Potential risk for the introduction of exotic mosquito species to Germany via transnational vessels

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Abstract
In a globalised world, different invasive, highly vector-competent mosquitoes like the Asian tiger mosquito or the Yellow fever mosquito are transported by human assistance all over the world. At the global scale, this spread can be attributed to trade and transport via oceanic vessels or international air traffic. To conform to the International Health Regulations (IHR), the city of Hamburg (Germany) was designated a Point of Entry. In order to assess the potential risk for the introduction of exotic mosquito species via transnational shipping, five mosquito traps (Biogents-Sentinel® 2nd trap with BG-lure) were installed on five different sites of a container vessel, starting in Hamburg shipping via West Africa to several South American Atlantic harbours with one to two days of stay in each port, returning to Hamburg via West Africa.

The traps were operated continuously from departure until the return of the vessel to Hamburg after 67 days. Samples were taken once at the end of the journey. Supported by a DNA barcoding approach (mitochondrial COI gene), seven of 128 collected dead mosquito specimens were identified as Aedes aegypti and four as Anopheles gambiae s.l. Additionally, 19 specimens were classified as Culex pipiens cf. quinquefasciatus using a DNA typing assay. These results indicate the potential risk for the introduction of exotic mosquito species to Germany via transnational vessels and highlight the need of monitoring of seaports as potential introduction sites of invasive mosquito species. However, the question of the mosquito survival probability on the ships needs further investigation.

Methods
We chose a shipping company where the contact from the Hamburg Port Health Center had intensified due to the Ebola virus 2014 in vessels (only 10 days from West Africa to Hamburg). The identified company serves South America and Hamburg on one single route. On a large Roll-on/Roll-off cargo ship which operates between Northern Europe, Hamburg, West Africa and Brazil, we were allowed to set up five adult mosquito traps (Biogents-Sentinel® 2nd trap with BG-lure, Fig. 1). These traps do not need Co2 gas to be run, which could be difficult to be on use on a travelling vessel. Locations were chosen that were at the same time in the vicinity of people and of electrical devices. Two traps were installed at the bottom opening of the stern ramp and three in the vicinity of the quarters in the superstructures: pantry, hospital room and navigation bridge (Fig. 2). The traps were operated continuously from departure until the return of the vessel to Hamburg. The only possibility to empty the traps was at the end of the trip. Mosquito specimens were morphologically identified (Becker et al. 2010). The specimens were strongly damaged and missed relevant characters for the species identification. Therefore, the specimens classified as Cx. pipiens s.l./torquatum were identified to the species level (Cx. pipiens pipiens form pipiens, Cx. pipiens form molestus and Cx. torrentium) using a molecular DNA typing assay (Rudolf et al. 2013).

For all other specimens, a molecular identification was conducted by the analysis of the mitochondrial cytochrome c oxidase subunit 1 gene (Kambhampati & Smith 1995). PCR products were sequenced by conventional Sanger technology (B&G, Berlin, Germany).

Results
The ship left Hamburg on August 18, 2016 and traveled in 67 days from Hamburg via West Africa to several South American Atlantic harbours with one to two days of stay in each port. The route is indicated in Table 1. The temperature ranges at most of the harbor stops in South America were between 19 and 27 degrees Celsius and were followed via www.wetter.de/brasilien. The 5 adult traps that had been installed on the ship were in good condition at the time of removal. Most of the mosquitoes were trapped at the open ramp. The specimens were strongly damaged. The distribution of the trapped mosquito species is shown in Table 2. All in all 128 specimens of dead mosquitoes could be sorted from the nets of the traps, 104 of them being Culex spp. Of them, 19 specimens were classified as Culex pipiens cf. quinquefasciatus. Eight Aedes mosquitos were caught in trap No. 1, located at the left bottom side of the stern ramp (7 Aedes aegypti and 1 Aedes sp.). Additionally 3 findings of Anopheles gambiae s.l. were recorded in trap No. 1. Another Anopheles gambiae s.l. was caught in trap No. 2, which was situated in a commando room at the right bottom side of the stern ramp, together with 8 Culex individu- als. Only in one of the three traps in the superstructures, mosquitoes were caught (2 Culex sp. in Trap No. 5 on the bridge).

Conclusion
This first trial resulted in evidence that mosquito vectors enter large vessels and give the prerequisites for them to be transported to Hamburg. They highlight the need of monitoring of seaports as potential introduction sites of invasive mosquito species. However, the question of the mosquito survival probability on the ships needs further investigation. Also there was no way of analyzing a possible viral load of the mosquitoes. Therefor a coming further investigation will trap more intensively and constantly on the journey of a vessel heading for Hamburg with the possibilities of a daily morphological analysis and freezing of the trapped species for later virological investigations. Choosing a traveling time more towards the mosquito season will result in more mosquito individuals.

References