Dengue Epidemic in Puerto Rico 2012-2013: Integration of Passive and Sentinel Surveillance to Characterize and Differentiate Dengue in the Peri-vaccination Era

By

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CERTIFICATE OF APPROVAL

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Glossary of Terms

AFI: Acute Febrile Illness

AIC: Aikike information criterion

BIC: Bayesian information criterion

CDC: Centers for Disease Control and Prevention

CDC-DB: Centers for Disease Control and Prevention-Dengue Branch

CIF: Case Investigation Form

DCIF: Dengue Case Investigation Form

DENV: Dengue virus

DF: Dengue Fever

DHF: Dengue Hemorrhagic Fever

DSS: Dengue Shock Syndrome

EMR: Electronic Medical Record

ICD: International Classification of Disease

IHC: Immunohistochemical

MAC-ELISA: Molecular antibody-capture enzyme-linked immunosorbent assay

PAHO: Pan American Health Organization

PDSS: Passive Dengue Surveillance System

PRDH: Puerto Rico Department of Health

RSV: Respiratory Syncytial Virus

RT-PCR: Real time polymerase chain reaction

SEDSS: Sentinel-Enhanced Dengue Surveillance System

WHO: World Health Organization

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Introduction

Dengue represents an increasingly important global health challenge, as recent estimates suggest that nearly 2.5 billion people worldwide are at risk for infection[1] and 390 million infections occurred in 2010.[2] The four dengue virus-types (DENV-1–4) that cause dengue are single-stranded, positive-sense RNA viruses of the family *Flaviviridae*. *Aedes aegypti* and *Ae. albopictus* mosquitoes are endemic throughout the tropics and subtropics and serve as the primary vector for DENV transmission. DENV infection can result in a range of outcomes, from asymptomatic infection, to self-limited acute febrile illness (AFI), to potentially fatal severe dengue.[1]

In 2009, the World Health Organization (WHO) revised the clinical classification of dengue from dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS)[3] to dengue, dengue with warning signs, and severe dengue.[1] A major impetus for this change was the observation that many life-threatening cases of dengue did not meet the definition of DHF/DSS, and the identification of clinical signs and symptoms present in some dengue cases were positively associated with the development of more severe illness.[4,5] Dengue is characterized by fever, anorexia, rash, aches and pains, and leucopenia.[1] Warning signs that signal development of severe dengue include abdominal pain, persistent vomiting, mucosal bleed, hepatomegaly greater than 2 centimeters, clinical fluid accumulation, lethargy or restlessness, and hemoconcentration concurrent with rapid decrease in platelet count. Severe dengue is characterized by plasma leakage that may lead to shock, severe bleeding, or severe organ impairment.

In Puerto Rico, clinical suspicion of dengue should be followed by collection of a serum specimen and completion of a Dengue Case Investigation Form (available at

www.cdc.gov/dengue/resources/dengueCaseReports/DCIF_English.pdf) to enable case reporting and diagnostic testing by either reverse-transcriptase-polymerase chain reaction (RT-PCR) to directly detect viral genome and/or antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) to detect anti-DENV immunoglobulin M (IgM) antibodies. Although primary DENV infection confers lifelong immunity to the infecting DENV-type, subsequent infection with another DENV-type confers a slight but statistically significant increased risk of developing more severe illness.[6]

Currently, no vaccine or anti-viral drug is available to prevent or treat dengue, although several vaccine candidates are in clinical trial.[7,8] The mainstay for treatment of dengue is therefore supportive care, which can reduce the case-fatality rate in hospitalized patients from approximately 10% to less than 0.1%.[1,9] Clinical management of patients depends on recognition of the three phases of dengue: the febrile phase, critical phase, and recovery phase. During the febrile phase, maintaining proper hydration and vigilance for warning signs of severe dengue are important. Defervescence, typically 3–7 days after illness onset, defines the start of the critical phase, which lasts 24–48 hours. Hemoconcentration may also occur as a result of plasma leakage in the critical phase, in which case judicious use of intravenous fluids and close monitoring of clinical status are needed to avert shock, organ impairment, and unnecessary morbidity. Corticosteroids, though once thought to benefit dengue patients, have not shown to decrease mortality or morbidity due to dengue and in fact may result in increased morbidity due to immunosuppression and/or increased risk of gastrointestinal bleeding.[10,11] The recovery phase reflects a return to normal capillary permeability, although continued monitoring of fluid status is important to avoid fluid overload. Detailed patient management protocols and best practice

guidelines elaborate on the appropriate clinical management of patients with suspected or confirmed dengue (Figure 1).[1]

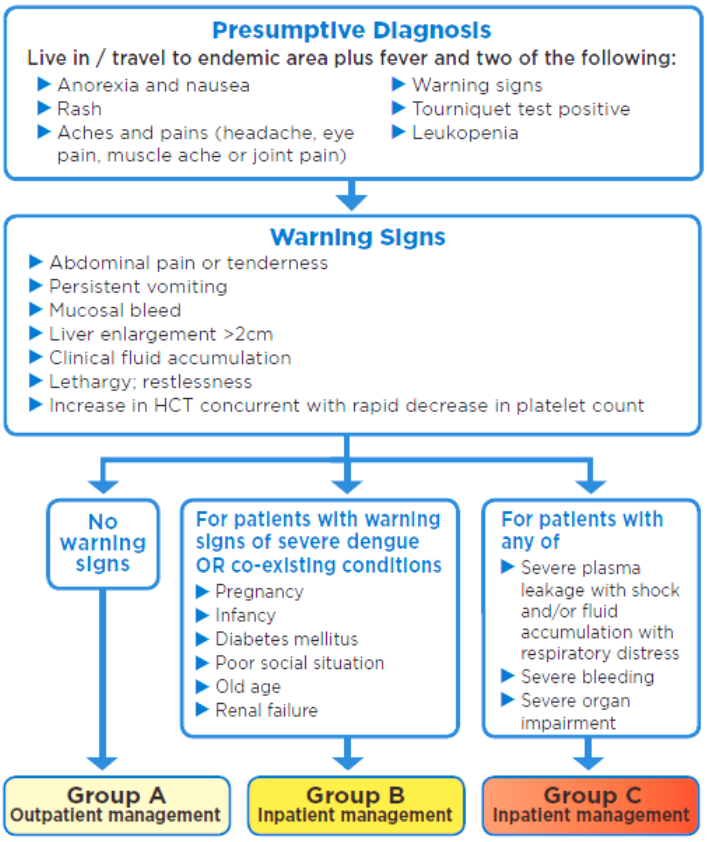


Figure 1. Schematic of World Health Organization guidelines[1] for clinical management of patients suspected to have dengue.

Dengue Epidemiology

Outbreaks of dengue-like illness were first reported in the 1600s and have been consistently reported from various regions of the tropics for more than a century. Although the Americas experienced a reprieve from dengue in the 1950s and 1960s following an extensive *Aedes aegypti* elimination program by the Pan American Health Organization (PAHO),[12] the resurgence of dengue in the region reflects global trends[1,13,14] in urbanization, migration, and inadequate preventive measures.[15,16] The number of dengue cases reported to WHO nearly doubled between the 1990s and the early 2000s,[17] and in 2010 an estimated 96 million dengue

cases occurred worldwide.[2] The need to evaluate the economic impact of dengue and novel prevention methods, such as vaccines, underscore the importance of maintaining disease surveillance to better understand changes in dengue epidemiology.

The seasonal and cyclic nature of dengue is due in part to environmental influences, in particular rainfall, temperature,[18] and weather indices such as El Niño Southern Oscillation,[19] all of which affect the proportion of individuals in the population that are susceptible to the DENV types in circulation. Transmission of the virus via the vector *Aedes aegypti* tends to increase as conditions favor the reproduction of mosquitos. Nevertheless, a recent review of the literature emphasizes the complex interactions between environment, the mosquito vector, and host factors in the propagation of DENV and cautions the limitations of current methods to predict spread of dengue with environmental models.[20]

Dengue in Puerto Rico

The first reported dengue outbreak in Latin America occurred in the early 1600s on the Caribbean island of Martinique.[21] Similar outbreaks of dengue-like illness spread throughout Latin America over the subsequent three centuries.[21] In Puerto Rico, outbreaks of dengue-like illness were reported in 1918[22] and 1945,[23] DENV-2 was isolated during the 1963–1964 outbreak, and endemicity was documented soon after.[24] Introduction of additional DENV-types were documented in outbreaks in the 1970s and 1980s, culminating in an outbreak with co-circulation of all four DENV-types in 1998.[25] Dengue epidemics occurred most recently in Puerto Rico in 2007,[26] 2010,[27] and 2012–2013 (PRDH, unpublished data) (Figure 2).

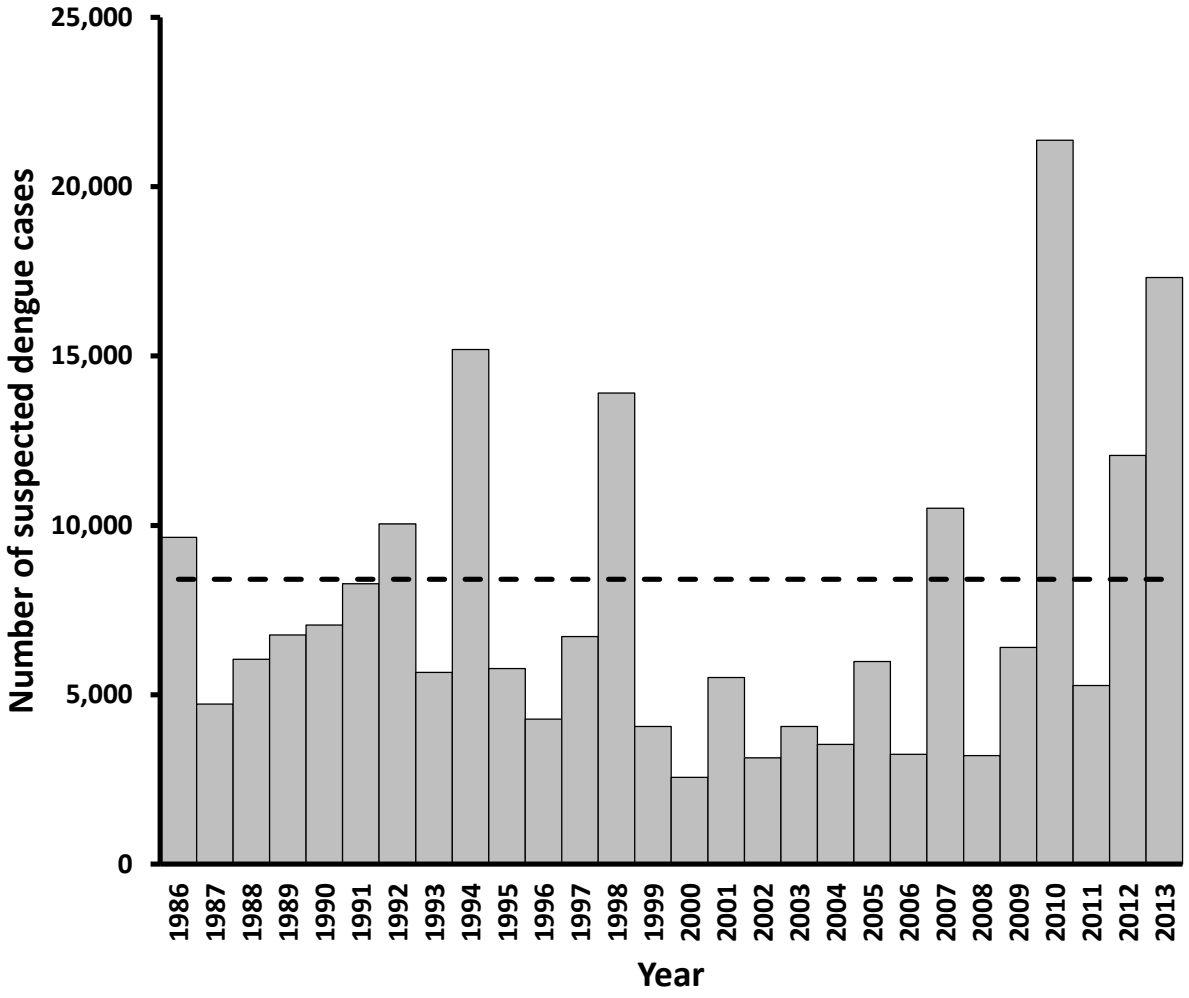


Figure 2. Suspected dengue cases reported to the passive dengue surveillance system during 1986–2013. The dotted horizontal line indicates the epidemic threshold.

Dengue epidemiology in Puerto Rico during epidemic and non-epidemic years consistently reveals a disproportionate burden of disease for individuals aged 10–19 years, followed by younger children and infants; nonetheless, adults consistently represent roughly one-half of all reported cases.[25-27] There have been no consistent differences in the incidence of dengue by sex or race. In 2007, a total of 10,508 suspected cases and 44 deaths were reported; however, only one-third of suspected cases and 11 fatal cases had laboratory evidence of DENV infection. Moreover, among all laboratory-positive dengue cases identified, the percentage of

individuals who had been previously infected with a DENV was >75%.[26] A separate investigation regarding the 11 lab-confirmed deaths revealed that less than half received a clinical diagnosis of dengue, more than half were given corticosteroids, and none were managed according to the WHO guidelines.[28] Subsequently, the 2010 epidemic in Puerto Rico documented nearly 27,000 suspected cases and 128 fatal cases, of which half and one-third were laboratory confirmed, respectively.[27] Similar to the 2007 epidemic, approximately 80% of dengue cases had been previously infected with a DENV. The 2007 and 2010 epidemics illustrated critical lessons for dengue epidemiology in Puerto Rico and revealed several aspects of dengue clinical case management in need of improvement (e.g., use of non-isotonic intravenous saline, frequency of vital sign monitoring, administration of corticosteroids).

Dengue Surveillance in Puerto Rico

Dengue in Puerto Rico is monitored with the Passive Dengue Surveillance System (PDSS), which was established in the late 1960s.[29] PDSS was for several decades a collaborative surveillance system co-operated between the Centers for Disease Control and Prevention-Dengue Branch (CDC-DB) and the Puerto Rico Department of Health (PRDH); however, since 2012, PDSS has been primarily operated by PRDH. A general overview of PDSS spans the initial interface of a patient with the health care system to the reporting of suspected dengue cases to the public health response (Figure 3). Overall goals of dengue surveillance[1] include: early detection of increased incidence to enable early intervention, measurement of disease burden, evaluation of programs to prevent and control dengue, and facilitation of appropriate resource distribution.

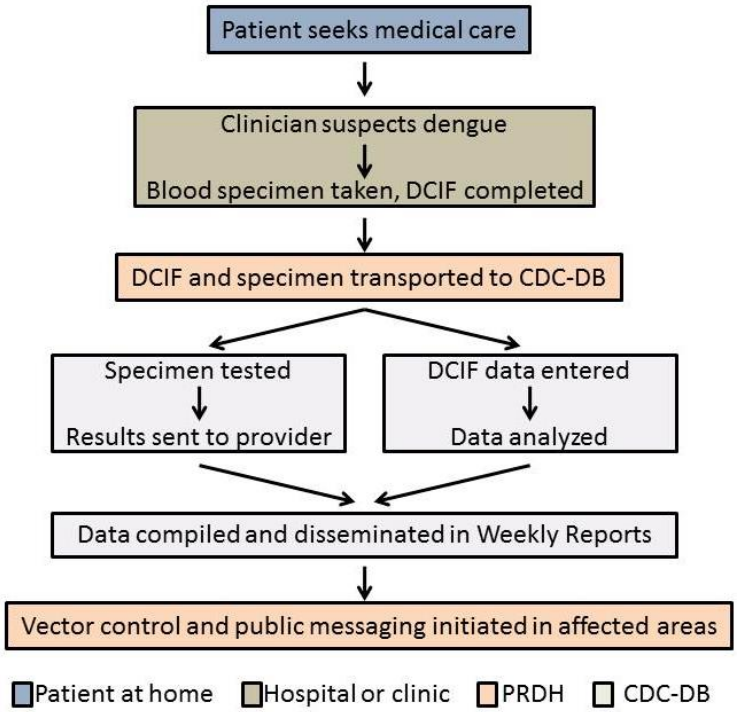


Figure 3 Schematic of how the passive dengue surveillance system (PDSS) was operated until 2012. PDSS is initiated when a patient seeks medical care, following which the patient's medical provider suspects dengue as a cause of the patient's illness. The clinician orders a blood specimen be collected from the patient and the Dengue Case Investigation Form (DCIF) is completed; both are transported by Puerto Rico Department of Health (PRDH) to Centers for Disease Control and Prevention, Dengue Branch (CDC-DB) for diagnostic testing. Specimens are tested and patient information from the DCIF is entered into a database at CDC-DB. Diagnostic test results are sent to the health care provider that reported the case, who then relays results to the patient and, if necessary, requests that the patient return to provide a convalescent serum specimen. Patient demographic information is compiled into weekly reports that CDC-DB and PRDH disseminate to stakeholders and the public via Weekly Reports. PRDH uses data from Weekly Reports to direct response activities in areas most affected by dengue. After 2012, all activities indicated as being conducted by CDC-DB were instead conducted by PRDH.

An evaluation of PDSS from 2009–2011, guided by the nine attributes of public health surveillance,[30] identified strength in the utility, flexibility, and stability of the system; however, timeliness, sensitivity, and acceptability represent attributes to be improved (CDC, unpublished data). Data quality, positive predictive value, and simplicity of the surveillance system were considered to be acceptable. The total time for specimens to be transported (Figure 4), processed, tested, and reported back to clinicians were 10 days in an epidemic period and 15 days during a non-epidemic period, thus reducing the clinical utility of diagnostic testing for health care providers. Nevertheless, the primary purpose of PDSS is to inform public health and not to

produce diagnostic test results. The stability of PDSS over the past several decades contributes to its utility to monitor dengue epidemiology and direct public health action in Puerto Rico.

An inherent limitation of passive surveillance is the difficulty of measuring the true burden of disease. A meta-analysis of surveillance systems throughout Latin America and Southeast Asia revealed significant underreporting of dengue cases, from 3–9 symptomatic cases not being reported for each case that was reported.[31] Studies in Puerto Rico in the 1990s estimated that for each case of dengue reported to PDSS, 10–27 additional cases were not reported.[32,33] Although recent estimates of underreporting are needed, much anecdotal evidence suggested that PDSS is biased towards hospitalized cases (CDC, unpublished data).

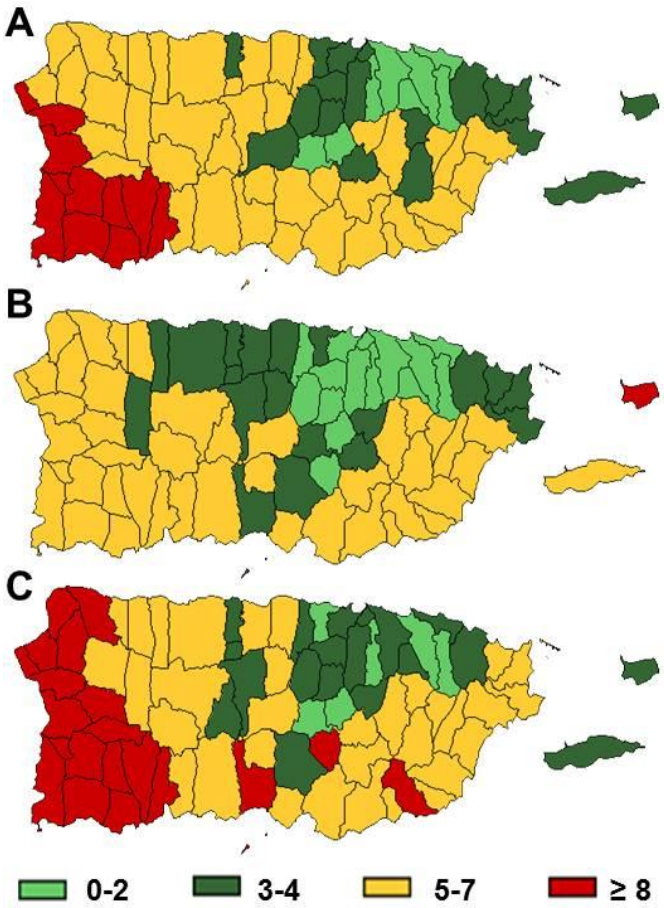


Figure 4. Median number of days needed for specimens to arrive at Centers for Disease Control and Prevention, Dengue Branch (CDC-DB) according to case-patients' municipality of residence in 2009 (**A**), 2010 (**B**) and 2011 (**C**). Light green, dark green, yellow and red regions indicate municipalities with an average transport time of 0–2, 3–4, 5–7 and >8 days, respectively.

To improve surveillance, a pilot enhanced surveillance system was implemented in 2005 in Patillas to encourage health care providers to report suspected cases.[34] In 2009, WHO recommended the addition of sentinel surveillance systems to complement passive surveillance.[1,31] To meet this need, in 2012 CDC-DB established the Sentinel Enhanced Dengue Surveillance System (SEDSS) in Ponce, and later expanded it to sites in Guayama and Carolina.[35] A major utility of SEDSS sites includes the ability to determine baseline levels of dengue, which will be needed to evaluate the efficacy of a dengue vaccine and more accurately quantitate the burden of all clinically-apparent dengue cases as opposed to mostly hospitalized dengue cases. This will enable a better understanding and evaluation of interventions to control dengue in Puerto Rico.

Dengue Diagnosis

Dengue surveillance systems in Puerto Rico, both passive and enhanced, depend on accurate diagnostic testing to identify DENV-infected individuals; however, the time between specimen collection and laboratory confirmation frequently approaches two or more weeks due primarily to delays in specimen transport and receipt of reports containing diagnostic test results (Figure 4; CDC, unpublished data). Consequently, surveillance-based diagnostic testing provides minimal clinical utility to the health care provider. Rapid diagnostic tests, in conjunction with a clinical diagnosis of probable dengue, promise greater utility in population-based surveillance for dengue, particularly in resource poor settings that necessitate instrument-independent laboratory diagnostics.[36,37] Despite this, rapid diagnostic tests have not yet been demonstrated to be sufficiently reliable to enable individual patient diagnosis and management. Alternatively, highly sensitive and specific laboratory-based diagnostic tests are now available that can accurately diagnose dengue patients in a single serum specimen. Both molecular[38] and serologic[39]

diagnostic tests that have been approved by the FDA and are available in Puerto Rico at PRDH and CDC Dengue Branch, and currently all submitted specimens are tested for evidence of DENV infection. However, until these or other tests are available in hospitals and clinics, clinical diagnosis by the health care provider will continue to be the primary method to diagnose and treat suspected dengue cases.

Clinical diagnosis of dengue in endemic areas is often complicated by the myriad of other endemic acute febrile illnesses (AFIs) and the dynamic epidemiologic trends of such diseases. Influenza, enterovirus, an array of respiratory pathogens, leptospirosis, and various other bacterial infections often muddle the picture of a non-differentiated AFI, which may be misdiagnosed as dengue during dengue epidemics.[40] The aforementioned WHO criteria for dengue demonstrate considerable overlap of non-specific symptoms with other AFIs. Furthermore, the recent emergence of chikungunya in Puerto Rico,[41] which has a clinical presentation similar to dengue and is also transmitted by *Aedes spp* mosquitos,[42] further complicates identification of dengue patients.

Historically, epidemiologic studies have primarily focused on differentiating laboratory-confirmed dengue patients from dengue-negative patients in regions with endemic dengue. One systematic review[43] and a study in Puerto Rico[44] observed associations between dengue cases and decreased platelets and white blood cell count in addition to an increased proportion of patients with myalgia, rash, and hemorrhagic signs. Specific comparisons between patients with dengue or influenza observed higher proportions of rash, hemorrhagic signs, and positive tourniquet test in addition to more pronounced thrombocytopenia and leukopenia among dengue patients.[45] The scientific literature regarding the clinical manifestations of dengue, though varied in the development of predictive models and likely influenced by circulation of different DENV-

types, provides a framework from which to evaluate the utility of clinical diagnoses and improve timeliness for diagnosis.

Advances in Dengue Prevention

In 2003, the Pan-American Health Organization (PAHO) developed the Integrated Management Strategy for Dengue Prevention and Control (i.e., *Patio Limpio*) and most countries in the region adopted this approach; however, the impact of this program has since been shown to be minimal.[21] The lack of effective approaches to primary prevention of dengue (e.g., a dengue vaccine, sustainable and effective vector control methods) therefore demonstrated the importance of secondary prevention (e.g., disease surveillance, clinical diagnosis, and management of cases) to mitigate the morbidity and mortality associated with dengue.

In example of this, after noting sub-optimal management of fatal dengue cases during the 2007 epidemic in Puerto Rico,[46] medical epidemiologists from CDC Dengue Branch utilized the 2009 WHO Dengue Guidelines[1] to design a four-hour physician clinical training course for physicians in the recommended management of dengue patients. When the 2010 epidemic was growing in magnitude and fatal cases began to be reported, the Secretary of Health of Puerto Rico mandated that all clinicians that see dengue patients take the course, and more than 11,000 clinicians were ultimately trained. An evaluation of clinical practices in 2009 compared to 2011 demonstrated significant increases in adherences to recommended clinical practices, such as use of isotonic intravenous saline, frequency of monitoring vital signs, and avoidance of corticosteroid administration (CDC, unpublished data). This course was subsequently developed into an online training (available at www.cdc.gov/dengue/training/cme.html) that clinicians can take to receive continuing medical education credit. Thus, although an effective and sustainable approach to

primary prevention of dengue is not yet available, improvements in clinical management of dengue patients can reduce the case-fatality rate to <0.1%.[9]

Despite the recognition of dengue as a neglected tropical disease, considerable attention by global, regional, and local stakeholders has produced invaluable resources to guide preventive efforts. The most recent initiative by WHO is comprehensive and includes diagnosis and case management, integrated surveillance and outbreak preparedness, sustainable vector control, future vaccine implementation, and basic operational and implementation research. These components are key actions to reduce dengue mortality by 50% and morbidity by 25% by 2020.[47] Recent advances in dengue vaccine development offer hope for control and prevention. One vaccine candidate reported an overall efficacy of 56% with an excellent safety profile from a phase III trial in Southeast Asia,[48] though the failure to protect against DENV-2 was consistent with previous studies.[8] Nevertheless, the potential to prevent dengue, especially severe cases,[49] with this vaccine and others[50] in development underscores the importance of accurate clinical diagnosis and surveillance to measure their impact. Therefore, until a vaccine or other sustainable and effective approach to dengue control becomes available, health professionals will continue to play the most critical role in the clinical management of cases with dengue and other AFIs in Puerto Rico.

Significance and Contribution of this Research

The continuing burden of dengue in Puerto Rico, as evidenced recent epidemics[26,27] and estimates of economic cost,[15,51,52] emphasizes the crucial role of epidemiology to guide public health initiatives. Within the continental United States, DENV transmission in Florida[53,54] and Texas[55] demonstrate the health impact of dengue on the populations that reside and travel in these states. In 2012, WHO recognized dengue as the fastest spreading vector-borne viral

disease worldwide and prioritized the implementation of sustainable preventive measures against dengue in the effort to overcome neglected tropical diseases.[56,57] Though current prevention strategies fail to curb such trends, recent progress in vaccine development demonstrates the possibility of a safe vaccine to prevent dengue.[8] Accurate epidemiologic surveillance is needed to evaluate the effectiveness of these new vaccines and to inform decisions on their introduction as well as monitor potential changes in the epidemiology of dengue.[58,59]

The robust clinical information collected by SEDSS and inclusion of other AFIs provides an excellent opportunity to contribute to the current understanding of the clinical presentation for dengue and the practices of health care providers in their provisional diagnosis of dengue. The clinical diagnosis of dengue, especially without widespread use of rapid diagnostic tests, is important to differentiate the disease from other AFIs with similar clinical presentations, such as influenza. Furthermore, SEDSS provides a unique opportunity to elucidate potential reasons for under-reporting of dengue cases by health care providers. The clinical manifestations and laboratory results that prompt a health care provider to diagnose a patient with dengue are crucial to understand the process of diagnostic reasoning through which cases become suspect. As such, research from the first year of operation of SEDSS informs both the differentiation of dengue from other AFIs and identifies current trends in diagnosis of dengue in an effort to improve future clinical detection and reporting of the disease.

This study also seeks to understand the integration of SEDSS and PDSS. Two goals of SEDSS as it expands to include additional sites are to provide an epidemiologic sample representative of Puerto Rico and to evaluate primary and secondary prevention methods.[60] Recent progress of dengue vaccine candidates, including ongoing Phase III clinical trials of one candidate and evidence to support the possibility of a safe dengue vaccine, underscores the need

for an adaptable surveillance system in the peri-vaccination era.[8,61,62] Such interventions on the horizon merit a thorough consideration of how SEDSS can work in conjunction with PDSS. The future role of SEDSS to measure the impact of primary prevention methods, such as a dengue vaccine and Phase IV clinical trials[59,62], is vital in the effort to reverse the ominous upward trend of dengue incidence in Puerto Rico and globally.

Specific Aims

Using data from the Sentinel Enhanced Dengue Surveillance System (SEDSS) (Kay Tomashek, PI), operated by the CDC-DB and the Saint Luke's Episcopal Hospital System staff, and data from the Passive Dengue Surveillance System (PDSS), jointly operated by the Centers for Disease Control and Prevention Dengue Branch (CDC-DB) and the Puerto Rico Department of Health (PRDH), we will:

1) Determine to what extent the dengue cases detected by SEDSS are representative of the data from the Ponce Health Region collected by the passive system (PDSS).

   Hypothesis: The data accuracy, completeness, and identification of cases will be greater in SEDSS; however, the demographic and serologic distributions will be the same.

2) Analyze the clinical and laboratory features that differentiate dengue, influenza, other viral upper respiratory infections, enterovirus, bacterial infections, and other acute febrile illnesses.

   Hypothesis: Identified clinical and laboratory markers for common AFIs will reflect previously established markers comparing dengue and influenza to other febrile illnesses.

3) Of patients who tested positive for dengue virus (DENV), use multiple logistic regression modeling to compare the distribution of demographic, clinical, and laboratory characteristics between those who were diagnosed as dengue and those with an alternative clinical diagnosis.

   Hypothesis 1: There will be a number of alternative, more symptom-based, provisional diagnoses used to classify patients who were DENV positive.

Hypothesis 2: Patients with alternative diagnoses who tested positive for DENV will have a different distribution of clinical and laboratory characteristics compared to patients who were diagnosed with dengue and were DENV positive.

Methodology

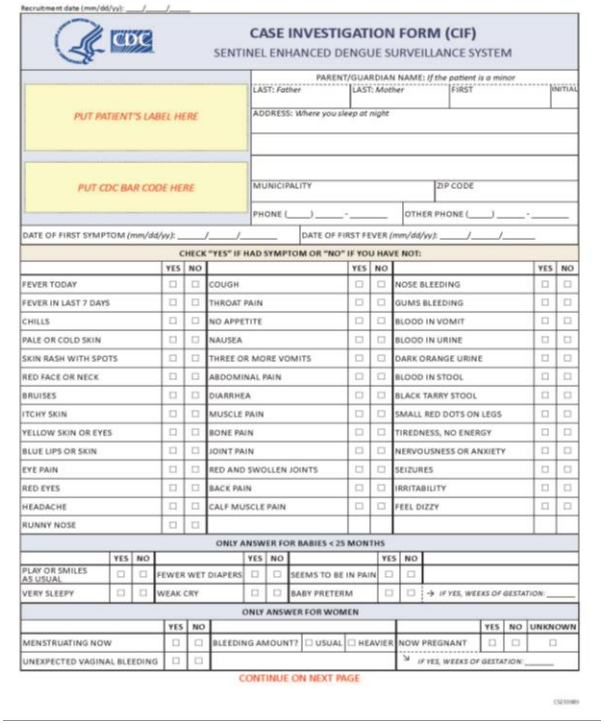
Study Population

The Commonwealth of Puerto Rico, an unincorporated territory of the United States located in the Northeastern Caribbean Sea, had an estimated population of 3,725,789 people in 2010 and a land mass of 8,870 square kilometers.[63] Suspected dengue cases are reported to PDSS from the 78 municipalities that comprise Puerto Rico. Ponce is the fourth largest municipality, is located on the southern coast of the island, and has a population of approximately 166,327. Within Ponce, the Saint Lucas Episcopal Hospital is a tertiary care, teaching hospital with a total of 425 inpatient beds and approximately 54,000 annual emergency department visits. The hospital served as the initial site for the implementation of SEDSS in 2012 with a goal to expand to other parts of the island. The study population consists of patients with an acute febrile illness (AFI) (i.e., ≤7 days of fever) who presented to Saint Luke's Episcopal Hospital between May 7, 2012 and May 6, 2013, met the eligibility criteria, and consented to participate in SEDSS. Surveillance data of suspected and laboratory-confirmed dengue cases in the Ponce Health Region collected by PDSS during this time period were also used for comparison of SEDSS and PDSS.

Collection of Data

SEDSS is the primary database presented and analyzed in this study. The study protocol for SEDSS was reviewed and approved by the Human Subject Institutional Review Board of the Centers for Disease Control and Prevention. A nurse-initiated system, SEDSS recruits patients seeking care at the ED or transferred for direct admission at Saint Luke's Hospital in Ponce. Inclusion criteria include having either a documented fever (>38ºC) or history of fever in the last seven days. Patients who meet the inclusion criteria are offered enrollment, and then complete

an informed consent form and are assigned a tracking code. The Case Investigation Form (CIF) includes demographic and symptoms that are filled out by the patient, vital signs recorded by the nursing staff, a provisional diagnosis completed by the physician, and laboratory results filled in by CDC-DB personnel (Figure 5). Along with the CIF, blood samples are collected and tested for DENV by reverse-transcriptase-polymerase chain reaction (RT-PCR) and antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) for detection of anti-DENV immunoglobulin M (IgM) antibodies. In addition, diagnostic testing is also performed to detect evidence of infection with *Leptospira* species bacteria, *Burkholderia pseudomallei*, *Rickettsia* species bacteria, and enterovirus. A nasopharyngeal and oropharyngeal swab is collected to test for a panel of respiratory viruses by RT-PCR including influenza A and B, respiratory syncytia virus, and parainfluenza viruses. All laboratory tests are performed at either CDC-DB or CDC-Atlanta.



CASE INVESTIGATION FORM (CIF)
SENTINEL ENHANCED DENGUE SURVEILLANCE SYSTEM

PUT PATIENT'S LABEL HERE

PUT CDC BAR CODE HERE

PARENT/GUARDIAN NAME: *If the patient is a minor*
LAST: Father | LAST: Mother | FIRST | INITIAL

ADDRESS: *Where you sleep at night*

MUNICIPALITY | ZIP CODE

PHONE (___) ___ - ____ | OTHER PHONE (___) ___ - ____

DATE OF FIRST SYMPTOM (mm/dd/yy): ___/___/___ | DATE OF FIRST FEVER (mm/dd/yy): ___/___/___

CHECK "YES" IF HAD SYMPTOM OR "NO" IF YOU HAVE NOT:

	YES	NO		YES	NO		YES	NO
FEVER TODAY	☐	☐	COUGH	☐	☐	NOSE BLEEDING	☐	☐
FEVER IN LAST 7 DAYS	☐	☐	THROAT PAIN	☐	☐	GUMS BLEEDING	☐	☐
CHILLS	☐	☐	NO APPETITE	☐	☐	BLOOD IN VOMIT	☐	☐
PALE OR COLD SKIN	☐	☐	NAUSEA	☐	☐	BLOOD IN URINE	☐	☐
SKIN RASH WITH SPOTS	☐	☐	THREE OR MORE VOMITS	☐	☐	DARK ORANGE URINE	☐	☐
RED FACE OR NECK	☐	☐	ABDOMINAL PAIN	☐	☐	BLOOD IN STOOL	☐	☐
BRUISES	☐	☐	DIARRHEA	☐	☐	BLACK TARRY STOOL	☐	☐
ITCHY SKIN	☐	☐	MUSCLE PAIN	☐	☐	SMALL RED DOTS ON LEGS	☐	☐
YELLOW SKIN OR EYES	☐	☐	BONE PAIN	☐	☐	TIREDNESS, NO ENERGY	☐	☐
BLUE LIPS OR SKIN	☐	☐	JOINT PAIN	☐	☐	NERVOUSNESS OR ANXIETY	☐	☐
EYE PAIN	☐	☐	RED AND SWOLLEN JOINTS	☐	☐	SEIZURES	☐	☐
RED EYES	☐	☐	BACK PAIN	☐	☐	IRRITABILITY	☐	☐
HEADACHE	☐	☐	CALF MUSCLE PAIN	☐	☐	FEEL DIZZY	☐	☐
RUNNY NOSE	☐	☐						

ONLY ANSWER FOR BABIES < 25 MONTHS

	YES	NO		YES	NO		YES	NO	
PLAY OR SMILES AS USUAL	☐	☐	FEWER WET DIAPERS	☐	☐	SEEMS TO BE IN PAIN	☐	☐	
VERY SLEEPY	☐	☐	WEAK CRY	☐	☐	BABY PRETERM	☐	☐	→ *IF YES, WEEKS OF GESTATION:* ____

ONLY ANSWER FOR WOMEN

	YES	NO			YES	NO	UNKNOWN
MENSTRUATING NOW	☐	☐	BLEEDING AMOUNT? ☐ USUAL ☐ HEAVIER	NOW PREGNANT	☐	☐	☐
UNEXPECTED VAGINAL BLEEDING	☐	☐		↘ *IF YES, WEEKS OF GESTATION:* ____			

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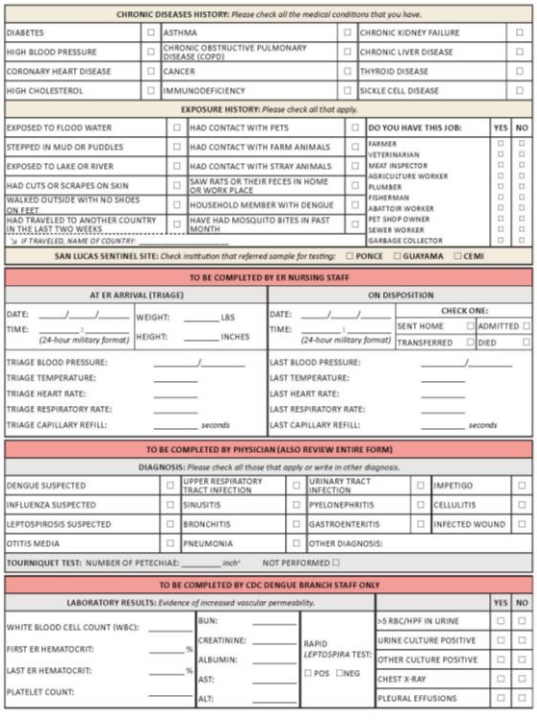

CHRONIC DISEASES HISTORY: *Please check all the medical conditions that you have.*

DIABETES	☐	ASTHMA	☐	CHRONIC KIDNEY FAILURE	☐
HIGH BLOOD PRESSURE	☐	CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)	☐	CHRONIC LIVER DISEASE	☐
CORONARY HEART DISEASE	☐	CANCER	☐	THYROID DISEASE	☐
HIGH CHOLESTEROL	☐	IMMUNODEFICIENCY	☐	SICKLE CELL DISEASE	☐

EXPOSURE HISTORY: *Please check all that apply.*

				DO YOU HAVE THIS JOB:	YES	NO
EXPOSED TO FLOOD WATER	☐	HAD CONTACT WITH PETS	☐	FARMER	☐	☐
STEPPED IN MUD OR PUDDLES	☐	HAD CONTACT WITH FARM ANIMALS	☐	VETERINARIAN	☐	☐
EXPOSED TO LAKE OR RIVER	☐	HAD CONTACT WITH STRAY ANIMALS	☐	MEAT INSPECTOR	☐	☐
HAD CUTS OR SCRAPES ON SKIN	☐	SAW RATS OR THEIR FECES IN HOME OR WORK PLACE	☐	AGRICULTURE WORKER	☐	☐
WALKED OUTSIDE WITH NO SHOES ON FEET	☐	HOUSEHOLD MEMBER WITH DENGUE	☐	PLUMBER	☐	☐
HAD TRAVELED TO ANOTHER COUNTRY IN THE LAST TWO WEEKS	☐	HAVE HAD MOSQUITO BITES IN PAST MONTH	☐	FISHERMAN	☐	☐
↘ *IF TRAVELED, NAME OF COUNTRY:* ____				ABATTOIR WORKER	☐	☐
				PET SHOP OWNER	☐	☐
				SEWER WORKER	☐	☐
				GARBAGE COLLECTOR	☐	☐

SAN LUCAS SENTINEL SITE: *Check institution that referred sample for testing:* ☐ PONCE ☐ GUAYAMA ☐ CEMI

TO BE COMPLETED BY ER NURSING STAFF

AT ER ARRIVAL (TRIAGE)		ON DISPOSITION	
DATE: ___/___/___	WEIGHT: ____ LBS	DATE: ___/___/___	CHECK ONE: SENT HOME ☐ ADMITTED ☐
TIME: ____ : ____ (24-hour military format)	HEIGHT: ____ INCHES	TIME: ____ : ____ (24-hour military format)	TRANSFERRED ☐ DIED ☐
TRIAGE BLOOD PRESSURE: ____/____		LAST BLOOD PRESSURE: ____/____	
TRIAGE TEMPERATURE: ____		LAST TEMPERATURE: ____	
TRIAGE HEART RATE: ____		LAST HEART RATE: ____	
TRIAGE RESPIRATORY RATE: ____		LAST RESPIRATORY RATE: ____	
TRIAGE CAPILLARY REFILL: ____ seconds		LAST CAPILLARY REFILL: ____ seconds	

TO BE COMPLETED BY PHYSICIAN (ALSO REVIEW ENTIRE FORM)

DIAGNOSIS: *Please check all those that apply or write in other diagnosis.*

DENGUE SUSPECTED	☐	UPPER RESPIRATORY TRACT INFECTION	☐	URINARY TRACT INFECTION	☐	IMPETIGO	☐
INFLUENZA SUSPECTED	☐	SINUSITIS	☐	PYELONEPHRITIS	☐	CELLULITIS	☐
LEPTOSPIROSIS SUSPECTED	☐	BRONCHITIS	☐	GASTROENTERITIS	☐	INFECTED WOUND	☐
OTITIS MEDIA	☐	PNEUMONIA	☐	OTHER DIAGNOSIS:			

TOURNIQUET TEST: NUMBER OF PETECHIAE: ____ *inch²* NOT PERFORMED ☐

TO BE COMPLETED BY CDC DENGUE BRANCH STAFF ONLY

LABORATORY RESULTS: *Evidence of increased vascular permeability.*

					YES	NO
WHITE BLOOD CELL COUNT (WBC): ____	BUN: ____	RAPID *LEPTOSPIRA* TEST: ☐ POS ☐ NEG		>5 RBC/HPF IN URINE	☐	☐
FIRST ER HEMATOCRIT: ____ %	CREATININE: ____			URINE CULTURE POSITIVE	☐	☐
LAST ER HEMATOCRIT: ____ %	ALBUMIN: ____			OTHER CULTURE POSITIVE	☐	☐
PLATELET COUNT: ____	AST: ____			CHEST X-RAY	☐	☐
	ALT: ____			PLEURAL EFFUSIONS	☐	☐

Figure 5. Complete CIF front and back.

From May 7, 2012 to May 6, 2013, 9,407 patients presenting to Saint Luke's were offered enrollment in the study, of which 2,213 (33%) agreed to participate and were enrolled in SEDSS at the Ponce site. Laboratory negative or indeterminate cases which lacked a convalescent sample were excluded from further analysis. Also, 54 cases without a provisional diagnosis and 19 with a co-infection of dengue and influenza were excluded. In total, 1,083 participants with a laboratory-identified etiologic agent of AFI were included for analysis.

Case Definitions

A laboratory-positive dengue case was defined as detection of: DENV nucleic acid sequence in a serum, cerebrospinal fluid, or autopsy tissue specimen by RT-PCR; seroconversion from negative to positive detection of anti-DENV IgM antibody in paired serum specimens; DENV antigen in an autopsy tissue specimen by IHC assays; or anti-DENV IgM antibodies in a serum specimen. A laboratory-positive influenza case was defined by detection of influenza A or B virus nucleic acid. Similar definitions were applied to all other upper respiratory pathogens (Adenovirus, Human coronavirus 229E, OC43, NL63, HKU1, Human metapneumovirus, respiratory syncytial virus, Parainfluenza virus 1,2,3) detected by RT-PCR.

Classification of Provisional Diagnoses

The CIF captures the physician's provisional diagnosis of the patient (Figure 6). A classification system was developed to group diagnoses into clinical syndromes defined as dengue, influenza, viral infection, gastrointestinal, respiratory, enterovirus, and genitourinary (Table 1). To classify and analyze cases with multiple diagnoses, a hierarchy was used to favor the most specific syndrome. For example, cases diagnosed as dengue and viral syndrome or dengue and thrombocytopenia were classified as dengue. Additional information on the provisional diagnosis for incomplete forms was obtained through Meditech, the electronic health

record used by Saint Luke's Health System, and from a review of patient charts using ICD-9 codes from diagnosis at admission.

TO BE COMPLETED BY PHYSICIAN (ALSO REVIEW ENTIRE FORM)							
DIAGNOSIS: *Please check all those that apply or write in other diagnosis.*							
DENGUE SUSPECTED	☐	UPPER RESPIRATORY TRACT INFECTION	☐	URINARY TRACT INFECTION	☐	IMPETIGO	☐
INFLUENZA SUSPECTED	☐	SINUSITIS	☐	PYELONEPHRITIS	☐	CELLULITIS	☐
LEPTOSPIROSIS SUSPECTED	☐	BRONCHITIS	☐	GASTROENTERITIS	☐	INFECTED WOUND	☐
OTITIS MEDIA	☐	PNEUMONIA	☐	OTHER DIAGNOSIS:			

Figure 6. CIF section for provisional diagnosis by physician.

Table 1. Classification of diagnoses and clinical syndromes.

Clinical Syndrome	Included Diagnoses
Dengue	Box checked on CIF for dengue suspected, Other diagnoses: r/o dengue, dengue-like syndrome, viral illness dengue-like, dengue fever
Influenza	Box checked for influenza suspected, Other diagnoses: r/o influenza, Flu-like syndrome
Viral Infx	Viral syndrome, viral infx, AFI, acute viral syndrome, suspected viral illness, viral exanthem
Respiratory	Sinusitis, pharyngitis, nasopharyngitis, otitis media, croup, rhinitis, tonsillitis, pneumonia, bronchitis, bronchiolitis
Genitourinary	UTI, pyelonephritis, cystitis, urethritis, prostatitis, orchitis, epididymitis
Gastrointestinal	Gastroenteritis, enteritis, esophagitis, mesenteric adenitis, Campylobacter
Other	Leptospirosis, cellulitis, abscess, asthma, symptoms (thrombocytopenia, myalgias, fever, etc), cholecystitis, pancreatitis, HUS, CHF/ESRD, Stroke

Statistical Analysis

All statistical analyses were performed using Stata version 12.1 (StataCorp, College Station, TX). The first aim of this study involves the comparison of data on suspected dengue collected by PDSS and SEDSS to determine to what extent dengue cases detected by SEDSS are representative of the Ponce Health Region. Common variables collected by the DCIF and laboratory data from CDC-DB were described and compared with regards to age, gender, DENV type, the proportion of hospitalizations, the number of cases with at least once hemorrhagic manifestation, and the number of cases with at least one warning sign. Statistical differences in proportions were tested by applying the chi-squared test and Fisher's exact test for categorical variables and Student's t-test and the Mann-Whitney U test for continuous variables. Additionally, a pre/post analysis was conducted using the four years of surveillance data from PDSS prior to introduction of enhanced surveillance at Saint Luke's Episcopal Hospital for a pilot study in 2009. A control hospital similar to Saint Luke's Episcopal Hospital was selected based on type of hospital, services, and average number of annual patient visits. Expected number of cases for the study period were then calculated using surveillance data collected from 2005-2008 to estimate the improved detection of suspected and confirmed cases of dengue. Among all laboratory-positive cases, summary statistics for clinical and laboratory features as well as provisional diagnoses were provided for dengue, influenza, and other upper respiratory infections. To differentiate dengue and influenza, simple logistic regression was conducted for each relevant covariate, identified in the literature and the WHO case definition for suspected dengue, as the initial step in model-building for multiple logistic regression. Purposeful selection[64,65] was employed to construct a multiple logistic regression model with dengue and influenza as the two primary outcomes. Variables with a p-value of <0.25 on univariate analysis

were selected for the initial model. A reduced model was formed using variables with a p-value <0.05 in the initial multivariate model. Assessment of confounding and interaction was performed in addition to the use of fractional polynomials to evaluate the scale for the continuous variable of age. The Hosmer-Lemeshow Test was used to test the goodness of fit and appropriate logistic regression diagnostics were performed for the final main effects model. Forward stepwise and backward stepwise procedures for multivariate model-building, both with a significance level of 0.2 for removal from model and 0.1 for addition to the model, were used for comparison with the model obtained from purposeful selection.

The final aim considered only laboratory-positive dengue cases to investigate clinical and laboratory factors associated with a provisional clinical diagnosis of dengue. For logistic regression modeling, the continuous variable platelets was dichotomized into thrombocytopenia with a threshold of <100,000 platelets/mm^3 and normal if platelets >100,000 platelets/mm^3. Similarly, white blood cell count was also dichotomized into leukopenia (total white blood cell count <4,500/mm^3) and normal (total white blood cell count >4,500). Simple logistic regression analysis was conducted for each relevant covariate, identified in the literature and the WHO case definition for suspected dengue, as the initial step in model-building for multiple logistic regression. Purposeful selection[64,65] was employed to construct a multiple logistic regression model with provisional diagnosis of dengue as the dichotomous outcome. Variables with a p-value of <0.25 on univariate analysis were selected for the initial model. A reduced model was formed using variables with a p-value of <0.05 in the initial multivariate model. Assessment of confounding and interaction was performed in addition to the use of fractional polynomials to evaluate the scale for the continuous variable of age. The Hosmer-Lemeshow Test was used to test the goodness of fit and appropriate logistic regression diagnostics were performed for the

final main effects model. Forward stepwise and backward stepwise procedures for multivariate model-building, both with a significance level of 0.2 for removal from model and 0.1 for addition to the model, were also used for comparison with the model obtained from purposeful selection. Stratification analyses using the aforementioned modeling procedures were performed for age groups ≤10 years-old, 10-19 years-old, and ≥20 years-old.

Results

Aim 1:

Between May 7, 2012 and May 6, 2013, a total of 621 laboratory-confirmed dengue cases were reported to the SEDSS site at Saint Lucas Episcopal Hospital and 1479 laboratory-confirmed dengue cases were reported to PDSS in the Ponce Health Region. The epi curves for both surveillance systems were roughly the same with peak cases at week 29 (Figure 7). Compared to PDSS, dengue cases detected by SEDSS were approximately six years younger ($p<0.0001$), less likely to be hospitalized ($p<0.0001$), and had a higher completion rate of the case investigation form ($p<0.0001$) (Table 2). The median day post onset of illness at which time the sample was collected also differed by one day (Rank Sum Test; $p<0.0001$). The distribution of DENV types (Figure 8) did not differ significantly between SEDSS and PDSS (Table 3) and there was no observed difference in the gender distribution ($p>0.05$). The total number of laboratory-positive dengue cases detected by PDSS between 2005 and 2008 at Saint Lucas Episcopal Hospital was 219 whereas 746 laboratory-positive dengue cases were detected during the first year of SEDSS at Saint Lucas Episcopal Hospital (Table 4). Using Damas Hospital as a control site, the number of additional laboratory-positive dengue cases detected was estimated to have increased by 314 (240%) by the implementation of SEDSS.

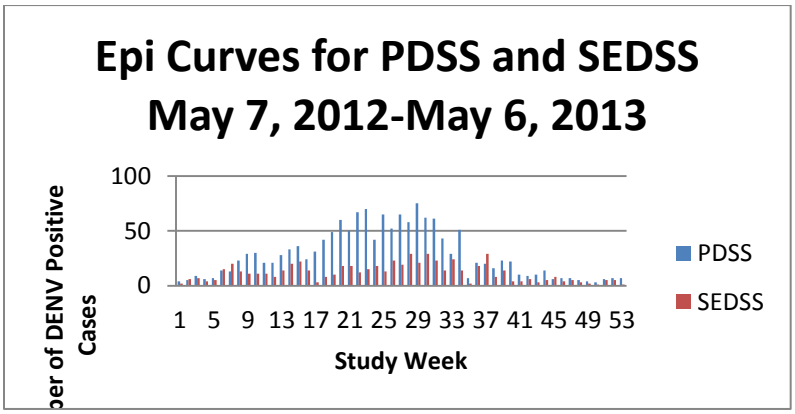

Figure 7. Epi Curves for PDSS and SEDSS by Study Week

Table 2. Demographic and Completion Variables for Comparison of SEDSS and PDSS

	PDSS (n=1479)	SEDSS (n=565)	Estimated Difference	P-Value
Age	24.94(24.02, 25.87)	18.86(17.58, 20.13)	6.09 (6.06, 6.14)	<0.0001
Gender (Proportion Male)	0.4429 (0.4175, 0.4682)	0.4767 (0.4373, 0.5160)	0.03	NS
Proportion Hospitalized	0.641 (0.602, 0.679)	0.4612 (0.4218, 0.5006)	0.179 (0.125, 0.234)	<0.0001
Day Post Onset of Illness (Median)	4	3	1	<0.0001
Completion of Case Investigation Form (%)	27.23(27.03,27.42)	61.47 (60.85, 62.09)	36.87 (36.84, 36.90)	<0.0001

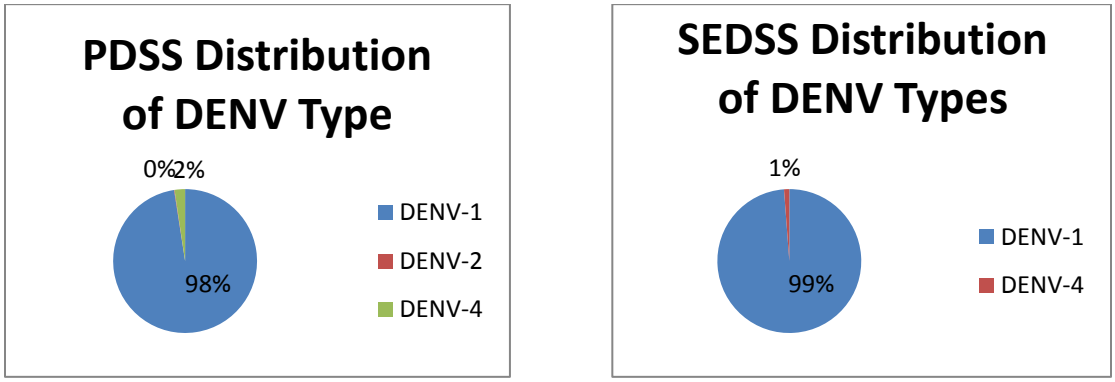

Figure 8. DENV Type Distribution

Table 3. DENV Type Distribution

	DENV-1	DENV-2	DENV-4	Unknown	Total
PDSS	874	1	21	215	1111
SEDSS	418	0	5	141	564
Total	1292	1	26	356	1675
p=0.288 (Pearson Chi-Squared test) and p=0.307 (Fisher's Exact test)					

Table 4. Estimation of increased reporting of suspected dengue cases

Hospital	**DENV-Positive Cases 2005-2008**	**DENV-Positive Cases 2012-2013**	**Expected Number of Cases based on Control Hospital**	**Estimated Increased Detection**
Saint Luke's Episcopal Hospital (SEDSS Site)	219	220 (PDSS) + 526 (SEDSS) = 746	314	2.4X
Damas Hospital (Control Site)	163	234		

Aim 2:

A closer look of the data collected by SEDSS permitted a comparison between the clinical presentation of dengue and other AFIs. Among the 1083 patients enrolled in the first year of SEDSS with a laboratory-identified etiologic agent of AFI included in the analysis, 269

(24.8%), 21 (1.9%), 302 (27.9%), 274 (25.3%), 217 (20.0%) received a provisional diagnosis of dengue, influenza, viral infection, respiratory infection, and other diagnosis, respectively (Figure 9). Of these, 579 (53.5%) were laboratory-positive dengue cases, 283 (26.1%) were laboratory-positive influenza cases, and 221 (20.4%) were laboratory-positive for another pathogen (Table 5). Compared to DENV-positive cases, influenza-positive cases were approximately four years older ($p<0.01$) and there were no statistically significant differences by gender (Table 6). Cases with headache, myalgia, rash, diarrhea, nausea, vomiting, lethargy, abdominal pain, leukopenia, and thrombocytopenia were less likely to be influenza-positive than DENV-positive. Odds ratios were the primary measures of associations reported. Of note, leukopenia and thrombocytopenia demonstrated a strong magnitude of association with odds ratios of 0.15 and 0.07, respectively. Therefore, the odds of presenting with leukopenia among influenza-positive cases were 0.07 times the odds of presenting with leukopenia among DENV-positive cases. Similarly, cases with cough, runny nose, and sore throat were more likely to be influenza-positive than DENV-positive.

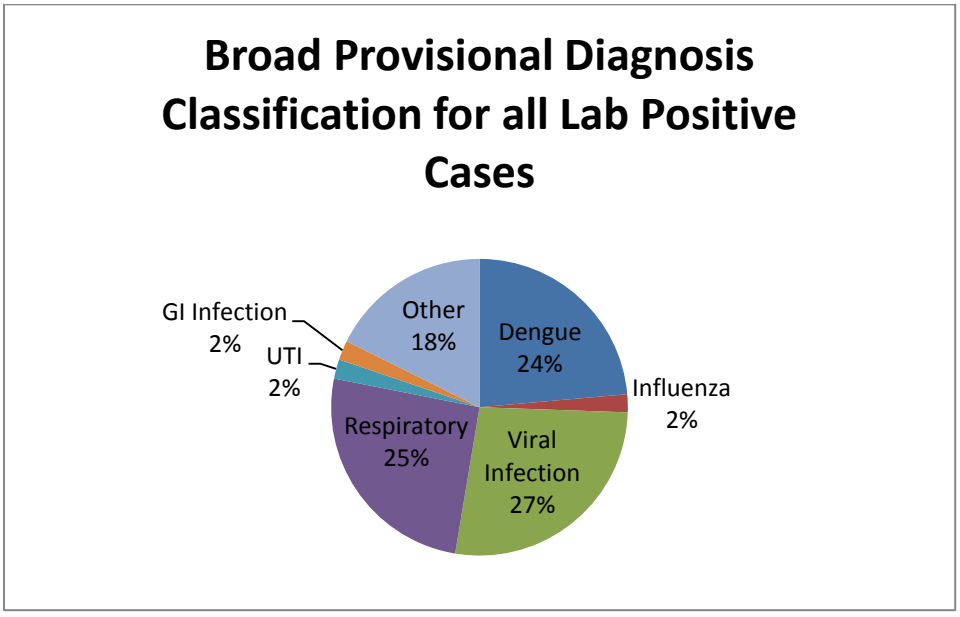

Figure 9. Distribution of provisional diagnoses for all laboratory-positive cases for the first year of SEDSS.

Table 5. Distribution of provisional diagnoses for all laboratory-positive cases for the first year of SEDSS.

Provisional Diagnosis	Laboratory Diagnosis			Total
	DENV-Positive	Influenza Positive	Other Positive	
Dengue	243	21	5	269
Influenza	3	14	4	21
Viral Infection	183	88	31	302
Respiratory	60	94	120	274
Other	90	66	61	217
Total	579	283	221	1083

Table 6. Descriptive statistics for DENV-positive and Influenza-positive cases

Clinical/ Laboratory Feature	Dengue (n=579)	Influenza (n=283)	
	Mean (SD, Range)	Mean (SD, Range)	OR (95%CI)*
Age ≤10 years-old 10-19 years-old ≥20 years-old	18.7 (16.1, 0-88) 153 (26.4%) 276 (47.7% 150 (25.9%)	22.8 (22.2, 0.2-91) 108 (38.2%) 61 (21.6%) 114 (40.3%)	0.011 (0.004, 0.019)
Gender (Proportion Male)	0.485	0.463	0.914 (0.687, 1.215)
Headache	0.841	0.761	0.601 (0.421, 0.858)
Retro-orbital pain	0.590	0.524	0.765 (0.572, 1.022)
Myalgia	0.692	0.617	0.718 (0.530, 0.974)
Arthralgia	0.593	0.532	0.779 (0.581, 1.044)
Rash	0.444	0.147	0.215 (0.148, 0.313)
Cough	0.373	0.888	13.40 (8.88, 20.21)
Runny Nose	0.304	0.798	9.05 (6.42, 12.77)
Diarrhea	0.370	0.263	0.608 (0.442, 0.836)
Nausea	0.720	0.562	0.499 (0.368, 0.674)
Vomiting	0.255	0.182	0.649 (0.453, 0.932)
Lethargy	0.869	0.770	0.506 (0.349, 0.732)
Abdominal Pain	0.607	0.460	0.551 (0.411, 0.738)
Sore throat	0.365	0.635	3.03 (2.24, 4.09)
Bruising	0.050	0.058	1.18 (0.626, 2.22)
Bleeding	0.088	0.078	0.873 (0.518, 1.47)
Hematocrit Hemoconcentration	40.2 (4.6; 26.8-55.5) 0.0098	39.9 (4.4; 26.3-52.9) 0	 -
WBC Leukopenia	3.94 (2.61; 1-19) 0.719	6.3 (2.7; 1.9-19) 0.273	 0.147 (0.106, 0.205)
Platelets Thrombocytopenia	128,469 (70,051; 100-511,000) 0.408	196,341 (68,834; 22,000-610,000) 0.049	 0.074 (0.041, 0.133)

* DENV-positive cases used as reference group for OR

Multiple logistic regression analysis utilized purposeful selection, forward stepwise, and backward stepwise procedures to determine significant laboratory and clinical features associated with testing positive for influenza compared to testing positive for dengue (Table 7). Provisional diagnosis, thrombocytopenia, leukopenia, lethargy, cough, sore throat, and sore throat were included in all three models. All three models demonstrated adequate goodness of fit by both the Hosmer-Lemeshow Test and the Pearson Chi-Squared ($p>0.05$). The most parsimonious model was that obtained by the forward stepwise procedure with comparable values for the Aikike information criterion (AIC) and the Bayesian information criterion (BIC).

Table 7. Comparison of Different Multiple Logistic Regression Model-building Procedures for DENV-positive and Influenza-positive Cases

Model-Building Strategy	Variables Included in Multiple Logistic Regression Model	AIC	BIC	Goodness of Fit Pearson Chi-Squared	Goodness of Fit H-L Chi-Squared
Forward/Forward Stepwise Procedure	Provisional diagnosis, thrombocytopenia, leukopenia, nausea, lethargy, cough, sore throat, rash	480.0	533.9	185.31 (0.9225)	11.16 (0.1930)
Backward/Backward Stepwise Procedure	Provisional diagnosis, thrombocytopenia, leukopenia, retro-orbital pain, lethargy, cough, sore throat, rash, arthralgia	479.8	538.1	261.15 (0.9097)	9.21 (0.3248)
Purposeful Selection	Provisional Diagnosis, thrombocytopenia, leukopenia, nausea, lethargy, cough, sore throat, rash, arthralgia	541.1	600.7	272.6 (0.9216)	12.02 (0.1504)

As compared to DENV-positive cases, laboratory-positive cases for other pathogens were younger by approximately eight years ($p<0.01$) and there were no statistically significant differences in gender (Table 8). Cases with headache, retro-orbital pain, myalgia, arthralgia, rash, diarrhea, nausea, lethargy, abdominal pain, leukopenia, and thrombocytopenia were less likely to be laboratory-positive for another pathogen than DENV-positive. Similarly, cases with cough, runny nose, and sore throat were more likely to be laboratory-positive for another pathogen than DENV-positive.

Table 8. Descriptive Statistics for DENV-positive and other lab-positive cases

Clinical/ Laboratory Feature	Dengue (n=579)	Other Pathogen (n=221)	
	Mean (SD, Range)	Mean (SD, Range)	OR* (95% CI)
Age ≤10 years-old 10-19 years-old ≥20 years-old	18.7 (16.1, 0-88) 153 (26.4%) 276 (47.7% 150 (25.9%)	10.9 (19.1, 0.1-85) 168 (76.0%) 15 (6.8% 38 (17.2%)	-0.036 (-0.050, -0.023)
Gender (Proportion Male)	0.485	0.471	0.943 (0.691, 1.286)
Headache	0.841	0.469	0.167 (0.117, 0.237)
Retro-orbital pain	0.590	0.248	0.229 (0.160, 0.327)
Myalgia	0.692	0.329	0.218 (0.155, 0.307)
Arthralgia	0.593	0.279	0.266 (0.187, 0.377)
Rash	0.444	0.175	0.267 (0.180, 0.395)
Cough	0.373	0.804	6.88 (4.73, 10.01)
Runny Nose	0.304	0.766	7.51 (5.23, 10.78)
Diarrhea	0.370	0.238	0.534 (0.373, 0.763)
Nausea	0.720	0.507	0.399 (0.289, 0.553)
Vomiting	0.255	0.280	1.14 (0.799, 1.62)
Lethargy	0.869	0.623	0.250 (0.173, 0.361)
Abdominal Pain	0.607	0.380	0.397 (0.286, 0.551)
Sore throat	0.365	0.519	1.88 (1.36, 2.60)
Bruising	0.050	0.033	0.654 (0.281, 1.52)
Bleeding	0.088	0.095	1.09 (0.638, 1.85)
Hematocrit Hemoconcentration	40.2 (4.6; 26.8-55.5) 0.0098	36.7 (4.2; 25.9-61.5) 0.010	 1.071 (0.206, 5.57)
WBC Leukopenia	3.94 (2.61; 1-19) 0.719	9.65 (4.56; 1-26.5) 0.070	 0.029 (0.017, 0.052)
Platelets Thrombocytopenia	128,469 (70,051; 100-511,000) 0.408	271,816 (101,960; 33,000-664,000) 0.0199	 0.029 (0.011, 0.080)

* DENV-positive cases used as reference group for OR

Multiple logistic regression analysis utilized purposeful selection, forward stepwise, and backward stepwise procedures to determine significant laboratory and clinical features associated with testing positive for another pathogen compared to testing positive for dengue (Table 9). Provisional diagnosis, thrombocytopenia, leukopenia, absence of cough, and rash were included in all three models. The two models obtained by forward stepwise and backward stepwise procedures violated tests for goodness of fit by both the Hosmer-Lemeshow Test and the Pearson Chi-Squared ($p<0.05$), whereas the model obtained by purposeful selection had appropriate

goodness of fit ($p>0.05$). Values for AIC and BIC were slightly larger for the purposeful selection model compared to forward and backward stepwise procedure models.

Table 9. Comparison of Different Multiple Logistic Regression Model-building Procedures for DENV-positive and Other Pathogen-positive Cases

Model-Building Strategy	Variables Included in Multiple Logistic Regression Model	AIC	BIC	Goodness of Fit Pearson Chi-Squared (p-value)	Goodness of Fit H-L Chi-Squared (p-value)
Forward/Forward Stepwise Procedure	Provisional diagnosis, thrombocytopenia, leukopenia, Age group, hemoconcentration, bleeding, retro-orbital pain, cough, myalgia, rash	296.1	362.1	542.29 (<0.0001)	77.21 (<0.0001)
Backward/Backward Stepwise Procedure	Provisional diagnosis, thrombocytopenia, leukopenia, age, hemoconcentration, bleeding, retro-orbital pain, cough, myalgia, rash, sore throat	295.8	366.2	658.58 (<0.0001)	23.08 (0.0033)
Purposeful Selection	Provisional Diagnosis, thrombocytopenia, leukopenia, nausea, lethargy, cough, sore throat, rash, arthralgia	327.3	384.6	261.99 (0.6252)	12.92 (0.1146)

Aim 3:

For the final aim, dengue cases were analyzed for features associated with receiving a provisional clinical diagnosis of dengue. Among the 1083 patients enrolled in the first year of SEDSS with a laboratory-identified etiologic agent of AFI included in the analysis, 579 (53.5%) were laboratory-positive dengue cases. Of these, 42% received a provisional diagnosis of dengue, and 58% received an alternative diagnosis, most commonly viral infection (32%) or respiratory infection (10%) (Figure 10). The sensitivity of a provisional diagnosis of dengue was 42% with a specificity of 95% (Table 10). The positive predictive value and negative predictive value were 76% and 81%, respectively.

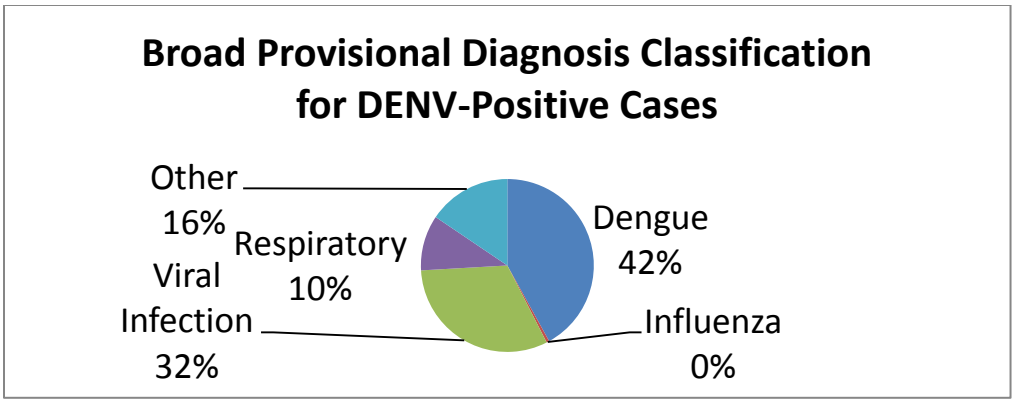

Figure 10. Provisional Clinical Diagnoses for DENV-Positive Cases

Table 10. Sensitivity and Specificity of Provisional Diagnosis of Dengue

Provisional Diagnosis	DENV(+)	DENV(-)	Total
Dengue	242	76	318
Not Dengue	337	1,468	1,805
Total	579	1,544	2,123

Dengue cases that received a provisional diagnosis of dengue were approximately three years younger (p=0.019) than dengue cases that received an alternative diagnosis, and more likely to meet the WHO criteria for dengue. There were no statistically significant differences in either of these by gender (Table 11). Cases with rash, diarrhea, and abdominal pain were more likely to receive a provisional diagnosis of dengue. Dengue cases that presented with leukopenia and thrombocytopenia were also more likely to receive a provisional diagnosis of dengue.

Table 11. Descriptive Statistics for Provisional Diagnosis of Dengue and Alternative Diagnosis among DENV-positive Cases

Clinical/Laboratory Feature	Provisional Diagnosis Dengue (n=243) Mean (SD; Range)	Provisional Diagnosis Not Dengue (N= 336) Mean (SD; Range)	OR (95%CI)
Met WHO criteria for dengue	0.918	0.747	3.78 (2.25, 6.35)
Age	16.87 (13.57; 0.16-71)	20.06 (17.67; 0-88)	
≤10 years-old	64 (26.3%)	89 (26.4%)	1.00
10-19 years-old	133 (54.7%)	143 (42.6%)	1.29 (0.87, 1.93)
≥20 years-old	46 (18.9%)	104 (31.0%)	0.615 (0.383, 0.987)
Gender (Proportion Male)	0.469	0.497	0.894 (0.64, 1.24)
Headache (n=573)	0.867	0.822	1.41 (0.885, 2.25)
Retro-orbital pain (n=568)	0.619	0.568	1.24 (0.88, 1.74)
Myalgia (n=558)	0.707	0.681	1.13 (0.78, 1.63)
Arthralgia (n=553)	0.685	0.584	0.96 (0.68, 1.36)
Rash (n=559)	0.530	0.381	1.83 (1.30, 2.57)
Cough (n=555)	0.349	0.390	0.838 (0.59, 1.19)
Diarrhea (n=560)	0.442	0.318	1.69 (1.20, 2.41)
Nausea (n=554)	0.750	0.699	1.29 (0.88, 1.89)
Vomiting (n=557)	0.289	0.231	1.35 (0.92, 1.99)
Lethargy (n=571)	0.867	0.870	0.979 (0.56, 1.60)
Abdominal Pain (n=557)	0.682	0.552	1.74 (1.22, 2.48)
Sore throat (n=556)	0.322	0.396	0.723 (0.508, 1.03)
Bruising (n=564)	0.055	0.046	1.21 (0.56, 2.59)
Bleeding (n=579)	0.115	0.068	1.77 (0.99, 3.16)
Hct (n=511)	40.29 (4.86; 28-53)	40.20 (4.48; 26.8-55.5)	
Hemoconcentration	0.00433	0.0143	0.3 (0.0333, 2.703)
WBC (n=540)	3.06 (1.86; 1-11)	4.61 (2.90; 1-19)	
Leukopenia	0.843	0.622	3.27 (2.15, 4.98)
Platelets (n=539)	93,203 (45,785; 18,000-276,000)	155,937 (73,361; 10,000-511,000)	
Thrombocytopenia	0.631	0.234	5.59 (3.85, 8.14)

Multiple logistic regression analysis utilized purposeful selection, forward stepwise, and backward stepwise procedures to determine significant laboratory and clinical features associated with a provisional diagnosis of dengue among all laboratory-positive dengue cases (Table 12). Both leukopenia and thrombocytopenia were significant in all three models. Headache was included in the forward and backward stepwise procedures with the addition of age and hemoconcentration in the backward stepwise model. All three models demonstrated adequate goodness of fit by both the Hosmer-Lemeshow Test and the Pearson Chi-Squared ($p>0.05$).

Forward and backward stepwise procedures yielded superior values for the AIC and the BIC compared to the model utilizing purposeful selection.

Table 12. Comparison of Different Multiple Logistic Regression Model-building Procedures

Model-Building Strategy	Variables Included in Multiple Logistic Regression Model	AIC	BIC	Goodness of Fit Pearson Chi-Squared	Goodness of Fit H-L Chi-Squared
Forward/Forward Stepwise Procedure	Thrombocytopenia, leukopenia, headache	518.6	539.0	190.96 (0.3472)	13.67 (0.0907)
Backward/Backward Stepwise Procedure	Thrombocytopenia, leukopenia, hematoconcentration, age, headache	517.4	542.0	192.21 (0.3620)	13.15 (0.1068)
Purposeful Selection	Thrombocytopenia, leukopenia, age, leukopenia*age	629.6	651.0	179.3 (0.2612)	12.48 (0.1309)

Stratification by Age Group

The statistical significance of age in univariate analysis and its inclusion as a variable in two of the multiple logistic regression models prompted a subanalysis with stratification by age to control for this variable. Among the 579 laboratory-positive dengue cases, 152 (26.3%) were ≤10 years-old, 276 (47.7%) were 10–19 years-old, and 148 (25.6%) were ≥20 years-old. Sensitivities of provisional diagnoses for children ≤10 years-old, 10–19 years-old, and adults ≥20 years-old were 41%, 48%, and 31%, respectively. For children ≤10 years-old, diarrhea, abdominal pain, thrombocytopenia, and leukopenia were all significantly associated with a provisional diagnosis of dengue (Table 13). Rash, abdominal pain, bleeding, thrombocytopenia, and leukopenia were significant for older children 10-19 years-old (Table 14). The only variable associated with a provisional diagnosis of dengue for adults ≥20 years-old was thrombocytopenia (Table 15).

Table 13. Descriptive Statistics for Provisional Diagnosis of Dengue and Alternative Diagnosis among DENV-positive Cases for ≤10 years-old

Clinical Feature	Provisional Diagnosis Dengue (n=63)	Alternative Diagnosis (n=89)	OR (95%CI)
Met WHO criteria for dengue	0.889	0.539	6.96 (2.86, 16.92)
Gender	0.524	0.573	0.82 (0.43, 1.57)
Rash	0.581	0.464	1.64 (0.85, 3.18)
Diarrhea	0.484	0.195	3.74 (1.81, 7.73)
Headache	0.806	0.663	1.96 (0.92, 4.17)
Retro-orbital Pain	0.413	0.352	1.34 (0.69, 2.60)
Myalgias	0.557	0.442	1.64 (0.85, 3.16)
Arthralgias	0.525	0.386	1.81 (0.93, 3.53)
Nausea	0.635	0.542	1.41 (0.72, 2.73)
Vomiting	0.246	0.233	1.05 (0.49, 2.27)
Lethargy	0.857	0.744	1.86 (0.81, 4.26)
Abdominal Pain	0.714	0.494	2.42 (1.22, 4.81)
Bruises	0.0635	0.068	0.91 (0.25, 3.37)
Bleeding	0.079	0.0562	1.45 (0.40, 5.23)
Cough	0.403	0.357	1.23 (0.60, 2.32)
Sore throat	0.397	0.369	1.12 (0.57, 2.20)
Thrombocytopenia	0.783	0.182	4.21 (1.95, 9.09)
Leukopenia	0.8	0.481	4.32 (1.99, 9.38)
Hemoconcentration	0	0	-

Table 14. Descriptive Statistics for Provisional Diagnosis of Dengue and Alternative Diagnosis among DENV-positive Cases for 10-19 years-old

Clinical Feature	Provisional Diagnosis Dengue (n=133)	Alternative Diagnosis (n=143)	OR (95%CI)
Met WHO criteria for dengue	0.925	0.797	3.13 (1.46, 6.71)
Gender	0.451	0.448	1.01 (0.63, 1.63)
Rash	0.543	0.400	1.78 (1.10, 2.89)
Diarrhea	0.413	0.348	1.32 (0.80, 2.17)
Headache	0.917	0.860	1.79 (0.82, 3.89)
Retro-orbital Pain	0.669	0.647	1.10 (0.67, 1.82)
Myalgias	0.722	0.703	1.10 (0.64, 1.87)
Arthralgias	0.545	0.540	1.02 (0.63, 1.66)
Nausea	0.790	0.710	1.54 (0.87, 2.71)
Vomiting	0.307	0.210	1.67 (0.95, 2.91)
Lethargy	0.893	0.922	0.71 (0.31, 1.62)
Abdominal Pain	0.690	0.568	1.69 (1.02, 2.81)
Bruises	0.047	0.029	1.65 (0.45, 5.97)
Bleeding	0.128	0.035	4.04 (1.45, 11.30)
Cough	0.315	0.331	0.93 (0.56, 1.56)
Sore throat	0.317	0.406	0.681 (0.41,1.13)
Thrombocytopenia	0.638	0.262	4.98 (2.92, 8.49)
Leukopenia	0.9	0.746	3.06 (1.52, 6.17)
Hemoconcentration	0.008	0.0088	0.903 (0.0558, 14.61)

Table 15. Descriptive Statistics for Provisional Diagnosis of Dengue and Alternative Diagnosis among DENV-positive Cases for ≥20 years-old

Clinical Feature	Provisional Diagnosis Dengue (n=46)	Alternative Diagnosis (n=102)	OR (95%CI)
Met WHO criteria for dengue	0.935	0.853	2.42 (0.66, 8.79)
Gender	0.457	0.5	0.84 (0.42, 1.69)
Rash	0.409	0.278	1.76 (0.83, 3.69)
Diarrhea	0.477	0.380	1.47 (0.72, 3.01)
Headache	0.826	0.901	0.51 (0.19, 1.39)
Retro-orbital Pain	0.756	0.65	1.69 (0.76, 3.72)
Myalgias	0.864	0.85	1.09 (0.39, 3.02)
Arthralgias	0.791	0.842	0.69 (0.28, 1.72)
Nausea	0.818	0.808	1.04 (0.42, 2.60)
Vomiting	0.302	0.253	1.25 (0.57, 2.75)
Lethargy	0.826	0.901	0.51 (0.19, 1.39)
Abdominal Pain	0.628	0.571	1.22 (0.59, 2.55)
Bruises	0.068	0.051	1.39 (0.32, 6.09)
Bleeding	0.130	0.118	1.05 (0.37, 2.96)
Cough	0.381	0.5	0.62 (0.29, 1.28)
Sore throat	0.233	0.414	0.43 (0.19, 0.97)
Thrombocytopenia	0.80	0.235	13.04 (5.48, 31.0)
Leukopenia	0.733	0.576	2.03 (0.937, 4.38)
Hemoconcentration	0	0.0309	-

Purposeful selection, forward stepwise, and backward stepwise procedures were all employed to build multiple logistic regression models for each of the age groups (Tables 16, 17, 18). For children ≤10 years-old, thrombocytopenia and leukopenia were consistent variables included in all three modeling strategies whereas the forward stepwise and backward stepwise procedures both added diarrhea to the model with superior values for the AIC and BIC. The most parsimonious model for clinical and laboratory features associated with a provisional diagnosis of dengue among children 10–19 years-old included thrombocytopenia, headache, and myalgias. The backward stepwise procedure and purposeful selection method also added leukopenia, absence of sore throat, and lethargy. All three models demonstrated adequate goodness of fit and yielded similar values for AIC and BIC. For adults ≥20 years-old, both the forward stepwise procedure and the purposeful selection method failed to produce a multivariate model and violated tests for goodness of fit. In contrast, the backward stepwise procedure produced a

model which included thrombocytopenia and bruises with appropriate goodness of fit and superior values for AIC/BIC.

Table 16. Comparison of Different Multiple Logistic Regression Model-building Procedures ≤10 years-old Age Group

Model-Building Strategy	Variables Included in Multiple Logistic Regression Model	AIC	BIC	Goodness of Fit Pearson Chi-Squared	Goodness of Fit H-L Chi-Squared
Forward Stepwise Procedure	Thrombocytopenia, leukopenia, diarrhea	132.7	143.4	6.67 (0.154)	6.56 (0.2556)
Backward Stepwise Procedure	Thrombocytopenia, leukopenia, diarrhea	132.7	143.4	6.67 (0.154)	6.56 (0.2556)
Purposeful Selection	Thrombocytopenia, leukopenia	169.2	178.0	0 (0.9699)	J<4, so HL inappropriate

Table 17. Comparison of Different Multiple Logistic Regression Model-building Procedures 10-19 years-old Age Group

Model-Building Strategy	Variables Included in Multiple Logistic Regression Model	AIC	BIC	Goodness of Fit Pearson Chi-Squared	Goodness of Fit H-L Chi-Squared
Forward Stepwise Procedure	Thrombocytopenia, headache, myalgias	255.4	268.7	1.49 (0.8285)	1.16 (0.7615)
Backward Stepwise Procedure	Thrombocytopenia, leukopenia, headache, myalgia, sore throat, lethargy, bleeding	255.3	282.0	38.11 (0.2481)	8.13 (0.4205)
Purposeful Selection	Thrombocytopenia, leukopenia, headache, sore throat, lethargy, myalgia, thrombocytopenia*leukopenia, thrombocytopenia*sore throat	276.8	308.2	0.54 (0.9973)	32.77 (0.1689)

Table 18. Comparison of Different Multiple Logistic Regression Model-building Procedures for ≥20 years-old Age Group

Model-Building Strategy	Variables Included in Multiple Logistic Regression Model	AIC	BIC	Goodness of Fit Pearson Chi-Squared	Goodness of Fit H-L Chi-Squared
Forward Stepwise Procedure	Thrombocytopenia	140.1	146.0	0	J<6
Backward Stepwise Procedure	Thrombocytopenia, bruises	114.9	123.3	0.43 (0.5134)	0.36 (0.5468)
Purposeful Selection	Thrombocytopenia	140.1	146.0	0	J<6

Discussion

The first year of SEDSS provided more robust clinical information, identified a larger proportion of non-hospitalized dengue cases, and increased the detection of DENV-positive cases by an estimated 2.4 times as compared to PDSS. The integration of these two surveillance systems better characterizes the epidemiology of dengue in the Ponce Region than PDSS alone. Furthermore, the data collected in first year of SEDSS permitted detailed analysis of clinical variables, such as provisional diagnosis, thrombocytopenia, leukopenia, nausea, lethargy, rash, absence of cough, and absence of sore throat, which differentiate dengue from influenza and other pathogens. Analysis of provisional diagnoses for DENV-positive cases detected by SEDSS highlights the utility of such diagnoses and potential applications of syndromic surveillance. Stratification by age demonstrated higher sensitivity and more complex multiple logistic regression models for provisional diagnoses of dengue among younger patients, which suggests opportunities for education of clinicians and the predictive value of such diagnoses for adults.

Aim 1: Determine to what extent the dengue cases detected by SEDSS are representative of the data from the Ponce Health Region collected by the passive system (PDSS).

The integration of SEDSS and PDSS is essential to optimize the utility of SEDSS to describe dengue epidemiology in the Ponce Health Region and eventually all of Puerto Rico with the addition of other sites. One of the primary purposes of SEDSS, in response to an appeal by the WHO to improve dengue surveillance through the establishment of sentinel sites, is to provide an epidemiologic sample representative of Puerto Rico. A comparison of the surveillance data for dengue gathered by PDSS and SEDSS provides an initial foundation from which to explore potential sources of bias. The unique properties of each system merit

individual consideration of bias followed by an integrated discussion of the inferences drawn by the data reported to SEDSS and PDSS for the Ponce Health Region. Overall, SEDSS appears to improve detection of laboratory-positive dengue cases and detects younger, non-hospitalized cases compared to PDSS.

Passive surveillance systems, such as PDSS, are especially prone to underestimation of the true burden of disease due to under-ascertainment, under-recognition, and underreporting by clinicians of suspected cases.[66] Under-ascertainment represents a type of selection bias in which only cases which seek medical attention are detected by the passive surveillance system.[67] Potential causes of this bias include the notion that sicker patients are more likely to seek care and healthcare access bias. Passive surveillance is also subject to the reporting patterns of physicians and non-random sampling bias another type of selection bias. A recent evaluation of PDSS comments on the limitations of passive surveillance due to lack of reporting by clinicians and hospitals as well as a tendency to view dengue as a pediatric illness (CDC, unpublished data). Comparison to SEDSS confirmed the suspected bias of PDSS to report a higher proportion of hospitalized patients. Consequently, a number of non-hospitalized cases of dengue are likely missed due to either under-recognition or underreporting by clinicians to PDSS. Diagnostic suspicion bias is both an example of selection bias and information bias[67] since it effects whether patients are tested for DENV and perhaps the quality of the clinical data reported. Nevertheless, the utility of PDSS to monitor trends in dengue epidemiology, despite a significant degree of underreporting, fulfills a major goal of dengue surveillance to detect outbreaks and initiate public health action.[1]

SEDSS tests all febrile patients for DENV and thus mitigates biases due to under-recognition and underreporting by clinicians. As a sentinel surveillance system based in a health

care facility, under-ascertainment persists, as only cases which seek medical attention are detected by SEDSS. Further considerations of bias in SEDSS involve both the study design and the calculation of an expansion factor with Damas as a control hospital. Of patients who presented to San Lucas with fever during the first year of SEDSS, only 33% participated in the study. The issue of non-response bias introduces important implications for the data collected by SEDSS. If non-differential, then the comparisons between SEDSS and PDSS should be relatively unaffected. However, the average age of study participants was 13 year-old, which suggests parents may have been more likely to enroll their child in the study rather than adults to volunteer for enrollment. Therefore, the younger cases detected by SEDSS may be the result of a differential recruitment bias rather than a reflection of the true burden of dengue. To ameliorate this potential bias, stratification by age would yield a more reliable expansion factor for comparison to PDSS and, as discussed later, permit a more valid analysis of DENV-positive cases with regards to clinical characteristics. In addition to age, other differences between study participants and non-participants could represent other biases in the comparison of PDSS and SEDSS to the true underlying burden of dengue in the Ponce Health Region.

The selection of Damas as a control hospital for San Lucas to calculate an expansion factor for the detection of DENV-positive cases in the Ponce Health region was based on the type of hospital, the services offered, and the average number of annual patient visits each year. The inclusion of multiple years of data prior to the implementation of SEDSS also helped establish a reliable baseline for the pre/posttest comparison of the two hospitals. Nevertheless, this analysis rests on the assumption that these two hospitals are comparable to one another. Calculation of incidence rates rather than absolute number of cases would have yielded a more precise estimate; however, the number of ED visits and total volume of the two hospitals before

and after the implementation of SEDSS was assumed to be the same. If San Lucas had experienced a significant rise in the total number of visits compared to Damas, then this would have led to an overestimation of additional cases detected by SEDSS. Similarly, any changes in reporting trends by clinicians could have influenced the number of DENV-positive cases detected at each site. After the 2010 dengue epidemic in Puerto Rico, a physician education program for dengue was initiated, yet it is reasonable to assume that such an intervention affected the reporting patterns at Damas and San Lucas equally.

Overall, SEDSS complements PDSS quite well in the characterization of dengue in the Ponce Health Region during the first year of its implementation. Although there are considerations for differential recruitment bias due to the low response rate in SEDSS, adjustment for such biases in age are possible. The reduction in underreporting and under-recognition of dengue by SEDSS represents a considerable advantage to detect cases. The calculation of an expansion factor serves as a useful tool to better estimate the true burden of dengue in Puerto Rico to guide future public health interventions, such as vaccines. In order to assess the impact of potential vaccines, a baseline incidence of dengue prior to vaccine implementation is needed to compare incidence rates afterward. SEDSS could serve as a platform from which to perform this analysis, either through a formal Phase IV clinical trial or through its integration with PDSS as part of routine public health surveillance. Additionally, the robust clinical information collected by SEDSS also permits a more detailed analysis of dengue cases in the Ponce Health Region.

Aim 2: Analyze the clinical and laboratory features that differentiate dengue, influenza, other viral upper respiratory infections, enterovirus, bacterial infections, and other acute febrile illnesses.

The differentiation of dengue from other AFIs represents an important challenge for health care professionals as considerable overlap exists in the clinical presentations of these disease entities. The detailed clinical information gathered by SEDSS provided sufficient data to describe the clinical characteristics and construct multivariate association models for laboratory-positive dengue, influenza, and other pathogen cases. Although the dynamic nature of infectious disease epidemiology and the aforementioned biases of different surveillance systems require careful consideration, the association models from SEDSS help elucidate key clinical features among dengue, influenza, and other AFIs. Furthermore, existing knowledge from other epidemiologic studies offers a framework from which to interpret such models for both clinical management and disease reporting purposes.

Dengue and influenza demonstrated the highest burden among laboratory-positive AFIs during the first year of SEDSS and frequently co-circulate in the population. This emphasizes the need to understand differences in clinical presentation to improve disease reporting. Among the three modeling strategies, the forward stepwise procedure yielded the most parsimonious multiple logistic regression model for laboratory-positive dengue and influenza cases which included provisional diagnosis, thrombocytopenia, leukopenia, nausea, lethargy, rash, absence of cough, and absence of sore throat. The decision to include provisional diagnosis as a variable reflects the utility of such diagnoses to improve case reporting and disease surveillance. In the modern era of electronic medical records (EMR), the potential for both initial diagnosis and relevant clinical features to enhance the timeliness of outbreak detection prior to laboratory

confirmation is quite promising. The next step for such an association model would be its validation on the subsequent years of data during epidemic and non-epidemic periods to predict laboratory-positive dengue and influenza cases.

The paucity of studies with multiple logistic regression modeling for dengue and influenza underscores the significance of this analysis. Despite a lack of appropriate comparison studies, univariate analyses from other studies lends support to these observations. A previous study at Saint Lucas reported that dengue cases were significantly more likely to present with rash, thrombocytopenia, leukopenia, and absence of cough or sore throat.[45] Additionally, a separate analysis of the same dataset considered the utility of the tourniquet test and leukopenia to differentiate dengue from other AFIs, including influenza, and concluded that the absence of the two was useful to rule out dengue.[68] The tourniquet test was not considered as a variable in this study; however, leukopenia demonstrated an OR of 0.15 for influenza and 0.03 for other pathogens. Finally, leukopenia, nausea, rash, and lethargy are all criteria for a presumptive diagnosis of dengue according to the WHO 2009 case definition.[1]

Of note, the final multiple logistic regression association model to differentiate dengue from other pathogen-positive cases included the same variables as that of the influenza model with arthralgias as an additional variable. Among the different modeling procedures, only the purposeful selection strategy met criteria for goodness of fit tests and only after the modification of the significance threshold from 0.05 to 0.1 for model building. This poor fit of the model to the data was likely a consequence of the decision to include multiple pathogens as a single comparison group due to small sample sizes for the various upper respiratory pathogens. For example, although Parainfluenza viruses, RSV, and adenovirus may exhibit considerable overlap in their clinical presentation, grouping them together may have resulted in a loss of granularity

needed to construct an appropriate model. Nevertheless, such subtleties reflect the necessary diagnostic acumen for physicians to distinguish between the various etiologies of AFIs.

The loss of specificity in the comparison of dengue to other AFIs encountered by our modeling mirrors the epidemiologic literature which primarily focuses on differences between laboratory-positive and laboratory-negative dengue cases. One analysis of surveillance data in Puerto Rico from 2007-2008 involved the construction of a multivariate model with retro-orbital pain, rash, platelets <240,000, absence of sore throat, and absence of cough as significant predictors.[44] Stratification by different age groups revealed differences in the clinical and laboratory features of dengue with retro-orbital pain as the only consistent variables across models. Other multivariate approaches to differentiate laboratory-positive and laboratory-negative dengue cases included conjunctivitis, rash, and leukopenia.[69] A systematic review of the literature prior to 2008 reported platelet count, white blood cell count, rash, and signs of liver damage as the most common variables included in published models; however, no model had been validated to test its utility to predict future cases of dengue.[43] Leukopenia, thrombocytopenia, and rash were included in models for both influenza and other pathogens whereas signs of liver damage were not included. Indeed, the primary difficulty is the loss of specificity due to the consideration of laboratory-positive and laboratory-negative dengue cases rather than specific pathogens. The variability of models in the literature undoubtedly reflects the dynamic changes in the epidemiology of AFIs which includes dozens of diseases.

The two models presented in this analysis build upon the current framework of laboratory-positive dengue cases compared to other AFIs, yet also provide a clearer picture to differentiate dengue from influenza and other upper respiratory pathogens. The generalizability of these results and the application of these models to improve disease surveillance greatly

depend on the relative incidence of each pathogen. Dengue epidemiology is cyclical, with epidemics occurring every several years, influenza epidemiology is seasonal, and the milieu of other pathogens changes over time. Of all study participants, only 62% had an identified etiology for their AFI which, as in other studies considering dengue-negative cases, contribute to the variability in the true distribution of AFIs. Nevertheless, the next steps would be to validate these models on the next year of data collected by SEDSS in order to determine their validity in both epidemic and non-epidemic years for dengue. The consideration of the incidence and seasonality of influenza would also refine the usefulness of a potential predictive model. Chikungunya and the arrival of other AFI etiologies underscore the fluid nature of the true disease distribution and diagnostic reliability; however, the longitudinal design for SEDSS offers considerable adaptability to improve the understanding of dengue epidemiology and its clinical presentation in Puerto Rico. The early clinical suspicion of a particular AFI etiology from such prediction models, informed by current epidemiologic trends and disease presentations specific to Puerto Rico, could also guide clinical management and decrease unnecessary morbidity.

Aim 3: Of patients who tested positive for dengue virus (DENV), use multiple logistic regression modeling to compare the distribution of demographic, clinical, and laboratory characteristics between those who were diagnosed as dengue and those with an alternative clinical diagnosis.

Provisional diagnosis of dengue by a health professional at the initial presentation provides unique insights with regards to the clinical utility of such diagnoses. In the absence of rapid diagnostic testing, the clinical suspicion for dengue as a provisional diagnosis guides treatment and initiates reporting for public health action. Much of the epidemiologic literature for the clinical presentation of dengue focuses on the differentiation of laboratory-positive and laboratory-negative dengue cases. These clinical and laboratory features serve as the basis for

the WHO classification of dengue and aid health care professionals in the timely diagnosis of dengue. This study sought to elucidate the clinical characteristics associated with whether or not a physician diagnosed a patient with dengue, given that the patient tested positive for DENV. Overall, the provisional diagnosis of dengue cases detected by SEDSS in its first year of operation yielded a sensitivity of 42% and specificity of 95%. However, nearly 75% of all cases which received an alternative diagnosis, such as viral syndrome, met the WHO 1997 criteria for dengue. For all laboratory-positive dengue cases, the sensitivity of the WHO 1997 case definition was 81%. The introduction of the new WHO 2009 case definition for dengue sparked great debate in its clinical utility for health care providers to diagnose, manage, and report suspected dengue cases. A recent review article highlighted the advantages of the new classification system to better characterize the severity of dengue cases, though no studies have evaluated the consequences for dengue surveillance and reporting.[70] In one study, the sensitivity and specificity for DF compared with other AFIs among adults were 95.4% and 36%, respectively, whereas the sensitivity and specificity for dengue were 79.9% and 57.0%.[71] Although this study primarily focused on diagnostic tests and its generalizability to the SEDSS population is limited due to the predominance of pediatric patients, the observation that the WHO 1997 case definition yields higher sensitivity and lower specificity is noteworthy and consistent across studies.[72] Furthermore, the patterns of provisional diagnoses by physicians at Saint Lucas reflect the newer trends in diagnosis which favor increased specificity over sensitivity.

The use of multiple logistic regression modeling techniques permits a deeper understanding of important clinical and laboratory variables associated with whether or not patients who tested positive for dengue received a provisional diagnosis of dengue or an

alternative diagnosis, both for the overall study and stratification by age groups. Leukopenia and thrombocytopenia represented the two variables consistently included in the multiple logistic regression models for all DENV-positive cases. Both the backward stepwise and forward stepwise procedure models yielded similar values for the AIC and BIC; however, the forward stepwise procedure model is preferred as the most parsimonious model to include thrombocytopenia, leukopenia, and headache in the association model. Consequently, this suggests that health professionals were more likely to provisionally diagnose a patient with dengue if the patient presented with leukopenia, thrombocytopenia, and headache, given that the patient eventually tested positive for dengue. Leukopenia and headache are both included in the revised case definition of dengue and the former case definition of dengue fever. Thrombocytopenia, however, was added as a warning sign in the new WHO case definition with the occurrence of concurrent hemoconcentration and it is consistently associated with acute dengue infection. Hemoconcentration was not very common for the initial hematocrit in the emergency department and, therefore, did not appear in any of the multiple logistic regression models. Although the final hematocrit in the emergency department showed a higher number of cases with hemoconcentration, this value was not included due to incomplete data collection and uncertainty whether physicians had this value for their diagnostic decision-making.

A comparison of the provisional diagnosis model for laboratory-positive dengue cases and those to differentiate dengue from influenza and other pathogens provides context for whether these clinical features were truly associated with dengue or merely a perception by physicians for this population. Both leukopenia and thrombocytopenia were included in the models to differentiate dengue from influenza and other pathogens; however, headache was not included in either of these models. Nevertheless, headache was significant on univariate analysis

for both influenza and other pathogens. Additional significant variables from the differentiation models not included in the provisional diagnosis model were nausea, lethargy, rash, and absence of cough or sore throat. Of these, only rash was significant on univariate analysis.

The statistically significant three year age difference between dengue cases that received a provisional diagnosis and those that received an alternative diagnosis suggests that physicians were more likely to diagnose dengue in younger patients. Either physicians recognize dengue more readily in younger patients or younger patients present a more classic clinical picture are two potential explanations for this observation. The sensitivities of a provisional diagnosis of dengue for the age groups ≤10 years-old, 10-19 years-old, and ≥20 years-old were 41.4%, 48%, and 31.1%, respectively. With regards to laboratory-positive dengue cases among the age groups ≤10 years-old, 10-19 years-old, and ≥20 years-old, 68.4%, 85.9%, and 87.8% met WHO 1997 criteria for dengue, respectively. Therefore, these data suggest that younger children who tested positive for dengue did not demonstrate a classic picture, which is a likely explanation for the lower sensitivity of the provisional diagnosis by a physician. Older children and adults, however, frequently met criteria with a much higher sensitivity observed in the 10-19 year-old category compared to adults, who displayed the lowest sensitivity for provisional diagnosis among all three age groups. These differences in sensitivities require careful consideration with regards to the complexity of the multiple logistic regression models built for each age group.

For young children ≤10 years-old the multiple logistic regression association model constructed by the forward and backward stepwise procedure included thrombocytopenia, leukopenia, and diarrhea. The addition of diarrhea added considerable value to the model as reflected in the AIC and BIC, which favors this as the final model over the more parsimonious model obtained by purposeful selection. All three of these variables are fairly objective as both

leukopenia and thrombocytopenia are laboratory values and diarrhea is more easily reported by parents. Other clinical symptoms included in the case definition for dengue and the logistic regression models for older children may be more difficult to report in younger children. For example, headache, myalgias, arthralgias, retro-orbital pain may be harder for younger, especially non-verbal, children to communicate to parents or health care providers. This may also provide an explanation for the lower proportion of laboratory-positive dengue cases which met WHO 1997 criteria for dengue fever compared to older children and adults. Alternatively, dengue may in fact present quite differently in younger children, as physicians were more likely to diagnose young children with dengue who presented with diarrhea, even though diarrhea is not included in the case definition for dengue.

Proposed multiple logistic regression models for older children ages 10-19 tended to display a more complex constellation of clinical features compared to the other two age groups. Nevertheless, the model with thrombocytopenia, headache, and myalgias, produced by the forward stepwise procedure, was both the most parsimonious and yielded superior values for the AIC and BIC. Leukopenia, absence of sore throat, and lethargy were significant covariates in other models; however, the addition of these variables failed to sufficiently improve the model to favor the added complexity. Despite the relatively high point estimate of 3.06 for the OR of leukopenia on univariate analysis and the inclusion of this laboratory value in the model for all laboratory-positive dengue cases, leukopenia was not included in the final model for older children ages 10-19. Once again, possible explanations for the significance of these variables for the provisional diagnosis of dengue by a physician include that thrombocytopenia, headache, and myalgias were regarded by physicians as being more associated with dengue than other variables or that this truly reflected the clinical presentation of dengue compared to other AFIs in this age

group. The next most common diagnoses for laboratory-positive dengue cases were viral infection and other alternative diagnoses, which may simply reflect the infectious disease milieu in which dengue is evaluated.

The difficulty to construct a multiple logistic regression model for adults ≥20 years-old in the setting of the poor sensitivity of the provisional diagnosis of dengue and good sensitivity of the WHO criteria for dengue suggests that physicians may not consider the more robust case definition for dengue and focus on thrombocytopenia as a defining criteria. Although thrombocytopenia was strongly associated with whether or not a physician provisionally diagnosed a case as dengue for all age groups, the point estimate for the OR of thrombocytopenia was much higher at 13.04. In fact, the only other statistically significant variable at the 95% confidence level in univariate analysis was the absence of a sore throat. Even meeting the WHO criteria for dengue was not significant for adults ≥20 years-old, though it was highly significant for the other two age groups. The backward stepwise procedure was the only strategy which produced a true multiple logistic regression model containing both thrombocytopenia and bruising. Both of these variables are closely related, as thrombocytopenia is associated with bruising, which suggests that physicians emphasize this aspect of dengue at the time of initial diagnosis.

Provisional diagnosis is not isolated to merely laboratory-positive dengue cases, but also depends greatly on the competing AFI etiologies and other potential diagnoses. The next most common provisional diagnosis was viral infection, which represents quite a broad category, followed by other and respiratory. Although these alternative diagnoses lack specificity, the tendency of physicians to report cases as a viral infection or a symptom rather than a more specific diagnosis may influence their diagnostic decision-making, likelihood to report suspected

dengue cases, and their overall clinical management of a patient. The study design for SEDSS included laboratory testing for dengue and all of the other AFI etiologies mentioned previously and this led to an increased detection of laboratory-positive dengue cases compared to PDSS; however, the next line of inquiry would be to determine which proportion of these cases with a provisional diagnosis of viral infection, other or respiratory would have been reported and tested for dengue in the community setting. Furthermore, among the 302 laboratory-positive cases, over 60% tested positive for DENV. The identification of clinical features that distinguish laboratory-positive dengue cases with a provisional diagnosis of viral infection from other etiologies for viral infection could improve detection of dengue in areas with only passive surveillance systems in place.

The classification of diseases, reflected in the provisional diagnosis, by the health care provider represents the first line of surveillance to raise clinical suspicion, manage the patient appropriately, send serum samples for diagnostic testing, and report suspected dengue cases. Nosology, as a foundational component of medicine, seeks to classify and identify distinct disease entities with the ultimate goal of improved management and patient outcomes.[73] The restructuring of the WHO criteria for dengue reflects the mutability and difficulties of such classification schemes in the context of scientific, clinical, and diagnostic advancement. Indeed, the International Classification of Disease (ICD), now in its tenth revision, hinges both upon specific etiologies of disease and a more general categorization of symptoms, often the result of diagnostic uncertainty. Symptomatology, though less precise than the etiologic taxonomy of the ICD, suggests an alternative method to study disease. Syndromic surveillance studies for influenza, as an AFI and suitable case study for dengue, utilize the potential of symptomatology to improve detection of cases during epidemic periods and aim to capture a more complete

burden of disease.[74,75] Dengue diagnosis and surveillance during epidemic periods could also benefit from syndromic approaches based on the WHO criteria for clinical diagnosis. The results from this study invite the possibility to harness the advances of an EMR and integrate information with regards to provisional diagnosis and clinical features associated with dengue to improve detection of cases.

The impact of a provisional diagnosis on the management of patients, clinical outcomes, and reporting patterns by clinicians represents another area of future research. This analysis primarily focused on the initial presentation of cases and the eventual laboratory diagnosis, which is important to establish associations between clinical features of dengue and provisional diagnoses. However, outcome measures such as hospital admissions, length of hospital stay, and whether the likelihood of the clinician to report suspected cases would provide useful information. Especially for alternative diagnoses for which dengue may not be suspected, a provisional diagnosis of viral syndrome accompanied by clinical features such as thrombocytopenia or leukopenia may benefit from more aggressive management. Likewise, if clinicians are less likely to test for and report dengue for cases with an alternative provisional diagnosis, then this would lend further support for the aforementioned syndromic surveillance strategy to improve detection.

Conclusion

The integration of PDSS and SEDSS adds greater clarity to the epidemiologic landscape of dengue in the Ponce Health Region and, with additional sites, the island of Puerto Rico. The calculation of an expansion factor permits a more sensitive measure of dengue burden in Puerto Rico and will allow for improved evaluation of future public health interventions, such as a dengue vaccine or novel vector control methods. The robust clinical information provided by SEDSS underscores its utility to differentiate dengue from other acute febrile illnesses. Analysis from ongoing data collection will adapt to the shifting epidemiology of various febrile illnesses to provide greater external validity and generalizability to inform physicians in Puerto Rico. Provisional diagnoses, in conjunction with clinical features associated with dengue, could guide syndromic surveillance to improve detection of dengue and prediction of outbreaks. Dengue continues to a pose a significant global health challenge; however, recent advances in dengue prevention, such as a efficacious vaccine candidate currently in the process of licensure,[76,77] emphasize the need for public health surveillance. SEDSS serves as a model in dengue surveillance to inform public health and clinical practice in Puerto Rico, Latin America, and globally.

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Additional Tables

Table 19. Multiple Logistic Regression Modeling for DENV-Positive and Influenza-Positive Cases

Covariate	Adjusted Odds Ratio (95% CI)	P-value
Provisional Diagnosis		
Dengue	1.00	
Influenza	9.84 (1.74, 55.51)	0.010
Viral Syndrome	2.64 (1.30, 5.37)	0.007
Respiratory	4.81 (2.20, 10.55)	<0.001
Other	2.68 (1.24, 5.78)	0.012
Thrombocytopenia	0.192 (0.089, 0.415)	<0.001
Leukopenia	0.345 (0.218, 0.546)	<0.001
Nausea	0.519 (0.323, 0.833)	0.007
Lethargy	0.475 (0.250, 0.900)	0.022
Cough	11.90 (6.85, 20.67)	<0.001
Sore Throat	1.69 (1.05, 2.71)	0.030
Rash	0.346 (0.202, 0.592)	<0.001
Arthralgia	0.732 (0.448, 1.19)	0.211

Table 20. Multiple Logistic Regression Modeling for DENV-Positive and Other Pathogen-Positive Cases

Covariate	Adjusted Odds Ratio (95% CI)	P-value
Provisional Diagnosis		
Dengue	1.00	
Influenza	9.84 (1.74, 55.51)	0.010
Viral Syndrome	2.64 (1.30, 5.37)	0.007
Respiratory	4.81 (2.20, 10.55)	<0.001
Other	2.68 (1.24, 5.78)	0.012
Thrombocytopenia	0.192 (0.089, 0.415)	<0.001
Leukopenia	0.345 (0.218, 0.546)	<0.001
Nausea	0.519 (0.323, 0.833)	0.007
Lethargy	0.475 (0.250, 0.900)	0.022
Cough	11.90 (6.85, 20.67)	<0.001
Sore Throat	1.69 (1.05, 2.71)	0.030
Rash	0.346 (0.202, 0.592)	<0.001
Arthralgia	0.732 (0.448, 1.19)	0.211

Table 21. Multiple Logistic Regression for DENV-positive Cases Receiving a Provisional Clinical Diagnosis of Dengue or an Alternative Diagnosis

Covariate	Adjusted Odds Ratio	95% Confidence Interval	P-value
Thrombocytopenia	5.57	(3.74, 8.30)	<0.001
Leukopenia	3.65	(1.87, 7.14)	<0.001
Age^	0.994	(0.975, 1.014)	0.547
Leukopenia*Age	0.975	(0.951, 0.99998)	0.050

Table 22. Multiple Logistic Regression for DENV-positive Cases with and without a Provisional Clinical Diagnosis of Dengue ≤10 years-old Age Group Using Purposeful Selection

Clinical Feature	OR (95%CI)	P-value (Wald Test)
Thrombocytopenia	3.41 (1.53, 7.60)	0.003
Leukopenia	3.56 (1.60, 7.94)	0.002

Table 23. Multiple Logistic Regression for DENV-positive Cases with and without a Provisional Clinical Diagnosis of Dengue 10-19 years-old Age Group Using Purposeful Selection

Clinical Feature	OR (95%CI)	P-value (Wald Test)
Thrombocytopenia	6.61 (3.52, 12.40)	<0.001
Leukopenia	2.42 (1.07, 5.44)	0.005
Headache	4.07 (1.53, 10.8)	0.005
Lethargy	0.37 (0.13, 1.08)	0.070
Sore Throat	0.59 (0.32, 1.08)	0.087
Myalgias	0.51 (0.25, 1.02)	0.056

Table 24. Multiple Logistic Regression for DENV-positive Cases with and without a Provisional Clinical Diagnosis of Dengue for ≥20 years-old Age Group Using Purposeful Selection

Clinical Feature	OR (95%CI)	P-value (Wald Test)
Thrombocytopenia	13.04 (5.48, 31.0)	<0.001