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Evidence of vertical transmission and co-circulation of chikungunya and dengue viruses in field populations of *Aedes aegypti* (L.) from Guerrero, Mexico

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Background: We report results of the entomo-virological surveillance system in *Aedes aegypti* local populations performed by the Ministry of Health of Guerrero.

Methods: Indoor-adult *Ae. aegypti* collected at Acapulco, Zihuatanejo, Coyuca de Benitez and Atoyac de Alvarez (dry season, 2015) were processed for dengue virus (DENV) and chikungunya virus (CHIKV) using RT-PCR.

Results: We identified different seroptypes of DENV (2, 3 and 4), CHIKV and their co-circulation in field-caught mosquitoes across a significant geographic area. Pools of males were positive for CHIKV and DENV 3 and 4 suggesting vertical transmission.

Conclusions: Entomo-virological surveillance in Guerrero has identified early circulation of CHIKV and DENV and provided a trigger for timely and focalized vector control actions.

Keywords: *Aedes aegypti*, Chikungunya, Dengue, Mexico, Surveillance

Introduction

Aedes aegypti is the primary vector associated with current dengue virus (DENV) and chikungunya virus (CHIKV) outbreaks in Southern Mexico. Given the arrival of CHIKV to the state of Guerrero, an active entomo-virological surveillance system (monitoring of DENV and CHIKV from field-caught female mosquitoes) was established to pro-actively detect the virus and preform rapid and high quality control.[1,2] Here we report evidence of CHIKV natural vertical transmission and co-circulation with DENV in wild populations of *Ae. aegypti* as well as additional results of the entomo-virological surveillance system.

Methods

Samples of adult *Ae. aegypti* (no *Ae. albopictus* were found) were collected indoors for a 15 min period per house with Prokopack aspirators[3] during the dry season 2015 (January–March) from 16 456 houses located in five cities: Acapulco (16°52N, 99°54'W), Zihuatanejo (17°38'N,101°33'W), Coyuca de Benitez (17°0'N,100°5'W) and Atoyac de Alvarez (17°12'N,100°26'W) (Table 1). At that time, there was confirmed DENV transmission across these four localities, but CHIKV transmission in humans had only been confirmed in Acapulco, with probable reports at Zihuatanejo. In Acapulco, three sequential monthly mosquito collections were performed in January–March, with tests for DENV performed in January–February collections and CHIKV on February–March. At the other localities, mosquito collections and DENV and CHIKV testing were only performed in February (Table 1).

Collected specimens were placed in vials together with BD Universal Viral Transport medium (Becton, Dickinson and Company, Sparks, MD, USA), kept at 4–8°C to avoid degradation and taken to the laboratory for their separation by collection locality, species and sex. Adult female and male mosquitoes were first separated by sex and then grouped in pools of one up to 25 specimens. Each pool was processed for the presence of RNA for DENV and CHIKV using existing a real-time reverse-transcription (RT)-PCR protocols.[2,4] To validate the test results, a positive control

Table 1. Prevalence of infection with chikungunya (CHIKV) and dengue (DENV) virus in *Aedes aegypti* mosquitoes collected from selected localities at the Mexican state of Guerrero during January March 2015

Locality	No. houses (neighbourhoods)	Collection round (date)	Sex	Total specimens	Total pools	Total positive pools [infection rate] (95% CI)						
						CHIKV	DENV 1	DENV 2	DENV 3	DENV 4	CHIKV & DENV 2	CHIKV & DENV 4
Acapulco	5000 (160)	1 (5 Jan to 5 Feb)	M	2574	155	0	0	0	2 [0.78] (0.14–2.53)	2 [0.78] (0.14–2.54)	0	0
			F	3203	211	3 [0.94] (0.25–2.55)	0	0	3 [0.94] (0.25–2.53)	5 [1.58] (0.59–3.48)	0	0
			Total	5777	366	3 [0.52] (0.14–1.41)	0	0	5 [0.87] (0.32–1.91)	7 1.22 (0.54–2.41)	0	0
Acapulco	5358 (160)	2 (5 Feb to 5 March)	M	2677	172	0	0	0	2 [1.06] (0.19–3.46)	0	0	0
			F	2953	203	10 [3.47] (1.77–6.16)	0	1 [0.58] (0.03–2.79)	2 [1.16] (0.21–3.80)	1 [0.58] (0.03–2.80)	0	1 [0.34] (0.02–1.64)
			Total	5630	375	10 [1.80] (0.92–3.20)	0	1 [0.28] (0.02–1.33)	4 [1.11] (0.36–2.67)	1 [0.28] (0.02–1.34)	0	1 [0.18] (0.01–0.86)
Acapulco	5581 (163)	3 (6 to 31 March)	M	963	76	2 [2.10] (0.38–6.88)	NA	NA	NA	NA	NA	NA
			F	893	78	3 [3.42] 0.90–9.25	NA	NA	NA	NA	NA	NA
			Total	1856	154	5 [2.74] (1.02–6.06)	NA	NA	NA	NA	NA	NA
Zihuatanejo	236 (12)	1 (2 February)	M	172	10	0	0	0	0	0	0	0
			F	369	21	2 [5.58] (1.01–18.44)	0	0	0	0	1 [2.72] (0.16–13.28)	0
			Total	541	31	2 [3.77] (0.68–12.41)	0	0	0	0	1 [1.85] (0.11–9.02)	0
Coyuca de Benitez	147 (10)	1 (7 February)	M	94	5	0	0	0	0	0	0	0
			F	65	4	0	0	0	0	0	0	0
			Total	159	9	0	0	0	0	0	0	0
Atoyac de Alvarez	134 (10)	1 (7 February)	M	144	6	0	0	0	0	0	0	0
			F	141	9	0	0	0	0	0	0	0
			Total	255	15	0	0	0	0	0	0	0
Totals			M	6594	424	2 [0.30] (0.05–1.00)	0	0	4 [0.83] (0.27–1.99)	2 [0.42] (0.07–1.36)	0	0
			F	7624	526	18 [2.41] (1.48–3.73)	0	1 [0.18] (0.01–0.88)	5 [0.91] (0.34–2.02)	6 [1.10] (0.45–2.28)	1 [0.18] (0.01–0.88)	1 [0.18] (0.01–0.88)
			Total	14218	950	20 [1.42] (0.90–2.16)	0	1 [0.10] (0.01–0.47)	9 [0.87] (0.43–1.60)	8 [0.78] (0.36–1.47)	1 [0.10] (0.01–0.47)	1 [0.10] (0.01–0.47)

NA: not applicable. No diagnostic tests were done on that survey.

(RNA from a reference strain provided by Instituto de Diagnostico y Referencia Epidemiológicos [InDRE]) and two negative controls were included in each RT-PCR run. The threshold (CT) was determined based on the positive and negative controls, with a detection limit of ≤40 cycles. Infection rates (IR) and 95% CIs were calculated with Excel PoolInfRate 4.0 from CDC using a bias corrected maximum likelihood estimation and the skewness-corrected score, respectively.[5]

Results and Discussion

We identified different DENV serotypes (2, 3 and 4), CHIKV and their co-circulation in field-caught male and female mosquitoes across a large geographic area (Table 1). Overall infection levels across all localities for the January–February samples was 1.9% (15/796 pools) for CHIKV, 2.7% (18/671) for DENV and 0.3% (2/671) for both (CHIKV and DENV 2 and CHIKV and DENV 4). In the March samples (exclusive for Acapulco and only testing CHIKV), infection increased to 3.2% (5/154 pools).

The overall (across all sampling dates) estimated infection rate was 1.41 per 1000 mosquitoes (95% CI 0.90–2.16%) for DENV 2; 0.87 per 1000 mosquitoes (95% CI 0.43–1.60%) for DENV 3; 0.78 per 1000 mosquitoes (95% CI 0.36–1.47%) for DENV 4; and 0.10 per 1000 mosquitoes (95% CI 0.10–0.47%) for CHIKV and DENV 2 and CHIKV and DENV 4. However, the infection rate increased to 3.42–5.58 per 1000 mosquitoes if calculated by locality and sex.

In Acapulco two pools of *Ae. aegypti* males were positive for CHIKV and were collected from nearby neighbourhoods (Palma Sola [IR 40.0; 95% CI 0.0–116.81 per 1000 mosquitoes] and Palma Sola Fovissste [IR 83.3; 95% CI 0.0–239.71 per 1000 mosquitoes]). Detection of CHIKV genomic fragments in male adults of *Ae. aegypti* suggests the occurrence of vertical (from a parent organism to its progeny) transmission. This represents, as far as we are aware, the first quantification of vertical transmission in Mexico and in the Americas. Evidence of vertical transmission of DENV 3 and 4 was also found in Acapulco (Table 1). Previous studies assessing field-collected populations of *Ae. aegypti* in the same area have reported evidence of vertical transmission of DENV 1.[6] We also identified the co-circulation of CHIKV and three different DENV serotypes (DENV 2, 3 and 4) and the presence of CHIK and DENV in the same pool. Concomitant transmission (and thus co-circulation) in humans and mosquitoes is a phenomenon observed in areas where CHIKV is spreading and that are already endemic for DENV in Asia and Africa,[6] and expected to occur in Mexico.

We acknowledge several limitations inherent to this observational study, as follows: with the exception of Acapulco, this was a cross-sectional study that prevented calculating temporal variability in infection rates as the CHIKV epidemic unveiled; infrastructure limitations in Acapulco hindered our capacity to perform advanced virological studies including virus isolation; although all efforts were made to limit contamination, we cannot fully rule out virus cross-contamination in male pools from infected females (thus, our estimates should be considered as an upper-limit); and due to the high number of mosquitoes tested we could not run separate tests for unfed and bloodfed females.

To date, the entomo-virological surveillance system in Guerrero reported here and previously[2,6] has been useful to: identify early co-circulation of CHIKV and DENV; quantify the magnitude of vertical transmission of CHIKV and DENV; and provide a trigger for vector control actions, which in Guerrero involved integrated and synchronized focal intervention (chemical control of breeding-sites and indoor and outdoor chemical control of adults) at the houses with reports and adjacent houses.[7,8] Our overall results with regard to the laboratory diagnosis and entomological surveillance of dengue using molecular techniques show that these approaches are sensitive and specific for CHIKV and DENV surveillance. Furthermore, and given the recent introduction of Zika virus into the Americas,[9,10] the elaboration of surveillance systems detecting virus activity in the vector could provide a cost-effective and rapid alternative to the detection of cases of symptomatic disease, as they allow identifying the co-circulation of multiple viruses with different pathogenicity and level of immunity in the human population.

Authors' contributions: FDM, NEM and CGC conceived the study; FDM, LLD and JIL designed the study protocol; AMJ, WBM, MCN and CTB carried out the sample collection and molecular experiments; FDM, GVP and PMS carried out analysis and interpretation of data; FDM, GVP and PMS drafted the manuscript. All authors read and approved the final manuscript. FDM is guarantor of the paper.

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