution of *Aedes albopictus* and *Ae. aegypti* in the Southern United States in 1997

(http://www.cdc.gov/ncidod/dvbid/dhspot98.htm)
Centers for Disease Control and Prevention, 2000

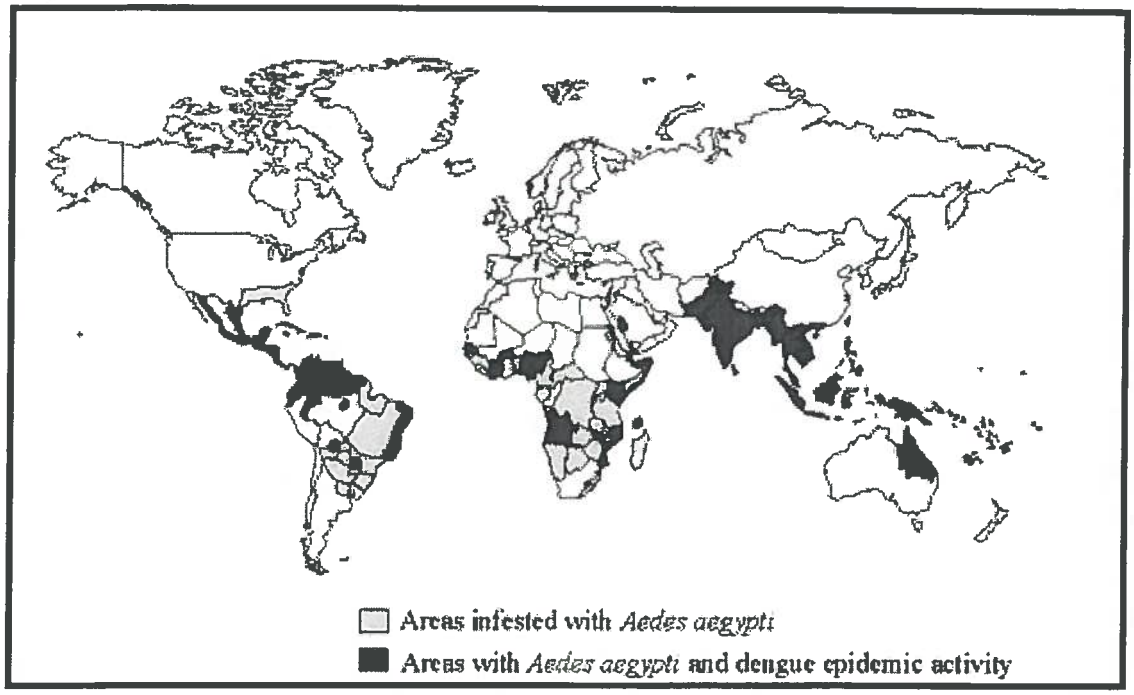

Figure 6. World distribution of Aedes aegypti and dengue epidemics in 1997

(http://www.cdc.gov/ncidod/dvbid/dhspot98.htm)
Centers for Disease Control and Prevention, 2000

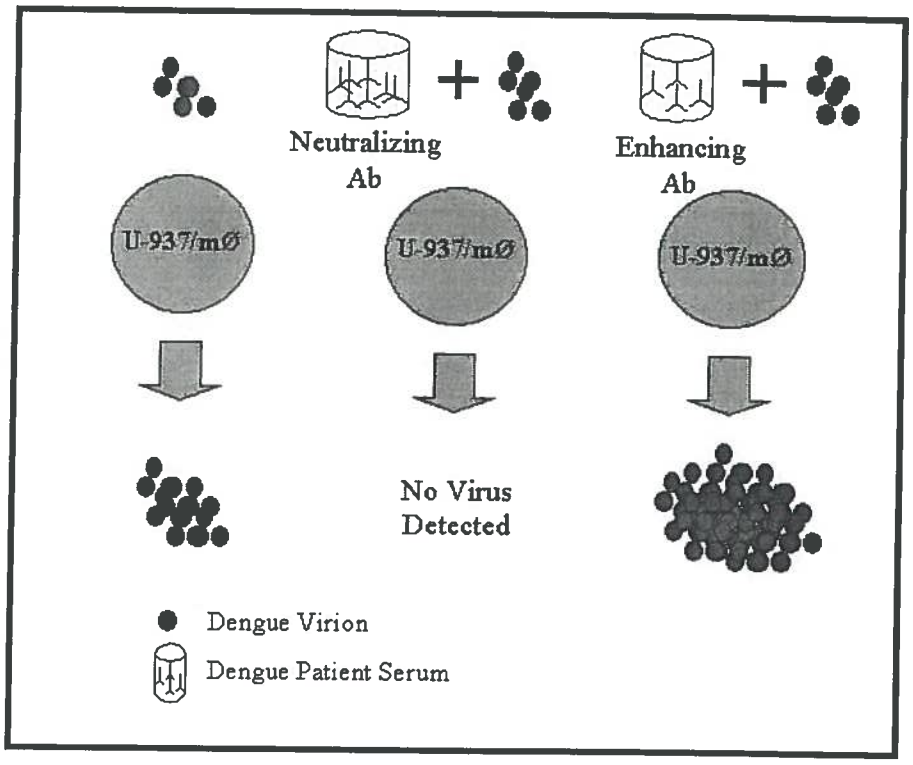

Figure 7. Antibody dependent enhancement

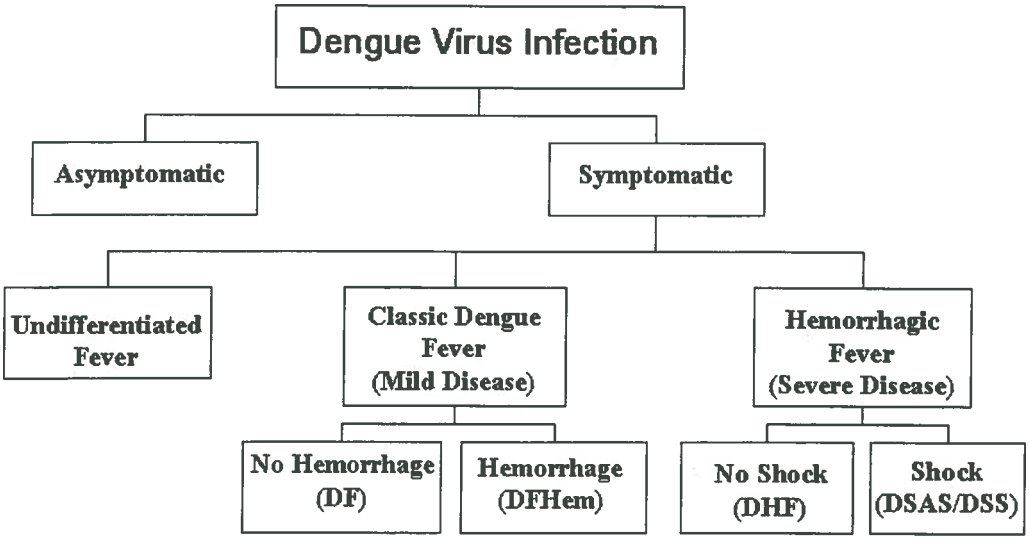

Figure 8. Dengue classification scheme

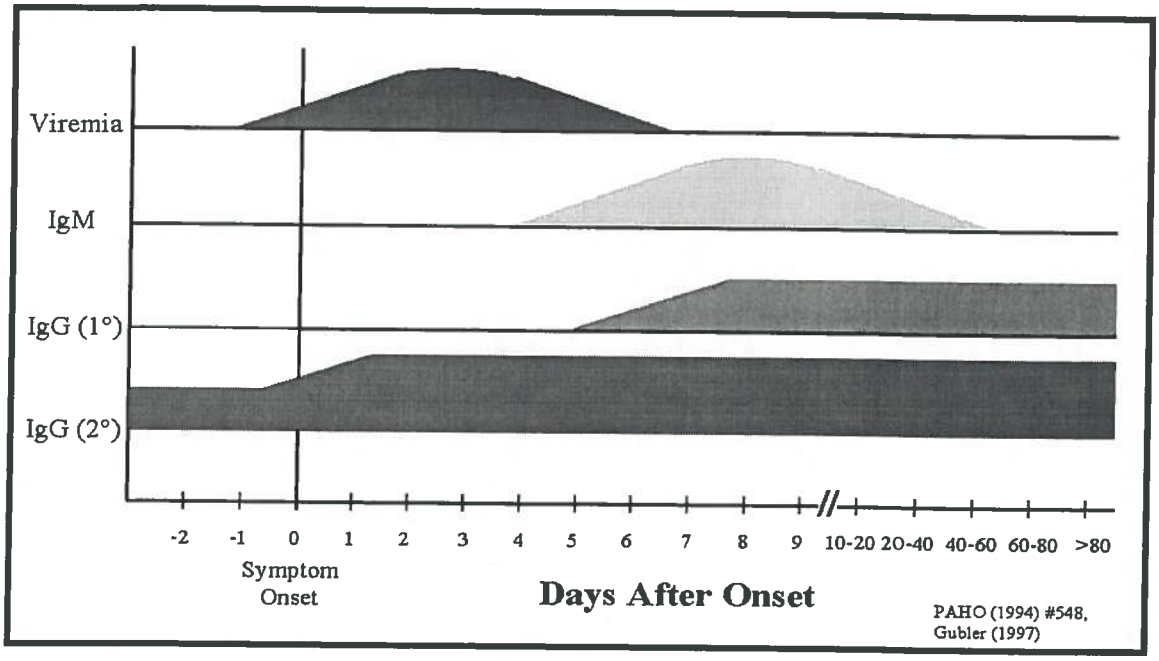

Figure 9. Viremia and antibody levels in patients with dengue virus

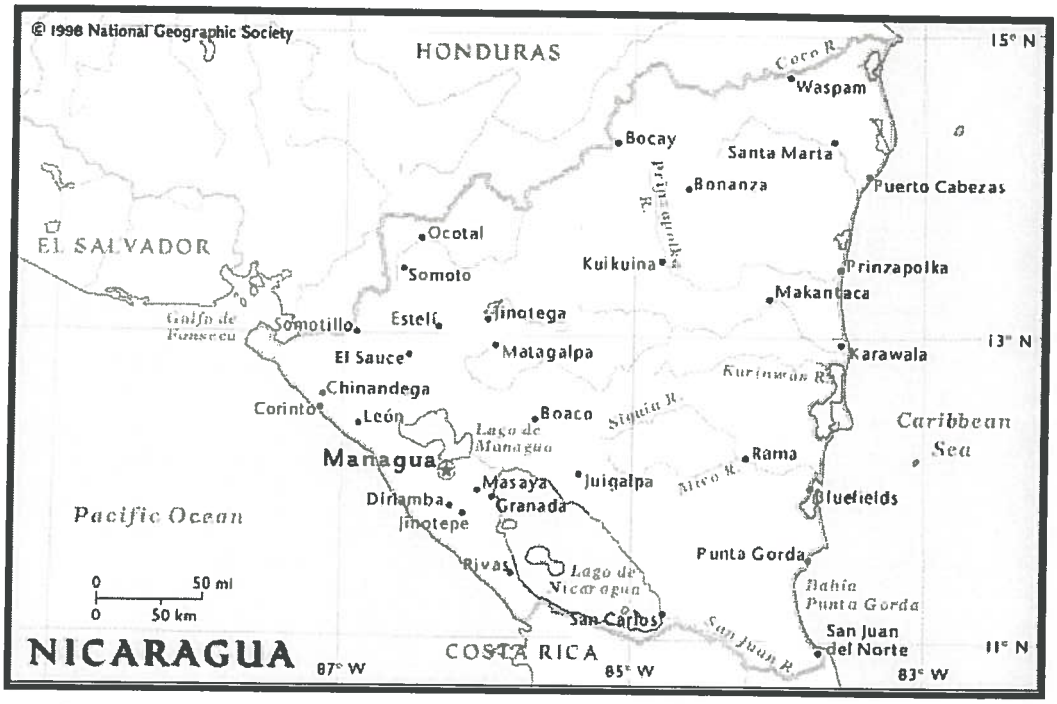

Figure 10. Map of Nicaragua

(www.nationalgeographic.com/expeditions)

National Geographic, 2000

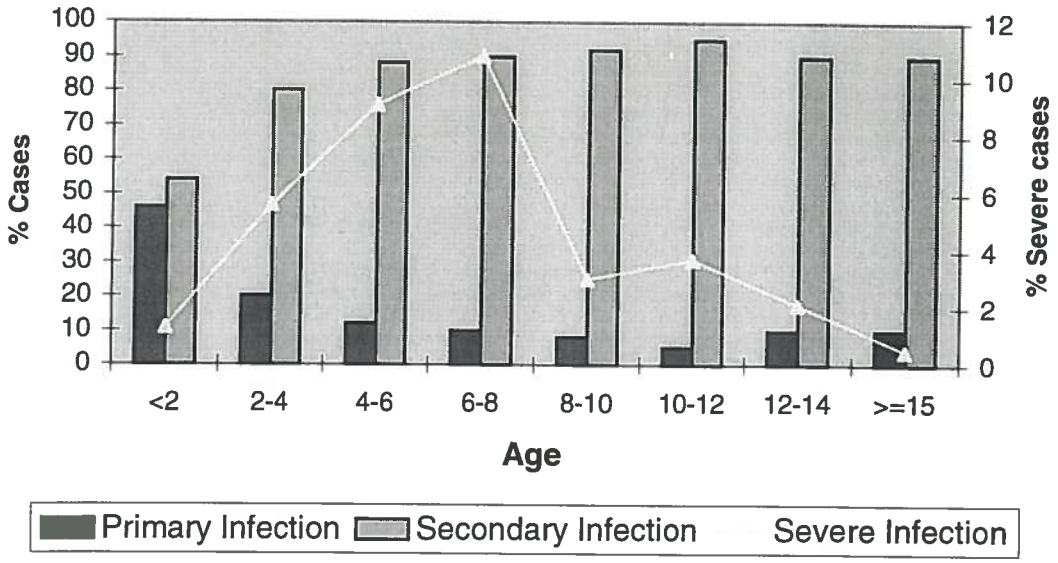

Figure 11. Relationship of severity to immune status

Appendices

Appendix 1. Letter of consent in English

LETTER OF CONSENT

Project Coordinators:
Dr. Angel Balmaseda, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud
Dr. Eva Harris, School of Public Health, University of California, Berkeley

Title of the Research Project:
Risk Factors for Severe Dengue in Nicaragua

You are invited to voluntarily participate in a research study about dengue in Nicaragua. The following describes the study and your participation in it. Please listen carefully and clarify any questions with the interviewer.

Background and Objectives:
Since 1985, Nicaragua has experienced annual outbreaks of dengue, infecting thousands of people with the virus. However, the true burden of the disease and why some people get severe dengue have not been well studied in Nicaragua. The goal of this study is to identify aspects of the virus and the patient that may influence the development of severe disease or contribute to a mild outcome, as well as to investigate the current situation of dengue in the country. New laboratory techniques will also be evaluated, with the goal of improving dengue diagnosis. Through this investigation, we hope to advance knowledge about dengue in order to improve its control and prevention.

Procedures: In Nicaragua, a blood sample is routinely taken from patients thought to have dengue fever, and a short questionnaire is filled out at the health post they attend. They are scheduled for an appointment 7 days later for a second blood sample to be drawn. If you voluntarily accept to participate in this study or accept that your child participates, the remainder of your blood sample will be used for additional laboratory tests. In addition, you will be administered a questionnaire that includes general information about you, what fluids and medicines you have taken, and what you have experienced during your illness. When you return for your second appointment, you will be administered a questionnaire very similar to the first, but which focuses on the course of your illness. If you are hospitalized, your medical records will be reviewed by investigators and notes taken regarding your physical signs, the results of your lab tests, and what fluids you have been given. This information will be stored in a computer to which only investigators associated with this study have access. Your name will not be used in any publications.

Risks: The blood sample is taken as part of routine diagnostic procedures; only the left-over portion will be used for this study. There are minimal risks associated with taking the blood sample; however, this procedure will be done in the hospital or health center by experienced personnel, and necessary measures will be taken to avoid complications.

Benefits: If you choose to participate in this study, you will receive a definitive diagnosis if your illness was caused by dengue virus. You will also receive information about the disease and its potential complications. Your participation will help scientific investigation aimed towards a better understanding of dengue and towards improving its control and prevention.

Alternatives: You can choose not to participate in the study without affecting the routine processing of your sample for dengue diagnosis.

Who to contact: If you have any questions or problems related to this study, please do not hesistate to contact Dr. Angel Balmaseda at the Centro Nacional de Diagnóstico y Referencia a the following numbers: 289 77 23 or 289 46 04.

Consent:

1) I recognize that my participation (or the participation of my child) in this study is voluntary. I have the freedom to participate or withdraw at any moment.

2) I permit the remainder of the blood sample collected during my medical visit to be used in this study. I also permit the investigators of this study to use the information collected in the questionnaires and grant them access to my hospital medical records that relate to this illness.

Signature of the patient or his/her guardian	Name	Date

Appendix 2. Letter of consent in Spanish

CARTA DE CONSENTIMIENTO

Coordinadores del Proyecto:
Dr. Angel Blamaseda, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud
Dra. Eva Harris, School of Public Health, University of California, Berkeley

Titulo del Proyecto de Investigación:
Estudio de Factores de Riesgos para Enfermedad Severa de Dengue en Nicaragua

Por este medio, le invitamos a participar voluntariamente en un estudio de investigación sobre dengue en Nicaragua. La información a continuación describe de forma resumida el estudio y su participación en él. Escuche con atención y aclare cualquier duda o pregunta con el entrevistador.

Objetivos y Antecedentes: Desde 1985 hasta la fecha, Nicaragua ha sufrido casi anualmente de brotes y epidemias de dengue, infectandose miles de personas con el virus. Sin embargo la real situación de esta enfermedad y los factores de riesgo que la caracterizan no han sido bien estudiados en Nicaragua. El objetivo de este estudio es determinar factores virales, inmunológicos y del paciente que puedan incidir en la evolución hacia enfermedad severa, asi como determinar la verdadera situación del dengue en el pais. Se investigará el manejo de los casos de dengue tanto en la casa como en el hospital para identificar potenciales factores de riesgo o factores prtoectivos.Además, se evaluará nuevas técnicas de laboratorio con el fin de mejorar el diagnóstico del dengue. A través de esta investigación, se espera avanzar los conocimientos de esta enfermedad para mejorar su control y prevención.

Procedimientos: En Nicaragua, a los pacientes sospechosos de dengue, se le toma una muestra de sangre para diagnóstico y se adminstra un cuestionario breve en la unidad de salud a las que ellos acudieron. Se le cita para la toma de una segunda muestra de sangre 7 días despues. Si usted de manera voluntaria acepta participar en el estudio o acepta que su hijo participe, se utilizará el restante de la muestra de sangre para análisis adicional en el laboratorio. También, le será llenado un cuestionario que incluye datos generales y sintomas que ha padecido durante su enfermedad. Cuando regresa para su segunda muestra, se le llenará otro cuestionario muy similar al primero, pero donde se enfocará fundamentalmente sobre la evolución de su enfermedad. La información adicional a la ficha nacional será almacenada en una computadora a la cual solo tienen acceso los investigadores asociados con este estudio. Su nombre no estará utilizado en ninguna publicación.

Riesgos: La muestra de sangre se toma como parte del diagnóstico rutinario; solo se utiliza los restantes en este estudio. Hay riesgos minimos relacionados con la extracción de la muestra; sin embargo, esta sera realizada en los centros hospitalarios o centros de salud por personal experimentados y se tomaran todas las medidas necesarias para evitar cualquier complicacion.

Beneficios: Si Ud. opta participar en el estudio, recibirá un diagnóstico definitivo de si su enfermedad fue causado por el virus dengue; tambien recibirá información sobre la enfermedad y sus potenciales complicaciones. Su participación ayudará a la investigación científica para entender mejor el dengue a nivel clínico, epidemiológico y biológico y asi mejorar su control y prevención.

Altnernativas: Ud. puede optar por no participar en este estudio sin que esto afecte el proceso rutinario de su diagnóstico.

A quien contactar: Para cualquier duda o problema relacionado con el estudio usted puede contactar al Dr. Angel Balmaseda en el Centro Nacional de Diagnóstico y Referencia a los telefonos 2 89 77 23 6 2 89 46 04.

Consentimiento:

1) Reconozco que mi participacion (o la participación de mi hijo) en este estudio es voluntaria. Tengo libertad de participar en el estudio o retirarme de él en cualquier momento.

2) Permito que se utilice en el estudio el restante de la muestra de sangre que se colectó como parte de mi visita médica. También permito que los investigadores del proyecto utilicen la información colectada en los cuestionarios y que tengan acceso a los expedientes médicos en el hospital que sean relevantes a mi enfermedad actual.

_____	_____	_____
Firma del participante o padre/tutor del menor	Nombre	Fecha

Appendix 3. Questionnaire in English

FIRST QUESTIONNAIRE

General Information: ID_____

Date ___/___/____ Silais_____ Municipality _____

Health Center _____First and Last

Names_____

Mother's and Father's

Name_____

Exact Address_____

Age:___ (Circle Years or Months) Sex ___ Ethnicity: White____ Black___ Mestizo___

Indigenous____ Urban:___ Rural: ____ Pregnant___ (Y/N) Months ____ Weeks ___ Weight ____ Height _____

Do you work outside your home? ____ (Y/N) Where? _____

Occupation: _____ Have you traveled in the last month? ____ (Y/N) If so, where? _____

Date of onset of symptoms:___/___/____ Date of sample collection: ___/___/___

Chronic disease: Asthma ____ (Y/N) Allergies ____ (Y/N) Respiratory? ____ Dermatologic? ____ Diabetes ____ (Y/N)

Other_____

Other acute illness: ____ (Y/N) Pneumonia: ___ Malaria: ____ Urinary tract Infection: ____

Other: _____

Mark with a Y, N, or U (Unknown)

Symptoms:		**Signs:**	
Fever	_____	Epistaxis	_____
Rash	_____	Petechiae	_____
Retro-orbital pain	_____	Melena	_____
Vomiting	_____	Hematemesis	_____
Arthralgia	_____	Vaginal Bleeding	_____
Abdominal pain	_____	Hematuria	_____
Diarrhea	_____	Gigivitis	_____
Constipation	_____	Pleural Effusion	_____
Cough	_____	Hepatomegaly	_____
Anorexia	_____	Cold skin	_____
Pos.Torn. Test.	_____	Ascites	_____

Temp _____ BP: ____/____ mm Hg Pulse: _____ /minute Capillary filling: _____ seconds

Hospitalized _____(Y/N) Observation ____ (Y/N) Ward ____ (Y/N) Deceased ___ (Y/N) Date: _____

Name of interviewer: _____

Laboratory:
Hematocrit: _____ Hemaglobin: _____ Platelets: _____ WBC: _____ Linf: _____ Band: _____
Mono: _____ AST: _____ ALT: _____ PT: _____ PTT: _____ Albumin: _____
Total Proteins: _____ Thick Smear: _____

Results:
ELISA IgM: _____ HI _____ Inhib.ELISA: _____ RT-PCR: _____ Viral Isol.: _____ Final Res.: _____

BACKGROUND (FIRST QUESTIONNAIRE)

How much liquid have you drunk in the last 24 hours? Less than 4 glasses ____ 4 or more glasses _____

Which liquids? (Mark Y or N)

Water: _____ Fruit juice _____ Soda _____ Lemonade: _____

Milk: _____ Coffee: _____ Homemade ORT ____ Commercial ORT ____

Beer: _____ Alcohol: _____ Herb tea: _____ Which? _____

Other fluids: _____ (Y/N) Which ones? _____

Have you used any of the following medications over the course of the disease?

	Who ordered it? Friend/family	HC	Private
Doctor			
Acetaminophen (Panadol, Tylenol): _____ (S/N)	_____	_____	_____
Aspirin: _____ (Y/N)	_____	_____	_____
Antibiotics: ___ (Y/N) Which ones? _____	_____	_____	_____
Diclofenac: _____ (Y/N)	_____	_____	_____
Ibuprofen: _____ (Y/N)	_____	_____	_____
Traditional medicine____ (Y?N) Which? _____	_____	_____	_____
Other? ____ (Y/N) What? _____	_____	_____	_____

Vitamin C: ___ (Y/N) Multivitamins: ___ (Y/N) Do you take vitamins regularly? _____ (Y/N)

Have you visited another health center for this disease? _____ (Y/N)

Health Center _____ (Y/N) Private Clinic ____ (Y/N) Other _____ (Y/N)

What?_____

Distance between your home and the hospital o C/S ____ km

How did you arrive? Car___Bus ___On foot ___ By horse __

During this illness:

(Mark as appropriate): Did you stop working? ___ (Y/N) For how many days? ____

Did you stop going to school? ____ (Y/N) For how many days? ____

Did you stop doing household chores? ___ (Y/N) For how many days? ____

Did you stay in bed? _____ (Y/N) For how many days? _____

Did you have to work despite feeling sick? _____ (Y/N) For how many days? _____

Last time that you ate? ____ hours ago Last time that you drank? _____ hours ago

Last time that you urinated? _____ hours ago

Measure of dehydration upon arrival:

Decreased urine output: _____ (Y/N) Poor lacrimation: _____ (Y/N) Dry mucusa: _____ (Y/N)

Skin turgor: _____ (Y/N) Sunken orbit: _____ (Y/N) Sunken fontanels: _____ (Y/N)

SECOND QUESTIONNAIRE

General Data: ID_____

Date ___/___/____ Silais_____ Municipality _____

Health Center _____

First and Last Names _____

Mother 's and Father's Name_____

Exact

Address_____

Age_____ Years____ Months Sex ____ Weight ____ Height _____

Date of onset of symptoms:___/___/____ Date of sample collection: ___/___/___

Additional acute illness: ____ (Y/N) Pneumonia: ___ Malaria: ____ Urinary Tract Infection: ____

Other: _____

During this illness did you experience any of the following signs or symptoms?

Mark with a Y, N, or U (Unknown)

Symptoms:		**Signs:**	
Fever	_____ Temp _____	Epistaxis	_____
Rash	_____	Petechiae	_____
Retro-orbital pain	_____	Melena	_____
Vomiting	_____	Hematemesis	_____
Arthralgia	_____	Vaginal Bleeding	_____
Abdominal pain	_____	Hematuria	_____
Diarrhea	_____	Gigivitis	_____
Constipation	_____	Pleural Effusion	_____
Cough	_____	Hepatomegaly	_____
Anorexia	_____	Cold , clammy skin	_____
Pos. Torn. test	_____	Ascites	_____

Laboratory:

Hematocrit: _____ Hemaglobin: _____ Platelets: _____ WBC: _____ Linf: _____ Band: _____

Hospitalized ____ (Y/N) Observation ____ (Y/N) Ward ___ (Y/N) Stay: ____ days

Diseased _____ (Y/N) Date: ___/___/____

Name of Interviewer: _____

BACKGROUND (SECOND QUESTIONNAIRE)

DURING THIS ILLNESS:

Have you drunk:

Water: _____ (Y/N) Every day? ____ (Y/N) Fruit juice _____ (Y/N) Every day? _____(Y/N)

Soda _____ (Y/N) Every day? ____ (Y/N) Lemonade: _____ (Y/N) Every day? _____ (Y/N)

Milk: _____ (Y/N) Every day? ____ (Y/N) Coffee: _____ (Y/N) Every day? ____ (Y/N)

Homemade ORT: ____ (Y/N) Every day? ____ (Y/N) Commercial ORT ____ (Y/N) Every day? _____ Beer: ____ (Y/N) Every day? ____ (Y/N) Alcohol _____ (Y/N) Every day? ____ (Y/N)

Chamomile tea: _____ (Y/N) Every day? _____ Other fluids: ____ (Y/N) Which ones?_____

Have you used any of the following medications :

	Who ordered it?	Friend/family	HC	Private
Doctor				
Vitamin C: ___ (Y/N) Multivitamins: ___ (Y/N)		_____	_____	_____
Acetaminophen _____ (Y/N)		_____	_____	_____
Aspirin: _____ (Y/N)		_____	_____	_____
Antibiotics: ___ (Y/N) Which ones? _____		_____	_____	_____
Diclofenac: _____ (Y/N)		_____	_____	_____
Ibuprofen: _____ (Y/N)		_____	_____	_____
Traditional medicine ____ (Y/N) Which ones? _____		_____	_____	_____

Have you visited another health center for this illness? _____ (Y/N)

Health Center _____ (Y/N) Private clinic____ (Y/N) Other _____ (Y/N) Which one?_____

Hospital _____ (Y/N) Admitted? _____ (Y/N)

(Mark as appropriate): Did you stop working? ___ (Y/N) For how many days? ____

Did you stop going to school? ____ (Y/N) For how many days? ____

Did you stop doing household chores? ___ (Y/N) For how many days? ____

Did you stay in bed? _____ (Y/N) For how many days? _____

Did you have to work despite feeling sick? _____ (Y/N) For how many days? _____

Did you eat normally? _____ (Y/N)

Appendix 4. Questionnaire in Spanish

ENCUESTA DE LA PRIMERA MUESTRA

Datos Generales:

ID_____

Fecha ___/___/____ Silais_____ Municipio _____ Unidad de Salud _____ Nombres y Apellidos_____

Nombre del papá y mamá _____

Dirección Exacta

_____

Edad_____ (Indicar Años o Meses) Sexo ____ Raza: Blanco___ Negro___ Mestizo___ Indigena____ Urbano:___ Rural: ____ Embarazada____ (S/N) Meses ____ Sem. ___ Peso ____ Talla _____

Trabaja fuera de la casa? ____ (S/N) Donde?

_____

Ocupación: _____Viajó en el último mes? ____ (S/N) Donde?

_____

Fecha de inicio de sintomas:___/___/____ Fecha de toma de Muestra: ___/___/___

Enfermedad Crónica: Asma ____ (S/N) Alergia ____ (S/N) Respiratorio? ____ Dermatológico? ____ Diabetes ____ (S/N)

Otro_____

Enfermedad aguda adicional: ____ (S/N) Neumonia: ___ Malaria: ____ Infección de via urinaria: ____ Otro:

_____

Marque con una S, N, o D (Desconocido)

Sintomas:

Fiebre	_____
Rash	_____
Dolor Retroobitario	_____
Vómitos	_____
Artralgias	_____
Dolor abdominal	_____
Diarrea	_____
Estrenimiento	_____
Tos	_____
Anorexia	_____
P.Torniquete Pos.	_____

Signos:

Epistaxis	_____
Petequias	_____
Melena	_____
Hematemesis	_____
Hemorragia vaginal	_____
Hematuria	_____
Gigivorragia	_____
Derrame pleural	_____
Hepatomegalia	_____
Piel fría	_____
Ascitis	_____

Temp _____ PA: ____/_____mm Hg Pulso: _____ /minuto Llenado capilar: _____ segundos

Hospitalizado _____ (S/N) Observación ____ (S/N) Sala ____ (S/N) Fallecido _____ (S/N)

Fecha: _____

Nombre del encuastador: _____

Laboratorio:
Hematocrito: _____ Hemaglobina: _____ Plaquetas: _____ GB: _____ Lin: _____ Segm: _____
Mono: _____ TGO: _____ TGP: _____ TP: _____ TPT: _____ Albuminas: _____
Proteinas Totales: _____ Gota gruesa: _____

Resultados:
ELISA IgM: _____ IH _____ ELISA Inhib.: _____ RT-PCR: _____ Aisl. Viral: _____ Res. final: _____

ANTECEDENTES (PRIMERA MUESTRA)

Cuanto líquido ha tomado en los últimos 24 horas? Menos que 4 vasos_____ 4 o mas vasos_____

Que tipo de líquido? (Marque S o N)

Agua: _____ Refresco _____ Gaseosa _____ Limonada: _____

Leche: _____ Cafe: _____ Suero oral casero ____ Suero oral comercial____

Cerveza: _____ Licor _____ Te de hierba: _____ Cual? _____

Otros liquidos: _____ Cuales? _____

Usó los siguentes medicamentos durante esta enfermedad?

	Quien lo ordeno? Amigo/familia	C/S	Médico privado
Acetaminofen (Panadol, Tylenol): _____ (S/N)	_____	_____	_____
Aspirina: _____ (S/N)	_____	_____	_____
Antibioticos: ___ (S/N) Cuales? _____	_____	_____	_____
Diclofenac: _____ (S/N)	_____	_____	_____
Ibuprofeno: _____ (S/N)	_____	_____	_____
Medicina tradicional ____ (S/N) Cual? _____	_____	_____	_____
Otro? ____ (S/N) Cual? _____	_____	_____	_____

Vitamina C: ___ (S/N) Multivitaminas: ___ (S/N) Usa vitaminas de manera rutinaria? _____ (S/N)

Ha visitado otra unidad de salud para esta enfermedad? _____ (S/N)

Centro de Salud _____ (S/N) Clínica privada ____ (S/N) Otro _____ (S/N)

Cual?_____

Distancia entre su casa y el hospital o C/S ____ km Como vino? Carro ___Bus ___A pie ___ A caballo __

Durante esta enfermedad:

(Segun sea apropiado): Dejo de trabajar? ___ (S/N) Cuantos dias? ____

Dejo de ir a la escuela? ____ (S/N) Cuantos dias? ____

Dejo de hacer labores en el hogar? ___ (S/N) Cuantos dias? ____

Ha estado en cama? _____ (S/N) Cuantos dias? _____

Tuvo que trabajar a pesar de sentirse mal? _____ (S/N) Cuantos dias? _____

Ultima vez que comió? Hace ____ horas Ultima vez que tomó líquidos? Hace _____ horas

Ultima vez que orinó? Hace _____ horas

Medición de deshidratacion al ingreso:

Reduccion de la orina: _____ (S/N) Llanto sin lagrima: _____ (S/N) Mucosa seca: _____ (S/N)

Pliegue cutanea: _____ (S/N) Globo ocular hundido: _____ (S/N) Fontanelas hundidas: _____ (S/N)

ENCUESTA DE LA SEGUNDA MUESTRA

Datos Generales:

Fecha ___/___/____ Silais_____ Municipio _____ Unidad de Salud _____ Nombres y Apellidos_____

Nombre del papá y mamá

_____

Direccion Exacta

_____

Edad_____ Años ____ Meses Sexo ____ Peso ____ Talla _____

Fecha de inicio de sintomas:___/___/____ Fecha de toma de Muestra: ___/___/___

Enfermedad aguda adicional: ____ (S/N) Neumonia: ___ Malaria: ____ Infeccion de via urinaria: ____ Otro:

_____

Durante su enfermedad tuvo los siguientes sintomas o signos?

Marque con una S, N, o D (Desconocido)

Sintomas:

Fiebre	_____ Temp _____
Rash	_____
Dolor Retroobitario	_____
Vómitos	_____
Artralgias	_____
Dolor abdominal	_____
Diarrea	_____
Estrenimiento	_____
Tos	_____
Anorexia	_____
P.Torniquete Pos.	_____

Signos:

Epistaxis	_____
Petequias	_____
Melena	_____
Hematemesis	_____
Hemorragia vaginal	_____
Hematuria	_____
Gigivorragia	_____
Derrame pleural	_____
Hepatomegalia	_____
Piel fría	_____
Ascitis	_____

Laboratorio:

Hematocrito: _____ Hemaglobina: _____ Plaquetas: _____ GB: _____ Lin: _____ Segm: _____

Hospitalizado _____ (S/N) Observacion ____ (S/N) Sala ____ (S/N) Estadia: ____

Fallecido _____ (S/N) Fecha ___/___/____

Nombre del encuastador: _____

ANTECEDENTES (SEGUNDA MUESTRA)

DURANTE SU ENFERMEDAD:

Ha tomado lo siguente (Marque S o N):

Agua: _____ Cada día? ____ (S/N)

Refresco _____ Cada día? _____(S/N)

Gaseosa: _____ Cada día? ____ (S/N)

Limonada: _____ Cada día? _____ (S/N)

Leche: _____ Cada día? ____ (S/N)

Cafe: _____ Cada día? ____ (S/N)

Suero oral casero: ____ Cada día? ____ (S/N)

Suero oral comercial____ (S/N) Cada día? _____ Cerveza: ____ Cada día? ____ (S/N)

Licor _____ Cada día? ____ (S/N)

Te de hierba: _____ Cada día? _____

Otros líquidos: ____ Cual? _____

Usó los siguentes medicamentos:

	Quien lo ordenó? Amigo/familia	C/S	Médico privado
Vitamina C: ___ (S/N) Multivitaminas: ___ (S/N)	_____	_____	_____
Acetominofen _____ (S/N)	_____	_____	_____
Aspirina: _____ (S/N)	_____	_____	_____
Antibioticos: ___ (S/N) Cual? _____	_____	_____	_____
Diclofenac: _____ (S/N)	_____	_____	_____
Ibuprofeno: _____ (S/N)	_____	_____	_____
Medicina tradicional ____ (S/N) Cual? _____	_____	_____	_____
Otro? _____ (S/N) Cual? _____	_____	_____	_____

Visitó otra unidad de salud para esta enfermedad? _____ (S/N)

Centro de Salud _____ (S/N) Clínica privada ____ (S/N) Otro _____ (S/N)

Cual?_____

Hospital _____ (S/N) Ingresado? _____ (S/N)

(Segun sea apropiado): Dejo de trabajar? ___ (S/N) Cuantos dias? ____

Dejo de ir a la escuela? ____ (S/N) Cuantos dias? ____

Dejo de hacer labores en el hogar? ___ (S/N) Cuantos dias? ____

Ha estado en cama? _____ (S/N) Cuantos dias? _____

Tuvo que trabajar a pesar de sentirse mal? _____ (S/N) Cuantos dias? _____

Comió normalmente _____ (S/N)

Appendix 5. Epi Info program - 419con.pgm

```
read c:\epi6\nica\nica1999\cen402.rec
relate id c:\epi6\nica\nica1999\hosp418.rec

define agelabel <AAAAAAA>
define age ###
DEFINE AGESTRAT <aaaaaaaaaaaaa>
define clinica <AAAAAAAAAAAAAAAAAA>
define race <AAAAAAAAA>
define res <A>
define seccon <A>
define secrel <A>
define timesamp ####
define window <AA>
define winRTPCR <AAA>
define plasleak <A>
define tromhemocon <AAAAAAAAA>
define hemsign <A>
define signshock <A>
define shksign <A>
define generalsx <A>
define leukopenia <A>
define tachycardia <A>
define fever <A>
define fevsum <A>
DEFINE DC <A>
DEFINE DCMH <A>
DEFINE DHF <A>
define DSAS <A>
DEFINE DSS <A>
define NODX <A>
define dxfinal <AAAA>
define severe <A>
define mild <A>
define dxsum <AAAAAA>
define serotype <A>
define distance <AAAA>
define dehyd <A>
define worksite <AAAAAAAAAA>
define black <A>
define white <A>
define mestizo <A>
define acutifx <A>
```

```
if enfagudhos ="D" or enfagudhos="M" or enfagudhos="M" then acutifx="S"
if enfagudhos <>"S" and hosp="S" then acutifx="N"

if ocupacion="abogado" or ocupacion="administradora" or ocupacion="ama de casa" or
ocupacion="artesana" or ocupacion="asistente administrativo" or ocupacion="auxiliar de
enfermeria" then worksite = "INDOORS"
if ocupacion="artesana" or ocupacion="cajero" or ocupacion="cerrajero" or
ocupacion="cocinera" or ocupacion="conserje" or ocupacion="contador publico" or
ocupacion="contadora" or ocupacion="costurera" then worksite="INDOORS"
if ocupacion="decoracion" or ocupacion="dependiente" or ocupacion="despachador" or
ocupacion="docente" or ocupacion="domestica" or ocupacion="economista" or
ocupacion="editor de tv" or ocupacion="empacador" then worksite="INDOORS"
if ocupacion="enfermera" or ocupacion="enfermero profesional" or
ocupacion="estadistica" or ocupacion="estilista" or ocupacion="estudiante" or
ocupacion="estudiante 1er grado" or ocupacion="pintor" then worksite="INDOORS"
if ocupacion="estudiante de medicina" or ocupacion="fisioterapista" or
ocupacion="fotomecanico" or ocupacion="joyero" or ocupacion="laboratorista" or
ocupacion="maestro" or ocupacion="manufactura" or ocupacion="medico" then
worksite="INDOORS"
if ocupacion="medico pediatra" or ocupacion="mesera" or ocupacion="modista" or
ocupacion="odontologo" or ocupacion="oficinista" or ocupacion="operador de caldera"
or ocupacion="panadero" or ocupacion="periodista" then worksite="INDOORS"
if ocupacion="prensista" or ocupacion="profesor" or ocupacion="psicologa" or
ocupacion="remodelador" or ocupacion="repartidor" or ocupacion="responsable de
bodega" or ocupacion="rotulador" or ocupacion="secretaria" then worksite="INDOORS"
if ocupacion="tecnico en estadisticas" or ocupacion="tecnico en refrigeracion" or
ocupacion="tipografo" or ocupacion="estudiante de preescolar" or ocupacion="medico
general" or ocupacion="tecnico en computacion" then worksite="INDOORS"
if ocupacion="admicionista" or ocupacion="dependienta" or ocupacion="electricista" or
ocupacion="facturador" or ocupacion="odontologia" or ocupacion="profesora"
ocupacion="conductor" or ocupacion="tecnico en anestesia" then worksite="INDOORS"
if ocupacion="odontologia" then worksite="indoors"

if ocupacion="agricultor" or ocupacion="albanil" or ocupacion="ayudante de albanil" or
ocupacion="ayudante de camiones" or ocupacion="ayudante de construccion" or
ocupacion="ayudante de fontaneria" or ocupacion=" then worksite="OUTDOORS"
if ocupacion="ayudante de maquina" or ocupacion="cargador" or ocupacion="cargador
de mariscos" or ocupacion="comerciante ambulante" or ocupacion="construccion" or
ocupacion="constructor" or ocupacion="obrero" then worksite="OUTDOORS"
if ocupacion="obrero agricola" or ocupacion="operador de equipo pesado" or
ocupacion="operador de maquina" or ocupacion="profesor de ed fisica" or
ocupacion="vaquero" or ocupacion="vendedor" then worksite="OUTDOORS"
if ocupacion="vigilante" or ocupacion="conductor" or ocupacion="zootecnista" or
ocupacion="vulcanizador" or ocupacion="taller de mecanica" then worksite="outdoors"
```

```
if ocupacion="mecanico" or ocupacion="taxista" or ocupacion="policia" or
ocupacion="jornalero" then worksite="outdoors"
if ocupacion="vendedor ambulante" or ocupacion="vendedor de tortillas" then
worksite="OUTDOORS"

if ocupacion="comerciante" or ocupacion="operador" or ocupacion="lava y plancha"
then worksite="D"
if (ocupacion="D" or ocupacion=.) then worksite="D"

if total <=4 and (reducorina="S" or llansinlag="S" or mucosaseca"S" or glbhundido="S"
or fonthundid="S" or skinturgor="S") then dehyd="S"
if total =. and reducorina="D" and llansinlag="D" and mucosaseca"D" and
glbhundido="D" and fonthundid="D" and skinturgor="D" then dehyd="D"
if dehyd <>"D" and dehyd <>"S" then dehyd="N"

if distancia >=0 and distancia <=10 then distance="near"
if distancia >10 then distance="_far"
if distance <>"near" and distance<>"_far" then distance="D"

if unidad="T" then clinica  ="Mateare"
if unidad="S" then clinica  ="Silvia Ferrufino"
if unidad="Z" then clinica  ="Morazan"
if unidad="F" then clinica  ="Buitrago"
if unidad="V" then clinica  ="Venezuela"
if unidad="M" then clinica  ="La Mascota"
if unidad="H" then clinica  ="HEODRA"

IF EDADMESes >= 36 OR (EDADMESes = .)  THEN age= EDAD
IF (EDADMESes < 12) AND (EDADMESes <> .) tHEN age = 0
iF (EDADMESes >= 12) AND (EDADMESes <24) or (edad >=1 and edad <2) THEN
age = 1
IF (EDADMESes >= 24) AND (EDADMESes <36) THEN age = 2
if edad > 2 then age = edad

if (age <15) and (age <> .) then agelabel ="child"
if age >=15 then agelabel = "ADULT"
IF age= . THEN agelabel ="D"

if age =0 then agestrat="0-1"
IF (age >=1 AND age <=4) THEN AGESTRAT="01 to 4"
IF (age > 4 AND age <=9) THEN AGESTRAT="05 to 9"
IF (age >9 AND  age <15) THEN AGESTRAT="10 to 14"
IF (age >=15) THEN AGESTRAT=">15"
if (age = .) then agestrat = "D"
```

```
if raza ="B" then race="blanco"
if raza ="M" then race="mestizo"
if raza ="N" then race="negro"
if raza ="I" then race="indigena"
if raza ="A" then race="asiatico"
if raza ="O" then race="otro"
if raza=. or raza="D" then race="D"

if race="NEGRO" then black="S"
if race="MESTIZO" or race ="blanco" then black="N"
if race="mestizo" then mestizo="S"
if race ="negro" or race="blanco" then mestizo="N"
if race="blanco" then white="S"
if race ="negro" or race="mestizo" then white="N"

let timesamp = tomademues - iniciosint
if (timesamp <=4) and (timesamp >=0) then window="S"
if (timesamp >4) then window="N"

if window="S" and ((igg="P") or (igm="P") or (av="P")) then winrtpcr="S"
if window="N" then winrtpcr ="N"

ESTAhosp = (FEGRESO - FINGRESO) + 1

PRESPULSO = SIShosp - DIAShosp
IF PRESPULSO < 20 THEN ESTRECH = "S"
IF PRESPULSO >= 20 THEN ESTRECH = "N"
IF PRESPULSO = . THEN ESTRECH = "D"

if ((IGM="P") OR (MEZCLA >=2560) OR (AV="P") OR (RTPCR="P")) THEN
RES="P"
if (igm=. or igm="D") and (mezcla=.) and (av=. or av="D") and (rtpcr=. or rtpcr="D")
then res="D"
if res <> "P" and res <> "D" then res = "N"

if rtserotipo =1 or avifi=1 then serotype="1"
if rtserotipo =2 or avifi=2 then serotype="2"
if rtserotipo =3 or avifi=3 then serotype="3"
if rtserotipo =4 or avifi=4 then serotype="4"

if (res="P") and (timesamp >=1 and timesamp <=4) and (mezcla >=20) then secrel ="S"
if (res="P") and (timesamp >=1 and timesamp <=4) and (mezcla <20) then secrel ="N"
if (res="P") and (timesamp >=5 and timesamp <=7) and (mezcla >20) then secrel ="S"
if (res="P") and (timesamp >=5 and timesamp <=7) and (mezcla <=20) then secrel ="N"
if (res="P") and (timesamp >=8) and (mezcla >=2560) then secrel ="S"
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if (res="P") and (timesamp >=8) and (mezcla <2560) then secrel ="N"
if (res="P") and ((mezcla =.) or (timesamp=.)) then secrel ="D"

if (res="P") and (timesamp >=1 and timesamp <=4) and (mezcla >20) then seccon ="S"
if (res="P") and (timesamp >=1 and timesamp <=4) and (mezcla <=20) then seccon ="N"
if (res="P") and (timesamp >=5 and timesamp <=7) and (mezcla >40) then seccon ="S"
if (res="P") and (timesamp >=5 and timesamp <=7) and (mezcla <=40) then seccon ="N"
if (res="P") and (timesamp >=8) and (mezcla >2560) then seccon ="S"
if (res="P") and (timesamp >=8) and (mezcla <=2560) then seccon ="N"
if (res="P") and ((mezcla =.) or (timesamp=.)) then seccon="D"

if mezcla >=2560 then igg="P"
if mezcla <2560 then igg="N"
if mezcla =. then igg="D"

IF age >= 5 AND SIShosp < 90 THEN HIPOTEN = "S"
IF age >= 5 AND SIShosp >= 90 THEN HIPOTEN = "N"
IF age < 5 AND SIShosp < 80 THEN HIPOTEN = "S"
IF age < 5 AND SIShosp >= 80 THEN HIPOTEN = "N"
if age = . or sishosp = . then hipoten = "D"

IF HTOMASALTO >= 1.2*HTOEGRESO THEN HEMOCON = "S"
iF HTOMASALTO < 1.2*HTOEGRESO THEN HEMOCON = "N"
IF (HTOMASALTO = .) OR (HTOEGRESO = .) THEN HEMOCON = "D"

IF (age = .) OR (HTOMASALTO = .) OR (sexo = "D") THEN HTOELEVADO = "D"
IF (age <=2) AND (age <> .) AND (HTOMASALTO >=40) THEN HTOELEVADO =
"S"
IF (age <=2) AND (age <> .) and (HTOMASALTO <40) and (htomasalto <> .) THEN
HTOELEVADO = "N"
IF (age < 12) AND (age >2) AND (HTOMASALTO >=43) THEN HTOELEVADO =
"S"
IF (age < 12) AND (age >2) AND (HTOMASALTO < 43) AND (HTOMASALTO <> .)
THEN HTOELEVADO = "N"
IF (age >= 12) AND (SEXO="F") AND (HTOMASALTO >= 45) THEN
HTOELEVADO = "S"
IF (age >= 12) AND (SEXO ="F") AND (HTOMASALTO < 45) and (htomasalto <> .)
THEN HTOELEVADO = "N"
IF (age >= 12) AND (age <18) and (SEXO = "M") AND (HTOMASALTO >= 46)
THEN HTOELEVADO = "S"
IF (age >= 12) AND (age <18) and (SEXO = "M") AND (HTOMASALTO < 46) and
(htomasalto <> .) THEN HTOELEVADO = "N"
if (age >=18) and (sexo="M") and htomasalto >=50 then htoelevado="S"
if (age >=18) and (sexo="M") and htomasalto < 50 then htoelevado="N"
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IF PLAQUETA <= 100000 THEN TROMBO = "S"
IF PLAQUETA > 100000 THEN TROMBO = "N"
IF PLAQUETA = . THEN TROMBO = "D"

if (manhem="S" or epistaxis="S" or petequias="S" or melena="S" or hematem="S" or
hemorvag="S" or hematuria="S" or gingivorra="S" or ptorniquet="S") then hemsign="S"
if manhem="D" and epistaxis="D" and petequias="D" and melena="D" and
hematem="D" and hemorvag="D" and hematuria="D" and gingivorra="D" and
ptorniquet="D" then hemsign="D"
if hemsign <> "S" and hemsign <>"D" then hemsign = "N"

if (derpleural="S" or derrame="S" or ascitis="S" or ascithosp="S") then plasleak ="S"
if derpleural="D" and derrame="D" and ascitis="D" and ascithosp="D" then
plasleak="D"
if plasleak <>"D" and plasleak <>"S" then plasleak="N"

if trombo="S" and ((hemocon="S") or (htoelevado="S") or plasleak="S") then
tromhemocon = "S"
if trombo = "N" and ((hemocon ="N") and (htoelevado="N") and plasleak="N") then
tromhemocon ="N"
if trombo="N" and ((hemocon="S") or (htoelevado="S")) then tromhemocon="NotBoth"
if trombo="S" and ((hemocon ="N") and (htoelevado="N")) then
tromhemocon="NotBoth"
if trombo ="D" or (hemocon ="D" and plasleak="D" and htoelevado="D") then
tromhemocon ="D"

if (pulso >=100) and (pulso <=200) then tachycardia ="S"
if (pulso >=60) and (pulso <100) then tachycardia ="N"
if pulso <60 and pulso >=20 then tachycardia ="B"

if (pielfria="S" or signoshock="S") then shksign="S"
if pielfria="D" and signoshock="D" then shksign="D"
if shksign <>"D" and shksign <>"S" then shksign="N"

if ((hipoten="S" and (shksign="S" or tachycardia="S")) or ((estrech="S" and
(shksign="S" or tachycardia="S"))) then signshock = "S"
if ((hipoten="D" or shksign="D") and (estrech="D" or tachycardia="D")) then
signshock="D"
if signshock <>"S" and signshock <>"D" then signshock="N"

if (gb <4000) and (gb >= 0) then leukopenia ="S"
if (gb >=4000) and (gb <=10000) then leukopenia ="N"
if (gb >10000) then leukopenia ="E"
if gb=. then leukopenia="D"
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if temp >=38 then fever="S"
if temp <38 and temp >=36 then fever="N"
if fever <>"S" and fever <>"N" then fever="D"

if fever="S" or fiebre="S" then fevsum="S"
if fever="N" and (fiebre="D" or fiebre=.) then fevsum="N"
if (fever="D" or fever=.) and fiebre="N" then fevsum="N"
if fever="N" and fiebre="N" then fevsum="N"
if (fever="D" or fever=.) and (fiebre="D" or fiebre=.) then fevsum="D"

if ((fevsum="S") or (cefalea="S") or (mialgias="S") or (artralgias="S") or
(dolorretro="S") or (rash="S") or (leukopenia="S")) then generalsx ="S"
if ((fevsum="D") and (cefalea="D") and (mialgias="D") and (artralgias="D") and
(dolorretro="D") and (rash="D") and (leukopenia="D")) then generalsx ="D"
if generalsx <> "S" and generalsx <>"D" then generalsx="N"

if (res="P") and (fevsum="S") and (tromhemocon = "NotBoth") and (hemsign="S") and
(signshock ="S") then DSAS ="S"
if res="P" and ((fevsum="N") or tromhemocon="S" or tromhemocon="N" or
hemsign="N" or signshock="N") then DSAS ="N"

if (res="P") and (fevsum="S") and (tromhemocon="S") and (hemsign="S") and
(signshock ="S") then DSS ="S"
if res="P" and ((fevsum="N") or tromhemocon="N" or tromhemocon="notboth" or
hemsign="N" or signshock="N") then DSS="N"

if (res="P") and (fevsum="S") and ((tromhemocon="S") and (hemsign="S")) and (DSS
<> "S") and (DSAS <> "S") then DHF ="S"
if res="P" and ((fevsum="N") or (tromhemocon="N") or (tromhemocon="notboth") or
(hemsign="N")) then DHF="N"

if (res="P") and ((DSS <> "S") and (DHF <> "S") and (DSAS <> "S") and (hemsign <>
"S")) and generalsx ="S" then DC ="S"
if res="P" and (hemsign="S" or generalsx="N") then DC="N"

if (res="P") and ((DSS <> "S") and (DHF <> "S") and (DSAS <> "S") and (DC <> "S"))
and (hemsign = "S") then DCMH ="S"
if res="P" and (hemsign="N") then DCMH="N"
if res="P" and ((DSS <> "S") and (DHF <> "S") and (DSAS <> "S") and (DC <> "S")and
(DCMH <>"S")) then NODX="S"
if res="P" and ((DSS = "S") or (DHF = "S") or (DSAS = "S") or (DC = "S") or (DCMH
="S")) then NODX="N"

if (DCMH="S" or DHF="S" or DSAS="S" or DSS="S") then DC="N"
if (DC="S" or DHF="S" or DSAS="S" or DSS="S") then DCMH="N"
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if (DC="S" or DCMH="S" or DSAS="S" or DSS="S") then DHF="N"
if (DC="S" or DCMH="S" or DHF="S" or DSS="S") then DSAS="N"
if (DC="S" or DCMH="S" or DHF="S" or DSAS="S") then DSS="N"

if DC = "S" then dxfinal ="DC"
if DCMH = "S" then dxfinal ="DCMH"
if DHF = "S" then dxfinal ="DHF"
if DSAS = "S" then dxfinal ="DSAS"
if DSS = "S" then dxfinal="DSS"
if NODX="S" then dxfinal="NODX"

if DHF="S" or DSAS="S" or DSS="S" then severe="S"
if DC="S" or DCMH="S" then severe="N"
if DC="S" or DCMH="S" then mild="S"
if DHF="S" or DSAS="S" or DSS="S" then mild="N"

if mild="S" then dxsum="MILD"
if severe="S" then dxsum="severe"
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