Mosquito collections on incoming intercontinental flights at Schiphol International airport, the Netherlands, 2010-2011

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Abstract: After a report of mosquito nuisance and collection of live *Culex quinquefasciatus* mosquitoes on board of a passenger aircraft from Africa to Amsterdam, a mosquito surveillance was carried out in 38 cabins of passenger aircraft from overseas airports immediately after landing at Amsterdam Schiphol airport, the Netherlands (2010 and 2011). Live mosquitoes were collected in 10 aeroplanes, belonging to the species *Culex quinquefasciatus* (n=9), *Culex antennatus* (n=2), and *Aedes mcintoshi* (n=1). All mosquitoes were collected in aeroplanes that came from airports located in Africa. Disinsection is discussed. ***Journal of the European Mosquito Control Association*** **32: 17-21, 2014**

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Introduction

Accidental transport of mosquitoes in aeroplanes and their associated risks have long been recognised (Griffitts, 1933; Hughes, 1961). Due to the globalisation of trade and travel, the geographical spread of vectors and vector-borne diseases has increased (Gratz, 1999; Ahmed *et al*., 2009), with air travel being a prime long-distance transporting factor (Gezairy, 2003; Tatem *et al*., 2006). Mosquitoes readily track their human hosts unnoticed, and enter aircrafts by following passengers that enter on board (Gratz *et al*., 2000). In areas where mosquito vectors may be infected with pathogens (e.g. malaria endemic areas), their presence on board poses a direct health risk to passengers by biting during the flight (Mangili & Gendreau, 2005). Upon arrival in another country, they may disembark with the passengers (Carlson *et al*., 2006), and may manage to colonise new areas or infect people locally in and around the airport (Gratz *et al*., 2000; Bataille *et al*., 2009). Such transport of mosquitoes on aeroplanes may be more common than previously expected. A 13 year study from 1947-1960, where mosquitoes were collected from incoming international flights in the USA, yielded a total of more than 20,000 mosquitoes, belonging to 92 different species (Hughes, 1961). Examples of pathogen-carrying mosquitoes that were transported via aircrafts, included cases where patients with malaria (n=7) and dengue (n=1) were traced to be infected on board of an aircraft during flight (Mangili & Gendreau, 2005). Cases of malaria occur in and around airports all over the world in people without travel history to malaria-endemic areas, also known as airport malaria. Prior to August 1999, a total of 89 cases of airport malaria had been reported, 83 of which were in Europe (Gratz *et al*., 2000). In the Netherlands, airport malaria was reported twice around Schiphol airport, in 1978 and 1983 (Wetsteyn & de Geus, 1995). Considering the chain of multiple events required to lead to infection of individuals locally, *viz. Anopheles* mosquitoes a) to be infectious with malaria, b) to embark aircraft, c) to survive the flight, d) to escape the aircraft and disperse into the surrounding area, and e) to infect a person, it must be assumed that transport of *Anopheles* mosquitoes (and undoubtedly also other Culicidae) on aircrafts is fairly common. In a French study (Giacomini *et al*., 1994), it was estimated that 8-20 *Anopheles* mosquitoes were imported into France on each flight from Africa. The substantial number of mosquito species introduced into countries in which they were not previously present indicates that accidental introductions are not unusual. Introductions may occur via all means of international transport. However, aircrafts can transfer mosquitoes from one place to another relatively rapidly, thus increasing the chance of their survival in receptive areas (Gratz *et al*., 2000).

In 2008, the Netherlands reported live mosquitoes on a flight from Dar es Salaam, Tanzania, to Schiphol airport, Amsterdam, with several passengers who complained of being bitten on board. One of the flight attendants collected four specimens that were later identified as *Culex quinquefasciatus* Say (Scholte *et al*., 2010). The reported case raised basic questions as to the frequency and number of mosquitoes on board of aircrafts that land at Schiphol airport, the mosquito species involved, the origin of these flights (species and origin defining the range of pathogens that might be introduced), and the risks of establishment of the species in the Netherlands. Here we describe a study where cabins from passenger aircrafts on intercontinental flights to Schiphol airport (Amsterdam, the Netherlands) were inspected for the presence of live mosquitoes. Special emphasis was given to flights that came from areas known for the presence of vector-borne diseases (e.g. malaria and/or dengue), or for the presence of invasive exotic mosquito species known to be competent vectors for human pathogens (e.g. *Aedes albopictus* (Skuse) or *Aedes aegypti* (Linnaeus)).

Materials and Methods

Sampling strategy

To investigate the presence of mosquitoes on intercontinental flights to Amsterdam Schiphol international airport, passenger cabins of these flights were inspected for the presence of live mosquitoes upon arrival. Inclusion criteria were 1) origin of the flight: intercontinental flights with emphasis on tropical areas in Africa, Asia, and South America, 2) informed consent of the aeroplane personnel, 3) accessibility with regard to security issues, 4) suitable working days (inspectors' agenda). Weather circumstances at port of departure, specific airlines, seasonality, and disinfection on departure were not taken into account. Inspections were carried out by an employee of the Dutch National Centre for Monitoring of Vectors (CMV) that received training in mosquito identification and ecology. Visual inspections took place immediately after the passengers had left the cabin, and took approximately 30-45 minutes per cabin. Mosquitoes were collected using a suction tube. Collected mosquitoes were kept in a separate tube for each cabin. The tubes were sealed and labelled, and taken to the CMV-laboratory for morphological identification.

Mosquito identification

Mosquitoes were killed by placing the tube in a freezer for one hour. From each collected mosquito, a leg was removed and kept separately in a RNA stabilizing buffer (RNA-later) to enable further future analysis if indicated (e.g. pathogen detection). The mosquitoes were morphologically identified to species level using the keys of Harbach (Harbach, 1988) and Huang (Huang, 2001; Huang, 2004). Mosquitoes that were identified as belonging to the *Culex pipiens* complex were subjected to PCR sequencing (Synergene, Schlieren, Switzerland) of the ACE locus (Smith and Fonseca, 2004) at the University of Zurich, Switzerland. For one *Culex* specimen it was not possible to morphologically identify to species level. Two legs of this specimen were sent to the Natural History Museum (NHM) in London and subjected to molecular diagnostic.

Results

In total, cabins of 38 aircrafts were inspected in the period August 2010 - October 2011. On two occasions, entrance to an aeroplane was rejected; one coming from Jakarta, Indonesia, and one from Dar es Salaam, both on the 7th of October 2010. Of the 38 inspected cabins, 21 flights had come from Africa, two from the Arabian Peninsula, seven from the Indian subcontinent, four from southeast Asia, three from South America, and one from North America (see Figure 1).

Mosquitoes were found in 10 of the 38 inspected cabins (26.3%). Fourteen specimens were found (13 collected, 1 escaped), all female, belonging to three species: *Cx. quinquefasciatus* (n=9), *Culex antennatus* (Becker) (n=2), and *Aedes mcintoshi* Huang (n=1) (Table 1). The molecular diagnostics of the single *Culex* spp. specimen that was sent to the NHM in London was unsuccessful: even after repeated trials, no identification to species level was achieved (Y. Linton, personal communication). No *Anopheles* specimens were found. On one occasion, a live mosquito was clearly observed but could not be caught. No dead mosquitoes were found. From 21 inspected flights from Africa, ten aircrafts were found mosquito-infested. Ten of the 14 mosquitoes were found on flights from Tanzania.

Figure 1. Origin of flights to Amsterdam Schiphol Airport, the Netherlands for cabin inspections for life mosquitoes, 2010-2011. Green dots: location origin of flights to Amsterdam-Schiphol, Red star: Amsterdam-Schiphol airport location.

Table 1. Results of cabin inspections for live mosquitoes on intercontinental flights arriving at Amsterdam Schiphol airport, 2010-2011. ([1] bloodfed; [2] two of three bloodfed).

City of Departure	Country of departure	Date inspection	Mosquito species (n)
Accra	Ghana	7-10-2010	no mosquitoes
Accra	Ghana	29-08-2011	*Culex quinquefasciatus* (n=1)[1]
Accra	Ghana	1-09-2011	no mosquitoes
Bangkok	Thailand	29-08-2011	no mosquitoes
Dar es Salaam	Tanzania	24-09-2010	*Culex quinquefasciatus* (n=1)
Dar es Salaam	Tanzania	30-09-2010	*Culex* spp. (n=1)
Dar es Salaam	Tanzania	13-10-2010	*Culex antennatus (*n=1)
Dar es Salaam	Tanzania	20-10-2010	1 detected, but not captured
Dar es Salaam	Tanzania	27-10-2010	*Culex quinquefasciatus* (n=2)
Dubai	United Arab Emirates	30-09-2011	no mosquitoes
Entebbe	Uganda	24-09-2010	*Culex antennatus* (n=1)
Entebbe	Uganda	20-10-2010	no mosquitoes
Entebbe	Uganda	27-10-2010	no mosquitoes
Entebbe	Uganda	3-10-2011	no mosquitoes
Hidd/Kuwait	Bahrein/Kuwait	29-08-2011	no mosquitoes
Karthoum	Sudan	13-10-2010	no mosquitoes
Kilimanjaro	Tanzania	30-09-2011	no mosquitoes
Kilimanjaro	Tanzania	4-10-2011	*Culex quinquefasciatus* (n=1)
Kilimanjaro/Dar es Salaam	Tanzania	2-09-2011	*Culex quinquefasciatus* (n=3)[2]
Kuala Lumpur	Malaysia	1-09-2011	no mosquitoes
Kuala Lumpur	Malaysia	30-09-2011	no mosquitoes
Lagos	Nigeria	27-10-2010	*Culex quinquefasciatus* (n=1)
Lagos	Nigeria	30-08-2011	no mosquitoes
Miami	USA	29-08-2011	no mosquitoes
Mumbai	India	30-09-2011	no mosquitoes
Mumbai	India	4-10-2011	no mosquitoes
Nairobi	Kenya	24-09-2010	no mosquitoes
Nairobi	Kenya	30-09-2010	*Aedes mcintoshi* (n=1)
Nairobi	Kenya	1-09-2011	no mosquitoes
New Delhi	India	30-09-2010	no mosquitoes
New Delhi	India	20-10-2010	no mosquitoes
New Delhi	India	27-10-2010	no mosquitoes
New Delhi	India	2-09-2011	no mosquitoes
New Delhi	India	4-10-2011	no mosquitoes
Paramaribo	Surinam	30-09-2010	no mosquitoes
Paramaribo	Surinam	29-08-2011	no mosquitoes
Paramaribo	Surinam	20-09-2011	no mosquitoes
Taipei	Taiwan	30-08-2011	no mosquitoes

Discussion and conclusion

All mosquitoes collected in this study were found in aeroplanes that came from Africa, and most of them were found in flights originating from Tanzania. All three collected mosquito species are known vectors of human and/or veterinary pathogens: *Cx. quinquefasciatus* (common southern house mosquito) is in Africa a competent vector of lymphatic filariasis nematodes, Rift Valley Fever virus and chikungunya virus (Foster & Walker, 2002). *Culex antennatus* is a reported vector of Rift Valley Fever virus, and *Ae. mcintoshi* is an important vector of Rift Valley Fever virus, Wesselsbron and Middelburg viruses. None of these species are known to be invasive for north-western Europe. Surprisingly, no *Anopheles* species were collected, although airport malaria was reported twice around Schiphol airport, in 1978 and 1983 (Wetsteyn & de Geus, 1995), and Schiphol airport was included in the year 2000 in a top 10 worldwide list of airports ranking the estimated risks routes of airport-malaria through importation of malaria-infected African mosquitoes in aeroplanes (Tatem *et al*., 2006). Considering that 16,007 air transport movements between Schiphol and Africa were recorded in 2010, and 15,235 air transport movements for 2011 (SchipholGroup, 2014), combined with the fact that 10 out of the 21 inspected aeroplanes that had flown from African countries and landed at Schiphol airport contained live mosquitoes, this poses to the Netherlands a significant health risk for importation of infected African mosquitoes.

The risks related to mosquitoes on board aeroplanes has long ago been recognised. The World Health Organization (WHO) has developed recommendations for reducing these risks as much as possible (WHO, 1995), of which aircraft insect control (disinsection) is an important tool. In fact, disinsection is recommended by the International Health Regulations in aircrafts travelling from countries with malaria and other vector-borne diseases. In many countries disinsection is viewed as the initial protective barrier against introduction and potential establishment of exotic mosquitoes (Russell & Paton, 1989), but relatively few countries act upon that view (Gratz *et al*., 2000). The current recommendations for aircraft disinsection, set out by WHO (WHO, 1995), include specifications for aerosols and approved insecticidal formulations, and include three preferred methods: disinsection before take-off ("blocks-away" disinsection), disinsection top-of-descent (spraying of the cabin is done immediately after the aircraft started its descent to destination), and disinsection with residual insecticide. Disinsection on the ground ("on-arrival" disinsection) may be retained as an acceptable back-up method if an aircraft, coming from areas of threat, has not been adequately disinsected by any of the preferred methods (WHO, 1995).

It is up to countries to decide if and what procedures are implemented. Some countries require disinsection on inbound flights using aerosol sprays with passengers on board, while other countries require a residual treatment, or the application of an aerosolised spray while passengers are not on board. The majority of countries, however, have no disinsection requirements on inbound flights. According to Ellis (1996), the Air France/Royal Dutch Airlines (KLM) consortium follows WHO recommendations ('blocks-away' aerosol spraying) to control possible disease vectors when leaving designated countries. The current policy of flying companies is unknown to the authors.

A serious concern regarding disinsection are the negative effects it may cause to passengers and especially aircraft crew members who are routinely subjected to pesticide exposure (Van Netten, 2002; Sutton *et al.*, 2007). A report (WHO, 1995), noted that some individuals might experience 'transient discomfort' after aircraft disinsection. In an earlier report, the WHO expressed concern that passengers who suffer allergic reactions to the aerosols of disinsection may not receive immediate appropriate medical attention (Russell & Paton, 1989). In fact, some authors describe a life-threatening allergic reaction of an otherwise healthy patient after flight cabin pyrethroid spraying (Vanden Driessche *et al.*, 2010). Thus, the need to protect passengers and aircraft crew from potentially negative effects of insecticide exposure and the need for assurance that an aircraft is free from mosquitoes represents a significant challenge (Carlson *et al.*, 2006). Considering the health concerns of pesticide exposure on one hand, and on the other the public health reasons for preventive disinsection, the development of alternative strategies is needed. One such method may be the use of air-currents at the entrance of aircraft, preventing mosquitoes from entering (Carlson *et al.*, 2006).

Although none of the collected species in this study are known or suspected invasive mosquito species for north-western Europe, the import of potentially invasive mosquitoes into the Netherlands via international air transport cannot be excluded. Preliminary mosquito surveillance for exotic and invasive mosquitoes at and in the surrounding area of Schiphol, and other international airports in the Netherlands, is currently being planned.

The observed presence of live non-indigenous mosquitoes aboard passenger aeroplanes arriving in the Netherlands from overseas, some of them actively biting passengers and/or crew-members, demonstrates the potential for individual and public health and veterinary risks of international flights. The collected species are known vectors of several serious human and veterinary exotic diseases. In addition, aeroplanes might introduce mosquito species that have potential for establishment in Europe. Adequate disinsection strategies need to consider the risk for allergic reactions upon exposure to pyrethroid aerosols.

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