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Vector competence of *Aedes japonicus* for chikungunya and dengue viruses

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Abstract

The Asian bush mosquito *Aedes japonicus japonicus* (Theobald, 1901) [= *Ochlerotatus japonicus* (sensu Reinert *et al.*, 2004) = *Hulecoeteomyia japonica* (sensu Reinert *et al.*, 2006)], has invaded large parts of North America and has recently started to spread in Central-Western Europe. The species is suspected to act as a bridge vector of West Nile virus but nothing or very little is known about its vector competence for Chikungunya and Dengue viruses. Here, we report on experiments of laboratory infections of *Ae. japonicus* with CHIKV and DENV, demonstrating that the species has a vector potential for both viruses. Considering the high abundance of the species in urban environments and its ability to feed on human, these results plead to include this species when processing risk assessments for mosquito-borne diseases.

Key Words: Culicidae, *Aedes japonicus*, *Ochlerotatus japonicus*, *Hulecoeteomyia japonica*, Arbovirus, Dengue, Chikungunya, Switzerland, Insect vector

The Asian bush mosquito *Aedes japonicus* was first recognized as an invasive species in North America in 1998 where it rapidly spread throughout the USA and parts of Canada (Fonseca *et al.*, 2010). This species recently became established in Central-Western Europe (Schaffner *et al.*, 2009, Versteirt *et al.*, 2009). *Aedes japonicus* is highly abundant in the newly colonized areas (Andreadis & Wolfe, 2010), larval stages being recovered in Europe from more breeding vessels than the most common resident culicid species, *Culex pipiens* (Schaffner *et al.*, 2009). There is indeed field evidence that *Ae. japonicus* reduces native mosquitoes (Andreadis & Wolfe, 2010).

The Asian bush mosquito was shown to readily feed on humans (Molaei *et al.*, 2009), and we regularly receive complaints about biting nuisance. *Aedes japonicus* was identified in the laboratory as competent vector of several zoonotic arboviruses such as flaviviruses of the Japanese encephalitis complex, including West Nile virus (WNV) (Turell *et al.*, 2001), and this virus is regularly detected in field-caught *Ae. japonicus* specimens in the USA (CDC, 2008).

In Europe, WNV is considered as re-emerging pathogen (Zeller *et al.*, 2010), and two other mosquito-borne viruses, Chikungunya (CHIKV) and Dengue (DENV), have surfaced in autochthonous transmissions in southern Europe, with another invasive species, the Asian tiger mosquito *Aedes albopictus* [= *Stegomyia albopicta* (sensu Reinert *et al.*, 2004)], being incriminated as the most likely vector. Nothing is known with regard to the vector competence of *Ae. japonicus* for CHIKV, whereas no replication of the DENV-2 New-Guinea strain was observed after intracranial injection into suckling mice of a suspension pool of orally infected mosquitoes incubated 20 days at 30°C post-infection (Eshita, 1982). *Aedes japonicus* eggs were collected in the field in Switzerland (in the suburban area of Zurich, by means of ovitraps, between July and September 2010) and raised to the adult stage under standard conditions (25°C, 80 % RH) at the Institut Pasteur in Paris. Adult females were exposed to CHIKV and DENV by oral feeding. The CHIKV 06.21 strain, isolated in November 2005 in La Réunion, exhibits the Alanine to Valine substitution at position 226 of the E1 glycoprotein. The DENV-2 strain was isolated from a human case in Bangkok, Thailand, in 1974. Viral titres of blood meals were 10⁷ ffu/mL (focus forming unit / mL) for both viruses, and females were allowed to feed through a chicken skin (Vazeille-Falcoz *et al.*, 1999). Engorged females were incubated 14 days at 28°C in a BSL3 insectarium, and the infectious status of surviving females was analyzed by (1) indirect fluorescent antibody assays on head squashes (Kubersky *et al.*, 1977) to assess the dissemination rate and (2) by titration of saliva collected by the capillary method (Dubrulle *et al.*, 2009) to assess the transmission rate.

The dissemination rate was high for DENV (10/11; 91.0%) and lower for CHIKV (2/15; 13.3%). Effective transmission potential was demonstrated for both viruses by isolation of infectious viral particles in collected saliva (DENV: 20 ffu/saliva, n=1; CHIKV: ~9 ffu/saliva, range 1-25, 5/13). Albeit the number of investigated mosquitoes was low due to the reluctance of the F-0 females to feed under artificial conditions, these data reveal that the invasive *Ae. japonicus* has a vector potential for both DENV and CHIKV.

To assess a vector's capacity under natural conditions, other parameters besides the vector competence need to be taken into account, such as population density, longevity, vector-host contact rate, and duration of the gonotrophic cycle. Considering the high densities of *Ae. japonicus* populations observed even in urban environments, its continuing spreading in Central-Western Europe (own unpublished data) and its ability to bite humans, this species deserves as much attention as *Ae. albopictus* when processing risk assessments for mosquito-borne diseases, including Dengue and Chikungunya fever.

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