Arboviral Surveillance among Pediatric Patients with Acute Febrile Illness in Houston, Texas

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Abstract. We instituted active surveillance among febrile patients presenting to the largest Houston-area pediatric emergency department to identify acute infections of dengue virus (DENV), West Nile virus (WNV), and chikungunya virus (CHIKV). In 2014, 1,063 children were enrolled, and 1,015 (95%) had blood and/or cerebrospinal fluid specimens available for DENV, WNV, and CHIKV testing. Almost half (49%) reported recent mosquito bites, and 6% ($N = 60$) reported either recent international travel or contact with an international traveler. None were positive for acute WNV; three had false-positive CHIKV results; and two had evidence of DENV. One DENV-positive case was an acute infection associated with international travel, whereas the other was identified as a potential secondary acute infection, also likely travel-associated. Neither of the DENV-positive cases were clinically recognized, highlighting the need for education and awareness. Health-care professionals should consider the possibility of arboviral disease among children who have traveled to or from endemic areas.

INTRODUCTION

Historically, arboviruses have rarely caused human illness; however, some mosquito-borne viruses have been associated with widespread disease. Texas has established populations of *Aedes* spp. and *Culex* spp. mosquitoes, which are capable of spreading multiple viral pathogens, including dengue virus (DENV), chikungunya virus (CHIKV), West Nile virus (WNV), and Zika virus. Current detection of DENV, WNV, and CHIKV infections in the United States is primarily based on passive surveillance, requiring clinical disease recognition. Given the emergence (CHIKV) or possible reemergence (DENV) of these viruses in the United States and infrequent clinical suspicion of cases, the true burden of disease may be underestimated.¹ Although no specific therapeutic options exist, a DENV vaccine has been licensed, and vaccines are under development for CHIKV and WNV.^{2–4} Given the absence of information about disease prevalence, the need for these vaccines among U.S. children is unclear. To understand the prevalence of arboviral diseases in an area at high risk for transmission, we instituted active surveillance in a large pediatric emergency department (ED) using the existing Centers for Disease Control and Prevention's (CDC) New Vaccine Surveillance Network's surveillance platform to determine the baseline burden of DENV, CHIKV, and WNV illness among febrile children.⁵

MATERIALS AND METHODS

Subject enrollment. We conducted active surveillance for acute febrile illness in the Texas Children's Hospital (TCH) ED during April through October 2014. Enrollment occurred using convenience sampling of patients who presented during evening and weekend hours, totaling 42 hours each week. Patients were eligible to participate if they were aged 15 days to 20 years, presented with subjective fever or fever at presentation $\geq 38^{\circ}\text{C}$, and required phlebotomy or cerebrospinal

fluid (CSF) collection. Patients were ineligible if they had been enrolled for the same illness within 7 days or if their parent/guardian did not speak English or Spanish.

Data collection and laboratory testing. Information about demographics, illness severity and duration, and travel history were obtained through parent interview using a standardized data collection tool. Residual plasma, serum, and/or CSF samples were retrieved from clinical laboratories after testing for clinical purposes was complete. Based on viremia and immunoglobulin M (IgM) response kinetics relative to the timing of symptom onset, our testing strategy was WNV IgM enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) for DENV 1–4, and PCR for CHIKV for all patients (specimens closest to symptom onset were selected for testing for patients with multiple samples available); and DENV IgM and CHIKV IgM testing for all patients with specimens ≥ 5 days post-symptom onset (specimens collected 6–10 days post onset of symptoms were preferred for DENV IgM testing) (Figure 1).

Samples were tested by using ELISA using the following kits: WNV IgM Capture DxSelect ELISA kit (Focus Diagnostics, Cypress, CA), DENV Detect IgM Capture (or immunoglobulin G [IgG]) ELISA kit (InBios, Seattle, WA), and Anti-CHIKV IgM Human ELISA kit (Abcam, Cambridge, MA). To detect DENV RNA in the samples, CDC DENV-1–4 Real Time RT-PCR Assay (provided by CDC) was used with strict adherence to the assay manual.⁶ Chikungunya virus TaqMan assay was run in parallel with the DENV PCR Assay using the same conditions and reagents.⁷ All samples that were positive or equivocal for CHIKV were sent to CDC's Division of Vector-Borne Diseases Arbovirus Diagnostic Laboratory for confirmatory testing. Samples positive by DENV PCR did not require confirmatory testing at CDC as the procedure was performed at the diagnostic test level, including completion of a proficiency panel from CDC. To remove PCR inhibition in samples derived from heparinized plasma, extracted RNA was precipitated with 0.5 M LiCl and 50% isopropanol, washed, and dissolved in the original volume of the RNA sample.

Institutional review board approval was obtained from Baylor College of Medicine and the CDC.

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FIGURE 1. Testing methodology for serum/plasma and CSF samples. CHIKV = chikungunya virus; CSF = cerebrospinal fluid; DENV = dengue virus; ELISA = enzyme-linked immunosorbent assay; WNV = West Nile virus.

RESULTS

A total of 5,783 patients were screened for eligibility. Of 1,627 eligible patients, 1,065 (65%) were enrolled. Common reasons for non-enrollment included inability to locate or approach the patient in the ED ($N = 275$; 49%), admission or discharge before recruitment ($N = 143$; 25%), and patient's parent/guardian declining participation ($N = 126$; 22%). Following exclusion of ineligible patients, 1,063 participants were included in analyses (Figure 2). Median age was 3.7 years (range: 0.5 months to 19.6 years), 53% of enrolled patients were Hispanic/Latino, and 97% were born in the United States (Table 1). The most common symptoms reported at enrollment were fever ($N = 1,063$; 100%), fussy and/or less playful behavior ($N = 941$; 89%), anorexia ($N = 750$; 71%), lethargy ($N = 737$; 69%), and nasal congestion ($N = 521$; 49%). Few patients presented with retro-orbital pain ($N = 81$; 15%), joint pain ($N = 38$; 4%), and rash ($N = 125$; 12%). Almost half ($N = 523$; 49%) reported a history of mosquito bites (median number of bites was 4; range: 1–50) and 19% ($N = 99$) reported ≥ 10 bites. Six percent ($N = 60$) of enrolled patients reported either international travel or contact with an international traveler. Comorbidities were common; almost half (46%; $N = 490$) had ≥ 1 chronic medical condition.

Residual serum, plasma, and/or CSF specimens were available for 1,015 (95%) enrolled patients. Some patients had insufficient specimen volume to complete all tests on our panel: 1,010 unique patients were tested for WNV IgM (995 plasma/serum samples and 90 CSF samples), and all were negative; 284 patients were tested for DENV IgM (272 plasma/serum samples and 21 CSF samples), and all but one were negative; 287 patients were tested for CHIKV IgM (270 in plasma/serum and 26 in CSF). Two patients tested positive and one equivocal for CHIKV IgM, but all three were determined to be negative on CDC confirmatory testing. Thus, we determined these were falsely positive, potentially because of interference of the assay with polyclonal stimulation during Epstein-Barr virus infections.⁸ A total of 988 patients were tested for DENV and CHIKV by using PCR (972 plasma/serum samples and 83 CSF samples). Two patients were found to be positive for DENV by using PCR. Serology testing was conducted on these patients: whereas one patient was dengue IgM positive, a second patient was IgM negative, and only positive for dengue IgG, suggesting prior infection and a potential second acute infection. Both of these patients presented to the ED with history of travel from endemic areas.

DISCUSSION

We did not detect significant DENV, WNV, or CHIKV activity in children within the greater Houston area. Although we identified five children whose tests were initially suggestive of acute arboviral disease, more specific laboratory analyses failed to confirm these findings in four of the five patients.

This surveillance program was designed to capture a convenience sample of febrile children who presented to the TCH ED for care, as fever is a hallmark symptom for all three of the arboviruses for which we tested. Interestingly, patients in this study reported otherwise uncommon symptoms, such as retro-orbital pain, bone pain, and joint pain, despite testing negative for arboviruses. Reliance on these symptoms as a marker for arboviral infection may prove unreliable; however, testing may still be warranted. Considering almost half the patients in this assessment presented with an average of four mosquito bites, the potential for contracting an arbovirus exists in our population.

We believe that the absence of disease detected in this study reflects either nonexistent or minimal disease transmission of WNV, DENV, and CHIKV among children in the Houston area. During the same timeframe, state health department data reveal that nearly all cases of DENV and CHIKV were travel associated.⁹ Of note, the only patient in this study with confirmed acute DENV infection had recently traveled to a DENV-endemic region. Furthermore, our study design may have contributed to a low capture rate of arboviral disease. Although a high proportion of CHIKV infections are symptomatic, the opposite is true for DENV and WNV infections, and, therefore, it is unlikely that otherwise healthy children would have had symptoms severe enough to present to an ED.^{10–12}

As children may present with a diverse array of symptoms, we suggest that future surveillance programs should target a wider variety of locations where children with mild illness could present for care (i.e., urgent care centers or outpatient pediatric care clinics). Future studies should identify feasible ways, such as through randomized community-based or clinic-based screening, to optimize screening of large populations for rare diseases to improve the ability to detect cases. In the context of emerging arboviruses, novel surveillance strategies are necessary to rapidly identify acute cases to implement control and prevention measures.

This single site study had limitations. Patients were identified through convenience sampling, and a sizeable

FIGURE 2. Flow diagram of participant inclusion. * Inadequate sample volume existed to test all patients for each arbovirus; 1,010 of 1,015 patients were tested for WNV whereas 994 of 1,015 patients were tested for CHIKV and DENV. CHIKV = chikungunya virus; DENV = dengue virus; WNV = West Nile virus.

number of eligible children who did not participate ($N = 418$ of 562; 74%) were unable to be approached for participation. These patients may have had milder illness courses with fewer comorbidities than patients whose parents were available to provide consent for participation, which may have introduced bias. Although the sensitivity of the various arboviral assays used ranged from 90% to 98%, misclassification may have occurred. However, all samples that were initially identified as positive were confirmed using additional assays.

TABLE 1
Demographic characteristics and social history of enrolled subjects

	Number of patients $N = 1,063$ (%)
Age (years; median, range)	3.7 (0, 19.6)
Male	553 (52.0)
Insurance type	
Public	631 (59.4)
Private	325 (30.6)
Public and private	65 (6.1)
None	42 (4.0)
Race	
White	816 (76.8)
Black	184 (17.3)
Asian	19 (1.8)
Other	44 (4.1)
Ethnicity	
Non-Hispanic	495 (46.6)
Hispanic	568 (53.4)
Patients born in the United States	1,030 (96.9)
History of travel outside the greater Houston area	174 (16.4)
Travel within the United States*	151 (86.8)
International travel*	19 (10.9)
National and international travel	4 (2.3)
Contact with an individual who traveled outside the greater Houston area	303 (28.5)
Contact traveled within the United States†	234 (77.2)
Contact traveled internationally†	55 (18.2)
Contact traveled nationally and internationally†	14 (4.6)

* Percentage calculated from patients reporting travel.

† Percentage calculated from patients reporting contact with a traveler.

CONCLUSIONS

In this active surveillance assessment, arboviral disease among acutely febrile patients with mosquito bites was rare, despite the known presence of mosquitoes capable of transmitting disease. Health-care professionals should consider the possibility of arboviral disease, especially among children who have traveled to or from areas with documented arboviral transmission.

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REFERENCES

1. Tomashek KM, Gregory CJ, Rivera Sanchez A, Bartek MA, Garcia Rivera EJ, Hunsperger E, Munoz-Jordan JL, Sun W, 2012. Dengue deaths in Puerto Rico: lessons learned from the 2007 epidemic. *PLoS Negl Trop Dis* 6: e1614.
2. Amanna IJ, Slifka MK, 2014. Current trends in West Nile virus vaccine development. *Expert Rev Vaccines* 13: 589–608.
3. Chang L-J et al., 2014. Safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: a phase 1 dose-escalation trial. *Lancet* 384: 2046–2052.
4. World Health Organization, 2016. *Immunization, Vaccines and Biologicals. Dengue Vaccine Research*. Available at: http://www.who.int/immunization/research/development/dengue_vaccines/en. Accessed August 5, 2016.
5. Centers for Disease Control and Prevention, 2017. *New Vaccine Surveillance Network (NVSNet)*. Available at: <http://www.cdc.gov/surveillance/nvsnet>. Accessed November 15, 2017.
6. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, Medina F, Colon C, Margolis H, Munoz-Jordan JL, 2013. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. *PLoS Negl Trop Dis* 7: e2311.
7. Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, Campbell GL, 2007. Chikungunya virus in US travelers returning from India, 2006. *Emerg Infect Dis* 13: 764–767.
8. Abcam, 2011. *Ab108717. Chikungunya Virus IgM Human ELISA Kit*. Available at: [http://www.abcam.com/ps/products/108/ab108717/documents/ab108717%20Chikungunya%20Virus%20IgM%20Human%20ELISA%20\(Website\).pdf](http://www.abcam.com/ps/products/108/ab108717/documents/ab108717%20Chikungunya%20Virus%20IgM%20Human%20ELISA%20(Website).pdf). Accessed October 7, 2016.
9. Texas Department of State Health Services, 2015. *Arbovirus Activity in Texas. 2014 Surveillance Report*. Available at: <https://www.dshs.texas.gov/WorkArea/linkit.aspx?LinkIdentifier=id&ItemID=8589999503>. Accessed October 5, 2016.
10. Nakkhara P, Chongsuvivatwong V, Thammaphalo S, 2013. Risk factors for symptomatic and asymptomatic chikungunya infection. *Trans R Soc Trop Med Hyg* 107: 789–796.
11. Bhatt S et al., 2013. The global distribution and burden of dengue. *Nature* 496: 504–507.
12. Mostashari F et al., 2001. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. *Lancet* 358: 261–264.