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## Temporal Correlations Between Mosquito-Based Dengue Virus Surveillance Measures or Indoor Mosquito Abundance and Dengue Case Numbers in Mérida City, México

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### Abstract

Surveillance of dengue virus (DENV) in *Aedes (Stegomyia) aegypti* (L.) females is of potential interest because human DENV infections are commonly asymptomatic, which decreases the effectiveness of dengue case surveillance to provide early warning of building outbreaks. Our primary aim was to examine if mosquito-based virological measures – monthly percentages of examined *Ae. aegypti* females infected with DENV or examined homes from which 1 DENV-infected *Ae. aegypti* female was collected – are correlated with reported dengue cases in the same or subsequent months within study neighborhoods in Mérida City, México. The study encompassed ~30 neighborhoods in the southern and eastern parts of the city. Mosquitoes were collected monthly over a 15-month period within study homes (average of 145 homes examined per month); this produced ~5,800 *Ae. aegypti* females subsequently examined for DENV RNA. Although monthly dengue case numbers in the study neighborhoods varied more than 100-fold during the study period, we did not find statistically significant positive correlations between monthly data for mosquito-based DENV surveillance measures and reported dengue cases in the same or subsequent months. Monthly average temperature, rainfall, and indoor abundance of *Ae. aegypti* females were positively correlated ( $P < 0.001$ ) with dengue case numbers in subsequent months with lag times of 3–5, 2, and 1–2 months, respectively. However, because dengue outbreak risk is strongly influenced by serotype-specific susceptibility of the human population to DENV, the value of weather conditions and entomological indices to predict outbreaks is very

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limited. Potential ways to improve the sensitivity of mosquito-based DENV surveillance are discussed.

## Keywords

*Aedes aegypti*; dengue virus; virological surveillance; México

## Introduction

Numerous previous studies have documented correlations between weather conditions (rainfall or temperature-related variables) or entomological indices for the dengue virus (DENV) vector *Aedes (Stegomyia) aegypti* (L.) (abundance measures based on surveys for immatures or trap catches) and the numbers of dengue cases in subsequent weeks or months (reviewed by Eisen and Moore 2013). Such linkages between weather or mosquito abundance and dengue disease are tenuous, however, because they are also strongly influenced by the constantly changing serotype-specific susceptibility of the human population to DENV (Scott and Morrison 2009). The lack of confidence in weather conditions and entomological indices as predictors of DENV transmission intensity, together with problems related to asymptomatic infections in humans and delays in diagnostic testing and reporting of dengue cases, raises the question of whether virological surveillance of DENV in mosquito vectors may be a useful complement to human-based surveillance. DENV is readily detected from field-collected *Ae. aegypti* females (reviewed by García-Rejón et al. 2008). The infected females can be linked to the spatial locations where they were collected, and very likely have far smaller spatial activity spaces compared with DENV-infected humans (Harrington et al. 2005, Vazquez-Prokopec et al. 2010).

As part of a study conducted in Mérida City, México over a 15-month period (May 2009–July 2010) to determine whether insecticide-treated curtains can be used to protect homes from intrusion by *Ae. aegypti* and intradomicillary DENV transmission (Loroño-Pino et al. 2013), we performed monthly indoor collections of *Ae. aegypti* females (typically in >125 homes/month) and determined their DENV infection status. Herein, we examine if mosquito-based virological measures (monthly percentages of examined *Ae. aegypti* females infected with DENV or examined homes from which 1 DENV-infected *Ae. aegypti* female was collected) are correlated with reported dengue cases in the same or subsequent months in our Mérida City study neighborhoods.

## Materials and Methods

The Mérida City study area, the selection of study homes, and the methods used to collect mosquitoes and detect DENV RNA in *Ae. aegypti* females were described previously (Loroño-Pino et al. 2013). Study homes distributed across ~30 neighborhoods (Colonias) in the southern and eastern parts of Mérida City were followed over a 15-month period from May 2009–July 2010. The study homes represent ~3% of the total number of homes in these neighborhoods. Individual neighborhoods included both intervention homes with insecticide-treated curtains and control homes with similar but non-treated curtains. Monthly data presented herein for *Ae. aegypti* females and their DENV infection status are based on

homes designated as control or intervention homes for the portion of the study period that preceded curtain installation (May–August 2009), whereas only the control homes were included for the months after curtains were installed (September 2009–July 2010). Weather data (rainfall and mean temperature) for the period from January 2009 – December 2010 were obtained from a weather station at the Mérida City airport operated by the Comisión Nacional del Agua.

Data on monthly numbers of laboratory-confirmed dengue cases occurring in Mérida City, and the subset of these cases occurring within our study neighborhoods, during 2009–2010 were obtained through a collaborative agreement with Servicios de Salud de Yucatán. The monthly numbers of dengue cases for the specific study neighborhoods and overall for Mérida City were strongly correlated (Spearman rank correlation;  $\rho = 0.812$ ,  $N = 24$ ,  $P < 0.001$ ); we therefore use data only for the specific study neighborhoods in the statistical analyses. The study was approved by the Institutional Review Board of Colorado State University and the Bioethics Committee of Universidad Autónoma de Yucatán.

Because of the skewed distribution for the number of reported dengue cases per month, the Spearman rank correlation coefficient (computed using the JMP statistical package; Sall et al. 2005) was used to examine correlations between dengue cases and the other measured variables (percentage of examined *Ae. aegypti* females infected with DENV, percentage of examined homes from which at least one DENV-infected *Ae. aegypti* female was collected, geometric mean number of *Ae. aegypti* per home, total rainfall, and average daily temperature). The Poisson approximation of the binomial distribution was used to evaluate the power to detect the presence of DENV in *Ae. aegypti* females for the monthly numbers of mosquitoes examined during the study period.

## Results

Monthly average daily temperatures in Mérida City ranged from 21–25 °C from November to March but exceeded 27 °C from April to September/October, and most rainfall occurred from May to September (Figure 1, Table 1). Consistent with our previous study from Mérida City (García-Rejón et al. 2008), the abundance of *Ae. aegypti* females indoors was low during the cooler and drier part of the year, started to increase in May/June, and peaked during July–September (Table 1, Figure 1). In contrast to our previous study, which focused on collection of mosquitoes specifically from the homes of dengue patients and found DENV-infected *Ae. aegypti* females from July–December but not from January–June (García-Rejón et al. 2008), we unexpectedly recorded lower percentages of examined homes with DENV-infected *Ae. aegypti* females collected from July–December 2009 (0–1.1%) compared with January–May 2010 (0.7–5.4%) (Table 1). The temporal distribution of reported dengue cases exhibited distinct seasonality, with most cases occurring from August/September to November/December (Figure 1, Table 1).

The strongest positive correlations with the monthly number of dengue cases in the study neighborhoods were observed for monthly average daily temperature 3 months prior ( $\rho = 0.844$ ,  $N = 21$ ,  $P < 0.001$ ), rainfall 2 months prior ( $\rho = 0.553$ ,  $N = 22$ ,  $P = 0.008$ ), and the monthly geometric mean number of *Ae. aegypti* females collected indoors per home 1 month

prior ( $\rho = 0.756$ ,  $N = 15$ ,  $P = 0.001$ ) (Table 2). In striking contrast, we did not find statistically significant positive correlations between monthly data for the mosquito-based DENV surveillance measures and dengue cases in the study neighborhoods (Table 2). Instead, the recorded significant correlations with dengue cases – occurring for each of the two mosquito-based DENV surveillance measures 2 months prior ( $\rho = -0.557$ ,  $N = 15$ ,  $P = 0.031$  in both cases) – were negative (Table 2).

## Discussion

Surveillance of DENV in *Ae. aegypti* females is of potential interest as a means for early detection of dengue outbreaks because human DENV infections are commonly asymptomatic or “silent” (Gubler 1998, Kyle and Harris 2008), which decreases the effectiveness of dengue case surveillance to provide early warning of building outbreaks. Improved methods for detection of DENV RNA or antigen in mosquitoes (Bangs et al. 2007, Chao et al. 2007, Chen et al. 2010, Tan et al. 2011, Muller et al. 2012, Voge et al. 2013) provide new opportunities for mosquito-based DENV surveillance, and studies focusing entirely or in part on dengue patient premises have shown promise with regards to the potential for linking DENV infections in *Ae. aegypti* to human dengue cases in space and time (Pinheiro et al. 2005, Urdaneta et al. 2005, García-Rejón et al. 2008, Guedes et al. 2010, Yoon et al. 2012). Despite a substantial effort – including monthly indoor backpack aspiration mosquito collections from a large number of homes (typically >125 per month) scattered over ~30 neighborhoods in Mérida City, and with nearly 5,800 *Ae. aegypti* females examined for presence of DENV RNA – we disappointingly failed to reproduce the strong linkage between DENV-infected *Ae. aegypti* females and dengue cases seen in our previous study from Mérida City that focused specifically on dengue patient premises (Table 2; García-Rejón et al. 2008). Instead, our study yielded the paradoxical result of infected mosquitoes being collected primarily in the winter and spring of 2010, whereas dengue cases peaked in the preceding late summer and fall of 2009 in the study neighborhoods (Table 1). The only other comparable study of which we are aware that used a similar approach, i.e., collection of adult mosquitoes in randomly selected “sentinel blocks”, similarly failed to find a statistically significant positive association between DENV infection rates of pooled mosquitoes and DENV infection rates in humans (Mendez et al. 2006).

The number of homes examined monthly was sufficient to detect a positive, significant correlation between the geometric mean number of *Ae. aegypti* females collected per home and dengue cases in the following month, but it likely was not sufficient for collection of DENV-infected females. Moreover, the study homes were selected at random within the study neighborhoods in southern and eastern Mérida City, and we cannot rule out the possibility that a more strategic selection of homes would have produced a different result. The selection of homes – including their number and locations – to target in mosquito-based DENV surveillance programs to ensure success in detecting building dengue outbreaks under a range of different DENV transmission intensities would seem to be a fruitful area for further research.

Greater numbers of *Ae. aegypti* females examined for DENV undoubtedly would have been beneficial, but there are indications that the sample size of examined females per se was not a critical issue in this study. First, we were able to detect a statistically significant, but unfortunately negative, correlation between monthly percentages of examined *Ae. aegypti* females infected with DENV or examined homes from which at least one DENV-infected *Ae. aegypti* female was collected and the number of dengue cases reported from the study neighborhoods 2 months later (Table 2). Second, a power analysis indicated that the number of examined females had greater than 90% power to detect the presence of DENV in *Ae. aegypti* females for each month during the study period except for May 2009 based on an overall DENV infection prevalence of 2%; for a DENV infection prevalence of 1%, the power ranged from approximately 80% to 99% except for August 2009 and May 2009 (75% and 19% power, respectively). Infection prevalence values for DENV in *Ae. aegypti* females in the 1–2% range were recorded in this study (Table 1) as well as previous studies from Mérida City (García-Rejón et al. 2008, 2011). Thus, our failure to document a positive correlation between mosquito-based DENV surveillance measures and dengue case numbers within the study neighborhoods likely resulted from an insufficient number of homes from which mosquitoes were collected rather than insufficient numbers of mosquitoes examined for DENV. Another potential criticism of the study is that it only covered a 15-month period. Although a study of extended duration certainly would have been beneficial, we point out that the study period during which mosquitoes were collected extended across times of the year with low and high DENV transmission intensity, and that the numbers of monthly reported dengue cases varied >100-fold for the study neighborhoods (range, 0–127) during our study period (Table 1).

Although the peaks for dengue cases in Mérida City occurred during the fall in both 2009 and 2010, substantial numbers of dengue cases were reported from January to April in both years, and we also collected DENV-infected *Ae. aegypti* females during all four of these months in 2010 (Table 1). This is intriguing because a study conducted in 1984 indicated that dengue cases were very rare in Mérida City during the cooler and drier part of the year from January to April (Loroño-Pino et al. 1993). Therefore, it would be interesting to examine the seasonal curves for dengue cases in Mérida City for selected years over the last decades to determine if DENV activity in the cooler and drier part of the year is a recently emerged phenomenon.

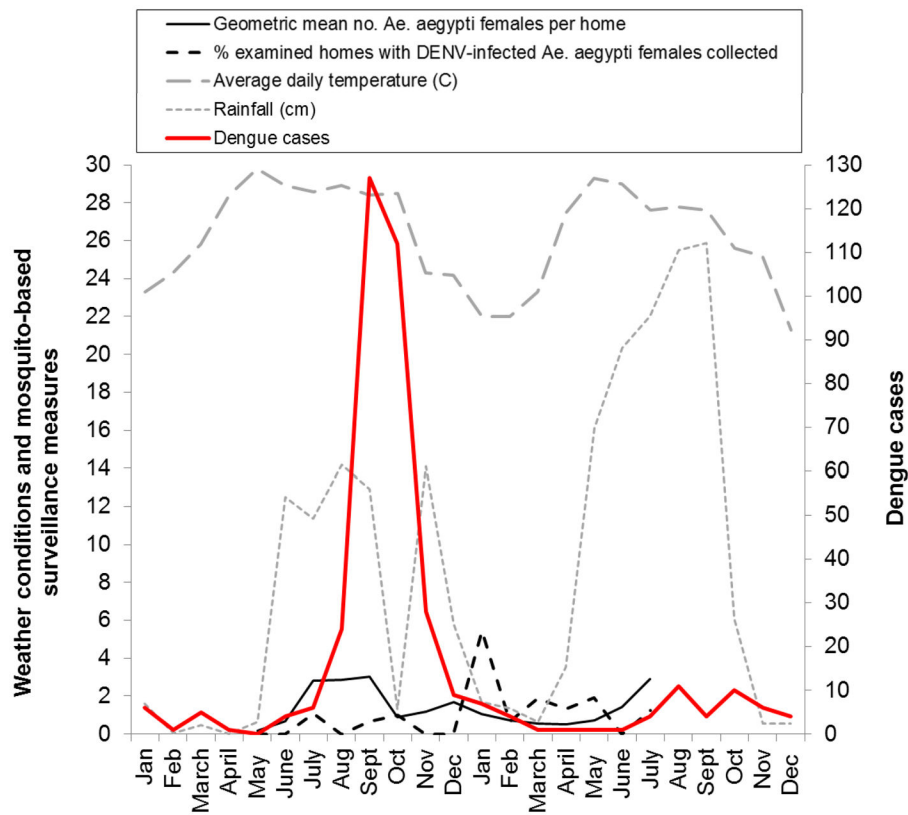
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**Figure 1.** Temporal curves for monthly numbers during 2009–2010 of reported dengue cases in the Mérida City study neighborhoods, weather conditions (total monthly rainfall and monthly average daily temperature), an entomological index (monthly geometric mean number of *Ae. aegypti* females collected per home) and a mosquito-based DENV surveillance measure (monthly percentage of examined homes from which at least one DENV-infected *Ae. aegypti* female was collected).



Table 1

Monthly numbers of reported dengue cases during 2009–2010 in relation to data for weather conditions, abundance of *Ae. aegypti* females, and surveillance for dengue virus (DENV) in *Ae. aegypti* females.

Month and Year	No. of reported dengue cases		Weather conditions			Abundance of <i>Ae. aegypti</i> females				Surveillance for DENV in <i>Ae. aegypti</i> females		
	Mérida City	Study neighborhoods within Mérida City	Total rainfall (mm)	Average daily temp. (°C)	No. of homes examined for mosquitoes	Total no. collected <sup>a</sup>	Geometric mean per home	Total no. DENV-infected females collected	DENV infection prevalence in examined females (%) <sup>a,b</sup>	Total no. homes with DENV-infected females collected	Percentage of examined homes with DENV-infected females collected	
Jan 2009	35	6	16	23.3	0							
Feb 2009	14	1	0.3	24.3	0							
March 2009	10	5	4.7	25.8	0							
April 2009	13	1	0	28.4	0							
May 2009	7	0	6.5	29.8	74	21	0.17	0	0	0	0	0
June 2009	22	4	12.5	28.9	128	182	0.68	0	0	0	0	0
July 2009	19	6	11.4	28.6	184	1,092	2.83	3	0.27	2	1.09	
Aug 2009	62	24	14.2	28.9	27	139	2.86	0	0	0	0	0
Sept 2009	363	127	12.9	28.4	152	836	3.04	1	0.12	1	0.66	
Oct 2009	782	112	13	28.5	203	288	0.87	2	0.69	2	0.99	
Nov 2009	527	28	14.1	24.3	180	441	1.17	0	0	0	0	0
Dec 2009	334	9	58	24.2	149	447	1.67	0	0	0	0	0
Jan 2010	210	7	17	22.0	166	319	1.05	16	5.02	9	5.42	
Feb 2010	99	4	14	22.0	150	258	0.73	7	2.71	1	0.67	
March 2010	38	1	6.4	23.3	160	158	0.54	4	2.53	3	1.88	
April 2010	7	1	35	27.5	151	160	0.50	9	5.63	2	1.32	
May 2010	9	1	161	29.3	157	226	0.71	6	2.65	3	1.91	
June 2010	24	1	203	29.0	143	423	1.45	0	0	0	0	0
July 2010	74	4	220	27.6	161	803	2.92	2	0.25	2	1.24	
Aug 2010	149	11	255	27.8	0							
Sept 2010	123	4	259	27.6	0							
Oct 2010	202	10	61	25.6	0							

No. of reported dengue cases		Weather conditions		Abundance of <i>Ae. aegypti</i> females			Surveillance for DENV in <i>Ae. aegypti</i> females				
Month and Year	Mérida City	Study neighborhoods within Mérida City	Total rainfall (mm)	Average daily temp. (°C)	No. of homes examined for mosquitoes	Total no. collected <sup>a</sup>	Geometric mean per home	Total no. DENV-infected females collected	DENV infection prevalence in examined females (%) <sup>a,b</sup>	Total no. homes with DENV-infected females collected	Percentage of examined homes with DENV-infected females collected
Nov 2010	142	6	5.7	25.2	0						
Dec 2010	52	4	5.5	21.3	0						

<sup>a</sup> All collected *Ae. aegypti* females were examined for presence of DENV RNA.

<sup>b</sup> Infection prevalence was calculated as: (total no. DENV-infected *Ae. aegypti* females/total no. collected *Ae. aegypti* females)\*100.

Correlations between dengue case numbers in the Mérida City study neighborhoods and data for weather conditions, abundance of *Ae. aegypti* females, or surveillance for dengue virus (DENV) in *Ae. aegypti* females in the same or subsequent months.

**Table 2**

Correlation with numbers of dengue cases in the study neighborhoods in the same or subsequent months based on the monthly data presented in Table 1 <sup>a</sup>																				
Lag time	Total monthly rainfall (mm)			Monthly average daily temperature (°C)			Geometric mean no. <i>Ae. aegypti</i> females per home			Percentage of examined <i>Ae. aegypti</i> females infected with DENV			Percentage of examined homes with DENV-infected <i>Ae. aegypti</i> females collected							
	Type <sup>b</sup>	N	ρ	P	Type <sup>b</sup>	N	ρ	P	Type <sup>b</sup>	N	ρ	P	Type <sup>b</sup>	N	ρ	P				
+ 0 mo	NA	24	0.272	0.198	NA	24	-0.137	0.525	Pos	15	0.666	0.007	NA	15	-0.178	0.525	NA	15	-0.199	0.477
+ 1 mo	Pos	23	0.476	0.022	NA	23	0.268	0.215	Pos	15	0.756	0.001	NA	15	-0.506	0.054	NA	15	-0.402	0.138
+ 2 mo	Pos	22	0.553	0.008	Pos	22	0.646	0.001	NA	15	0.512	0.051	Neg	15	-0.641	0.010	Neg	15	-0.557	0.031
+ 3 mo	NA	21	0.364	0.105	Pos	21	0.844	<0.001	NA	15	0.112	0.691	NA	15	-0.386	0.156	NA	15	-0.255	0.359
+ 4 mo	NA	20	0.017	0.944	Pos	20	0.750	<0.001	NA	15	-0.170	0.545	NA	15	-0.291	0.292	NA	15	-0.258	0.354
+ 5 mo	NA	19	-0.280	0.245	Pos	19	0.508	0.027	NA	15	-0.400	0.140	NA	15	-0.184	0.511	NA	15	-0.077	0.785

<sup>a</sup>Based on Spearman's rank correlation (ρ) along with the number of data points (N) and the p-value for the test of significance (P).

<sup>b</sup>Pos – Positive correlation (P<0.05); Neg – Negative correlation (P<0.05); NA – non-significant correlation (P 0.05).