Scientific Note

First establishment of *Aedes japonicus japonicus* (Theobald, 1901) (Diptera: Culicidae) in France in 2013 and its impact on public health

Thomas Krebs[1], Philippe Bindler[1], Grégory L'Ambert[2,3], Céline Toty[3,4], Yvon Perrin[3], and Frédéric Jourdain[3]✉

[1]*Brigade Verte du Haut-Rhin, Service de démoustication, 68360 Soultz, France*
[2]*Entente Interdépartementale pour la Démoustication, 34184 Montpellier, France*
[3]*Centre National d'Expertise sur les Vecteurs, 34394 Montpellier, France, frederic.jourdain@ird.fr*
[4]*Institut de Recherche pour le Développement (UMR MIVEGEC UM1-CNRS 5290-IRD 224), 34394 Montpellier, France*

Aedes japonicus japonicus is an invasive mosquito species native to eastern Asia. The species has been detected in a number of France's neighboring countries and is considered as established in Austria, Belgium, Croatia, Germany, Netherlands, Slovenia, and Switzerland (Kampen and Werner 2014).

For several years, the French Ministry of Health has been coordinating a national plan for preparedness and response to autochthonous circulation of dengue and chikungunya viruses. The entomological component of this integrated strategy is intended to monitor the dispersion of *Aedes albopictus* (Skuse, 1895). However, a secondary objective of entomological surveillance aims to detect the introduction of other invasive species, such as *Ae. j. japonicus*. The introduction of the species has been monitored in eastern France since 2010 from May to November for that purpose. The main criteria for active surveillance implementation are the distance to the colonized area, the presence of at-risk activities, the importance of points of entry, and human density (Centre national d'expertise sur les vecteurs 2012).

Some specifics of the geographic and socioeconomic context can be highlighted, such as the presence of an international airport (Basel-Mulhouse), three borders (France, Germany, and Switzerland), a highly industrialized and densely populated urban area with more than 200,000 inhabitants in Basel (S), Weil-Am-Rhein (Ge), and Saint-Louis (F), and an alluvial wetland classified as natural reserve (Petite Camargue Alsacienne). Therefore, surveillance and potential risk management measures must work within regulatory, organizational, and environmental limitations.

In 2013, nine cemeteries were selected for surveillance of *Ae. j. japonicus* close to the areas in Switzerland and Germany where the mosquito has been established since 2008 (Schaffner et al. 2009, Huber et al. 2012). Priority was given to cemeteries of municipalities located in the vicinity of cross-border forested areas and major traffic links. A search for larva was carried out once a month from July to September. Mosquito Magnet® Liberty Plus (MM) lured with octenol and BG-Sentinel® traps (BG; BioGents, Regensburg, Germany) baited with CO_2 at a flow rate of 500 g/24 h and BG-Lure® were used for trapping adults. Pupae were reared to the adult stage for morphological identification. Larvae and adults were identified using determination keys (Schaffner et al. 2001, European Centre for Disease Prevention and Control 2012). Identification was confirmed by PCR amplification and sequencing of the cytochrome c oxidase subunit 1 (COI) gene with primers designed by Simon et al. (1994). The sequences obtained were aligned and compared with sequences of Genbank.

During the first inspection in early July, 2013, eight larvae and ten pupae were collected in a dark stone vase in the Hesingue cemetery (Haut-Rhin, France). Mosquitoes were morphologically identified as *Ae. japonicus*. An additional 51 larvae and 54 pupae were collected during routine surveys in 11 breeding sites throughout the summer until mid-October within a radius of 6 km (Figure 1). The main breeding sites were 100 liter barrels. Larvae were also collected in smaller containers such as buckets or tires containing water that ranged from clear to loaded with organic matter. Associated species were *Anopheles plumbeus* Stephens, 1828, *Culex pipiens* Linnaeus, 1758, *Culiseta annulata* (Schrank, 1776), and *Aedes geniculatus* (Olivier, 1791) in forest areas and *Cx. pipiens* and *Cx. hortensis* Ficalbi, 1889 in urban areas. One BG and one MM trap ran for two weeks in a cemetery hedge. Traps were collected every 48 h but failed to capture any adult specimens. The identification of specimens as *Ae. j.* japonicus was confirmed by molecular analysis. Molecular work generated eight sequences of 703 bp available in Genbank under accession numbers KF874592 to KF874599. These sequences showing 99% similarity with sequences of the COI gene of *Ae. j. japonicus* species (accession numbers: USA: HQ978777, HQ978778, JX259639 to JX259646, GQ25793 to GQ54801; Germany: JX888952 to JX888994 and JQ404435; Canada: GU907917 to GU90922; China: JQ728068, JQ728069, JQ728181; Belgium: FJ641869; and Japan: AB690836). Two haplotypes were found in samples from France, in addition to the 12 previously described haplotypes.

Previous reviews on the risks of invasive mosquitoes in Europe exist (Medlock et al. 2012, Schaffner et al. 2013). However, any detection should (1) update knowledge, (2) prioritize risks in a local context, and (3) redefine the objectives of entomological surveillance in relation to specific detection. To address these issues, the following main parameters are considered herein: distribution, density, population dynamics, trophic preferences, human biting rate, and vector competence.

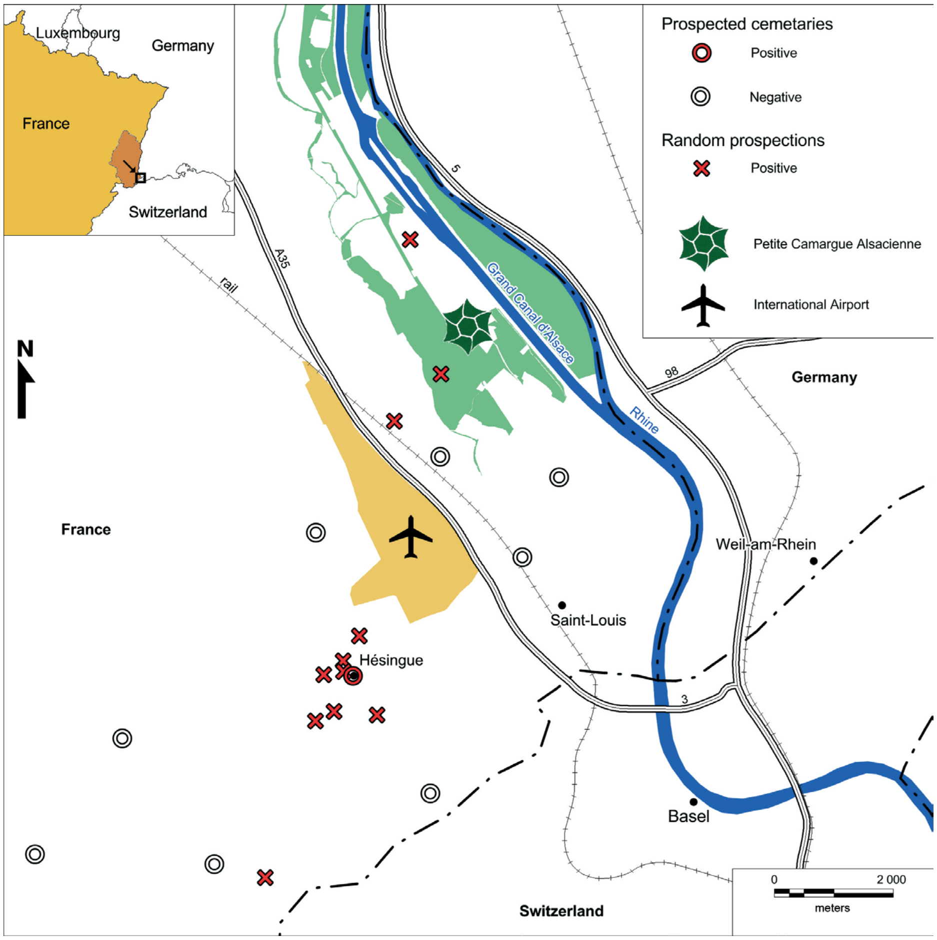

Figure 1. Collection sites of *Aedes japonicus japonicus* in eastern France.

Aedes j. japonicus is considered to be mainly mammalophilic and there is strong evidence for their significant proportion of blood meals on humans (Apperson et al. 2004, Molaei et al. 2008, Molaei et al. 2009). However, evidence is also scarce for feeding on birds. No specimen with an avian blood meal has been caught in the field and only laboratory observations suggest the occurrence of this behavior (Sardelis et al. 2002a, Sardelis et al. 2003, Williges et al. 2008). Detection of West Nile virus (WNV) in field-collected *Ae. japonicus* does not constitute conclusive evidence of avian feeding, whereas the existence of mammal reservoirs for WNV in North America is currently being discussed (Root 2013).

In its native range, the species is not considered as particularly aggressive toward humans (Tanaka et al. 1979). However, some nuisances in sylvan areas were observed in Switzerland and Germany, thereby detecting the establishment of this species (Schaffner et al. 2011, Kampen et al. 2012).

Vector competence has been demonstrated for different arboviruses. *Aedes japonicus* appears to be a more efficient vector than *Cx. pipiens* for WNV in laboratory studies (Turell et al. 2001). Experimental transmission has also been shown for eastern equine encephalitis virus (Sardelis et al. 2002a), La Crosse virus (Sardelis *et al.* 2002b), St. Louis encephalitis virus (Sardelis *et al.* 2003), dengue 2 virus and Chikungunya virus (Schaffner et al. 2011), Rift Valley fever virus (Turell et al. 2013) and Getah virus (Takashima and Hashimoto 1985). Nonetheless, *Ae. j. japonicus* has never been involved with the transmission of these pathogens in the field (Kampen and Werner 2014, Kaufman and Fonseca 2014).

The species is suspected to be a vector of Japanese encephalitis virus (JEV) in the field and vertical transmission has been demonstrated under laboratory conditions for this virus (Grascenkov 1964, Takashima and Rosen 1989). Several field-collected specimens were positive to WNV (Centers for Disease Control and Prevention 2012), suggesting a potential role of bridge vector. However, field data indicate a minor role for such a species in the transmission of WNV (Andreadis 2012).

Establishment of *Ae. j. japonicus* in France

Specimens were collected over a continuous and wide area of France, with respect to an exotic species. Based on the current distribution of *Ae. j. japonicus* in Europe, it is reasonable to hypothesize that populations established in Germany and Switzerland since 2008 spread to France. Thus, the introduction of this mosquito in France is not considered an introduction as such, usually defined as an invasion process (Juliano and Lounibos 2005), but follows an introduction stage that has already taken place across the national borders where an establishment phase has also occurred. This means that eradication of the population is not considered a realistic option (Simberloff 2003).

Epidemiological considerations

Aedes j. japonicus is an invasive species and a pest, but is not recognized as a primary vector of arboviruses. Its vector role remains unclear and uncertain, especially in the United States and Europe (Kaufman and Fonseca 2014). On the other hand, scientific evidence (vector competence, aggressiveness, and trophic preferences) suggest a potential threat for arbovirus transmission, in particular for flaviviruses of the Japanese encephalitis complex (WNV, JEV, and SLE). This raises the question of Usutu virus transmission by *Ae. j. japonicus* since this virus is emerging in Europe and it is part of the JEV complex. Moreover, the introduction of this species in a new environment may trigger changes in the transmission dynamics of some pathogens (endemic or regularly imported), but it is obviously impossible to forecast what may happen. This potential for arbovirus transmission argues in favor of the implementation of vigilance and response preparedness. The current state of knowledge seems insufficient to implement a specific epidemiological surveillance targeting a particular virus. This vigilance should be based on entomological surveillance, science watch, and improving knowledge so as to reduce uncertainty. In particular, species spread needs to be accurately known and monitored.

Aedes j. japonicus can be currently seen as a pest and its control should be mainly based on reducing breeding sites by mechanical control or larviciding when necessary as well as public information and awareness campaigns for the adoption of protective behavior. The vector status of the species may evolve. In this case, risk management measures would be similar to those implemented against *Ae. albopictus*: destruction and treatment of breeding sites (in public and private spaces) and adulticide treatments around imported and potential autochthonous cases. The option of mosquito population elimination should be considered whenever any new introduction, rather than following a spreading process, is detected.

Regarding environmental impacts, the main effect is likely to be on native species sharing similar larval habitats, mainly through interspecific competition (Juliano and Lounibos 2005). Thus, indigenous treehole mosquitoes such as *Oc. geniculatus* and *An. plumbeus* could be the most vulnerable. Interactions with *Ae. albopictus* could also occur when the establishment areas of these two invasive species overlap.

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