First report of the Asian tiger mosquito, *Aedes (Stegomyia) albopictus* Skuse, 1984 (Diptera, Culicidae) in Cordoba (southern Spain). New challenges for the administration and citizens of Cordoba

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Abstract: The first record of the presence of the invasive Asian tiger mosquito *Aedes albopictus* in the province of Cordoba is presented. Adults, larvae, pupae and exuviae have been located at different points of housing estates in the northeastern part of the city. One possible origin is the coast around Malaga, much frequented by the citizens of Cordoba. Its detection has been possible due to the programme of monitoring, surveillance and control of hematophagous Dipterans, initiated in 2016 by our research group (Terrestrial Ecology, RNM232) in collaboration with the Sanitation Public Company of Cordoba (SADECO).
The arrival of the tiger mosquito in Cordoba is a relevant issue due to its implications for welfare and public health. In addition to the discomfort and injuries caused by its bites, it is a competent vector of non-endemic arboviruses such as Chikungunya fever, Dengue fever and Zika. Therefore, with the information gathered by our group, the SADECO technicians acted appropriately, with the focus being to control it and try to prevent it from spreading and causing public health problems. However, given the large flow of people between Cordoba and the coast around Malaga, a vacation area very popular among the people of Cordoba, it is foreseeable that the inadvertent transport of mosquitoes by car to Cordoba will generate new outbreaks. Therefore, it will be important to continue with the programme of early detection of this invasive species through coordinated work involving researchers from the University of Cordoba, SADECO technicians, and the local and provincial administrations, with the collaboration of neighbourhood associations and the population in general. The information presented in this article is relevant due to its implications for welfare and public health.
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Introduction

The Asian tiger mosquito (*Aedes* (*Stegomyia*) *albopictus* Skuse) (Diptera, Culicidae) is the most widespread and monitored invasive Diptera species in Europe. Originally from Southeast Asia, it was introduced into Europe in 1979 in Albania, in a rubber factory adjacent to the port of Durrës (Durazzo), where it arrived in a shipment of used tyres (Adhami & Reiter, 1998). Also, the cultivation in homes of the lucky bamboo (genus *Dracaena*) has facilitated the accidental entry of pre-imaginal stages into water reservoirs in Europe (Scholte *et al*., 2008). Since then it has colonised practically all the European territory, from east to west (Adhami & Reiter, 1998; ECDC, 2018a). The presence of urban habitats with abundant vegetation and accumulated water, on occasion, with artificial conditions simulating tropical gardens, has favoured its rapid expansion. Although the dispersal capacity of this mosquito is very low (Lacroix *et al*., 2009; Marini *et al*., 2010), its accidental transport has led to it being detected in 19 countries in Europe, and it is already present in 17 provinces of Spain, in 11 of which it maintains stable populations (ECDC, 2018a). In the Iberian Peninsula, its presence was detected for the first time in 2004, in Barcelona, and since then it has spread widely along the Mediterranean coast to the south, reaching the province of Cadiz (Giménez *et al*., 2007; Aranda *et al*., 2006; Roiz *et al*., 2007; Delacour *et al*., 2014; ECDC, 2018a). Until 2016 there were only records of *Ae. albopictus* in the coastal provinces of eastern and southern Spain - all of them with mild, frost-free climates - but recently it has been detected in inland provinces such as Zaragoza, Huesca, Madrid, Seville (Lucientes & Molina, 2017), Badajoz and Caceres (Bravo-Barriga *et al*., 2019).

It is a species able to take advantage of any reserve of natural or artificial water and the eggs can withstand drying for long periods of time, in the diapause phase. This ability to survive under unfavourable conditions (e.g., prolonged drought) gives it a great adaptive capacity (Hawley, 1988). In addition to the discomfort and injuries that can result from the reaction produced by the compounds of its saliva (antiaggregants, anticoagulants, anaesthetics, etc.), it is a vector for arboviruses that represent a concern for public health in tropical and temperate regions worldwide, such as Dengue, Zika and Chikungunya. The presence of these introduced vectors and the intense flux of travellers on a global scale may favour the appearance of non-endemic infectious diseases, and may even trigger a pandemic, outside their native areas (Lucientes & Molina, 2017).

Since 2010 there have been incidents regarding nuisance biting in different residential areas of the periphery of the city of Cordoba. The problems were mostly due to Dipterans of importance for public health belonging to the families Ceratopogonidae, Simuliidae and Culicidae - such as *Leptoconops bezzii*, *Simulium ornatum*, *S. pseudequinum* and *Culex pipiens* - that appeared in high densities at the end of spring and in early summer. This motivated the start-up in 2016 of a project aimed at the surveillance and control of hematophagous Dipterans in the city of Cordoba, financed by the pest control service of SADECO, which is dependent on the city council. Therefore, although this project has been led by the Department of Ecology

of the University of Cordoba, since its inception it has had a multidisciplinary nature because it has combined the work and collaboration of ecologists, entomologists, veterinarians and technicians specialised in the control of urban pests.

In addition, the proximity of provinces with already-established populations of tiger mosquitoes, such as Malaga (Delacour-Estrella *et al*., 2014; Lucientes & Molina, 2017), and the movement of large numbers of people between Cordoba and the coast of this province during the summer months forced the setting-up of an early detection programme for these mosquitoes in anticipation of their possible arrival in Cordoba.

Materials & Methods

During the summer and autumn of 2016 to 2018 intense sampling was carried out for the surveillance of invasive hematophagous Dipterans, especially aimed at the search for the tiger mosquito (*Aedes albopictus*) in any of its development phases. Surveillance was carried out using oviposition traps (ovitrap type) and by periodic visits to potential breeding habitats, of public or private ownership, such as: manholes, scuppers, water register boxes, accumulations of water due to faults in the public network or irrigation, containers of different types where water accumulates, and drinking stations for pets.

Ovitrap surveillance was conducted in the city of Cordoba in order to detect the early presence of invasive mosquito species. Fifty-six oviposition traps were placed in 28 sites (2 traps per site, Fig. 1a), being operational between May and October. These mimic traps attract females, because they offer a suitable place for them to lay eggs and constitute a very effective indirect method to study the presence/absence of both *Ae. albopictus* and other invasive species present in Europe such as *A. aegypti*, *A. japonicus* and *A. koreicus*. These types of trap have been used as an effective tool in surveillance programmes, and insecticides, such as *Bti*, can be added to the trap substrate to prevent them from becoming potential breeding sites (Sutter *et al*., 2016; Johnson *et al*., 2017).

If these species are present in a locality being sampled, egg-laying will occur in the ovitraps, where eggs and larvae are easily detected. The homemade oviposition traps used in this study were based on common lethal ovitraps (Johnson *et al*., 2017). They are very cheap, which allows high coverage to be achieved by using a lot of them simultaneously across many optimal sites. They comprised a black container (a flower pot of 12 cm in diameter) with 300-400 ml of water, and a partially-submerged strip of porous wood (15 × 3 × 0.5 cm) as the laying substrate (Fig. 1b).

The traps were primed with an aqueous infusion of dehydrated hay (5% by volume) (Allan *et al*., 1995; Obenauer *et al*., 2009). The infusion was prepared by boiling 200 g of hay in 5 l of water over a low heat for 30 minutes. Subsequently, it was filtered and kept in a refrigerator at 6ºC until its use. Every 10 days, during the time the traps were operational, 10 ml of infusion were added to each trap. No larvicide was added. The traps were checked every two weeks to see if there were eggs on the strips or hatched larvae in the water. If eggs and/or larvae were present, they were transferred to the laboratory for study and identification; they were then kept in adequate conditions until they completed their development, according to the protocol established in Alarcon-Elbal *et al*. (2010).

The oviposition traps were placed outdoors in selected urban gardens, public (83.9%) or private (16.1%), near hedges or bushes, which the insects use as corridors (ECDC, 2014). The choice of sites was based on several criteria, such as the availability of irrigation and the occurrence of abundant vegetation, places where water can accumulate and highly-populated areas with private housing estates nearby. The traps were installed at a height of 0.5-2 metres above the ground, taking advantage of different supports (in a tree, in a bush, on a window ledge, etc.). At some sites, they were placed directly on the ground due to the absence of supports. The main reason for installing traps in elevated positions on some type of support was to reduce their visibility to humans, since we have suffered numerous losses, when people detect them and overturn the content, break them or take them away. All the traps were geo-referenced with a Garmin 60Csx GPS device.

The strategic sites selected for the sampling offer suitable conditions for the tiger mosquito: the Botanical Garden, the Municipal Zoo, the Sotos de la Albolafia Natural Monument, the public gardens of the suburbs of Encinarejo, Alcolea and Villarrubia, the University Campus of Rabanales and the urban

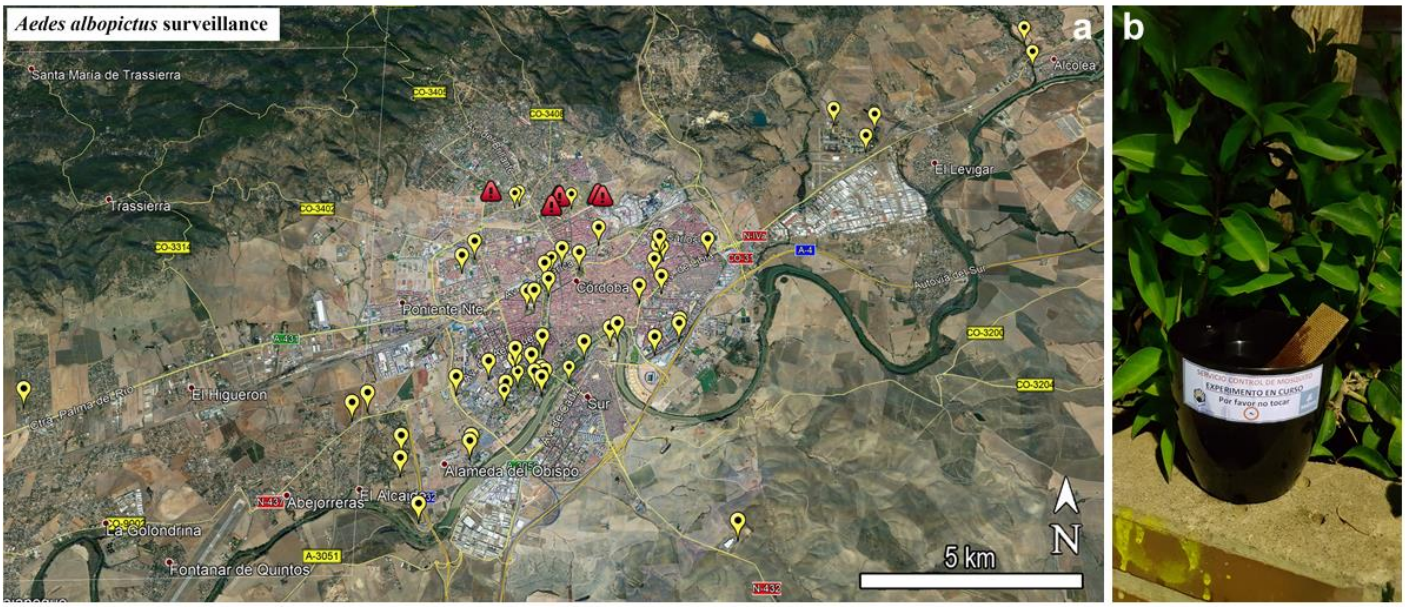

Figure 1: (a) Study area and ovitrap (N = 56) locations in Cordoba, southern Spain (yellow marks) and confirmed presence of *Ae. albopictus* during October and November 2018 due to notifications by citizens (red triangles). (b) Oviposition trap for the surveillance of *Aedes albopictus*.

public gardens of Miraflores, Cruz Conde Park, Fidiana, Santuario, Avda. Barcelona, Vallellano, Alameda del Obispo, Asomadilla-south, Avda. Brillante, Vial Norte, Figueroa, the Faculty of Educational Sciences, the Rectorate and Duque de Rivas (Fig. 1a).

In addition, other surveillance traps were installed at the highway services in Los Visos (A4 highway, km 406), a usual stop for travellers on the way to/from Seville, Huelva or Cadiz, and at the highway services in Lucena (A45 highway, km 51), for the surveillance of travellers coming from Malaga. These last two traps were checked every three weeks, due to the long distances from Cordoba city (24 and 73 km, respectively).

To complement the traps, numerous breeding habitats (scuppers, manholes, water containers, animal water stations, etc.) were surveyed in sensitive areas of the city of Cordoba and in peripheral neighbourhoods. This was done more intensively between July and October, coinciding with the maximum levels of tiger mosquito activity in coastal areas of Andalusia and the high number of journeys made by holidaymakers to and from the coastal zones in which the species is well established.

For the identification of the *Ae. albopictus* specimens, the keys of Schaffner *et al.* (2001) and MosKeyTool v. 1.2. (Gunay *et al.*, 2017) were used.

Results

The samplings made with the oviposition traps did not give any positive result regarding the presence of the species. Neither did the samplings carried out in parks and gardens in the city or in peripheral neighbourhoods, in the active search for both adults and larvae, the latter in containers of all kinds with accumulated water.

In the month of May 2018, we received several telephone calls alerting us to the possible presence of tiger mosquitoes in a housing estate located in the hills of Cordoba (north of the city). Of the three warning calls, two were negative while the other was considered doubtful because the species could not be confirmed owing to the deterioration (in particular, a lack of scales) of the specimen captured by the person who contacted us. It was not possible to perform a molecular analysis of this specimen during the monitoring season. A month later, the Tiger Mosquito Dataset of the Mosquito Alert application (CREAF) recorded a probable sighting in the area of La Aguardentera (not confirmed by a specialist). Three oviposition traps were placed there several days later. After 10 days they were collected and there were no eggs on the wooden strips.

However, on October 2, 2018, we received a warning call of mosquitoes in a housing estate located northeast of the city. An adult specimen captured by a resident was identified in the laboratory as *Ae. albopictus*, confirming our suspicions. On October 4 we visited the housing estate and, in several places (Fig. 2), both in common areas and in private gardens, we found larvae - in various stages of development - and pupae of the tiger mosquito (up to 12 larvae/dipper, vol: 300 ml). According to the information provided by several residents of the housing estate, the discomfort and bites caused by these mosquitoes began at the end of the summer. This is when many citizens of Cordoba return from their summer holidays, so it seems very likely that the tiger mosquito was passively transported by car, inadvertently, by a resident or residents of this housing development. The importance of the passive dispersion of the tiger mosquito in cars has been well documented and demonstrated in other regions of Spain (Eritja *et al.*, 2017).

Figure 2: Breeding habitat of tiger mosquito (*Aedes albopictus*), where eggs, larvae or pupae have been found, in the city of Cordoba.

On October 9, two CDC traps baited with attractant were installed in order to estimate the abundance of adults in this housing estate. Although the CDC traps were not designed for the monitoring of *Ae. albopictus* and do not provide good results, they were taken advantage of since they are used for the monitoring of Simuliidae and Ceratopogonidae within the hematophagous Diptera programme. The traps were installed in the common area (a garden next to a swimming pool) and in the back garden of one of the residents. They were collected 24 hours later, with ten adult females captured (6 and 4 from the common area and back garden, respectively). Over the subsequent days - as a consequence of press releases in local media (radio, television and newspapers) - we received several notifications from housing estates near the first captures, all located in the northern sector of the city of Cordoba. Four additional confirmed localities were found, from which adults, eggs, larvae and pupae were collected, expanding the known distribution of the species, which had occupied a larger area than expected.

After press reports of the presence of the invasive mosquito in Cordoba, Dr. Francisco Collantes (University of Murcia) contacted to inform us that, in their routine sampling for the surveillance of tiger mosquitoes at a national scale, at the end of August they had placed an oviposition trap near the Avda. del Brillante, in the city of Cordoba. Several days later they collected the trap and it gave a positive result, with 68 eggs (F. Collantes pers. comm.)

The presence in the housing estate of abundant water, in manholes and drainage channels of the common areas as well as in private terraces and patios (plates underneath pots, plastic pools and containers of all kinds with stored water), has provided a suitable substrate for the reproduction of *Ae. albopictus* after its arrival. The authors of this work, recommend some preventive practices to avoid the reproduction of the species, included in an informative leaflet for the citizens (https://www.sadeco.es/storage/files/medias/2018/10/tripticomosquitotigre-baja.pdf). In addition, the SADECO technicians performed a treatment with *Bti* (Vectobac G, Kenogard®) in the water-collecting vessels of the common areas in the localities where the presence of the mosquito was confirmed.

Discussion

Since *Ae. albopictus* was introduced into Europe, via Albania, it has spread throughout the continent, Portugal being the most recent European country cited, in 2017 (Osório *et al*., 2018; Marabuto & Rebelo, 2018). Although the dispersal capacity of *Ae. albopictus* females is very limited, the maximum dispersion being 400-600 m according to capture-tag-recapture studies (Rosen *et al*., 1976; Niebylski & Craig, 1994), the rapid and constant expansion of this species throughout the European continent, especially in Mediterranean countries, can be explained by its enormous capacity to travel as a passive dispersant in private vehicles (Eritja *et al*., 2017) or in cargoes transported by aeroplane or by sea (Lucientes & Molina, 2017). Therefore, it is likely that the species arrived in Cordoba from the coast of Malaga, which is very popular among the population of Cordoba as a holiday area and for second homes. Its confirmed presence poses an important challenge for the local administration. The increase in people travelling to countries in Africa and Latin America - where *Ae. albopictus* and/or *A. aegypti*, as well as several pathogens they transmit (Chikungunya, Dengue and Zika), are endemic - has produced a considerable number of cases of travellers returning infected to their countries of origin (in Europe) in the last few decades. In addition, some studies, in both natural and experimental conditions, have shown that *Ae. albopictus* can act as a secondary vector of West Nile virus (WNV), in areas with a high population density of this mosquito (Holick *et al*., 2002; Paupy *et al*., 2009; Fortuna *et al*., 2015; Vogels *et al*., 2017). The unusually large number of autochthonous human WNV infections reported in Europe and neighbouring countries in 2018 (ECDC, 2018b) and the geographical location of Spain, on a usual route of migration for many species of bird (the primary hosts of WNV), are a cause for concern.

In recent years the tiger mosquito has invaded and established populations in different parts of Europe (ECDC, 2018a). The occurrence in these areas of people infected during travel and of the tiger mosquito has led to outbreaks of Chikungunya disease originating from autochthonous transmissions of the virus; that is, people infected in European countries - like Italy (102 cases in the Lazio region in 2017), France (11 cases in the Department of Var) (ECDC, 2017) or Spain (5 cases confirmed in October and November 2018) (ECDC, 2018c) - by mosquito bites without having travelled to countries where the virus is indigenous. If we add the growing migratory flow from Africa to Spain, then we must consider the risk of indigenous transmission of viruses by tiger mosquito bites in areas where it maintains viable populations. Therefore, the expansion of this invasive species has serious implications for public health. It is not certain that *Ae. albopictus* will establish viable populations in Cordoba that may persist in the medium and long term, since it may not survive the cold winter months. However, winters are becoming shorter and milder here and this trend will continue in the future due to climate change. Also, it is likely that recolonisations from the coast of Malaga will occur during the summer, giving rise to new populations that would persist at least until the arrival of the first cold snap of winter.

The artificial conditions in which vegetation and turf are maintained, in the common areas of the housing estates where the foci have been located, favour the presence and establishment of this invasive anthropophilic mosquito, and of other exotic species such as the ant *Tetramorium bicarinatum* (Nylander, 1846), also from southeast Asia (Reyes & Espadaler, 2005; Reyes & Obregón, 2018).

Once again, the importance of citizen collaboration in the detection of invasive mosquito species has been demonstrated. In the case of Cordoba, the ovitrap surveillance within the programme of early detection of invasive mosquitos did not allow detection of *Ae. albopictus* in the first instance; it was detected due to the local citizen advice network and the information campaigns launched in 2016 in this city. For this reason, we would like to highlight the importance of the involvement of local communities in early detection programmes.

Therefore, it is of the utmost importance to continue working to achieve the early detection of new outbreaks and the application of measures to prevent and control the expansion of this invasive species and the risks related to public health. This article will be useful for researchers, administrators and pest control and management companies.

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