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SHORT COMMUNICATION

Repeated introduction of *Aedes albopictus* into Germany, July to October 2012

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Abstract During a small-scale surveillance project to identify possible routes of entry for invasive mosquitoes into Germany, 14 adult *Aedes* (*Stegomyia*) *albopictus* (Skuse) were discovered between July and October 2012. They were trapped at three different service stations in Bavaria and Baden-Wuerttemberg located along two motorways that connect Germany with southern Europe. This indicates regular introduction of *A. albopictus* into Germany and highlights the need for a continuous surveillance and control programme.

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Blood-feeding mosquitoes play an important role in the transmission of pathogens. Changes in climate and ecology as well as globalisation of international trade can influence their distribution (Reiter 1998; Becker 2008). During the last decades, various mosquito species, in particular *Aedes albopictus* have spread expeditiously from their tropical origin and colonised temperate zones. Some of these invasive species are capable to transmit chikungunya or dengue virus and autochthonous clinical cases caused by these viruses have been already reported from several European countries within the last few years (Gould et al. 2010; Gjenero-Margan et al. 2011).

Until recently, routine surveillance of mosquitoes in Germany was restricted to specific areas in the Southwest (Hesse, Rhineland-Palatinate, Baden-Wuerttemberg). Occasional findings of eggs and adults of *A. albopictus* along the Upper Rhine Valley have been reported in 2007 and 2011 (Pluskota et al. 2008; Werner et al. 2012) and the establishment of *Ochlerotatus japonicus*, another invasive species, has been recently discovered in wider areas of southern Germany (Becker et al. 2011). As a consequence, at the end of 2011, a surveillance network was initiated in close collaboration of scientific, traffic, and governmental institutions in order to assess the risk for introduction and spread of invading mosquitoes in Germany. The project focuses on selected possible hotspots that may act as introduction sites or stepping stones for invasive vectors. These sites include main international airports, harbours, and train stations with a high turnover of imported goods, as well as service stations and reloading sites at main motorways connecting Germany with countries in

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which *A. albopictus* and other invasive mosquito species have already been reported.

A total of 29 sites were selected (Fig. 1) and each site was equipped with one to three BG-Sentinel CO_2 traps (Biogents) and three traditional ovitraps (Becker et al. 2010). In addition, to map the distribution of *O. japonicus*, a considerable number of cemeteries in southern Germany were sampled for mosquito larvae. From April 2012, all traps were inspected every second week. Until the end of the observation period in October 2012, invasive mosquito species were discovered repeatedly but only in traps placed in southern Germany. During the entire observation period, *O. japonicus*, eggs, larvae, and adults, respectively, were collected at various trapping sites, supporting previous observations that this species is widely distributed in southern Germany (Becker et al. 2010; Huber et al. 2012; Werner et al. 2012). In addition, in July and August 2012, *A. albopictus* adult females were discovered for the first time in Bavaria. The mosquitoes were found in traps located at two different service stations along the same motorway about 25 km apart from each other (Fig. 1). At the same time, two *A. albopictus* females were trapped at a motorway station in Baden-Wuerttemberg several hundred kilometres apart from the two infested sites in Bavaria. The finding prompted us to intensify surveillance, in that the period for inspection was reduced to weekly intervals and additional traps were set up in a perimeter of 200–500 m from the *A. albopictus*-positive traps according to European Centre for Disease Prevention and Control (2012) guidelines. During the intensified follow up, further *A. albopictus* adults were trapped at two of the three sites. In total, one male and 13 female mosquitoes were collected, comprising three to eight adults per infested site (Table 1). However, no *A. albopictus* oviposition was detected despite the distribution of

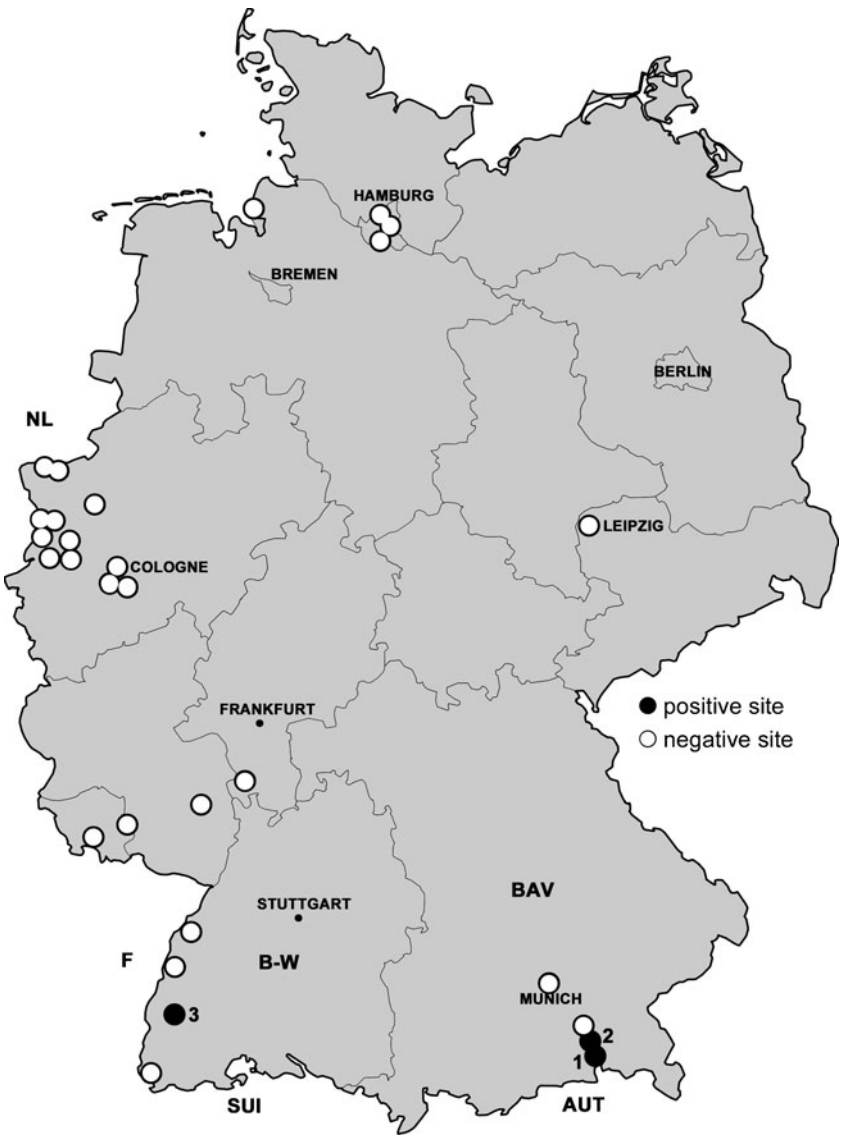

Fig. 1 Map of Germany showing the locations positive (n=3) and negative (n=26) for adult *A. albopictus* in the 2012 surveillance campaign. Numbers of positive sites refer to Table 1. *BAV* Bavaria, *B-W* Baden-Wuerttemberg

Table 1 Inspections and findings of *A. albopictus* for each location per week, April–October 2012

Location	Week																										
	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
Site 1												1										1	1					
Site 2																						3						
Site 3																			2		1		4			1		

No inspection | No *Ae. albopictus* found | *Ae. albopictus* found and number of specimen

considerable numbers of ovitraps and the fact that three out of 13 discovered females were gravid. The species *A. albopictus* was confirmed for all 14 specimens by PCR sequencing the mitochondrial cytochrome oxidase subunit 1 (CO1) gene (Folmer et al. 1994). To further determine whether the various mosquitoes represent siblings or independent introductions, a total of six microsatellite DNA markers were analysed according to previously published methods (Porretta et al. 2006; Kamgang et al. 2011). The results were not fully conclusive as only two of the markers were informative. However, the results were in favour of independent introductions at the three sites and the presence of siblings at the site in Baden-Wuerttemberg (Table 2).

Although the detection of *A. albopictus* (eggs or adults) in southwest Germany has been previously reported (Pluskota et al. 2008; Werner et al. 2012), the discovery of a considerable number of this invasive species concomitant in Bavaria and Baden-Wuerttemberg was unexpected. All mosquitoes were collected at service stations along main motorways connecting Germany with southern European countries either via Austria or Switzerland. Thus, it is likely that the mosquitoes originated from southern Europe and were introduced into Germany by transit road traffic. To our knowledge, there is no published report on the discovery of *A. albopictus* in Austria. However, there is information on a recent detection of *A. albopictus* larvae in September 2012 in the Lower Inn Valley in Tyrol (Franz Allesberger and Bernhard Seidel, personal communication), just 15–40 km apart from the infested sites in Bavaria. Thus, introduction from Austria to Bavaria cannot be excluded and intensified surveillance along the Inn Valley on both the German and the Austrian side is recommended.

All *A. albopictus* were collected in the second half of the trapping season suggesting that they were introduced into Germany in 2012 and not present before. Moreover, microsatellite DNA analysis suggests that the mosquitoes at the different sites represent independent introductions. Given the fact that we have discovered *A. albopictus* in traps at three out of seven service stations located along main motorways that connect Germany with southern European countries, it is evident that *A. albopictus* is regularly introduced into Germany. This however, poses the risk for establishment of this species at least in southern Germany, where conditions might be suitable for colonisation (Becker 2008).

The fact that relatively few adults and no other life stages of *A. albopictus* were found at the two locations in Bavaria indicates low levels of infestation. However, this might not be true for the infested site in Baden-Wuerttemberg where eight adults were collected and which is in close proximity to a site where *A. albopictus* was recently collected (Kampen et al. 2012). Thus, it will be important to continue intensified surveillance at all sites and beyond from early spring next year, to analyse whether *A. albopictus* is still present and whether control measures are indicated.

In conclusion, Germany is at risk for being colonised by *A. albopictus* due to regular and repeated introduction of this species by substantial ground vehicle transportation from southern European countries. To avoid establishment of this

Table 2 Allele size variation of microsatellite markers from all *A. albopictus* trapped at the three different sites between July and October 2012

Location[a]	Week[b]	Microsatellite A9[c]		Microsatellite D2	
		Allele 1	Allele 2	Allele 1	Allele 2
Site 1	27	146[d]	150	160	175
	37	146	152	173	174
	38	146	150	174	175
Site 2	37/1	140	140	174	174
	37/2	144	144	173	175
	37/3	150	150	174	174
Site 3	34/1	148	150	173	177
	34/2	142	148	173	173
	36	146	150	175	177
	38/1	146	150	175	175
	38/2	150	150	173	177
	38/3	150	150	173	177
	38/4	150	150	173	177
	41	148	148	173	177

[a] Sites where *A. albopictus* adults were discovered according to figure

[b] Week in which *A. albopictus* were found

[c] Microsatellite markers according to reference 12

[d] Numbers represent lengths of DNA fragments in base pairs

invasive mosquito species that poses a potential threat to public health, surveillance at least in southern Germany has to be expanded and suitable control measures should be in place.

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References

Becker N (2008) Influence of climate change on mosquito development and mosquito-borne diseases in Europe. Parasitol Res 103(Suppl 1): S19–S28

Becker N, Petrić D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A (2010) Mosquitoes and their control, 2nd edn. Springer, Heidelberg

Becker N, Huber K, Pluskota B, Kaiser A (2011) *Ochlerotatus japonicus japonicus*—a newly established neozoan in Germany and a revised list of the German mosquito fauna. Eur Mosq Bull 29:88–102

European Centre for Disease Prevention and Control (2012) Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm: ECD

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Gjenero-Margan I, Aleraj B, Krajcar D, Lesnikar V, Klobučar A, Pem-Novosel I, Kurečić-Filipović S, Komparak S, Martić R, Duričić S, Betica-Radić L, Okmadžić J, Vilibić-Čavlek T, Babić-Erceg A, Turković B, Avsić-Županc T, Radić I, Ljubić M, Sarac K, Benić N, Mlinarić-Galinović G (2011) Autochthonous dengue fever in Croatia, August–September 2010. Euro Surveill 16:pii019805

Gould EA, Gallian P, De Lamballerie X, Charrel RN (2010) First cases of autochthonous dengue fever and chikungunya fever in France: from bad dream to reality! Clin Microbiol Infect 16:1702–1704

Huber K, Pluskota B, Jöst A, Hoffmann K, Becker N (2012) Status of the invasive species *Aedes japonicus japonicus* (Diptera: Culicidae) in south Germany. J Vec Ecol 37:1–3

Kamgang B, Brengues C, Fontenille D, Njiokou F, Simard F, Paupy C (2011) Genetic structure of the tiger mosquito *Aedes albopictus* in Cameroon (Central Africa). PLoS One 6:e20257

Kampen H, Kronefeld M, Zielke D, Werner D (2012) Further specimens of the Asian tiger mosquito *Aedes albopictus* (Diptera, Culicidae) trapped in southwest Germany. Parasitol Res 5:250 (Epub ahead of print)

Pluskota B, Storch V, Braunbeck T, Beck M, Becker N (2008) First record of *Stegomyia albopicta* (Skuse) (Diptera: Culicidae) in Germany. Eur Mosq Bull 26:1–5

Porretta D, Gargani M, Bellini R, Calvitti M, Urbanelli S (2006) Isolation of microsatallite markers in the tiger mosquito *Aedes albopictus* (Skuse). Mol Ecol Notes 6:880–881

Reiter P (1998) *Aedes albopictus* and the world trade in used tires, 1988–1995: the shape of things to come. J Am Mosq Control Assoc 14:83–94

Werner D, Kronefeld M, Schaffner F, Kampen H (2012) Two invasive mosquito species, *Aedes albopictus* and *Aedes japonicus japonicus*, trapped in south-west Germany, July to August 2011. Euro Surveill 17:pii020067