PŮVODNÍ PRÁCE

The invasive mosquito *Aedes albopictus* (Diptera, Culicidae) firstly recorded in Bohemia, Czech Republic

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ABSTRACT

Study objective: In 2016–2017, the monitoring of possible introduction of an invasive mosquito species, the Asian tiger mosquito *Aedes (Stegomyia) albopictus* (Skuse, 1894) (Diptera, Culicidae), was conducted in eastern, southern, central and western parts of Bohemia, Czech Republic.
Material and Methods: The focus was placed on local major traffic arteries (motorways D1, D3 and D5 and an expressway E49), which connecting South Europe and some of Balkan countries, infested by *Ae. albopictus*, with the Czech capital Prague. In total, more than 100 ovitraps were placed on 16 study sites - close surroundings of refuelling gas stations and neighbouring parking lots.
Results: In August and September 2017, totally eight specimens of *Ae. albopictus* were collected at the ovitrap site near Mezno/Mitrovice, Central Bohemia on D3 motorway and other two specimens were recovered at the gas station near Rozvadov, West Bohemia on D5 motorway. On the other hand, Ae. albopictus was not recorded on a main Czech motorway D1 connecting Prague and Bratislava capitals during the monitoring.
Conclusion: The introduction of this mosquito into the Czech Republic is known since 2012 from surroundings of Mikulov town (South Moravian Region), our records were then the first in the region of Bohemia. Moreover, the distance between positive localities shows the potential for *Ae. albopictus* to be introduced by ground transport anywhere within the Czech Republic.

KEYWORDS

***Aedes albopictus* – Asian tiger mosquito – Culicidae – monitoring – arbovirus vector**

SOUHRN

První záznamy o introdukci invazivního komára *Aedes albopictus* (Diptera, Culicidae) na území Čech
Cíl: V letech 2016 a 2017 bylo prováděno monitorování výskytu invazivního komára *Ae. albopictus* na předpokládaných místech jeho vstupu na území Čech. Tito komáři mohou být zavlečeni na území republiky silniční dopravou ze zemí s jejich masivním výskytem (jihoevropské státy).
Materiál a metodika: Monitorování výskytu invazivních komárů bylo prováděno celkem na 16 stanovištích – nejbližší okolí parkovišť a zařízení k odpočinku řidičů u benzinových stanic na dálnicích D1, D3, D5 a odpočívadla na státní silnici E49. K zachycení byly použity pasti, tzv. ovitrapy, což jsou nádobky s vodou lákající gravidní samice komárů *Ae. albopictus*. Po expozici pastí v terénu byl jejich obsah transportován do laboratoře, kde bylo sledováno líhnutí larev a později líhnutí dospělců (pro snadnější identifikaci druhu komára).
Výsledky: V roce 2016 nebyli podél dálnic v Čechách zachyceni žádní invazivní komáři. K prvnímu záchytu došlo začátkem srpna 2017 v blízkosti parkoviště benzinové stanice na 64. km dálnice D3 v blízkosti obcí Mezno/Mitrovice poblíž Miličína (Středočeský kraj). *Ae. albopictus* zde byl zachycen opakovaně (celkem 8 exemplářů). Další výskyt tohoto druhu (2 exempláře) byl pak zaznamenán na odstavném parkovišti u benzinové pumpy u dálnice D5 nedaleko Rozvadova (Plzeňský kraj) a státní hranice s Německem.
Závěr: Zavlečení *Ae. albopictus* na území ČR je známo od roku 2012 z okolí Mikulova, blízko hranic s Rakouskem (Jihomoravský kraj). V tomto článku je popsán první záchyt komára na dvou různých lokalitách na území Čech. Ukazuje se tak, že tento invazivní druh může být, díky své schopnosti využít sílící silniční dopravu k pasivnímu transportu, zavlečen kamkoliv na území státu.

KLÍČOVÁ SLOVA

***Aedes albopictus* – komár tygrovaný – Culicidae – monitorování – vektor arbovirů**

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INTRODUCTION

The Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894) (Diptera; Culicidae), is a native of Southeast Asia from where it spread artificially to tropical and temperate climate parts of the world (India subcontinent, Pacific and Indian ocean Islands, both Americas, Europe, northern Australia, parts of Africa) [1]. Thanks to its ability to use passive transport and its ecological plasticity, this species is currently listed among the 100 most invasive species in the world [2]. In Europe, the first establishment of *Ae. albopictus* was documented in 1979 in Albania, where these mosquitoes had been probably introduced from China. Used tyres became their principal larval habitat in Albania [3]. In eighties and nineties of the last century global trade with used tyres was a main cause of *Ae. albopictus* dramatic spread of formerly tree-hole mosquito species (its larvae breed in rain water accumulated in

tree holes and e.g. in bromeliad plants. Used tyres transported by uncovered trans-ocean ships or tyres stored in the field provide suitable larval habitats similar to *Ae. albopictus* original natural breeding sites. Gravid females are attracted to lay eggs into the tyres. Rain water accumulated inside tyres and a little of organic material secure further development of larvae and pupae. In larval stage, the species has great capacity to colonize also other man-made environments (rain water catch basins, rain barells, flower vases, used tyres tins etc.).

Since *Ae. albopictus* establishment and massive development after the turn of the century in Italy, (later on in France or south Switzerland), firmly established populations of this species have been reported from altogether 21 European countries. Moreover, passively introduced specimens of *Ae. albopictus* were captured in other five countries including the Czech Republic [4]. The first evidence of *Ae. albopictus* occurrence in this country was proved in the South Moravian Region in 2012 [5].

Ae. albopictus is an aggressive, anthropophilic, daytime biting mosquito, which was proved to be competent vector of more than 25 arboviruses [6] from which Chikungunya virus, Dengue virus, Zika virus, West Nile virus, Usutu virus and few other viruses were isolated in the field as well [6, 7]. It is also known as a vector of the filarial nematode species, *Dirofilaria*. Its transmission was detected primarily between dogs and mosquitoes, but it can affect humans as well [8]. The first serious outbreak (epidemics) of Chikungunya fever unexpectedly occurred in Italy (Ravenna, region Emiglia Romana) in 2007 [9]. Some cases of Dengue vectored by *Ae. albopictus* followed in Croatia in 2010 [10]. Considering those epidemics as well as recent autochthonous Chikungunya virus outbreaks reported in South European countries [11, 12], the presence of *Ae. albopictus* mosquitoes could pose a serious threat to public health. The future monitoring of its occurrence (introduction and possible establishment) in the Czech Republic is thus essential.

MATERIAL AND METHODS

During 2016, the research was performed at 11 sites (numbers 5–16 on Figure 1) located on the D1 and D3 motorways. In 2017, the monitoring was extended to the D5 motorway (sites 1–4) and the E49 state road. All the above mentioned roads connect the Czech Republic with neighbouring (Austria, Germany) or Mediterranean (Italy, Slovenia and Croatia) countries already infested by *Ae. albopictus*. These roads were thus expected to be suitable for entry of the mosquito to Bohemia. All the selected sites serve as refuelling and resting places for personal, bus, and truck traffic. Therefore, they are appropriate for the compulsory breaks as well as longer stays (e.g. during public holidays or weekends) for professional drivers. These places may be the first stop of the trucks, cars or caravans in the country during the journey from

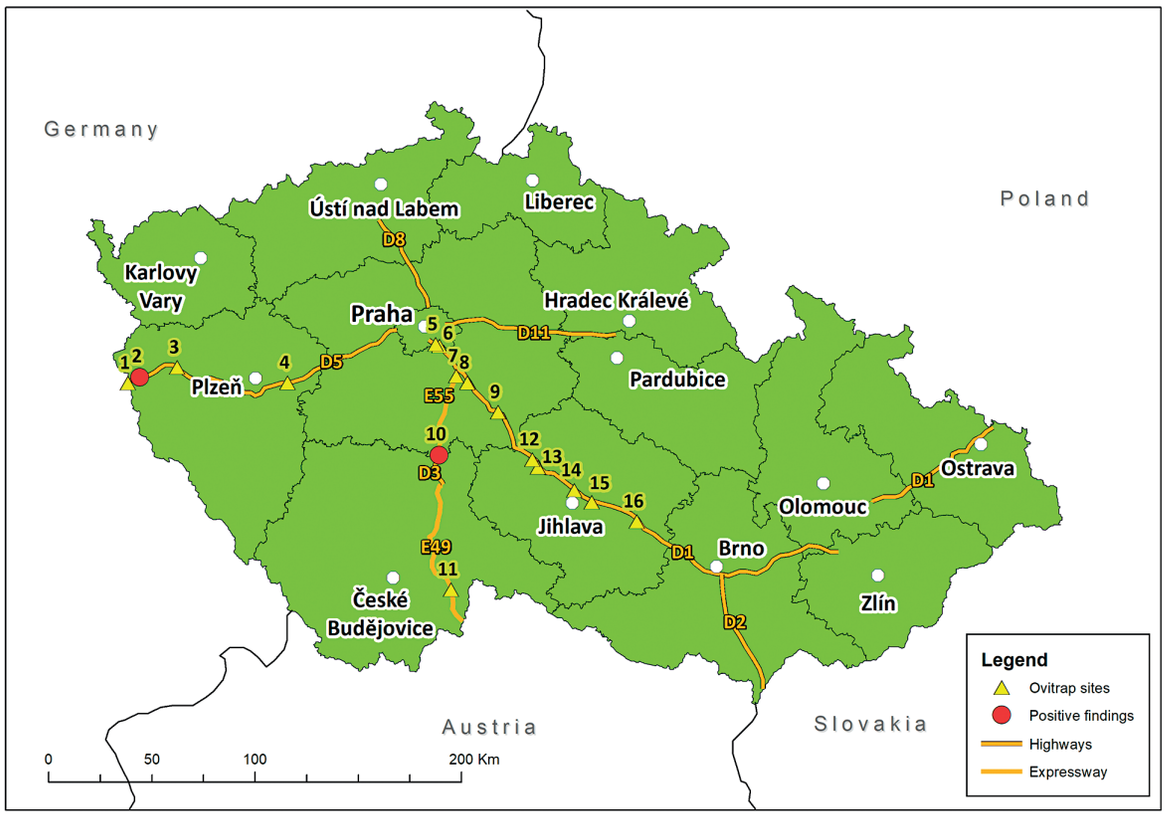

Figure 1. Map of the Asian tiger mosquito (*Ae. albopictus*) monitoring in Bohemia

countries infested by *Ae. albopictus* and could thus be the first possibility for the mosquitoes to leave the vehicles. For *Ae. albopictus* monitoring, oviposition traps (ovitraps) were used. These devices are dark containers containing water and substrate, which simulate natural *Ae. albopictus* breeding sites and attract gravid mosquito females to lay eggs. Ovitraps are considered to be the most effective traps for tiger mosquito monitoring [13]. The ovitraps used in the purpose of this study were home-made, prepared from black conical plastic containers (flower pots) 1.0–1.5 litre in capacity or cut white plastic bottles (originally used for chemicals) covered by thin black shield. Ovitraps were hanged on trees 50 cm above ground and in shaded places in bushes and hedges around selected parking lots. The traps were then filled with 500 ml of tap water. A wooden tongue depressor paddle was immersed into each trap to serve as substrate for oviposition. Based on the recommendation of Moravian colleagues [5], paddles were wrapped into a cotton fabric to attract gravid mosquito females to the trap and ensure better grip for oviposition. Ovitraps were checked once in 7–14 (21) days interval for the presence of eggs or larvae (pupae). The wooden paddles were collected, labelled, and put in the plastic bags (to maintain sufficient air humidity). Water from traps from each site was collected and accumulated in 10 litres plastic canisters. All the obtained samples were subsequently transported to laboratory where the paddles were firstly incubated in 100% relative humidity in ambient atmosphere of a dessicator outfitted with a plastic cup with soaked cotton wool for 3–5 days and then immersed into the containers with water. Water collected from traps was put into the aquariums (10 litres in capacity) immediately after arrival to the laboratory. The temperature in the laboratory was set to 26 °C and maintained throughout the whole incubation period. The samples collected were checked daily, and the presence of larvae was recorded. The hatched larvae were reared in the same conditions to L4 or to the adult stage, when they were euthanized for identification.
At study sites No. 4, 10, 11, 13 and 14, two used tyres per a site filled with hay infusion were placed close to ovitraps sites. Tyres were positioned in a shady places as hedgerow, bushes, trees etc. from August 2017 until the end of September 2017, then they were removed.
Morphological identification of mosquitoes was carried out using the key by Becker et al. [14]. For determination of captured Culex mosquitoes, preimaginal stages were reared to adults and males hypopygia were then used for species identification.

RESULTS

In 2016, *Ae. albopictus* mosquitoes were not captured at any of the study sites.
During monitoring in 2017, totally 10 specimens of *Ae. albopictus* were collected at two study sites on D3 and D5 motorways: No. 10 , in the Central and No. 2 in the West Bohemia (See Figure 1).
Totally 8 specimens of *Ae. albopictus* were recorded at study site No. 10 on D3 highway near Mezno/Mitrovice, Central Bohemia, (49.5332 N, 14.6647 E, 559 m a.s.l.) during three collecting periods:

– samples collected 2nd August, 2 adults hatched (2 ♂) on 4th August
– ovitraps material collected 22nd Aug: 2 adults hatched (1 ♀ and 1 ♂) on 24 th August
– ovitraps material collected 6th Sept: 4 adults hatched (2 ♀ and 2 ♂) on 14th September.

Two *Ae. albopictus* larvae (one reared to ♂ adult, one euthanized in L4) were then found in water collected from ovitraps at site No. 2 on D5 motorway near Rozvadov/Svatá Kateřina, West Bohemia close to the Czech-German border (49.6768 N, 12.6028 E, 526 m a.s.l.) on 11th Sept 2017.

DISCUSSION

The Czech Republic is a landlocked country with limited import of used tyres, principal way of *Ae. albopictus* introduction to a new environment, and negligible import of lucky bamboo ornamental plants, which are also considered as possible introduction way of *Ae. albopictus* [15, 16]. On the other hand, the importance and density of freight transportation by road trucks has recently increased. Personal travelling (cars, buses, caravans) between southern part of Europe and the Czech Republic has become very frequent in the last summer seasons as well. Monitoring of *Ae. albopictus* introduction was therefore aimed on the surface transport, resp. on the parking lots as ideal places for possible passive escape of *Ae. albopictus* females.
Firstly, tiger mosquito larvae were captured at parking lot placed on 64th km of D3 motorway, which will (once completed) connect the Czech capital Prague with Linz, and is one of a few resting places with a restaurant, shop, petrol station and other services, and thus it is very often visited by truck drivers or travellers on their way from Croatia, Slovenia or Italy (through Austria). Due to known establishment of *Ae. albopictus* populations in the above mentioned countries, its introduction to these sites was expected as probable.
Similarly, the other positive locality (site 2) is close to the former border cross and now offer wide range of services including gas stations, restaurants, fast foods, showers, entertainment industry and shops. Therefore, these parking lots often serve as the first place to rest for many drivers coming from southwestern European (Italy, Switzerland, France and Germany) countries, with firmly established populations of the tiger mosquito [17, 18, 19, 20, 21, 22].
Surprisingly, no *Ae. albopictus* individuals were captured on any of monitored parking lots near D1 motorway (sites 5–16), although the traffic of long distance trucks and travellers from Balkan countries is much more dense in comparison with other monitored motorways. The reason why *Ae. albopictus* was not recorded here might be that the mosquitoes had left the vehicles before they reached Czech Republic or, owing to frequent traffic jams on the D1 motorway, the mosquitoes could also escaped through open windows.
The sites, where *Ae. albopictus* mosquito was captured, in Bohemia are 230 and 413 km far from Mikulov, where the first *Ae. albopictus* import to the Czech Republic was recorded by Šebesta et. al. [5]. The same authors also

considered southern Moravia region as suitable for successful development of this mosquito. In the light of mild winters in the recent years and tiger mosquito´s ability to adapt and overwinter in the egg stage [23], this paper indicates that the regions with mild climate throughout the Czech Republic may face tiger mosquito colonisation in the near future.
Regarding the native mosquito species, only *Culex* spp. larvae were occasionally found in ovitraps. In 2016, the preimaginal population of *Cx. pipiens* was found at ovitrap site no. 6. Even though the used tyres are considered to be very attractive breeding sites for *Ae. albopictus* gravid females oviposition, only *Cx. pipiens* (91.0 %) and *Cx. torrentium* (9.0 %) in average were recorded at the tyres intentionally placed to the study sites No. 4, 10, 13.

Conclusion
The Asian tiger mosquito (*Aedes albopictus*) is very invasive species, whose passive introduction or establishment has been reported from almost all the neighbouring countries of the Czech Republic during recent years. So, the evidence of its import into Bohemia region was just rather the question of time. This paper confirmed introduction of *Ae. albopictus* to the Central and West Bohemia. Additionally, the distances between positive records (183 km) indicates that this species can appear basically anywhere within this country.

REFERENCES

1. Kraemer MUG, Sinka ME, Duda KA, et al. The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. eLife 2015;4:e08347.
2. Invasive Species Specialist Group. Global Invasive Species Database. 2017.
3. Adhami J, Reiter P. Introduction and establishment of Aedes (Stegomyia) albopictus Skuse (Diptera: Culicidae) in Albania. J Am Mosq Cont Ass 1998;14(3):340–343.
4. European Centre for Disease Control. Aedes albopictus – current known distribution in Europe, April 2017.
5. Šebesta O, Rudolf I, Betášová L, et al. An invasive mosquito species Aedes albopictus found in the Czech Republic, 2012. Euro Surveill 2012;17(43):6–8.
6. Paupy C, Delatte H, Bagny L, et al. Aedes albopictus, an arbovirus vector: from the darkness to the light. Microb Infect 2009;11(14):1177–1185.
7. Calzolari M, Bonilauri P, Bellini R, et al. Usutu virus persistence and West Nile virus inactivity in the Emilia-Romagna region (Italy) in 2011. PLoS One 2013;8(5):e63978.
8. Medlock JM, Hansford KM, Schaffner F, et al. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector-borne zoonot 2012;12(6):435–447.
9. Rezza G, Nicoletti L, Angelini R, et al. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 2007;30:1840–1846.
10. Schmidt-Chanasit J, Haditsch M, Schöneberg I, et al. Dengue virus infection in a traveller returning from Croatia to Germany. Euro Surveill 2010;15:19677.
11. Calba C, Guerbois-Galla M, Franke F, et al. Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017. Euro Surveill 2017;22(39):5–10.
12. Venturi G, Di Luca M, Fortuna C, et al. Detection of a chikungunya outbreak in Central Italy, August to September 2017. Euro Surveill 2017;22(39):17–00646.
13. European Centre for Disease Prevention and Control. Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm: ECDC;2012.
14. Becker N, Petrić D, Boase C, et al. Mosquitoes and their control. New York: Kluwer Academic;2003.
15. Knudsen A. Global distribution and continuing spread of Aedes albopictus. Parassitol 1995;37(2–3):91–97.
16. Hofhuis A, Reimerink J, Reusken C, Scholte E-J, et al. The hidden passenger of lucky bamboo: do imported Aedes albopictus mosquitoes cause dengue virus transmission in the Netherlands? Vector-Borne and Zoonotic Dis 2009;9(2):217–220.
17. Urbanelli S, Bellini R., Carrieri P, et al. Population structure of Aedes albopictus (Skuse): the mosquito which is colonizing Mediterranean countries. Heredity 2000;84:331–337.
18. Suter TT, Flacio E, Farna BF, et al. Surveillance on control of Aedes albopictus in Swiss-Italian border Region: Difference in egg densities between intervention and non-intervention areas. PloS One 2016;10(1):e0004315.
19. Baldacchino F, Marcantonio M, Manica M, et al. Mapping of Aedes albopictus Abundance at a Local Scale in Italy. Remote Sens 2017;9(7):749–763.
20. Carrieri M, Albieri A, Urbanelli S, et al. Quality control and data validation procedure in large-scale quantitative monitoring of mosquito density: the case of Aedes albopictus in Emilia-Romagna region, Italy. Pathog Glob Health 2017;111(2):83–90.
21. Roche B, Léger L, L'Ambert G, et al. The Spread of Aedes albopictus in Metropolitan France: Contribution of Environmental Drivers and Human Activities and Predictions for a Near Future. 2015. PLoS One 2015;10(5):e0125600.
22. Weren D, Kampen H. Aedes albopictus breeding in southern Germany, 2014. Parasitol Res 2015;144(3):831–834.
23. Thomas SM, Obermayr U, Fischer D, Kreyling J, Beierkuhnlein C. Low-temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, Aedes albopictus (Diptera: Culicidae). Parasit. Vectors 2012;5(1):100–107.

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