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Source: Journal of Medical Entomology, 48(4):956-960. 2011.

Published By: Entomological Society of America

DOI: 10.1603/ME11016

URL: <http://www.bioone.org/doi/full/10.1603/ME11016>

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## Host-Feeding Patterns of Native *Culex pipiens* and Invasive *Aedes albopictus* Mosquitoes (Diptera: Culicidae) in Urban Zones From Barcelona, Spain

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J. Med. Entomol. 48(4): 956–960 (2011); DOI: 10.1603/ME11016

**ABSTRACT** The feeding patterns of haematophagous arthropods are of major importance in the amplification and transmission of infectious disease agents to vertebrate hosts, including humans. The establishment of new vector populations in nonnative range might alter transmission networks. The Asian tiger mosquito *Aedes albopictus* (Skuse) represents an example of how an invasive species can alter the risk of viral transmission to humans. Blood meal molecular identification from two sympatric mosquito species (the invasive *Ae. albopictus* and the native *Culex pipiens*) was carried out by polymerase chain reaction-based methods. Samples were collected in Barcelona metropolitan area, Spain, from June to October 2009 as part of a monitoring-control program. Blood meals were identified to the species level in 30 *Ae. albopictus* and 43 *Cx. pipiens*. *Ae. albopictus* acquired blood exclusively from human hosts (100%), whereas *Cx. pipiens* fed on a diversity of avian and mammalian hosts, including 35.7% of blood meals from humans. Based on mosquito diet, our results suggest that the *Ae. albopictus* invasion in Spain might increase the risk of virus transmission to humans and could support local outbreaks of imported tropical viruses such as dengue and chikungunya. However, in the studied area, the presence of this invasive species would have a negligible effect on the transmission of zoonotic agents such as West Nile virus. However, *Cx. pipiens* could amplify and transmit West Nile virus, but avian contribution to its diet was lower than that reported in North America. Feeding patterns of these mosquito species may help to understand the flavivirus outbreaks recently reported in southwestern Europe.

**KEY WORDS** host-feeding patterns, metropolitan areas, invasive species, disease transmission

Feeding patterns of haematophagous arthropods have important consequences for the amplification and transmission of diseases to vertebrate hosts. Knowledge of the feeding patterns of these vectors can help to define efficient disease-control policies, decrease the risks of outbreaks, and attain better knowledge of ecoepidemiology in both humans and other species of interest (Dye and Hasibeder 1986). The Asian tiger mosquito *Aedes albopictus* (Skuse) and the northern house mosquito *Culex pipiens* are considered impor-

tant vectors transmitting pathogens that affect both human and animal health (Becker et al. 2003). *Ae. albopictus* is native to East Asia and is a major vector of human arboviruses such as chikungunya and dengue in tropical and nontropical areas (Aranda et al. 2006, Martin et al. 2010), where it has caused several outbreaks in recent years (e.g., the island of La Reunion, Renault et al. 2007; Italy, Rezza et al. 2007). This mosquito species has invaded ~10 European countries over the last 20 yr (Aranda et al. 2006). *Cx. pipiens*, however, is a cosmopolitan species, although its invasiveness in North America and other areas around the world is currently still under discussion (Vinogradova 2000). It is an important vector for West Nile virus (WNV) transmission (Turell et al. 2001, Fonseca et al. 2004, Kilpatrick et al. 2005, Hamer et al. 2008), but it is not competent for dengue and some other tropical viruses (Vazeille et al. 2008, Reiter 2010). Currently, both mosquito species coexist in several European countries, including Spain, since 2004 (Aranda et al. 2006). Additionally, several WNV outbreaks have been detected in different parts of Europe over the last two decades (Hubalek and Halouzka 1999, Hayes et al. 2005), and its circulation has been recurrently re-

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ported in Spain in recent years (Figuerola et al. 2007, Jiménez-Clavero et al. 2008).

The incorporation of molecular techniques such as polymerase chain reaction (PCR) and sequencing into the study of mosquito ecology, as a complementary tool to the serological methods, has revealed a broad range of host species, and important local and seasonal differences in diet composition in the wild (Kilpatrick et al. 2007, Gómez-Díaz and Figuerola 2010). Particularly, the feeding patterns of *Ae. albopictus* are unclear. In some cases it has been identified as a human feeder species (e.g., Thailand, Ponlawat and Harrington 2005), but it has also been reported as a generalist (e.g., the United States, Savage et al. 1993, Richards et al. 2006; Japan, Kim et al. 2009; Italy, Valerio et al. 2010). The contrasting feeding patterns found at different nonnative geographical regions, and its role as a vector of several emergent diseases, make necessary assessing its host-feeding composition in newly invaded areas as a means of understanding the local risk of exotic arbovirus outbreaks in humans. However, studies addressing the feeding patterns of *Ae. albopictus* in Europe are still scarce (see Valerio et al. 2010).

To our knowledge, only Sawabe et al. (2010) have compared *Ae. albopictus* and *Cx. pipiens* feeding patterns in their native range. The objective of the current study was to estimate the feeding patterns and compare the potential role of *Ae. albopictus* and *Cx. pipiens* in the amplification and transmission to humans of mosquito-borne diseases (e.g., WNV, dengue, and chikungunya) in Barcelona metropolitan areas.

## Materials and Methods

**Sampling Areas and Mosquito Collection.** Mosquitoes were collected from five municipalities located within the Barcelona metropolitan area (Table 1) between June and October 2009 as part of a monitoring-control program carried out by the Barcelona Agency of Public Health, and a study of human social behavior and the presence of mosquitoes conducted by the Baix Llobregat Regional Council, the University of Barcelona, and the Barcelona Provincial Council. Sampling sites included private and public gardens where humans, domestic animals (i.e., dogs, cats, chickens), and free-living garden bird species (i.e., house sparrow, common blackbird, common wood pigeon, Eurasian collared dove) are common. All samples except one (the single female captured in El Prat de Llobregat, which was hand collected) were collected outdoors using BG Lure attractant traps (BioGentsAG, www.biogents.com, Regensbourg, Germany). Traps were operational continuously for 24 h every day throughout the whole study period, and were sampled on a weekly basis without being removed. Samples transported to the laboratory were killed by freezing, sorted, and identified using specific descriptive keys (Schaffner et al. 2001). Blood-fed females were sorted by visual clues that differed between species. Whereas *Cx. pipiens* females are easy to identify by distended abdomens containing reddish or dark blood meals,

**Table 1.** Samples tested and host vertebrate species identified from blood meals (scientist–common name) captured in Barcelona metropolitan area

Mosquito species	No. tested (no. identified)	Host species
<i>Aedes albopictus</i>	172 (30)	<i>Homo sapiens</i> –human
<i>Culex pipiens</i>	65 (43)	<b><i>Canis lupus familiaris</i>–dog (14.3%)</b>
		<i>Columba palumbus</i> –pigeon (4.8%)
		<b><i>Felis catus</i>–cat (21.4%)</b>
		<i>Gallus gallus</i> –chicken (2.4%)
		<b><i>Homo sapiens</i>–human (35.7%)</b>
		<i>Myiopsitta monachus</i> –monk parakeet (2.4%)
		<i>Passer domesticus</i> –house sparrow (4.8%)
		<i>Streptopelia decaocto</i> –Eurasian collared dove (9.5%)
		<i>Turdus merula</i> –common blackbird (4.8%)

No. tested, number of blood-fed females detected visually; no. identified, number of blood-fed females detected by the PCR. Percentages correspond to the number of identified fed females (i.e., those PCR amplified and sequenced). In *Culex pipiens*, the most frequent host species are indicated in bold.

blood-fed females of *Ae. albopictus* could only be detected by their relatively enlarged abdomens, as the dark natural tegument prevents spotting of any digestive content. Blood-fed females were transferred into individual 1.5-ml Eppendorf tubes and stored at  $-80^{\circ}\text{C}$  until genetic analyses. A total of 237 *Cx. pipiens* and *Ae. albopictus* females was tested for blood meal composition.

**Blood Meal Molecular Identification and Disease Transmission.** Mosquito abdomens were individually removed from the rest of the body and placed into individual PCR tubes using two sterile tips. DNA isolation and PCR and sequencing of the cytochrome *c* oxidase subunit I (COI) were performed in an identical way, as described Alcaide et al. (2009). As the PCR protocol from Alcaide et al. (2009) produced two PCR products (i.e., one unspecific extra locus) for *Ae. albopictus*, sequencing reactions were performed using the newly designed primer BCVINT-RV (5'-ATRTANACYTCN GGR TGN CC-3'). This de novo primer was designed from a sequence alignment containing both mosquito and vertebrate sequences to avoid the sequencing of the Asian tiger mosquito DNA. We validated the new primer through blood meal samples obtained from different mosquito species with different host vertebrate species. Sequences were analyzed and annotated using the software Sequencher v.4.5 (Gene Codes, ©1991–2005, Ann Arbor, MI), and identified by comparison with the GenBank DNA sequence database (National Center for Biotechnology Information Blast) and the BOLD Systems platform (<http://www.boldsystems.org/views/login.php>) to assign unknown COI sequences to particular vertebrate species. Positive identification and host species assignment were accomplished when exact or nearly exact matches (>98%) were obtained.

Additionally, to check for the presence/absence of mixed blood meals of both avian and mammal blood, we designed two de novo avian-specific primers (Avian-CYTB-FW 5'-GAY AAA ATY CCM TTY CAC C-3' and AvianCYTB-RV 5'-TGT TCD ACD GGY TGG CT-3') that targeted the mitochondrial cytochrome *b* gene. Primers were validated using several blood meals originating from bird species according to the identification of the COI locus. A fragment of 389 bp was amplified by PCR under the following conditions: a cycle of 3 min at 94°C, followed by 35 cycles of 45 s at 94°C, 60 s at 45°C, and 60 s at 72°C, with a final step of 5 min at 72°C.

### Results

We successfully amplified and sequenced vertebrate COI locus in 30 *Ae. albopictus* and 43 *Cx. pipiens* blood-fed individuals. Amplification success was lower for *Ae. albopictus* because blood-engorged females are difficult to identify (Ponlawat and Harrington 2005), as most of them already contained eggs and no sign of fresh vertebrate blood. All blood meals came from a single vertebrate species, and no mixed blood meal or contamination (i.e., extraction and PCR blank that were used during the molecular analyses) was detected. All blood ingested by *Ae. albopictus* was identified as originating from humans. By contrast, *Cx. pipiens* blood meals came from both mammals and birds (up to nine different species; see Table 1). Human and common pets (e.g., dogs and cats) were the main hosts detected. The screening for the presence of mixed blood in both *Cx. pipiens* and *Ae. albopictus* abdomens only resulted in PCR amplification for those samples labeled as bird by the COI locus. No amplification was obtained from blood meals assigned to mammals, which confirmed the absence of mammalian/avian mixed blood meals.

### Discussion

*Cx. pipiens* is a very common mosquito species in the Barcelona metropolitan area, recently invaded by *Ae. albopictus*. In contrast to the generalist behavior reported for *Ae. albopictus* in previous studies from its native (Kim et al. 2009, Niebylski et al. 1994, Savage et al. 1993) and invaded areas (Sawabe et al. 2010, Valerio et al. 2010), our preliminary results indicate that this invasive species feeds mainly on human blood in Barcelona metropolitan area. Even though mosquitoes were trapped with BG Lure traps (an attractant that contain several substances present on human skin), we are confident that the use of this type of traps has not biased our results toward more antropophilic females. In an independent field trapping experiment, capturing different mosquito species, we have not found any effect of the attractant used on the frequency of avian- or mammal-fed females in Doñana Natural Space (SW Spain; D. Roiz, M. Roussel, J. M., S. Ruiz, R. Sorignier, and J. F., unpublished data). Although the Asian tiger mosquito is considered a secondary vector for dengue virus in its native range

(Rodhain and Rosen 1997), our findings are important because this mosquito species has been identified as the vector of the 2005–2007 chikungunya epidemic outbreak on La Reunion (French territories), which resulted in >250,000 clinical cases and >400 fatalities. In addition, a local chikungunya outbreak in continental Europe occurred in Ravenna, Italy, in 2007. Cases of dengue fever have been detected in travelers returning to France (Gautret et al. 2010), and the first modern case of local transmission of this disease has recently been reported in Nice, France (La Roche et al. 2010). All these recent cases highlight the fact that established *Ae. albopictus* populations in Europe represent a real risk when they overlap in space and time with imported nonnative viruses. The replication of dengue and chikungunya in *Ae. albopictus* and its anthropophilic feeding behavior in Barcelona would indicate that a risk of transmission to humans exists given the likelihood that *Ae. albopictus* feeds on a susceptible human reservoir (see also Sawabe et al. 2010).

*Cx. pipiens* is considered an important enzootic vector in North America and the main vector in WNV outbreaks in Europe (Dauphin et al. 2004, Fonseca et al. 2004). Among other factors, its feeding preferences have been identified as a potential factor explaining WNV dynamics in the United States (Kilpatrick et al. 2006a,b) both by its effects on virus amplification through bird-to-bird transmission and through the potential to act as a bridge when feeding on humans after having fed on birds. Pathogen population growth rate ( $R_0$ ) depends on factors such as the host infectious period, vector survival, and host and vector infections, as well as on vector-feeding patterns (Wonham et al. 2004). Assuming that all the other parameters remain constant, the relative impact of mosquito diet on the  $R_0$  parameter varies with the square of the proportion of vectors feeding on competent hosts (see Kilpatrick et al. 2007). Consequently, mosquito populations more extensively feeding on birds would have a larger capacity to amplify WNV because humans and other mammals do not support high enough viremias to infect mosquitoes (see Platt et al. 2007).

The *Cx. pipiens* feeding patterns reported in our study area (i.e., Barcelona) indicate a diverse diet with a lower frequency of avian blood meals (28.6%) than those reported in other areas, and consequently, a more reduced potential for WNV amplification. For instance, in the United States, bird blood conforms 69–97% of the diet of *Cx. pipiens* (Gómez-Díaz and Figuerola 2010). In Europe, in the Doñana Natural Space, a natural wetland in southern Spain where WNV circulation has been recorded for several years (Figuerola et al. 2007, Vázquez et al. 2010), the contribution of birds to the diet of *Cx. pipiens* was ~64% (Alcaide et al. 2009). Although the proportion of avian blood meals was relatively low in Barcelona, human-derived blood meals form an important proportion of *Cx. pipiens* diet (35.7%). This value was higher than in other regions such as Chicago, Illinois or Tokyo, Japan (Hamer et al. 2009, Kim and Tsuda 2010), but lower than in Japan urban/suburban areas (43.3–82.6%, Sawabe et al. 2010). Both birds and humans form part of

the diet of *Cx. pipiens*, meaning that although amplification potential of WNV is lower than in other areas, transmission of the virus from birds to humans in Barcelona is possible. This preliminary observation could have important implications as a zoonosis like WNV might become an epizoonosis in this region.

In conclusion, the diet composition of *Ae. Albopictus* in Barcelona suggests that the presence of this invasive species represents a high risk for local transmission of dengue and chikungunya from imported cases, but it seems not to have altered the risk of WNV local transmission. In fact, our preliminary results on the diet of *Cx. pipiens* could allow a reduced amplification of WNV, but suggest that transmission of virus from birds to humans is possible. We believe that the role of mosquito diet in explaining differences of WNV in Europe merits further attention in the future.

### Acknowledgments

This work was the result of an agreement between the Consejo Superior de Investigaciones Científicas and Agència de Salut Pública de Barcelona. Samples from the town of Castelldefels were collected during a study supported by the Diputació de Barcelona and the municipality (Project Code 09/Y/48172). The Generalitat de Catalunya Departament de Medi Ambient collaborated in the sampling of the city of Barcelona. David Roiz provided constructive criticism of an earlier version of this manuscript. J.F., J.M., and R.C.S. were supported by the Junta de Andalucía research project on West Nile virus ecology in relation to mosquito feeding patterns (P07-RNM-02511 and RNM-118) and the European Commission EuroWestNile FP7 Project (261391).

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Received 25 January 2011; accepted 12 May 2011.