Infection of *Aedes* mosquitoes by native *Wolbachia* in urban cemeteries of Southern Mexico

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Abstract

Objective. To evaluate the prevalence of *Wolbachia* infections in *Aedes spp.* field populations from cemeteries of Southern Mexico. **Materials and methods.** Six cemeteries were selected to be sampled in the central part of the Soconusco region, Chiapas. *Aedes albopictus* and *Ae. aegypti* mosquitoes were collected during the rainy season of 2015. Females were analyzed individually by PCR to determine the presence of *Wolbachia*. **Results.** A field overall prevalence of 38% was found; only *Ae. albopictus* mosquitoes were positive. **Conclusion.** Local strains of *Wolbachia* were detected and have the potential to be applied as a biological method for vector control.

Keywords: *Wolbachia*; *Aedes albopictus*; Soconusco; Mexico

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Resumen

Objetivo. Evaluar la presencia de *Wolbachia* en poblaciones de campo de *Aedes spp*. en cementerios del Sur de México. **Material y métodos.** Se seleccionaron seis cementerios como sitios de colecta para las poblaciones silvestres de *Aedes albopictus* y *Ae. aegypti*, en la región del Soconusco, Chiapas, durante la época de lluvias 2015. Se determinó la infección por *Wolbachia* en hembras individuales por PCR. **Resultados.** Se obtuvo una infección de 38% por *Wolbachia* en *Ae. albopictus*. **Conclusión.** Existen cepas locales de *Wolbachia* en los mosquitos y poseen el potencial de aplicarse como medida de control biológico de vectores.

Palabras clave: *Wolbachia*; *Aedes albopictus*; Soconusco; México

Successful application of *Wolbachia* in insect vector control depends on its ability to invade at high frequency under field conditions. The transfection of *Wolbachia* in mosquitoes can cause resistance for a variety of pathogens.[1,2] In this study, we examined the field infection frequency of native *Wolbachia* in the vector *Aedes* spp. mosquitoes of the Soconusco region, Chiapas, Mexico. *Aedes albopictus* and *Ae. aegypti* mosquitoes were sampled during the rainy season of 2015 (June to November) in six selected cemeteries in the central part of the Soconusco, Chiapas (15°19′ N, 92°44′ W) (figure 1).

Wild females of both species were preserved separately at -20 °C. Larvae and pupae obtained from the breeding sites were maintained until adult emergence;

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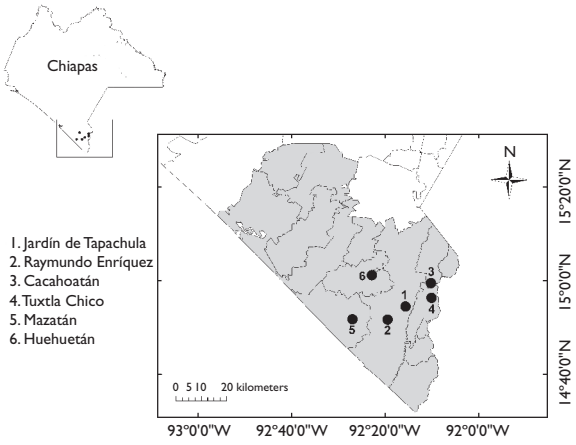

Figure 1. Study area. Cemeteries: 1) Jardín de Tapachula, 2) Raymundo Enríquez, 3) Cacahoatán, 4) Tuxtla Chico, 5) Mazatán, and 6) Huehuetán. These sites were sampled for mosquitoes from May to July, 2015

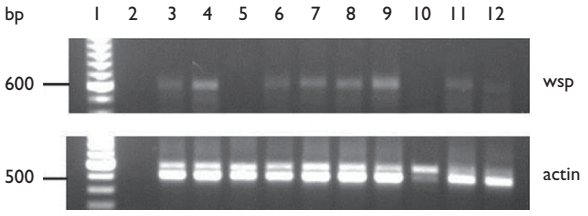

Figure 2. *Wolbachia wsp* gene amplification in mosquitoes *Aedes spp.* Gene *wsp* was amplified by PCR. In the lower panel the actin gene amplification of the same DNA samples is shown as control. 1) 100 bp ladder; 2) negative control; 3) and, 4) *Ae. albopictus* collected using an entomological net (Jardín de Tapachula); 5) *Ae. aegypti* collected using an entomological net (Jardín de Tapachula); 6), 7), 8), 9), and 10) *Ae. albopictus* larvae (Huehuetán); 11), 12) *Ae. albopictus* larvae (Cacahoatán). Collections were performed from May to July, 2015

newly emerged females were preserved as previously described. Genomic DNA was extracted using DNAzol kit and analyzed by PCR to determine the presence of *Wolbachia* by means of amplifying the surface protein gene (*wsp*) reported previously.[3] Integrity of the DNA was analyzed using primers to amplify the actin gene of *Ae. aegypti* and *Ae. albopictus*.[4] To confirm the presence of *Wolbachia*, PCR products from the *wsp* gene were purified using the QIAquick PCR purification kit (QIAGEN) for sequencing. The sequences were used for Blast analysis using the GenBank database (http://blast.ncbi.nlm.nih.gov).

A total of 215 mosquitoes were collected, 49.8% of which were identified as *Ae. aegypti* and 50.2% as *Ae. albopictus*. Adults were collected only in the cemeteries Jardín de Tapachula (13% *Ae. albopictus*, and 56% *Ae. aegypti*), and Raymundo Enríquez (26% *Ae. albopictus*, and 5% *Ae. aegypti*). Larvae and pupae sampling was performed in all cemeteries (55% *Ae. albopictus* and 45% *Ae. aegypti*) (table I). Positive samples for *Wolbachia* infection were detected by PCR amplification of a 600 bp fragment corresponding to *wsp* gene (figure 2, upper panel), showing that *Wolbachia* infections only occurred in *Ae. albopictus* (lanes 3, 4, 6, 7, 8, 9, 11, and 12) and is absent in *Ae. aegypti* (lane 5). Not all the *Ae. albopictus* were infected with *Wolbachia* (lane 10). Actin gene was used as quality control for DNA and PCR reactions (figure 2, lower panel).

In general, *Wolbachia* infection rate was 38% in *Ae. albopictus*. *Wolbachia* infection in collected adult mosquitoes from Jardín de Tapachula and Raymundo Enríquez cemeteries was 12.5 and 25%, respectively. In addition, *Wolbachia* infection in mosquitoes from collected larvae was greater in *Ae. albopictus* from Huehuetán cemetery (81%), followed by 32% of infected mosquitoes from Raymundo Enríquez (table I). Sequencing results of three randomly selected PCR amplicons were aligned using Clustal W algorithm. Two samples, named W5 (accession no. KX118690) and W83 (accession no. KX118691) showed 100% identity with *Wolbachia* strain previously reported in *Ae. albopictus*; and W4 (accession no. KX118692) showed 99% identity.

This is the first study in the Soconusco region, Chiapas, that shows the infection of *Wolbachia* in *Ae. albopictus* and its absence in *Ae. aegypti* local populations. This evidence may be relevant from the epidemiological point of view because *Wolbachia* infection in *Ae. albopictus* has different effects in the mosquito life cycle as well as a blocking effect on the development of pathogens, which strengthens the idea of using *Wolbachia* as a biological control strategy for emerging and reemerging arboviruses in the country.

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Table I

WOLBACHIA INFECTION RATES OF *AEDES SPP.* MOSQUITOES. LOCAL POPULATIONS OF *AEDES AEGYPTI* AND *AE. ALBOPICTUS* WERE SAMPLED IN THE SOCONUSCO REGION, CHIAPAS, AND ANALYZED FOR THE PRESENCE OF *WOLBACHIA* THROUGH AMPLIFICATION OF *WSP* GENE. ADULT MOSQUITOES WERE BOTH CAPTURED AS ADULTS OR AS LARVAE AND GROWN TO ADULTS DURING THE RAINY SEASON (MAY TO JULY) OF 2015

Cementery	*Individuals*	*Ae. aegypti*			*Ae. albopictus*		
		Analyzed	*wsp (+)*	*% Infection*	*Analyzed*	*wsp (+)*	*% Infection*
Jardín de Tapachula	45	36	0	0	9	1	11.1
Raymundo Enríquez	47	3	0	0	44	13	29.5
Huehuetán	33	2	0	0	31	25	80.6
Mazatán	63	62	0	0	1	0	0.0
Cacahoatán	16	2	0	0	14	2	14.3
Tuxtla Chico	11	2	0	0	9	0	0.0
Total	215	107	0	0	108	41	37.9

Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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