Surveillance and findings of exotic mosquitoes in used tires in The Netherlands: a methodological approach

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In 2010 an extensive national exotic mosquito survey was conducted at companies that import used tires. Three exotic mosquito species were found at used-tire companies at five locations in The Netherlands: the Asian tiger mosquito (*Aedes albopictus*), the American rock-pool mosquito (*Ae. atropalpus*), and the yellow fever mosquito (*Ae. aegypti*). The survey set-up included a qualitative risk assessment of the companies, meant to gain insight in possible factors that are indicative of the risk that exotic mosquitoes are imported. Details on the methodology of the tire inspections are presented together with the results of the survey. Continuous surveillance of import pathways of exotic mosquitoes is crucial to facilitate early detection. This study may help in setting up new surveys or to improve existing mosquito surveillance.

Keywords: exotic mosquitoes, *Aedes albopictus*, *Aedes aegypti*, *Aedes atropalpus*, risk assessment, surveillance, tires

The worldwide spread of exotic mosquitoes is a threat to public health, because they may act as vectors of pathogens, causing diseases in animals and humans. Together with international travel, the increasing international trade is the main reason why several important mosquito vector-species have expanded their native distributional range (Enserink 2010).

In Europe we have witnessed multiple examples of the introduction of exotic mosquitoes such as the Asian tiger mosquito (*Aedes albopictus*) in Italy (Sabatini *et al.* 1990), the American rock-pool mosquito (*Ae. atropalpus*) in The Netherlands (Scholte *et al.* 2009), the Asian rock-pool mosquito (*Ae. japonicus*) in Switzerland and Germany (Schaffner *et al.* 2009) and *Ae. japonicus* in Belgium (Versteirt *et al.* 2009). In some countries, the exotic species *Ae. albopictus* has also shown to be able to transmit pathogens between humans, causing dengue and chikungunya cases in France (Gould *et al.* 2010, La Ruche *et al.* 2010), and even a chikungunya outbreak in Italy (Rezza *et al.* 2007).

Mosquito larvae occur in a wide-range of natural aquatic habitats including ditches, ponds, tree holes and bromeliad plants. Besides natural, also artificial containers with stagnant water such as used tires, cemetery vases and buckets are known to be a suitable habitat (Vezzani 2007). Used tires are abandoned, dumped, recapped, or stored for trading and eventually re-used. Abandoned or outdoor stored tires are nearly ideal mosquito incubators. Exposed to the elements tires can accumulate persistent water, organic material and microbes (Yee 2008). In addition they offer high humidity, high temperatures, and lack of natural enemies. Female mosquitoes can lay their eggs inside these tires, using them as a 'vehicle' to get transported and invade new areas (Reiter & Sprenger 1987). Eggs of *Aedes* spp. are drought resistant and are oviposited above the water level (*e.g.*, in tires the area where the mosquito species is indigenous). When the water level inside tires raises due to rain (*e.g.*, in a new area where that species did not previously occur) the eggs can hatch.

Some *Aedes* spp. that are associated with tires such as the invasive species *Ae. albopictus* and *Ae. japonicus* are important vectors for human diseases such as dengue and chikungunya. When these mosquito species breed in the vicinity of human activities, there is a potential risk of disease transmission, indicated by the examples described above. This can be either because eggs itself may already be infected with a virus at their arrival (Rosen 1987, Baqar *et al.* 1993), or via infectious human travelers (virus carriers) that bring a disease to an area where the vector is present, that can transmit pathogens to other people, as shown in the chikungunya outbreak in Italy (Rezza *et al.* 2007).

Until 2009 it was not reported that exotic mosquitoes are imported into The Netherlands via tires (Scholte *et al.* 2009), although data collected in 2009 and 2010 suggest that it has happened before. Apart from this route, it was already reported from 2006 onwards that the exotic mosquito *Ae. albopictus* occasionally enters The Netherlands at companies that import Lucky bamboo (*Dracaena sanderiana*) plants (Scholte *et al.* 2007).

The finding of *Ae. atropalpus* in 2009 at two companies that import used tires illustrated that The Netherlands are also exposed to the risk of exotic mosquitoes imported via the international used tire trade, and formed the basis for the work that is presented here: results of an extensive national surveillance (2010) of companies that trade in used tires. In addition this paper describes a qualitative method to divide trading tire companies in different categories and their corresponding risk to import exotic mosquitoes. It is meant to gain better insight in possible factors that are indicative of the risk and to help design or improve mosquito surveillance on tire companies in general.

MATERIALS AND METHODS

Survey set up

In cooperation with representatives of the tire industry, thirty-four companies that were registered to be trading in used tires, were sent a questionnaire to acquire information regarding the imported tires. Companies that had not responded, were phoned and asked the same questions as in the questionnaire. All the approached companies that imported used tires were included in the survey. Based on the obtained information a qualitative risk assessment was performed for each company to assess the risk on introduction of exotic mosquito species. The following risk factors were taken into account: (1) the type of tires that are imported, (2) the origin of the tires and (3) the method of storage. Risk factors were quantified by giving them a score (Table 1). The total score was calculated for each company. Companies that had a total score ≥4 were assigned to the high risk category (n = 14), with a score of 3 to the medium risk category (n = 3), and with a score ≤2 to the low risk category (n = 17).

Inspections were conducted to determine if exotic mosquitoes were present at a company. This was done from April through October with varying frequency based on the risk category, ranging from every 2 weeks (high risk), 3 times per season (medium risk) to once per season (low risk). At least one visit to the medium and the low risk companies was paid during summer, when mosquitoes are most abundant.

Table 1. Risk factors, values and their relative scores, that were used to assess the risk that exotic mosquitoes are imported at tire companies. For each risk factor, one value and corresponding score was selected. The total score was the sum of the individual scores of the three risk factors.

Risk factor	Value	Relative score
1. Type of tires	Car/van	0
	Truck	1
	Airplane	1
	Agricultural vehicles	1
	A combination of tire types above	1
2. Origin	From EU country without reports of exotic mosquitoes (negative)	0
	From EU country reported as positive	2
	From Dutch company reported as positive	2
	From non-European country	2
	A combination of origins above	2
3. Storage method	Indoors (dry)	0
	Outdoors (exposed to rain)	2
	A combination of storage methods above	2

Tire inspections

Tires with water were visually inspected for mosquito larvae with an even intensity over the tire storage site. If present, mosquito larvae were collected from tires with a plastic cup and transferred with a pipette in tubes with 70% ethanol. To decrease the effect of disturbance and risk that larvae were more difficult to collect, tires were approached cautiously and the cup was immersed fast at the water surface instead of slowly 'scooping' the water. At least 10 tires with larvae were sampled per inspection, if less tires contained larvae, up to 100 tires were inspected. Water in tires of which the opening was too narrow was sucked up with a manual pump.

Adult mosquitoes, sometimes present inside the tires, were disturbed by kicking tire piles. Flying mosquitoes were collected with a net, and mouth aspirator. Adult mosquitoes were killed by placing them in a tube in a freezer. All samples were labeled, sealed, and sent to the CMV laboratory for diagnostics. Mosquitoes were diagnosed either morphologically by using the diagnostic keys from FS (Schaffner *et al.* 2001, Schaffner 2003) or molecularly by PCR sequencing the mitochondrial cytochrome oxidase subunit 1 (CO1) gene (Simon *et al.* 1994). In some cases also macro-photographs were made and sent to the associate mosquito taxonomist (FS) for confirmation. Immediately after morphological diagnosis, exotic mosquitoes were stored in RNA-stabilizing buffer to allow virus detection later on. A few adult mosquitoes were preserved to serve as voucher specimens.

Shortly after the finding of an exotic mosquito at a company, all potential breeding sites in a 500m buffer around the company were investigated and removed if possible. Additionally, 4-5 adult traps (BG-sentinel, Biogents) and 10-15 oviposition traps were placed in this buffer zone, and emptied weekly. Oviposition traps consisted of a black plastic container (height: 15 cm, diameter: 15 cm), with hay-infusion water and a piece of polystyrene. If exotic specimens were found in a buffer zone, a new buffer zone of 500m was defined with the site of finding as buffer-centroid and new traps were place inside the buffer zone. All trap locations and findings outside company premises were geo-referenced using a GPS-device (GPSMAP 60C, Garmin). All locations, samplings and diagnoses were stored in an Oracle database.

Both larvicides and adulticides were used to for mosquito control. Details on the control methodology are presented in Scholte *et al.* (2010). If a company was found to be positive, from that moment inspections were performed weekly at that location to determine the effectiveness of the control.

RESULTS AND DISCUSSION

At five of the 34 companies that were included in the survey exotic mosquitoes were found. One of these companies occupied two sites, both of which were infested. In another case, two adjacent companies were both found to be 'positive'. In these cases, the infestations were obviously linked to each other, and therefore the two companies were treated as one in the results. For these reasons, we speak of five positive locations hereafter. A number of 360 specimens of three exotic species were found at the five locations: *Ae. albopictus* was found at four locations, *Ae. aegypti* at two locations and *Ae. atropalpus* at four locations. Overall, *Ae. atropalpus* larvae and adults were found in highest numbers. Only 13 adults of *Ae. aegypti* were found and no larvae. No mosquito eggs were found in any of the oviposition traps. A more detailed overview on the findings of exotic mosquitoes can be found in Scholte *et al.* (2010).

In total, 180 visits were paid to the 34 tire companies and their surroundings. At 27 visits at least one of the three exotic species was found. The first exotic specimen was found at 21 July 2010 (larval *Ae. atropalpus* at location 1) and the last on 5 October 2010 (adult *Ae. albopictus* at location 5).

At three of the five locations exotic mosquitoes were found in the surrounding areas of the company premises. *Aedes albopictus* adults were found between 25m and 300m from the tire platform in BG-Sentinel traps at three locations. *Aedes atropalpus* larvae were found between 15m and 150m from the tire platform in a water-filled tarpaulin, a wheelbarrow and a plant pot respectively, at one location. Other life-stages of these species were not found in the surroundings. *Aedes aegypti* was never found outside company premises.

Besides exotic mosquito larvae also larval specimens of three indigenous species were found in tires of various companies: *Culex pipiens*, *Cx. territans* and *Anopheles plumbeus*, of which *Cx. pipiens* was found in highest numbers. Also hundreds of non-biting midges (Chironomidae) larvae were found inside the tires.

The finding of *Ae. albopictus* in the outdoor environment is important because it is a competent vector of chikungunya and dengue viruses (Gratz 2004), and is probably able to survive the Dutch climate (Takumi *et al.* 2009). *Aedes aegypti* was found in relatively low numbers, and at 2 locations only. Although *Ae. aegypti* is not directly associated with the international trade in tires, several studies report this species to be a tire breeder (reviewed by Yee 2008). This is the first time that *Ae. aegypti* was reported in tires in The Netherlands and it is more than 50 year ago that this species was seen in Europe (Enserink 2010). Since it is a tropical species it is unlikely that it would survive the winter here.

The reported finding of *Ae. atropalpus* was not the first in The Netherlands; the species was also found in 2009 (Scholte *et al.* 2009). Other European reports go back to 2003 and 2006 from France (Scholte *et al.* 2009 and references therein) and in 1996 from Italy (Romi *et al.* 1997) from where it has been successfully eliminated (Romi *et al.* 1999; F Schaffner, pers. comm.). Therefore it is likely that the found *Ae. atropalpus* originates from the USA since this is the only place where this species naturally occurs.

Companies on which the exotic species were found, imported used tires from countries that are colonized by *Ae. aegypti* and *Ae. albopictus*, including Italy and

USA or from countries where the species naturally occurs; in case of *Ae. atropalpus* from the USA.

It is likely that exotic mosquitoes have been introduced before 2009 and 2010, but since this was the first extensive survey at these high risk introduction locations it is the first time that they were noticed, together with the findings in 2009.

Using the risk factors and their scores, described above, has shown to be an effective method to assess the variation in risk that exotic mosquitoes are imported: all positive companies belong to the high risk category, although recognizing that the likelihood of positive findings were higher for these high risk companies because they were inspected more often than companies of other categories. In future surveys at Dutch tire companies, the categorization of companies will be evaluated based on interviews with company owners and observations of inspectors.

The concern that exotic mosquitoes might spread from a tire company to its surroundings is reasonable, indicated by the finding of *Ae. atropalpus* larvae and *Ae. albopictus* adults up to 150 and 300 m from the company premises respectively, and in breeding sites other than used tires (*Ae. atropalpus* only). Up to now, no exotic species were found further than these distances from tire-platforms. However, unless measures are taken to reduce the risks, mosquito eggs will continue to be imported via the used tire trade. In case some of these species establish, it will increase the risk of mosquito-borne pathogen transmission.

Therefore continuous monitoring of import-pathways including used tire companies is crucial to facilitate early detection and subsequent control. Additional measures such as roofed tire storage, arrangement of tire stacks, and preventive control could reduce the spread of exotic mosquitoes via tires even more. It would be helpful not to limit this to The Netherlands, but take measures in all countries involved in the international tire-trade.

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