

First report of established population of *Aedes japonicus japonicus* (Theobald, 1901) (Diptera, Culicidae) in the Netherlands

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Abstract: In January 2013, a female mosquito collected during the week 18th-25th July 2012 in Lelystad (The Netherlands) during routine national vector surveillance was morphologically identified and genetically confirmed as the Asian bush mosquito, *Aedes japonicus japonicus*. In order to assess the extent of the infestation area, subsequent extensive mosquito surveillance in the surrounding area during 2013 consisted of visual inspection of potential habitats and adult trapping in increasing radially around the location of the initial finding. This surveillance confirmed the existence of a widely established population of *Ae. j. japonicus* in the municipality of Lelystad. Despite this detection, it was decided not to implement any mosquito control measures for two reasons: this would require large scale biocidal treatment and community participation in order to be effective, and this species is not a confirmed vector of disease agents in the field. As an alternative, it was decided that community information would be provided to enable management measures such as larval habitat source reduction. *Journal of the European Mosquito Control Association* 32: 9-13, 2014

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Introduction

Since the first European interception of *Aedes japonicus japonicus* (Theobald) [= *Ochlerotatus japonicus* sensu Reinert *et al.*, 2004 = *Hulecoeteomyia japonica* sensu Reinert *et al.*, 2006] in France in 2000 (Schaffner *et al.*, 2003), this species has established populations in Belgium (Versteirt *et al.*, 2009), Switzerland (Schaffner *et al.*, 2009), southern Germany (Becker *et al.*, 2011), Austria and Slovenia (Seidel *et al.*, 2012), western Germany (Kampen *et al.*, 2012) and northern Germany (Werner & Kampen, 2013). Since the 1990s, it has successfully established in numerous states of the USA, and in 2000 it was reported from south-eastern Canada (Kampen & Werner, 2013).

Aedes j. japonicus originates from Japan, Korea and Southern China (Tanaka *et al.*, 1979) and has also been found in south-eastern Russia (Gutsevich & Dubitskiy, 1987). This species has never been proven to be an efficient vector of pathogens in the field. Under laboratory conditions, however, it has been shown to be a competent vector of West Nile virus (Sardelis & Turell, 2001), Japanese encephalitis virus (Takashima & Rosen, 1989), La Crosse virus (Sardelis *et al.*, 2002a), St. Louis encephalitis virus (Sardelis *et al.*, 2003), Eastern equine encephalitis virus (Sardelis *et al.*, 2002b), chikungunya virus and dengue virus (Schaffner *et al.*, 2011).

Since 2009, the Centre for Monitoring of Vectors (CMV) has carried out various surveys focussing on vectors (indigenous and exotic) throughout the Netherlands. During exotic mosquito surveys, invasive mosquito species have been

routinely found in known risk locations such as used tyre storage facilities, Lucky bamboo greenhouses, and airports. Exotic species found were *Aedes albopictus* (Skuse) [= *Stegomyia albopicta* sensu Reinert *et al.* 2004], *Aedes aegypti* (Linnaeus) [= *Stegomyia aegypti* sensu Reinert *et al.* 2004], *Aedes atropalpus* (Coquillett) [= *Ochlerotatus atropalpus* sensu Reinert *et al.* 2004, = *Georgecraigius atropalpus* sensu Reinert *et al.* 2006] (Scholte *et al.*, 2007; Scholte *et al.*, 2010), and *Culex quinquefasciatus* Say and *Culex antennatus* Becker (Scholte *et al.*, unpublished). National Vector Surveys (NVSSs) are implemented in order to determine the composition and geographic distribution of indigenous mosquito fauna. From 2010 to 2013, mosquitoes were collected in more than 700 locations (unpublished data). In the NVSSs, species with established populations are generally anticipated in the catch, since the likelihood of finding a recently introduced exotic mosquito specimen is rather small with this sampling strategy. However, this random survey serves also as a passive surveillance for invasive mosquitoes.

Because of the rapid spread of *Ae. j. japonicus* throughout Europe, the presence of established populations in Belgium and Germany close to the Dutch border (Figure 1), its vector competence for various viruses, and the severe biting nuisance to mammals that it can generate, a survey of the possible introduction, establishment and spread of *Ae. j. japonicus* in the Netherlands was considered necessary. This was performed not only to detect the presence of this invasive species, but also to evaluate the efforts and potential costs associated with an effective population elimination plan.

Materials and Methods

Sampling protocol

During the NVS in 2012, locations all over the Netherlands were randomly selected from three categories, natural habitats agricultural and urban areas, and sampled once for a single week (cross-stratified sampling) during the active period of adult mosquitoes (April-October), using the CO₂-baited Mosquito Magnet Liberty Plus trap (Woodstream® Co., Lititz, USA; hereafter MM trap).

Mosquito samples from NVSs were sent to the laboratory where they were stored at -20°C until analysis. Morphological identification of the collected material is performed during the winter months by CMV specialists on European mosquitoes. Data are used to develop predictive mosquito distribution maps using stochastic distribution models. When invasive mosquitoes are found, additional monitoring is performed.

Extensive mosquito surveillance in the vicinity of the first *Ae. j. japonicus* 2012 collection site was implemented from April until October 2013, both by visual inspection of potential larval habitats and by adult trapping (MM traps) in increasing circles around the location of the initial finding, to assess the size of the infestation.

Additional municipality-wide mosquito surveillance in Lelystad was implemented from August until early October 2013 after the finding of one specimen 7 km away from the first *Ae. j. japonicus* finding location. Additionally, BG-Sentinel traps (Biogents AG, Regensburg, Germany) are routinely run near to risk sites for introduction of exotic mosquito (e.g. a tyre trade company at Lelystad).

Mosquito diagnostics

Species identification was done on morphological features in the field and in the laboratory, followed by laboratory genetic confirmation (COI barcoding) of at least one larva or adult per collection site. For indigenous mosquitoes collected during the NVSs, an identification key specifically designed for the rapid identification of Dutch adult female culicids was used (Scholte, 2009; based on Schaffner *et al.*, 2001, Becker *et al.*, 2003 and Verdonschot, 2002). Exotic and invasive species were identified using dichotomic and electronic identification keys (Harbach, 1988; Schaffner *et al.*, 2001). To confirm by molecular analysis the morphological identification of an invasive mosquito specimen, body parts are processed for COI (cytochrome oxidase subunit I) barcoding: DNA is extracted using the High Pure PCR Template Preparation Kit (Roche) following the mammalian tissue protocol. Primers LCO1490 (GGTCAACAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) as described by Folmer *et al.* (1994) are used to amplify a segment of the mitochondrial COI gene. The PCR product is cycled in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions and sequenced using a 3500 Genetic Analyzer (Applied Biosystems). Lasergene v 9.0 (DNASTAR) is used to assemble the two trace files and create a consensus sequence. Obtained sequence is compared to reported sequences using both the BLAST tool in NCBI (megablast, nucleotide collection (nr/nt) database) and the BOLD Animal Identification System (Species Level Barcode Records).

Results

In January 2013, a female mosquito specimen collected during the NVS in Lelystad (the Netherlands) between the 18th-25th of July 2012 (Figure 1) was morphologically identified as belonging to the Asian bush mosquito, *Ae. j. japonicus*. The trap was situated inside a forested area near to a parking ground. Besides *Ae. j. japonicus*, the same sample comprised 126 *Coquillettidia richiardii*, 2 *Anopheles plumbeus*, 26 *Anopheles claviger*, 147 *Culiseta annulata*, 11 *Aedes rusticus*, 1 *Aedes annulipes/cantans*, 1 *Culex pipiens/torrentium* and 3 indigenous *Aedes* damaged specimens not identifiable to species.

The morphological identification of the *Ae. j. japonicus* specimen was confirmed by COI barcoding. Sequence comparisons resulted in 100% matches with *Ae. j. japonicus* voucher specimens (NCBI: GQ254797, BOLD: NEONU116-11) and in clustering with *Ae. j. japonicus* specific clades in Fast Minimum Evolution and Neighbour Joining tree views.

Because identification of the *Ae. j. japonicus* female was performed during the winter, an extensive surveillance in the surroundings of the 2012 collection site in the municipality of Lelystad was implemented from early spring to early autumn 2013.

No larvae were found or adults encountered during the visual inspections of potential breeding sites in early spring or early summer in 2013 in the surroundings of the 2012 collection site. However, on 1st of August 2013, five *Ae. j. japonicus* adult females were trapped at the same site where the specimen has been caught in 2012 in a MM trap, and on August 20th 2013 this same site trap (n=2) as well as two MM traps situated almost 500m westwards (n=1) and southwards (n=1), respectively, were also found positive. On 29th August 2013,

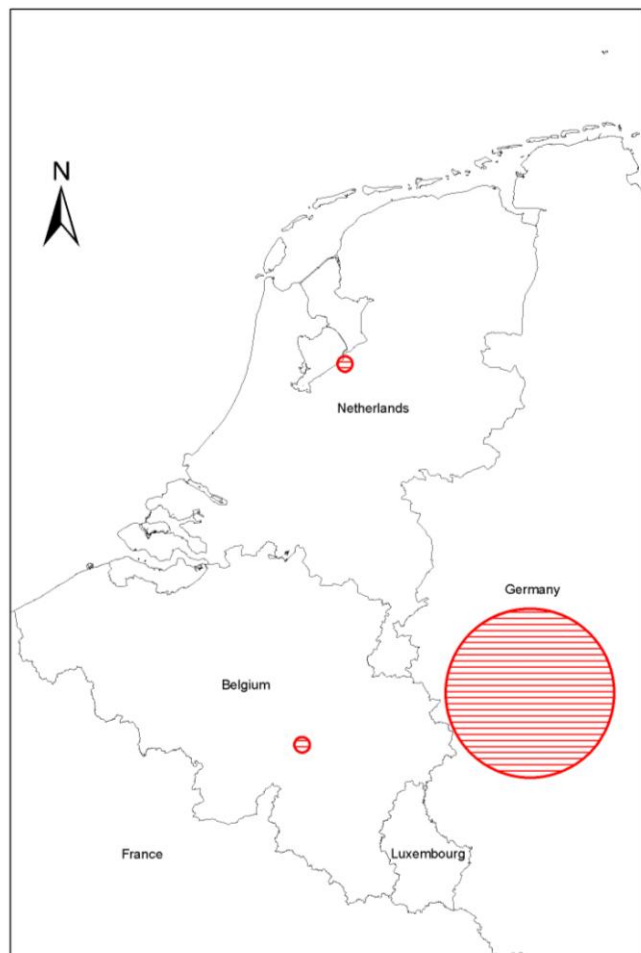


Figure 1. *Aedes j. japonicus* collection area in the Netherlands and the closest infested areas in Belgium and western Germany

the BG-Sentinel trap operated near to the tyre trading company in Lelystad also contained one *Ae. j. japonicus* female. The distance between the 2012 collection site and the company was approximately 7 km.

Finally, the municipality wide survey in September 2013 (Figure 2) revealed the presence of *Ae. j. japonicus* larvae, pupae, eggs and adults at allotment gardens, forest areas and in the cemetery of Lelystad. Larvae of all instars, pupae and eggs were found in barrels used for rain water harvesting in allotments (Figure 3). Larvae were also present in manholes and other artificial containers. During that survey, a small batch of larvae was also collected from a plastic toy containing rainwater in the surroundings of the site where the first adult had been trapped in 2012 and in 2013 (Figure 4). In addition, adults were caught by human landing catches in the forested areas surrounding the allotment gardens.

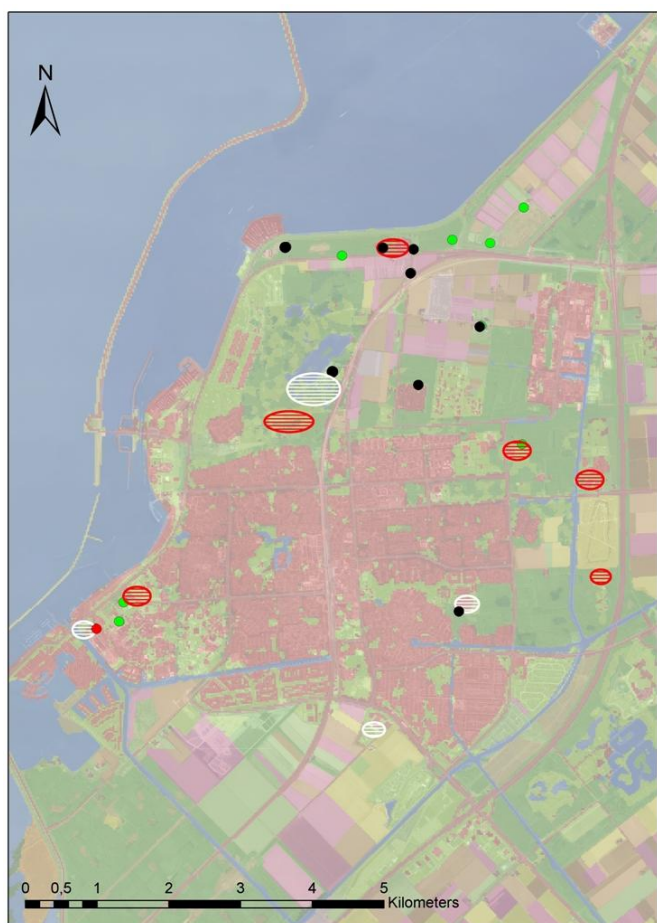


Figure 2. Sampling locations in Lelystad. Red patches: sampling sites positive for *Ae. j. japonicus* larvae; white patches: sampling sites negative for *Ae. j. japonicus* larvae; black dots: MM traps positive for *Ae. j. japonicus* adults; red dot: BG-Sentinel trap positive for *Ae. j. japonicus* adult; green dots: MM traps negative for *Ae. j. japonicus* adults.

Discussion and conclusion

Here we report the detection of an established population of *Ae. j. japonicus* in the municipality of Lelystad in the Netherlands. Since the detection of *Ae. j. japonicus* populations in Belgium and western Germany, the arrival of this invasive species was anticipated in the Netherlands. However, the detection of a well-established population in a limited Dutch region at more than 100 and 150 km from the Belgium and German borders respectively was surprising. The presence of eggs, larvae, pupae and adults in the infested area suggests that

Ae. j. japonicus has been present in this municipality for several years.

The initial point of entry could not be identified. Although a tyre trading company exists in the area, no relationship could be established with the origin of the infestation since *Ae. j. japonicus* specimens have never been found on the premises of this company where standard monitoring for exotic mosquitoes has been carried out since 2010. On 29th August 2013, a single *Ae. j. japonicus* specimen was found in the vicinity of this company.

In addition to the COI region, the ND4 (NADH dehydrogenase subunit 4) gene region of the mitochondrial DNA of the mosquito collected in 2012 was sequenced for preliminary population genetic analysis (Fonseca *et al.*, 2001), as a descent from mosquito populations in neighbouring Germany was assumed. Sequence analysis revealed the widely distributed haplotype H1, which had also been found in the *Ae. j. japonicus* population in western Germany, among others, and is common in the US, Japan and New Zealand as well (Fonseca *et al.*, 2001).



Figure 3. Barrels used for rain water harvesting in allotments, positive for *Ae. j. japonicus* larvae.



Figure 4. Plastic toy containing rainwater and *Ae. j. japonicus* larvae at the site where the first adult was trapped.

Conclusions on a putative relationship between the Dutch specimen collected in 2012 and the German or Belgian population cannot be drawn at this stage. The genetic haplotype found in this Dutch specimen is a widely distributed haplotype for *Ae. j. japonicus* making it difficult to characterise a population. Moreover, an analysis of a single specimen (collected in 2012) is difficult to interpret. The results of combined microsatellite and ND4 analyses may display the interrelationships and origins of the various *Ae. j. japonicus* geographic populations in Europe. Such analyses are in progress.

In response to the finding of *Ae. j. japonicus* in the municipality of Lelystad, an ad-hoc assembled team of entomologists and public health experts advised not to implement mosquito control measures. They reasoned that the extensive efforts required and the associated high costs for effective population elimination would not be compensated by the benefits that could be gained, while the species is not a confirmed vector of disease agents in the field. To eliminate this well established mosquito population, cost-intensive and environmentally questionable large scale biocidal treatment as well as intensive community participation efforts would be needed. As an alternative, it was decided that community information would be provided to enable private parties to implement management measures such as larval habitat source reduction.

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