National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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Risk assessment of *Aedes japonicus* in the Netherlands

RIVM Letter report 2018-0091

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Synopsis

Towards a policy decision on *Aedes japonicus*
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The Dutch government wants to limit the risk for local transmission of mosquito-borne diseases and therefore aims to limit the establishment of invasive exotic mosquitoes in the Netherlands. At the request of the Ministry of Public Health, RIVM and CMV have investigated which approach is appropriate for the Asian bush mosquito, *Aedes japonicus*.

An effective approach requires a tailor-made method and requires choices. Which choices are made depends on the chance of transmission of diseases by a specific mosquito species, the effect of the control and the costs thereof. In the Netherlands, mosquitoes do not form a major problem as vectors of human diseases at the moment. To become problematic, two preconditions are required. There must be human biting mosquitoes that are able to transmit pathogens, and there must be pathogens that can be transmitted by mosquitoes. In the Netherlands such pathogens are rare. In addition, in the Netherlands mosquitoes are not well able to transmit pathogens. The establishment of specific exotic mosquitoes could increase the risk of transmission of pathogens in the Netherlands.

The Asian bush mosquito was discovered in the Netherlands in 2012 and was found present in large parts of Lelystad. In recent years, the area, where this species is present, has expanded. This makes it increasingly difficult to fight the mosquito and the costs for the control increase. On the other hand, the risk of transmission of pathogens by Asian bush mosquito is small. This mosquito does not play an important role in outbreaks of mosquito-borne diseases. Only under specific conditions in the laboratory and field can Asian bush mosquito transmit pathogens. Worldwide only a few patients are known who may have become ill through this mosquito. The risk of the Asian bush mosquito in the Netherlands is comparable to that of a number of native mosquito species. The Asian bush mosquito provides a small added risk of spreading diseases in the Netherlands.

Keywords: risk assessment, Asian bush mosquito, *Aedes japonicus*, Netherlands,

Publiekssamenvatting

Op weg naar een beleidsbeslissing over de Aziatische bosmug:
Risicobeoordeling van de Aziatische bosmug in Nederland

De overheid wil de kans op ziekten, die door muggen overgedragen worden, beperken en daarom de vestiging van invasieve exotische muggen in Nederland beperken. Het RIVM en het CMV hebben op verzoek van VWS onderzocht welke aanpak gekozen kan worden voor de Aziatische bosmug *Aedes japonicus*.

Een effectieve aanpak vergt maatwerk en vraagt om keuzes. Een goede aanpak hangt af van de kans op overdracht van ziekten door een specifieke muggensoort, het effect van de bestrijding en de kosten daarvan. In Nederland zijn muggen als overbrenger van ziekten op dit moment geen groot probleem. Voordat muggen op grote schaal ziekten kunnen verspreiden zijn er twee randvoorwaarden nodig. Er moeten muggen (die van nature ook vaak mensen bijten) aanwezig zijn die ziekten kunnen overbrengen, en er moeten ziekteverwekkers aanwezig zijn die door muggen overgebracht kunnen worden. In Nederland komen zulke ziekteverwekkers niet of nauwelijks voor. Bovendien komen in Nederland vooral muggen voor die niet goed in staat zijn om ziekten over te dragen. De vestiging van specifieke exotische muggen zou het risico op overdracht van ziekten in Nederland kunnen vergroten.

De Aziatische bosmug is in 2012 aangetroffen in Nederland en bleek al in grote delen van Lelystad voor te komen. In de afgelopen jaren is het leefgebied van deze soort uitgebreid. Daarmee wordt het moeilijker om de mug te bestrijden en stijgen de kosten voor de bestrijding. Daartegenover staat dat het risico van overdracht van ziekten door de Aziatische bosmug klein is. Deze mug speelt geen belangrijke rol bij uitbraken van door muggen overdraagbare ziekten. Alleen onder specifieke omstandigheden in het laboratorium en veld kan Aziatische bosmug ziekteverwekkers overbrengen. Wereldwijd zijn slechts enkele patiënten bekend die mogelijk via deze mug ziek zijn geworden. Het risico van de Aziatische bosmug in Nederland is vergelijkbaar met dat van een aantal inheemse muggensoorten. De Aziatische bosmug zorgt voor een klein toegevoegd risico op verspreiding van ziekten in Nederland.

Kernwoorden: risicobeoordeling, Aziatische bosmug, *Aedes japonicus*, Nederland,

Contents

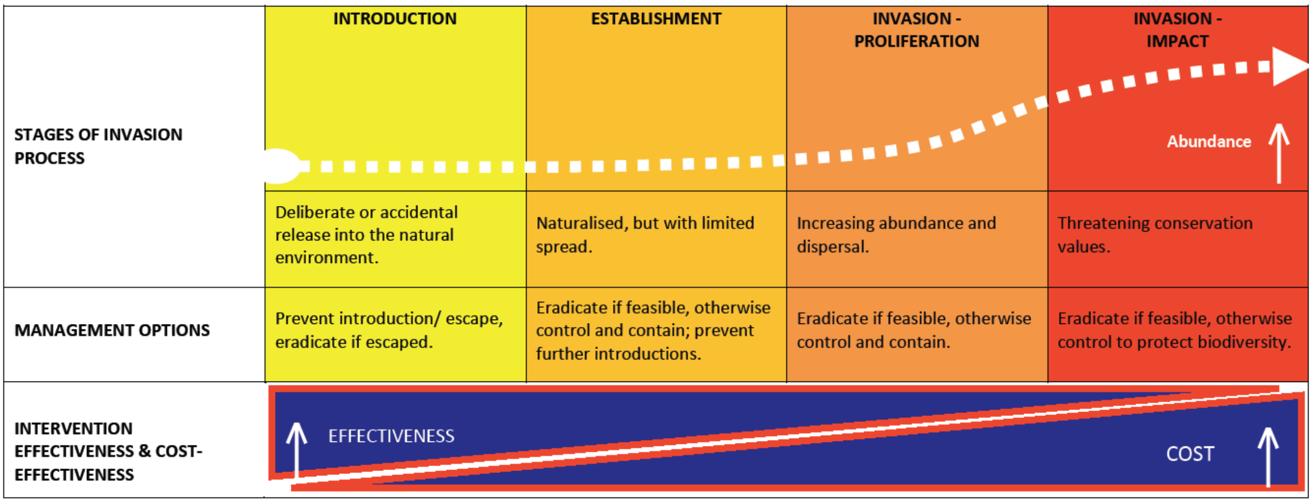
Summary

New establishments of invasive mosquito species can increase the transmission risk of mosquito-borne diseases. Depending on the risk posed by a particular invasive species and the stage of the invasion, management scenarios may change and differ between invasive species. The *Aedes japonicus* population recently expanded beyond the city limits of Lelystad and entered a new stage of invasion. To support the policy decision making process about the proportionality of future strategies in regard to the control of *Ae. japonicus*, an assessment of the risks of mosquito species for the transmission of pathogens important for public health was requested by the ministry of Health, Welfare and Sport.

A broad establishment of *Ae. japonicus* in the Netherlands would pose a previously non-existing, but low risk for transmission of six pathogens, since *Ae. japonicus* is the only potential vector present. It may also introduce an additional, but also low risk of transmission of five pathogens currently posed by native mosquito species. Despite the fact that *Ae. japonicus* has been shown to have biological potential for spreading eleven mosquito-borne pathogens, the actual risk for transmission of these pathogens in day to day practice in the Netherlands is considered (very) low.

1 Background

New establishments of invasive mosquito species can increase the transmission risk of mosquito-borne diseases. It is Dutch policy to counter this with a set of interventions. The management options depend on the stage of the invasion. As rule of thumb, as the invasion progresses, the effectiveness of interventions decreases while their cost increases (Figure 1).



	INTRODUCTION	**ESTABLISHMENT**	**INVASION - PROLIFERATION**	**INVASION - IMPACT**
STAGES OF INVASION PROCESS	Deliberate or accidental release into the natural environment.	Naturalised, but with limited spread.	Increasing abundance and dispersal.	Threatening conservation values.
MANAGEMENT OPTIONS	Prevent introduction/ escape, eradicate if escaped.	Eradicate if feasible, otherwise control and contain; prevent further introductions.	Eradicate if feasible, otherwise control and contain.	Eradicate if feasible, otherwise control to protect biodiversity.
INTERVENTION EFFECTIVENESS & COST-EFFECTIVENESS				

Figure 1: Invasion processes and management options (source https://invasives.org.au/strategy-invasive-species-australia/)

Different scenarios with respect to management options towards invasive mosquito species in the Netherlands are presented in Figure 2. Depending on the stage of the invasion and the risk posed by a particular invasive species, management scenarios may change and differ between invasive species.

In the Netherlands, current policy on invasive mosquitoes is to prevent establishment of introduced invasive species, and in the event that (local) establishment preceded detection, to control, contain and prevent further introduction. For the Asian bush mosquito *Aedes japonicus* (in Dutch known as “Aziatische bosmug”) this entails to eradicate new introductions and to control and contain the local established population in Lelystad. After *Ae. japonicus* was found in 2017 to have spread from Lelystad to Zeewolde, having several breeding habitats in-between, the question arose to what extent the various management options towards this invasive mosquito species are proportional to the risks it poses. The current policy towards *Ae. japonicus* is depicted as Scenario 1 (Figure 2).

To support the policy decision making process about the proportionality of future strategies in regard to *Ae. japonicus*, an assessment of the risks of mosquito species for the transmission pathogens important for the public health was requested by the ministry of Health, Welfare and Sport.

The risk assessment of *Ae. japonicus* will be build up in the following three sections:

1. Invasion status in the Netherlands
2. Vector status worldwide
3. Risk status for public health in the Netherlands

The risk assessment will, together with the supporting data, provide a solid basis for policy decision on the management of *Ae. japonicus*. The decision making itself and its result do not form part of this document. Specific risks for veterinary health are not taken into consideration.

The document has been written by Arjan Stroo and Adolfo Ibañez-Justicia, CMV and Marieta Braks (PhD), RIVM. Valuable input has been provided by Sander Koenraadt, Associate professor at the Laboratory of Entomology (WUR) and Chantal Reusken, Assistant Professor Public Health Virology, Preparedness and Response (Erasmus MC).

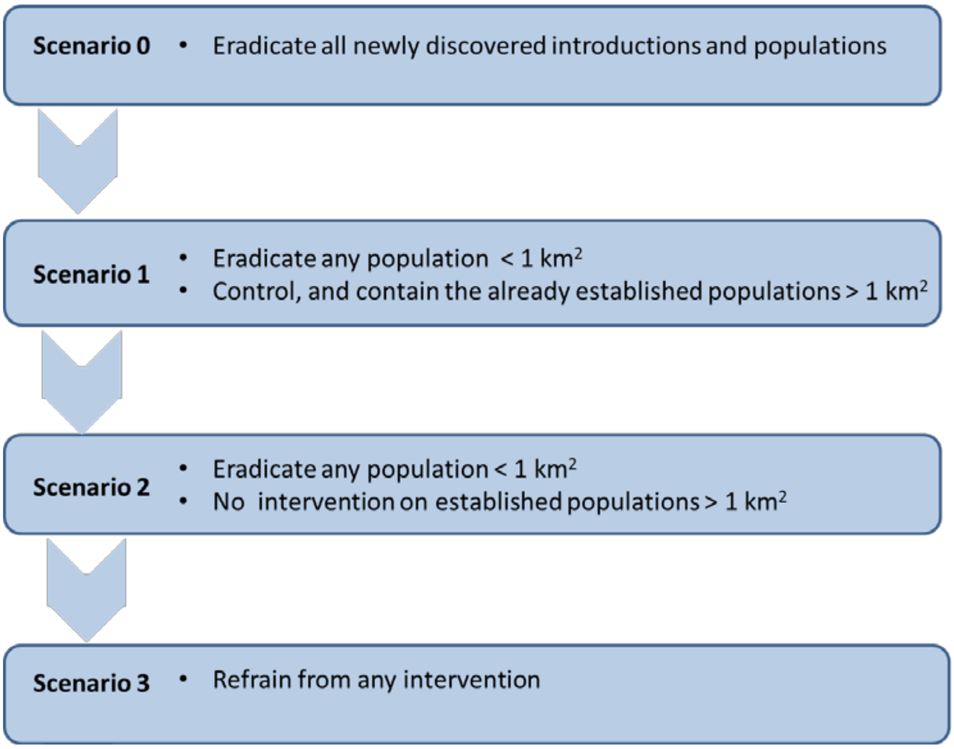


Figure 2: Different scenarios with respect to management options.

2 Risk assessment

2.1 Invasion status of *Aedes japonicus* in the Netherlands

In 2012, the Asian bush mosquito (*Aedes japonicus japonicus* (Theobald)) was detected for the first time in the Netherlands, in the municipality of Lelystad. *Aedes japonicus* (also known under its formally correct name as *Hulecoeteomyia japonica*, or under another synonym as *Ochlerotatus japonicus*) is an invasive mosquito species that originates from Japan, Korea, and Southern China (Tanaka et al. 1979) and has also been found in south-eastern Russia (Gutsevich and Dubitskyi 1987). The first interception of the species in Europe occurred in France in the year 2000 (Schaffner et al. 2003). Afterwards, established populations of this species have been found in Belgium (Versteirt et al. 2009), Switzerland (Schaffner et al. 2009), Germany (Becker et al. 2011, Kampen et al. 2012, Werner and Kampen 2013), Austria, and Slovenia (Seidel et al. 2012). Since the first interceptions in the 1990s, *Ae. japonicus* has also successfully colonized numerous states of the United States, and by 2000, it was already reported from south-eastern Canada (Kampen and Werner 2014).

After the initial catch in 2012 in the city of Lelystad, subsequent research revealed hundreds of specimens, mostly found in the peri-urban areas of the city in 2013 (Ibañez-Justicia et al. 2014). Allotment gardens turned out to be important reproductive areas (Ibañez-Justicia et al. 2018).

Initially, the National Coordination Centre for Communicable Disease Control, informed by CMV about the situation, advised the Ministry of Public Health not to engage in active control of the population, since at the time no human disease cases vectored by *Ae. japonicus* were known. Active control was only started after novel information on the possible role of *Ae. japonicus* in the transmission of La Crosse virus in the USA. Given the fact that the species was limited to the municipality of Lelystad, a targeted mosquito control campaign was initiated in 2015 with the objective of reducing populations in locations with the highest concentrations of *Ae. japonicus* breeding sites (allotment gardens). Mosquito control consisted of source reduction combined with application of the larvicide Vectomax® in breeding sites. Source reduction, also known as physical control, is one part of Integrated Pest Management (IPM) and is accomplished by eliminating mosquito breeding sites, preferably permanently. This can be as simple as discarding containers which hold water capable of producing mosquitoes. Source reduction is usually considered the most effective and economical of the mosquito control techniques.

At all eight allotment garden locations, mosquito control effectiveness has been systematically measured by sampling larvae from breeding sites. Six measurements were performed in 2015 and 2016. Results showed that the effectiveness of the mosquito control actions was similar in all treated allotment gardens and resulted in a significant reduction (around 80 %) in *Ae. japonicus* larval abundance. Rain barrels at the allotments represent the most frequent breeding site in Lelystad, but every water filled artificial container is a potential breeding site for the species. The species has never been found in surface water and naturally occurring container-like breeding sites like tree holes were never found occupied. Until September 2017, *Ae. japonicus* was not found in samples from a surveillance that assessed potential expansion of the distribution into other towns besides Lelystad in the province of Flevoland.

In the fall of 2017, however, *Ae japonicus* was found in an allotment in Zeewolde (NVWA 2018), a municipality approximately 19 km south of the positive allotment gardens of Lelystad. In a subsequent surveillance of 27 locations (farms) in a transect between Lelystad and Zeewolde, four were positive for *Ae. japonicus* (Figure 3).

Figure 3: Findings of Ae. japonicus in the province of Flevoland, since its first detection in 2012.

Since the discovery of *Ae. japonicus* in Lelystad, not a single complaint about biting nuisance was received by the NVWA, notwithstanding the fact that the hotspot are intensively used allotment gardens where people are also around at times when it will bite (at dusk).

2.2 Vector status of *Aedes japonicus* worldwide

The vector status of *Ae. japonicus* is determined by the current knowledge of its ability to transmit pathogens either under laboratory conditions (vector competence) or in field situations (vector capacity). Its most recent has been assessed in a literature review performed in January 2018 using the literature database PubMed (https://www.ncbi.nlm.nih.gov/pubmed/). In the following section, we summarize the information on the vector status of *Ae. japonicus* as well as its feeding behaviour and nuisance. Detailed report of the literature search can be found in the Appendices 1-4.

Of the 153 papers found, a total of 30 unique papers contained information related to the vector status of *Ae. japonicus.* In the laboratory, vector competence has been shown for CHIKV and DENV and the following wildlife-borne zoonotic pathogens CCV, EEEV, JEV, LACV, SLEV, WNV, GETV, and *Dirofilaria immitis / repens*. Among the wildlife zoonotic pathogens, *Ae. japonicus* has been found infected in the field with CVV, WNV and LACV, but was only shown to play a role in the transmission cycle of the latter (Appendix 2).

Table 1: Pathogens for which Ae. japonicus has shown vector competence and/or vector capacity.

Pathogen	***Vector competence***	***Vector capacity***
Viruses, abbreviation		
Cache Valley virus, CVV	√	
Chikungunya virus, CHIKV	√	
Dengue virus, DENV	√	
Eastern equine encephalitis virus, EEEV	√	
Getah virus, GETV	√	
Japanese encephalitis virus, JEV	√	
La Crosse encephalitis virus, LACV	√	√
Rift Valley virus, RVF	√	
Saint Louis encephalitis virus, SLEV	√	
West Nile virus, WNV	√	
Parasite, abbreviation		
Dirofilaria immitis/ repens, Diro	√	

Aedes japonicus displays a rather opportunistic feeding behaviour and has been found feeding on mammals, including humans and bird species (Appendix 3). Although based on a small number of field studies, a high variation in the percentages of human blood in the available blood meal analysis also indicates a rather opportunistic feeding behaviour (Appendix 4).

Aedes japonicus colonises urbanised and suburban environments (Schaffner et al. 2009) and females are active during the late afternoon and evening (Turell et al. 2005). Reports from Switzerland mention moderate to severe nuisance (Seidel et al. 2016).

2.3 Risk status of *Aedes japonicus* in the Netherlands

New establishments of invasive mosquito species can increase the risk of transmission of mosquito borne pathogens currently posed by native mosquito species. There is no current Dutch policy for the control or management of native mosquitoes, irrespective of whether they are potential vectors. An assessment as to what extent the risk will increase by a well-established *Ae. japonicus* population in the future is an important aspect in the policy decision making process about the proportionality of future (control) strategies in regard to *Ae. japonicus.* In 2016, the vector status of 26 native mosquito species in the Netherlands (Ibañez-Justicia et al. 2015) was reviewed and summarized (Braks and Stroo 2016), using the following coding system for vector potential developed by ECDC (ECDC 2014):

Empty cell = Absence of information for the species
0 = Refractory to infection in the laboratory
1 = Species infected in nature only
2 = Species competent in the laboratory only (at low, moderate or high level)
3 = Species infected in nature and competent (fulfilling 1 and 2)
4 = Species known as vectors in regions and countries outside Europe only
5 = Species known as vectors in Europe

For 14 of these 26 native mosquito species, evidence for some level of vector potential exists (Braks and Stroo 2016). It is important to realise that no information on vector potential of the 12 remaining species is available in the literature, because they either have never been tested or are never found infected. The information on the vector status of *Ae. japonicus* reported in paragraph 2.2. is transferred into vector potential codes using the same coding system.

In Table 2, the vector potential of 26 native mosquitoes with respect to 11 pathogens (Braks and Stroo 2016) is combined with that of *Ae. japonicus* with respect to 12 pathogens. With the addition of the recent publication on Zika virus detection in field collected *Ae. vexans* (Elizondo-Quiroga et al. 2018), the table contains the vector potential of 27 species for 16 pathogens. A more visual representation of the added risk of *Ae. japonicus* is shown in Figure 4.

Four of the 16 pathogens shown in Table 2, CHIKV, DENV, ZIKV and *Plasm* circulate strictly between humans via mosquitoes. The pathogen can be introduced by travellers with viremia or parasitemia. The remainder include zoonotic pathogens for which humans play no or a subordinate role in the transmission ecology. For the majority of these zoonotic pathogens, amplification in an enzootic wild cycle precedes transmission to humans.

Table 2: Vector potential of Ae. japonicus and 26 native mosquito species to various pathogens (adapted from (ECDC 2014, Braks and Stroo 2016))

Mosquito species	Viral pathogens													Other pathogens*		
	CHIKV	CVV	DENV	EEEV	GETV	JEV	LACV	RVFV	SINV	SLEV	USUTV	WNV	ZIKV	Diro	F.tula	Plasm
Ae. japonicus	2	1	2	2	2	2	4	2		2		3		2		
Ae. annulipes/cantans																
Ae. caspius								**4**				1		**5**		
Ae. cinereus				1					**5**			1			**5**	
Ae. communis									**5**							
Ae. detritus											1					
Ae. dorsalis												3				
Ae. flavescens																
Ae. geniculatus																
Ae. leucomelas																
Ae. nigrinus																
Ae. punctor																
Ae. riparius																
Ae. rusticus																
Ae. sticticus																
Ae. vexans				1	2			**4**				3	1	3		
An. claviger														1		**4**
An. maculipennis s.l.												1		3		3
An. plumbeus												2				3
Cq. richiardii												1		3		
Cx. modestus												**5**		**5**		
Cx. pipiens/torrentium								**4**			1	**5**		**5**		
Cx. territans																
Cs. annulata																
Cs. fumipennis																
Cs. morsitans									**5**			1		3		
Cs. subochrea																

~ abbreviation of pathogens see table 1 and appendix 2, *F. tula refers to Fransicella tularensis, Plasm refers to malaria parasites

For 11 pathogens, vector potential of *Ae. japonicus* has been shown. A broad establishment of this invasive mosquito species in the Netherlands would pose a previously non-existing risk for transmission of six pathogens (CHIK, CVV, DENV, JEV, LACV, SLEV), since *Ae. japonicus* is the only potential vector present. In addition it would introduce an additional risk of transmission of five pathogens (EEEV, GETV, RFV, WNV and Diro) currently posed by native mosquito species.

The vector potential of *Ae. japonicus* for 10 of the 11 pathogens is low, 1 or 2. For the latter, it means that proof of the ability to transmit these pathogens is only available from laboratory studies (Figure 4). Extrapolation of laboratory results to field situations is difficult. Laboratory studies examine the vector competence under artificial, controlled, often tropical, conditions, often outside the natural range of condition in the Netherlands. This adds to the uncertainty of a possible role in transmission in more temperate areas. Knowledge of the context and limitations of the laboratory experiments is essential when interpreting the results. Unfortunately, conclusions are often drawn outside the validity range of the assumptions. The validity of laboratory data in the outside world is questionable as responses to varying conditions or key parameters can be missed. On the other hand, while the absence of autochthonous cases of for example of dengue in areas where *Ae. japonicus* is abundant, might suggest that *Ae. japonicus* might not be considered a good vector, this observation does not provide conclusive evidence as non-entomological factors determining the vector capacity (a.o. density of susceptible hosts, temperature) might be missing.

Due to the recent large scale invasions of *Ae. japonicus* in USA and Europe, the number of investigations into its vector competence is increasing, while investigations into various non-invasive *Aedes* species are lacking. Haphazard comparisons of *Ae. japonicus* with invasive important vector species, such *Ae. aegypti* and *Ae. albopictus,* that belong to another subgenus, may lead to erroneous conclusions.

Only for La Crosse encephalitis virus, *Ae. japonicus* appears to interact in the natural transmission cycle, which is predominantly driven by the American tree hole mosquito *Aedes triseriatus.* Upon invasion and establishment into the LACV endemic area, *Ae. japonicus* appeared to act as one of the bridge vectors from the wildlife reservoirs for LACV to humans. For LACV to arrive to Europe is highly unlikely, given that chipmunks and squirrel species, that are the dominant hosts in the sylvatic cycle in the endemic area in the United States, are not present in nature in the Netherlands and are not even allowed as pets here. Other squirrel species, but within the same genus, do occur, as does the susceptible red fox, but at much lower densities than the reservoir species in the US. Chances of introduction, establishment and spread in a sylvatic transmission cycle are therefore considered extremely small for this virus. An important lesson from the LACV epidemiology in the US might be that upon establishment of one of the above mentioned zoonotic pathogens in the Netherlands, the host preferences of *Ae. japonicus* may ease bridging with humans.

After LACV, WNV represents the next highest added score from *Ae. japonicus*. However, in contrast with LACV, other (potential) native vectors for WNV are already present in the Netherlands.

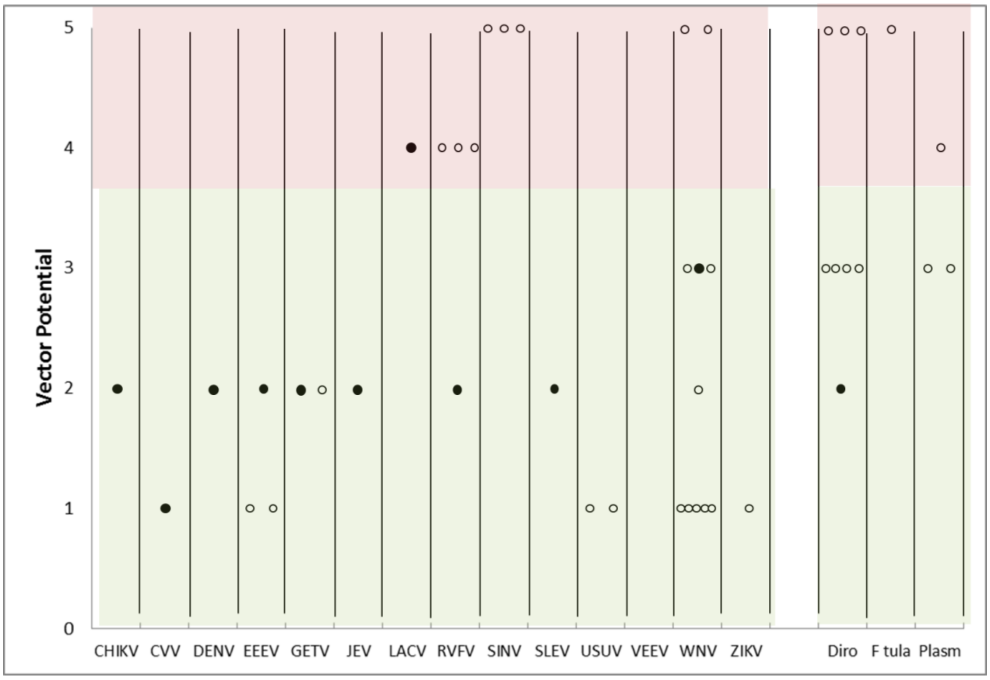


Figure 4: Risk potential. Added risk potential from Ae. japonicus upon establishment (solid circles) to the risk potential from individual native mosquito species (open circles) in the Netherlands . Circles in green area indicate at mosquito species with evidence for vector competence; circles in pink area indicate at mosquito species with evidence for vector capacity.

In the Netherlands, *Ae. japonicus* will most probably flourish in peri-urban and rural areas, being dependent on man-made breeding places and favouring relatively natural resting places. Basically nothing is known about its feeding behaviour in Lelystad, except for anecdotal information indicating that some human biting occurs in and along forest edges and in the evening. The relative low nuisance level in Lelystad compared to other European locations remains unexplained. A miss-match between the vector activity and human activity in the heavy infested locations in Lelystad, namely the allotment gardens is not likely, many people are present during the biting hours.

3 Conclusion

- *Ae. japonicus* has been shown to have vector potential for 11 mosquito-borne pathogens
- A broad establishment of *Ae. japonicus* in the Netherlands would
    - Pose a previously non-existing, but low risk for transmission of six pathogens (CHIK, CVV, DENV, JEV, LACV, SLEV), since *Ae. japonicus* is the only potential vector present.
    - Introduce an additional, but low risk of transmission of five pathogens (EEEV, GETV, RFV, WNV and Diro) currently posed by native mosquito species.

4 Appendices: Literature search

4.1 Appendix 1: Search strings for literature search

Keyword	Total number of records	Number of records used	References
Aedes japonicus	153	24	(Takashima and Hashimoto 1985, Sucharit et al. 1989, Takashima and Rosen 1989, Sardelis and Turell 2001, Turell et al. 2001b, Turell et al. 2001a, Sardelis et al. 2002b, Medlock et al. 2005, Styer et al. 2007, Molaei et al. 2009, Leisnham and Juliano 2012, Schaffner et al. 2013, Turell et al. 2013, Andreadis et al. 2014, Huber et al. 2014, Krebs et al. 2014, Harris et al. 2015, Westby et al. 2015, Bara et al. 2016, Muturi et al. 2016, Schonenberger et al. 2016, Silaghi et al. 2017, Urquhart et al. 2017, Wagner et al. 2018)
Ochlerotatus japonicus	51	12	(Sardelis and Turell 2001, Turell et al. 2001a, Sardelis et al. 2002b, Sardelis et al. 2002a, Andreadis et al. 2003, Sardelis et al. 2003, Medlock et al. 2005, Andreadis and Wolfe 2010, Andreadis et al. 2014, Huber et al. 2014, Bara and Muturi 2015, Bara et al. 2016)
Hulecoeteomyia japonica	6	3	(Huber et al. 2014, Schonenberger et al. 2016, Wagner et al. 2018)

4.2 Appendix 2: Vector potential of *Aedes japonicus* for various mosquito-borne pathogens

Pathogen Abbreviation	**Ref.**	**Information provided**	**Author results / risk perception**	**Disease occurrence**	***Vector potential***
Cache Valley Virus CVV	(Andreadis et al. 2014)	Isolation virus from field populations	One CVV infected *Ae. japonicus (1 pool out of 3120).* If proven to be a competent vector, *Oc. japonicus* could contribute to local transmission as regional studies have shown that females feed exclusively on mammalian hosts that include mostly white-tailed deer and humans	Enzootic throughout Americas (Andreadis et al. 2014)	***1***
Chikungunya virus CHIKV	(Schaffner et al. 2011)	Oral feeding through a chicken skin, incubated 14 days. The dissemination rate was lower than for CHIKV (2/15; 13.3%).	*Ae. japonicus* has a vector potential for both DENV and CHIKV. 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. The vector competence study shows low dissemination rate for CHIKV.	Endemic in Africa and Asia; Freq. imported into Europe (Schaffner et al. 2013) Epid in Americas	***2***
Dengue virus DENV	(Schaffner et al. 2011)	Oral feeding through a chicken skin IP 14 days. The dissemination rate was high for DENV (10/11; 91.0%)	*Ae. japonicus* has a vector potential for both DENV and CHIKV. 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. The vector competence study shows high dissemination rate for DENV.	Endemic in Africa, Americas, Asia; Freq. imported into Europe (Schaffner et al. 2013)	***2***
Dirofilaria immitis/ Dirofilaria repens Diro	(Silaghi et al. 2017)	Anticoagulated microfilariaemic blood was fed at a density of 3000 mf/ml to mosquitoes with a hemotek system	In field-collected *Ae. japonicus* infectious L3 larvae of both *D. immitis* and *D. repens* developed, rendering this mosquito species an efficient vector for both filarial species, and indicating that this invasive and locally highly abundant species may contribute to a transmission of filarial worms. our findings suggest that there is an increased risk of Dirofilaria transmission in areas populated by this species	Endemic in southern Europe and the Americas (Schaffner et al. 2013)	***2***
Eastern equine	(Sardelis et al. 2002b)	Chickens were inoculated	Although not a highly efficient vector under laboratory conditions (15-25% transmission rate), *Oc. j. japonicus* can become infected with	Americas; No evidence of	***2***

Pathogen Abbreviation	**Ref.**	**Information provided**	**Author results / risk perception**	**Disease occurrence**	***Vector potential***
encephalitis virus EEEV		subcutaneously and mosquitoes were allowed to feed on them 24 h later. IP 14 days.	EEE virus and transmit it by bite. The results of this study combined with the limited knowledge of the distribution and bionomics of this species in the United States suggest that *Oc. j. japonicus* could function as a bridge vector for EEE virus between the enzootic avian cycle and susceptible mammalian hosts. *Ae. japonicus* is not a highly efficient vector under laboratory conditions.	introduction into Europe to date (Schaffner et al. 2013)	
Getah virus GETV	(Takashima and Hashimoto 1985)	Several species of mosquitoes, were examined for infection rates following 21 days' incubation at 28°C after ingesting of virus-blood mixture	44 to 50% of *Ae. japonicus* were infected	At present, GETV has spread across Eurasia and Southeast Asia, including mainland China, Korea, Japan, Mongolia, and Russia (Li et al. 2017)	***2***
Japanese encephalitis virus JEV	(Takashima and Rosen 1989)	Wild caught adult mosquitoes. Mosquitoes were fed upon a virus-blood mixture soaked on cotton or were fed upon a viremic chick that was inoculated with virus.	*Ae. japonicus* had vector competence for Japanese encephalitis virus and the capability to transmit the virus vertically to its offspring. This mosquito species was able to transmit the virus to suckling mice after feeding on a virus-blood mixture as well as on a viremic chick. Vertical transmission of the virus was demonstrated in *Ae. japonicus* after intrathoracic inoculation; the highest minimum infection rate was 0.7%.	Endemic in Asia; No clear evidence of introduction into Europe to date (Schaffner et al. 2013)	***2***
	(Huber et al. 2014)	Oral feeding, human blood, cotton stick soaked. only 8 % of females accepted the	All females challenged with JEV were positive for JEV-RNA (100 % infection rate) on day 14 post-infection.		

Pathogen Abbreviation	**Ref.**	**Information provided**	**Author results / risk perception**	**Disease occurrence**	***Vector potential***
		artificial blood meal.			
La Crosse virus LACV	(Sardelis et al. 2002a)	After *Oc. j. japonicus* ingested virus from hamsters with viremia, its estimated transmission rates were 35-88%	Virus had disseminated from the midgut in 100% (8/8) of these specimens. *Oc. j. japonicus* could function as an additional vector of LAC virus.	Endemic in northern America; No evidence of introduction into Europe to date (Schaffner et al. 2013)	***4***
	(Westby et al. 2015)	**Field collected** adults tested for LCV	The single positive *Ae. japonicus* pool was collected in May. The future role of *Ae. japonicus* in the maintenance or transmission of LACV in this, and other endemic regions, is unknown, but it is an aggressive invader and has become established in many parts of the country where LACV is known to occur. In some areas of the USA, *Ae. japonicus* is replacing the native species.		
	(Bara et al. 2016)	Field collected pupae delivered adults in the lab, which were fed via the Hemotek with LACV infected bovine blood	*Oc. japonicus* had the highest Effective Vector Competence value (2.92), followed by *Ae. albopictus* (EVC=1.55), *Ae. aegypti* (EVC=0.88), and *Oc. triseriatus* (EVC=0.64). while **LACV-positive field** collections of Oc. japonicus have been reported in Tennessee, Virginia, and West Virginia (Gerhardt et al. 2001, Lambert et al. 2010, Westby et al. 2011, Harris et al. 2015). For both species, LACV-positive samples were obtained from mosquito eggs or larvae, indicating active vertical transmission. This study conclusively demonstrates that *Oc. japonicus* and *Ae. albopictus* develop disseminated infections within an epidemiologically relevant timeframe that should allow them to be efficient vectors of LACV.		
	(Harris et al. 2015)	**Field infected**	The authors report 2 independent isolations of LACV from adult *Ae. japonicus* mosquitoes in southwestern Virginia and 7 field detections of LACV RNA from adults (Virginia and West Virginia) and adults		

Pathogen Abbreviation	**Ref.**	**Information provided**	**Author results / risk perception**	**Disease occurrence**	***Vector potential***
			reared from eggs (Virginia). Suggesting that Ae. japonicus mosquitoes may play a major role in transmission of LACV to humans.		
Rift valley virus RVF	(Turell et al. 2013)	Fed on infected adult hamsters, infection rates >90% and dissemination rates >84%	*Ae. japonicus* proved to be highly efficient laboratory vectors of RVFV. *Ae. j. japonicus* should be targeted for immediate control should RVFV be introduced into an area where this mosquito is now present	Africa, Arabian peninsula (Turell et al. 2013)	***2***
St. Louis encephalitis virus SLEV	(Sardelis et al. 2003)	after feeding on chickens with viremias	Infection rates in *Oc. j. japonicus* were 0-33%, Estimated transmission rates at viral doses sufficient to infect the tested species were 29-84%. Because of its continued geographic expansion, field and laboratory evidence incriminating it as a vector of the closely related West Nile virus, and its ability to transmit SLE in the laboratory, *Oc. j. japonicus* should be considered as a potential enzootic or epizootic vector of SLE.	Endemic in the Americas; Not seen in Europe to date (Schaffner et al. 2013)	***2***
West Nile virus WNV	(Turell et al. 2001b)	Fed on chickens infected with WN virus (from 1999 outbreak). IP 14 d.	*Aedes japonicus* (Theobald) were highly susceptible to infection, and nearly all individuals with a disseminated infection transmitted virus by bite.	Worldwide (Schaffner et al. 2013)	***3***
	(CDC 2000)	Field infected	However, the recent isolation of WNvirus from three pools of *Ae. japonicus* collected in New York (CDC 2000b) indicates that this species may be involved in nature.		
	(Styer et al. 2007)		The amount of WNV inoculated by *Ae. japonicus* [1]during feeding were not significantly different from those inoculated by *Cx. tarsalis*, *Cx. pipiens*, and *Ae. triseriatus*.		
	(Huber et al. 2014)	Oral feeding, human blood, cotton stick soaked. only 8 % of Ae. j. japonicus	Of 67 *Ae. j. japonicus* females challenged by feeding of WNV-containing blood, none had measurable amounts of WNV-RNA (0 % infection rate) on day 14 post-infection. The authors suggest that differences in genetic background between		

Pathogen Abbreviation	**Ref.**	**Information provided**	**Author results / risk perception**	**Disease occurrence**	***Vector potential***
		females accepted the artificial blood meal.	*Ae. j. japonicus* populations from North America and Germany are most likely responsible for the differences in vector competence for WNV. Author suggest, possible contribution as enzootic vector.		
	(Wagner et al. 2018)	Wild caught adults, hemotek with chicken skin.	This study confirms that *Ae. japonicus* is highly susceptible to infections with WNV, seemingly even more so than *Cx. pipiens*, as has been shown in previous studies from the U.S.A. (Turell et al., 2001). This study shows that a field population of Ae. japonicus collected in northeastern Switzerland is susceptible to two WNV strains under Central European summer conditions. Given its high local abundances and its opportunistic feeding behavior, including both mammals and birds as hosts, Ae. japonicus is a potential key bridge vector of WNV in Europe. Thus far, WNV has not occurred in areas that are inhabited by this mosquito species.		

4.3 Appendix 3: Anthropophily from blood meal analyses of *Aedes japonicus*

Area	Area type	Percentage human blood	Reference
New Jersey	suburban/nature	36%	(Molaei et al. 2009)
Belgium	rural	60%	(Damiens et al. 2014)
Switzerland	zoo	3%	(Schonenberger et al. 2016)
New York state	nature	0%	(Apperson et al. 2004)

4.4 Appendix 4: Hosts for *Aedes japonicus*

Host	Observed feeding habits	Reference
Birds & Mammals	*Aedes japonicus* fed readily on chicks and mice but did not feed on both reptiles and amphibians.	(Miyagi 1972)
Birds & Mammals	Mammals: New World camelid , human, sheep, donkey, harbour seal, Indian lion, nilgai, Avian: chicken, Darwin's rhea, blackbird, house, sparrow, Humboldt's penguin, unknown bird species Mixed: house sparrow and New World camelid *H. japonica* and *Aedimorphus vexans* (=*Aedes vexans*) are the most likely candidate bridge vectors for WNV transmission in Switzerland.	(Schonenberger et al. 2016)
Humans	readily feed on humans,	(Molaei et al. 2009)
Humans	readily feed on humans, regularly receive complaints about biting nuisance.	(Schaffner et al. 2011)
Cat	The single bloodfed *Ae. japonicus* collected fed on a domestic cat (*Felis catus* L.).	(Westby et al. 2015)
Chickens	Feed on chickens infected with WN virus isolated from a crow that died during the 1999 outbreak.	(Turell et al. 2001b)

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