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DISTRIBUTION OF *AEDES ALBOPICTUS* AND *AEDES JAPONICUS* IN URBAN AND PERI-URBAN ENVIRONMENTS IN THE BASQUE COUNTRY OF NORTHERN SPAIN

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Abstract Invasive mosquitoes' distribution has increased in recent decades throughout Europe. In 2014 *Aedes albopictus* was identified for the first time in the Basque Country (northern Spain) and in 2020 *Aedes japonicus* was detected in the three Basque provinces. This study aimed to evaluate the association of invasive mosquito presence with factors related to urbanization. In 2021, a total of 568 ovitraps were deployed in 113 sampling sites located in 45 municipalities, and tablets (wooden sticks) were fortnightly replaced and examined for *Aedes* spp. eggs detection from June to November. A selection of positive tablets with eggs compatible with *Aedes* spp. were hatched, and emerged adult mosquitoes were morphologically or molecularly identified. *Aedes albopictus* and *Ae. japonicus* showed to be widespread in the studied area, and co-occurrence of both species was observed in eight municipalities. The presence of *Ae. albopictus* was associated with municipalities with higher population density. *Ae. japonicus* was associated with peri-urban areas and municipalities with lower population density. According to our results, surveillance programs should be designed for different urbanization gradients.

Key words Invasive species, urbanization, mosquitoes, entomological surveillance

INTRODUCTION

Mosquitoes (*Culicidae*) are of medical and veterinary significance due to their blood-feeding behavior. In addition to the impact of mosquitoes as biting and annoying pests, their role as vectors of several diseases is extremely important for public health. Globalization, human travelling and global trade, facilitate the spread of vectors, such as invasive mosquitoes (Medlock et al., 2012). Also, climatic and environmental changes, increase the possibility of the spread and establishment of some species of *Aedes* spp. to new regions (Medlock et al., 2012).

Among invasive *Aedes* mosquitoes (AIM), *Aedes albopictus*, *Aedes aegypti*, *Aedes japonicus*, and *Aedes koreicus* have known established populations in Europe. Also, *Aedes atropalpus* has been detected (ECDC 2021). The Asian tiger mosquito (*Ae. albopictus*) is the most widely distributed AIM in Europe. The welfare of citizens may be altered due to the aggressive biting behavior of *Ae. albopictus*. Moreover, this species is a well-known vector of some arboviruses and filarial nematodes (Medlock et al. 2012). In areas of Europe where *Ae. albopictus* is well established, autochthonous outbreaks of arboviruses have been reported (Monge et al., 2020).

In Spain, after the first detection of the tiger mosquito in Catalonia in 2004, the Spanish Ministry of Health launched a surveillance campaign in 2007 in several Mediterranean Spanish regions (Collantes et al., 2015). The surveillance program in the Basque Country started in 2013 and in 2014 *Ae. albopictus* was identified for the first time in this region (Goiri et al. 2020). Since 2014, the distribution of this invasive species has been expanding towards new territories of the Basque Country until it has been considered as well established in some municipalities in the region (Goiri et al., 2020). Moreover, in 2020 *Ae. japonicus* was identified in the three Basque provinces (Eritja et al., 2021). This study aimed to evaluate the association of the presence and expansion of these two invasive mosquito species regarding factors related to urbanization.

MATERIAL AND METHODS

This study took place in the Basque Country, a region in the North of Spain divided in three administrative provinces: Gipuzkoa, Bizkaia, and Araba (Figure 1). The presence of *Aedes* spp. eggs was investigated using oviposition traps (ovitraps) and the sampling strategy was focused on urban and peri-urban areas of the municipalities with more than 10,000 inhabitants (n=42). In addition, three municipalities with less than 10,000 inhabitants were included due to their epidemiological interest (surroundings of an airport; a tourist town located in the furthest south of the Basque Country; and a small village with AIM presence suspicions. In total, 113 sampling areas located in 45 municipalities were selected, and 568 ovitraps were deployed. Ovitraps consisted of a dark container (250 mL) filled with non-chlorinated water and a wooden stick (Masonite) (15 cm long and 2 cm width) submerged inside as a support for egg-laying, and were placed protected from the sun and wind and hidden in the vegetation The examination of the ovitraps and the replacement of the tablets were carried out fortnightly. Thus, each municipality and area was sampled between 11 and 12 times over a 23 weeks period (June-November).

When eggs compatible with *Aedes* spp. were detected, these were counted and some selected positive tablets were immersed in a Petri dish with dechlorinated water. Once two-three stage larvae developed, were transferred into a mosquito breeder to molt to adult stage. Adult specimens were identified using taxonomic keys (Becker et al., 2020). In the case of unsuccessful egg hatching, 2-10 eggs were collected from each tablet and DNA extraction was performed using a commercial kit (NZYtech, Portugal), followed by PCR targeting cytochrome c oxidase I subunit (COI) gene (Simon et al., 1994). Amplicons were sequenced using Sanger technique and obtained sequences were then compared with those available in GenBank by BLAST analysis to confirm the species.

Ovitrap Index (OI) (percentage of positive traps of inspected traps) was estimated for each sampling point. A sampling point was considered positive when the presence of *Aedes* spp. eggs was detected on at least one ovitrap in at least one sampling fortnight. Associations between the presence of *Ae. albopictus* and *Ae. japonicus* (as a dependent binomial variable) with the following independent variables: urbanization (urban/peri-urban), type of environment (city center, green parks, parking, industrial and residential areas), and human population density were evaluated. The analyses were carried out using Generalized Linear Models or Mann-Whitney U test in R software.

RESULTS

A total of 5,896 (2,853 from Gipuzkoa, 2,484 from Bizkaia; 559 from Araba) tablets were examined. Eggs of *Aedes* spp. were detected in 84% of the municipalities (38/45) (Figure 1), in 66% of the sampling points (75/113) and 33% of the ovitraps (186/568) distributed in the three territories of the Basque Country. *Aedes* spp. eggs were not detected in seven municipalities. It is noteworthy that 100% of the municipalities of Gipuzkoa were positive to the presence of *Aedes* spp., while in Araba *Aedes* spp. eggs were detected only in one out of four municipalities investigated. It should also be noted that in three municipalities only one positive ovitrap (one tablet) was detected throughout the surveillance period.

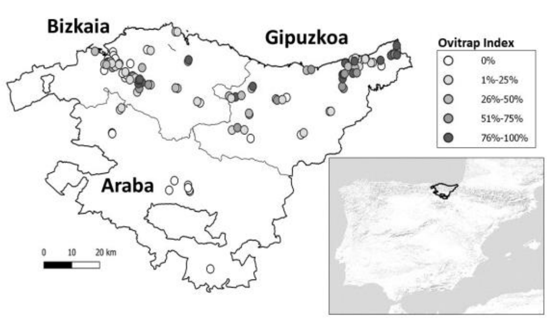

Figure 1. Map with Ovitrap Index of *Aedes* spp. eggs for each sampling point.

Adult hatching and complementary PCR and sequencing analyses from the eggs detected in 75 tablets from Bizkaia, 128 tablets from Gipuzkoa, and one from Araba, confirmed the identity of *Ae. albopictus* in 38 sampling areas from 22 municipalities, while *Ae. japonicus* has been detected in 38 sampling areas from 24 municipalities. In addition, co-occurrence of both species was observed in eight municipalities.

The presence of *Ae. albopictus* was associated with municipalities with higher population density (mean= 3,236 inh./ km2) (p<0.001) but not with urbanization category or type of environment (p=0.3). On the other hand, *Ae. japonicus* was associated with peri-urban areas (p=0.01) and municipalities with lower population density (mean= 1,245 inh./ km2) (p<0.001).

DISCUSSION

The wide distribution of invasive mosquitoes of the genus *Aedes* spp. in the Basque Country is increasingly evident, thus confirming the spread and establishment of invasive mosquitoes in the region. In some municipalities, all the selected zones and all the locations of the ovitraps have been positive, which indicates that the species is fully established in some geographical areas. The sampling areas with a greater establishment of invasive mosquitoes are found around the two provincial capitals (Bilbo and Donostia) with larger populations and with important industrial ring roads, favoring introduction and movement of invasive *Aedes* spp. In contrast, the presence in some sampling areas (those with one positive ovitrap throughout all study period), could be explained by anecdotal specific entries of invasive *Aedes* suggesting that the species might not be yet established in those areas. While establishment in Gipuzkoa seems to be obvious, in Araba it seems that in general terms there are no stable populations in the areas studied. It must be considered that the number of municipalities studied in Araba province is low. Even so, it should be remembered that both *Aedes* species had already been detected in this province previously (Goiri et al., 2020; Eritja et al., 2021). In some cities, although the presence of invasive *Aedes* had already been detected (Goiri et al. 2020), by increasing the number of ovitraps it has been possible to detect the presence of invasive *Aedes* in new areas of these cities, thus demonstrating the expansion and generation of new stable populations year after year.

Until 2019, the surveillance program focused on areas with high concentration of traffic, which favored the detection of *Ae. albopictus* (Goiri et al., 2020). In 2020, the sampling was extended to municipalities less populated and less industrialized areas, which made possible the detection of *Ae. japonicus* in four localities (unpublished data). The sampling strategy carried out in 2021 has shown that *Ae. japonicus* is widespread, and is well established in the region, at least in Bizkaia and Gipuzkoa provinces. As shown, the distribution patterns of both species vary depending on the degree of urbanization as demonstrated by the factor analysis. *Aedes albopictus* appears concentrated in large urban areas, whereas *Ae. japonicus* is found in less populated areas. This species, like the tiger mosquito, uses a wide variety of larval habitats, both natural and artificial containers (Kaufman and Fonseca 2014; Mogi et al., 2020). Although some authors have considered *Ae. japonicus* an urban species, larvae is found more frequently in forested or rural areas than in highly urbanized environments (Kaufman and Fonseca 2014). Indeed, it has been suggested that hot and dry summer conditions increased by the "urban warming effect" and a decrease in preferred mammal hosts could be the reasons for the preference of *Ae. japonicus* in peri-urban and rural settings (Mogi et al., 2020). In contrast, *Ae. albopictus* can adapt to climatic conditions and has a wider blood-feeding host range (Medlock et al., 2012).

CONCLUSION

Due to the potential impact on public health, surveillance of invasive *Aedes* species used to be biased to urban areas and municipalities with high population density. However, according to our findings, surveillance programs should be designed taking into account different environments, including municipalities with low population density and peri-urban areas. Thus, the results may have practical applications regarding to the design for AIM surveillance. Even though *Ae. japonicus* is not a high-risk mosquito for public health, the role it could play is still unknown.

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