Between July and September 2012, seventeen larvae of the invasive mosquito species Aedes (Stegomyia) albopictus (Skuse) were discovered using 60 ovitraps at four study sites alongside two main road exits in South Moravia, Czech Republic. This is the first report of imported Ae. albopictus in the Czech Republic. The findings highlight the need for a regular surveillance programme to monitor this invasive species throughout western and central Europe.

Background
Of the invasive mosquitoes discovered in Europe recently, the Asian tiger mosquito Aedes albopictus (Skuse) represents the major threat to public health. Historically, this species originated in South-East Asia, but it has spread to the Americas, parts of Africa, northern Australia, and 19 European countries (Albania, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, France, Germany, Greece, Italy, Malta, Monaco, Montenegro, the Netherlands, San Marino, Serbia, Slovenia, Spain, Switzerland, Vatican City State) during the last decades. The species is now widely established and reportedly a nuisance mosquito in Italy, parts of France and Spain [1]. Ae. albopictus is globally an important vector of human pathogens such as chikungunya and dengue viruses as well as filarial nematodes represented by Dirofilaria spp., and an experimentally proven vector of eastern equine encephalitis, Venezuelan equine encephalitis, La Crosse encephalitis, Japanese encephalitis, West Nile and several other viruses [2,3].

Its eggs are frequently transported via used tire trade or by importation of lucky bamboo [2]. However, the most important mode of long-distance dispersal of Ae. albopictus in Europe in the last decade seems to be transportation by ground vehicles (i.e. lorries, cars, caravans) from southern Europe [4,5].

While two frequently used main roads connecting the Czech Republic with southern European countries cross the border in South Moravia, no systematic surveillance of invasive mosquito species has been conducted until present. This led us to periodically monitor invasive mosquito species at this so-called ‘Moravian entrance gate’ using ovitrap installations.

Trapping of mosquitoes
To monitor the presence of Ae. albopictus we used traditional ovitraps [6]. These were constructed from a dark blue 800 ml plastic cup and supplemented with 500 ml of dechlorinated tap water and a floating wooden tongue depressor paddle wrapped into rough cotton fabric that was in contact with the water line to ensure Ae. albopictus oviposition. Ovitraps were placed on shrubs, columns or public lighting in close proximity to parking spaces about 50 cm above the ground. Wooden paddles and water were periodically replaced (every 7 days) and transported in closed containers to the laboratory. The paddles were incubated at 25°C in humid atmosphere for three days and then kept immersed below the water surface at 25°C for another 12 days. Additionally, water from the ovitrap containers was incubated in the laboratory at 25°C for one week. Both components were daily examined for the presence of hatching eggs or larvae. Larvae and adults reared from larvae were morphologically identified according to recent entomological keys [6,7].

Study sites and findings
Several ovitraps were placed at four study sites (parking lots) in close proximity to exits of two main roads respectively connecting Austria and Slovakia with the Czech Republic (Figure). A total of 60 ovitraps were installed between the beginning of July and the end of September 2012.

The first two ovitrap sites (study sites 1 and 2) were situated near the main road E65, a transit route for goods to the Czech Republic from Slovakia and Hungary as well as from Balkan countries (Romania, Bulgaria, Croatia, Serbia, Greece). Individual and collective transport between western (e.g. Germany, Belgium, the Netherlands), central and southern Europe also operates through this main road. Study site 1 (22 ovitraps) was in Lanžhot (N 48°43.554’, E016°59.041’, 155 m above sea level (a.s.l.)), at a one km distance from Slovakia. The location is used for refreshment and
Study site 2 (17 ovitraps) was at Ladná (N 48°48,669’, E016°53,600’, 177 m a.s.l.) and situated approximately 16 km north of the first study site alongside the same main road. The site serves mainly as a refueling and rest area with a parking capacity of about 40 spaces. Two additional ovitrap sites (study sites 3 and 4) were chosen beside main road E461, where this road enters the Czech Republic from Austria. The main road E461 is frequently used for transit of goods from southern Europe (Italy, Slovenia, Croatia, Serbia, Montenegro, Macedonia, Albania) to the Czech Republic. Study site 3 (9 ovitraps) was Mikulov II (N 48°47,424´, E016°38,154´, 198 m a.s.l.), a former customs’ house now solely intended for refreshment. It is located on the Czech–Austrian border and has a parking capacity of about 10 spaces. Study site 4 (12 ovitraps) was Mikulov I (N 48°47,845’, E016°37,970’, 207 m a.s.l.), at the periphery of the town of Mikulov about 1.2 km north of study site 3. It serves a rest and refueling purpose and has a parking capacity of about 20 spaces.

From study site 4, we found 16 larvae of *Ae. albopictus*. Eight larvae in stage IV were euthanised for identification while the remaining eight were left to rear to adult stage (five females and three males) and also subsequently identified. Interestingly, all mosquito larvae developed from ovitraps set up within two subsequent intervals (20 August and 27 August 2012). Furthermore, one larva of *Ae. albopictus* developed from an ovitrap situated at the study site 3, on 10 September 2012, while no deposited eggs were detected in the study sites 1 and 2.

**Conclusion**

South Moravia is owing to its mild climate the most favourable habitat for breeding of mosquitoes within the Czech Republic [8]. Massive broods of mosquitoes (predominantly *Aedes* spp.) periodically occur here along the rivers Dyje and Morava. This area has been known for a long time as a natural focus of several mosquito-borne viruses: mainly Ťahyňa virus, the etiologic agent of Valtice fever, and since 1997 also...
West Nile virus lineage 3 – Rabensburg [9,10]. Many mosquito species occurring in the Czech Republic were only recorded in this region, e.g. Anopheles atroparvus, An. hyrcanus, An. labranchiae, Aedes nigrinus, Uranotaenia unguiculata, Culex martini [11,12]. We should take this region into consideration when searching for a suitable habitat for possible introduction and subsequent establishment of invasive mosquito species in central Europe. Our findings suggest that *Ae. albopictus* may be able to complete its developmental cycle in this region, and in case of a mild winter might also survive in the stadium of eggs [13]. Our results also indicate that ovitraps are a suitable tool for monitoring invasive mosquitoes on parking lots alongside main roads where alternative egg depositing water is likely less available.

In conclusion, we provide the first evidence of import of *Ae. albopictus* in the Czech Republic. Interestingly, *Ae. albopictus* has not yet been reported from the neighbouring central-European countries Austria, Slovakia, Hungary or Poland.

Acknowledgments

We are grateful to František Rettich and Francis Schaffner for confirmation of the mosquito species identity. This study was partially funded by the EU grant FP7-261504 EDENext, and it is catalogued as EDENext072. The input for the study was given by VBORNET (European network of medical entomologists and public health experts) under ECDC service contract ECD 2009/018. The work was also supported by the Institutional Research Plan of the Institute of Vertebrate Biology AS CR, v.v.i. (RVO:68081766).

References