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ARTICLE

First confirmed outdoor winter reproductive activity of Asian tiger mosquito (*Aedes albopictus*) in Europe

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Resumen

Primer registro de reproducción al aire libre en Europa del mosquito tigre (Aedes albopictus)

Es la primera vez que se registra la continuidad en invierno del ciclo reproductivo de *Aedes albopictus* en Europa. Se discute sobre el empleo de la variable precipitación en los modelos de riesgo de implantación de mosquito tigre. Pensamos que la disponibilidad local de agua para estos insectos no depende siempre de la lluvia.

Palabras clave: Reproducción invernal, Clima, Culicidae, Modelo, Lluvia.

Abstract

This is the first record about the continuity of reproductive cycle of *Aedes albopictus* in winter for Europe. Also the variable rainfall included in the risk models for suitability of Asian tiger mosquito is discussed. We think that local water availability for this insects is not always rainfall dependent.

Key words: Winter Reproduction, Climate, Culicidae, Model, Rainfall.

Introduction

In the 21st century, prevention and control of vector-borne diseases emergence and resurgence represent a critical challenge for the global health system. This issue arises from the phenomenon of economic globalization and it is affected by multiple anthropogenic factors which predispose to its occurrence. The most relevant of these factors are the increase of international travels, migration phenomena and climate change (Busquets 2010).

Some species of invader mosquitoes from remote areas are playing an undeniable role in this context, and *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) is, maybe, one of the leading actors. During the last decades, this mosquito has been able to expand its global range of distribution to a great extent, so that nowadays it is considered as one of the one hundred worst invasive alien species of the world by the Global Invasive Species Database (Lowe et al. 2000).

In Spain, this species has colonized the majority of the coastal Mediterranean area ever since it arrived in 2004 (Aranda et al. 2006). At present, this mosquito is established in the provinces of Gerona, Barcelona and Tarragona (Catalonia) (Torrell-Sorio & Fernàndez-Rodríguez 2008), Castellón, Valencia and Alicante (Valencian Community) (Delacour et al. 2009, Delacour-Estrella et al. 2010, Alarcón-Elbal et al. 2013, Bueno-Marí et al. 2013, Lucientes-Curdi et al. 2014), the Region of Murcia (Collantes & Delgado 2011) and the

Balearic Islands (Miquel et al. 2013), and it is threatening to colonize the province of Valencia and the region of Andalusia (including the Atlantic watershed).

Oddly enough, risk establishment models proposed up to now do not identify the Mediterranean coast of the Iberian Peninsula as a suitable area for *Ae. albopictus*. Also, in these models it has not been taken into account that this species was able to keep its cycle in mild winters, which are typical of this area. As far as we know, the ability of *Ae. albopictus* to grow in artificial water microhabitats in conjunction with some environmental factors may have not only exacerbated the establishment of this species in new sites, but also originated phenology and ecological niche shifts (Medley 2010, Caminade et al. 2012). This seems to be what it is happening with the studied populations in the present work. The aforementioned phenology shifts would be outstanding for epidemiology, since this new situation could entail an extension of the potential risk period for arbovirus transmission.

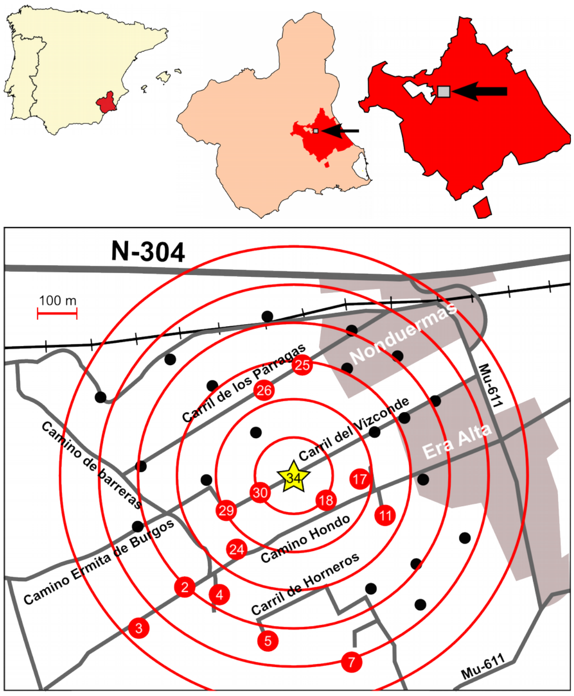

Figura 1. Ovitrampas estudiadas durante el invierno (n=14) (sólo las positivas antes del invierno). ☆: Punto inicial de 2011. ●: trampas negativas en 2012 antes del invierno.

Figure 1: Studied ovitraps on winter (n=14) (Positive ones before winter). ☆: First point 2011. ●: Negative traps in 2012, before winter.

Materials and methods

At the beginning of September 2011, some specimens of *Ae. albopictus* were caught in a semi- detached house located in the peri-urban area of Murcia city (Southeastern Spain). When they were identified, at the end of September, an emergency sampling was made around the house although no more specimens were collected (Collantes & Delgado 2011). The following year, in the same house, a BG-SentinelTM trap was working during July, August and September and females were also caught by the owner using human bait. In this second sampling period, ten larvae were collected in an adjoining house. As the BG-Sentinel trap was continuously colleting adult specimens, we planned a new extensive sampling with ovitraps so as to know if *Ae. albopictus* had enlarged its local range and also to keep the study for a longer period.

Concentrical circles were drawn around the initial place, increasing their radius by 100 m in each circle up to 580 m, which was the maximum distance. 33 ovitraps were placed (35 at the beginning) as can be seen in Figure 1. These ovitraps were larger than usual, with an 11 cm diameter at the opening and a capacity of 1 l. Also, some larvae were collected from rain barrels in some sampling places. The survey began in October 3rd-5th 2012 (3 days were required for placing the ovitraps) and the last collection took place in March 1st 2013. At the beginning, the collecting frequency was about every two weeks. From the 19th November on, only the 14 positive ovitraps were kept active (Fig.1), and the collecting frequency was decreased.

Results

During the months of July, August and September 2012, female specimens collected using human bait and BG-Sentinel trap were not abundant though almost constant (20 collections with 33 specimens).

In the ovitraps sampling, 14 traps were positive and 4 of them were repetitive. The maximum distance from the initial detection point to the furthest positive trap was the max. length initially planned (580 m). The max. distance between positive traps was 840 m. This seems to indicate that the real colonized area is larger than the studied area, which could not be larger due to inadequate budget.

Throughout the unfavorable winter time (December 2012 to March 2013), only the 14 positive and 7 of them were repetitive. The max. distance from the initial detection point to the furthest positive trap was the max. length initially planned (580 m). The max. distance between positive traps was 840 m. This seems to indicate that the real colonized area is larger than the studied area, which could not be larger due to inadequate budget. As can be seen in Table 1, during the unfavorable winter time (December 2012 to March 2013), only the 14 positive ovitraps were monitored and we surprisingly found eggs (some times already opened) or larvae in 5 points (the point No. 18 was considered as positive since, although the ovitrap was broken, alive larvae were found in rain barrels there). Some of positive ovitraps (No. 2, 3 and 4) were removed on December by owners, so it may not be asserted if they are negative or positive on winter.

Point	19/IX/2012	19/XII/2012	17/I/2013	01/III/2013
2	-	R	R	R
3	-	R	R	R
4	1 larva	R	R	R
5	-	50 eggs	20 eggs	25 eggs
7	-	-	-	-
11	4 eggs	*	-	-
17	2 larvae	*	5 eggs	-
18	+	+	+	-
24	-	-	-	-
25	-	-	-	-
26	-	*	*	-
29	11 eggs	-	-	41 eggs
30	-	-	R	R
34	+	-	-	-

Tabla 1: Ovitrampas estudiadas durante el invierno (n=14) (Sólo las que fueron positivas antes del invierno). + Larvas en barriles; * Muestras perdidas (trampa vacía, rota, etc.,); R removidas por el propietario.

Table 1: Studied ovitraps on winter (n=14) (Positive ones before winter). + Larvae in rain barrels; * Lost samples (empty, broken, etc.); R Removed by owner.

Some eggs were stimulated for hatching and the larvae were identified following the identification key provided by Schaffner et al. (2001).

This interesting result does not only show the existence of established populations in the Region of Murcia, but it is also the first record of an extended winter outdoor reproductive activity of *Ae. albopictus* in Europe. Previous to our finding, Romi et al. (2006) reported the first data on female overwintering and ovoposition activity in Europe, but the authors do not collected hatching larvae in ovitraps during the monitored winter season and were not able to stimulate hatching in the lab. These authors considered two hypotheses for the origin of the active females: a long-lived adult generation, which could emerge even in the cold December of Rome or several reproductive cycles, occurring in indoor habitats. Our results suggest that, at least under the favorable winter conditions of Southern Europe, the first of these situations is a fact.

Our results suggest that risk models do not fit with that observed at the studied area. Likewise, the capacity of *Ae. albopictus* for enlarging its populations has been underestimated compared with those populations which only reproduce in summer. For these reasons, this matter requires a careful discussion.

Discussion

Recently, there have been proposed several risk models for the suitability of *Ae. albopictus* in Europe (ECDC 2009, Carminade et. al. 2012, ECDC 2012a) or in certain areas of this continent (UK: Medlock et al. 2006; Italia: Neteler et. al. 2011, Roiz et al. 2011; Riviera, France: Tran et al. 2013) as well as in other places of the world (Northeastern USA: Rochlin et al. 2013). In all of them, temperature and rainfall are considered as the most important climatic variables. Rochlin et al. (2013) also include a non-climatic variable, “land use/cover”, which does not predict the suitability for *Ae. albopictus* but which contributes the model to have the best goodness-of-fit. In the discussion, Carminade et al. (2012) only indicate that other socio-economic factors should be considered, but these are not specified. That is precisely the point we want to highlight, because recent risk models, all of them developed using climatic variables, show a low o very low suitability for *Ae. albopictus* in Southeastern Spain. There should be noted the differences between the successive versions of ECDC models (2009 and 2012). In the first one, the estimated risk for Spanish coastal area, from Catalonia to Valencian Community, is very low. After the new records of Asian tiger mosquito in Spain, the model of 2012 estimates a high risk up to the northern half of Alicante province (Valencian Community) but it continues showing a very low risk for the southern half of this province and for the Region of Murcia, because it is an arid area, with low annual rainfall. The recent model of Caminade et al. (2012) also estimates an even lower risk for Southeastern Spain.

However, as it has been stated in the introduction, in spite of these estimations, the spread of *Ae. albopictus* throughout Spanish Mediterranean coastal area seems unstoppable. In the specific case of the Region of Murcia, new southern localities (Bueno-Marí et al. 2012) have been added to the first recorded population around city of Murcia, and this fact confirmed our suspicions about a larger range in the Region of Murcia. In fact, in 2013, many positives localities have been found along nearly all coastal municipalities of Region of Murcia (data unpubliseh yet).

Then, the successive changes in European risk models suggest that the lack of information about *Ae. albopictus* in certain areas is assumed as absence of them. Maybe, models based in maximum entropy (Maxent) could show fitter hypotheses about potential distributions of established invasive species (Gormley et al 2011) and niche shifts in the new localities (Medley 2010).

In our opinion, the apparent contradiction between suitability models and the observed reality lies in the fact that rainfall is overly important in the proposed models for *Ae. albopictus*. We think that both predictive and suitability models have to include water availability as a limiting factor but, even though in many places of Europe it depends on rainfall, this is not always the case. This is not a novel idea and it is surprising that, whereas in many campaigns against Asian tiger mosquito (or dengue and yellow fever) authors always consider that educational programs are indispensable for local people to remove the potential artificial breeding sites (Winch et al. 2002, Heintze et al. 2007, Richars et al. 2008, Abramides et al. 2011, 2013, Fonseca et al. 2013; Diéguez-Fernandez et al. 2013), this aspect is not incorporated as a variable in current models, but it is directly “translated” as the rainfall variable.

In the Southeastern area of the Iberian Peninsula at least (and maybe in other areas of the Mediterranean coast), the so-called “water culture” makes traditional practices and customs to supply water to reproductive cycle of *Ae. albopictus* without the need for high rainfall. As Aguilera-Klink (1995) stated, from the sociocultural perspective, water is more than a natural resource in Southern Spain, it is also a social asset. So, in peri-urban areas as the Huerta area of Murcia (=extensive market garden area) or the residential zones there may be infinite objects which can serve as breeding sites for *Ae. albopictus* and which are deliberately placed by people. In these cases, temperature, not rainfall, is the only limiting factor as long as there are artificial water resources. Evidently, this variable must be included in future predictive models.

The continuous reproductive cycle of *Ae. albopictus* all winter long in the Region of Murcia seems to depend on the permanent water availability and the specially mild winter temperatures. In other places of Europe (Roiz et al. 2010), the temperature threshold for reproductive cycle was found to be 13 ºC and 9 ºC for adult activity. In our research, though there were no thermometers with the ovitraps, we have temperature data from the nearby weather station of Alcantarilla (37.9538ºN 1.2314ºW; distance ±5km). During the survey, monthly mean temperatures were 20.8 to 12.8 ºC and monthly absolute minimum temperatures were 4.2 to -0.5 ºC. By and large, monthly temperatures were slightly warmer than usual (about 2 ºC higher), compared to weather data series, but not extraordinary. As a result, considering not only mean usual temperature but also extreme recorded data, this winter was slightly warmer but it cannot be regarded as an exceptional anomaly. The question which remains unanswered is whether this small peak temperature has exceptionally allowed the continuation of reproductive cycle of *Ae. albopictus* this winter in Southeastern Spain or if, in the following years, the Asian tiger mosquito could continue its reproductive cycle in winter. The second scenario would dangerously increase the epidemiological risk, inasmuch as it increases the chance for populations of *Ae. albopictus* to go up too and spread more than they would in an overwinter scenario (ECDC 2012b).

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