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PANORAMA EPIDEMIOLÓGICO DE ARBOVIRUS Y SUS VECTORES EN SAN LUIS POTOSÍ 2021-2022

TESIS QUE PRESENTA

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PARA OBTENER EL GRADO DE MAESTRA

EN CIENCIAS BIOMÉDICAS BÁSICAS

CODIRECTORES DE TESIS

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Molecular survey of mosquito populations and arbovirus in San Luis Potosí Mexico, 2021.

Running title: Mosquito and arbovirus surveillance in Mexico

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ABSTRACT

Medically relevant arboviruses can be transmitted by *Anophelinae* (*Anopheles* genus) or *Culicinae* (*Aedes* and *Culex* genera) mosquitos. Ecological and socio-demographic factors such as urbanization, poverty, access to health systems and social inequality determine vector density and risk of disease transmission. Effective surveillance of vectors and arboviruses in "at risk" areas are crucial for guiding public health strategies. We developed a low-cost molecular approach to assess mosquito and arbovirus prevalence in the city of San Luis Potosí, Mexico in 2021. Our results provide evidence of the centripetal expansion of mosquito populations originating in city outskirts post-rainy season. *Culex* was the most abundant genus (63.3%) followed by *Aedes* (26.6%) and *Anopheles* (4.7%). DENV was detected in mosquitoes seven weeks before the first local human report, highlighting the epidemiological utility of this strategy. Four different arboviruses were identified in FTA cards: DENV (6.5%), ZIKV (5.6%), CHIKV (1.6%) and WNV (3.2%).

Keywords: Arbovirus, Mosquito, Vector, Aedes, Anopheles, Culex, Dengue, Zika, Chikungunya

INTRODUCTION

Mosquito-borne diseases (MBD) are transmitted by members of the Culicidae family comprising 43 genera and 3,500 species of mosquitos classified into the *Anophelinae* (*Anopheles*) and *Culicinae* (*Aedes* and *Culex*) subfamilies (1). Malaria is the most important MBD, causing 249 million estimated cases during 2022 and 608,000 deaths in 85 countries, of which approximately 94% (233 million) of the cases and 95% (580,000) of deaths occurred in the WHO African Region (2). The *Anopheles* genus includes 460 species, of which more than 100 can transmit *Plasmodium falciparum*, the etiologic agent of malaria (3). The *Aedes* genus contains several subgenera (*Aedes, Stegomyia*, etc.) and over 700 species (*aegypti, albopictus,* etc.) (4). The *Culex* genus includes over 20 subgenera and more than 1,000 species (*annulirostris, erythrothorax,* etc.) (5). Arboviruses (**ar**thropod-**bo**rne **viruses**) are a diverse group of viruses transmitted through insect bites (mosquitoes, ticks, sand flies and midges). After mosquitoes ingest a blood meal from an infected host, arboviruses multiply in the insect's mid-gut (extrinsic

incubation period), resulting in high viral titers in the salivary gland which are then passed on to humans, where viral replication continues (intrinsic incubation). Most arboviral diseases are zoonotic, infections of animal vertebrates that can cause infection and disease in humans (6). The three main mosquito genera, *Anopheles*, *Aedes*, and *Culex*, can transmit Arboviruses (7). Dengue virus (DENV) is a flavivirus represented by four distinct serotypes transmitted by *Aedes* mosquitoes. It is the predominant arbovirus affecting humans, with 3.6 billion people at risk of transmission and hundreds of million clinical cases reported each year (8). Zika virus (ZIKV) is a flavivirus transmitted by *Aedes aegypti* and *Ae. albopictus* mosquitoes with ongoing epidemics in Latin America and the Pacific and currently perceived as one of the most serious public health threats (9,10). Chikungunya virus (CHIKV) is an Alphavirus transmitted by *Aedes albopictus* and *Aedes aegypti* which typically occurs in Africa and Asia but also affecting Europe and America since the year 2000 (11). West Nile virus (WNV) is, along with Dengue, Zika, Rift Valley fever, yellow fever, and Japanese encephalitis viruses, a Flavivirus that causes severe neurological disease in humans and horses of Africa, Europe, the Middle East, North America, and West Asia (12). A comprehensive list of the different mosquitoes of medical importance is provided in Appendix A. In 2021, 1.3 million cases of mosquito bite associated fever occurred in Latin America, 88.6% of which were due to Dengue, 10% to Chikungunya, and 1.4% to Zika. During 2021, 6,746 human cases of Dengue were reported in Mexico, as well as 35 cases of Zika, 4 of Chikungunya, and none of West Nile Virus. Only 80 cases of Dengue occurred in the state of San Luis Potosi during that same year (13). Several environmental factors determine the survival and reproduction of mosquitoes, including temperature, humidity, and precipitation (14,15). Socio-demographic factors such as urbanization, poverty, access to health systems, and social inequality determine vector density and the risk of disease transmission (16–18). Impoverished rural communities have long been known to be at higher risk of MBD, given that unfavorable socioeconomic conditions converge with mosquito-favorable ecological conditions to cause disease (19). In addition, human factors such as diet, pregnancy, metabolic diseases, genetics, skin microbiota and human volatile footprint (lactic acid, CO_2, ammonium, etc.) modulate mosquito attractiveness (20). Mexico's current MBD surveillance system is largely based on the detection of human cases with limited monitoring of mosquito larvae in endemic regions (21). This approach precludes the implementation of timely vector control strategies, early identification of circulating arbovirus, and prompt risk communication, management, and

response. Our research group addressed these challenges and set forth to develop a molecular mosquito and arbovirus surveillance strategy specially adapted for resource-limited and remote settings. This system relies on locally designed, adult mosquito, passive traps coupled to an ambient-temperature viral RNA preservation approach to provide PCR based diagnosis of mosquito genus and arbovirus. This manuscript summarizes the results of applying this system in the city of San Luis Potosí Mexico during 2021.

MATERIALS AND METHODS

Yoy mosquito trap

A passive adult mosquito trap known as the “Yoy trap” (after the Tenek indian word for mosquito) was developed locally incorporating several design criteria (22–27). The trap is constructed from generic, recycled PET water bottles and tubing, and exploits female hematophagous mosquito chemical cues such as a dry-ice based CO_2 generator, a chemical attractant, and a source of humidity to lure and retain live mosquitoes (28–30). The trap incorporates a honey-soaked food-coloring impregnated FTA® card to provide an alternate food source for trapped mosquitoes. Such cards allow mosquitoes to survive in captivity for more than 72 hours and preserve regurgitated viral RNA for up to 1 week at ambient temperature (31,32). By extending captive mosquito survival, the number of mosquitoes probing the FTA card in search of food and depositing viral RNA due to regurgitation increases. The mosquito collection performance of this trap was either comparable or surpassed that of commonly used mosquito collection traps tested (CDC light trap, SUNA, SMART, etc.) (33–35).

Mosquito collection sites

The metropolitan area of the city of San Luis Potosí is located near the geographic center of Mexico (22°9′4″N 100°58′34″W) at an altitude of 1,864 meters and has a population of 1’221,526 inhabitants (See figure 1). Ten collection sites were chosen to sample city outskirts, suburban and urban locations, see Table 1. The city of San Luis Potosí has at least ten large water reservoirs capable of hosting mosquito populations, seven of which are year-round permanent bodies of water (San Jose dam, Cañada de Lobo dam, Mexquitic dam, San Antonio dam,

Tangamanga Park lake, Tenorio basin and Rio Españita brook), four exhibit seasonal variations of water level (IMMSA basin, Tangamanga Park basin, Rio Santiago brook, Rio Santiago basin). Only two bodies of water exhibit a year-round steady water flow (San Jose dam and Rio Españita). Rio Españita brook is fed by Cañada de Lobos dam. IMMSA basin is a metallurgy-polluted basin uninhabited by mosquitoes. Single 10-liter Yoy mosquito traps were placed at each sampling site except at San Jose dam which was sampled with two traps.

Mosquito collection

Mosquito collections were conducted weekly from epidemiological week (EW) 16 to 23 and every fortnight from EW 25 to 46 of 2021. Traps were deployed on Mondays by 17:00 hours and retrieved on Tuesdays by 08:00 hours. Traps were sealed before transport and transported to our laboratory where they remained in a biocontained and secluded area under natural daylight cycles with an additional humidity supply for 48 hours. After 48 hours, mosquitoes were euthanized by placing the trap in a -80 °C freezer for 15 minutes.

Assessment of mosquito feed rates and mosquito counts

The total number of mosquitos captured per trap was counted manually. The percent feed rate was assessed for each trap and sampling date through stereomicroscope observation of mosquitos with visible abdominal green food-coloring for a representative sample of 100 collected mosquitos per trap.

Mosquito genomic DNA extraction

Genomic DNA from individual mosquitoes per trap was extracted using the phenol-chloroform-isoamyl alcohol (PCI) method (36). After discarding mosquito abdomens, the remains were homogenized in 25 µl of cell lysis buffer (50 mM Tris, 5 mM EDTA, 100 mM NaCl and 1% SDS, pH 8.0) using a glass rod in a 1.5 mL microcentrifuge tube, supplemented with of 12.5 µl of Proteinase K (10 mg/ml) followed by 437 µl of lysis buffer and vortexed. Homogenate was centrifuged at 15,000 G for 30 sec, incubated for 1 hr at 55°C at 350 rpm, supplied with 500 µl

of PCI, incubated for 10 min at 25 °C at 550 rpm, then centrifuged at 15,000 G for 5 min and the upper phase ultimately transferred to a new tube. PCI extraction of this supernatant was then repeated and transferred to a new microtube and precipitated with 14.5 µl of 5M NaCl (for a final concentration of 0.2 M) and 700 µl of -20°C 96% ethanol. This solution was left overnight at -20 °C and then centrifuged at 15,000 G for 10 min, the supernatant discarded, and the pellet washed with 70% ethanol, incubated for 30 min at -20°C and then centrifuged at 15,000 G for 5 min. Finally, the supernatant was discarded, and the pellet was allowed to air dry and then resuspended in 50 µl of 10mM Tris-HCl, pH 8.0, for 30 min at 40°C at 350 rpm and kept stored at 4°C until further use.

Mosquito molecular taxonomy

Identification of *Aedes*, *Anopheles,* and *Culex* mosquitoes was performed through a two-reaction endpoint sequence-specific primer PCR (PCR-SSP). The first PCR employed previously published primers (Mosq-F:5'-TgT-gAA-CTg-CAg-gAC-ACAT-3' and Mosq-R: 5'-TAT-gCT-TAA-ATT-CAg-ggg-gT -3') to generate amplicons of 325 bp for *Aedes* mosquitos, a 500 bp for *Anopheles* mosquitos and 400 bp for *Culex* mosquitos (37). A second PCR geared towards better discriminating amplicon sizes of *Culex* and *Aedes* species included locally developed reverse oligonucleotides Aedes-R: 5'-gAg Agg gAg gCA CAC gTA TA-3' and Culex-R: 5'- gTC TTg AAT gTT TTg CCA gC-3' together with Mosq-F to generate a 125 bp amplicon for *Aedes* mosquitos and a 200 bp amplicon for *Culex*. Reaction components included 1x buffer, 2 mM MgCl2, 10 mM dNTPs, 300 mM each primer, 1 IU Taq DNA Polymerase (Vivantis Technologies Sdn. Bhd. Malaysia) in a final reaction volume of 25 µl. Amplification conditions included an initial 94°C for 2 min, followed by 30 cycles of 30 sec at 94°C, 40 sec at 50°C and 45 sec at 72°C and a final step of 5 min at 72°C. Gel electrophoresis was performed on 3% agarose for 90 min at 90 VDC (3.6 V/cm). PCR-based molecular taxonomical assignment was performed on a representative set of 48 mosquitoes per trap for logistical reasons.

Arboviral RNA extraction from honey-impregnated FTA cards

Flinders Technology Associates (FTA) cards recovered from each trap were placed in 1.5 ml microtubes, supplied with 500 µl of 10:1 TE buffer and vortexed (38). Microtube bottoms were

pierced with a sterile hypodermic needle and then placed in a sterile test tube, centrifuged at 3,000 G for 30 min and the eluent recovered. 200 µl of the eluent were placed in a new microtube, 1 ml of TRIzol (TRIzol Thermo Fisher Scientific Inc. Waltham, Massachusetts USA) was added and mixed by pipetting. The remaining eluent was stored at -80° C for future use. This mix was then supplied with 200 µl of chloroform, vortexed and incubated at room temperature for 5 min. This solution was then centrifuged at 15,600 G for 5 min and the upper aqueous phase was transferred to a new microtube to which 350 µl of 100% isopropanol was added to precipitate the RNA for 12 hours at -20°C. Samples were centrifuged at 15,600 G for 5 min and washed with 70% ethanol with DEPC-treated water and the final RNA was then resuspended in 50 µl of DEPC-treated water. Samples were stored at -80°C until further use. In the case of viral RNA extraction from mosquito heads, pools of 10 heads were prepared in 1.5 ml tubes and homogenized with 200 µl PBS and subsequently subjected to the same procedures described above for FTA cards.

Arbovirus vRNA screening through RT-qPCR

Arboviral RNA was detected using an EVA green based RT-qPCR with SCRIPT One-Step RT-qPCR EvaGreenMaster kit (Jena Bioscience, Thuringia, Germany) in individual reactions. CHIKV sequences were detected using CHIKV-F (5´-AAg-CTC-CgC-gTC-CTT-TAC-CAA-3´) and CHIKV-R (5´-CCA-AAT-TgT-CCT-ggT-CTT-CCT-3´) to generate a 209 bp amplicon, DENV with DENV-F (5´-AAg-gAC-TAg-Ag-TTA-KAg-gAg-ACC-C-3´) and DENV-R (5´-ggC-gYT-CTg-TgC-CTg-gAW-TAg-Tg-3´) to generate a 111 bp amplicon, WNV with WNV-F (5´-CAg-ACC-ACg-CTA-Cgg-Cg-3´) and WNV-R (5´-CTA-ggg-CCg-CgT-ggg-3´) to generate a 101 bp amplicon and ZIKV with ZIKV-F (5´-gAg-TgT-gAT-CCA-gCC-gTT-ATT-3´) and ZIKV-R (5´-CAg-CCT-CCA-TgT-gTC-ATT-CT-3´) to generate a 105 bp amplicon (39). The CHIKV RT-qPCR reaction components consisted of 150 nM of each oligonucleotide, 1x ROX, 1x SCRIPT and 3 µl of RNA in a final reaction volume of 10 µl. The DENV and ZIKV components consisted of 100 nM of each oligonucleotide, 1x ROX, 1x SCRIPT and 3 µL of RNA for DENV and 1 µL for ZIKV in a final reaction volume of 10 µL. The WNV RT-qPCR reaction components consisted of 400 nM of the forward and 200 nM of the reverse oligonucleotides, 0.5x ROX, 1x SCRIPT and 3 µL of RNA in a final reaction volume of 10 µL. Thermocycling (Applied Biosystems 7500) conditions

for the four viruses consisted of 50 °C for 15 minutes, 95 °C for 3 minutes, followed by 40 cycles of 95 °C for 10 seconds and 60 °C for 60 seconds. Data was acquired during the annealing step. After amplification, amplicon size was assessed with a dissociation curve spanning from 60 to 95 °C.

Statistical analysis

GraphPad Prism 8.0.1 was used for statistical analysis. For the analyses of two groups showing normality, student's t-test was used treating a $p < 0.05$ as significant. Comparisons of groups having non-parametric data relied on either the Wilcoxon or Kruskal-Wallis test. To assess the normality of the data the D' Agostino & Person, Shapiro-Wilk and Kolmogorov-Smirnov tests were used, if two of the three tests exhibited a $p > 0.05$ the data was considered as having a normal distribution.

RESULTS

During mosquito collections, from April 19 (EW 16) to November 21 (EW 48) of 2021, the city of San Luis Potosí recorded a median daily temperature of 18°C (IQR 14, 22°C), a median daily humidity of 63.6% (IQR 40.8, 81.9%), and an average daily precipitation of 0.13 ± 0.51 cm (detailed weekly weather conditions are available on Appendix B). The rainy season for San Luis Potosí began on the second week of May (EW 19) and lasted until the first week of October (EW 40), see Figure 2. A total of 16,319 mosquitoes were collected, 98% (16,078) of them in the city outskirts. The San Jose dam (city outskirt site) yielded 60.6% (9893) of all mosquitoes collected, while suburban and urban sites represented only 2.8% (243) of the total. Mosquito collections per day were higher during the rainy season than during the dry season (1139 ± 566.4 mosquitoes, vs 332.4 ± 384.8 mosquitoes, p=0.0025). Nearly 65% (10,558) of all mosquitoes were collected from June (EW 25) to September (EW 38), see Figure 3, panel A (appendix C provides detailed weekly mosquito collections). A total of 2804 (17.4%) mosquitoes were subjected to molecular taxonomical classification; 63.3% (1775) were found to be *Culex*, 26.6% (745) *Aedes*, and 4.7% (133) *Anopheles*. Only 5.4% (151) of the mosquitoes collected

failed to yield taxonomical results. On average, 3.33 ± 1.47 µg of total DNA was extracted from individual mosquitoes having an $A^{260/280}$ and $A^{260/230}$ index of 1.95 ± 0.07 and 2.27 ± 0.93, respectively. Non-target animals were trapped on 13 occasions (2 house flies, 1 grasshopper, and 10 land snails). Average weekly genus abundance was 24.9% ± 16.7 for *Aedes*, 4.4% ± 3.9 for *Anopheles* and 65.5 ± 18.7 for *Culex*. *Aedes* abundance was statistically different between dry and wet season (13.8% ± 14.9 vs 30.1% ± 15.4, p=0.0452) as was that of *Anopheles* (1.5% ± 1 vs 5.7% ± 4.1, p=0.0260) and of *Culex* (79.2% ± 14.7 vs. 59.2% ± 17.3, p=0.0261). Interestingly, *Culex* was more abundant during spring (April to May) in comparison to the rest of the year (90.4% ± 3.4 vs. 56.6% ± 12.6, p=<0.0001). The four collection sites located in the city outskirts exhibited similar mosquito population dynamics where *Culex* mosquitoes dominated from April to May (EW 16 to 20), see Figure 4. *Aedes* mosquito population size remained high from the fourth week of May to the second week of August (EW 21 to 31), closely following local rainfall patterns. *Anopheles* mosquitoes exhibited a similar behavior but with fewer numbers. The two suburban collection sites exhibited an increase in mosquito populations after EW 25 and seven weeks after the first rainfall. Arboledas site (which is closer to urbanized areas) exhibited a greater abundance of *Aedes* mosquitoes than the Rivera site (which is closer to rural areas) which had a higher abundance of *Culex* mosquitoes, see Figure 5. The three urban sites failed to yield significant numbers of mosquitoes during the sampling period. However, *Aedes* mosquitoes were the most abundant (between 67% and 78%) genus at urban sites. The abundance of mosquito populations is highly dependent on weather conditions. Therefore, fluctuations in weather patterns play a crucial role in influencing the prevalence and density of mosquitoes.

A total of 124 FTA card samples, as well as 324 pools of mosquito heads, were screened for the presence of arboviral RNA (DENV, ZIKV, CHIKV, and WNV) through RT-qPCR. A total of 21 FTA cards (16.9%) and 2 mosquito-head pool samples (0.62%) tested positive for arboviruses. Eight (6.5%) FTA cards were positive for DENV of which 6 corresponded to city outskirts (two traps were placed on San Jose dam and both were DENV positive), one corresponding to a suburban site, and another one to an urban site. Seven (5.6%) cards and two (0.61%) pools were positive for ZIKV vRNA, all corresponding to city outskirt sites. Two (1.61%) cards were positive for CHIKV vRNA corresponding to a suburban and an outskirt site. Finally, four (3.22%) FTA cards were positive for WNV vRNA, three corresponding to city outskirt

sites and one corresponding to an inner-city site. In all, outskirt sites made up 81% of all arboviral detections, San Jose dam providing 28.6% (6/21) of all arbovirus-positive FTA cards, Rio Españita brook 23.8% (5/21), Tangamanga Park basin 19% (4/21) and CIACYT 9.5% (2/21). Arboledas housing complex contributed to all suburban site arboviral detections comprising 9.5% of the total FTA cards found positive (2/21). Interestingly, the city center also contributed with 9.5% of arboviral detections. DENV was detected in FTA cards between EW 27 to 42, ZIKV from EW 16 to 29, CHIKV from EW 31 to 36 and WNV from EW 27 to 42. Arboviral detections did not correlate with mosquito population dynamics, genus abundance or collection sites (Figures 3 to 5). Based on the feeding ratio of mosquitoes during captivity, we estimate that nearly 3,923 (24.04%) of all collected mosquitoes contributed to the FTA card regurgitate and to the arboviral screening.

DISCUSSION

Local precipitation was the single most important weather variable determining mosquito population density (40). City outskirt sites provided the most informative data on mosquito population size and genus abundance, especially San Jose dam, which is by far the closest and largest perennial water reservoir having clean flowing water as well as stagnant water ponds, lush vegetation, and ample shade (41). That city outskirt sites, and the Rivera housing complex exhibited similar *Culex* genus abundance is likely due to the proximity of this suburban site to agricultural lands and its distance from densely inhabited areas. As is well known, *Culex* mosquitoes prefer natural stagnant water reservoirs and the proximity of the Rivera housing complex to the Tenorio basin (which was not sampled for logistical reasons) might also explain these similarities. The *Aedes* and *Culex* mosquito genus abundance seen in outskirt sites is very similar to previously published frequencies for other Mexican states in which 65% of mosquitoes were *Culex* and 33% *Aedes* (42). While mosquito population density showed an early surge in the city outskirts almost immediately after the first rainfall (April), the density of mosquito populations in suburban and urban sites only increased 8 weeks after the city outskirts surge (by the end of June). Analysis of mosquito population waves for each genus by sampling site revealed that *Aedes* mosquitoes were detected in city outskirts 3 weeks prior to suburban sites and 7 weeks before they were seen in urban sites (see Figure 6). Similarly, *Culex* mosquitoes

were detected in city outskirts 4 weeks prior to suburban sites and between 8 and 12 weeks before urban sites. Although similar, *Anopheles* mosquitos were detected in city outskirts only 2 weeks before being identified in suburban sites and 3 weeks before urban sites.

Our observation of a high prevalence of *Culex* mosquitoes during winter months align with a study conducted in Florida, where a similar dominance of Culex was noted during colder months (43). A variety of *Culex* mosquito overwintering mechanisms has been known to enhance their resilience, enabling them not only to better withstand dry, and cold seasons but to easily adapt to less conspicuous effects of climate change (44). FTA card arboviral screening proved to be a logistically and technically efficient way to assess MBD burden by allowing the screening of between 25% and 50% of all mosquitoes collected (considering variations in feeding rates between traps). The use of FTA cards yielded greater analytical sensitivity at detecting arboviral RNA sequences than mosquito head pools, which were much more laborious to process. The prevalence of ZIKV, CHIKV, and WNV arboviral RNA in FTA cards was unexpected, as no human cases of these diseases have ever been reported in San Luis Potosi city. The 6.5% positivity seen for DENV is higher than that reported previously for the city of Merida, but this likely stems from methodological differences (42,45). The 5.6% positivity seen for ZIKV in our study is perhaps, an underestimation of true positivity, as a previous study carried out in 29 Mexican states reported a 10.6% positivity in mosquito pools by using the high-resolution CDC Trioplex real-time RT-PCR (46). The frequency of the remaining arboviruses (CHIKV and WNV) among mosquitoes has not been established for other Mexican states other than in birds and bats (47,48). Analysis of the temporal appearance of DENV, CHIKV, and WNV arboviral detections showed a gradual progression in which arboviruses were detected first in city outskirts, then in suburban areas and subsequently in the city's interior. As such, DENV was detected by EW27 in an outskirt site, by EW 31 in a suburban site, and by EW 42 in the inner city. CHIKV was first detected in city outskirts by EW 31 and subsequently on suburban sites by EW 36. Finally, WNV was first detected in city outskirts by EW 27 and in the city center by EW 42. Whether this phenomenon represents individual mosquito inward migrations or solely the propagation of arbovirus among mosquitoes of increasingly distant areas (from city outskirts) remains unknown. Although no cases of ZIKV, CHIKV, and WNV have been reported in the city of San Luis Potosi, 6 human cases of DENV infection were reported by the state public health laboratory as of EW 34 (personal communication). Our arbovirus surveillance approach

successfully identified the presence of circulating DENV in local mosquitoes seven weeks before human cases manifested in the same city. This underscores the effectiveness of comprehensive molecular mosquito and arbovirus surveillance strategies, as outlined in this study, in furnishing real-time, high-resolution information. Such data is invaluable for guiding proactive public health measures and ensuring the effective management of emerging infectious diseases transmitted by mosquitoes.

CONCLUSION

The main objective of this study was to assess the reliability of an integrated vector and arboviral detection strategy based on robust molecular methods to serve as a public health guidance tool for assessing MBD risk and, ultimately, for the surveillance of MBD in resource-limited settings. In addition, our results provide an insight into the mosquito population dynamics as well as evidence of the circulation of four arboviral species in the city of San Luis Potosí. An ever-increasing human population is likely to require more natural resources in the coming decades, further accelerating climatic change and disturbing natural habitats, all of which are likely to increase the frequency, severity, and distribution of MBD. Realtime mosquito and arboviral surveillance through tools such as that reported are likely to represent a major scientific contribution to public health capable of positively impacting the lives of the world’s most vulnerable and neglected populations.

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Tables

Table 1. Collection site information

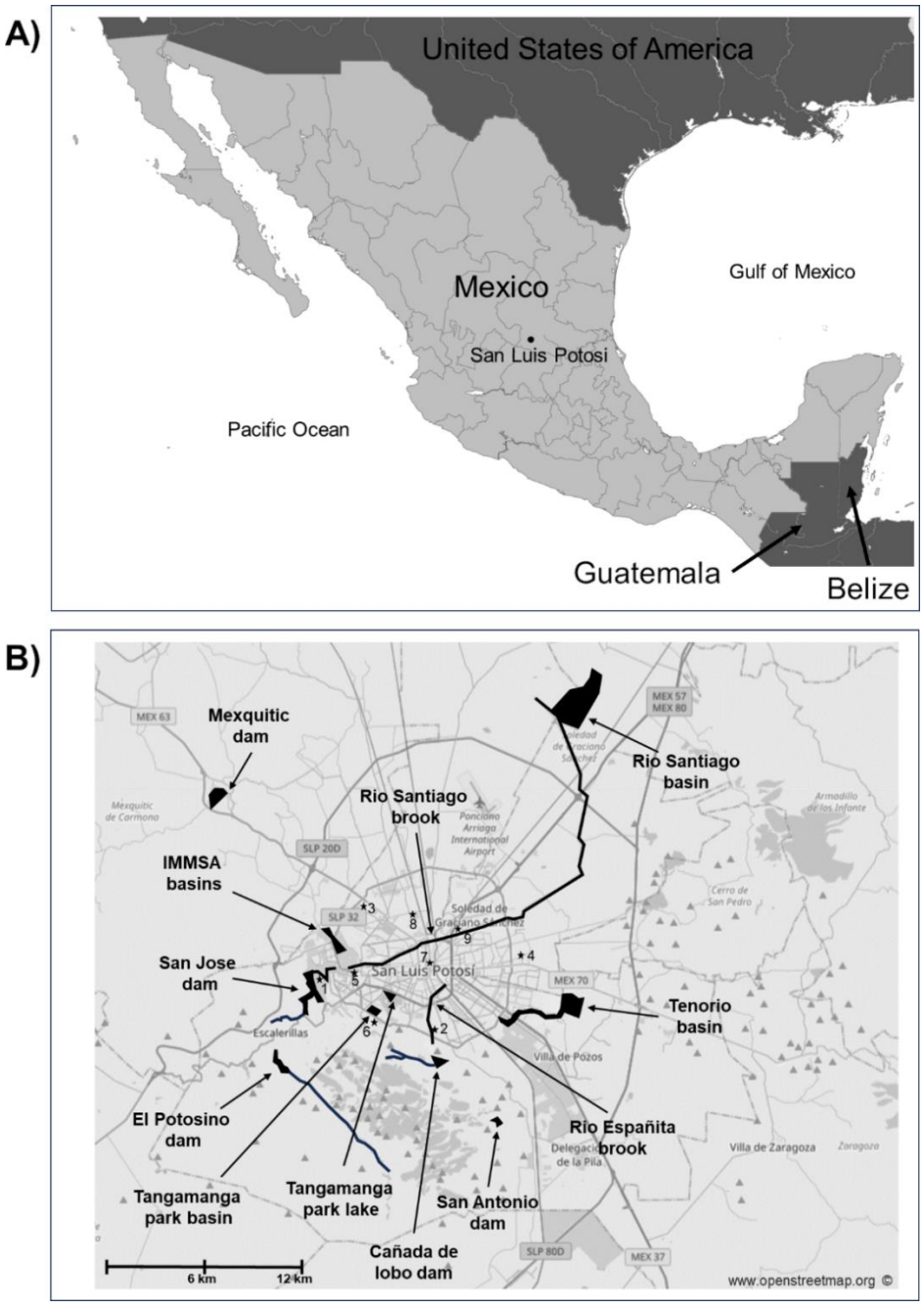
Site	Type	Collection site location	Flowing water	Stagnant water	Vegetation	Shade
1	City outskirts	San Jose dam	Abundant	Abundant	Abundant	Abundant
2	City outskirts	CIACYT	None	Moderate	Abundant	Abundant
3	City outskirts	Tangamanga Park basin	None	Abundant	Abundant	Some
4	City outskirts	Rio Españita brook	Moderate	Abundant	Abundant	Moderate
5	Suburban	Rivera housing complex	Moderate	Moderate	Garden	Some
6	Suburban	Arboledas housing complex	None	None	Garden	None
7	Urban	City center	None	None	Flowerpots	Moderate
8	Urban	FOVISSSTE housing complex	None	None	Flowerpots	None
9	Urban	Pavon housing complex	None	None	Flowerpots	None

Table 2. Arboviral RNA detection by epidemiological week (EW) and collection site

Arbovirus	Date		Site type	Site	Sample	Mosquitos	Abundant	Feed rate
DENV	EW27	July 6	City outskirts	Rio Españita brook	FTA	6		50%
	EW29	July 20	City outskirts	CIACYT	FTA	322	Aedes	26%
	EW31	August 2	City outskirts	CIACYT	FTA	336	Aedes	30%
			City outskirts	San Jose dam	FTA	1173	Culex	15%
			City outskirts	Rio Españita brook	FTA	37	Culex	54%
			Suburban	Arboledas HC	FTA	2	Aedes	50%
	Ew42	October 19	Inner city	City center	FTA	2	Aedes	50%
ZIKV	EW16	April 19	City outskirts	Tangamanga Park	FTA	3	Aedes	67%
	EW17	April 26	City outskirts	San Jose dam	FTA	315	Culex	21%
	EW20	May 17	City outskirts	Rio Españita brook	FTA	16	Culex	13%
	EW21	May 24	City outskirts	San Jose dam	FTA	609	Culex	20%
	EW23	June 7	City outskirts	San Jose dam	FTA	354	Culex	28%
			City outskirts	Tangamanga Park	Pool	14	Aedes	50%
	EW25	June 21	City outskirts	San Jose dam	FTA/Pool	1104	Culex	26%
	EW29	July 20	City outskirts	Tangamanga Park	FTA	336	Aedes	46%
CHIKV	EW31	August 2	City outskirts	Rio Españita brook	FTA	37	Culex	54%
	EW36	September 6	Suburban	Arboledas HC	FTA	26	Culex	50%
WNV	EW27	July 6	City outskirts	Tangamanga Park	FTA	72	Aedes	46%
	EW31	August 2	City outskirts	San Jose dam	FTA	1173	Culex	15%
	EW36	September 6	City outskirts	Rio Españita brook	FTA	35	Culex	20%
	EW42	October 19	Inner city	City center	FTA	2	Aedes	50%

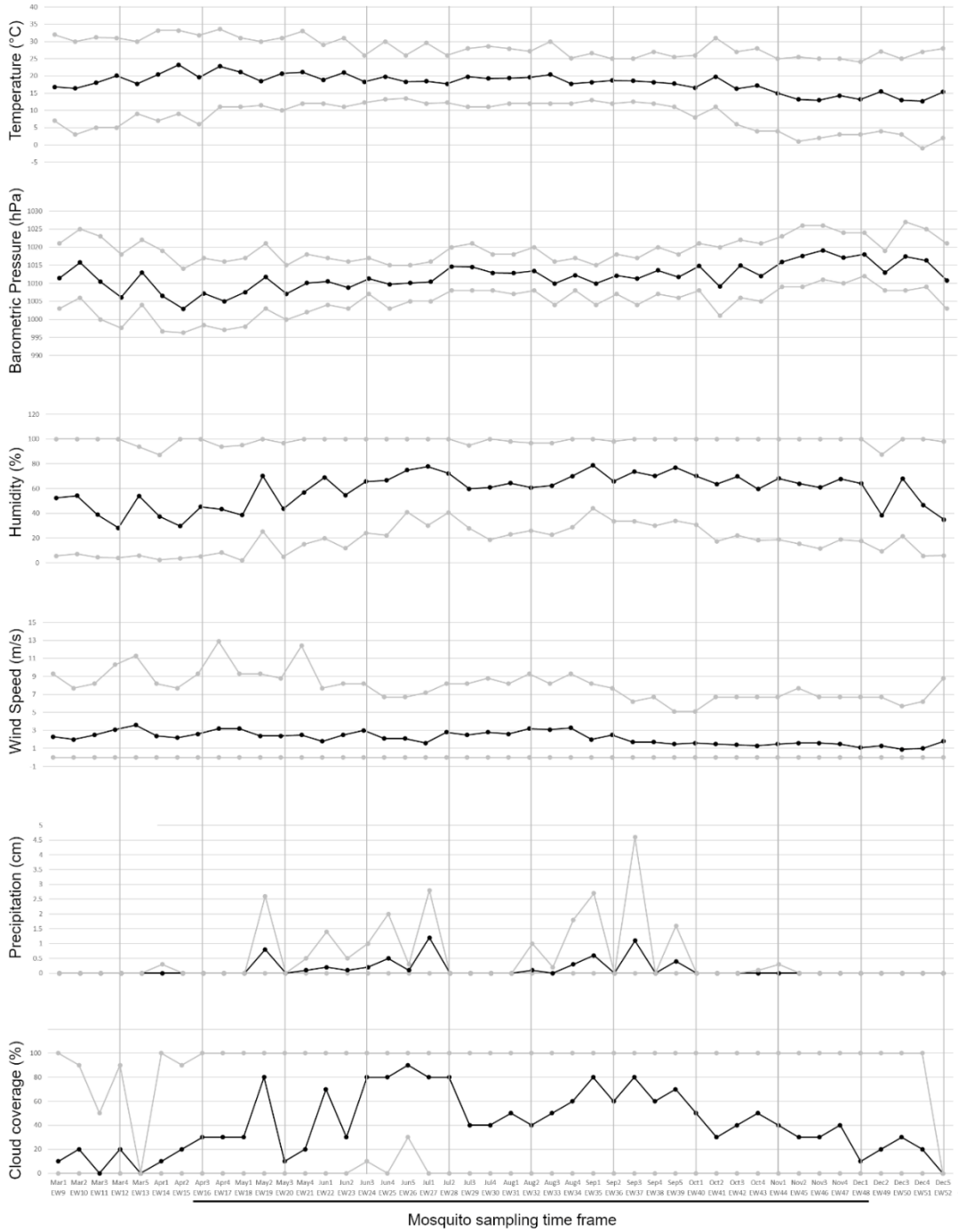
Figures

Figure 1. Geographic location, main water features and mosquito collection sites of San Luis Potosí.



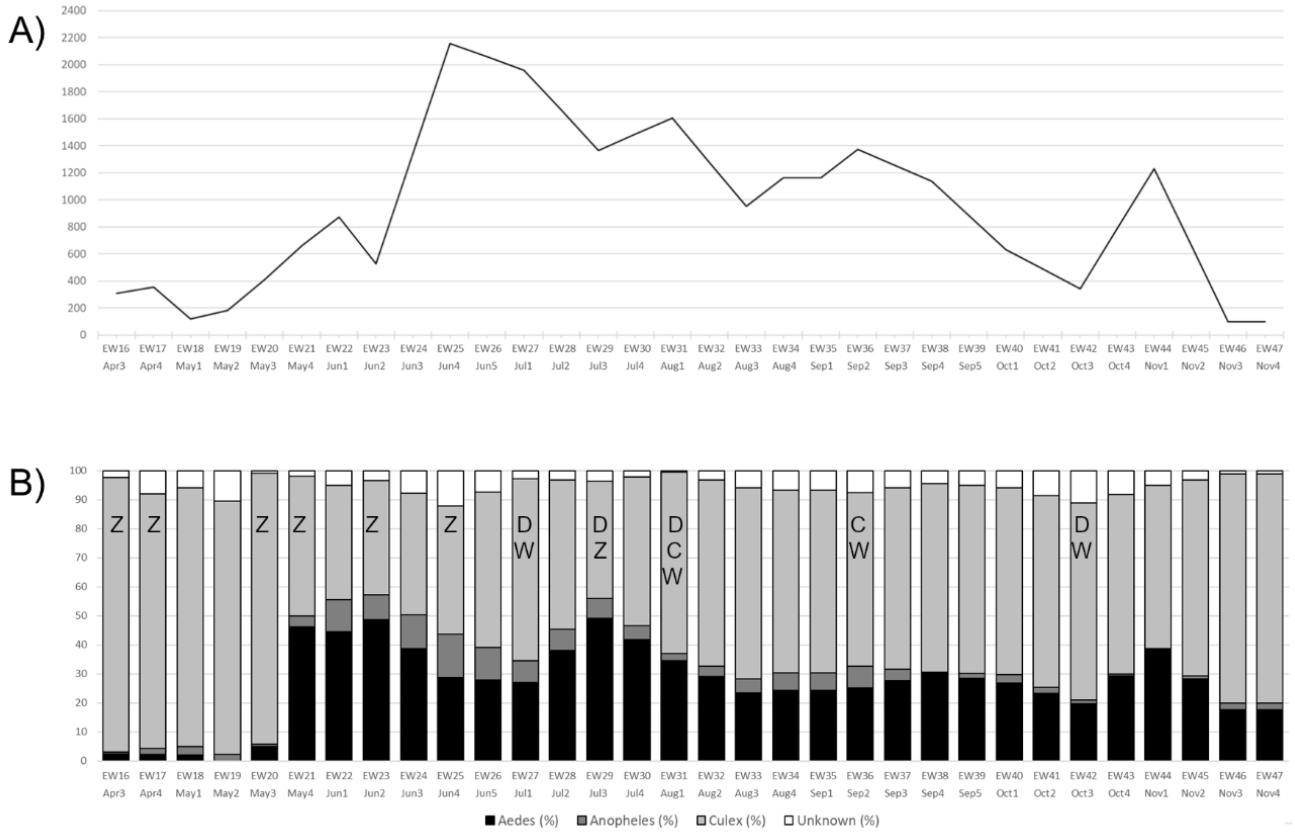
San Luis Potosi is located near the geographical center of Mexico (panel A). Mosquito collection sites (numbered stars) included city outskirt (1 through 4), suburban (5 and 6) and urban (7 through 8) locations.

Figure 2. Weather variables for San Luis Potosí during 2021.



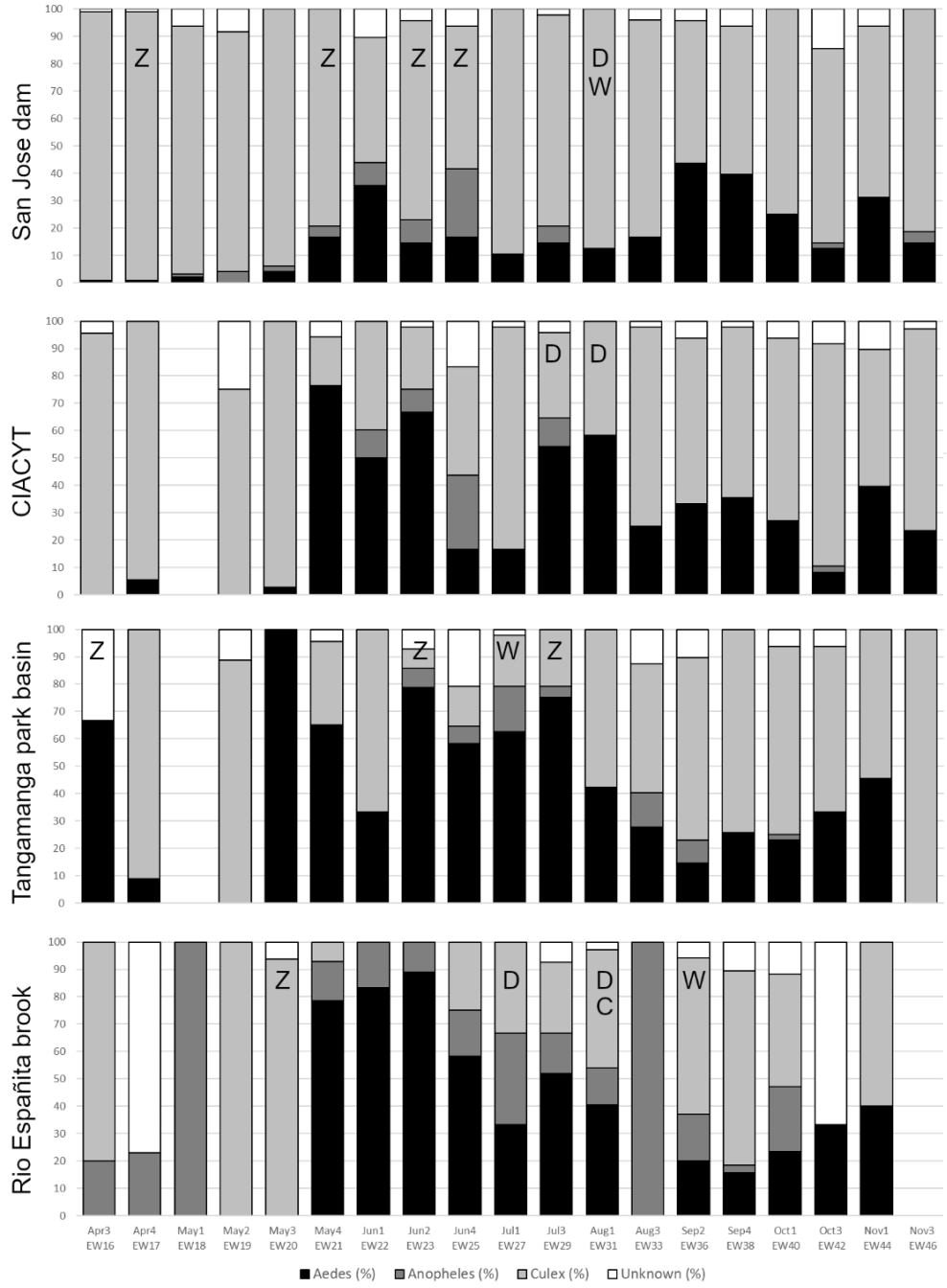
The black histogram line corresponds to average, grey lines indicate minimum and maximum values, black line below dates on horizontal axis corresponds to the time during which mosquitos were collected.

Figure 3. Total mosquito collections (A) and genus abundance (B) in all collection sites. San Luis Potosi, Mexico 2021.



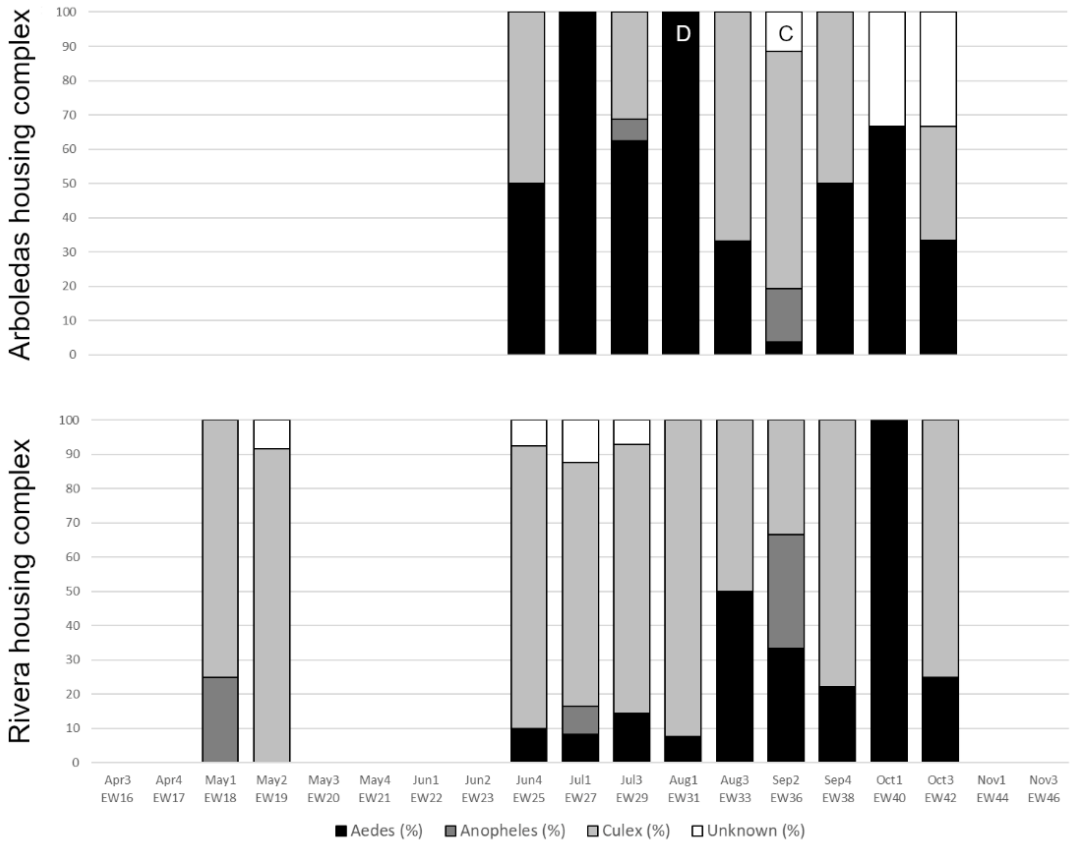
Panel A depicts mosquito numbers collected from all sites; Panel B depicts mosquito genus abundance as percentage. Arboviral RNA sequences detected in this study are shown as D (DENV), Z (Zika), C (Chikungunya) and W (West Nile Virus) for their corresponding epidemiological week.

Figure 4. Mosquito genus abundance in city outskirts. San Luis Potosí, Mexico 2021.



Percent mosquito genus abundance per week for each of the four city outskirt collection sites. Arboviral detections corresponding to each specific site is indicated as D=DENV, Z=ZIKV, C=CHIKV and W=WNV

Figure 5. Mosquito genus abundance in suburban San Luis Potosí, Mexico 2021.



Percent mosquito genus abundance per week for the two suburban collection sites. Arboviral detections corresponding to each specific site are indicated as D=DENV and C=CHIKV.

Figure 6. Mosquito population wave off-set observed between city ouutskirt, suburban and urban sites of San Luis Potosi Mexico during 2021.