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Mosquito Exposure and Chikungunya and Dengue Infection among Travelers during the Chikungunya Outbreak in the Americas

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Abstract. Travelers are at risk for arbovirus infection. We prospectively enrolled 267 Department of Defense beneficiaries traveling to chikungunya-outbreak regions in the Americas between December 2013 and May 2015 and assessed travel characteristics and serologic exposure to chikungunya virus (CHIKV) and dengue virus (DENV). Ten ill-returning travelers were also assessed retrospectively. Self-reported mosquito exposure was common (64% of 198 evaluable travelers saw mosquitoes; 53% of 201 reported ≥ 1 bite). Increased exposure was associated with active-duty travelers (odds ratio [OR] = 2.6 [1.3–5.4] for seeing mosquitoes) or travelers visiting friends and relatives (VFR) (OR = 3.5 [1.0–10.0] for high-intensity bite exposure). Arbovirus infection was defined as seroconversion on plaque reduction neutralization testing (PRNT) of pre- and posttravel sera. For ill subjects enrolled posttravel, infection was defined by a positive convalescent PRNT and/or a positive reverse transcription polymerase chain reaction for CHIKV or DENV. We identified seven cases of arbovirus infection: four with CHIKV, five with DENV, and two with both. The composite attack rate for CHIKV and DENV infection was 3.7% of 108 evaluable, immunologically naïve, prospectively assessed travelers; there was serologic and/or polymerase chain reaction evidence of arbovirus infection in three of four evaluable (three of 10 total) ill-returning travelers. We identified both symptomatic and asymptomatic cases. Military purpose of travel and VFR travel accounted for five of seven cases. Pretravel counseling is important and should target higher risk groups. Given a shared vector between CHIKV, DENV, and Zika virus (ZIKV), this study can also help guide counseling for travelers to ZIKV-outbreak regions.

INTRODUCTION

International travelers are at risk for arbovirus infection during travel to endemic and epidemic regions. Although the specific arbovirus risk varies with geography, dengue virus (DENV) and chikungunya virus (CHIKV) are among the most commonly diagnosed arbovirus infections in travelers.[1–3] More recently, Zika virus (ZIKV) has also emerged as an infectious disease risk to travelers.[3] All three are transmitted by the same *Aedes* mosquito vector. Infection may be asymptomatic but may also present with an overlapping clinical syndrome of fever, arthralgia, and rash.[1,3–6]

Prior to 2013, autochthonous transmission of CHIKV was limited predominantly to Africa and Asia.[6,7] However, local spread was first described in the Caribbean in December 2013,[7,8] followed by epidemic spread throughout the tropical Americas (i.e., Caribbean, Central America, and tropical South America). Between December 2013 and June 17, 2016, the Pan American Health Organization reported greater than 1.9 million suspected autochthonous transmission cases of CHIKV in the Americas and more than 3,400 travel-related cases in the United States.[9–11] Autochthonous ZIKV transmission has occurred in Brazil since at least March 2015, though clusters of an acute exanthematous illness have been reported since late 2014.[3,12,13] As of June 24, 2016, autochthonous ZIKV transmission had been confirmed in 40 countries and territories in the Americas, with greater than 398,000 suspected autochthonous cases; over 800 travel-related cases have been reported in the United States.[14–16]

In contrast, DENV has followed an endemic-epidemic pattern in the Americas, with reemergence in the mid-2000s.[4,17] This trend has continued with greater than 5.1 million probable cases of DENV reported in the Americas between January 1, 2014 and June 17, 2016, including over 1,200 cases in the United States (82% imported).[18–20] GeoSentinel surveillance data implicate DENV as the cause of approximately one-third of febrile illnesses among returned travelers presenting to clinic sites after travel to Latin America and the Caribbean.[2]

Large existing surveillance networks describe traveler demographics and travel patterns in patients presenting for pretravel health care (Global TravEpiNet)[21] and for presumed travel-related illness (GeoSentinel Surveillance Network).[2] However, evaluation of military travelers is limited in these large databases (< 1% of GeoSentinel enrollees; military association is not evaluated in the Boston Area Travel Medicine Network),[2,22,23] and they do not assess paired pre- and posttravel travel serology. Prospective assessments of travelers that include pre- and posttravel evaluation are limited in scope, vary by country of departure, and are typically focused on the civilian population.[24–28] While a single-center, prospective survey of military-beneficiary travelers has been performed at Brooke Army Medical Center (now San Antonio Military Medical Center, San Antonio, TX), it did not include serologic data.[29]

The TravMil study prospectively enrolls Department of Defense (DoD) beneficiaries traveling outside the continental United States to evaluate the epidemiology of deployment-relevant infectious diseases and the effectiveness of

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preventive measures. We describe traveler demographics, travel characteristics, and personal protective measure (PPM) use to assess factors associated with 1) mosquito exposure and 2) CHIKV and/or DENV infection in a military-medical-system cohort traveling to CHIKV-outbreak regions in the Americas during the recent CHIKV epidemic.

MATERIALS AND METHODS

Study design. This study is a subset of the larger TravMil study: Deployment and Travel Related Infectious Disease Risk Assessment, Outcomes, and Prevention Strategies Among Department of Defense Beneficiaries, which is approved by the Infectious Disease Institutional Review Board of the Uniformed Services University of the Health Sciences (Bethesda, MD). TravMil is a prospective, observational cohort of DoD beneficiaries traveling outside the continental United States for $\leq$ 6.5 months. Consenting adult and pediatric travelers are enrolled pretravel at five military travel clinics (Madigan Army Medical Center, Tacoma, WA; Naval Medical Center Portsmouth, Portsmouth, VA; Naval Medical Center San Diego, San Diego, CA; San Antonio Military Medical Center, San Antonio, TX; and Walter Reed National Military Medical Center, Bethesda, MD) and in the predeployment setting. Travel medicine physicians and independent duty corpsmen counsel travelers and deployers, but no standardization of counseling is performed as part of the study. Subjects who did not enroll prior to travel but who presented to these clinics for a possible travel-related illness within 2 months of their return are enrolled posttravel. For this analysis, we selected subjects who departed the United States for CHIKV-outbreak regions (i.e., Mexico, the Caribbean, and Central and South America) between December 1, 2013 and May 14, 2015, and had submitted their travel itineraries by May 21, 2015.

Survey data. Travel questionnaires were assessed to determine characteristics associated with mosquito exposure. Pretravel enrollees completed a pretravel survey regarding their demographics and anticipated travel characteristics. Travelers were also provided a diary to record episodes of fever during travel. Enrollees were asked to complete a posttravel survey within 2 months of their return from travel, confirming travel characteristics and also discussing mosquito exposure, PPM use, and febrile illnesses encountered during travel. Optimal PPM use was defined as regular (i.e., "often/everyday") application of repellent to exposed skin and treatment of outer clothing separately with repellent (e.g., permethrin). For posttravel enrollees, a survey collecting the same demographic and travel-related information was conducted at the time of enrollment. Posttravel surveys of seropositive travelers were also assessed for the following symptoms: fevers not associated with diarrhea or a respiratory infection, myalgias, arthralgias, headaches, and rashes. The primary outcome of interest from the survey component was mosquito exposure, which was defined as seeing mosquitoes or receiving mosquito bites during travel. Bite exposure was further characterized as low intensity (0–5 bites) or high intensity ($\geq$ 6 bites).

Laboratory data. Paired blood samples were collected from travelers prior to travel and within 8 weeks after their return from travel. For posttravel enrollees, paired blood samples were collected at the time of enrollment during acute illness and 3–8 weeks later. Paired sera were sent to the Naval Infectious Diseases Diagnostic Laboratory in Silver Spring, MD, for analysis. Screening for CHIKV and DENV infection was performed using an enzyme-linked immunosorbent assay (ELISA). Infection was confirmed using a plaque reduction neutralization test (PRNT). In seroconverted posttravel enrollees, infection was also confirmed using a real-time reverse transcription polymerase chain reaction (RT-PCR).

Posttravel or convalescent sera were tested for the presence of anti-CHIKV and anti-DENV IgM and IgG using indirect ELISAs.[30] Polyethylene-glycol-precipitated CHIKV and DENV (a mixture of four DENV serotypes) antigens were used. Uninfected Vero cell antigen was used to subtract background absorbance. Horseradish-peroxidase-labeled antihuman IgM and IgG were used for detection. A sample was considered positive if its net optical density (OD) value exceeded the mean plus three standard deviations of the normal control sera. A positive immunoglobulin level on posttravel or convalescent sera prompted complementary ELISA testing of the pretravel or acute sera.

Positive CHIKV and/or DENV antibodies were confirmed by PRNT using Vero cells and the following viruses: CHIKV (Vaccine), DENV1 (Western Pacific 74), DENV2 (OBS8041), DENV3 (CH53489), and DENV4 (341750). The serum was serially diluted starting at 1:10, and an equal volume of diluted virus yielding 400–600 plaque-forming units per milliliter was added, as described previously.[31] The $PRNT_{50}$ titer was the reciprocal of the serum dilution that reduced the number of plaques by 50%. The titer was determined by probit analysis using SPSS software (IBM SPSS Statistics Version 16, Chicago, IL). A $PRNT_{50}$ titer $\geq$ 20 was considered positive.

Acute samples from seroconverted posttravel enrollees were further analyzed by a laboratory-developed multiplex CHIKV and DENV real-time RT-PCR.[32] Viral RNA was extracted from the serum using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Two sets of primers and probes were used to detect CHIKV and DENV (all serotypes) RNA. Primers and probes were synthesized by Integrated DNA Technologies (Coralville, IA). The assay was performed by using the SuperScript III Platinum One-Step Quantitative RT-PCR Kit with ROX Reference Dye (Invitrogen, Carlsbad, CA), with amplification in the 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems, Foster City, CA). A standard setup of 40 cycles was run. The RNA was considered detected if the cycle threshold value was 35 or less.

The primary outcome of interest from the laboratory component was infection with CHIKV and/or DENV during the studied travel. A case of arbovirus infection was defined as PRNT seroconversion in any traveler, a positive convalescent PRNT in a posttravel enrollee, and/or a positive RT-PCR in an acute sample from a posttravel enrollee. Travelers with a positive posttravel or convalescent PRNT were assessed for receipt of the yellow fever and/or Japanese encephalitis vaccine prior to or during a pretravel study visit (DENV) and/or for prior travel to developing regions within 5 years (CHIKV, DENV). Testing of serum samples for cross-reactive antibodies to non-DENV flaviviruses was not performed.

Statistical analysis. Pearson's χ^2 test or Fisher's exact test were performed for univariate analysis of categorical variables, and Mann–Whitney *U* was performed for continuous variables. Variables with a *P* value of ≤ 0.1 on univariate analysis were incorporated into a logistic regression model for multivariate analysis to determine independent risk factors for each outcome. Results of the multivariate analysis were reported as odds ratios (ORs) with 95% confidence intervals (CIs). A *P* value of ≤ 0.05 was considered significant on the multivariate analysis. Statistical analyses were performed using SPSS software (IBM SPSS Statistics Version 22).

RESULTS

Baseline characteristics. During the study period, 277 travelers met inclusion criteria for the destinations of interest, including 10 (3.6%) who enrolled posttravel (Table 1); however, the number of respondents to individual survey questions was variable (Tables 1–3). Nearly half of travelers were activity-duty military (43%), though only 56% of 118 active-duty members traveled for a military purpose (39% traveled for vacation and 7.6% to visit friends and relatives [VFR]). For all comers, the most frequent purpose of travel was vacation (51%), followed by missionary work (29%), and military purpose (26%), whereas few travelers went to VFR (10%).

Mosquito exposure and PPM use. Mosquito exposure was common among travelers to CHIKV-outbreak regions, with 64% of travelers reporting that they saw mosquitoes, and 53% reporting at least one mosquito bite, though only 6.5% reported > 15 bites (Table 1). Travelers reported variable use of insect repellent on exposed skin: 48% reported using repellent often, 29% rarely, and 24% never. Only

TABLE 1

Travel characteristics, PPM use, and mosquito exposure in travelers to Mexico, the Caribbean, and Central and South America from December 2013 to May 2015

Characteristic	No. of total travelers (%) or value (*N* = 277)	No. of travelers infected with CHIKV and/or DENV (%) or value (*N* = 7)*
Male gender	141 (51)	3 (43)
Age, median years (IQR)	40 (29–60)	49 (25–60)
Active duty military	118 (43)	1 (14)
Posttravel enrollment	10 (3.6)	3 (43)
Region of travel		
Caribbean	78 (28)	3 (43)
Mexico/Central America	114 (41)	1 (14)
South America	85 (31)	3 (43)
Type of location		
Rural	138 (50)	3 (43)
Peri-urban	78 (28)	3 (43)
Urban	193 (70)	6 (86)
Port	29 (10)	0 (0)
Duration of travel, median days (IQR)	11 (8–17)	18 (5–45)
Type of accommodation		
Military	33 (12)	0 (0)
Dormitory	30 (11)	0 (0)
Hotel	179 (65)	3 (43)
Hotel without AC	32 (12)	0 (0)
Purpose of travel		
Adventure	31 (11)	1 (14)
Cruise	32 (12)	0 (0)
Medical support	34 (12)	0 (0)
Military	73 (26)	2 (29)
Missionary	79 (29)	1 (14)
Vacation	141 (51)	3 (43)
VFR	28 (10)	3 (43)
Saw mosquitoes	127 (64)†	6 (86)
Total mosquito bites		
0	95 (47)‡	1 (14)
1–5	72 (36)‡	3 (43)
6–10	17 (8.5)‡	1 (14)
11–15	4 (2.0)‡	0 (0)
> 15	13 (6.5)‡	2 (29)
PPM use		
Frequency of repellent use		
Never	47 (24)§	1 (14)
Rarely	57 (29)§	1 (14)
Often/every day	96 (48)§	5 (71)
Treated outer clothing with a repellent	30 (11)	2 (29)
Optimal PPM use	21 (11)§	2 (29)
Symptoms during/after travel	–	5 (71)

AC = air condition; CHIKV = chikungunya virus; DENV = dengue virus; IQR = interquartile range; PPM = personal protective measure; VFR = visiting friends and relatives.

*CHIKV and/or DENV infection is defined as plaque reduction neutralization test (PRNT) seroconversion, a positive convalescent PRNT in a posttravel enrollee, and/or a positive reverse transcription polymerase chain reaction. Due to the small sample size of travelers who acquired these viruses, calculations to determine statistical significance were not performed.

†Of 198 evaluable travelers.

‡Of 201 evaluable travelers.

§Of 200 evaluable travelers.

TABLE 2
Travel characteristics and PPM use in 198 travelers according to whether they saw mosquitoes while traveling

Characteristic	Saw mosquitoes, no. of travelers (%) or value		*P* value		Multivariate OR (95% CI)
	No	Yes	Univariate	Multivariate	
Gender			0.41		
Male	39 (39)	62 (61)			
Female	32 (33)	65 (67)			
Age, median years (IQR)	52 (34–66)	40 (29–56)	0.01	0.66	-
Active duty	19 (25)	56 (75)	0.02	0.01	2.6 (1.3–5.4)
Region of travel			0.53		
Caribbean	17 (33)	35 (67)			
Mexico/Central America	29 (34)	57 (66)			
South America	25 (42)	35 (58)			
Type of location					
Rural	33 (32)	70 (68)	0.24		
Peri-urban	21 (32)	44 (68)	0.47		
Urban	52 (36)	94 (64)	0.91		
Port	11 (52)	10 (48)	0.10	0.85	–
Duration of travel, median days (IQR)	11 (7–18)	12 (8–16)	0.57		
Type of accommodation					
Military	5 (28)	13 (72)	0.45		
Dormitory	6 (33)	12 (67)	0.82		
Hotel	54 (40)	80 (60)	0.06	0.33	–
Hotel without AC	10 (45)	12 (55)	0.32		
Purpose of travel					
Adventure	13 (57)	10 (43)	0.03	0.13	–
Cruise	11 (61)	7 (39)	0.02	0.24	–
Medical support	6 (25)	18 (75)	0.24		
Military	12 (27)	32 (73)	0.18		
Missionary	12 (22)	42 (78)	0.01	0.32	–
Vacation	47 (43)	63 (57)	0.02	0.57	–
VFR	7 (32)	15 (68)	0.68		
PPM use					
Frequency of repellent use			< 0.01	< 0.01	3.3 (2.2–5.0)
Never	31 (67)	15 (33)			
Rarely	23 (40)	34 (60)			
Often/every day	16 (17)	78 (83)			
Treated outer clothing with a repellent	6 (20)	24 (80)	0.05	0.69	–
Optimal PPM use	1 (5)	20 (95)	< 0.01	0.15	–

AC = air condition; CI = confidence interval; IQR = interquartile range; OR = odds ratio; PPM = personal protective measure; VFR = visiting friends and relatives.

11% of travelers treated their outer clothing separately with repellent (e.g., permethrin); this practice was typically accompanied by regular application of repellent to exposed skin, providing optimal PPM use.

On multivariate logistic regression, only active-duty status (OR = 2.6 [95% CI = 1.3–5.4]) and more frequent repellent use on the skin (OR = 3.3 [95% CI = 2.2–5.0]) were associated independently with seeing mosquitoes (Table 2). Active-duty status was most strongly correlated with a military purpose of travel ($\rho = 0.578$, $P < 0.01$). On a separate multivariate regression, VFR (OR = 3.5 [95% CI = 1.2–10.0]) and increased frequency of repellent use on the skin (OR = 2.4 [95% CI = 1.3–4.4]) were associated independently with more intense bite exposure, whereas older age correlated negatively with bite exposure (OR = 0.98 [95% CI = 0.95–1.0]) (Table 3).

Arbovirus exposure. Paired sera were available in 122 travelers, but only 121 pairs (44% of the total enrollment) were analyzed; one pair was excluded, as both available samples for that traveler were collected pretravel. Pretravel enrollees with pre- and posttravel sera accounted for 117 of the pairs; posttravel enrollees with acute and convalescent sera accounted for the remaining four. ELISAs of the posttravel/convalescent sera revealed disproportionately high rates of anti-CHIKV IgM positivity compared with rates of anti-CHIKV neutralizing antibodies on confirmatory testing with PRNT (Table 4); a similar disproportionality was observed between ELISAs for anti-DENV IgG and PRNTs for anti-DENV neutralizing antibodies. Overall, 16 travelers were seropositive by posttravel/convalescent PRNT (nine for CHIKV, nine for DENV, and two for both); 11 (69%) had previously traveled to developing regions within the prior 5 years, including four of those who also had a positive pretravel PRNT (Table 5). Five travelers with a positive posttravel/convalescent PRNT for DENV received the yellow fever and/or Japanese encephalitis vaccine prior to ($N = 2$) or during the pretravel study visit ($N = 3$).

Of the 16 seropositive travelers on posttravel/convalescent PRNT, seven cases were identified (four with CHIKV, five with DENV, and two with both viruses) (Table 5). Two CHIKV-infected travelers, including one posttravel enrollee, represented PRNT seroconversions. Two additional posttravel enrollees were also positive for CHIKV by PRNT. Three DENV-infected travelers represented seroconversions, all of whom were prescribed either the yellow fever or Japanese encephalitis virus vaccine at the pretravel study visit. Two posttravel enrollees were also positive for DENV by PRNT. Of the three posttravel enrollees with a positive convalescent PRNT for CHIKV alone ($N = 1$) or both CHIKV and DENV ($N = 2$), only one (traveler 8) enrolled within a week after returning from travel (3 days), whereas the other (travelers 7 and 9) enrolled 15–22 days after

TABLE 3
Travel characteristics and PPM use in 201 travelers according to intensity of bite exposure

Characteristic	Bite exposure, no. of travelers (%) or value		*P* value		
	Low intensity (0–5 bites) (*N* = 167)	High intensity (≥ 6 bites) (*N* = 34)	Univariate	Multivariate	Multivariate OR (95% CI)
Gender			0.36		
Male	88 (85)	15 (15)			
Female	79 (81)	19 (19)			
Age, median years (IQR)	46 (31–65)	37 (28–50)	0.01	0.05	0.98 (0.95–1.0)
Active duty	61 (78)	17 (22)	0.14		
Region of travel			0.15		
Caribbean	39 (75)	13 (25)			
Mexico/Central America	74 (84)	14 (16)			
South America	54 (89)	7 (11)			
Type of location					
Rural	89 (85)	16 (15)	0.51		
Peri-urban	54 (80)	13 (19)	0.51		
Urban	125 (84)	23 (16)	0.39		
Port	21 (100)	0 (0)	0.03	0.16	–
Duration of travel, median days (IQR)	11 (8–17)	12 (7–15)	0.77		
Type of accommodation					
Military	15 (79)	4 (21)	0.54		
Dormitory	17 (94)	1 (6)	0.32		
Hotel	115 (85)	21 (15)	0.42		
Hotel without AC	22 (92)	2 (8)	0.38		
Purpose of travel					
Adventure	19 (79)	5 (21)	0.57		
Cruise	17 (94)	1 (6)	0.32		
Medical support	23 (88)	3 (12)	0.58		
Military	37 (82)	8 (18)	0.86		
Missionary	47 (84)	9 (16)	0.84		
Vacation	95 (86)	16 (14)	0.29		
VFR			0.02	0.02	3.5 (1.2–10.0)
No	153 (85)	26 (15)			
Yes	14 (64)	8 (36)			
PPM use					
Frequency of repellent use			< 0.01	< 0.01	2.4 (1.3–4.4)
Never	45 (96)	2 (4)			
Rarely	50 (88)	7 (12)			
Often/every day	71 (74)	25 (26)			
Treated outer clothing with a repellent	23 (77)	7 (23)	0.31		
Optimal PPM use	17 (81)	4 (19)	0.76		

AC = air condition; CI = confidence interval; IQR = interquartile range; OR = odds ratio; PPM = personal protective measure; VFR = visiting friends and relatives.

returning from travel. Traveler 8 was positive for both viruses by PRNT, but the acute serum multiplex RT-PCR was positive only for CHIKV; no other evaluated travelers were viremic. The attack rate was 0.89% for CHIKV (1/112), 2.7% for DENV (3/113), and 3.7% for composite arbovirus infection (4/108) among evaluable, immunologically naïve pretravel enrollees. In contrast, there was serologic and/or PCR evidence of CHIKV/DENV infection in three of four evaluable (3 of 10 total) posttravel enrollees.

Among travelers who acquired CHIKV and/or DENV during the studied trip, there were fewer active-duty members compared with the total population (14% versus 43%), though military travel accounted for two of the four cases in pretravel enrollees (Table 1). There was an increased proportion of VFR travelers in cases (43% versus 10%). Military purpose of travel and VFR together accounted for five of seven (71%) of cases compared with 36% of the total population; the three posttravel cases were diagnosed in VFR travelers.

TABLE 4
Results of CHIKV and DENV serologies in 121 travelers with paired sera

Posttravel/convalescent result	No. positive (%)
CHIKV IgM ELISA (+)	31 (26)
CHIKV IgG ELISA (+)	9 (7)
CHIKV PRNT (+)	9 (7)
DENV IgM ELISA (+)	8 (7)
DENV IgG ELISA (+)	71 (59)
DENV PRNT (+)	9 (7)

CHIKV = chikungunya virus; DENV = dengue virus; ELISA = enzyme-linked immunosorbent assay; PRNT = plaque reduction neutralization test.

Of the seven cases, five (71%) were symptomatic, including both coinfected patients, one of two infected by CHIKV alone, and two of three infected by DENV alone. Three of the five symptomatic travelers were posttravel enrollees, and reported fever (*N* = 2), rash (*N* = 2), headache (*N* = 2), joint pain (*N* = 1), and pedal edema (*N* = 1). Headache (*N* = 2) was the only symptom reported by pretravel enrollees who acquired infection during travel; no pretravel enrollee reported a fever that was not associated with diarrhea or a respiratory infection. By comparison, only two of the other nine PRNT-seropositive travelers reported symptoms: one each with headache and rash.

DISCUSSION

Our assessment of mosquito exposure and CHIKV and DENV infection in travelers to CHIKV-outbreak regions in

TABLE 5
Results of 16 individual travelers positive for CHIKV and/or DENV by PRNT

Traveler	Pretravel or acute CHIKV $PRNT_{50}$ titer	Posttravel or convalescent CHIKV $PRNT_{50}$ titer	Pretravel or acute DENV $PRNT_{50}$ titer	Posttravel or convalescent DENV $PRNT_{50}$ titer*	Prior travel to developing regions within 5 years
1	QNS	73			Yes
2	56	124			Yes
3	33	20			No
4	27	22			Yes
5	20	176			No
6†	< 20	28			Yes
7†‡	4,611	4,762			Yes
8†‡§	< 20	4,808	QNS	299	No
9†‡	8,821	6,407	QNS	1,588	Yes
10†			< 20	807‖	Yes
11†			< 20	106‖	Yes
12†			< 20	20‖	Yes
13			936	1,120‖	Yes
14			758	950	No
15			39	25‖	Yes
16			QNS	626	No

CHIKV = chikungunya virus; DENV = dengue virus; $PRNT_{50}$ = plaque reduction neutralization test, reciprocal of the serum dilution that reduced the number of plaques by 50%; QNS = quantity of sample not sufficient to perform test.
*Highest posttravel/convalescent titer among the four DENV serotypes assessed (DENV-1, 2, 3, and 4); the pretravel/acute titer reflects the corresponding serotype.
†Meets case definition of CHIKV and/or DENV infection.
‡Posttravel enrollee.
§Reverse transcription polymerase chain reaction positive for CHIKV at a cycle threshold value of 22.3.
‖Received yellow fever and/or Japanese encephalitis vaccine prior to or during a pretravel study visit.

the Americas is the first prospective assessment of the CHIKV attack rate in travelers and highlights the increased frequency of the outcomes of interest in military and VFR travelers as well as the importance of subclinical infection in travelers. Our findings support the need for mosquito-avoidance education in select groups of travelers.

We found that mosquito exposures were common among travelers to CHIKV-outbreak regions. Particularly, younger travelers, active-duty travelers, and VFR travelers were more likely to report mosquito exposure. More frequent repellent use was also associated with mosquito exposure for both seeing mosquitoes and bite intensity. This may reflect pretravel anticipation of mosquito exposure or an in-travel reaction to exposure, though a definitive explanation for this phenomenon cannot be determined from the available data. PPM use has demonstrated effectiveness in preventing mosquito exposure, and the combination of skin repellent and permethrin-treated clothing has optimal reduction in bites.[33] Overall, self-reported PPM use was suboptimal in this study, though similar to that reported in the prior travel survey at Brooke Army Medical Center.[29] Given historically suboptimal use of PPM even among attendees at pretravel clinics, this is an area on which those who practice travel medicine can focus their educational efforts (e.g., providing a demonstration of permethrin use or making skin repellent and/or permethrin available through their clinics).

In addition to military and VFR travel being associated with mosquito exposure, our study found higher rates of these purposes of travel in those with CHIKV and DENV infection as well, though formal statistics were not performed due to the small sample size of infected travelers. Over two-thirds of cases were traveling for a military purpose or to VFR, whereas these groups together accounted for just over one-third of the total study population. This supports the previously established increased risk of VFR travelers to acquire potentially preventable travel-related infections compared with tourist travelers.[2,34] Our study is unique in highlighting a potential association between military travel and arbovirus infection in a cohort of prospectively enrolled military and civilian travelers. Indeed, two of the four infected pretravel enrollees traveled for a military purpose.

Although a true denominator was lacking for posttravel enrollees, 3.7% of immunologically naïve, prospectively enrolled travelers were infected with CHIKV and/or DENV compared with three of four evaluable (3 of 10 total) posttravel enrollees. Given that all posttravel cases were in VFR travelers and VFR travelers historically have a low rate of attendance at pretravel clinics and a higher rate of acquiring preventable illnesses,[34] this may suggest a benefit to pretravel counseling, though the study did not assess whether posttravel enrollees sought pretravel advice outside of the study setting. While the military can mandate predeployment or pretravel counseling in its active-duty members, such an approach is not possible for civilian travelers. Outreach to VFR travelers to encourage attendance at pretravel clinics or some discussion of travel at primary care clinics may help narrow this gap, though adherence to pretravel counseling in VFR travelers is also reported to be low.[34,35] Optimizing effective pretravel education remains a challenge, especially in this higher risk group.

Our attack rate for arbovirus infection in pretravel enrollees was comparable to other studies evaluating arbovirus risk in travelers when accounting for duration and destination of travel, inclusion of asymptomatic cases, differences in methodology, and our evaluation for both CHIKV and DENV. To our knowledge, our study is the first prospective assessment of the CHIKV attack rate in travelers. A mathematical modeling study determined an attack rate for chikungunya fever of 0.31–1.23% in travelers to southeast Asia.[36] A prospective evaluation of Israeli travelers found that 6.7% of travelers seroconverted for DENV over a median travel duration of 6.1 months,[28] whereas two prospective studies of Dutch travelers reported an attack rate of 1.2–2.9% over a median travel duration

of 3–4 weeks.[24,25] A French military study reported an attack rate of 0.43% for confirmed symptomatic DENV infection and 1.3% for possible symptomatic infection over a 2-year period.[37] CHIKV and DENV coinfections have been described, with coinfection rates of 2.5–10% of total infections in local populations with cocirculation.[38,39] Our higher coinfection rate may reflect our small sample size or geographic differences in transmission, though concurrent infection with both viruses cannot be proven definitively without the simultaneous presence of RNA from both CHIKV and DENV. Thus, it is possible that one or both of our coinfections actually represented discrete infectious events within the individual travelers.

Among our serologically defined cases, five of seven reported symptoms consistent with CHIKV and/or DENV during travel or at the time of acute presentation. Our small sample size and the inclusion of coinfected and posttravel enrollees does not allow for a clear denominator to determine the true frequency of symptomatic infection for each arbovirus in comparison to the literature. Additionally, the literature often defines suspected CHIKV or DENV as a fever-plus syndrome involving fever plus arthralgias/arthritis (CHIKV) or fever plus headache, retro-orbital pain, myalgias, rash, or hemorrhagic signs (DENV) in the appropriate epidemiologic context.[1,11,20,37] Because we defined cases serologically rather than syndromically, we identified cases that would not have met the suspected clinical case definition (i.e., only half of the infected pretravel enrollees reported any symptoms consistent with CHIKV or DENV and none of them reported fever). Within those limits, one of the two CHIKV monoinfected travelers was asymptomatic. By contrast, most studies quote a 3–25% rate of asymptomatic CHIKV infections,[6,40] though there is a prospective study in a nonnaïve population in the Philippines that revealed an 82% rate of subclinical infection.[41] In our study, only one of three DENV monoinfected travelers was asymptomatic, whereas the literature predominantly indicates that over half of DENV infections are asymptomatic.[42,43] A study of Israeli travelers with similar sample size as our study, though, did demonstrate that 43% of cases were asymptomatic.[28] Host susceptibility, population seroprevalence, age, strain virulence, and geography may impact rates of clinical versus subclinical arbovirus infection.[40–42,44] Reported rates of arbovirus infection looking only at ill-returning travelers may underestimate actual arbovirus exposure in all travelers; travelers with subclinical infection may be at particular risk for introducing new pathogens into nonendemic regions leading to autochthonous transmission.[42,45]

The most significant strength of our study is that it is a multicenter, prospective assessment of arbovirus risk in travelers that includes clinical and serologic data. It thus provides the first prospective assessment of the risk for CHIKV infection in travelers and demonstrates the risk for subclinical infection in afebrile travelers returning to nonendemic regions. It also provides a unique perspective on the mosquito and arbovirus exposure risk associated with military travel. Follow-up evaluations of risk factors and seroconversion rates for ZIKV in the same geographic area are planned.

Our study also has multiple limitations. First, its small sample size limits its power and prevents meaningful statistical analysis to compare infected versus noninfected travelers. Second, PPM use and exposure history were self-reported and collected retrospectively up to 2 months after return from travel without traveler foreknowledge that these elements would need to be recalled. Future studies could consider evaluation of human antibody response to species-specific mosquito salivary antigens as a biomarker to better quantify mosquito exposure risk in travelers.[46,47] Third, symptoms not associated with febrile illness were also collected retrospectively, and posttravel surveys did not specifically query the prominent symptoms of arthralgias and myalgias. Fourth, the inclusion of posttravel enrollees introduces heterogeneity into diagnosed cases. While posttravel enrollees had minimal impact on the overall survey of travel characteristics (represented 3.6% of total enrollees and 3.4% of enrollees with paired sera), they were disproportionately represented in arbovirus cases (43%). Posttravel enrollees may have had recall bias in their mosquito exposure, PPM use, and symptom histories. Fifth, this study focused on the Americas, so application to worldwide travel must consider the potential for variability in CHIKV and DENV transmission in different epidemiologic contexts.[40,42,45]

Finally, laboratory methods for assessing serologic response to CHIKV and DENV infection are not standardized, and the optimal breakpoints for defining positive ELISAs and PRNTs are unknown.[48–50] We found a high rate of apparently false-positive anti-CHIKV IgM and anti-DENV IgG, which may have reflected cross-reactivity with other alphaviruses or flaviviruses (respectively) or prior exposure.[51,52] Although we assessed for prior Japanese encephalitis and yellow fever virus vaccinations and prior travel that would have increased risk for antecedent arbovirus exposure, we did not perform laboratory assessment for cross-reactive antibodies to non-DENV flaviviruses; thus, our cases may include false-positive, cross-reactive signals. Our definition of a positive ELISA (OD that exceeded the mean plus three standard deviations from normal sera) has been described.[44,53] However, other laboratories have reported disparate breakpoints both above and below ours.[24,28,30,40,41,49] Because PRNTs were only performed on positive ELISAs, our data does not allow for the determination of the false-negative rate for CHIKV or DENV ELISAs.

We defined a positive $PRNT_{50}$ titer as ≥ 20 and applied that definition to a dichotomous, qualitative decision tree in which a PRNT seroconversion in any traveler or a positive convalescent PRNT in a posttravel enrollee was considered a case, irrespective of the quantitative change in pretravel/acute to posttravel/convalescent titer. This impacted both our sensitivity (e.g., traveler 5 was not considered a case despite 4-fold increase in titer from pre- to posttravel) and specificity (e.g., travelers 6 and 12 were considered cases despite demonstrating borderline-positive seroconversions) (Table 5). As with the ELISA, other laboratories have reported disparate breakpoints both above and below ours, and results have been shown to vary widely with varied testing conditions; interpretation is further confounded by host/epidemiologic factors including primary versus secondary exposure, time since exposure, patient vaccination status, and exposure to non-DENV flaviviruses (e.g., ZIKV).[49,50,54–56] If we had increased the PRNT breakpoint to ≥ 30, we would have decreased our case count by 2, eliminating one CHIKV case (traveler 6) and one DENV case

(traveler 12). If we considered the borderline posttravel CHIKV PRNT a false positive, potential explanations could be infection with a non-CHIKV alphavirus during the trip or variable testing conditions.[52,54] If we considered the borderline posttravel DENV PRNT a false positive, it would likely reflect cross-reactivity with the traveler's receipt of the Japanese encephalitis virus vaccine at the pretravel study visit; less likely explanations would include cross-reactivity with the traveler's remote receipt of the yellow fever virus vaccine, cross-reactivity due to infection with a non-DENV flavivirus, or variable testing conditions.[49–51,56,57] Increasing the positive breakpoint to 30, though, would not have significantly affected the implications of our study given that four of the five high-titer cases were traveling either to VFR ($N = 3$) or on a military purpose ($N = 1$).

CONCLUSION

Military and VFR travelers are at increased risk for mosquito exposure and arbovirus infection. Although none of our pretravel enrollees would have met a clinical case definition for suspected CHIKV or DENV infection, subclinical infection has implications for secondary transmission in nonendemic regions and needs to be prevented. Although the incidence of CHIKV infection is declining and the incidence of DENV continues to wax and wane, ZIKV has emerged as the latest arbovirus threat in the Americas.[3,4,9–11] Given their shared mosquito vector, these cocirculating arboviruses share some similar risk factors. Thus, the risk factors and preventive measures described herein can potentially be applied to pretravel counseling for those traveling to current ZIKV-outbreak regions and to other regions with endemic or epidemic arbovirus infection.

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