

Essays on Science and Society

GLOBAL VOICES OF SCIENCE:

Deciphering Dengue: The Cuban Experience

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María G. Guzmán, head of the virology department at the Tropical Medicine Institute Pedro Kourí (IPK), in Havana, Cuba, has more than 20 years of experience working on virology. With her husband and a group of distinguished collaborators, her work on dengue viruses in Cuba and abroad has contributed to knowledge of the pathogenesis, diagnosis, epidemiology, and clinical progression of this disease. She is a member of the Cuban Academy of Science, a Fellow of the Academy of Sciences for the Developing World (TWAS), and director of the PAHO/WHO

Collaborating Center for Viral Diseases. More recently, she has taken over the helm of the PAHO/WHO Collaborating Center for the Study of Dengue and Its Control and become a member of the Foundation Council of the Global Forum for Health Research. She is a member of several expert committees at the Pan American Health Organization (PAHO), World Health Organization (WHO), and the Special Programme for Research and Training in Tropical Diseases (TDR).

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By the 1960s, vaccines and antibiotics had so reduced the incidence of such deadly diseases as smallpox, poliomyelitis, and acute rheumatic fever that the public health community was basking in a "comfort zone." This comfort was shattered beginning in the 1980s with the emergence of new infectious diseases, among them HIV/AIDS, severe acute respiratory syndrome (SARS), and avian flu, and the reemergence of diseases once considered scourges of the past,* including West Nile fever and dengue, a devastating disease to which I have devoted much of my professional life.

Factors involved in the emergence of infectious diseases are complex and interrelated. Epidemiological evidence shows that social and economic factors such as poverty, social exclusion, health systems, environments, food security, water and sanitation, and education are of utmost importance.* Public health infrastructure, including disease surveillance, disease prevention, communication, and financial support, are crucial for facing threats posed by emerging infectious disease.

Almost 50 years ago, a small Caribbean country, the Republic of Cuba, embarked on a plan to accelerate development of its education, public health, and science sectors, a policy that has prepared the country for the new global context of emerging and returning diseases. At the end of the 1950s, Cuba had approximately 10 million inhabitants and no more than 3000 physicians. Now a population of about 11 million is served by a health system that includes more than 70,000 medical doctors.

This buildup has been serving the population well. Poliomyelitis and malaria were eradicated in 1962 and 1967, respectively. In the 1970s, tetanus neonatorum (which afflicts newborns) and diphtheria became a worry of the past for Cubans. A national regimen of 13 vaccines led, in the mid-1990s, to the elimination of measles, rubella, and mumps and to the control of tetanus, meningococcal disease, hepatitis B, leptospirosis, and other diseases. The rates of contracting meningococcal disease and dying from it diminished by 93% and 98%, respectively, and the rate of hepatitis B infection has been cut by 97% in children younger than 15 years of age. Although the incidence of tuberculosis is increasing worldwide, with some countries having reported rates in recent years well above 100 cases per 100,000 inhabitants, Cuba has a low rate of 6.6 cases per 100,000 inhabitants, and the cases that we do see are treatable. Deaths due to diarrhea were reduced by more than 95%.

In general, mortality from infectious and parasitic diseases in Cuba is only 6.5%, with most of the deaths due to influenza and pneumonia. According to the World Health Organization's World Health Report, 1998, infectious and parasitic diseases caused one-third of all deaths in the world in 1997 and 43% of deaths in the developing world. The low rate in Cuba is possible because of the high educational and health levels of the country.

The steady improvement of Cuba's health system over the past half-century has been complemented by a buildup of the country's scientific strength, particularly in the biomedical sciences. At the beginning of the 1960s, there were only four experimental stations (all of them dedicated to the study and improvement of sugar cane) and three universities, and illiteracy was widespread. Today, there are more than 700,000 graduates at 60 universities, which are host to 220 science and technology centers. In 2003, these institutions employed nearly 78,000 Cubans, almost two-thirds of them scientists and technicians and 52% of them women.

A Scientist Grows in Havana

As a little girl, I found myself within this improving health system and educational infrastructure, and I grew up with the idea that I could become a scientist. Early on, I dreamed about studying astronomy. I was impressed by the planets that I could see in the sky, as well as the other worlds I couldn't see. However, I was caught up in the rapid changes of Cuban society, particularly those related to the development of biomedicine. As a result, I shifted my studies toward medicine.

After I received my medical degree from Havana University in 1975, I started working as an investigator at the virology laboratory of the Centro Nacional de Investigaciones Científicas (CNIC) in Havana. This scientific institution was founded in 1965 and it has been crucial for the development of Cuban science ever since. Many of the scientific leaders of the country's main research centers--including the Centro de Ingeniería Genética y Biotecnología (CIGB), Instituto Finlay (for vaccine development), Centro de Inmunoensayos (for the development of diagnosis technology), and Instituto de Medicina Tropical "Pedro Kouri" (IPK) (for the surveillance, research, and control of infectious and parasitic diseases)--were trained at CNIC.

At CNIC, I honed my skills in research and analytic thinking and in 1980 I moved to the virology laboratory at IPK. There was no better place for a person who wanted to devote her efforts to fighting infectious diseases.

In May 1981, my country was befallen with a public health crisis, one that would decide my professional future. The first epidemic of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) anywhere in the Americas took hold in Cuba. DHF/DSS is the most lethal form of disease resulting from infection with the dengue virus ([see the figure](#)), a member of the viral family Flaviviridae that is transmitted from person to person via mosquitoes, mostly in the Tropics where these vectors thrive. Most people who get infected develop a fever and a rash, but recover in about 5 days. About 1 in 20 of those who develop a hemorrhagic form of the fever die, and of those who develop dengue shock syndrome, 40% die.

Previously, only 60 DHF/DSS cases had been observed in the Americas. In the 1981 epidemic in Cuba, more than 344,000 cases were reported, of which 10,000 were deemed severe and very severe. There were 158 fatalities. All but 57 of these were children, a chilling factor that only added to the national dread elicited by this epidemic. Once the first cases were detected, I found myself--despite my youth and inexperience--playing a crucial national role in the diagnosis of a severe viral disease that had been relatively unknown in Cuba or the region before. (Although a milder dengue epidemic had been reported in Cuba in 1977-1978, I was not involved in the country's scientific and medical response at that time.)

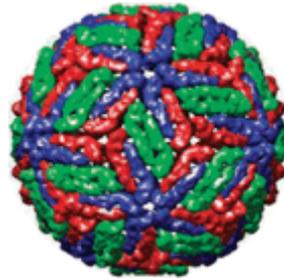
Our serological and virological examinations quickly revealed that the 1981 outbreak was due to the dengue 2 virus, one of the four serotypes known. In addition to studying the severe cases during the epidemic, we developed protocols for diagnosing the disease, which can be confused with other infections, and we established disease surveillance methods.

Even as Cuban health care workers were succeeding in checking the spread of the virus, using mosquito eradication and other techniques, a new phase in my scientific career was just beginning. Rather than leaving the dengue virus behind, I devoted my research to it. After all, this outbreak that I had just witnessed represented a globally significant turning point in the disease's history.

The Whole Dengue

Among my first tasks were to clinically describe DHF/DSS in the adults who contracted the disease, define and confirm some risk factors for progressing to the more severe form of the disease, and conduct a genetic study of the dengue 2 strain. I would later find this work to be helpful, during DHF/DSS epidemics in Cuba in 1997 (dengue 2) and 2001-2002 (dengue 3). Dengue disease is considered one of the best contemporary examples of the emergence or reemergence of a viral infectious disease. First described in 1780 by the Philadelphia physician Benjamin Rush during an epidemic in his city at that time, many epidemics have been reported since then. Currently, the distribution of dengue is worldwide. Caused by any of the four dengue serotypes and mainly transmitted by the *Aedes aegypti* mosquito, the disease is observed in two main clinical forms: the mild disease called dengue fever (DF) and the severe syndrome, DHF/DSS.

¶ **Bad ball.** A computer portrait of the dengue virus indicates several types of protein constituents in the virus's shell.



In the last 30 years, the incidence of the disease has been increasing. The first cases of DHF/DSS were reported in Southeast Asia and the Western Pacific (during the 1950s and the 1960s) and then later in the American region. Factors such as substandard housing, poor water supplies, and the spread of dengue viruses between populations have directly contributed to the reemergence of the disease.

Soon after the clinical recognition of DHF/DSS, Scott. B. Halstead, then at the University of Hawaii's School of Medicine, and others argued that those most at risk for developing this severe form of dengue disease are those who already had been infected by one dengue serotype and then subsequently become infected with a different serotype. Others proposed that viral virulence is the key risk factor.

Following the 1981 outbreak in Cuba, I, along with Gustavo Kourí, my collaborator and husband (and son of the founder of IPK), and a group of distinguished scientists, including Susana Vásquez, dedicated our work to uncovering risk factors for DHF/DSS. Our epidemiological, virological, and clinical investigations have led to important new observations. For one thing, in studies of three well-defined DHF/DSS epidemics in Cuba--in 1981, 1997, and 2001-2002--we confirmed that secondary infection was a significant factor in more than 97% of the severe cases. We made two other particularly important epidemiological observations that support the role of the secondary infection. One of them was that no severe and fatal DHF/DSS cases were observed among 1- to 2-year-olds during the 1981 epidemic. Because they were not born until after the first epidemic of DF caused by the dengue 1 virus in 1977, they could only have experienced a primary infection during the 1981 epidemic. We also found that no cases of DHF/DSS were observed among children during the 1997 and 2001-2002 epidemics. These children were born in a period free of dengue transmission (1982-1996) and so also were only at risk of a primary dengue infection.

Another relevant finding, which our group reported in 2000, is the influence of the interval between dengue infections. In contrast with early epidemiological studies that predicted that DHF/DSS would ensue only if the primary and secondary infections occurred within an interval of 5 years, our studies have demonstrated a marked increase in severity with longer intervals between an initial dengue 1 and a secondary dengue 2 infection. Supporting this result is our recent demonstration that certain lymphocytes, a type of immune cell, can retain a "memory" of a dengue infection that occurred 20 years earlier. These observations suggest that once an individual is infected by dengue 1 virus, that person could be susceptible to developing the severe disease for decades. The message to vaccine developers is clear: A dengue vaccine needs to elicit long-lasting protection against the four dengue serotypes, or else the vaccine itself could sensitize individuals who are subsequently infected to mount a severe immune response.

In some of our other research into risk factors for severe dengue, we have found that individuals with chronic diseases such as bronchial asthma, diabetes mellitus, and sickle cell anemia have a higher likelihood of developing DHF/DSS. Age too is a risk factor. We have demonstrated a much higher risk of developing the severe disease during a secondary infection in infants and children as compared with adults. For children aged 3 to 14 years with secondary infections, the death rate was 14.5-fold higher than in young adults aged 15 to 39 years ([see the figure](#)).

Not many researchers have looked into how ethnicity and genetics relate to the risk of developing DHF/DSS. Our investigations into these issues have suggested that whites are at particular risk as compared with blacks. In my country's three epidemics since the late 1970s, DHF/DSS was predominantly observed in whites. Currently, Beatriz Sierra and Gissel Garcia, two of our immunologists, are studying the genes that may predispose individuals to the development of DF and DHF/DSS.

Meanwhile, others in our group are working on biological and genetic characterizations of the viruses that have been isolated in the Cuban dengue epidemics. With the help of Delfina Rosario and Rosmari Rodríguez Roche, we have demonstrated that viruses linked to DHF epidemics belong to an Asian genotype. In a more detailed study of genetic changes in the dengue 2 virus during the 1997 epidemic, we documented a pattern of sequence evolution in some genes, and a remarkable conservation both of genes coding for structural proteins, as well as of the noncoding regions in the genome. We are currently trying to decipher the implications of these findings.

In addition to the human and viral genes important in dengue infections, we are looking into the role of the humoral and cellular immune response in the development of DHF/DSS. With the collaboration of Ana B. Perez and Mayling Alvarez, two young researchers in our group, we have obtained preliminary data on the influence of heterotypic neutralization--in which antibodies elicited against one dengue serotype can react also with another serotype--to mitigate the severity of the severe form of the disease. Our results suggest that heterotypic dengue antibodies decline over time, a phenomenon that could explain why secondary infections often appear worse as more time passes since the primary infections. Also, we demonstrated the association of increased levels of interleukin-10 in dengue patients with a secondary infection, suggesting an important role of this cytokine in the pathogenesis of dengue. This observation provided the first "in vivo" evidence of a direct relationship between secondary dengue infection and the development of a noninflammatory immune response, opening yet another new avenue of research.

We have made several attempts to synthesize what is known about dengue pathogenesis into testable hypotheses about why some outbreaks lead to DHF/DSS epidemics. In one of these, published in 1987, my husband and I integrated epidemiological factors (high vector density, high virus circulation, and a susceptible population at risk of a secondary dengue infection), host factors (age, gender, ethnicity, chronic diseases, preexistence of dengue antibodies, interval between infections, and genetics), and viral factors (serotype, strains, and genotypes) into one multifactorial analysis to facilitate the evaluation of the risk of a given population.

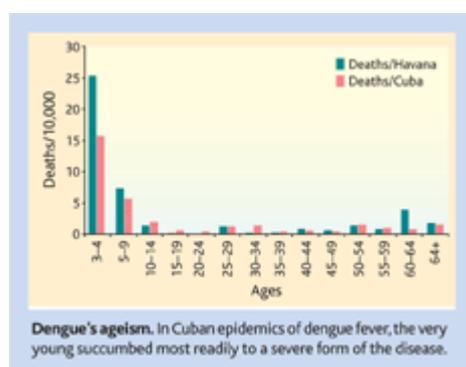
More recently, I, my husband, and Scott Halstead--now working for the South Korea-based Pediatric Dengue Vaccine Initiative--published a hypothesis in an attempt to explain the significant monthly increases in severity during the Cuban dengue epidemics. Specifically, a significant increase in the proportion of DHF/DSS cases and in fatality rates for both DF and DHF/DSS was observed during the 1981 and 1997 epidemics. In our "escape mutant" hypothesis, we conjecture that the occurrence of heterotypic dengue neutralizing antibodies after a primary dengue 1 infection later serves, during a subsequent infection with dengue 2 virus, as a

selection mechanism that favors "neutralizing-escape mutants" of the dengue 2 virus. This can bring on more severe sickness.

Dengue to Come

The world needs a dengue vaccine. Our group is now collaborating with CIGB on a project whose goal is to obtain a recombinant vaccine candidate to dengue viruses. With the collaboration of Mayra Mune, a molecular immunologist, for the first time we have evaluated in monkeys the usefulness of a recombinant protein expressed in yeast *Pichia pastoris*. We observed a rise both in neutralizing antibodies against dengue and partial protection to challenges with the wild-type virus. Also, our preliminary evaluations of a dengue protein fragment are showing promise in eliciting protective immune responses in animals.

Dengue's ageism. In Cuban epidemics of dengue fever, the very young succumbed most readily to a severe form of the disease.



CREDIT: M. GUZMAN ET AL.

Although dengue has dominated my research portfolio, I have been able to collaborate with my colleagues in the study of a number of the most medically important infectious diseases. As the national reference center for viral diseases, our virology department at IPK is charged with the diagnosis and surveillance of, and research into, hepatitis, measles, rubella, and mumps, as well as respiratory, enteroviral, and sexually transmitted diseases, among others. Current international events have obliged us to include in our portfolio new viral infections, such as West Nile fever, SARS, and avian flu, among others.

Founded in 1937 by Pedro Kourí, the famous Cuban parasitologist, IPK now gathers in one place the main disciplines involved in the study of infectious and parasitic diseases. In this setting that combines high scientific quality and collegiality, we have been able to assemble a multidisciplinary group for dengue research that has recently been recognized as a new PAHO/WHO Collaborating Center for the Study of Dengue and Its Control. It is gratifying to be able to share our insights and discoveries with others in what is becoming a global fight against this disease.

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¶ Image produced using the UCSF Chimera visualization package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (www.cgl.ucsf.edu/chimera).

Acknowledgement: As a woman scientist in Cuba, I have received official, social, and family support for my scientific development. I am especially grateful to my mother for attending to my family with love and care, my husband for encouraging me to pursue my dreams, and my son, who has often felt my absence.

10.1126/science.1115177

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Volume 309, Number 5740, Issue of 2 Sep 2005, pp. 1495-1497.

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