European Mosquito Bulletin 30 (2012), 1-14
Journal of the European Mosquito Control Association
ISSN 1460-6127; www.e-m-b.org
First published online 17th February 2012

Findings and control of two invasive exotic mosquito species, *Aedes albopictus* and *Ae. atropalpus* (Diptera: Culicidae) in the Netherlands, 2011

E.J. Scholte (e.j.scholte@minlnv.nl)[1], M. Dik[1], A. Ibañez Justicia[1], W. Den Hartog[1], B. Schoelitsz[2], M. Brooks[2], M. Braks[3], M. Steeghs[1]

[1]National Centre for Monitoring of Vectors (CMV), New Food and Consumer Product Safety Authority (nVWA), Dutch Ministry of Economic Affairs, Agriculture and Innovation, Wageningen, the Netherlands [2]Kenniscentrum Dierplagen (KAD), Wageningen, the Netherlands [3]Laboratory for Zoonoses and Environmental Microbiology, Centre for Infectious Disease Control (RIVM), the Netherlands, Bilthoven, the Netherlands

Abstract

In July 2011, during routine invasive exotic mosquito surveillance inspections at companies that import used tyres, two invasive exotic mosquito species were found at three locations in the Netherlands: the Asian tiger mosquito (*Ae. albopictus*) [*Stegomyia albopicta* sensu Reinert *et al*., 2004] and the American rock-pool mosquito (*Ae. atropalpus*) [*Georgecraigius atropalpus* sensu Reinert *et al.,* 2006]. Mosquito control was initiated one week after the first invasive mosquito was found, using adulticides and larvicides, and continued until the second week of November. Despite control, findings of these species continued in August and September, with the last finding in the first week of October. The same species were also found at these sites in 2010, followed by control until the end of the season. The available surveillance data suggest, although not conclusive, that the findings of 2011 are the result of unrestricted import of used tyres from *Ae.albopictus*- and *Ae. atropalpus*-endemic areas in 2011, rather than from surviving specimens found in 2010.

Introduction

Aedes albopictus, indigenous in Asia, has been expanding its geographical distribution around the world for more than three decades (Benedict *et al*., 2007; Enserink, 2008). It is known to hitchhike in used tyres, shipped around the world in containers. In Europe, the first reports came from Albania in 1979, followed by Italy in 1990, and many others since then, reviewed by (Scholte & Schaffner, 2007; Enserink, 2008; Schaffner *et al*., 2009). By 2011, the species had been reported from 14 countries in Europe (ECDC, 2011), mostly around the Mediterranean basin. Among other species, *Ae. albopictus* is a known vector of multiple arboviruses and nematodes, including DENVs and CHIKV, reviewed by (Mitchell, 1995; Gratz, 2004; Paupy *et al.,* 2009), which is why its ongoing global spread is viewed with argus-eyes.

In the Netherlands, the species is occasionally found (2005 until present) in glasshouses where so-called 'Lucky bamboo' plants, imported from China, are stored (Scholte *et al*., 2007; Scholte *et al.,* 2008), although surveillance data suggest that the species has not established itself in the surrounding area. In 2009, after the creation of the Dutch National Centre for Monitoring of Vectors (CMV), other exotic mosquito surveillance activities were initiated to identify as early as possible the presence of invasive exotic mosquito species with the aim to prevent the establishment of invasive exotic mosquito species (through control), especially those that are known to be vectors of pathogens of public health importance such as DENV and CHIKV.

In 2009, relatively large populations of the exotic mosquito species *Ae. atropalpus,* a North American species that had been encountered several times in Europe (Schaffner & Van Bortel, 2010) but had never established here, were found for the first time in the Netherlands (Scholte *et al.,* 2009*)* at two used-tyre platforms that were under invasive mosquito surveillance. As a result of these findings in 2009, surveillance for exotic mosquitoes at used-tyre platforms was increased to 34 companies in 2010 (Beeuwkes *et al*., 2011). In that surveillance, three exotic species were found: *Aedes albopictus*, *Aedes atropalpus*, and *Ae. aegypti* (Enserink, 2010) and successfully controlled at five locations (Scholte *et al*., 2010).

Here we report the findings and the subsequent control of two invasive mosquito species, *Ae. albopictus* and *Ae. atropalpus* in the Netherlands in 2011.

Methods

Surveillance

A total of 36 used tyre platforms, belonging to 33 different companies importing used tyres into the Netherlands were included in the invasive mosquito survey. Routine inspections consisted of collecting mosquito larvae from a minimum of 100 tyres per tyre platform, and manual collection of adult mosquitoes (if present) using a suction tube. A qualitative risk assessment on the introduction of invasive mosquito species was performed to determine the frequency of inspection of a company (Scholte *et al*., 2010; Beeuwkes *et al*., 2011), with bimonthly inspections of the tyre platforms/companies that were defined as 'high risk'. Routine inspections at companies that were assigned as 'low and middle risk levels' were carried out from April to mid October 2011, and to the beginning of November for the high risk level companies. On each tyre-platform that had been positive for exotic mosquitoes in 2010, one trap for adult mosquitoes (BG sentinel trap, BioGents™) was placed (5 locations), and was checked at the bimonthly inspection.

Around each tyre platform where invasive exotic mosquitoes were found, 500m diameter bufferzones (hence called '500m zones') were created to monitor potential spread. This consisted of checking all potential larval habitats for the presence of mosquito larvae. These were either removed, disposed of water where possible, or treated with biocides (Scholte *et al*., 2010) repeatedly until the end of the season. Each sample with larvae in the bufferzone was geo-referenced. On each of the exact sites where in the previous year one or more exotic mosquito

specimen(s) in the bufferzone were found (n=12), a trap was placed in the 2011 survey. Initially, these were oviposition traps (Fay & Elisasen, 1966) until September 2011. Due to increasing doubts about their efficacy in collecting exotic mosquito eggs under the circumstances at hand (i.e. too many alternative potential breeding sites available), these were changed for BG sentinel traps. Ad hoc, additional samples were taken by the employees of the control companies, prior to commencing control activities at a location (both on the tyre platform as in de 500m zone).

Following the discovery of an exotic species at a tyre platform, several changes in the surveillance took place ('intensified surveillance'). First, the infestation level on the tyre platform was assessed by one extensive search for larvae and imagos before control activities commenced. Second, to assess if the species had spread to the surrounding area, one extensive search for adults and larvae was carried out in the 500m zone surrounding the tyre platform, also before the onset of the control activities. Third, to check for the presence of exotic mosquitoes in the surrounding area after control had started, traps were placed (3-5 traps for adult mosquitoes and 10 oviposition traps and 5 drainage pits/manholes (for larvae) were selected in the 500m zone. Fourth, the frequency of the surveillance activities increased (compared with the routine inspections) from bi-monthly to weekly inspections. These weekly inspections not only allowed for early detection of exotic mosquito species on the tyre platform and in the surrounding 500m zone, but also to monitor the effectiveness of the control activities.

Diagnostics

Samples were labelled with a unique number, sealed, and sent to the laboratory for diagnostics. Collected larvae and adult mosquitoes were diagnosed morphologically by trained personnel, using the diagnostic keys from Schaffner *et al.*, 2001; (Schaffner, 2003)]. In those cases where damaged mosquitoes hampered morphological diagnoses, the sample was tested molecularly by PCR sequencing the mitochondrial cytochrome oxidase subunit 1 (CO1) gene. Each mosquito that was morphologically diagnosed as an invasive exotic mosquito was double-checked by another trained colleague. In case an invasive exotic mosquito was diagnosed morphologically that was collected from a location where no exotic mosquitoes had been collected before in 2011, the sample was triple-checked, by the molecular test described above.

After the finding of exotic mosquito(es), the sampling method was altered: living larvae and dead larvae were separated and sent in two different vials to the laboratory, in order to check on the effectiveness of the control activities. Also when no mosquitoes (larvae or adults) were found, an (empty) sample with unique number was sent to the laboratory and entered into the data-base.

Control

Within a week after the first finding, infested tyre-platforms mosquito control activities started. consisting of three methods: 2-3 treatments of deltamethrin (aqua K-Othrine, Bayer Environmental Sciences) against adult mosquitoes, with the second/third treatment 4-7 days after the previous, and larval treatments with *Bacillus thuringiensis israelensis (B.t.i.)* serotype H14 (Vectobac™) every three weeks until the end of the season (week 46; i.e. early November). Adult control was carried out in the evening hours. Larval control of the surrounding area (500 m

zone) consisted of removal of potential larval habitats for container-breeding *Aedes* spp. when possible, or treatment with *B.t.i.* space spray (VectoBac WG, Valent BioSciences), with an intended frequency of every three weeks. Due to the unavailability of data and subsequent uncertainties in a risk-assessment on the use of *B.t.i.* in close proximity of humans that was carried out by the National Institute of Public Health and the Environment (RIVM), larval control in the 500m zone was suspended for 5 weeks in locations Weert and Oss and for 8 weeks in the location Oosterhout (Table 2).

Communication

All companies in the surveillance had agreed after oral informed consent of the mosquito surveillance. They were informed via the branch organisation (Vaco), as well as via the inspectors that carried out the surveillances. After the finding of an exotic mosquito at a location, but before commencing control activities, the company was informed through the inspector, and information leaflets were distributed to the tyre company and the companies and inhabitants in the 500m zone to inform about the found species, its biology, the upcoming control, and the rationale behind the control activities, with references and contact information of the institute dealing with the surveillance (nVWA), and the responsible authorities regarding health related issues (GGD and RIVM), as well as links to their websites. After the first finding of exotic mosquitoes on a location, the mayor (as responsible health authority) of the affected municipality was informed, who in turn posted information related to the finding(s) on their websites. After the first finding, a press release was launched and several (mostly radio) interviews were given.

Results

In total, 1466 samples were taken, of which 223 from tyres on tyre platforms (larvae), 234 from other (potential) larval breeding sites, 271 from adult traps (BG Sentinel), 723 from oviposition traps, and 15 samples from manually collected adults mosquitoes, by using a suction tube. Fourteen of the samples contained one or more specimens of exotic mosquitoes (larvae, pupae, and/or adults), of which 2 samples derived from larval samples taken from tyres, 5 from manually collected adult mosquitoes using a suction tube, and 7 from BG Sentinel traps.

Two invasive exotic mosquito species (*Ae. albopictus*, and *Ae. atropalpus*) were found, in three locations (four companies[1]) in the Netherlands. The first sample that contained exotic mosquitoes was taken during a routine inspection on July 13th (week 28), from an adult trap on a tyre platform in the municipality of Weert[1], province of Brabant, the Netherlands (Table 1, Figure 1), whose collection-net had been placed on the 29th of June (week 26). This sample contained 16 adult (10♀, 6♂) *Aedes atropalpus*. Unfortunately, this sample was delayed in its delivery, arriving at the laboratory only on Friday afternoon the 22nd of July, and was not handled until after the weekend, on Monday the 25th (week 30), by then almost 2 weeks after the sample had been taken. Control was initiated one week after the diagnosis (week 31). In that same week (31)

[1] The location in Weert actually consists of two adjourning tire companies, but since both companies are separated only by a fence, and exotic mosquitoes were collected from both companies, they are considered as one 'location'.

the last adult exotic mosquito was found at this location, a female *Ae. albopictus* in an adult trap (the only *Ae. albopictus* specimen found at this location). Despite the relatively large numbers of adults in this adult trap sample, no immature stages of invasive mosquito species were found at that company. Only once, on the 29th of July, larvae (n=5) of *Ae. atropalpus* were found at that location, on the neighbouring company (two of these larvae were also molecularly tested and confirmed as *Ae. atropalpus*). In the same tyre where these *Ae. atropalpus* larvae were found, also 3 *Aedes* spp. pupae were collected, but the available morphological diagnostic keys (Schaffner *et al*., 2001; Schaffner, 2003) did not allow to determine if these pupae were *Ae. albopictus* or *Ae. atropalpus*. One of these pupae was tested molecularly, and diagnosed as *Ae. atropalpus*. In the intensified inspections in the surrounding area (500m zone), a total 369 samples, from 29 different sites were taken. Since invasive mosquitoes had been found in this area in the previous year (2010), the surveillance in this 500m zone had initiated from the start of the season (mid-April) with bimonthly samples, but increased to weekly samples after the findings of invasive species on the tyre platform, until mid-November 2011 (a total period of 31 weeks). During this extensive surveillance in the 500m zone, no exotic mosquitoes (larvae, pupae, nor adults) were found, suggesting that the exotic mosquito species at this location (Weert) had not spread.

However, Weert was not the only location where exotic mosquitoes were collected. During a routine inspection at a tyre platform in the municipality of Oss on July 19th (week 29), 7 female *Ae. albopictus* were manually collected using a suction tube. From this relative large number of active *Ae. albopictus* that had been collected at this tyre platform in only half an hours time, it was deduced that larvae should be present. However, despite intensive inspection, no larvae of invasive exotic mosquito species were found. Two days later, on July 21st, the intensified surveillance was started, where three more adult *Ae. albopictus* specimens were collected, but (again), no larvae of this species. Control at this location (and 500m zone) started on Tuesday, July the 26th. Just prior to the control, another five *Ae. albopictus* females were caught. No findings of exotic mosquitoes were done until 8 weeks later, when one female *Ae. albopictus* was collected from an adult trap on the tyre company (week 38). The last specimen at this location was collected one week later on September 29th (week 39), a female *Ae. albopictus* that was caught from an adult trap placed approximately 450 meters from the tyre company. From all the previous samples that were taken in the surrounding 500m zone (from week 29 onwards) no exotic mosquitoes had been found, suggesting that there was no population of *Ae. albopictus* in the surrounding area. Assuming that the species is not established in the area, this final finding showed that at least one adult tiger mosquito at this location in Oss, had actively dispersed from the tyre platform.

The third location where exotic mosquitoes were found, was in the municipality of Oosterhout. Here, one larva of *Ae. albopictus*, as well as one male *Ae. atropalpus* (manual collection with suction tube) were collected on August the 15th (week 33). Two more collections of single female *Ae. albopictus* specimens from the adult trap ón the tyre platform would follow; the first one in week 37, and the second (last) *Ae. albopictus* specimen was collected as late as the 5th of October (week 40). As in the location Oss had happened, also in the location Oosterhout *Ae. albopictus* was found outside the tyre platform. In this case, one female *Ae. albopictus* was collected from

the camping directly neighbouring the tyre platform, just prior to control activities (week 34), once again showing that the adult *Ae. albopictus* don't restrict themselves to the tyre platform alone, but actively disperse to the surrounding area.

All infested companies described here belong to the 'high risk'-category for importing exotic mosquito species, based on the type, origin and storage of the tyres that are imported, presence/absence of exotic mosquitoes in previous year(s), and 'on-site' judgement, and are therefore inspected every two weeks. No invasive mosquito species were found at any of the other companies that were included in the survey.

TABLE 1. Summary of the results of the invasive mosquito survey at used tyre companies by location (municipality), the Netherlands, July-October 2011.

Location	Adults collected		Larvae collected		Pupae collected			*Total* # specimens invasive mosquitoes
	Ae. albopictus	*Ae. atropalpus*	*Ae. albopictus*	*Ae. atropalpus*	*Ae. albopictus*	*Ae. atropalpus*	*Ae. spp.*	
on tyre platform								
Oss	16	0	0	0	0	0	0	***16***
Weert(n=2)	1	21	0	5	0	1	(2)	***28***
Oosterhout	2	1	1	0	0	0	0	***4***
in surrounding 500m bufferzone								
Oss	1	0	0	0	0	0	0	***1***
Weert	0	0	0	0	0	0	0	***0***
Oosterhout	1	0	0	0	0	0	0	***1***
Total	***21***	***22***	***1***	***5***	***0***	***1***	***(2)***	***50***

TABLE 2. Inspections, mosquito control, and findings of at least one of the two exotic mosquito species for each location per week, the Netherlands, April-November 2011.

Location	Week (2011)																														
	16	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**
Weert, tyre platforms (n=2)																x	x	x	x			x			x			x			
Weert, 500m zone (n=1)																x			x			x						x	x		
Oss, tyre platform (n=1)															x	x		x			x			x				x			
Oss, 500m zone															x			x			x			x				x			
Oosterhout, tyre platform (n=1)																			x			x				x			x		
Oosterhout, 500m zone																			x			x									

no inspection
no exotic species found (negative)
larvae and/or adults found of one of the two exotic mosquito species
x mosquito control carried out

TABLE 3. Overview of locations and species of exotic mosquito species that were found in the used tyre surveillance in the Netherlands between the years 2009 and 2011. Numbers of used tyre platforms included in this surveillance differed between years; n = 3 (2009), n = 36 (2010 & 2011).

mosquito spp.	Location														
	Oss			Heijningen			Oosterhout			Weert[1]			Montfoort		
	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009[2]	2010	2011	2009[2]	2010	2011
Aedes atropalpus	x	x		x	x			x	x		x	x			
Ae. albopictus		x	x		x				x		x	x		x	
Ae. aegypti					x			x							

[1] location consists of two adjacent tyre importing companies

[2] location not included in surveillance in 2009

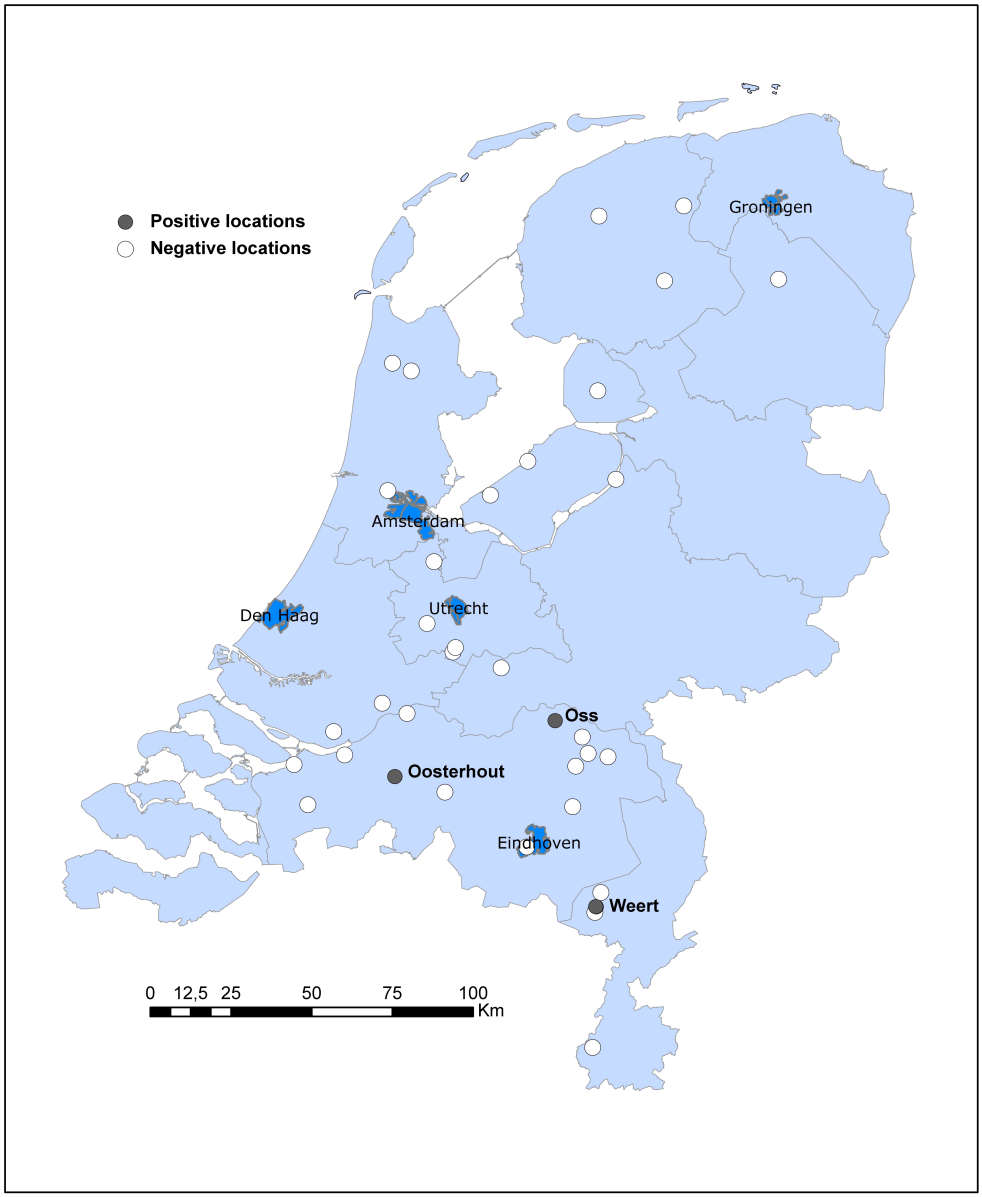

FIGURE 1. Locations of tyre companies that were incorporated in the survey for invasive mosquitoes in the Netherlands, 2011. Dark dots indicate positive locations (Oss, Oosterhout and Weert) for at least one of the invasive mosquito species. White dots indicate negative (n=32) locations.

Discussion

All three locations where exotic mosquitoes were found in 2011 (this manuscript) had also been positive for exotic mosquitoes in 2010 (Table 3). A justified question is whether the control activities that were carried out last year (2010) and at that time considered effective (Scholte *et al., 2010)*, were indeed effective, retrospectively. Our hypothesis is that it was effective. An argument that does nòt support this hypothesis, is that all three locations that were positive for exotic mosquitoes in 2011, also had been positive for exotic mosquitoes in 2010 (Table 3), although the species found in 2010 per location do not 'translate' perfectly to the findings in 2011: For example, *Ae. albopictus* and *Ae. atropalpus* were found in Oss in 2010, but the latter was not found there in 2011. Also, *Ae. albopictus*, absent in Oosterhout in 2010, was found there in 2011, suggesting a new introduction rather than a relict of last years' population.

One argument is that does not support, nor goes against the hypothesis is about the timing of the first findings of invasive exotic mosquitoes: No exotic mosquitoes were found until the end of July, despite inspections from April onwards. In case eggs from the 2010 findings would have survived the winter, it would be reasonable to expect findings in spring at one (or more) of the 5 locations that had been positive for exotic mosquitoes in 2010: Seasonal activity of *Ae. albopictus* (hatching of eggs) starts with 11.25 hours daylight (reached around the second week of March (www.meteopagina.nl) for NL), and a mean temperature of 10.5°C (Toma *et al*., 2003; Medlock *et al*., 2006), which in 2011 was reached on the 31st of March, with in the whole month of April dropping under this 10.5°C for not more than three days. However, precipitation was exceptionally low in spring 2011 in the Netherlands, with national averages of 13mm of rain in March, 11 mm in April, and 25mm in May (against long-year averages of 60, 44, and 61mm for March, April and May respectively). It started to rain considerably only in June (96mm in June 2011, against a long-year average of 68mm), with the first heavy rains on the 16th of June (source (http://www.knmi.nl/klimatologie/geografische_overzichten/maand/index6.htm). So if eggs would have survived the winter, it is likely that, based on these precipitation levels, they would have hatched not earlier than mid June. With relatively high average temperatures (day and night) of 16.1°C in that month, it is reasonable that they would have hatched after the heavy rains in mid June, and completed larval/pupal development by the end of June. The first findings of adults are of mid July, two weeks later, which is not unlikely in this scenario. However, in that case, considerable numbers of larvae would have been expected to be found in the second part of June and the first part of July, which was not the case.

An argument supporting the above hypothesis is the differences between 2010 and 2011 at the location 'Heijningen'. In 2010, three exotic mosquito species were found there; *Ae. albopictus*, *Ae. aegypti* and *Ae. atropalpus*), with an initial high infestation level of *Ae. atropalpus*, findings of adults of all three species in the surrounding 500m zone, and larvae of *Ae. atropalpus* at one site in the surrounding 500m zone, indicating spreading from, and population build-up outside of the tyre platform. In that year, control had started in week 30, and from week 35 onwards, despite intensive surveillance, no specimens of either of the three species was collected again at that location; not at the tyre platform, and not in the 500m zone. If control had not been effective, one would have expected to find exotic mosquitoes at this location (either on the tyre platform

itself, or in the surrounding area). However, in 2011, not a single specimen of an exotic mosquito species was found at this location anymore, despite thorough inspections and continuously running mosquito traps throughout the season.

Also supporting the above hypothesis is that *Aedes atropalpus*, found in Oss both in 2009 (when nó control was carried out) and in 2010 (when control wàs carried out), has not been found in Oss in 2011. However, this does not account for *Ae. albopictus*, in Oss, that was found both in 2010 and 2011. Its presence in 2011 could be attributed to introduction in 2011.

Finally, although model predictions suggest the opposite (Schaffner *et al*., 2009; Takumi *et al*., 2009), it might be that the harsh weather conditions in the Netherlands of the winter of 2010-2011 killed *Ae. albopictus* and *Ae. atropalpus* eggs that were (potentially) laid in 2010. This most likely was the case for any *Ae. aegypti* eggs that were laid.

Following the above considerations, it is more likely that the control activities of 2010 have been successful, and that instead, repeated introductions are a more likely explanation for the findings of exotic mosquitoes at these sites in 2011, although recognizing that the data are not conclusive. Data from 2010 (Scholte *et al*., 2010; Brown *et al*., 2011) and 2011 (personal communication with the affected companies), confirm that the companies in the described surveillance were known to import used tyres from abroad some including from Italy and from overseas areas, such as the USA. Since no (national nor EU) regulations are in place that reduce the risk of importing 'infested' used tyres from any geographical origin, and no guidelines exist on how to handle/store imported used tyres to reduce the risks regarding invasive exotic mosquitoes, it was not unexpected to find invasive exotic mosquitoes again in 2011. If import of used tyres from *Ae. albopictus* (and other invasive mosquito species for north-western Europe) endemic areas continues, these mosquitoes will 'keep knocking on the door'. The extensive surveillance in the surrounding 500m zones around tyre platforms where invasive exotic mosquitoes have been found strongly suggest that, at least until now, none of the invasive exotic mosquito species managed to establish in the Netherlands, most likely thanks to active mosquito control in an early stage of population development both on the tyre platforms, as in the surrounding areas. This strongly supports the active surveillance, allowing for early detection and subsequent control. It remains uncertain how long this strategy will remain effective in preventing establishment of invasive exotic mosquitoes. Unless and until regulations and/or guidelines are developed, put in place, and complied to, effective biocides are regulated/registered and available for use, invasive exotic mosquitoes are likely to be found again in the Netherlands, with the risk that at some point intervention may be too late to prevent establishment.

Acknowledgements

Roel Coutinho (RIVM), Marleen Kraaij-Dirkzwager (Ministry of VWS), Cindy Schenk (Ministry of VWS), Traas ongediertebestrijding, Corien Swaan (RIVM), André Jacobi (RIVM), Peter de Vries (VROM), Martin van den Homberg (nVWA), Ad Dilven (GGD-en Brabant), Annemiek Backx (GGD-ent Brabant), Roel Vincken (nVWA), Nicole Bouwer (nVWA), Arie Verhoef (VACO), Robert Luttik (RIVM), Heiko Kotter (Sumitomo).

References

Beeuwkes, J., Den Hartog, W., Dik, M. & Scholte, E. (2011) Surveillance and findings of exotic mosquitoes in used tires in the Netherlands; a methodological approach. *Proceedings of the Netherlands Entomological Society Meeting* **22**, 31-37.

Benedict, M.Q., Levine, R.S., Hawley, W.A. & Lounibos, L.P. (2007) Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector-Borne and Zoonotic Diseases* **7**, 76-85.

Brown, J., E.,, Scholte, E., Dik, M., Den Hartog, W., Beeuwkes, J. & Powell, J.R. (2011) *Aedes aegypti* mosquitoes imported into the Netherlands, 2010. *Emerging Infectious Diseases* **17**, 2335-2337.

ECDC (2011) Exotic mosquitoes distribution map. In. Enserink, M. 2008. A mosquito goes global. *Science* **320**(5878), 864-866.

Enserink, M. (2010) Yellow Fever Mosquito Shows Up in Northern Europe. *Science* **329**(5993), 736.

Gratz, N.G. (2004) Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology* **18**, 215-227.

Medlock, J.M., Avenell, D., Barrass, I. & Leach, S. (2006) Analysis of the potential for survival and seasonal activity of *Aedes albopictus* (Diptera: Culicidae) in the United Kingdom. *Journal of Vector Ecology* **31**, 292-304.

Mitchell, C.J. (1995) Geographic spread of *Aedes albopictus* and potential for involvement in arbovirus cycles in the Mediterranean basin. *Journal of Vector Ecology* **20**, 44-58.

Paupy, C., Delatte, H., Bagny, L., Corbel, V. & Fontenille, D. (2009) *Aedes albopictus*, an arbovirus vector: From the darkness to the light. *Microbes and Infection* **11**, 1177-1185.

Reinert, J.F., Harbach, R.E. & Kitching, I.J. (2004) Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. *Zoological Journal of the Linnean Society* **142,** 289–368.

Reinert, J.F., Harbach, R.E. & Kitching, I.J. (2006) Phylogeny and classification of *Finlaya* and allied taxa (Diptera: Culicidae: Aedini) based on morphological data from all life stages. *Zoological Journal of the Linnean Society* **148**, 1-101.

Schaffner, F. (2003) Mosquitoes in used tyres in Europe: species list and larval key. *European Mosquito Bulletin* **16**, 7-12.

Schaffner, F., Angel, G., Geoffrey, B., Hervy, J.-P., Rhaiem, A. & Brunhes, J. (2001) *The mosquitoes of Europe*. Montpellier: Institut de Recherche pour le Développement/Entente interdépartementale pour la démoustication du littoral (EID) Méditerrannée.

Schaffner, F., Hendrickx, G., Scholte, E., Ducheyne, E., Medlock, J.M. & Avenell, D. (2009) *Aedes albopictus* distribution maps. European Centre for Disease Prevention and Control.

Schaffner, F. & Van Bortel, W. (2010) Current status of invasive mosquitoes in Europe. ECDC, *VBornet Newsletter* **2**:6-8.

Scholte, E., Den Hartog, W., Dik, M., Schoelitsz, B., Brooks, M., Schaffner, F., Foussadier, R., Braks, M. & Beeuwkes, J. (2010) Introduction and control of three invasive mosquito species in the Netherlands, July-October 2010. *Eurosurveillance* **15**(45): pII-19710.

Scholte, E., Jacobs, F., Linton, Y., Dijkstra, E., Fransen, J. & Takken, W. (2007) First record of *Aedes* (*Stegomyia*) *albopictus* in the Netherlands. *European Mosquito Bulletin* **22**, 5-9.

Scholte, E. & Schaffner, F. (2007) Waiting for the tiger: establishment and spread of the *Aedes albopictus* mosquito in Europe. In *Emerging pests and vector-borne diseases in Europe* 241-260. Wageningen: Wageningen Academic Publishers.

Scholte, E.J., Den Hartog, W., Braks, M., Reusken, C., Dik, M. & Hessels, A. (2009) First report of a north american invasive mosquito species *Ochlerotatus atropalpus* (Coquillett) in the netherlands, 2009. *Eurosurveillance* **14**(45), 24-26.

Scholte, E.J., Dijkstra, E., Blok, H., De Vries, A., Takken, W., Hofhuis, A., Koopmans, M., De Boer, A. & Reusken, C. (2008) Accidental importation of the mosquito *Aedes albopictus* into the Netherlands: a survey of mosquito distribution and the presence of dengue virus. *Medical And Veterinary Entomology* **22**, 352-358.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87**(6): 651-701.

Takumi, K., Scholte, E.J., Braks, M., Reusken, C., Avenell, D. & Medlock, J.M. (2009) Introduction, Scenarios for Establishment and Seasonal Activity of *Aedes albopictus* in The Netherlands. *Vector-Borne and Zoonotic Diseases* **9**, 191-196.

Toma, L., Severini, F., Di Luca, M., Bella, A. & Romi, R. (2003) Seasonal patterns of oviposition and egg hatching rate of Aedes albopictus in Rome. *Journal of the American Mosquito Control Association* **19**, 19-22.