Monitoring of the Asian tiger mosquito (*Aedes albopictus*) in high risk locations in southern Hesse and development of DNA-based rapid tests for the early diagnosis of eggs and larvae of *A. albopictus*

Dr.Ulrich Kuch

The Asian tiger mosquito (*Aedes albopictus*) is one of the most dangerous invasive species worldwide and especially feared as a vector of chikungunya and dengue fever viruses. As a consequence of the globalization of trade and travel, *A. albopictus* has been introduced to numerous regions of our planet and was able to establish rapidly expanding populations in many of these. For example, in addition to large parts of Asia it is now also widespread in North, Central and South America, West Africa and the northern and eastern Mediterranean region. Used car tires and certain ornamental plants (*Dracaena* cuttings known as „lucky bamboo“) have been recognized as high-risk goods in the long-distance dispersal of the cold- and drought-resilient eggs of this mosquito species. Global warming is expected to render additional regions in higher latitudes or altitudes suitable for colonization by *A. albopictus*, and corresponding range expansions are being observed, e.g., in Europe and Japan.

In Germany no established population of *A. albopictus* has been reported yet. However, model projections suggest that large parts of the country will soon be suitable for a possible colonization by this species from a climatic point of view (e.g., Fischer et al. 2011). The geographically closest large established populations of *A. albopictus* exist south of the Alps, e.g., in southern Ticino (Switzerland) and northern Italy. Along the major south-north highways, *A. albopictus* mosquitoes can be transported in a few hours across the Alps and into southern Germany, using cars, mobile homes and trucks as vehicles. The first records of *A. albopictus* eggs and an adult mosquito of this species near a rest area of the German federal highway No. 5 in the State of Baden-Württemberg in the years 2007 (Pluskota et al. 2008) and 2011 (Werner et al. 2012), respectively, are attributed to this mode of dispersal, also additional records of adult *A. albopictus* that were made in the context of mosquito monitoring activities in the German States of Baden-Württemberg and Bavaria (Becker et al. 2013; Kampen et al. 2013).

An additional invasive mosquito species with similar habits but higher cold resistance, the Asian bush mosquito or Asian rock pool mosquito (*Aedes japonicus*) is now widely distributed and established in northern Switzerland and adjacent areas of Germany as well as in the greater Stuttgart area and upper Rhine valley (State of Baden-Württemberg) and in large parts of the western German States of Rhineland-Palatinate and North-Rhine-Westphalia (Schaffner et al. 2009; Becker et al. 2011; Schneider 2011; Kampen et al. 2012). It is expected that this species will also spread to the State of Hesse from these areas and/or from more distant locations, by means of vehicle traffic and trade (e.g., in used car tires).

The objectives of this research project were (1) to perform periodic surveillance of the possible introduction of *A. albopictus* into southern Hesse in the form of a „low-intensity monitoring“, in high-risk locations and during times of elevated risk or likelihood of introduction; (2) to test and develop molecular genetic identification methods to allow for a faster, observer-independent identification of *A. albopictus* and its different life stages (eggs, larvae, pupae, adults).

For surveillance, *Aedes* ovitraps were set up on highway parking lots, rest houses and service stations of the German federal highway No. 5 from the border to Baden-Württemberg to an area just south of Frankfurt am Main. The ovitraps were operated at the end of the summer holiday vacations in the years 2009, 2010 and 2011 which are the times with peak numbers of car and mobile home traffic in south-north direction. In addition, mosquito larvae were sampled from a wide variety of natural and artificial containers and other small bodies of water across a large area of south-central Hesse, and raised to adult emergence in the laboratory for detailed taxonomic identification. Adult mosquitoes were also collected at used tire deposits and other locations in Hesse using BG-Sentinel traps with carbon dioxide as an attractant. *Aedes albopictus* was not found during any of these activities.

DNA barcoding was established as the „gold standard“ of the molecular genetic identification of *A. albopictus* allowing for a reliable species diagnosis based on eggs, larvae and parts of adult mosquitoes like wings, legs or smashed and dried remains. In the case of this method, diagnostic speed is determined mainly by the time needed for DNA extraction from the animal material, PCR (polymerase chain reaction) for the amplification of the 'barcode region' of the mitochondrial cytochrome oxidase subunit I gene, and the determination of the nucleotide sequence of this gene region. This is followed by an automated comparison of the obtained DNA sequence with reference sequences in public or internal databases.

To obtain an even faster species diagnosis for *A. albopictus* a specific rapid diagnostic test was developed which requires neither PCR nor DNA sequencing and thus does not need the corresponding instruments. This test uses the LAMP method (‚loop mediated isothermal amplification') which, within 25 minutes, produces a colour reaction that can be read by eye if *A. albopictus* DNA is present. The specificity of the test was initially examined using multiple samples from ten different mosquito species. If needed, the LAMP test allows for a much faster identification of *A. albopictus* material with less laboratory equipment. Both molecular genetic methods are suitable for the rapid identification of those *A. albopictus* materials (e.g., eggs, smashed remnants of mosquitoes) that are either not reliable for morphological identification or only after extended periods of time (e.g., hatching and rearing larvae until adult emergence).

Adaptation measures of primary importance for the State of Hesse include the design and implementation of a long-term vector surveillance programme and the reduction of *A. albopictus* breeding habitats in high-risk localities. As the most important short-term goal, an improved waste disposal at highway parking lots, service stations and gas stations is recommended. The most important mid-term goal should be the introduction and enforcement of a law or regulation prohibiting the commercial transportation and storage of used and new vehicle tires or parts thereof, if these are not adequately protected by roofs from rain, and from flooding. Used tires are globally recognized as the most important breeding grounds and dispersal devices of *A. albopictus*.

In addition, flower vases on cemeteries represent important artificial breeding habitats for invasive and autochthonous container-breeding mosquitoes and should therefore be included in all risk assessments. Strategies for the control of mosquito populations on cemeteries and their ecological and societal impact and acceptability should be evaluated experimentally and in field studies well before the first “exotic” mosquito species colonize these highly suitable settings.

Recently imported or punctually established *A. albopictus* in Germany are still likely to be eliminated in a cost-effective manner by the local application of insecticides and ancillary measures. Thus, studies of the temperature-dependent and species-dependent as well as resistance-conferred effects of insecticides on *A. albopictus* and their autochthonous and invasive competitors should play an important role in addition to vector surveillance in integrative climate change adaptation strategies.