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Acute Febrile Illness Surveillance in a Tertiary Hospital Emergency Department: Comparison of Influenza and Dengue Virus Infections

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Abstract. In 2009, an increased proportion of suspected dengue cases reported to the surveillance system in Puerto Rico were laboratory negative. As a result, enhanced acute febrile illness (AFI) surveillance was initiated in a tertiary care hospital. Patients with fever of unknown origin for 2–7 days duration were tested for *Leptospira*, enteroviruses, influenza, and dengue virus. Among the 284 enrolled patients, 31 dengue, 136 influenza, and 3 enterovirus cases were confirmed. Nearly half (48%) of the confirmed dengue cases met clinical criteria for influenza. Dengue patients were more likely than influenza patients to have hemorrhage (81% versus 26%), rash (39% versus 9%), and a positive tourniquet test (52% versus 18%). Mean platelet and white blood cell count were lower among dengue patients. Clinical diagnosis can be particularly difficult when outbreaks of other AFI occur during dengue season. A complete blood count and tourniquet test may be useful to differentiate dengue from other AFIs.

BACKGROUND

The infectious causes and epidemiology of acute febrile illness (AFI), defined as illness of < 1 week duration with no identified source, remain poorly characterized in many parts of the world.[1,2] Previous studies performed in Egypt showed that infections, such as salmonellosis (5%), typhoid fever (18%), and brucellosis (11%), were common causes of AFI.[3] In South America, infections with *Leptospira*, malaria, *Rickettsia*, dengue virus, and Venezuelan equine encephalitis virus were identified as major AFI causes.[4] In some regions, such as sub-Saharan Africa and Southeast Asia, sentinel hospital-based studies have been established to obtain clinical and public health data about the causes of AFI throughout the year and to identify susceptibility patterns and clinical predictors.[2,5–9] The burden of dengue is uncertain, although believed to be substantial throughout the tropics,[10] and the importance of other infectious diseases such as leptospirosis is undefined.[2] Lack of information about the specific etiologies that make up the differential diagnosis of dengue slows our ability to make accurate diagnoses, provide effective treatment, and effectively target public health measures.[4]

In Puerto Rico, dengue is endemic throughout the year; however, dengue virus (DENV) transmission increases during the period of increased ambient temperatures and rainfall, which begins in June and extends until November. The Centers for Disease Control and Prevention (CDC), in collaboration with the Puerto Rico Department of Health (PRDH), has been conducting passive dengue surveillance (PDSS) for more than 3 decades. This system records ~3,000–5,000 suspect cases in non-epidemic years and up to 25,000 in recent epidemics. However, what is not known is the degree to which dengue is under-identified or reported among cases of AFI, and the incidence of confounding cases such as leptospirosis or influenza. Fortunately, malaria is no longer endemic in Puerto Rico as it is in neighboring islands,[11] and diseases associated with poor sanitation, such as typhoid fever, are uncommon.

In April 2009, novel influenza A (H1N1) was detected in the United States[12] and rapidly spread worldwide, including to Puerto Rico. The first confirmed case was detected in the southern part of Puerto Rico in June of 2009, and by early 2010, the island was one of the most heavily affected areas in the United States, with 59 influenza-related deaths among its 3.8 million inhabitants.[13] The PDSS was able to detect that an outbreak of AFI was occurring in Puerto Rico.[14] In May of 2009, an increase in suspected dengue cases with negative dengue diagnostic test results was detected by PDSS and these cases had an increased frequency of respiratory symptoms.

The introduction of influenza A (H1N1) 2009 virus corresponded with the onset of dengue season, which made the clinical diagnosis and differentiation between dengue fever (DF) and mild cases of influenza difficult. As a result, enhanced surveillance for AFIs was initiated at the emergency department (ED) of a large, tertiary care hospital in southern Puerto Rico. Enhanced surveillance was conducted to assess the etiology of AFI and to identify demographic and clinical features that might assist clinicians to distinguish between these different etiologies.

PATIENTS, MATERIALS, AND METHODS

Study site. An enhanced surveillance system for AFI was established at the Saint Luke's Episcopal Hospital, in Ponce, Puerto Rico. Located ~45 miles southwest of San Juan, Ponce is one of the 78 administrative municipalities of Puerto Rico and the second largest city (Figure 1). This hospital is a tertiary, acute-care facility that provides healthcare to patients from over 20 municipalities, has more than 54,000 patient visits to its ED annually,[15] and is the main teaching affiliate of the Ponce School of Medicine.

Study population. Patients of all ages were eligible to be enrolled if they met the following case definition of AFI:

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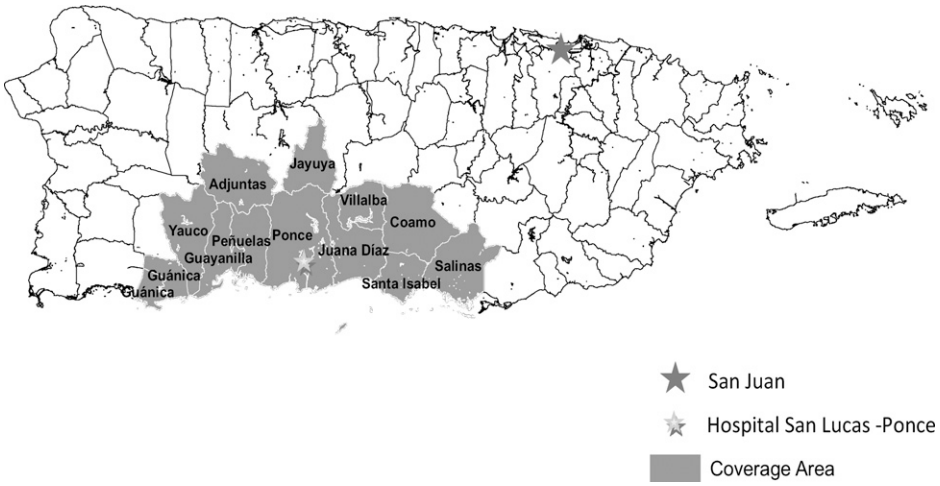

FIGURE 1. Map of Puerto Rico showing 78 municipalities.

documented fever of 38.0°C or higher at presentation to the ED or history of fever that had persisted for 2–7 days without an identified source. Patients were excluded if after the initial physical examination by hospital physicians they had an identifiable source of fever including, but not limited to diagnoses of otitis media, sinusitis, bronchitis, pneumonia, cellulitis, impetigo, wound infection, urinary tract infection, osteomyelitis, or varicella.

Study protocol. From September 29, 2009 through December 18, 2009, patients seeking medical care in the ED of St Luke's Episcopal Hospital who met the case definition for AFI were enrolled in the enhanced surveillance system (Figure 2). This surveillance included all elements of the PDSS established for decades and the addition of on-site CDC staff to help identify and collect data from AFI patients, including completion of the dengue case investigation form (DCIF), which accompanied specimens submitted to CDC's Dengue Branch for diagnostic testing. After physical examination, study personnel explained the purpose of the surveillance project and obtained verbal consent for participation. Participants were interviewed to collect DCIF data that included demographic, medical and clinical information, signs and symptoms at onset, and specimen collection dates. A tourniquet test was performed and results recorded by trained study personnel using standardized techniques as previously described.[16] Two nasopharyngeal samples were collected to perform rapid antigen and reverse transcriptase-polymerase chain reaction (RT-PCR) testing for influenza. Attending physicians and patients were informed immediately of the results of the rapid influenza test that was performed on-site. One blood sample was collected for dengue diagnostic testing. Additional laboratory tests such as a complete blood count and urinalysis were performed at the discretion of the attending physician in the course of routine patient care but were not part of the study. If available, laboratory results, including white blood cell (WBC) count, platelet count, hematocrit, and albumin, were recorded on the DCIF. This protocol was reviewed and approved by the Human Subjects Institutional Review Board of the CDC and the Institutional Review Board of Ponce School of Medicine.

Laboratory testing. Two nasopharyngeal samples were obtained at the same time. The first was tested on-site with the QuickVue Influenza A + B rapid influenza test (Quidel Corporation, San Diego, CA), and the second sample was placed in viral transport medium and refrigerated until transported to the CDC's Dengue Branch, San Juan, PR, for influenza testing by the CDC RT-PCR assay.[17] As per PDSS protocol, 5 to 10 mL of venous blood was collected, immediately refrigerated at 4°C, centrifuged on-site to separate serum, and transported on ice within 3 days to the CDC's Dengue Branch for further testing. Serum samples were initially tested for DENV by serotype-specific RT-PCR,[18] dengue-specific non-structural protein-1 (NS1), and by DENV-specific immunoglobulin M (IgM) antibody-capture enzyme-linked immunosorbent assay (anti-DENV MAC-ELISA).[19]

Serum samples from patients with severe disease (i.e., hospital admission or clinical signs including plasma leakage, fluid accumulation, respiratory distress, bleeding, and organ impairment) and from those who were influenza PCR-negative were subsequently transported on ice to CDC's Bacterial Zoonosis Branch in Atlanta for *Leptospira* testing. Specimens were screened for IgM antibodies to *Leptospira* by using the rapid dipstick ELISA ImmunoDOT kit (GenBio, Inc., San Diego, CA). Specimens with positive or borderline results with the ImmunoDOT kit were further tested by using the microscopic agglutination test (MAT).[20] Patient sera were serially diluted in the MAT and mixed with a panel of 20 *Leptospira* reference antigens representing 17 serogroups. Resulting agglutination titers were read by using dark field microscopy, and the final titer was expressed as the reciprocal of the last well that agglutinated 50% of the antigen. Samples drawn within the first 3 days from symptom onset and with sufficient serum volume were shipped frozen to the Picornavirus Laboratory at CDC, Atlanta, and tested for enteroviruses by a pan-enterovirus (EV) RT-PCR that targets the conserved 5′ non-translated region of the genome.[21]

Definitions: Acute febrile illness was defined as a patient with fever of 38°C or higher at presentation to ED or history of fever that persisted for 2–7 days with no localizing source.

Laboratory-positive dengue case: a patient with one or more of the following: 1) DENV RNA detected in serum by RT-PCR, 2) negative to positive anti-DENV IgM seroconversion in paired serum specimens, 3) a single positive anti-DENV IgM result in an acute-phase or convalescent-phase specimen (positive/negative antibody ratio ≥ 2.0),[22] or 4) a positive DENV antigen detection by NS1 rapid test.

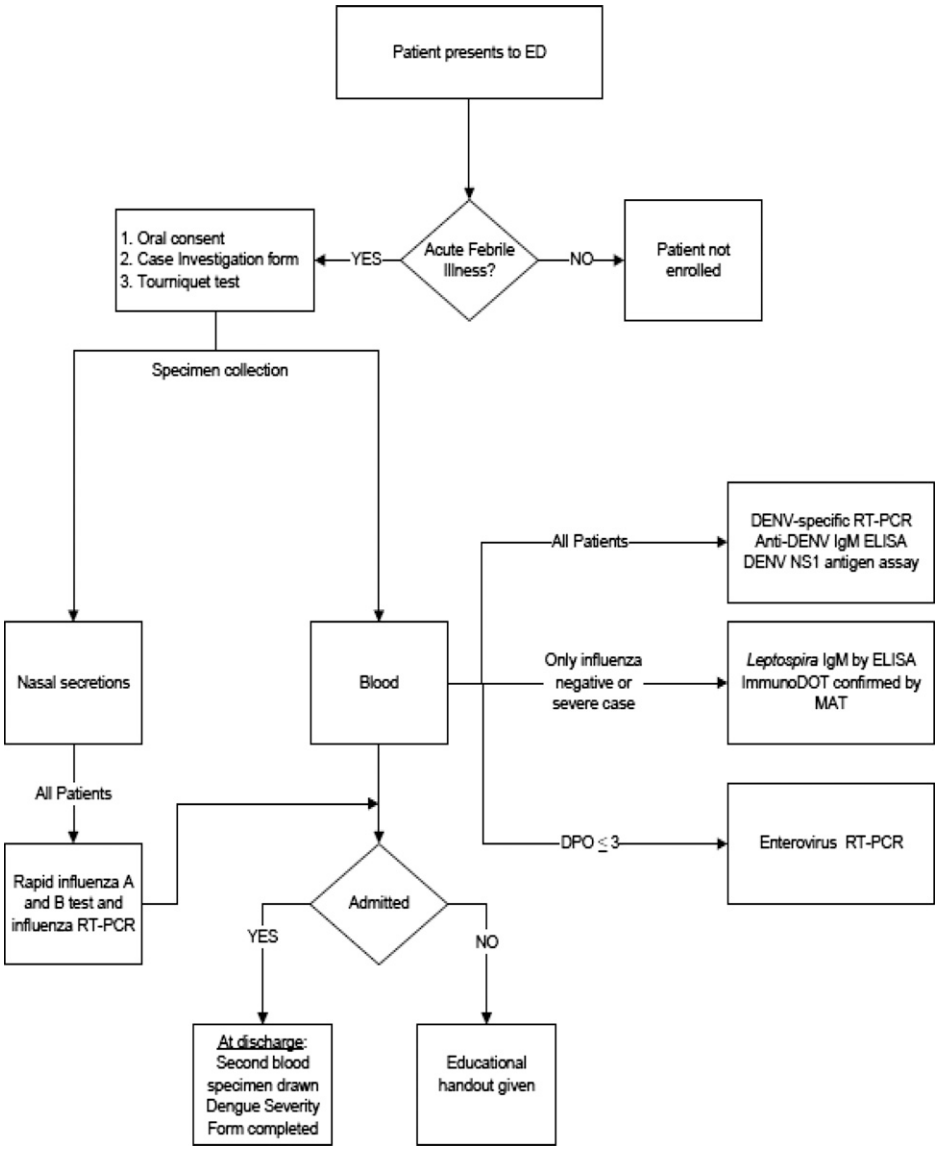

FIGURE 2. Enrollment procedure for the Enhanced Acute Febrile Illness Surveillance Project, Ponce, Puerto Rico, 2009.

Laboratory-negative dengue case: a patient who does not meet criteria for a laboratory-positive dengue case and has no anti-DENV IgM detected in a serum specimen collected 6 or more days after onset of fever.

Laboratory-indeterminate dengue case: a patient with no DENV RNA, NS1 antigen or dengue virus or anti-dengue IgM antibodies detected in the acute sample submitted for diagnostic testing and no convalescent sample submitted for diagnostic testing.

Dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) were defined by using the World Health Organization (WHO) criteria.[23–27]

Warning signs for severe dengue were defined as those that occurred within 48 hours of defervescence (about 3 to 7 days after symptoms onset) and included: severe abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, hemorrhagic manifestation, lethargy or restlessness, and liver enlargement.[28]

Laboratory-positive influenza case was defined as a patient with laboratory-confirmed infection detected by the CDC RT-PCR assay.[17]

Laboratory-positive leptospirosis case was defined by the following: 1) having positive IgM antibodies to *Leptospira* with the rapid dipstick ELISA ImmunoDOT kit and 2) a positive MAT for a single titer of > 1:400 or a fourfold rise in titer between acute and convalescent phase samples.[20]

Laboratory-positive EV case was defined as a patient with positive EV real-time RT-PCR assay and genotype identification by EV VP1 semi-nested RT-PCR and amplicon sequencing.[21,29,30]

Anemia was defined as a deficiency in the total number of erythrocytes in blood. A patient was considered to be anemic if its hemoglobin was less than the 2.5 percentile for age and sex.[23,24]

The WBC counts were classified as leucopenia (total WBC < 5,000/mm^3) or leukocytosis (WBC > 11,000/mm^3).[31]

Hemoconcentration was defined as an increase in hematocrit ≥ 20% above average for age or a decrease in hematocrit ≥ 20% of baseline following fluid replacement therapy.[22]

Analyses. Data were entered into a Microsoft Access database (Microsoft Access 2007; Microsoft, Redmond, WA) and

exported to SAS version 9.2 (SAS Institute, Cary, NC) for analysis. Outcome groups were identified as having confirmed dengue, confirmed influenza, confirmed leptospirosis, confirmed EV infection, or other AFI. Categorical variables were compared by using the χ^2 test or Fisher's exact test as appropriate and continuous variables were compared by using the Student's *t* test and the Mann-Whitney *U* test (Wilcoxon rank-sum test) where applicable. Results with values $P \leq 0.05$ were considered statistically significant.

RESULTS

A total of 284 patients were enrolled (Table 1), and 172 (61%) met the case definition for influenza, dengue, leptospirosis, or enteroviral disease. Influenza A was identified in 138 (49%) patients, and all except two had a positive RT-PCR for pandemic 2009 influenza A H1N1 virus. No influenza B was identified. Dengue virus was identified in 32 (11%) patients; one of these patients had a dual infection with DENV-2 and influenza A H1N1 virus. Among patients laboratory positive for dengue, 20 were DENV RNA positive; 18 had DENV-4, 1 had DENV-1, and 1 had DENV-2. DENV-3 was not found. The remaining dengue patients were NS1 antigen positive (7 [23%]) or had a single positive anti-DENV IgM positive (4, 13%). *Leptospira* was detected in one patient; however, this patient also had influenza A H1N1 detected by RT-PCR. Enterovirus was detected in three patients and the genotype was determined in 2 patients (coxsackievirus A2 and coxsackievirus B4). Blood cultures, urine cultures, or both were performed in only 10 of all enrolled patients. Two patients had a positive urine culture; one positive for *Escherichia coli* and one for *Staphylococcus saprophyticus*. The two patients with dual diagnoses were excluded from the rest of the analysis.

Influenza cases were more likely to be detected earlier in the study period than laboratory-positive dengue cases; most influenza cases were detected from October 1 through November 18, 2009, versus November 5 through December 16, 2009, for dengue (Figure 3). The three EV cases occurred during October. Overall, the patients ranged in age from 6 months to 82 years with a median age of 17.9 years (Table 2). Median age for patients with other AFI was 15.4 years compared with 20.3 years for dengue patients and 18.7 years for influenza patients. Overall, 55% of enrolled patients were female; however, more than half (55%) of those with dengue were male. Most enrolled patients lived in the neighboring municipalities of Ponce (128 [45%]), Villalba (40 [14%]), and Juana Diaz (38 [13%],). However, dengue cases were predominately residents of Villalba (18 [58%]), whereas most influenza cases were from Ponce (74 [54%]).

In addition to fever, most enrolled patients reported headache (81%), cough (79%), body pain (78%), nasal congestion (66%), eye pain (61%), arthralgia (59%), and sore throat (57%) (Table 3). The most common symptoms among influenza patients were cough (92%), headache (85%), nasal congestion (83%), and body pain (82%). Cough and sore throat were more common among influenza patients than among patients with other etiologies ($P \leq 0.0004$). Although most (120 [88%]) influenza patients met the CDC criteria for influenza-like illness (i.e., fever with cough or sore throat), so did half (15 [48.4%]) of the dengue cases and most (93 [83%]) of patients with unidentified febrile illness. In addition, most (107 [79%]) influenza patients met WHO criteria for DF; 15 (11%) presented with clinical bleeding, and 21 (18%) had a positive tourniquet test.

Dengue patients most commonly had headache (29 [94%]); body pain (26 [84%]); a positive tourniquet test or other hemorrhagic manifestations (25 [81%]); arthralgia (23 [74%]); and eye pain (21 [68%]) (Table 3). Rash was less common than other mentioned signs and symptoms, however more likely to be present among dengue patients than among patients with influenza (39% versus 9% $P \leq 0.0001$) or with other AFI (39% versus 15% $P \leq 0.003$). Dengue patients were more likely than influenza patients to have a positive tourniquet test (52% versus 18%, $P \leq 0.0001$) or other hemorrhagic manifestations (29% versus 11%, $P \leq 0.0001$). Other hemorrhagic manifestations included petechiae (48% versus 7%, $P \leq 0.0001$), purpura (13% versus 2%), epistaxis (6% versus 2%), bleeding gums (19% versus 3%, $P \leq 0.003$), blood in stool (3% versus 2%), hematemesis (6% versus 2%), menorrhagia (10% versus 0%), and hematuria (23% versus 4%). All but one dengue patient (30 [97%]) met WHO criteria for DF; 18 (58%) patients met criteria for DF with at least one warning sign for severe dengue, and 4 (13%) met criteria for DHF or DSS. Dengue patients were also more likely than influenza patients to report warning signs for severe dengue such as persistent vomiting (16% versus 11%), abdominal pain (58% versus 28%, $P \leq 0.003$), and liver enlargement (3% versus 0.8%).

Overall, patients sought medical care early after symptom onset (mean = 3.3; SD = 2.7 days), which was especially true for influenza patients who sought care a mean of 2.8 days after symptom onset. Nearly one in five (19%) enrolled patients were admitted to the hospital, including 22 (71%) of the dengue patients. Dengue patients were more likely than

TABLE 1
Pathogens identified among enrolled patients, Enhanced Acute Febrile Illness Surveillance Project, Ponce Puerto Rico, 2009*

Infectious agent	Number of positive cases	Number of negative cases	Number of not tested, indeterminate, or invalid*	Percent positive of tested	Percent positive of all samples	Total tested
Influenza virus only	136	144	2	48.2	47.8	283†
Dengue virus only	31	249	3	10.9	10.9	283†
Leptospirosis only	0	133	150	0.7	0.3	283†
Enterovirus only	3	73	208	4.1	1.4	
Dual infection	2†	–	–	–	0.7	
None‡	112	–	–	–	–	
Total	284					

*A total of 282 were considered for further analysis. Two dual infections were excluded; one with influenza A H1 N1 and dengue virus and the other with influenza A H1 N1 and *Leptospira*. All initial participants could have been tested for more than one test.

†Not tested: no sample volume available for testing; indeterminate: negative results in the acute sample but no convalescent sample were submitted for diagnostic testing; invalid: insufficient sample volume for testing.

‡None, no etiological agent was identified after testing.

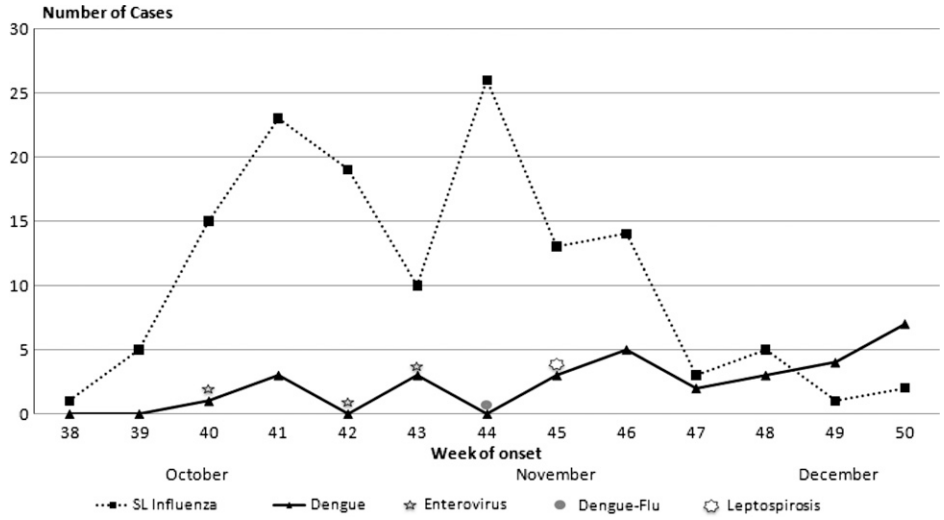

FIGURE 3. Number of laboratory-positive cases of influenza A, dengue, enterovirus, and leptospirosis by week of symptom onset; Enhanced Acute Febrile Illness Surveillance Project, Ponce Puerto Rico, 2009.

influenza patients to present later and to be admitted to the hospital. The median length of hospital stay was 4 days (range: 2–77 days). There was no statistically significant difference in length of hospital stay between patients with dengue and with those with influenza. Ten percent of dengue patients were admitted to an intensive care unit, which was a higher rate than for all admitted patients (3.2%; $P \leq 0.008$).

Upon initial presentation, the median platelet count of participants was 198,000 (range 10,000–449,000), and 13% had thrombocytopenia (platelet count ≤ 100,000) (Table 4). Patients with dengue had a lower median platelet count of 52,000 (range 10,000–227,000), whereas influenza patients had a median platelet count of 189,000 (range 39,000–369,000) ($P \leq 0.0001$). Most (22 [71%]) dengue patients were thrombocytopenic and nearly all (28 [90%)] of them had platelet counts ≤ 150,000 compared with 8 (6%) and 28 (21%) of influenza patients, respectively ($P \leq 0.0001$). Overall, the median WBC count of enrolled patients was $6.2 \times 10^3/mm^3$

TABLE 2

Patient demographic characteristics by pathogen identified at initial presentation, Enhanced Acute Febrile Illness (AFI) Surveillance Project, Ponce Puerto Rico, 2009

	All patients		Laboratory-positive Dengue		Laboratory-positive Influenza		Laboratory-positive Enterovirus		Other AFI		Significance
Characteristic	(N = 282)*		(N = 31)		(N = 136)		(N = 3)		(N = 112)†		P value
% Female	54.9		45.2		55.9		33.0		57.1		0.08
Age, years											
Median (range)	17.9 (0.5–82.4)		20.3 (3.3–64.4)		18.7 (0.8–61.5)		5 (0.64–7.6)		15.4 (0.5–82.4)		0.81
	n	%	n	%	n	%	n	%	n	%	
0–4	41	14.5	1	3.2	9	6.6	1	33.3	30	26.8	0.47
5–9	40	14.2	3	9.7	21	15.4	2	66.7	14	12.5	0.41
10–14	41	14.5	5	16.1	25	18.4	–		11	9.8	0.76
15–19	27	9.6	6	19.4	18	13.2	–		3	2.7	0.37
20–24	16	5.7	5	16.1	8	5.9	–		3	2.7	0.05
25–29	23	8.2	2	2.5	15	11.0	–		6	5.4	0.14
30–34	24	8.5	2	6.5	8	5.9	–		14	12.5	0.90
35–39	23	8.2	4	12.9	10	7.4	–		9	8.0	0.32
40–44	13	4.6	–		9	6.6	–		4	3.6	
45–49	11	3.9	2	6.5	3	2.2	–		6	5.4	0.21
50–54	7	2.5	–		5	3.7	–		2	1.8	
55–59	8	2.8	–		4	2.9	–		4	3.6	0.34
60–64	4	1.4	1	3.2	1	0.7	–		2	1.8	0.24
65–69	2	0.71	–		–		–		2	1.8	–
70+	2	0.71	–		–				2	1.8	–
Residence, municipality											
Ponce	128	45.1	1	3.2	74	54.4	0		53	47.8	< 0.0001
Villalba	40	14.1	18	58.1	9	6.6	1	33.3	12	10.7	< 0.0001
Juana Diaz	38	13.4	2	6.5	18	13.6	1	33.3	17	15.2	0.28
Coamo	21	7.4	5	16.1	11	8.1	0		5	4.5	0.17
Peñuelas	16	5.7	0	0.0	7	5.2	0		9	8.0	0.19
Santa Isabel	10	3.5	1	3.2	5	3.7	1	33.3	3	2.7	0.89

P value: compares laboratory positive dengue with laboratory positive influenza patients.
*Excludes two patients with dual infections: dengue/influenza and influenza/leptospirosis.
†Includes two positive urine cultures.

TABLE 3
Signs and symptoms at initial presentation, Enhanced Acute Febrile Illness (AFI) Surveillance Project, Ponce, Puerto Rico, 2009

Characteristic	All patients (*N* = 282)†		Laboratory-positive Dengue (*N* = 31)		Laboratory-positive Influenza (*N* = 136)		Laboratory-positive Enterovirus (*N* = 3)		Other AFI (*N* = 112)		Significance *P* value*
	n	%	n	%	n	%	n	%	n	%	
Signs and Symptoms											
Fever	248	87.9	30	96.8	120	88.2	3	100	95	84.8	0.15
Rash	41	14.7	12	38.7	12	8.8	1	33.3	16	14.7	< 0.0001
Headache	229	81.2	29	93.6	115	84.6	3	100	82	73.2	0.19
Eye pain	170	60.5	21	67.7	89	65.4	0		60	54.1	0.81
Body pain	221	78.4	26	83.9	112	82.4	1	33.3	82	73.2	0.84
Arthalgia	167	59.2	23	74.2	85	62.5	0		59	52.7	0.22
Cough	224	79.4	11	35.5	125	91.9	1	33.3	87	77.7	< 0.0001
Nasal congestion	187	66.3	7	22.6	112	82.5	1	33.3	67	59.8	< 0.0001
Sore throat	161	57.1	8	25.8	83	61	1	33.3	69	61.6	0.0004
Hemorrhagic manifestations											
Positive tourniquet test (TT)	54	21.9	16	51.6	21	17.8	0		17	17.7	0.0001
Hemorrhage with TT	96	34.0	25	80.6	36	26.5	2	66.7	33	29.5	< 0.0001
Hemorrhage without TT	42	14.9	9	29.0	15	11.0	2	66.7	16	14.3	< 0.0001
Warning signs for severe disease											
Persistent vomiting	35	12.6	5	16.1	15	11.2	0		15	13.6	0.45
Abdominal pain	95	33.7	18	58.1	38	27.9	0		39	34.8	0.003
Liver enlargement	2	0.7	1	3.2	1	0.8	0		0		0.28

* *P* value: compares laboratory-positive dengue with laboratory-positive influenza patients.
† Excludes two patients with dual infections: dengue/influenza and influenza/leptospirosis.

(range 0.9–27.2 × 10^3/mm^3). Dengue patients had significantly lower median white cell counts when compared with influenza patients (3.1 × 10^3/mm^3 versus 5.6 × 10^3/mm^3, $P \leq 0.0001$). A higher proportion of dengue patients than influenza patients were leucopenic (87.1% versus 44.1%, $P \leq 0.0001$), and none of the dengue patients had elevated WBC counts, whereas 5% of influenza patients and 21% of other patients had elevated white cell counts. Overall, the initial median hematocrit value for enrolled patients was 39.0 (range: 12.5–56.4); dengue patients had higher median hematocrit values (43.2, range: 26.2–56.4) than influenza patients (39.3, range: 12.5–50.6) ($P \leq 0.010$). However, only 3% of dengue patients and < 1% of influenza were hemoconcentrated. Hyponatremia (Na^+ concentration < 135 mEq/L) was detected in nearly 40% of dengue patients at initial presentation, a proportion significantly higher ($P \leq 0.0001$) in comparison with patients with influenza. Finally, dengue patients had higher median alanine aminotransferase levels (119 versus 56 U/L, $P \leq 0.01$) and asparate aminotransferase (181 versus 40 U/L, $P \leq 0.001$) levels than patients with influenza.

DISCUSSION

In this study of the etiology of AFI during a ~3-month period in the fall of 2009, we found that most patients presenting to a tertiary hospital in southern Puerto Rico had influenza A (H1N1) or dengue. We found that in the context of these two diseases occurring at the same time, clinical diagnosis was challenging because most influenza patients met the case definition for dengue and vice versa. This was not the first time that dengue and influenza have occurred simultaneously in Puerto Rico[31–33] or in other locations.[34] In Puerto Rico

TABLE 4
Laboratory findings at initial presentation, Enhanced Acute Febrile Illness (AFI) Surveillance Project, Ponce Puerto Rico, 2009.

Characteristic	All patients (*N* = 282)*		Laboratory-positive Dengue (*N* = 31)		Laboratory-positive Influenza (*N* = 136)		Laboratory-positive Enterovirus (*N* = 3)		Other AFI (*N* = 112)		Significance *P* value*
Platelet count per mm^3											
Mean (SD)	198,281 (84,000)		74,484 (58,000)		189,639 (57,400)		290,000 (74,100)		241,118 (80,600)		0.0001
Median (range)	198,000 (10–449 × 10^3)		52,000 (10–227 × 10^3)		189,000 (39–369 × 10^3)		256,000 (239–375 × 10^3)		240,500 (43–449 × 10^3)		0.0001
< 100,000 (no. per %)	35	12.4	22	71.0	8	5.9	0	–	5	4.6	0.0001
< 150,000 (no. per %)	71	25.2	28	90.3	28	21.1	0	–	15	13.6	0.0001
White cell count per mm^3											
Mean (±2 SD)	6.28 (3.8)		3.3 (1.5)		5.8 (2.8)		8 (3.6)		8.9 (4.1)		0.0001
Median (range)	6.2 (0.9–27.2)		3.1 (1.9–6.7)		5.6 (0.9–19.9)		5.9 (5.9–12.2)		8.4 (1.7–27.2)		0.0001
< 5000 (no. per %)	102	36	27	87.1	60	44.1	0		13	11.8	0.0001
> 11000 (no. per %)	32	11	0		7	5.2	2	50	24	21.4	0.0001
Hematocrit											
Median (min-max)	39 (12.5–56.4)		43.2 (26.2–56.4)		39.3 (12.5–50.6)		37.6 (34.3–43.9)		37.5 (28.8–48.2)		0.01
Hemoconcentrated (no. per %)	2	0.7	1	3.2	1	0.7	0	0	0	0	–
Chemistry (no. per %)											
Hyponatremia (< 135)	18	14.9	10	38.5	3	6	0	0	5	11.1	0.004
Hyperkalemia (> 5)	13	10.8	2	7.7	5	10.2	0	0	6	13.3	0.72

* *P* value: compares laboratory-positive dengue with laboratory-positive influenza patients.

in 1977, simultaneous outbreaks of influenza and dengue caused considerable diagnostic confusion as nearly one in four suspected dengue cases reported to PDSS were found to have influenza A/Texas/1/77, and only nasal congestion/rhinitis distinguished influenza from dengue patients.[33] In addition, hemorrhagic manifestations were reported in an equal proportion (~10%) of laboratory-confirmed and laboratory-negative dengue cases.[33]

One striking characteristic of our patients with influenza H1N1 was a relatively high proportion with a hemorrhagic manifestation or thrombocytopenia. In addition, the mean platelet count was considerably lower in our group of H1N1 patients in comparison with patients in previous investigations.[34–37] Influenza, however, has been associated with hemorrhagic manifestations, including a positive tourniquet test.[32,38–40] In 1971, during an investigation of suspected DHF among children hospitalized in Burma, influenza A was found to be the etiologic agent in 58% of the 278 cases investigated.[38] Of the 139 influenza cases for which clinical features were described, most had at least one hemorrhagic manifestation including hematemesis (89%), positive tourniquet test (47%), epistaxis (22%), and melena (12%). However, the authors of that study hypothesized that their findings may have been due in part to the wide-spread use of a traditional Burmese medicine containing salicylates, as patients with Chikungunya, measles, and other AFIs also had hemorrhagic manifestations.[39] In 1975, several influenza A cases with hemorrhagic manifestations were identified during a DF outbreak in Fiji.[40] And more recently, a simultaneous outbreak of dengue and influenza A (H1N1) was detected in rural Thailand.[34]

Although there can be considerable diagnostic confusion among dengue-like AFIs, an enhanced surveillance system can be useful to test for diseases that are not routinely surveyed and obtain laboratory confirmation to differentiate between etiologies during low incidence periods in endemic areas. On the other hand, knowledge of local dengue outbreaks can assist clinicians by increasing their index of suspicion for dengue among patients presenting from the affected sites. In our case, most laboratory-confirmed dengue cases resided in Villalba, a small municipality that is physically isolated from Ponce and the surrounding municipalities by a mountain range. Interestingly, DENV-4 was the most common serotype identified in our study, whereas DENV-1 was the most common serotype detected islandwide in 2009. This was probably because before 2009, DENV-4 had not been detected in southern Puerto Rico (and specifically Villalba) for almost 10 years,[33,35] and because of this, residents < 10 years of age may have been particularly susceptible. In this study, most dengue patients ranged in age from 2 to 18 years.

Early clinical manifestations of leptospirosis are non-specific and similar to other AFI including dengue. In Puerto Rico, the burden of leptospirosis is not well defined. Surveillance is passive and intermittent, case confirmation with laboratory tests is uncommon, and only a small proportion of suspected cases are thought to be reported to the Puerto Rico Department of Health.[41] A study conducted in 1997 estimated an overall annual incidence of 1 case per 100,000 inhabitants, and municipalities in the Ponce area, including Orocovis, Patillas, Las Marias, and Adjuntas, were thought to have the highest incidence of leptospirosis.[41] In our study, we identified one case of leptospirosis. One limitation was that samples with insufficient volume remaining after dengue diagnostic testing and those that were positive for influenza virus were not tested for *Leptospira.* Nevertheless, leptospirosis is thought to be an important AFI in the differential diagnosis of dengue in Puerto Rico, as unrecognized leptospirosis is often identified as the cause of laboratory-negative dengue deaths.[35] Because rapid diagnostic testing is limited for *Leptospira*, it is important for clinicians to identify risk factors for leptospirosis, such as contact with contaminated water, which may help to distinguish this condition from dengue.

This study has a number of limitations: 1) hospital-based surveillance underestimates the disease incidence, and the true burden of disease cannot be well characterized because not all patients seek medical attention at a hospital; 2) recruitment was limited in duration of time, which could have affected the observed incidence of some illnesses whose frequency vary throughout the year; 3) leptospirosis and EV could be underdiagnosed because only a subgroup of our patients were tested for these two pathogens; 4) patients with < 48 hours of fever were excluded; therefore, patients in the early stage of the disease were not recruited, with the exception of those who subsequently returned to the ED; 5) data are representative of only one hospital that receives patients mostly from one health region and does not represent the entire Puerto Rican population; and 6) data concerning the tourniquet test and laboratory test results were missing for a small group of patients.

In Puerto Rico, dengue seasonality follows a pattern with a high transmission period that begins as early as June and lasts until the end of November however information about the seasonality of other AFI is limited. The underlying causes of AFI are likely to vary substantially throughout the year and between years and in different geographic areas. One of our next steps is to establish an enhanced surveillance system for dengue-like illness in several hospitals in Puerto Rico during a longer period of time to determine the differential diagnosis of dengue and to establish the incidence, seasonality, and burden of these diseases. This system will be used as a platform to conduct epidemiologic, clinical, and laboratory research about pathogenesis, clinical outcomes, and approaches to therapy. Although many dengue-endemic countries have malaria, measles, rubella, and typhoid on the differential diagnosis of dengue, these conditions are uncommon in Puerto Rico because of eradication, immunization, improvements in water and sanitation.[11,31,32,42–45] However, there are other conditions in which information is limited, including co-infections. Recently, a study conducted in Cambodia identified unique co-infections such as dengue and *Burkholderia pseudomallei* and *Salmonella typhi*[46]; through an enhanced surveillance system co-infections could be identified and data could be used to better understand clinical outcomes of patients who were laboratory positive for multiple etiologies. In addition, surveillance and improved knowledge among clinicians of patients' final diagnoses can be useful in improving physicians' clinical diagnosis skills and broadening their differential diagnosis, and assisting public health authorities in prioritizing limited resources.

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