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RESEARCH ARTICLE

Imported arboviral disease cases and entomological surveillance of *Aedes albopictus* in Valencian Autonomous Region (Spain) along 2022

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Abstract

The Laboratory of Entomology and Pest Control (LEPC) of the University of Valencia, hired by the regional government of the Generalitat Valenciana, carried out the entomological surveillance of the Asian tiger mosquito along 2022 in the Valencian Autonomous Region, excepting the city of Valencia for which the surveillance was conducted by a private pest control company. The main purpose of this plan of action was both to continue the detection and monitoring of *Aedes albopictus* in the Valencian municipalities free of its presence until the end of 2021, and to carry out the entomological surveys related to imported arbovirus cases notified by the Regional Health System. The active search and detection of the mosquito breeding sites in both urban and peri-urban environments throughout the provinces of Alicante, Castellón and Valencia, has revealed the absence of new populations of this allochthonous mosquito in all those municipalities free of this species in December of 2021. Besides, the LEPC has conducted the mosquito surveys in 31 municipalities of the three provinces, in relation to the declaration of 33 cases of arthropod-borne diseases due to Chikungunya, dengue or Zika viruses, which were reported in travellers returning from countries of Latin America, Asia, or West Africa. The methods used to minimise the risk of local transmission of these viruses by *Ae. albopictus* were focused on detecting the presence of the tiger mosquito and other common native mosquito populations, at both adult and larval stages, in the surroundings of the residence of imported cases to guide the treatments of the detected resting and breeding sites. As result, tiger mosquito populations were recorded in 18 of the 33 municipalities where imported arbovirus cases were reported in 2022.

Keywords

public health – vector borne diseases – tiger mosquito – monitoring – Eastern Spain

1 Introduction

Viruses transmitted by arthropods, also known as arboviruses (arthropod-borne viruses), have accompanied humanity throughout its evolution. Currently, diverse factors such as the movement of population and goods, globalisation and global warming, all triggered directly or indirectly by humans, are favouring emerging and re-emerging diseases. This represents a significant and growing threat to human communities in temperate regions (Jiménez Peydró *et al.*, 2023; López-Vélez and Molina Moreno, 2005). Among the arthropods vectors of pathogens such as viruses, bacteria and helminths, mosquitoes transmit the agents that cause the higher

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human deaths numbers year after year (OMS, 2020). This happens during bloodmeal, when transferring saliva to humans after having previously acquired the pathogen from the blood of another infected vertebrate hosts, including humans for a number of them.

The Valencian Autonomous Region benefits from a temperate climate where the Asian tiger mosquito, *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894), an invasive species, was detected for the first time, in the Alicante town of Orihuela in 2005 at the adult stage (Roiz *et al.*, 2007), and in the year 2009 at preimaginal stages (Bueno-Marí *et al.*, 2010). Since then, it has been colonising and establishing itself in new urban and peri-urban areas until reaching a dispersion that covers the vast majority of the territory of this autonomous region (López-Peña *et al.*, 2022).

Aedes albopictus shows great physiological and ecological plasticity (Kramer *et al.*, 2021) that allows it to survive, establish and reproduce in urban environments where it finds favourable conditions to develop, in both some natural places, such as tree cavities and anthropic places, such as scuppers or pot plates, where they can carry out their aquatic life cycle phase. Likewise, as it is a competent vector of several viruses, it represents a significant risk to public health. Among the viruses that tiger mosquito is capable of transmitting are Chikungunya fever virus (CHIKV), dengue virus (DENV) and Zika virus (ZIKV). These three viruses are of mandatory report since 2015 for CHIKV and DENV (BOE, 2015), and since 2016 for ZIKV (MSSSI, 2016). Until a few years ago, all the reported cases were imported, i.e. cases of citizens who had contracted one of these viruses in endemic overseas places during work or leisure trips. However, the establishment of the tiger mosquito in Spain has already led to autochthonous DENV infections (CCAES, 2018, 2019a, 2023). Besides, the health system has to face not only the difficulty of its diagnosis due to the poor specificity of the symptoms they cause, but also the characteristics of the infections, for which 80% of those due to DENV (Bhatt *et al.*, 2013) and ZIKV (ECDC, 2021), and between 17 and 40% of those caused by CHIKV (ECDC, 2024), are asymptomatic or present only mild symptoms, thus representing a serious issue to the prevention sector since these cases remain undetected.

In light of the above-mentioned facts, the general directorate responsible for public health of the region hired the services of the Laboratory of Entomology and Pest Control (LEPC) of the University of Valencia in 2015, to carry out the detection, surveillance and monitoring of the *Ae. albopictus* populations to (1) determine its presence and geographical distribution in the Valencian territory and (2) to prevent the appearance of autochthonous transmissions of CHIV, DENV and ZIKV by carrying out entomological surveys around any imported cases.

We here report the results of the entomological surveillance carried out throughout 2022, in light of the imported cases that have been reported, and the outcomes of the required entomological surveys.

2 Materials and methods

Detection and surveillance of Ae. albopictus

The search, detection and surveillance of established tiger mosquito populations was carried out by visiting those municipalities of the three Valencian provinces in which the mosquito absence was confirmed at the end of 2021 after completing the detection and monitoring tasks of that same year. The detection consisted of the active search by prospecting all places present in urban and peri-urban environments that were likely to harbour water and therefore enable egg-laying and development of the tiger mosquito. Among them, the rainwater collection scuppers, non-recirculated water fountains whether ornamental or for public consumption, flowerpot plates and vases located on public roads or in cemeteries, and cavities in trees. In cases positive for the presence of mosquitoes, immature specimens were collected using the sampling technique called '*dipping*' (Service, 1993). They were then transferred to the laboratory facilities where they were processed for conservation and subsequent identification. Morphological identification was performed with the help of a stereoscopic microscope and standard identification keys (Schaffner *et al.*, 2001).

Entomological surveys around imported case

Following the communication from the General Directorate of Public Health (GDPH) about the arbovirus cases notified by the Epidemiological Surveillance and Control Service, the LEPC prepared a map with the delimitation of the areas to be inspected, both around the patients' homes and the areas frequented by the patients during the days prior to the diagnosis and confirmation of the imported arbovirus infection case. The entomological survey was performed in not more than 24 h after each case report. The zone to survey consisted of an area of approximately 150 m in radius, since the average flight distance of a tiger mosquito is 119 m per day (Marini *et al.*, 2010), taking as the epicentre the

patient's home and/or other frequented areas to carry out a thorough inspection of any structure potentially suitable for the development cycle of the tiger mosquito (Figure 1). Once this map was prepared, the laboratory staff went to the areas and methodically and exhaustively inspected all potential larval habitats. Once the presence of mosquitoes at any stage of development was detected, representative aquatic samples were collected by *dipping* and/or adult samples by using a manual entomological aspirator. Following the sample identification, detailed results were sent to the GDPH for each entomological survey, confirming the presence or absence of *Ae. albopictus* on an attached map and providing recommendations on the need or not to carry out larvicidal and/or adulticidal treatments at the places where its presence was detected. It is worth to mention that the urban areas inspected and colonised by *Ae. albopictus*, were treated by technical services hired by the municipalities. These services also carried out an entomological survey in these areas 7 days after the first treatment and if the mosquito presence persisted, more treatments were conducted until their total disappearance was achieved.

Map creation

The location and status of potential tiger mosquito breeding sites, such as fountains or scuppers with or without water and with or without mosquitoes in any phase of development, were represented on a pdf map that was submitted to the competent entity. These maps were created with the geographic information system (GIS) programme ArcMapTM 10.5, ESRI's ArcGIS® software (Redlands, CA, USA).

3 Results

Detection and surveillance of established populations of Ae. albopictus

The LEPC has reported the distribution and expansion of *Ae. albopictus* throughout the Valencian territory since 2009 to 2021, within the framework of a vector surveillance programme aimed at detecting the presence of established populations of this mosquito species, and in collaboration with the GDPH in the last 6 years.

At the end of 2021, 464 municipalities, out of a total of 542, revealed positive for the presence of *Ae. albopictus*, specifically 133 municipalities of Alicante province, 244 municipalities of Valencia province and 87 municipalities of Castellón province. In 2022, the survey continued, with the aim of assessing and updating the geographical distribution of the species with the highest possible resolution and in order to establish a control management of its populations and thus minimise the risks to the citizen health. However, as of December 2022, no additional municipality revealed positive for the mosquito presence, suggestions 78 municipalities (8 from Alicante, 22 from Valencia, and 48 from Castellón) not being colonised by the tiger mosquito. The absence of established populations of *Ae. Albopictus* in these municipalities depicted in white colour in the map (Figure 2), may be due to several factors such as the highs altitudes where they are located, the scarce inhabitants living there and visiting other places, and the lack of urban public structures suitable for mosquito breeding.

Imported cases and entomological surveys

During 2022, a total of 33 imported cases of arboviral infections were reported in areas of the region where the tiger mosquito is established. These cases affected citizens from municipalities of Alicante, Castellón, and Valencia provinces, without taking into account cases located in the city of Valencia which were reported through other protocols designed by the Valencia City Council.

Thus, the arboviral cases notified by GDPH and reported to the LEPC for its entomological surveillance consisted, in increasing order, of 27 cases of DENV, 4 cases of ZIKV, and 2 cases of CHIKV (Figure 3), revealing a higher incidence of the first mentioned arbovirus. The province with the highest number of affected people was Alicante with a total of 18 reported cases, of which 14 were due to DENV infection, 3 to the ZIKV, and 1 to CHIKV. Second is the province of Valencia with 9 cases, 8 diagnosed as DENV fever and 1 as CHIKV fever. Finally, Castellón showed a total of 6 cases, 5 due to DENV, and 1 to ZIKV.

The notification of these imported cases spanned from March to December but with a differently distribution (Figure 4). September showed a total of 14 notifications, 11 due to DENV and 3 to ZIKV, followed by October with 8 reported cases, 6 of DENV, 1 of ZIKV and 1 of CHIKV. Besides, August and November showed a total of 4 cases each mostly due to the DENV. Last, the months of March, June and December notified only one case each, due to the CHIKV in the first month, and DENV in the other months. The notification of cases by province is as shown in Figure 4.

All these 33 cases were notified from a total of 31 municipalities. Tiger mosquito populations of both preimaginal and imaginal stages were detected in

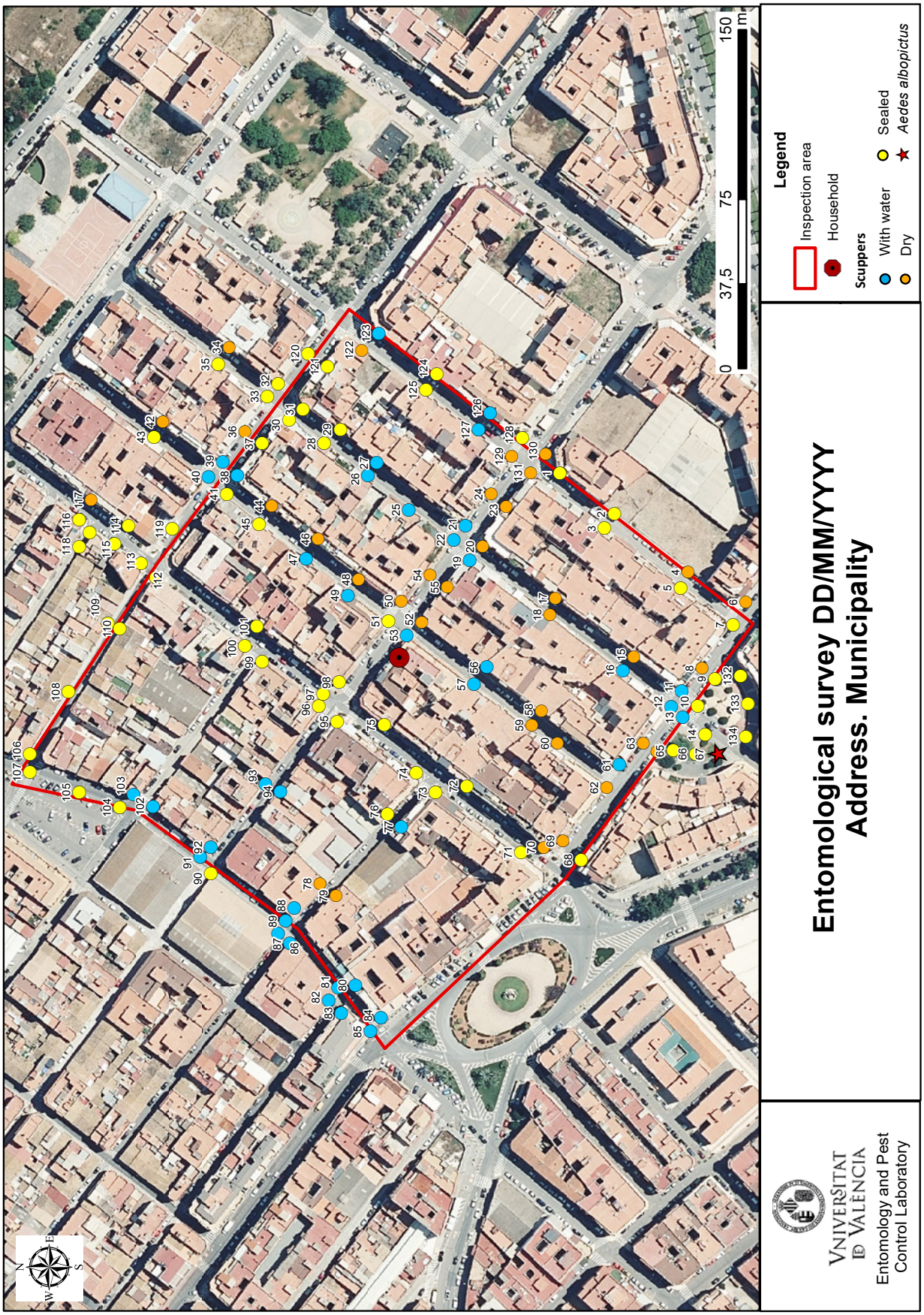

FIGURE 1 Example of data map resulting from an entomological survey.

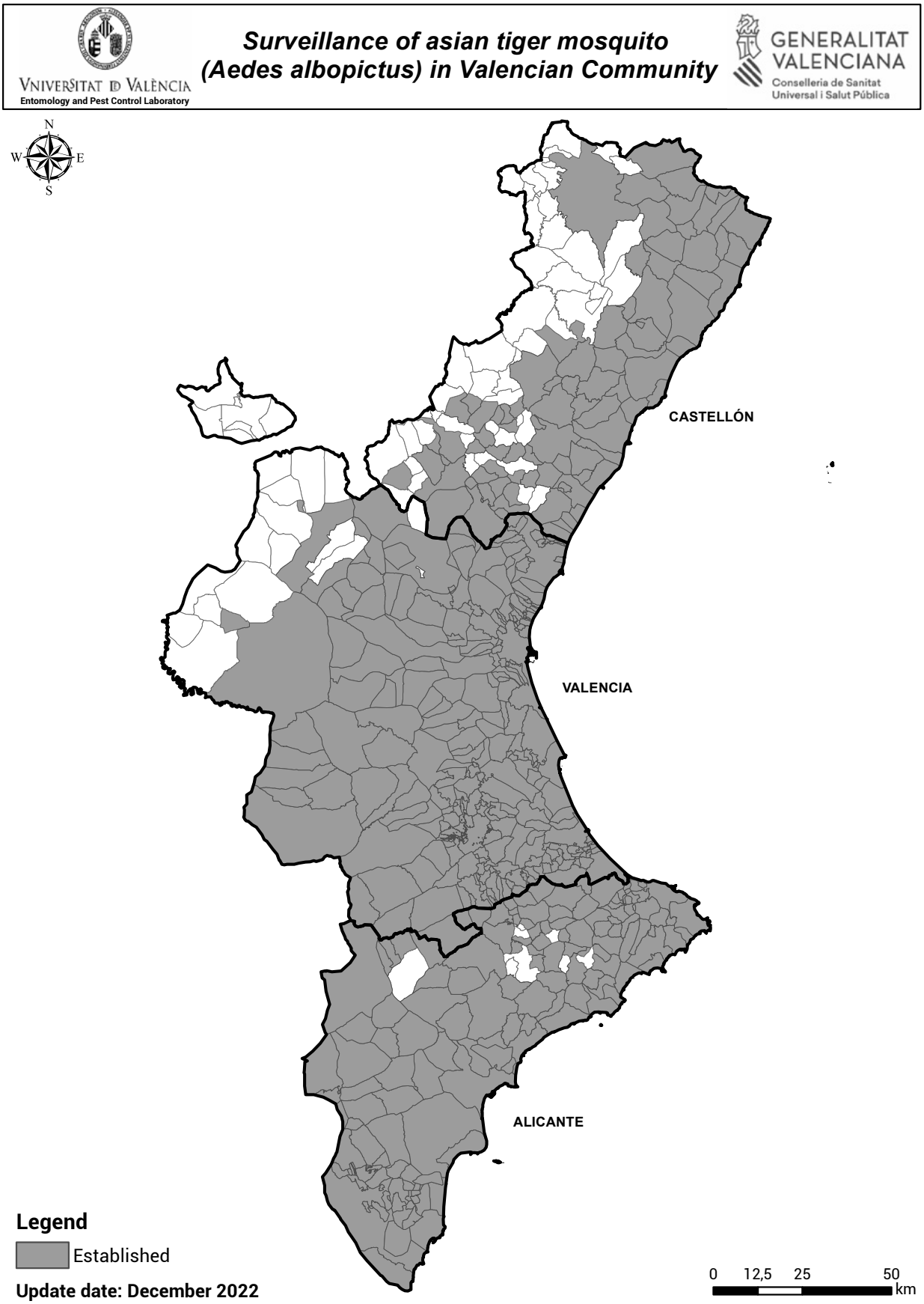

FIGURE 2 Geographical distribution of *Aedes albopictus* all over the three provinces of the Valencian Autonomous Region as of January 2023.

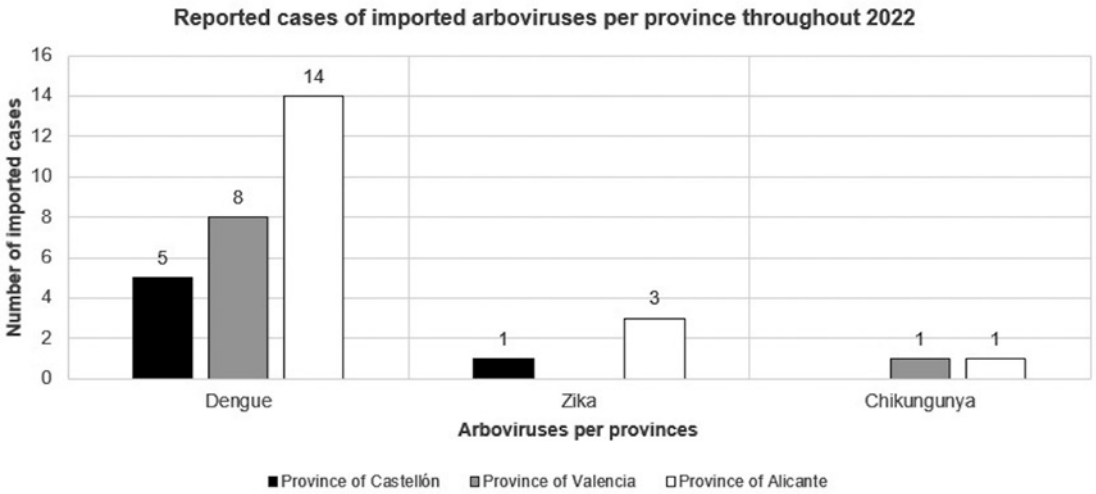

FIGURE 3 Reported cases of imported arboviruses per province of the Valencian Autonomous Region throughout the year 2022.

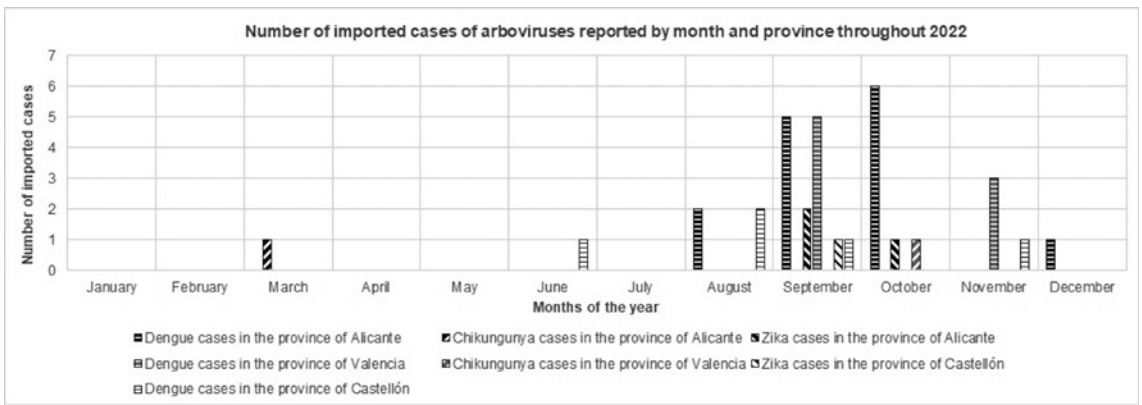

FIGURE 4 Number and type of cases reported by month and province of the Valencian Autonomous Region in 2022.

18 municipalities out of them. Subsequently, the adequate larvicidal treatment, as well as the use and management of proper adulticides were recommended at these locations, based on registered biocides.

The geographical origin of these imported arbovirus infection cases includes countries of Africa, Asia, and Latin America (Figure 5). The highest number of imported cases originated from American continent with a total of 23 infections. The most numerous were DENV infections, with a total of 20 cases, 15 from Cuba, 2 from Costa Rica, 2 from Mexico, and 1 from the Dominican Republic. The Asian continent provided 6 cases, 1 due to the ZIKV from Vietnam, and 5 to DENV, 2 of them from India, 1 from Philippines, 1 from Pakistan, and 1 from Vietnam. Finally, 4 cases originated from the African continent, including 3 cases of CHIKV from Chad, Ivory Coast and Mauritania, and 1 case of ZIKV from Uganda.

4 Discussion

Since the arrival of the tiger mosquito in Spain through Barcelona in 2004 (Aranda *et al.*, 2004), and into the Valencian region in 2005 (Roiz *et al.*, 2007), efforts to detect established populations emerged as a crucial need. A first detection of a stable population of this mosquito species was reported in 2009 by the LEPC (Bueno *et al.*, 2010). At that time, the justification for these efforts was based on the hematophagous habits of *Ae. albopictus* and its marked anthropophilly, and consequently the discomfort, itching and allergies it generates.

However, the expand of its distribution throughout the Valencian Mediterranean arch over the years, together with the report of imported arbovirus infection cases originating from endemic areas, increased the concern of public health authorities due to the risk of autochthonous outbreaks of DENV, CHIKV or ZIKV.

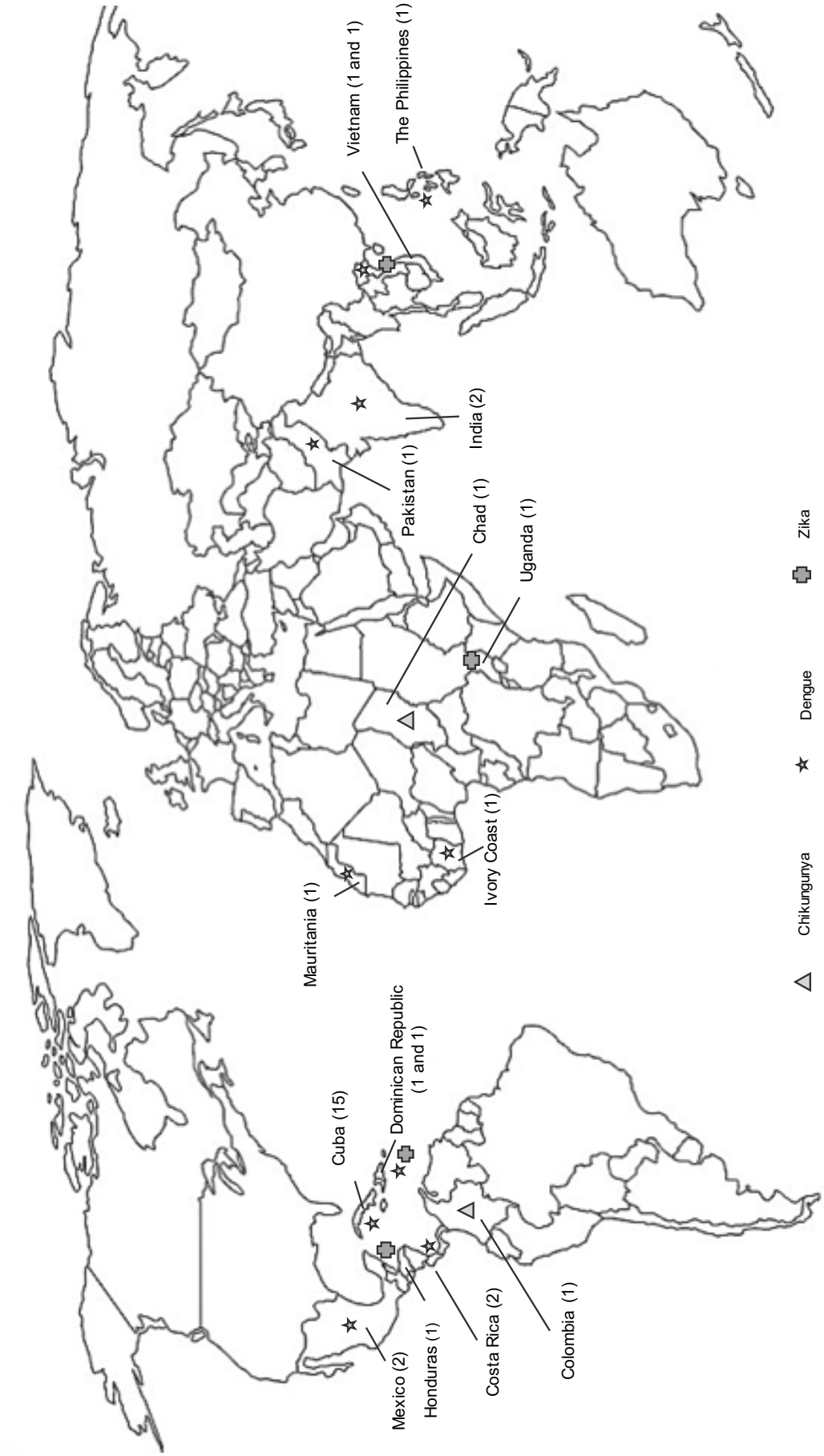

FIGURE 5 Countries from where arboviral disease cases were imported in 2022.

Therefore, the LEPC conducted the surveillance it was asked to perform since 2016 (López Peña *et al.*, 2019), periodically reporting the species' expansion and performing entomological surveys around each reported imported arbovirus case reported within 24 h after the report (Barberá Riera and López Peña, 2017). During this period, concern about the emergence of local arbovirus outbreaks has not ceased. Autochthonous cases of DENV have been described in 2018 in other regions of Spain, specifically 5 cases in the Region of Murcia and one in Cataluña (CCAES, 2023). Also in 2019, with one DENV case was reported again from Cataluña and another one from Madrid, the latter being likely due to a sexual transmission (CCAES, 2019b; CCAES, 2023), as well as 6 cases in Balearic Islands in 2022 (MS, 2024; CCAES, 2023). Furthermore, the possibility that an *Ae. albopictus* female mosquito can meet an infected human host and feed on is favoured by its preferential exophilic diurnal activity, but also by some endophilic activity that fits with the range of human activity. For this reason, the virus-specific viraemia period is considered for each reported cases, with the rapid implementation of control measures during this period. Hence, if the exposure time of the infectious human to mosquitoes is reduced, the probability of getting mosquitoes infected and later on capable of transmitting these viruses can be minimised.

During the eight years of entomological surveillance, differences were observed both in the detection and expansion of *Ae. albopictus* populations and in the reported number of imported arbovirus cases. The detection data revealed an increase of municipalities with established tiger mosquito populations in 2021 (López-Peña *et al.*, 2022). In 2022 the situation has remained stable, but the species has colonised 86% of the municipalities of the Valencian region and only 78 municipalities out of 592 remained free of *Ae. albopictus* populations. These municipalities are located in the interior of the region, where the environmental conditions are not as favourable for the mosquito as in the municipalities located in pre-coastal and coastal areas (López-Peña *et al.*, 2022).

Besides, a total of 46 imported arbovirus cases were reported in the six-year period of 2016 to 2022, 2 of them due to the CHIKV, 35 owing to DENV, and 9 to ZIKV (Barberá Riera *et al.*, 2022). Fifteen cases were reported in 2016 (Barberá Riera and López Peña, 2017), but 33 cases in 2022, which shows an increase of more than double. There are also differences in the type of virus, i.e. in 2016 there were 1 case of CHIKV, 3 of DENV and 11 of ZIKV (Barberá Riera and López Peña, 2017), while there were 2 cases of CHIKV, 27 of DENV and 4 of ZIKV in 2022, revealing that DENV infections have become the predominant arboviral cases. With regards to the territorial distribution of cases, 2 of them were diagnosed in citizens from Alicante province, 2 more from Castellón province and the remaining 11 from Valencia province in 2016 (Barberá Riera and López Peña, 2017). In 2022, 18 cases were reported from the Alicante province, 6 from the Castellón province and 9 from Valencia province. Thus, the province with the highest number of cases was Valencia in 2016, whereas it was the Alicante province in 2022.

The case notification period extended from May to October in 2016 (Barberá Riera and López Peña, 2017), whilst it extended from March to December in 2022. This reveals a period prolonged for 4 months, ranging from 6 in 2016 to 10 months in 2022. This may be due to the worsening of climate change, global warming and effects of globalisation. Likewise, differences also exist for the months in which the more cases were registered in both years, being August, May and October in 2016 with 5, 4, and 3 cases respectively (Barberá Riera and López Peña, 2017), and September and October in 2016 with 14 and 8 cases. This highlights not only the increase in reported cases, but also a change in notifications in spring, summer and autumn months, mainly towards the end of summer and autumn.

Finally, according to the LEPC's entomological surveys implemented around cases, the presence of *Ae. albopictus* was detected in 4 out of the 15 inspections carried out in 2016 (Barberá Riera and López Peña, 2017), while it was recorded in 18 out of the 31 surveys performed in 2022. This corresponds to an increase of detections of 31%, for a range of 27% of detections in 2016 to 58% in 2022.

5 Conclusions

Our entomological surveys reveal that *Ae. albopictus* maintained the same geographical distribution in the territory of the Valencian region in 2022 as compared to 2021. Besides, the 33 entomological surveys carried out around imported arbovirus cases revealed the presence of the species in 18 municipalities. Control measures were applied in 18 municipalities, and no secondary arbovirus cases were reported. These results were possible thanks to the close collaboration between academic and research institutions and health authorities, and should be pursued for the benefit of all Valencian citizens.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

The data supporting this article will be made available upon reasonable request to the authors.

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