Monitoring and Pathogen-Screening of Mosquitoes (Diptera: Culicidae) in Germany

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Zusammenfassung: Veranlasst durch den Eintrag nicht-einheimischer, vektorkompetenter Stechmückenarten, wie *Aedes albopictus* und *Ae. aegypti*, nach Südeuropa sowie assoziierter Ausbrüche von Dengue- und Chikungunya-Fieber, wurde 2011 in Deutschland ein Monitoringprogramm gestartet, um native und invasive Stechmückenarten zu kartieren und Risikoabschätzungen für Stechmücken-assoziierte Krankheiten zu ermöglichen. Das Programm besteht aus einem aktiven Teil, in dem adulte Stechmücken per Falle gefangen und Larven in ihren Brutgewässern gesammelt werden, und einem passiven Teil, der durch das Citizen Science-Projekt ‚Mückenatlas' repräsentiert wird. Die gesammelten Mücken werden nach Spezies oder Spezies-Komplex/-Gruppe morphologisch identifiziert und – im Falle von beschädigten oder kryptischen Arten – genetisch weiter aufgearbeitet. Parallel zu diesen Stechmückenmonitoring-Aktivitäten wurden in den letzten Jahren im Rahmen diverser Forschungsprojekte zusätzlich gesammelte Stechmückenweibchen, die zwecks Konservierung von Virus-RNA unmittelbar nach dem Fang tiefgefroren worden waren, auf Pathogene untersucht. Alle Monitoringdaten wurden in die deutsche Stechmückendatenbank CulBase eingegeben, die u.a. genutzt wird, um Verbreitungskarten zu erstellen und Mückenausbreitungen zu modellieren. Weiterhin werden Trocken- und DNA-Sammlungen der in Deutschland vorkommenden Stechmückenarten erstellt. Seit Beginn des Monitorings wurden ca. 800 000 Stechmücken gesammelt und analysiert, darunter nahezu 200 000 über das ‚Mückenatlas'-Projekt. Einundfünfzig Spezies wurden registriert, sechs mehr als zuvor für Deutschland beschrieben. Nach einem Jahrhundert ohne Dokumentation muss eine Spezies (*Cx. cyprius*) als in Deutschland nicht mehr vorkommend betrachtet werden, während eine Art (*An. daciae*) nachgewiesen wurde, die inzwischen genetisch von einer anderen separiert wurde. Fünf neue Spezies wurden entdeckt: zusätzlich zu den Asiatischen Buschmücken *Aedes japonicus* und *Ae. koreicus*, die sich im Labor als vektorkompetent für einige Pathogene zeigen, sowie den vektorinkompetenten wärmeliebenden Arten *An. petragnani* und *Cs. longiareolata*, hat sich die Asiatische Tigermücke *Ae. albopictus*, ein höchst effizienter Überträger zahlreicher gefährlicher Pathogene, fest in Deutschland etabliert. Während die klimatisch gut adaptierte und hochexpansive Japanische Buschmücke *Ae. japonicus* seit ihrem Erstnachweis im Jahr 2008 in Deutschland bereits die komplette südliche Landeshälfte besiedelt hat, konnte die thermophile und eher stationäre Tigermücke, die das erste Mal im Jahr 2007 in Deutschland beobachtet wurde, mehr als 20 lokale Populationen, meist im Südwesten des Landes, aufbauen. Von der Koreanischen Buschmücke *Ae. koreicus* ist bislang nur das Vorkommen einer Kolonie im westlichen Mitteldeutschland bekannt.

Dem Pathogen-Screening zufolge sind einige Stechmücken-übertragene Viren (Batai, Sindbis, Usutu und West-Nil-Virus) und Fadenwürmer (*Cardofilaria pavlovskyi, Dirofilaria repens, Setaria tundra*) endemisch in Deutschland. Die potenziell lebensbedrohlichen Dengue- und Chikungunya-Viren, die in den letzten Jahren wiederholt in Südeuropa auftraten und von *Ae. albopictus* übertragen werden, werden zwar regelmäßig durch Reisende nach Deutschland eingetragen, sind aber hier bisher weder in hiesigen Stechmücken nachgewiesen, noch mit autochthonen Erkrankungsfällen in Zusammenhang gebracht worden. Im Gegensatz dazu kommt das West-Nil-Virus (WNV) seit mindestens 2018 in Deutschland vor und hat in den folgenden Jahren zu zahlreichen symptomatischen Infektionen bei Vögeln und Pferden sowie beim Menschen geführt. Ein Mensch verstarb an der Infektion. WNV-Hotspots befinden sich v.a. in Nordostdeutschland. Hauptvektoren des Virus sind bestimmte Varianten der gemeinen Hausmücke *Culex pipiens*, der häufigsten und am

weitesten verbreiteten Stechmückenart Deutschlands. Das Virus wurde in mehreren Pools des *Cx. pipiens*-Komplexes, einschließlich eines aus überwinternden Mückenweibchen bestehenden, nachgewiesen, so dass davon auszugehen ist, dass das WNV sich dauerhaft in der deutschen Stechmückenfauna festgesetzt hat.

Globalisierung und Klimaveränderung sind die treibenden Kräfte der Einschleppung, Etablierung und Ausbreitung von Vektoren und Pathogenen, wobei die Erwärmung zusätzlich das Populationswachstum und die Verlängerung der saisonalen Aktivität von Vektoren sowie die extrinsische Entwicklung von Pathogenen fördert. Beide Phänomene zusammen werden in der Zukunft zu erhöhten Risiken von Stechmücken-assoziierten Krankheiten in Deutschland führen. Monitoring/Surveillance, Risikoanalysen und Aufklärung sind daher wichtige Aufgaben des Öffentlichen Gesundheitsdienstes. Das Stechmückenmonitoring ist inzwischen z.T. institutionalisiert worden, eine routinemäßige Pathogensurveillance existiert in Deutschland aber noch nicht.

Key words: Disease agents, distribution, Germany, monitoring, mosquitoes, transmission, vector

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Introduction

Decades after the disappearance of yellow fever, dengue and, finally, malaria (Bruce-Chwatt & de Zulueta 1980, Schaffner & al. 2013), mosquitoes and mosquito-borne diseases have regained considerable public health attention in Europe (Zeller & al. 2013). Starting in the late last millennium, not only vector-competent alien mosquito species, such as *Aedes albopictus* (Skuse, 1895) and *Ae. aegypti* (Linnaeus, 1762), have emerged and widely spread in parts of Europe, but mosquito-borne pathogens, such as dengue and chikungunya viruses, have been transmitted in southern Europe once their vectors had become established (Table 1). In addition, Usutu virus emerged for the first time outside of Africa (Weissenböck & al. 2002), and West Nile virus outbreaks, mainly vectored by native *Culex* species, such as *Culex pipiens* Linnaeus, 1758 or *Cx. modestus* Ficalbi, 1890, have become more widely distributed, more frequent and more aggressive (Camp & Nowotny 2020). Dirofilarial infections have also increasingly often been observed north of their traditional distribution range in the Mediterranean area (Genchi & Kramer 2020).

All these events of emerging and resurging mosquito species or mosquito-borne diseases must doubtlessly be attributed in one way or the other to globalisation and climate warming. Globalisation produces increasing numbers of introductions both of arthropods, including potential vectors, by intercontinental trade and of infection sources of vector-borne pathogens by mass tourism. Increasing temperatures facilitate establishment and spread of thermophilic vector species, accelerate blood digestion and egg production by them and thus increase the blood feeding frequency, and eventually lead to increased population densities (Ratte 1984). Moreover, they will allow for extended seasonal activities of vectors. Pathogens, which are likewise dependent on ambient temperatures as long as they pass through the vector, benefit from higher temperatures in that their extrinsic development accelerates or is made possible at all. In summary, elevated temperatures will generally cause intensification of natural transmission cycles (Mordecai & al. 2022).

Probably due to its climate, Germany has never been a country infamous for mosquito-borne diseases, although malaria had historically been widely distributed, particularly in the northern lowlands (Eichenlaub 1979). *Plasmodium* parasites, however, have never been documented in mosquitoes. Similarly, avian malaria did and, in all probability, still does occur in Germany (Wiersch & al. 2007, Schmid & al. 2017), but mosquito collections had only been checked for its causative haemosporidian agents during the monitoring programme (Heym & al. 2019).

Table 1: Major recent events of emergence of invasive mosquitoes and outbreaks of mosquito-borne disease in southern Europe and Germany.

Year	Event	Special notes	Reference
1990	*Ae. albopictus* starts spreading across Europe	first detection in Genoa, Italy	Scholte & Schaffner (2007)
1990s ff.	increase in number and severity of West Nile fever outbreaks in Europe	emergence of WNV lineage 2	Hernández-Triana & al. (2014)
2001	first outbreak of Usutu fever (Austria)	mass mortality among blackbirds	Weissenböck & al. (2002)
2004	establishment of *Ae. aegypti* on Madeira (Portugal)		Almeida et al. (2014)
2007	outbreak of chikungunya in northern Italy	ca. 200 people infected 1 fatal case	Rezza & al. (2012) Casolari & al. (2008)
2007	first detection of *Ae. albopictus* in Germany	eggs detected in south-western Germany	Pluskota & al. (2008)
2008	first detection of *Ae. japonicus* in Germany	adults detected on the border to Switzerland	Schaffner & al. (2009)
2012	outbreak of dengue on Madeira	ca. 2,000 people infected	Sousa & al. (2012)
2015	first detection of *Ae. koreicus* for Germany	specimens detected in archived '*Ae. japonicus*' collections	Werner & al. (2016) Hohmeister & al. (2021)
2016	outbreak of West Nile fever	1548 autochthonous human cases in EU, including 166 fatalities	ECDC (2019)
2022	outbreak of West Nile fever	1108 autochthonous human cases in EU, including 112 fatalities	ECDC (2023)

By contrast, various surveys found mosquitoes collected in Germany to be infected with Ťahyňa virus, Sindbis virus (SINV), Batai virus (BATV) and Usutu virus (USUV) (Pilaski 1987, Schüssler 2000, Jöst & al. 2010, 2011a, 2011b). The first three viruses may cause intermittent and self-limiting flu-like symptoms in humans (Hubálek 2008), while USUV, which is primarily bird-pathogenic, has been reported several times to cause neuroinvasive disease in immunocompromised humans (Cadar & Simonin 2023).

Authochthonous human cases of mosquito-borne disease had never been diagnosed in Germany in the post-malaria era until the West Nile fever outbreak in 2018. However, the emergence of the Asian tiger mosquito *Ae. albopictus* in 2007 (Pluskota & al. 2008) and of the Japanese bush mosquito *Ae. japonicus* (Theobald, 1901) in 2008 (Schaffner & al. 2009) before the background of dengue and chikungunya outbreaks in southern Europe (Tomasello & Schlagenhauf 2013), together with the unprecedented ceratopogonid-borne bluetongue epidemic among ruminants in central and northern Europe in 2006 (Saegerman & al. 2008), alerted German authorities and called for action. Vector monitoring and research programmes were initiated, including mosquito monitoring activities, hoping to fill the huge gap of data on the occurrence and spatiotemporal distribution of mosquito species as well as on the circulation of mosquito-borne pathogens.

Not so regularly, additional mosquito females were collected over night at selected places by BG-Sentinels and EVS traps as well as by aspiration in hibernation sites for pathogen screening. To conserve viral RNA, these mosquitoes were immediately put on dry ice after each trapping night or operation.

All data linked to a mosquito catch were entered into the German mosquito database (CulBase). They are meant to map the German mosquito fauna, identify circulating mosquito-borne pathogens and model the future risks of mosquito-borne diseases in Germany.

Results and discussion

Mosquito occurrence

From 2011 to 2022, about 800,000 mosquitoes were collected, some 200,000 of them submitted by citizens to the 'Mueckenatlas' scheme. While the number of mosquito species documented for Germany was still 46 in 2006 (Dahl & al. 1999), the monitoring activities produced six new species for Germany: *Ae. albopictus, Ae. japonicus, Ae. koreicus* (Edwards, 1917), *An. petragnani* del Vecchio, 1939, *Culiseta longiareolata* (Macquart, 1838) and *An. daciae* Linton, Nicolescu & Harbach, 2004 (Table 2). The first three of them originate from Asia and are supposed to have been introduced via their eggs, larvae and adults by trade and travel (Medlock & al. 2015). The pronounced thermophily of *Ae. albopictus* is a major reason why this mosquito species struggles to overwinter and establish in Germany. Notwithstanding, it succeeds here and there, predominantly in warmer regions, such as the Upper Rhine Valley (Walther & al. 2017). Most detections of *Ae. albopictus* colonies can be attributed to reports from citizens whereas trapping specimens of this species was successful only in areas already known to be colonized. At present, *Ae. albopictus* has established populations at about 20 locations.

In contrast to *Ae. albopictus*, the two closely related Asian bush mosquito species *Ae. japonicus* and *Ae. koreicus* are well adapted to the moderate central European climate and easily cope with below zero winter temperatures. The Japanese bush mosquito *Ae. japonicus* is an example for an extraordinarily successful invader: from its first detection in Germany in 2008 to 2022, it spread from the German-Swiss border over the complete southern part of the country and also colonized the Hanover region (federal state of Lower Saxony) in the north (Werner & Kampen 2013, Koban & al. 2019). It stays where it once has emerged and reaches high population densities in many areas. From Germany, it has crossed the borders to Belgium and the Czech Republic (Smitz & al. 2021, Vojtíšek & al. 2022). In the near future, it is expected to have colonized the whole of Germany and to cross borders to Poland in the east and to the Netherlands in the west.

For unknown reasons, the Korean bush mosquito *Ae. koreicus* appears to be hardly expansive in most of its distribution areas in Europe. The first detection in Germany was made in Augsburg (federal state of Bavaria) in 2015 (Werner & al. 2016), but the only established German population now exists in the Wiesbaden municipality (federal state of Hesse) (Pfitzner & al. 2018). Interestingly, it was initially thought to have colonized that area in 2016, but re-analysis of bush mosquito collections from previous years, considered to be *Ae. japonicus*, produced evidence of *Ae. koreicus* invasion already in 2015 (Hohmeister & al. 2021).

Culiseta longiareolata and *An. petragnani* are again thermophilic invasive species, widespread in Europe in the Mediterranean basin. They were first detected in Germany in 2011 and 2015, respectively (Becker & Hoffmann 2011, Kampen & al. 2013, Becker & al. 2016, Kampen & al. 2017). While the monitoring successively revealed more and more sites of *Cs. longiareolata* occurrence in Germany, *An. petragnani* did not occur at additional places but remained present over the years at one single site in the federal state of Baden-Württemberg (Werner & al. 2020).

Another thermophilic species from southern Europe, *Uranotaenia unguiculata* Edwards, 1913, first detected in Germany in 1994 in Baden-Württemberg (Becker & Kaiser 1995) and repeatedly found afterwards at the same site but not elsewhere, was observed at several places in northeastern areas during the monitoring activities (Tippelt & al. 2017). *Culiseta longiareolata, An. petragnani* and *Ur. unguiculata* are not considered vectors of disease agents, but their occurrence and spread in Germany clearly indicate improving climatic conditions for thermophilic mosquito species.

A sixth species detected during the monitoring programme was *An. daciae*, a sibling species of the *An. maculipennis* complex, separated from *An. messeae* Falleroni, 1926 only in 1994, based on consistent genetic differences in the ITS2 DNA region (Nicolescu & al. 2004). It has probably been present in Germany as long as mosquito species were recorded, but just not recognized.

In addition, the monitoring re-discovered several rare species, some of which had not been documented anymore for decades: *Ae. refiki* (Medschid, 1928), *An. algeriensis* Theobald, 1903 and *Cx. martinii* (Medschid, 1930) (Kuhlisch & al. 2017, 2018, Tippelt & al. 2018). Three other species occurring quite infrequently, *Cs. alaskaensis* Ludlow, 1906, *Cs. glaphyroptera* (Schiner, 1864) and *Cs. ochroptera* (Peus, 1935), could also be demonstrated (Kampen & al. 2013, Kuhlisch & al. 2019).

Table 2: Mosquitoes detected in Germany during the monitoring scheme (the formerly documented *Ae. cyprius* (Ludlow, 1920) is now considered extinct in Germany; additional species documented for Germany and considered present, but not detected in the monitoring programme, are *Ae. nigrinus* (Eckstein, 1918) and *Ae. pionips* (Dyar, 1919). Red: Species recently invaded with vector potential; blue: species recently invaded without known vector potential.

Aedes	*Anopheles*	*Coquillettidia*	*Culex*	*Culiseta*	*Uranotaenia*
Ae. albopictus (Skuse, 1895)	*An. algeriensis* Theobald, 1903	*Cq. richiardii* (Ficalbi, 1889)	*Cx. hortensis* Ficalbi, 1889	*Cs. alaskaensis* (Ludlow, 1906)	*Ur. unguiculata* Edwards, 1913
Ae. detritus (Haliday, 1833)	*An. atroparvus* van Thiel, 1927		*Cx. martinii* Medschid, 1930	*Cs. annulata* (Schrank, 1776)	
Ae. diantaeus (Howard, Dyar & Knab, 1913)	*An. claviger* (Meigen, 1804)		*Cx. modestus* Ficalbi, 1890	*Cs. fumipennis* (Stephens, 1825)	
Ae. dorsalis (Meigen, 1830)	*An. daciae* Linton, Nicolescu & Harbach, 2004		*Cx. pipiens* Linnaeus, 1758	*Cs. glaphyroptera* (Schiner, 1864)	
Ae. excrucians (Walker, 1856)	*An. maculipennis* Meigen, 1818		*Cx. territans* Walker, 1856	*Cs. longiareolata* (Macquart, 1838)	
Ae. flavescens (Müller, 1764)	*An. messeae* Falleroni, 1926		*Cx. torrentium* Martini, 1925	*Cs. morsitans* (Theobald, 1901)	
Ae. geniculatus (Olivier, 1791)	*An. petragnani* del Vecchio, 1939			*Cs. ochroptera* (Peus, 1935)	
Ae. intrudens (Dyar, 1919)	*An. plumbeus* Stephens, 1828			*Cs. subochrea* (Edwards, 1921)	
Ae. japonicus (Theobald, 1901)					
Ae. koreicus (Edwards, 1917)					
Ae. leucomelas (Meigen, 1804)					
Ae. pullatus (Coquillett, 1904)					
Ae. punctor (Kirby, 1837)					
Ae. refiki (Medschid, 1928)					
Ae. riparius (Dyar & Knab, 1907):					
Ae. rossicus Dolbeskin, Gorickaja & Mitrofanova, 1930					
Ae. rusticus (Rossi, 1790)					
Ae. sticticus (Meigen, 1838)					
Ae. vexans (Meigen, 1830)					

It is not clear whether *Cs. subochrea* (Edwards, 1921) was collected. This species is considered native to Germany but is not widely distributed and can reliably neither be distinguished morphologically nor genetically from *Cs. annulata* (Schrank, 1776) in its female stage. The monitoring programme produced no *Cs. subochrea* males (Werner & al. 2020).

Two other species listed in historic inventories of the German culicid fauna were definitely not encountered: *Ae. cyprius* and *Ae. nigrinus*. The first had been found last in the 1920s (Peus 1937), but never after and therefore must now be regarded extinct. The latter was documented only four times in Germany, the last time in 1993 (Becker & Kaiser 1995).

In summary, 49 culicid species could be detected during the monitoring activities of 52 species ever documented for Germany until 2021. In 2022, another species, *Ae. pionips*, was found outside the monitoring scheme (Kuhlisch 2022), bringing the total number of German culicids to 52, provided *Ae. cyprius* is not existent anymore.

Pathogen screening

In various studies carried out within the framework of the monitoring programme, some 30,000 mosquitoes collected throughout Germany were screened for filarial worms. Several worms pathogenic to humans or other vertebrates were found: the dog skinworm, *Dirofilaria repens* Railliet & Henry, 1911, the dog heartworm, *D. immitis* (Leidy, 1856), a worm pathogenic to cervids, *Setaria tundra* (Issaitshikoff & Rajewskaya, 1928), and another one pathogenic to birds, *Cardiofilaria pavlovskyi* Strom, 1937 (Table 3). In addition, numerous filarial worms were detected in the mosquitoes that could not be assigned to a species. Of the two *Dirofilaria* species, which are potentially human-pathogenic, *D. immitis* was found in Germany for the first time during the monitoring while *D. repens* had been observed in dogs already in 2007 (Sassnau & al. 2009). The latter also caused a symptomatic autochthonous human infection in 2014 (Tappe & al. 2014). *Setaria tundra* and *C. pavlovskyi* are not human-pathogenic, and at least *S. tundra* had been found in its vertebrate animal hosts in Germany before (Böhm & Supperer 1955).

Table 3: Viral, protozoan and helminth pathogens found in mosquitoes collected in Germany during the monitoring programme (2011 – 2022).

Viruses	Protozoa	Helminths
Ťahyňa virus	*Plasmodium relictum*	*Dirofilaria repens*
Batai virus	*Plasmodium matutinum*	*Dirofilaria immitis*
Sindbis virus	*Plasmodium vaughani*	*Setaria tundra*
West Nile virus	*Plasmodium vaughani*	*Cardiofilaria pavlovskyi*
	Haemoproteus majoris	

With respect to viruses, SINV, BATV and USUV were found among close to 100,000 examined mosquitoes collected from 2011 to 2016 throughout Germany (Scheuch & al. 2018). In another major study from 2019 to 2021, including 26,000 mosquitoes primarily collected in the eastern part of Germany, SINV and USUV were found again, but not BATV. In the same study, WNV was detected several times in *Cx. pipiens* biotype *pipiens* mosquitoes and *Cx. pipiens* biotype *pipiens* x *molestus* hybrids from zoos in the outbreak regions of northeastern Germany (Rau & al. 2023). WNV, however, had been detected already before in mosquitoes in the course of the monitoring activities. In 2019, mosquitoes trapped in the surroundings of various locations where WNV had emerged and been isolated from dead birds, were checked for WNV. Seven pools of mosquitoes of the *Cx. pipiens* complex originating from the 'Tierpark Berlin' were tested positive for the virus (Kampen & al. 2020). Later on, this virus could also be identified in a pool of overwintering *Cx. pipiens* females collected in northeastern Germany, confirming overwintering of the virus in their vectors (Kampen & al. 2021). *Culex pipiens* is considered the major vector of WNV in Europe (Brugman & al. 2018).

Finally, some 9,000 mosquitoes collected all over Germany from 2020 to 2022 were checked for haemosporidian parasites (Köchling & al. 2023). Next to *Haemoproteus majoris* (Laveran, 1902), a species of a genus believed to be transmitted by biting midges (Ceratopogonidae) rather than mosquitoes, several *Plasmodium* species and strains were detected, including three for the first time in Germany. Infected mosquitoes were identified as *Cx. pipiens* s.l., *Cs. morsitans* (Theobald, 1901)*/fumipennis* (Stephens, 1825) and *Ae. cinereus* Meigen, 1818/*geminus* Peus, 1970.

Conclusion

Introduction, establishment and spread of mosquitoes are clearly supported by globalisation and climate warming. Mosquitoes are displaced intercontinentally in their various developmental stages through global trade while mosquito-borne disease agents are introduced by migratory birds and infected travellers returning from endemic areas. Both mosquitoes and pathogens benefit from increasing temperatures since physiological processes and development are accelerated or facilitated, making natural transmission cycles more efficient.

Not only the invasive Asian *Aedes* species are vector-competent for disease agents (Paupy & al. 2009, Kampen & Walther 2014, Jansen & al. 2021) and are therefore reason of concern. Of the 52 mosquito species occurring in Germany, several indigenous ones have vector competence, too (Kampen & Walther 2018) and should carefully be observed.

Except for WNV, the identified filarial worm and viruses were detected in various mosquito species, including species never described before as potential vectors. However, these findings are not necessarily indicative to vector species since whole mosquitoes were homogenized and examined for the pathogens. These certainly could have been ingested by non-vector-mosquitoes without the capability of continuing their development and being transmitted to a vertebrate host.

Acknowledgments

The authors are grateful to numerous students, technicians, trap attendants and countless citizens for collecting submitting, identifying and screening mosquitoes as well as processing and analysing the data. This work was financially supported by the Robert Koch Institute, grant number RKI_Fo1362/1-982, the FLI, grant number HR-0054, and the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), grant numbers RKI-Fo_1362/1-982, 2810HS022, 2818SE001, 2819104615, 2819104915, 2819105315 and 2819113919.

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