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ADDRESSING THE WEST NILE VIRUS HEALTH CRISIS: SURVEILLANCE AND INTEGRATED VECTOR MANAGEMENT IN SPAIN

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Abstract West Nile Virus (WNV) is an emerging global health threat, with increasing incidence across Europe. In Spain, WNV has been endemic since 2004, with most human cases in the southwestern region. In 2024, the largest outbreak to date occurred, with around 138 confirmed cases and fifteen fatalities, primarily in small urban centers. In response, authorities rapidly launched emergency measures at the beginning of the summer. We conducted a comprehensive study to target both adult and immature stages across six municipalities (around 270.00 inhabitants) in southern Spain, where ca. 1/3 of all Spanish WNV cases were reported. A total of 729 sampling sites were identified and regularly visited over four months, involving a total sampling effort of 4,026 visits. Adult mosquitoes were monitored using suction traps, while immature stages were sampled using dipping techniques. Eleven mosquito species were identified, with high abundance of two primary urban WNV vectors: *Culex perexiguus* and *Culex pipiens*. The former was the most abundant based on adult collection (47.1%) and the latter by examination of larvae (90.8%). These mosquitoes were found breeding in 136 sites (18.6% of the total sites) with diverse waterlogged habitats, particularly rice fields, ditches, and canals near urban zones. A total of 190 blood-fed *Culex* mosquitoes were collected and analyzed to determine their host-feeding preferences. Our multi-strategy control approach targeted immature mosquitoes using *Bacillus thuringiensis* var. *israelensis* (*Bti*) and adult mosquitoes using cypermethrin-based adulticides at 138 positive points resulting in 266 control interventions. We assessed the effectiveness of the control measures by comparing the number of larvae before and after larviciding, and we found that *Bti* successfully eliminated and/or reduced immature stages in 80% of the treated sites; however, mosquito populations quickly rebounded if the interventions were not maintained constantly. Recommendations for future actions should include an earlier start of monitoring/control actions in spring season (triggered by authorities) and enhanced monitoring to better evaluate the effectiveness of integrated vector control strategies. We emphasize the critical role of specialized control companies in rapid, coordinated responses to vector-borne disease outbreaks and highlight the importance of timely interventions and mitigate public health risks.

Key words Mosquito control, *Culex* species, surveillance, Public Health outbreaks

INTRODUCTION

West Nile Virus (WNV) is a mosquito-borne flavivirus that has emerged as a significant global public health concern. Since its first identification in Uganda in 1937, WNV has expanded its geographical range, causing outbreaks across Africa, the Middle East, Europe, and the Americas (Kramer et al. 2019). The virus primarily cycles between mosquitoes and birds but can infect humans and other mammals, leading to a spectrum of clinical manifestations from mild febrile illness to severe neuroinvasive disease (Constant et al. 2022).

In Europe, WNV has become increasingly prevalent, with notable outbreaks reported in countries such as Greece, Italy, and Serbia. Spain has also experienced a rise in WNV cases, particularly in southern Spain. In 2020, Spain reported 77 human cases, and eight fatal attributed to WNV, marking a significant increase compared to previous years (Figuerola et al. 2022). However, in 2024 human WNV cases reached unexpected higher levels with up to 155 cases and 15 deaths, most of these cases concentrated in urban areas of different municipalities within the province of Seville, an area which features extensive water-flooded habitats (i.e. rice fields) that provide ideal breeding habitats for mosquito vectors such as *Culex pipiens* and *Culex perexiguus* (González et al. 2024). The escalation of WNV cases in Spain has heightened public health concerns and underscored the critical need for proactive surveillance and effective integrated control measures.

Recent studies highlight the importance of early-season surveillance, targeted larviciding, and the application of adult mosquito control measures to interrupt transmission cycles and reduce public health risks (ECDC, 2012). Regular surveillance provides essential insights into vector ecology, including the abundance, species composition, and seasonal dynamics of primary vectors. This information supports risk assessment by identifying high-risk areas and times, enabling the prioritization of resources and targeted interventions. On the other hand, Integrated Vector Management (IVM) is a comprehensive approach that combines multiple control methods, including environmental management, biological control, chemical interventions, and public education, to reduce mosquito populations and interrupt disease transmission. Implementing IVM requires coordinated efforts among public health authorities, entomologists, ecologists, and the community.

Recent studies have highlighted the effectiveness of various vector control methods in preventing and controlling WNV outbreaks. For instance, the use of larvicides in breeding habitats and adulticides during peak mosquito activity periods has been shown to significantly reduce mosquito populations (Bellini et al. 2014; González et al. 2024). In Spain, the implementation of integrated control programs has shown promise, particularly in addressing outbreaks in urban and agricultural areas. For instance, larvicidal treatments using *Bacillus thuringiensis var. israelensis* (Bti) and pyrethroid barriers around residential zones have demonstrated effectiveness in reducing vector populations during peak transmission seasons (García San Miguel et al. 2020).

Therefore, adaptive and proactive vector management strategies, grounded in scientific evidence and community engagement, are essential to mitigate the impact of WNV and safeguard public health. This study underscores the critical importance of strengthening WNV surveillance and response systems in Spain. By combining entomological monitoring, molecular analysis of mosquito feeding preferences, and integrated control measures, we aim to provide insights into the dynamics of WNV transmission and offer practical recommendations for future vector-borne disease management. This integrative approach is essential not only for mitigating the immediate risks posed by WNV but also for building resilience against other emerging vector-borne diseases in the face of global environmental changes.

MATERIAL AND METHODS

Study area. This study is part of a service implemented by Athisa Medio Ambiente (Grupo SASTI) under a contract with the public administration of Andalusia (southern Spain). The contract was initiated in response to a significant increase in human WNV cases during the summer of 2024 and become operational in August. The study area is located in the Bajo del Guadalquivir district (Sevilla, southern Spain) and encompass six major municipalities: Dos Hermanas (DH), Las Cabezas de San Juan (LC), Utrera (UT), Los Palacios y Villafranca (PV), Alcala de Guadaira (AG), and Lebrija (LE), with a combined population of around 270.000 inhabitants (**Figure 1**). Together, these municipalities reported a total of 42 WNV cases in 2024 (representing about one-third of all cases in Spain). In accordance with protocols established by local authorities, our company participated in the surveillance and control efforts in areas surrounding the urban zones of the selected municipalities.

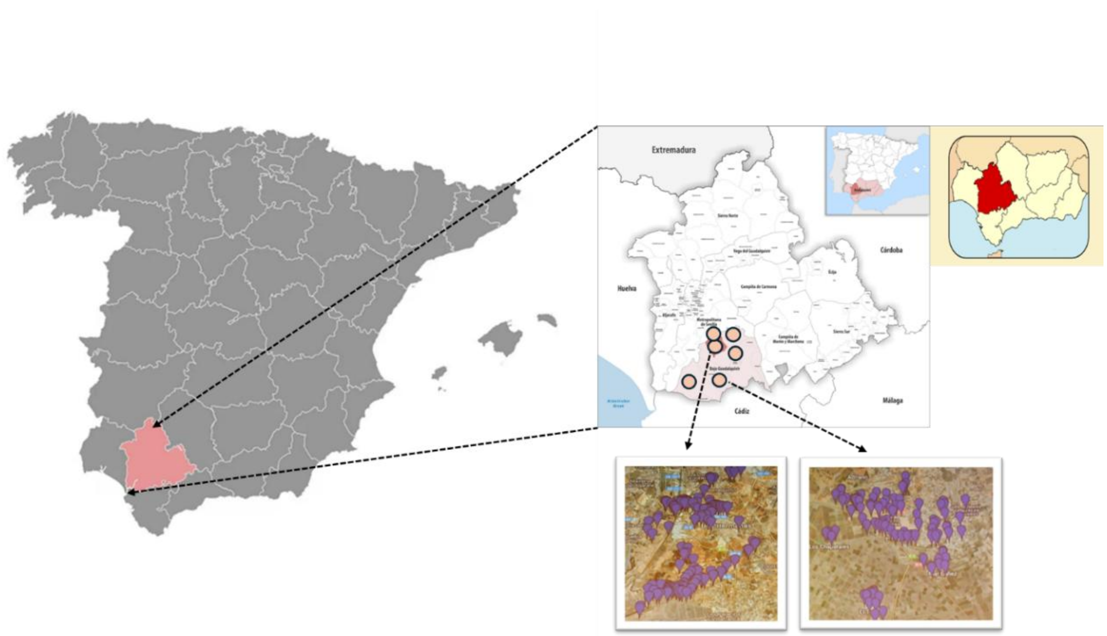

Figure 1. Map of the study area. On the left, the map of Spain highlights the province of Sevilla in southern Spain. On the right, the Bajo del Guadalquivir district with pink circles denoting the six study areas (municipalities), which is an extensive area of rice fields and agriculture fields severely affected by WNV cases.

Surveillance approaches. Surveillance activities included the collection of mosquitoes at both adult and immature stages. Prior to initiating fieldwork, the selected areas were mapped using QGIS, and potential mosquito breeding and high-risk zones were identified. Subsequently, a minimum of two teams surveyed these zones to characterize mosquito breeding sites. Immature stages were collected using a standard dipping method (10 dips per habitat = ca. 4-liter volume), with data georeferenced and plotted using IGEO software. These areas were visited periodically (twice per month). Larval samples were preserved in ethanol and transported to the laboratory for further analysis. To monitor adult mosquito populations, four to six permanent BG-Sentinel traps baited with odor lures were installed in urban areas, with insect content being replaced weekly. Bothoth adult and larval specimens were identified using taxonomic keys (Becker et al. 2020). This comprehensive approach allowed for the identification and characterization of mosquito populations in the region, providing crucial data for targeted control measures.

Control approaches. At breeding sites, habitats containing mosquito aquatic stages were treated with *Bti* (Aquabac 200G and/or Vectobac 12 AS) applied either manually (for small areas), by vehicle-mounted sprayer cannon (for large areas), or by drones (for inaccessible sites). Vegetated areas with high adult mosquito densities were treated with pyrethroid based-adulticides (Massocide® Ciper 100 and/or FORTEX NEXT) applied by sprayer cannons. All treatments adhered strictly to the corresponding safety and operational protocols.

Treatment assessment. Treatment success was evaluated by comparing the number of larvae within the same points, before and after interventions (R Core Team 2020).

RESULTS

Surveillance allowed us to characterize both adult and immature stages fauna of mosquitoes (eleven species in total) in the critical area of WNV outbreaks.

A total of 729 potential breeding sites (AG =116, DH = 146, LC = 64, LE = 86, PV = 102, UT = 215) (4,026 of trapping effort) were inspected over almost four months (06/08/2024 to 15/11/2024) of intensive effort. A total of 136 different points (266 visits) were positive to immature stages of mosquitoes (729/136 = 18.6%). We estimated the abundance and species richness from 132 sub-samples (which represents ca. 50% of the total sampling size) and accounted for 44,761 specimens and nine mosquito species (**Figure 2**). Among them, *Culex pipiens* (90.8%, frequency of collections: 41.4%) was the dominated species in all the habitat categories (n = 7) except rice fields, where *Cx. perexiguus* was predominant (**Figure 3**). The most productive larval sites were ditches/canals that accounted for more than half of the total mosquito collections (estimation, n = 24,574) (**Figure 4**).

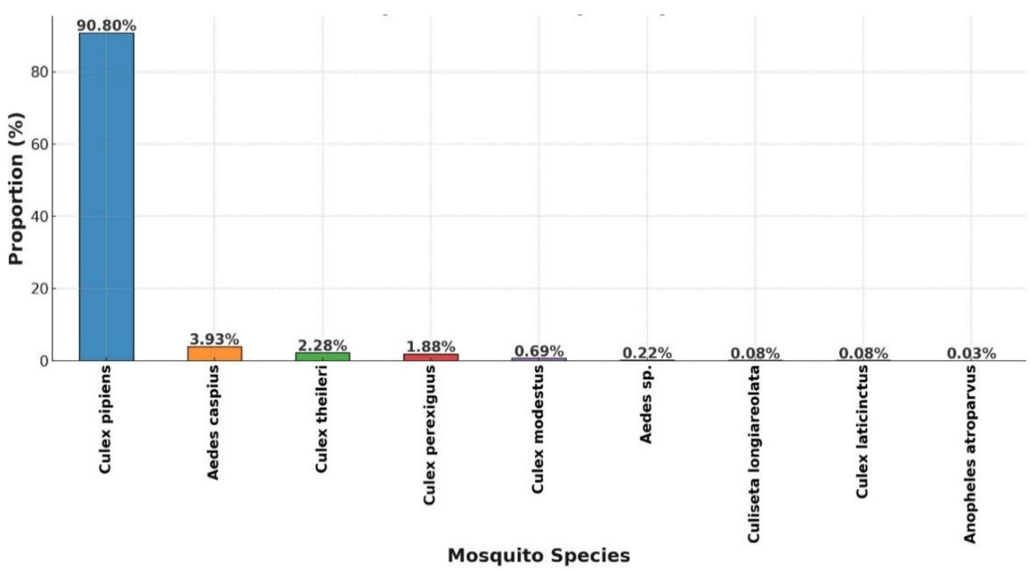

Figure 2. Proportion of mosquito species identified based on immature stages.

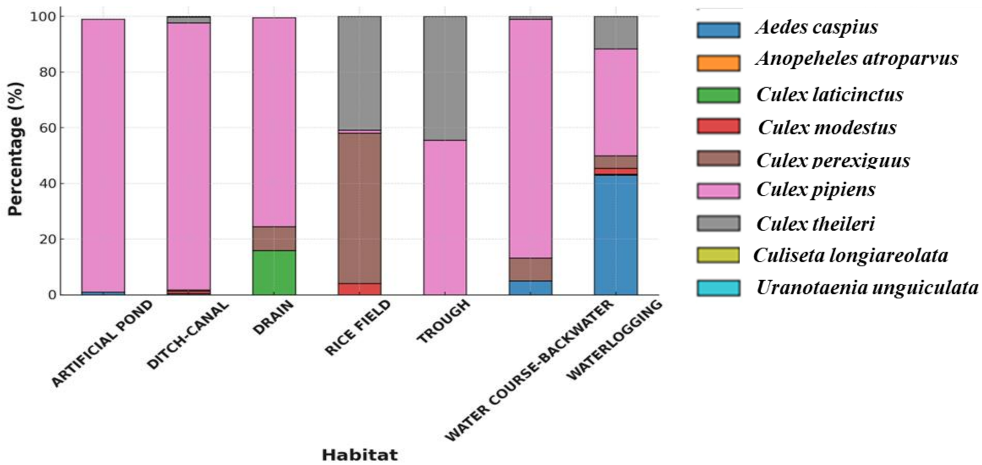

Figure 3. Immature mosquito abundance at seven different habitat categories in the study area (Sevilla province, southern Spain).

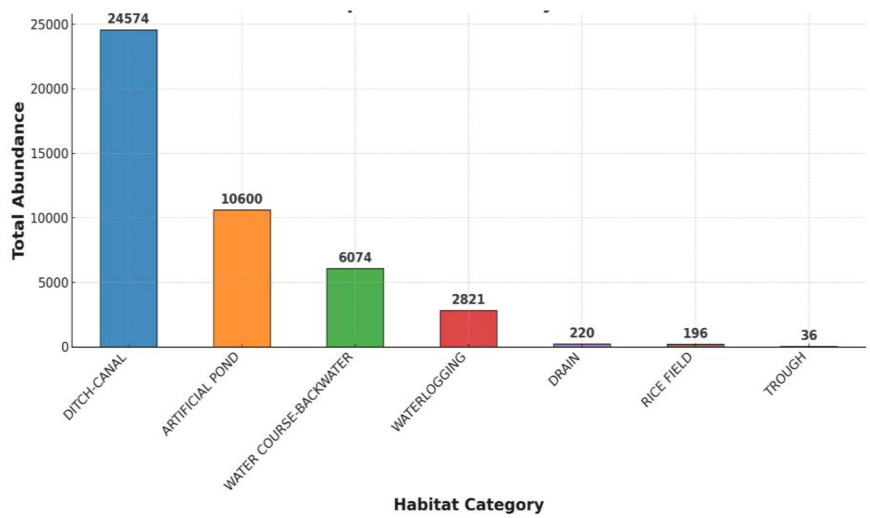

Figure 4. Total production of immature mosquito stages at the seven different habitat categories in the study area (Sevilla province, southern Spain).

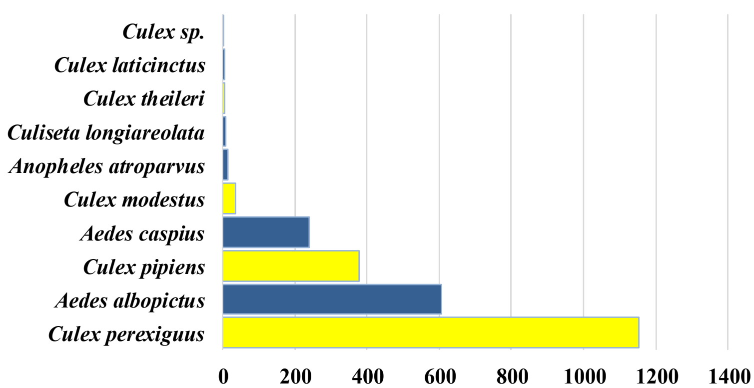

Figure 5. Counts of adult mosquitoes collected by BG-Sentinel traps in Sevilla province. In yellow vectors are denoted and in blue other non-target mosquito species.

Based on adult collections, a total of 2,553 adult mosquitoes of nine species were collected with the primary vectors of WNV and other arbovirus (i.e. dengue), such as Culex perexiguus, Aedes albopictus and Culex pipiens being predominant (Figure 5). Adult collections peaked in August, also with intense flight activity in September and October. However, adult mosquitoes were recorded throughout the entire year. The elucidation of the host-meals of the females collected are under analysis and will be reveled in the oral presentation.

Regarding control measures, our integrated control approach implemented across 138 positive points, resulted in a total of 266 control interventions for both immature (43%) and adult mosquitoes (56%). *Bacillus thuringiensis var. israelensis* (Bti) was applied to target larvae while cypermethrin-based adulticides were used for adult mosquitoes. Our preliminary analysis showed that in approximately 79.8% of the visits (with larvicide interventions), the number of larvae decreased in the subsequent visit. However, in the remaining 19.8%, the number of larvae did not decrease.

DISCUSSION

Our study provides a comprehensive analysis of an intensive and sustained effort that combined both surveillance and control measures to mitigate the impact of mosquito vectors of public health importance in a major WNV hot spot. We identified the most critical larval sites for key WNV mosquito vectors (*Cx. pipiens, Cx. perexiguus,* and *Cx. modestus*) and other mosquito species in one of the regions most affected by WNV in Spain.

Based on larval collections, *Cx. pipiens* species was widely recorded across various habitats, while *Cx. perexiguus* was also prevalent, particularly in environments such as rice fields, as well as in drains, water course backwater and other flood-prone areas. These findings are significant because larval habitats for *Cx. perexiguus* have not been thoroughly studied in our country. This highlights the need to expand larval control efforts beyond rice fields to include a wide range of waterlogged sites such as ditches, canals, ponds, and other stagnant water bodies to effectively target both critical vector species.

Based on adult collections, we observed a different and unusual trend. Surprisingly, high densities of adult *Cx. perexiguus* were found in urban areas; when, typically, *Cx. pipiens* is the predominant species in urban foci (Goiri et al. 2024). This observation underscores the importance of expanding control strategies to residential zones. It would be valuable to investigate whether *Cx. perexiguus* breeds in urban arear or migrates from other habitats to these locations.

Our findings also demonstrate that adult mosquito activity persists well into late autumn. This indicates that control measures should be implemented throughout most of the year to effectively reduce mosquito populations.

Furthermore, our results highlight that the application of *Bti* larvicides is effective in eliminating and/or reducing the populations of immature mosquito stages in the treated areas. However, these populations tend to recover quickly. Previous studies have stressed the need to re-treat affected areas within 8 to 10 days after the initial application, due to the biological life cycle of mosquitoes (Bonds, 2012). One limitation of our study is that we were unable to evaluate mosquito population reduction using consistent and comparable time intervals, which would be necessary to make statistically robust inferences. This was due to our team prioritizing coverage across all study sites, which limited the ability to standardize sampling schedules.

CONCLUSIONS

Our study reinforces the need for a multifaceted and adaptive approach to mosquito control in both rural and urban settings. Our study highlights the critical importance of comprehensive mosquito control strategies that address diverse breeding sites beyond rice fields, including urban areas and other waterlogged habitats. Persistent mosquito activity into late autumn necessitates year-round control measures with intensified use of *Bti*. These findings underscore the need for targeted, adaptive interventions to mitigate the public health risks posed by mosquito vectors in WNV hotspot regions. We also recommend that Pest Control Companies strengthen their efforts to rigorously and methodologically evaluate the effectiveness of mosquito control interventions, rather than relying solely on straightforward population reductions or simple field observations that lack scientific rigor.

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