RECOMMENDATIONS FOR STANDARD OPERATING PROCEDURES (SOPs) DEVELOPMENT FOR VECTOR (MOSQUITO) SURVEILLANCE AND CONTROL ACTIVITIES AT PORTS AND AIRPORTS

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Content

1 INTRODUCTION 6

2 BACKGROUND 8

2.1 Vector invasions and vector-borne diseases related to airports and ports.......................................8

2.2 Existing published relevant guidelines 9

3 PURPOSE AND SCOPE 11

4 COMPETENT AUTHORITIES: ROLES AND RESPONSIBILITIES 12

4.1 Inter-sectoral cooperation 12

4.2 Capacities, roles and responsibilities required in IHR (2005) 12

4.3 Communication.. 15

5 DECISION-MAKING PROCESS AND RAPID RISK ASSESSMENT 16

5.1 Preparedness, prevention and emergency phases 16

5.2 Risk assessment for vectors at ports 17

5.2.1 Risk analysis... 17

5.2.2 Risk categorization.. 20

5.2.3 Competent authorities: roles and responsibilities at ports 22

5.2.4 Problems/constraints at ports 23

5.3 Risk assessment for vectors at airports.. 25

5.3.1 Risk analysis... 25

5.3.2 Risk categorization.. 26

5.3.3 Competent authorities' roles and responsibilities at airports.. 29

5.3.4 Problems/constraints at airports 29

6 ROUTINE AND EMERGENCY MEASURES...31

6.1 Vector surveillance.. 31

6.1.1 General principles for ports and airports.. 31

6.1.2 Routine surveillance of EMS.. 31

6.1.3 Emergency surveillance after detection of EMS 32

6.1.4 Specificities for vector surveillance at ports 32

6.1.5 Integrated Pest Management (IPM) plan on ships... 32

6.1.6 Inspections for vectors on ships... 33

6.1.7 Inspections for vectors on aircrafts 33

6.2 Vector control measures 34
6.2.1 General principles for vector control measures at ports and airports 34
6.3 Vector surveillance and control activities at POE in response to a vector-borne disease case 35
7 ANNEXES 38

Annex 1: Guidelines for developing Standard Operating Procedures (SOPs)

Annex 2: Guidance documents from European and international organisations relevant to vector surveillance and control at points of entry

Annex 3: Bibliography related to vector surveillance and control at points of entry for maritime and air transport sectors

Annex 4: Memorandum of Understanding (MoU) among authorities sharing responsibilities for management of public health events at ports and at airports

Annex 5: Example of activities and methods for vector surveillance

Annex 6: Example of activities and methods for vector control with emphasis on the prevention of accumulation of stagnant water at the airport and at the port areas

Annex 7: Case study – Recommended response to the hypothetical scenario of *Aedes aegypti* introduction in a Southern European airport

Annex 8: Case study – A case of dengue fever among seaport staff

Abbreviations

ECDC	European Centre for Disease Prevention and Control
EMS	Exotic Mosquito Species
EU	European Union
IVM	Integrated Vector Management
MoU	Memorandum of Understanding
PCO	Pest Control Operators (subcontractors)
PoE	Points of Entry
SOPs	Standard Operating Procedures
VBD	Vector-Borne Disease
WHO	World Health Organization

Definitions

Active vector surveillance: A system employing staff members to visit surveillance sites and collect (often on a regular basis) information on vector-specific data[1].

Disinsection: the procedure whereby health measures are taken to control or kill the insect vectors of human diseases present in baggage, cargo, containers, conveyances, goods and postal parcels[2].

Enhanced surveillance: an expansion of surveillance activities, e.g. in the number, type and extent of mosquito monitoring traps, an increased frequency of inspection of the traps, and increased inspections of potential breeding sites. Any exotic mosquito detection generally requires enhanced surveillance[3].

Establishment of mosquito species: establishment is the perpetuation, for the foreseeable future, of an invasive species within an area following the species' introduction[4].

Exotic plant or animal species (synonyms: alien, foreign, non-indigenous, non-native): a species that is not native to an ecosystem and, if present, has been introduced[5].

Introduction of mosquito species: an introduction is the process of bringing a species from its endemic range into a biogeographic area to which it is completely foreign[6].

Invasive mosquito species: an invasive species is an exotic species that establishes and proliferates within an ecosystem and whose introduction causes, or is likely to cause, economic or environmental impact or harm to human health[4].

Native or indigenous mosquito species: a native or indigenous species is a species that occurs within its natural geographical range (past or present) and dispersal potential (i.e. within the range it occupies naturally, or could occupy, without direct or indirect introduction or other human intervention)[4].

Passive surveillance: a system by which the responsible authority/agency receives vector-specific data from all potential sources. Citizen science is considered passive surveillance[1].

Point of entry: a passage for international entry or exit of travellers, baggage, animals, plants, cargo, containers, conveyances, goods and postal parcels as well as agencies and areas providing services to them on entry or exit[2].

(Routine) vector surveillance: continuous, systematic collection, analysis and interpretation of vector-specific data that can be used in planning, implementing and evaluating public or veterinary health practice[1].

Vector control: measures of any kind against pathogen-transmitting arthropods (vectors), intended to limit their presence, abundance or their ability to transmit the pathogen[1].

Vector: an insect or other animal which can transport an infectious agent that can constitute a public health risk[2].

1 INTRODUCTION

This is deliverable 9.2 "Recommendations for Standard Operating Procedures (SOPs) for vector (mosquito) surveillance and control activities at Points of Entry (PoE)" of Work Package 7 "Maritime transport" of the European Union (EU) HEALTHY GATEWAYS Joint Action. The EU HEALTHY GATEWAYS Joint Action has received funding from the EU, in the framework of the Third Health Programme (2014-2020).

The general objective of the EU HEALTHY GATEWAYS Joint Action (Grant Agreement Number 801493) is to support cooperation and coordination between EU Member States in order to improve their capacities at points of entry (PoE), in preventing and combating cross-border health threats affecting or inherently coming from the transport sector. This document was produced to support efforts at international ports and airports to keep them free of both invasive and native mosquito vectors, as required by the International Health Regulations (IHR 2005)[2].

These recommendations are a consensus opinion among the members of the expert working group established to compose the recommendations, and whose names are listed at the end of the document.

According to the Grant Agreement (Number 801493), EU HEALTHY GATEWAYS has developed recommendations to support the development of SOPs for specific vector surveillance and control activities at airports and ports, for the implementation of an integrated vector control programme and based on the existing World Health Organization (WHO) and European Centre for Disease Prevention and Control (ECDC) guidelines for control of native and invasive mosquitoes in Europe (**Annex 1, Annex 2, Annex 3**). Moreover, issues about the inspection of ships and aircrafts for vectors have been incorporated in the document.

Materials and methods used to develop these recommendations included: a) literature review with the aim to identify guidelines, reports, practices and research articles describing vector surveillance and control activities at PoE and/or inspection of conveyances for vectors, b) conduct of site visits at ports and on ships to review practices for vector surveillance and control, c) expert group consultation and d) review of survey results for best practices implementation under Work Packages 6 and 7 of the Joint Action.

Exotic mosquito species (EMS) carrying pathogens or not, can be introduced in the EU through airplanes, ships, ground means of transport or freight. Mosquitoes can be introduced as adults or immature stages (eggs, larvae, pupae). Human cases of vector-borne disease (VBD) can be introduced into the EU via travel with airplanes, ships or ground transportation.

Mobility of people and goods by airplanes, ships and ground means has contributed to the introduction and establishment of vector species in EU countries as well as globally[7]. Regarding mosquitoes, several invasive mosquito species (IMS) have been inadvertently introduced into Europe, where they often, enhanced by climate change, find favourable environmental and climatic conditions, for the establishment of permanent populations[8].

The points of introduction of vectors into new areas could be ports, airports or ground crossings; therefore, it is important to have measures in place to prevent the introduction, as well as exportation of vector species to other areas. However, it should be noted that containers can be opened for inspection (e.g. phytosanitary or other type) at the port or airport facilities, or pallets can be separated for transportation with trucks. Freight containers are rarely opened at ports. Moreover, AKE containers carrying luggage are opened upon arrival at the airport facilities.

Other means of introduction include through vehicles arriving on ferries which may carry EMS. Vessels docking at marinas have been assumed to play a role in the dispersal of EMS along the Balkan Adriatic coast. ECDC advises that marinas in the Mediterranean and Black Sea basins should be considered for surveillance during the summer season[5]. Furthermore, ECDC advises the following in regards to ports, airports and ground crossings:

- *"Ports may be surveyed with ovitraps and/or routine adult trapping. It may be possible to evaluate the risk related to these sites by analysing a port's international traffic routes and the volume of goods passing through[5].*
- *Airports may also be surveyed by these methods, both in order to check for possible introductions and to demonstrate that an airport is 'free of IMS', as requested by IHR in the event of an MBD outbreak (IHR 2005)[5].*
- *Train stations can be surveyed for IMS, introduced by trains that originate in infested areas, although modern air-conditioned trains are not favourable to mosquito survival. Train terminals that serve so-called 'rolling highways' (RoLa), a combined transport system to transport trucks by rail, could be exposed to similar risks as ports and airports[5].*

Train terminals have been found as entry point for *Aedes albopictus* species[9]. The current document could be used in conjunction with other guidelines issued by WHO and ECDC: WHO guidelines "Vector Surveillance and Control at Ports, Airports, and Ground Crossings"; "WHO aircraft disinsection methods and procedure"[10]; ECDC technical report "Organisation of vector surveillance and control in Europe" and other relevant documents from ECDC[*], as well as national or local policies and practices.

[*] https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/guidelines-mosquito

2 BACKGROUND

2.1 VECTOR INVASIONS AND VECTOR-BORNE DISEASES RELATED TO AIRPORTS AND PORTS

Imported and autochthonous vector-borne diseases including malaria, dengue, chikungunya virus disease and Zika virus disease have occurred in EU countries in recent years[1], some in larger outbreaks with cases exceeding 200 (Italy) or even 2000 (Madeira)[11,12]. Cases of airport and port malaria have been documented in the past in the EU, and worldwide. Aircrafts infested with adult mosquitoes and infested airfreight containers have been found at airports; live adult mosquitoes have been found on board ships or inside containers transported by ships, and mosquito eggs and larvae have been found on board of ships and/or sea freight[13-15]. Mosquito species that have been found globally in sea freight include: *Aedes albopictus, Ae. aegypti, Ae. togoi, Ae. japonicus, Ae. atropalpus, Ae. bahamensis, Ae. togoi, Anopheles gambiae, An. albimanus, Culex pipiens* complex*, Tripteroides bambusa, Uranotaenia bimaculata* and others[13-15].

In Europe, EMS such as *Ae. albopictus* (Skuse, 1895), *Ae. japonicus* (Theobald, 1901), and *Ae. koreicus* (Edwards, 1917) have been introduced and have colonized large areas, spreading across multiple countries globally and to our concern, the process is still ongoing[16]. The main routes for the introduction of EMS are the import of products such as used tyres or Lucky bamboo (Dracaena sanderiana), the passive transport of mosquitoes in vehicles (traffic by road, train, air and sea) and their dispersal from regions where they are established in Europe[13-15]. EMS surveillance on these introduction routes is therefore critical to promptly detect and control introductions in non-established areas.

Due to globalisation and increased air connectivity, air travel has been considered to play a major role in long-distance dispersal of vectors as well as VBD pathogens[17]. In areas with high mosquito densities, there is a greater probability that mosquitoes can enter aircrafts at airports following their human hosts[18]. These aircrafts can then rapidly transfer mosquitoes to another country, thus increasing the chance of mosquitoes surviving the trip and reaching a location where the mosquitoes are non-native[17]. Upon arrival, mosquitoes may colonise new areas. In Europe, mosquitoes have also been introduced from abroad with airplanes. Recent reports indicate that aeroplanes (commercial and freight) and cargo boats from mainland Ecuador represent the main routes for insect introductions to Galápagos[19]. Once introduced to an island, insects may invade the rest of the archipelago through natural dispersal or via human-aided transport, such as aeroplanes and boats. In 2008, The Netherlands reported live mosquitoes on a flight from Tanzania to Schiphol airport in Amsterdam, with several passengers complaining of being bitten on board[20]. A follow-up study at Schiphol showed that exotic mosquitoes were transported on 10 of the 38 aircrafts inspected[21]. Repeated introductions of *Ae. aegypti* and *Ae. albopictus* specimens at Schiphol international airport in The Netherlands have shown that transportation of EMS in aircrafts is possible in Europe[22,23]. Over the past years, the species *An. crucians, An. subpictus* and *Mansonia titillans* have been detected at Schiphol airport in The Netherlands, and *Anopheles pharoensis* has been detected at the airport of Liege in Belgium[24], demonstrating that surveillance at airports can be crucial to detect the introduction of EMS. In 2015, *Ae. koreicus* was found inside the premises of the international airport of Genova[25]. No clear relationship was established with accidental aircraft mediated transport; however, the presence of an EMS at the airport - nearby an important commercial port - is concerning regarding the potential further spread of this EMS via aircrafts or boats in Italy and beyond. Depending on the mosquito species, the maximum flight distance may be from a few meters to several kilometres[26].

Imported used tyres and ornamental plants (mainly plant cuttings requiring water during transportation) can: a) re-introduce and establish *Ae. aegypti* in Europe, which is the primary vector of Zika virus disease and other diseases such as yellow fever and dengue fever; b) introduce *Ae. albopictus* and other EMS in areas where they are not currently present or established; c) import additional *Ae. albopictus* mosquito populations and other mosquito species in areas where they are currently established, influencing the mosquito population dynamics and density[27].

Several countries worldwide implement mosquito surveillance at PoE, to allow for early detection and control of invading species, preventing their development in the country. In many cases the surveillance at the PoE is part of a broader surveillance program taking place within the country, while in other cases surveillance is restricted only to the PoE. The IHR (2005), the WHO Handbook for Vector Surveillance and Control at Ports, Airports, and Ground Crossings and the ECDC's Guidelines for the surveillance of invasive mosquitoes in Europe along with the Zika virus, have supported some countries to initiate or further enhance surveillance at PoE.

Different countries follow different approaches and methodologies for surveillance of PoE. Many reports of mosquito surveillance at ports and airports highlight the need for use of proper surveillance tools and methods, as well as more standardized surveillance practices, since this improves the quality of data collected and facilitates sharing and comparison of surveillance results[28,29]. Certain practices currently implemented are not appropriate to collect both native and invasive mosquito species; therefore, sharing of expert knowledge, as well as good and effective practices would help authorities to ensure that target species are collected. Standardization of procedures also opens the road to centralized databases and GIS systems[30,31]. There are different collection methods that can be used in PoE. The most appropriate method is up to the competent authority to decide, considering different criteria such as mosquito species, financial and capacity aspects, climate conditions etc. In this document, we have selected certain methods which are included in ECDC guidelines for both native and invasive mosquito species[5,32].

2.2 EXISTING PUBLISHED RELEVANT GUIDELINES

In 2012 ECDC published comprehensive guidelines for the surveillance of mosquitoes[5]. This document details the strategic process that needs to be implemented by the EU Member States and provides guidance at operational level. The WHO's technical guidance "Vector Surveillance and Control at Ports, Airports, and Ground Crossings" published in 2016 covers specific guidelines for ports, airports and ground crossings[33]. This guidance document was developed in the framework of IHR, aiming to assist PoE to achieve the required capacity and provide comprehensive advice for establishing effective surveillance for disease vectors, including mosquitoes.

Guidelines for vector surveillance and control of conveyances on international voyages are provided in various WHO guidelines (e.g. "Guide to ship sanitation", "Aircraft disinsection methods and procedures", etc.). In Europe, the "European Manual for Hygiene Standards and Communicable Disease Surveillance on Passenger Ships" provides standards for vector control on board passenger ships sailing in Europe.

In accordance with IHR, a public health emergency contingency plan at PoE should address issues for public health risks related to vectors. Competent authorities may consider developing SOPs for vector surveillance and control at PoE. Detailed, recommended instructions for SOP development can be found in **Annex 1**.

The Australian Department of Health has published a guide for mosquito surveillance at PoE in Australia[3]. This guide provides detailed procedures for detection and control of exotic mosquitoes, specifically for ports and airports.

A list of other related guidance documents from European and international organisations relevant to vector surveillance and control at PoE is presented in **Annex 2**. A list of bibliography related to vectors at PoE and on board conveyances can be found in **Annex 3**.

3 PURPOSE AND SCOPE

The current document refers to information for consideration when developing procedures for surveillance and control mosquito populations at airports and sea ports. It focuses on European Union countries including islands (e.g. Madeira, Canary Islands), but not on areas outside geographic Europe (e.g. Reunion, French Guiana etc.). Vector control activities at ground crossings as well as vectors other than mosquitoes are not covered in this document.

The current document provides information for port and airport competent authorities to consider when developing their own SOPs for mosquito surveillance and control, as part of the PoE vector management programme and the public health emergency contingency plan of the PoE.

The current document focuses on: i) risk-analysis of the possibility of EMS introduction at specific ports/airports; ii) recommendations for SOPs development for implementing EMS surveillance at the port/airport facilities; iii) recommendations for SOPs development for implementing immediate control measures, in case of detection of EMS introduction. Entomological surveillance will be a useful tool to inform national authorities regarding the risk of introduction of vector species through airports and ports. The document also provides examples/case studies on surveillance/control at port/airports. This document also aims to support competent authorities and conveyance operators related to decision making about disinsection measures to be taken on board ships and aircrafts.

4 COMPETENT AUTHORITIES: ROLES AND RESPONSIBILITIES

4.1 INTER-SECTORAL COOPERATION

Vector surveillance and control activities are part of routine operations at points of entry. They are also part of countries' preparedness and contingency planning in the event of imported mosquito borne diseases, EMS introduction or establishment. Vector surveillance and control activities are a shared responsibility among authorities at central and local levels, as well as various actors from private and public sectors. Moreover, for effective response to any potential introduction of EMS of public health concern, cooperation and coordination of local authorities with central level public health surveillance centres and other institutions is essential. Response measures depend on the national and local context, including epidemiological surveillance findings, climate conditions and others. Inter-sectoral cooperation and communication is required for effective control of mosquitoes at PoE. It is essential that roles and responsibilities of all involved parties have been defined and agreed before developing SOPs for vector surveillance and control at the local PoE level. **Annex 4** provides a template for an Memorandum of Understanding (MoU) among actors involved in vector surveillance and control activities at PoE.

Examples of authorities that share responsibilities include:

- *Airport or port operators, terminal operators, container loading areas contractors*
- *Aircraft or ship operators*
- *Medical entomologist team*
- *Public health authorities at central and local levels*
- *Environmental health authorities at central and local levels*
- *Infectious diseases surveillance authority at central and local levels*
- *Instructed airport or port fellow workers*
- *Pest control operators (subcontractors)*
- *Food preparation facilities at airports*
- *Caterers*

4.2 CAPACITIES, ROLES AND RESPONSIBILITIES REQUIRED IN IHR (2005)

The IHR (2005) requires that designated PoE, including airports and ports, maintain certain capacities at all times and for responding to events that may constitute a public health emergency of international concern. In particular, IHR (2005) requirements, summarized in

, and briefly are: a) designated PoE, b) responsibilities of competent authorities, c) responsibilities of conveyance operators and d) responsibilities of competent authorities for container and container loading areas**.**

Box 1*: IHR (2005) requirements relevant to vector surveillance and control at PoE*
IHR (2005) requirements for designated PoE • *provide a programme and trained personnel for the control of vectors and reservoirs in and near points of entry;* • *establish and maintain a public health emergency contingency plan;* • *establish programmes to control vectors that may transport an infectious agent that constitutes a public health risk to a minimum radius of 400 metres from those areas of point of entry facilities that are used for operations involving travellers, conveyances, containers, cargo and postal parcels, with extension of the minimum distance if vectors with a greater range are present;* • *apply recommended measures to disinsect, derat, or otherwise treat baggage, cargo, containers, conveyances, goods or postal parcels including, when appropriate, at locations specially designated and equipped for this purpose.*
Competent authorities, in relation to health measures applied on aircrafts or ships, are responsible for: • *monitoring ships or aircrafts, baggage, cargo, containers, goods, postal parcels and human remains departing and arriving from affected areas (geographical location specifically for which health measures have been recommended by WHO under IHR) to ensure they are free from sources of infection or contamination (including vectors and reservoirs);* • *performing inspection and issuing Ship Sanitation Certificates after ship inspection (IHR Articles 22, 27, 39);* • *ensuring, as far as practicable, that facilities used by travellers at PoE are maintained in a sanitary condition and are kept free of sources of infection or contamination, including vectors and reservoirs;*
• *be responsible for the supervision of any deratting or disinsection of baggage, cargo, containers, conveyances, goods, postal parcels and human remains or sanitary measures for persons, as appropriate under IHR;* • *advise ship or aircraft operators, as far in advance as possible, of their intent to apply control measures to a conveyance, and shall provide, where available, written information concerning the methods to be employed.*

A conveyance (ship or aircraft) may be regarded as suspect and should be inspected for vectors and reservoirs if a possible or confirmed case of vector-borne disease has occurred on board during an international trip; or it has left an affected area within a period of time where on board vectors could still carry disease (IHR Annex 5). Inspection will identify infestation and conditions favouring harbourage. Disinsection, deratting, disinfection, decontamination and other sanitary procedures shall be carried out so as to avoid injury and as far as possible discomfort to persons, or damage to the environment in a way which impacts on public health, or damage to baggage, cargo, containers, conveyances, goods and postal parcels.
Ship operators or officers of ships, as well as aircraft operators are responsible for: • *permanently keeping the ships or aircrafts for which, they are responsible free from sources of infection or contamination, including vectors and reservoirs (the application of measures to control sources of infection or contamination may be required if evidence is found);* • *facilitating inspections of the cargo, containers and ships or aircrafts;* • *facilitating application of health measures including disinsection;* • *providing of relevant public health information requested by the State Party.*
Competent authorities for container and container loading areas are responsible for: • *ensuring that container shippers use international traffic containers that are kept free from sources of infection or contamination, including vectors and reservoirs, particularly during the course of packing;* • *ensuring that container loading areas are kept free from sources of infection or contamination, including vectors and reservoirs;* • *whenever, in the opinion of a State Party, the volume of international container traffic is sufficiently large, the competent authorities shall take all practicable measures consistent with IHR, including carrying out inspections, to assess the sanitary condition of container loading areas and containers in order to ensure that the obligations contained in IHR are implemented;* • *ensuring that facilities for the inspection and isolation of containers, as far as practicable, be available at container loading areas.* Container consignees and consignors shall make every effort to avoid cross-contamination when multiple-use loading of containers is employed.

The Annex 5, in IHR, describes measures for vector control. The competent authority can either implement vector control measures or supervise their application. Preventive applications of disinsection and deratting are also suggested by WHO when the ship leaves from a port where vector control is recommended. The WHO International Travel and Health book can be referenced to identify areas where vector-borne diseases are endemic.

Every conveyance leaving a point of entry situated in an area where vector control is recommended should be disinsected and kept free of vectors. When there are methods and materials advised by the WHO for these procedures, these should be employed. The presence of vectors on board conveyances and the control measures used to eradicate them shall be included:

- *in the case of aircraft, in the Health Part of the Aircraft General Declaration, unless this part of the Declaration is waived by the competent authority at the airport of arrival;*
- *in the case of ships, on the Ship Sanitation Control Certificates.*

WHO State Parties should accept disinsecting, deratting and other control measures for conveyances applied by other States if methods and materials advised by the WHO have been applied.

A WHO State Party should not prohibit the landing of an aircraft or berthing of a ship in its territory if the control measures are applied. However, aircraft or ships coming from an affected area may be required to land at airports or divert to another port specified by the competent authority for that purpose.

A competent authority may apply vector control measures to a conveyance arriving from an area affected by a vector-borne disease if the vectors for the foregoing disease are present in its territory.

4.3 COMMUNICATION

The IHR (2005) requires that competent authorities inform WHO within 24 hours of receipt, of evidence of a public health risk identified outside their territory that may cause international disease spread, as manifested by exported or imported: (a) human cases; (b) vectors which carry infection or contamination.

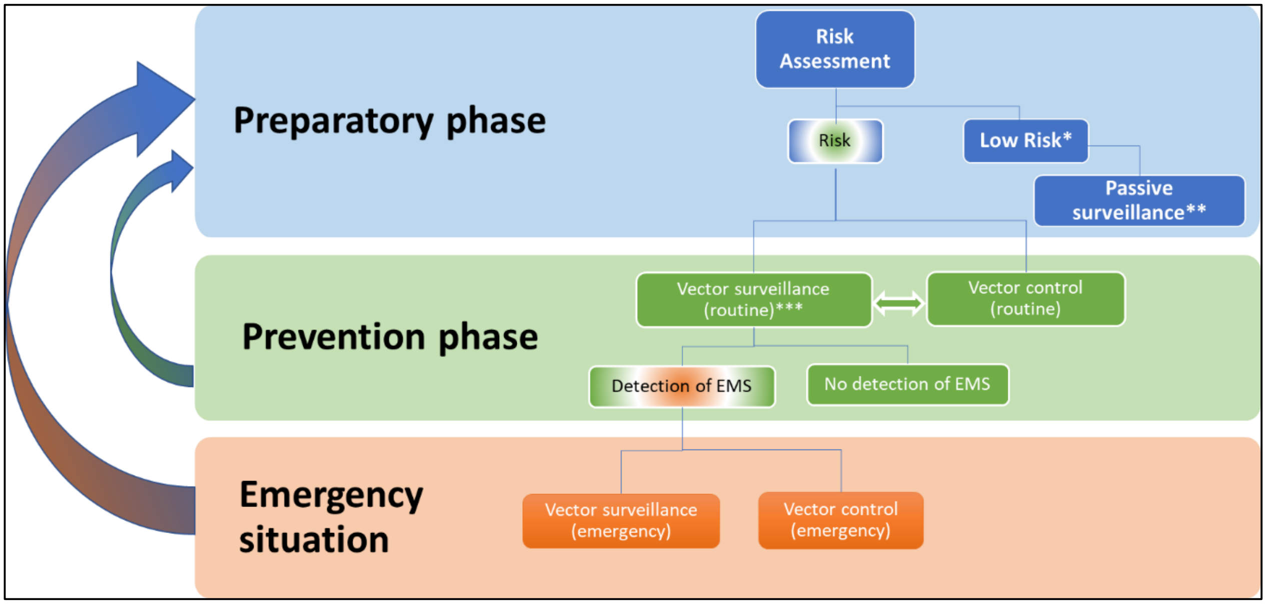
Each state party of WHO shall (c) furnish to WHO, as far as practicable, when requested in response to a specific potential public health risk, relevant data concerning sources of infection or contamination, including vectors and reservoirs, at its points of entry, which could result in international disease spread. Immediate reporting of an event to the IHR National Focal Point is necessary in these circumstances.

If a follow-up inspection is required to determine the success of the vector control measures applied on board an aircraft or a ship, the competent authorities for the next known port or airport of call with a capacity to make such an inspection, shall be informed of this requirement in advance by the competent authority advising such follow-up. In the case of ships, this shall be noted on the Ship Sanitation Control Certificate.

5 DECISION-MAKING PROCESS AND RAPID RISK ASSESSMENT

5.1 PREPAREDNESS, PREVENTION AND EMERGENCY PHASES

Rapid risk assessment results can be used to determine vector surveillance and control measures for each PoE. The **Figure 1** summarises the activities to be conducted at the PoE, depending on possible scenarios, presented in three phases: preparatory, prevention and emergency. In the first "preparatory phase", the risk assessment for introduction of EMS takes place. If the assessment indicates no risk, only passive surveillance will be implemented. This consists of a notification system for possible interception of EMS at the airport or port, complemented with EMS information campaigns and awareness for the airport or port personnel. If a risk is identified, then actions in the "prevention phase" may be considered for implementation. During the "prevention phase", vector surveillance on a routine basis may be considered to be conducted. Results from EMS surveillance are necessary to decide about the adequacy of vector control activities, and also to evaluate the effectiveness of control activities (e.g. mosquito density before and after mosquito control). The presence of local mosquitoes is inevitable; therefore, routine vector control is suggested to keep the native mosquitoes at a low level, or to keep PoE free of vectors (as recommended by IHR 2005). If EMS are detected during routine surveillance, then the "emergency phase" follows where implementation of intensive vector surveillance and control measures are implemented, to avoid proliferation at the airport or the port. In **Annex 5** and **Annex 6**, there is a detailed description of the activities and methods for routine vector surveillance and control (prevention phase), as well as for strengthening vector surveillance and control activities (emergency phase). Two case studies for *Ae. aegypti* introduction in an airport and management of a dengue fever case among seaport staff can be found in **Annex 7** and **Annex 8**, respectively.



**During preparatory phase when no risk is identified, re-evaluation takes place at least once every two years.*
***Passive surveillance describes all the actions that are related to information activities, stakeholders' awareness, any kind of notification system (e.g. send a sample or a photo to an expert for an opinion) and collection of public health surveillance data for vector-borne diseases (e.g. airport or port malaria).*
****The results from vector surveillance are used to decide on the kind of vector control measures that will be implemented and their frequency.*

Figure 1. Vector surveillance and control activities in the preparatory, prevention and emergency phases.

5.2 RISK ASSESSMENT FOR VECTORS AT PORTS

5.2.1 Risk analysis

Risk analysis may consider the following factors at ports:

1. *Presence and abundance of EMS on ships or cargo*
   - *Country of origin and countries visited during the voyage*
   - *Season at origin ports and ports visited by the ship*
2. *Survival of EMS on ships and cargo*
   - *Type of ship and cargo*
   - *Travel length*
   - *Conditions on board (temperature, availability of water and nutrients)*
3. *Volume of ships and cargo arriving at the port*
   - *Volume of ships and cargo arriving at the port*
4. *Prevention measures*
   - *Disinsection measures applied on ships and cargo*
5. *Survival and establishment of EMS*
   - *Season at destination ports (climate conditions such as rainy season, dry season, summer, winter etc.)*

- *PoE facilities and activities (in relation with possible breeding sites)*

6. *Local entomological situation*
    - *EMS mosquito surveillance in place*
    - *Previous findings of EMS*
    - *Reports for nuisance related to mosquitoes*
7. *Local epidemiological situation*
    - *Mosquito-borne diseases at destination area (imported or locally transmitted)*

Country of origin and countries visited during the voyage. It is suggested that the previous year's itineraries and possibly any scheduled itinerary for the upcoming years of ships visiting their port are reviewed, to determine the origin of cargo and port areas that ships visit during their voyages. Assessment of whether the itineraries include areas where EMS are present or have colonised is advised to be follow. Information on establishment of mosquito species in EU countries and other countries in the Western Palearctic region can be found here: https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps.

Season at ports of origin and visiting ports (rainy season, dry season). This information is important to evaluate possible mosquito introductions depending on the month and the location of the port of origin, as well as the ports visited. Mosquito densities will probably depend on the season.

[Reference: Optimal mosquito season for sub-countries https://journals.plos.org/plosntds/article/figure?id=10.1371/journal.pntd.0005604.g003]

Type of ship visiting the port and types of cargo carried or offloaded at the port. Specific types of vessels and specific types of cargo have been associated with movement of mosquitoes from one country to another. The risk for ships to carry mosquitoes differs depending on the type of freight carried on board the ship, as well as the presence of potential breeding sites (stagnant water). Potentially infested cargoes include used tyres, ornamental plants (mainly plant cuttings), stone fountains, repatriated construction and military material and vehicles, as well as other goods and equipment that can retain water and are stored outdoors in their country of origin. Specifically, used tyres that are stored in open areas before transportation pose a risk. Re-treated or new tyres do not pose a risk since they are not stored in open areas, as sunlight damages the tire gum. General cargo and particularly used vehicles/boats, used machinery, containers (including fresh fruit and vegetable containers, manufactured goods containers, empty containers, etc.) have also been reported to be of major importance[29,31,34-36]. Also containers transporting live animals should be taken into account, since all blooded creatures can serve as a source of a blood meal for mosquitoes.

Evidence also exists of interceptions of mosquitoes in fishing vessels and vessels carrying refugees[37]. In fact, most of the fishing vessels intercepted were involved in illegal fishing. It is important to note that some of these vessels did not visit a foreign port, but were fishing in water close to endemic areas. Mosquitoes have also been intercepted in yachts.

The port may also consider the origin of cargo from trucks carried by RO-RO vessels, as some of these trucks may carry high risk cargo[35].

Volume of ships and cargo arriving at the port. The volume (magnitude and frequency) of ships and cargo arriving at the port could be estimated by taking into consideration the previous year's traffic, if the schedule for the upcoming year is not available.

Disinsection measures applied on ships and cargo. Disinsection of the ships and cargo is a very important barrier for preventing mosquitoes from entering an area[31]. The port may consider reviewing whether disinsection measures are applied on high risk ships and cargo or not. This can be done by checking the disinsection certificates of ships and cargoes that arrived previous year from EMS colonised areas. If possible, the Ship Sanitation Certificates of incoming ships should also be checked to ensure that there is no evidence of vectors on board the ship visiting the port. The responsible port authorities can consider inspecting high risk ships and cargo for evidence of mosquitoes. Any container holding water is a potential breeding site of EMS on board ships (see paragraph 6.1.6).

Season at destination ports (rainy season, dry season). Although EMS may be introduced in an area, their establishment depends, among other, on the climate conditions of the specific area for the specific species. If possible, the port may consider to evaluate whether establishment of the specific EMS is possible in the area during the seasons that high-risk ships and cargo arrive.

Port facilities and activities. Survival of the EMS will also depend on the suitability of the environment for breeding. Any container that can hold water is a potential breeding site for mosquitoes. According to the WHO "Handbook Vector Surveillance and Control at Ports, Airports, and Ground Crossings", potential breeding sites of mosquitoes include ponds, puddles, ditches, surface drains, grassy and marshy land, pits and depressions, scrap and water containing depressions in sheeting, containers of different varieties and shapes, water chambers, hydrants, tyres including fenders, ground and overhead water tanks, septic tanks, terraces/roof tops/lintels, curing waters in construction/development sites, wells, iron ore loader buckets and potential resting places of mosquitoes include human dwellings/restrooms, sheds, indoor hanging objects, crevices, bushes/vegetation-wild and in gardens, underneath furniture, curtains, underneath or on the sides of tanks, cargo boxes/holds, workstations, walls of buildings and underneath roofs, open luggage/cargo boxes or containers, cartons and containers, wrapped crafts, vehicles, vessels, etc., tire dumps, abandoned structures/buildings, tree holes)[33]. It is important that the port and surrounding areas (at minimum radius of 400 meters) are inspected regularly, in order to identify and remove or manage such containers. In addition to this, it is advised the flight distance of targeted mosquitoes be considered when designing a mosquito surveillance program.

Studies have shown that some materials are preferred over others. For example, earthen containers were the most common breeding site of *Ae. aegypti*, and rubber containers were generally preferred over plastic containers[38]. One study showed that cemented containers and iron drums were preferred by *Ae. aegypti* over plastic containers[39]. Finally, another study showed that the only type of container where *Ae. aegypti* was systematically found with mosquito larvae was fire extinguisher buckets[6].

The type of facilities and activities inside a port may also facilitate the survival of EMS. In order to assess the facilities, the port may consider collecting information on the environment of the port with a minimum radius of 400 meters area[33]. GIS tools or other cartographic documents are advised to be used, in order to describe the site and facilities of interest. It is advised that maps contain delimitation of the closed zone, buildings, accesses, loading/unloading areas, cargo, warehouses, handling and maintenance, the sanitation and drainage systems and the hydraulic structures[33]. It is important to identify the areas that are of higher risk for EMS breeding. For example, the cold container parking areas have been shown to have a large number of *Aedes* species in a port, followed by parking areas, storage of petroleum products and customs areas[40]. Note that different species may be found in different locations of the port as well.

5.2.2 Risk categorization

In the "preparatory phase", the risk for introduction of EMS at the ports could be evaluated. For the quantitative categorization of risk for ports, a methodology to assign ports to different risk categories is introduced (**Figure 2**, **Table 1** and **Table 2**). This methodology is based on the risk for EMS importation. The different categories can be utilized to suggest different routine or strengthen vector surveillance and control efforts. This categorization needs to be done in cooperation with representatives of the national authorities and port representatives, which will provide information on the port calls, frequency of arrival from origins and port statistics, and information on previous EMS introductions. After the first risk assessment, competent authorities in collaboration with entomologists and others who share responsibilities, could perform the quantitative risk assessment once a year for each port, to detect eventual risk changes and accordingly adapt the vector surveillance. The factor scores are relative values used to quantify the risk of importing EMS for the different ports.

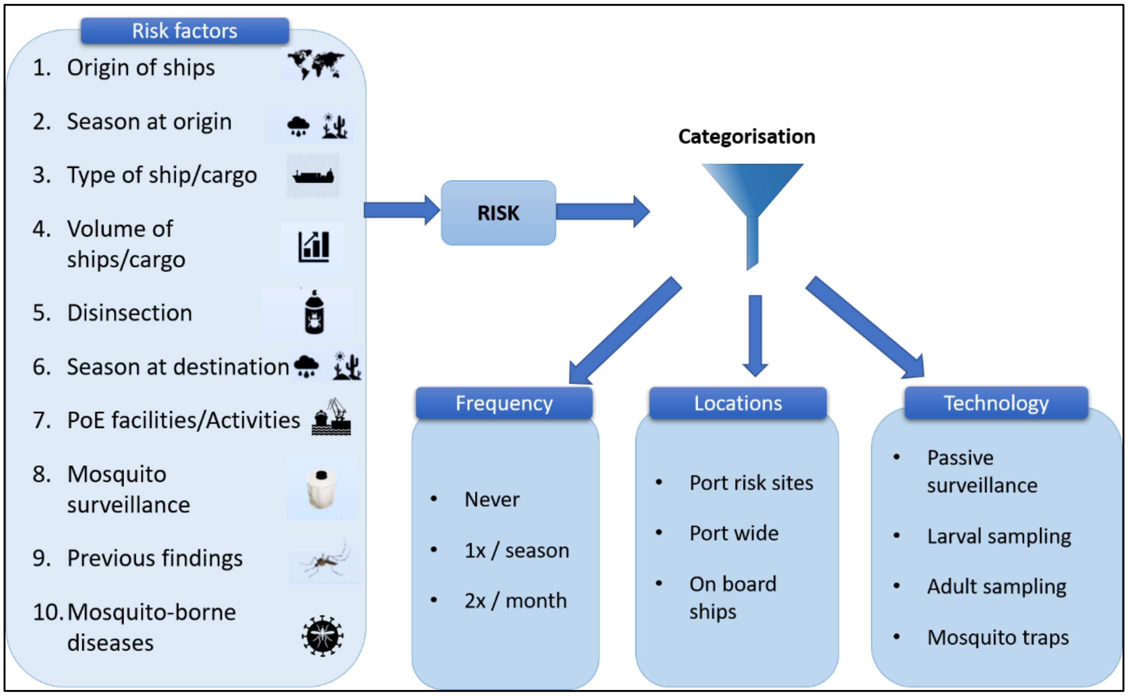


Figure 2*. Flowchart for categorization of introduction risk of EMS at ports.*

Table 1. *Risk factors and their relative scores for categorizing the risk of introduction of EMS at ports.*

Risk factor	Description	Relative score*
Country of origin and countries visited during the voyage	Internal EU from non-EMS colonized areas	0
	Internal EU from EMS colonized areas	1
	External from non-European countries	2
Season at origin ports and ports visited by the ship	Ship arriving or visiting countries outside the optimal mosquito season	0
	Ship arriving or visiting countries during the optimal mosquito season	1
	Unknown	1
Type of ship and cargo	Other types of ships and cargo	0
	Cargo ships containing ornamental plants, tyres, used machinery, containers (including fresh fruit and vegetable containers, manufactured goods containers, empty containers, etc.), RO-RO ships, fishing vessels fishing in water of colonised areas or where other fishing vessels are fishing with a colonised area as home port	2
Volume of ships and cargo arriving at the port	No ship or cargo is expected from EMS colonised areas	0
	A few ships and cargo are expected from EMS colonised areas during the next year	1
	There will be systematic traffic of ships and cargo from EMS colonised areas during the next year	2
Disinsection measures applied on ships and cargo	Documentation of disinsection applied in cargo and on board the ship	1
	Documentation of disinsection applied only to the cargo or only on board the ship	2
	No preventive measures applied	3

Season at destination ports (rainy season, dry season)	Destination area is outside the optimal mosquito season	0
	Destination area is in optimal mosquito season	2
	Unknown	2
PoE facilities and activities	The port and the surrounding area (minimum radius of 400 meters) have only a few containers that can hold water which is treated appropriately	1
	The port and the surrounding area (minimum radius of 400 meters area) have many containers that can hold water and are not managed appropriately, rubbish and other potential breeding sites.	2
EMS Mosquito surveillance active Max 2	EMS surveillance and no mosquito vectors were detected at a minimum radius of 400 meters area	0
	EMS surveillance and mosquito vectors present at minimum radius of 400 meters area	1
	No EMS surveillance and invasive mosquitoes present in the region	2
Previous findings of EMS of nuisance related to EMS Max 3	No EMS findings or nuisance reported at the port	0
	EMS findings or nuisance reported at the port	3
Mosquito-borne diseases at destination area	No cases of non-endemic mosquito-borne diseases were reported the last year	0
	Cases of non-endemic mosquito-borne diseases were reported the last year	3

*The total score of a port is the sum of the individual scores of the risk factors. Scores can take values from 0 (minimum-no risk identified) up to 22 (maximum). This total score is used in Table 2 for risk categorization of the port [Adaptation of the published table used for categorization of used tire locations: Table S1- https://meridian.allenpress.com/jamca/article/36/2/89/446440/Risk-Based-and-Adaptive-Invasive-Mosquito].

Table 2. *Risk category assignment for ports, and the suggested sampling method/frequency. The relative score is the sum of the individual scores of the five risk factors in Table 1.*

Relative Score*	**Risk category**	**Sampling method/ effort/ frequency inspections**
>17	High	Adult trapping, adult sampling, larval inspections and passive surveillance / port wide and on board ship / 2x month
8-16	Medium	Adult trapping, larval sampling and passive surveillance / port main identified risk sites / 2x month
0-7	Low	Passive surveillance and larval sampling / port main identified risk sites / 1x season

*The relative score is the sum of the individual scores of the risk factors listed in Table 1.

5.2.3 Competent authorities: roles and responsibilities at ports

It is important that in each port, the responsibilities and duties are clearly defined (see paragraph 4.2). It is strongly suggested that responsible authorities/stakeholders for each PoE collaborate with experts in entomological surveillance.

Table 3. *List of activities related to vector surveillance and control at ports, and examples of responsible authorities/stakeholders for implementing the activities.*

Activity	**Example of responsible stakeholder**
Risk analysis	Team of medical entomologists supported by public health authorities
Data port documentation (maps, statistics)	Port authorities
Permissions (pass) for entry port facilities	Port authorities
Permission for use of batteries, CO_2 at port facilities	Port authorities
Planning entomological activities	Team of medical entomologists supported by public health authorities
Management of adult traps, larval sampling, ship inspection	Team of medical entomologists, instructed port fellow workers, public health authorities
Samples evaluation, mosquito identification	Team of medical entomologists
Mosquito control	Instructed port fellow workers or external mosquito control company, supervised by the public health or competent authority

5.2.4 Problems/constraints at ports

Effective measures to reduce the risk of the introduction of IMS require coordinated efforts of all European Union Member States (EU MS) in cooperation with importers of high-risk goods, container loading area operators, shippers and other stakeholders involved in the importation and exportation of freight, which make efforts challenging. Other potential problems and constraints for vector surveillance and control activities at ports are listed in **Table *4***.

Table 4. *Constraints for vector surveillance and control at ports.*

Category	Constraint	Surveillance	Control	Arguments
Governmental and managerial level	Limited resources, understaffed authorities, lack of training, awareness, prioritization and commitment	X	X	Government commitment, managerial commitment, allocation of resources
Human resources	Availability of experienced medical entomologist in surveillance team and for identification of insect sampled	X		Recommendation that organization of surveillance is done by expert medical entomologist, training of staff supporting surveillance
	Availability of trained staff in day-to-day surveillance implementation	X		
	Availability of experienced team in surveillance and control nearby port	X	X	Team ready for the work and available (near port and timely available when needed) to provide the analysis and to react promptly in case of EMS detection
Materials	Availability of authorized biocides for implementing IVM		X	Stock of materials needed for IVM
	Availability of entomological equipment, traps	X	X	Availability of materials for surveillance (traps) and/or control (e.g. biocides)
Permissions, documentation	Access to facilities with permission	X	X	Surveillance and control team it is advised to have access to the necessary locations
	Availability of data collected by the port	X		e.g. origin ports, the volume of ship calls
Budget	Availability of budget (euros and hours) for entomological fieldwork, entomological laboratory work	X		Government commitment, managerial commitment, allocation of resources
	Availability of budget (euros and hours) for mosquito control and for implementing IVM		X	

5.3 RISK ASSESSMENT FOR VECTORS AT AIRPORTS

5.3.1 Risk analysis

In areas with high mosquito densities, there is a greater probability that adult mosquitoes can enter aircrafts at airports following their human hosts[18]. These aircrafts can then rapidly transfer mosquitoes to another country, thus increasing the chance of mosquitoes surviving the trip, and reaching a location where the mosquitoes are non-native[17].

Hypothetically, the higher the frequency of flights from airport origins with high mosquito density, the higher the probability of EMS introduction.

The probability of EMS introduction into an airport will vary depending on several factors. The major factors to be considered for the risk assessment include the following:

Volume passengers/cargo arriving at the airport. It is recommended to directly contact the airport authorities that will provide this specific information, probably already published or reported to the national authorities. To prioritize the main airports within a country it is recommended to check the national statistics service. [Suggested search source: Eurostat website; https://ec.europa.eu/eurostat/databrowser/explore/all/transp?lang=en&subtheme=avia.avia_pa&display=list&sort=category&extractionId=AVIA_TF_APAL; for EU domestic flights connection principal airports: https://ec.europa.eu/eurostat/cache/infographs/airports/]

Origin of flights arriving at the airport. Similarly to the volume of passengers, airport authorities can provide this information in advance. It is recommended to have access to the data each year (data can be provided monthly) from the origin airports, including the IATA code. [Suggested search source for the locations of airports of origin : https://datahub.io/core/airport-codes, https://www.arcgis.com/home/item.html?id=9508e68045764699a8857de7add4d168]

Previous experiences, notifications, etc. involving findings of EMS. Similarly to the volume of passengers, airport authorities can provide this information in advance. Suppose this information has been published because of the importance of the finding. In that case, it could be found in bibliographic databases or on internet search tools (e.g. Google), typing for example the name of the airport, country and keywords (such as airport and mosquitoes). Since this type of information is not always publicly available, and if so probably with a significant time delay, internal communication and cooperation among involved stakeholders at different levels (local, regional, national) is critical.

Disinsection measures applied or not on aircrafts. Different policies are implemented worldwide for aircraft disinsection, including requirements for disinsection from all in-bound flights or selected flights. There are four application methods: 1 pre-embarkation cabin (without passenger); 2) pre departure cabin (with passenger); 3) pre-departure cargo holds; and 4) residual long-term[41]. The treatment is offered by the competent airport authority or by others under the supervision of a competent authority. Insecticides used can include: (1R)-trans-phenothrin technical grade material and permethrin technical grade material. Insecticides and spray operations should be applied in accordance with the rules and regulations of the country of destination and WHO specifications[41].

[Suggested search source about countries requirements for aircraft disinfection as a condition for entry: The policies required by countries worldwide can be found on the website of the US Department of Transportation: https://www.transportation.gov/airconsumer/spray. The Aircraft Disinsection Requirements are presented in the following categories: i) countries requiring the disinsection of all in-bound flights with an aerosolized spray while passengers are on board; ii) countries requiring the disinsection of all in-bound flights but allowing, as an alternative to the above approach, either (a) the residual method or (b) the application of an aerosolized spray while passengers are not on board; and iii) countries that require disinfection of selected flights].

Season at origin airports (rainy season, dry season). This information is important to evaluate the possible mosquito introductions depending on the month and the location of the airport of origin. Mosquito densities at origin airports will probably depend on the season. [Suggested search source: Optimal mosquito season for sub-countries https://journals.plos.org/plosntds/article/figure?id=10.1371/journal.pntd.0005604.g003]

Mosquito species present at origin airports. Several mosquito species at origin airports can be transported to Europe in aircrafts. A list of all the species potentially risky for a country can be difficult to evaluate. Generally, upon arrival, subtropical species (e.g. *Ae. albopictus, Ae. aegypti*), could potentially establish in southern European countries, and temperate species (*Ae. japonicus, Ae. koreicus*) could be a risk for western/central Europe. *Aedes albopictus* has already been established in many European and Middle Eastern countries, while *Ae. aegypti* has since colonised Madeira and reappeared in parts of southern Russia and Georgia[42]. Recently, in 2022, an increased number of autochthonous transmissions of dengue virus were recorded in mainland France. These autochthonous transmissions, due to AIM, in correlation with the recent invasion of *Ae. aegypti* in Cyprus, appears to be an alarm for all the European and Mediterranean countries (https://www.ecdc.europa.eu/sites/default/files/documents/Communicable-disease-threats-report-7-october-2022.pdf). Distribution maps, including models, could help to evaluate the risk of importing EMS from airport origins.

[Suggested search source: The global distribution of *Aedes albopictus* and *Aedes aegypti*. https://elifesciences.org/articles/08347/figures; Global occurrence data improve potential distribution models for *Ae. japonicus* in non-native regions https://onlinelibrary.wiley.com/doi/10.1002/ps.5710]

5.3.2 Risk categorization

According to the WHO "Handbook for Vector Surveillance and Control at Ports, Airports, and Ground Crossings", capacity building is advised for vector surveillance and control at points of entry and up to (at least) a 400-metre perimeter around them (a minimum radius of 400 metres area). However, depending on the above-mentioned factors, routine surveillance and control of EMS vectors will be different at different airports.

In the "Preparatory phase", we evaluate the risk for introduction of EMS at the airports. For the quantitative categorization of risk for airports, we suggest a methodology to assign airports to different risk categories (**Table 5**, **Table 6 Error! Reference source not found.**and **Figure 3**). This methodology is based on the risk of importation of EMS. The different categories can be utilized to suggest different routine or strengthen vector surveillance and control efforts. This categorization needs to be done in cooperation with representatives of the national authorities and airport representatives, which will provide information on the volume of movements at the airport, frequency of arrival from origins, airport statistics and information on previous EMS introductions. After the first risk assessment, it is recommended to perform the quantitative risk assessment once a year for each airport, to detect eventual risk changes and accordingly adapt vector surveillance. The factor scores are relative values used to quantify the risk of importing EMS for the different airports.

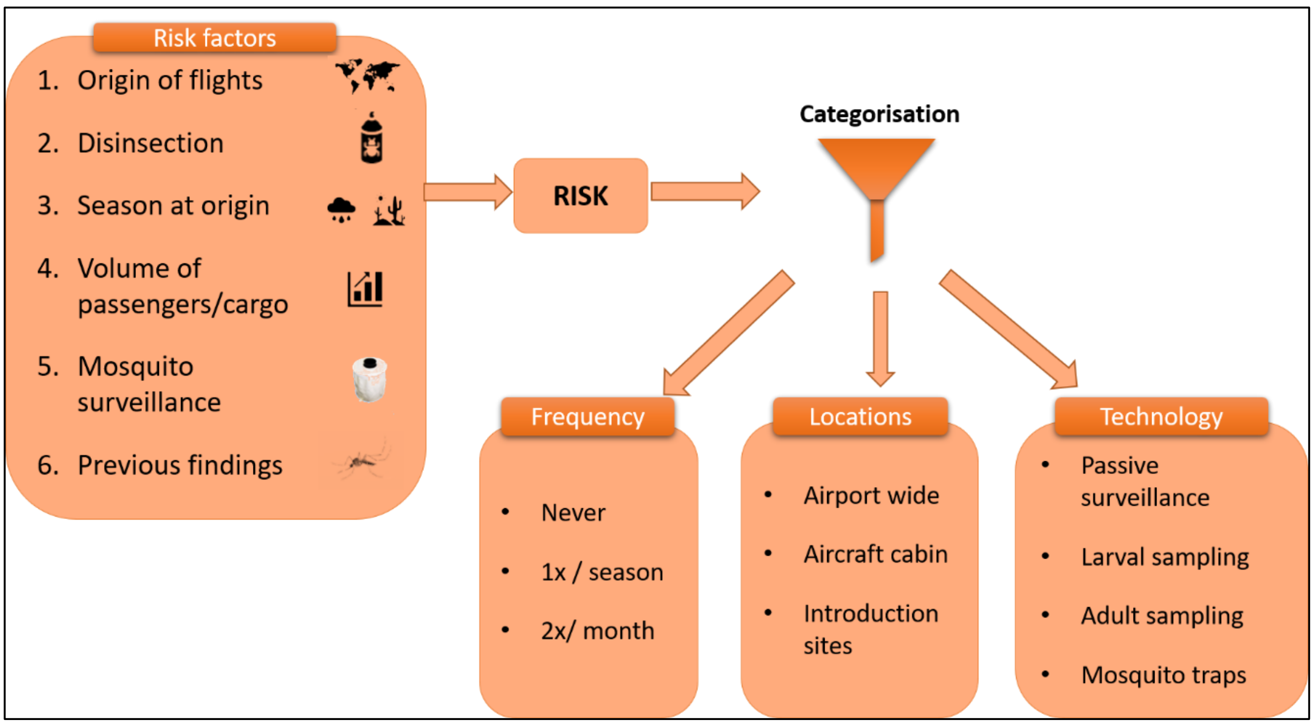


Figure 3. *Flowchart for risk categorization for risk of introduction of EMS at airports.*

Table 5. *Risk factors and their relative scores for categorizing the risk of introduction of EMS at airports [Adaptation of the published table used for categorization of used tire locations: Table S1[43]].*

Risk factor	Description	Relative score
Origin of flights arriving at the airport Max 2	Internal EU from non-EMS colonized areas	0
	Internal EU from EMS colonized areas	1
	External from non-European countries	2
Disinsection measures applied on aircrafts Max 2	Documentation of disinsection applied in cargo and cabin	0
	Documentation of disinsection applied only in cabin	1
	No preventive measures applied	2
Season at origin airports (rainy season, dry season) Max 1	Flight arriving from countries outside the optimal mosquito season	0
	Flight arriving from countries during the optimal mosquito season	1
	Unknown	1
Volume of aircrafts/airport, /type of cargo arriving at airport (international arrivals) Max 2	Low (for example: airport not in the top 5 airports at national level)	0
	Medium (for example: airport in the top 5 airports at national level)	1
	High (for example: airport in the top 3 airports at national level)	2
EMS surveillance active Max 2	EMS surveillance and no mosquito vectors detected at minimum radius of 400 meters area	0
	EMS surveillance and mosquito vectors present at minimum radius of 400 meters area	1
	No EMS surveillance and invasive mosquitoes present in the region	2
Previous findings of EMS of nuisance related to EMS Max 3	No EMS findings reported at the airport	0
	EMS findings reported at the airport	3

Table 6. *Risk category assignment for airports, and the sampling method/frequency applied based on this assignment. The relative score is the sum of the individual scores of the five risk factors in Table 5.*

Relative Score	Risk category	Sampling method/ effort/ frequency inspections
>9	High	Adult trapping, adult sampling, larval inspections and passive surveillance / airport wide / 2x month, inspection of aircraft cabins for adult mosquitoes could be considered
5-8	Medium	Adult trapping, larval sampling and passive surveillance / airport main identified risk sites / 2x month
0-4	Low	Passive surveillance and larval sampling / airport main identified risk sites / 1x season

5.3.3 Competent authorities' roles and responsibilities at airports

At each airport, it is advised that the responsibilities and duties be clearly defined as described in paragraph 4.2 and

Table 7. It is strongly suggested that responsible authorities/stakeholders for each PoE collaborate with experts in entomological surveillance.

Table 7. *List of activities related to vector surveillance and control at airports, and examples of responsible authorities/stakeholders for implementing the activities.*

Activity	**Example of responsible stakeholder**
Risk analysis	Team of medical entomologists, supported by public health authorities
Data airport documentation (maps, statistics)	Port authorities
Permissions (pass) for entry airport facilities	Port authorities
Permissions for use of batteries, CO_2 at airport facilities	Port authorities
Planning entomological activities	Medical entomologist team
Management of adult traps, larval sampling, aircraft inspection	Surveillance and medical entomologist teams (instructed airport fellow workers), public health authorities
Samples evaluation, mosquito identification	Team of medical entomologists
Mosquito control	Instructed port fellow workers or external mosquito control company, supervised by the public health or competent authority

5.3.4 Problems/constraints at airports

Constraints for vector surveillance and control for airports are listed in **Table 8**.

Table 8: *Constraints for the vector surveillance and control at airports*

Category	**Constraint**	**Surveillance**	**Control**	**Arguments**
Governmental and managerial level	Limited resources, understaffed authorities, lack of training, awareness, prioritization and commitment	X	X	Government commitment, managerial commitment, allocation of resources
Human resources	Availability of experienced medical entomologist in surveillance team and for identification of samples	X		Recommendation that organization of surveillance is done by expert medical entomologist
	Availability of experienced team in surveillance and control nearby the airport	X	X	Team ready for the work and available (nearby) to provide the analysis to react promptly in case of detection
Materials	Availability of authorized biocides for implementing IVM		X	Availability of materials for surveillance (traps) and/or control (e.g. biocides)

	Availability of entomological equipment, traps	X	X	
Permissions, documentation	Access to facilities with permission	X	X	Surveillance and control team is advised to have access to the necessary locations
	Availability of data collected by the airport	X		
Budget	Availability of budget (euros and hours) for entomological fieldwork, entomological laboratory work	X		
	Availability of budget (euros and hours) for mosquito control		X	
	Availability of authorized biocides for implementing IVM		X	

6 ROUTINE AND EMERGENCY MEASURES

6.1 VECTOR SURVEILLANCE

6.1.1 General principles for ports and airports

Routine surveillance for EMS detection at PoE mainly consists of placing adult mosquito traps and/or performing manual larval and adult samplings. If an EMS is identified during routine surveillance, an emergency surveillance action follows. This is done to evaluate if detection is an accidental introduction, or if an EMS is reproducing or established at the airport/port or in its surroundings (**Annex 5**). When designing a mosquito surveillance programme for a port or an airport, it is important to define the species of interest, and select the appropriate sampling methods (e.g. traps, larval samplings). It is important to note that not all adult mosquito trap types effectively be used to capture all different mosquito species of medical importance.

6.1.2 Routine surveillance of EMS

Specific routine surveillance activities are selected after considering the results of the risk assessment and risk category identified, as described in **Table 2** and **Table 6**. At PoE, it is important for EMS surveillance to be implemented throughout the year indoors (at risk location sites), and outdoors during the mosquito season (which depends on the climatic conditions and surveillance findings). In northern/central Europe, sampling is only performed indoors from November until April, while traps are placed indoors and outdoors from May to October. In southern Europe, outdoor sampling could be performed from April until November, and indoors throughout the year. Surveillance mainly consists of placing mosquito traps at the most likely locations for the introduction of EMS at PoE. Different types of sampling methods (e.g. traps) can be used to collect mosquitoes.

At airports, indoor traps can be placed at locations where sealed containers carrying baggage and cargo are opened for the first time after arrival.

Locations with higher probability to find imported EMS at the airport are the locations where containers (e.g. AKE, AKH) with luggage or other goods are for the first time opened at the airport facilities. These locations can be found indoors or outdoors. Outdoors, traps can also be placed in the vicinity of areas where aircraft doors (passengers and cargo) are opened (next to the gates). Other locations where traps can be placed are: near airport canteens, cafeterias or other places where female mosquitoes may seek their blood meals, near areas with vegetation, in places where accumulated items could serve as larval habitat and others. Recommended activities and methods for vector surveillance at ports and airports with an emphasis on EMS can be found in **Annex 5**.

6.1.3 Emergency surveillance after detection of EMS

If an EMS specimen is identified, intensified surveillance action follows. It is recommended that this action last for at least four weeks, depending on climatic conditions. The frequency of the inspections is then increased from fortnightly to weekly collections. Furthermore, depending on climatic conditions, additional larval and adult searches are implemented in the surroundings of the collection site. If necessary, additional traps are deployed (mosquito species such as *Ae. aegypti* can reproduce inside if the temperatures are good). The main objective of this intensive surveillance is to evaluate if the detection is an accidental introduction, or if an EMS is reproducing (or is already established) at the PoE or its surroundings. After four weeks without other EMS detections, the frequency of inspections returns to the routine plan (based on **Table 6**), and the additional deployed traps are removed.

6.1.4 Specificities for vector surveillance at ports

Ship operators must permanently keep ships free of sources of infection or contamination, including vectors and reservoirs (IHR article 24). Inspection at port facilities for potential breeding sites and application of control measures for their elimination could help improve the conditions at port facilities. For example, a regular inspection programme could take place every month, where all activities and port areas could be inspected for conditions favourable to mosquito resting areas and breeding sites.

6.1.5 Integrated Pest Management (IPM) plan on ships

Both cargo ships and passenger ships have pest management plans in place, as described in the WHO "Handbook for Inspection of Ships and Issuance of Ship Sanitation Certificates", as well as in the "European Manual for Hygiene Standards and Communicable Disease Surveillance"[44]. Preventing access of mosquitoes to the ship can be achieved through: screen/self-closing doors, windows, ramps, cabin balconies, air curtains, checks of incoming supplies, application of protocols for standards to containers' and cargoes' conditions (this is recommended for crew areas, not for cargo holds and/or Ro-Ro decks). Monitoring can be implemented by placing light sticky traps close to ship openings (including windows, doors, etc.). Surveillance can be conducted by trained ship board crew for mosquitoes and conditions favourable to support mosquitoes' infestations. Other measures include:

- *Elimination of accumulated standing rainwater indoors and at the open decks.*
- *Drying up all water collections on board that could act as breeding sites for mosquitoes, if possible, on every week.*
- *Application of vector control measures if mosquitoes are found, follow-up inspections and recording of actions and results.*
- *To have insecticides available for commonly found pests on board, including mosquitoes*
- *Recording of insect bites and complaints from travellers and crew.*

- *Training of personnel to implement the IPM plan.*

6.1.6 Inspections for vectors on ships

To investigate the presence of mosquitoes on board ships, inspectors should identify the high-risk areas, also considering the cargo that is carried and whether it is at high risk for vector infestations.

The risk for ships to carry mosquitoes differs depending on the type of freight carried on board, and the presence of potential breeding sites (stagnant water). Potential areas on board ships where mosquito larvae or adults may be present include: lifeboats (open type not allowing draining), mooring rope areas, safe valve opening points, containers, lifeboat covers, bilges, scuppers, awnings and gutters, fan rooms, any plastic sheets or open drums, paint drums, manifolds (tankers) and free space between deck and containers, laundry areas if window openings or doors to open deck exist, toilets and bathrooms, galleys, standing water around recreational water facilities and others. All ships on an international voyage must be inspected once every six months for public health risks, including presence of mosquitoes. Results of inspections should be noted in the Ship Sanitation Certificate[2].

6.1.7 Inspections for vectors on aircrafts

If possible, it is strongly recommended to investigate the presence of mosquitoes on intercontinental flights arriving from EMS risk areas. Passenger cabins and cargo hold spaces can be inspected for the presence of live mosquitoes upon arrival. Recommended selection of flights and inclusion criteria for inspection of flights are:

1) Origin of the flight: for example, intercontinental flights with emphasis on tropical areas in Africa, Asia, and South America.
2) Informed consent of the aeroplane personnel.
3) Accessibility about security issues.
4) Suitable working days (inspectors' agenda)

Weather conditions at the airport of departure, specific airlines, seasonality, and disinsection on departure can also be considered.

It is preferable for inspections to be carried out by an employee of the surveillance team that received training in mosquito identification and ecology. Visual inspections searching for living mosquitoes take place immediately after passengers have exited the cabin. These visual inspections take approximately 30-45 minutes per cabin and hold space. Mosquitoes can be collected using an entomological sweep net and suction tubes. Collected mosquitoes are kept in a separate tube for each cabin/hold. The tubes are sealed, labelled and taken to the laboratory for morphological identification.

6.2 VECTOR CONTROL MEASURES

6.2.1 General principles for vector control measures at ports and airports

After their introduction, EMS population development at the airport or port strongly depends on the availability of water-holding containers, stagnant water available for oviposition and larval development, and weather conditions. Water salinity is also of importance in the case of ports. On a routine basis (preparatory and prevention phases), larval treatment is the principal management method. Therefore, water containers (breeding sites with stagnant water) need to be sampled for mosquito immatures, followed by elimination or treatment with larvicides. Wells with stagnant water, holes, and open PVC cable tubes containing water must be closed with insulation foam or concrete. Silicone-based liquid for mosquito control can be used on stagnant water locations inside buildings at the underground level. These strategies allow for ruling out an established population of EMS at the port/airport.

If surveillance shows the presence of EMS, immediate mosquito control operations may be considered for the area (for surveillance it is advised to specify the size of the area). Depending on the specific environmental situation, the following actions can be adopted (**Annex 6**):

1. Larval control treatments in any drains, gutters, etc. with fast acting larvicide (e.g. Bti, diflubenzuron) on a weekly basis during favourable climatic conditions (not in winter).
2. A quality control plan on the efficacy of mosquito control activities is advised(based on the national plan for quality control of mosquito control programs)[45].
3. Adulticide treatments are advised only in case of high presence of adults (this should be defined based on surveillance data).
4. Eventually expand the area under control, in strict coordination with the evidence provided by the surveillance.
5. Specific to container breeding mosquitoes (e.g. *Aedes* invasive mosquitoes or *Anopheles stephensi*), larval treatments will be continued until surveillance data provided by the mosquito traps show no more EMS specimens during four consecutive weeks

Below are the key points to be considered by the competent authority regarding vector control measures on board ships:

1. All activities must be carried out safely according to the:

   a) United Nations International Maritime Organisation (IMO) Safety of Life at Sea (SOLAS) Convention.

   b) The Recommendations on the Safe Use of Pesticides in Ships published by the IMO.

   c) Requirements of national or regional regulations.

   d) The IMO International Maritime Dangerous Goods (IMDG) Code, fumigation of packaged goods.

e) The International Maritime Fumigation Organisation (IMFO) Code of Practice.

2. Licensed and competent specialists must apply the vector control measures.

3. The vector control method and the pesticides used must be appropriate for the location and type of cargo.

4. Written documentation must be provided by the pest control company, to ensure the implementation of the planned mosquito control actions.

The ship master must apply all required measures (e.g. in case of fumigation application, ensure that crew is briefed on the application process and appoint crew members to act as representatives of the ship master, etc.).

6.3 VECTOR SURVEILLANCE AND CONTROL ACTIVITIES AT POE IN RESPONSE TO A VECTOR-BORNE DISEASE CASE

At PoE where vectors are present and a mosquito-borne disease case (suspected or confirmed) has been confirmed, it is recommended to urgently perform mosquito control activities. It is important to start all the activities within 24 hours from case reporting, with the aim to eliminate possibly present infected mosquitoes (see **Error! Reference source not found.** for all the actions/activities). In a case where the situation regarding l ocal vector density is unknown, mosquito surveillance using traps baited with attractant and CO_2 should be used, and it is important to be conducted immediately.

The SOPs for emergence vector control operations in case of mosquito-borne disease cases detected, as described in this paragraph have considered previous work published by Bellini and colleagues[8†].

Area definition

At ports/airports, vector surveillance (e.g. traps, larval samplings) could be implemented during the whole year indoors. For surrounding areas outdoors within a radius of at least 400m from the airport perimeter, surveillance could be conducted during mosquito season, depending on the climatic conditions and surveillance data. In case of an emergency, a risk assessment could be conducted to determine if the area to conduct vector control operation is the port/airport and the surrounding area. Vector density, climate conditions, potential breeding sites, areas visited by the patient (indoors or outdoors), whether the patient was infectious etc. could be considered in the risk assessment. Risk assessment can be conducted by a multi-disciplinary team consisting of competent authority representatives, epidemiologists, entomologists, public health officers and others. It should be noted that IHR (2005) requires that *"States Parties shall establish programmes to control vectors that may transport an infectious agent that constitutes a public health risk to a minimum distance of 400 metres from*

[†] *Annex 5 in "Bellini, R., Michaelakis, A., Petrić, D., Schaffner, F., Alten, B., Angelini, P., Aranda, C., Becker, N., Carrieri, M., Di Luca, M. and Fălcuţă, E., 2020. Practical management plan for invasive mosquito species in Europe: I. Asian tiger mosquito (Aedes albopictus). Travel medicine and infectious disease, 35, p.101691"*
https://www.researchgate.net/publication/340815018_Practical_management_plan_for_invasive_mosquito_species_in_Europe_I_Asian_tiger_mosquito_Aedes_albopictus_-_ANNEX_5_Standard_Operational_Procedures_for_emergency_vector_control_in_case_of_dengue_chiku

those areas of point of entry facilities that are used for operations involving travellers, conveyances, containers, cargo and postal parcels, with extension of the minimum distance if vectors with a greater range are present".

Mosquito control execution

Mosquito control activities include three actions to be conducted in a synergistic manner: treatments with adulticide(s), with larvicide(s) and larval breeding sites removal.

The optimal sequence in which these processes may consider to be conducted is:

- *adult treatments in public areas during the hours that the vector is active;*
- *adult and larval treatments and source removal, this is also necessary for the surrounding area of port/airport (in private areas, door-to-door strategy).*

Contextual larval treatment in public catch basins

Products and equipment: pyrethroids are particularly suitable for adulticide interventions. For larvicide interventions, diflubenzuron, *Bacillus thuringiensis* var. *israelensis* and liquid surface films (e.g. Aquatain™) may be chosen. Depending on the accessibility of the areas to be treated, an atomizer for vehicle-mounted spraying (when inaccessible) or handheld sprayer (when accessible) can be used. Only authorised biocides (regulation differs depending on the country) must be used, following their label's directions for safe use and warnings. It is strongly recommended to contact the relevant national authorities annually for more details regarding registered biocides.

Treated places: adulticide treatment must be directed to the vegetation (hedges, bushes, shrubs) on public and private areas, up to three to four metres in height. In case of treatments performed on roads, it is advised the treatment on both the left and right side, possibly with the double pass, be carefully conducted.

Repetitions: it is advised that larval treatments on public roads be repeated frequently and as recommended by the producer. The number of repetitions is a minimum of two (2), with the maximum depending on the vector presence/density (surveillance data), on the label's directions and any regulatory constraints. Larval management must be conducted again in storm drains and catch basins (not necessarily in containers) in case of heavy rain after treatment.

Table 9. *Emergence control measures in the event of suspected or confirmed mosquito-borne disease case in the area of the PoE.*

Measure	Process	Description
Determination of area to be treated	Risk assessment to decide about the control measures to be implemented	Entomological surveillance and mosquito control activities in the PoE and the surrounding area, in accordance to the risk assessment results.
Mosquito control activity	Adult and larval treatment and source removal	Pyrethroids are particularly suitable for adulticide interventions. For larvicide interventions, diflubenzuron, *Bacillus thuringiensis* var. *israelensis* and liquid surface films (e.g. Aquatain™). The adulticide treatments in public and private areas are advised to be applied in the foliage (bushes, fences) up to approximately 3-4 metres in height. In addition, the treatments which are applied from the roads should ensure that the biocide is spread consistently on both sides. Special attention is advised to be paid in cases of adulticide treatment, in the vicinity of apiaries and surface waters. It is advised operators be protected to avoid being bitten by mosquitoes. For private areas (e.g. PoE surrounding area at a minimum radius of 400 meters), a door-to-door strategy must be implemented. In case that the local vector density is unknown, mosquito surveillance using traps baited with attractant and CO_2 must be conducted immediately (less than 24 hours after the record of the imported case).
Biocidal products	Adulticide & larvicide	It is essential to use only registered biocides, in accordance with the label's instructions.

7 ANNEXES

Annex 1: Guidelines for developing Standard Operating Procedures (SOPs)

Guidelines for developing SOPs can be found in the World Health Organization (WHO) document "Coordination of public health surveillance between points of entry and the national public health surveillance system: implementation toolbox". World Health Organization (2018)[46]

The specific tool for developing SOPs can be downloaded at the following link:

https://apps.who.int/iris/handle/10665/112855

Annex 2: Guidance documents from European and international organisations relevant to vector surveillance and control at points of entry

Organisation	**Title**	**Year Published**	**Link**
European Centre for Disease Prevention and Control (ECDC)	Guidelines for the surveillance of invasive mosquitoes in Europe[5]	2012	https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf
European Centre for Disease Prevention and Control (ECDC)	Zika virus disease epidemic: Preparedness planning guide for diseases transmitted by *Aedes aegypti* and *Aedes albopictus*[4]	2016	https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/zika-preparedness-planning-guide-aedes-mosquitoes.pdf
EU SHIPSAN ACT Joint action	European Manual for Hygiene Standards and Communicable Disease Surveillance on Passenger Ships[47]	2016	https://www.shipsan.eu/Home/EuropeanManual.aspx
International Maritime Fumigation	Code of Practice (COP) On Safety And Efficacy For Marine Fumigation[48]	2010	https://www.imfo.com/assets/files/IMFO_Code_of_Practice.pdf

Organisation (IMFO)			
World Health Organization (WHO)	International Health Regulations (2005)[2]	2008	https://www.who.int/publications/i/item/9789241580496
World Health Organization (WHO)	Guide to ship sanitation[49]	2011	https://www.who.int/publications-detail-redirect/9789241546690
World Health Organization (WHO)	Guidelines for testing the efficacy of insecticide products used in aircraft Control of Neglected Tropical Diseases[50]	2012	http://apps.who.int/iris/bitstream/handle/10665/44836/9789241503235_eng.pdf;jsessionid=67AA17354B843D9D3C7097BE437CB0F2?sequence=1
World Health Organization (WHO)	Aircraft disinsection insecticides[51]	2013	https://www.who.int/publications-detail-redirect/9789241572439
World Health Organization (WHO)	Strengthening health security by implementing the International Health Regulations (2005) Zika virus: Aircraft disinsection for mosquito control[52]	2016	
World Health Organization (WHO)	Vector Surveillance and Control at Ports, Airports, and Ground Crossings[33]	2016	https://apps.who.int/iris/rest/bitstreams/909095/retrieve
World Health Organization (WHO)	Aircraft disinsection methods and procedures[41]	2021	https://www.who.int/publications-detail-redirect/9789240014459
World Health Organization European Region	Manual on prevention of establishment and control of mosquitoes of public health importance in the WHO European Region (with special reference to invasive mosquitoes)[53]	2018	https://apps.who.int/iris/handle/10665/343056

Australian Government Department of Health	Response guide for exotic mosquito detections at Australian first points of entry[3]	2017	https://www1.health.gov.au/internet/main/publishing.nsf/Content/E4A2B36B23CBBF64CA257F6A001B058B/$File/Exotic-Mosquito-Detections-Australian-Borders.pdf
US CDC	Guidelines for *Aedes aegypti* and *Aedes albopictus* surveillance and insecticide resistance testing in the united states[54]	2016	https://www.cdc.gov/zika/pdfs/Guidelines-for-Aedes-Surveillance-and-Insecticide-Resistance-Testing.pdf
Public Health England	National contingency plan for invasive mosquitoes - Detection of incursions[55]	2020	https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/887925/National_contingency_plan_for_invasive_mosquitoes.pdf
World Health Organization (WHO)	Guide to Hygiene and Sanitation in Aviation[56]	1977	
World Health Organization (WHO)	Methods and operating procedures for aircraft disinsection Report of a WHO consultation[57]	2018	https://apps.who.int/iris/handle/10665/279702
World Health Organization (WHO)	Report of the WHO Ad-hoc Advisory Group on aircraft disinsection for controlling the international spread of vector borne diseases[58]	2016	
EUROPEAN COMMISSION. HEALTH AND FOOD SAFETY DIRECTORATE-GENERAL	Public Health Crisis management and preparedness in health. Flash report from the meetings with the transport, tourism and health[59]		

Annex 3: Bibliography related to vector surveillance and control at points of entry for maritime and air transport sectors

Relevant bibliography can be found at the EU HEALTHY GATEWAYS Bibliography Tool: https://www.healthygateways.eu/Bibliography-Tool

Annex 4: Memorandum of Understanding (MoU) among authorities sharing responsibilities for management of public health events at ports and at airports

EUHG_M6.8_Model MoU_airports_V1.doc

EUHG_M7.6_Model_ MoU_ports_V1.docx

Annex 5: Example of activities and methods for vector surveillance at an airport with emphasis on EMS

Before designing the surveillance program and installing traps, it is recommended that the team of medical entomologists prioritise sites with a higher probability for EMS to be introduced and detected. Before selecting the sites, it is strongly advised to consider the WHO "Handbook for Vector Surveillance and Control at Ports, Airports, and Ground Crossings" and specifically Table 10 in the WHO document. This preparation is required to install the traps and perform sampling at the most appropriate points/areas at the airport. Selection of the types of traps will depend on the mosquito species that the surveillance programme targets (native, EMS). Before selecting the types of traps, it is strongly advised to consider the ECDC guidelines for surveillance of invasive mosquito species in Europe, specifically Table 5 in the ECDC guidelines[5]. This Annex provides information on certain traps that have been used previously at PoE (e.g. in Greece, The Netherlands). It should be noted that the traps listed below should be considered as examples only, and not as guidelines or recommendations to be used exclusively.

Description of the environment

- GIS map delineating the 400m radius from the perimeter of the airport that will be included in the surveillance.
- Detailed information (GIS file) including the features present at the airport, such as buildings, gates, cisterns, ponds etc.

- Water system GIS file (drains, sewage, catch basins, water tanks etc.) to identify potential breeding sites.
- Previous data available for climatic conditions (temperature, rainfall, wind direction, speed).

Sites at airport with higher probability for arrival of EMS

- Arrival gates with high volumes of international flights landing from overseas.
- Platforms of gates for European flights arriving from areas where invasive mosquitoes are present.
- Indoor locations where suitcases are unloaded.
- Near locations where sealed containers (e.g. AKE, AKH) are opened for the first time at the airport (inside/outside).
- Animal hotels, quarantine facilities.
- Temporary storage locations for arriving cargo (warehouse).

Routine:

- Mapping the larval breeding sites.
- Regularly checking the mapped breeding sites.
- Recommendation of use of traps with CO_2 for a wider spectrum of mosquito species that could be detected. Lures can be more specific for certain invasive species (*Ae. albopictus, Ae. aegypti*).

Traps are continuously operated (24/7) and placed in shaded, wind-protected moist areas. Specifically, the monitoring methods used can include the following. One or more types of monitoring methods may be chosen, but at least standard oviposition traps (ovitraps) must be in operation at the airport:

- Standard oviposition traps (ovitraps) controlled weekly or fortnightly[8]. The trap consists of a black plastic container (12.5 cm high and 14 cm diameter) filled with water. A floating piece of polystyrene or a tongue depressor can serve as oviposition substrate. If placed outdoors, the top of the OT is covered with stainless steel bird netting to prevent the polystyrene from being blown away by the airplane engines or wind.
- BG-Mosquitaire (BG-M) and/or BG-Sentinel traps (BG-S) (Biogents AG, Regensburg, Germany). These traps have been specifically developed for capturing *Aedes* mosquitoes (*Ae. albopictus*, *Ae. aegypti* and related species) and use a patented mix of artificial skin emanations (BG-Sweetscent), in combination with air convection and light-and-dark contrasts.
- Mosquito Magnet (Independence or Liberty models) (MM trap) (Woodstream® Co., Lititz, USA). The MM trap produces a continuous stream of CO_2, heat and moisture into the air, while at the same time the counter flow system sucks the biting insects into a net where they die from dehydration. Octenol is added as a lure, and this trap has been evaluated successfully against a variety of mosquito genera and species for trapping and surveillance of biting culicids[60]. It has been successfully used during past nationwide monitoring of Culicidae in Belgium and The Netherlands[60].

- BG Gravid *Aedes* Trap (BG-GAT) (Biogents AG, Regensburg, Germany). This trap uses water and organic material to create attractant cues for ovipositing female mosquitoes. After being attracted into the trap, mosquitoes cannot reach the water in the trap and typically get stuck on an adhesive panel inserted into the trap.
- Larval sampling using fine mesh aquarium nets (e.g. drainage holes), and adult sampling using mouth aspirators (pooters) Larval sampling nets are emptied in a white tray/bowl, and the larvae are collected in tubes using a pipette. Ethanol (70%) is added to the tubes to kill and preserve the larvae.

Collected specimens (eggs on polystyrene, larvae and adults) are sent in isolated sealed plastic bags to the laboratory for morphological and molecular identification. All data from each sampling location is added into a database of the surveillance team (VecMap, for example). Finally, for the trapping/surveillance plan (related to the risk identified in **Table 6**), it is highly recommended to check Table 4 in ECDC guidelines for surveillance of invasive mosquito species[5].

Mosquito species identification

In the laboratory, mosquitoes will be sorted from other trapped insects, counted and morphologically identified using appropriate Culicidae morphological keys. It is recommended that each mosquito identified as exotic is double-checked by a second culicid taxonomist of each respective institution. One sample represents the collection of specimens after a visit. Samples can contain mosquito specimens (present), be empty, or only contain other arthropods only (absent). Additionally, molecular identification (PCR, qPCR, sequencing) of EMS can be also performed and it is strongly recommended.

Annex 6: Example of activities and methods for vector control with emphasis on the prevention of accumulation of stagnant water at the airport and at the port areas

Prevention of accumulation of stagnant water

In case of permanent breeding sites such as drains and gutters, preventive use of physical barriers for the aquatic cycle of mosquitoes (e.g. Aquatain) is strongly recommended. Additional activities may include the following tasks:

- Collapse holes with sand or other fillers (e.g. foam)
- Water aspiration or evaporation
- High pressure heat water/air
- Infiltration
- Container removal or well-sealed
- Flowerpots kept without open water

Larval treatment in public areas

All the catch basins and other possible permanent breeding sites present in the public area declared as infested by EMS can be considered for larval treatments. It can be considered to conduct larval treatments frequently - depending on the residual activity of the larvicide - and during the favourable season between April to October of each solar year. If present in a minimum radius of 400 m area, all streets, squares, parks and gardens as well as in municipal facilities, mosquito control activities could be carried out in all road drains (manholes and basement window cells, square and parking lot grids), even those which appear to be dry, including the ones present along median strips dividing road lanes, along the perimeters of roundabouts, cycle lanes and pedestrian areas. Manholes which appear to be closed but with possible access for rainy water shall be treated as well. It is advised that the operational units in charge of treatments draft a daily report on the activities that have been carried out. Furthermore, it is advised that all circumstances hindering normal progress of work be notified.

Adults' treatments

If necessary, adult control must be performed in public roads, green areas, parks and all areas accessible by atomizer mounted on vehicles.

Adulticide biocide formulations against mosquitoes should be based on active ingredients registered in the country (to be used following the doses shown on the label for specific use against mosquitoes).

Operational units equipped with Ultra Low Volume (ULV) or Low Volume (LV) nebulizer mounted on a motor vehicle are advised to be used in the treatment of large areas (e.g. urban streets, public parks), and/or motor-driven shoulder nebulizers for treatment on foot of limited areas and/or areas that cannot possibly be reached by the spraying device installed on the motor vehicle. Nebulizers mounted on a motor vehicle or motor-driven shoulder nebulizers must produce cold aerosol, with a particle diameter of ≤ 50 microns.

All operational units must be equipped with GPS instruments, to carry out adequate control of sites and treatment timing. To achieve maximum efficacy, it is advised that adult control operations are conducted during the night or early morning.

Local and regional authorities' involvement

- Stimulate the organization of a coordination team (e.g. crisis unit) including all entities such as regional and local public health authorities, epidemiology services, municipalities, port and airport authorities, and expert entomologists.
- The coordination team (e.g. crisis unit) must define responsibilities, money allocation, coordination of activities, and adapt the strategy to the observed evidence.

Quality control methods

To achieve high efficacy in the elimination campaign, it is advised that operators' work is checked for quality by independent technicians. It is important that quality control is conducted by skilled technicians, following a standardized protocol including control of treatment of public catch basins and on private properties. The role of quality control is fundamental in the prompt identification of possible constraints, which may affect the final success of the elimination campaign. Additionally:

- The coordination team (e.g. crisis unit) must commission an independent body, to be assigned the task of quality control activities checking the efficacy of vector control
- It is advised that 10% of the total road drains in the treated area be sampled for larvae/pupae following each treatment.
- Sampling will be conducted with a water net, with the number of larvae/pupae recorded and kept for species determination.
- Regular reporting on the treated roads by the designated staff (e.g. pest control operator).
- Weekly reporting on the findings to the coordination team (e.g. crisis unit).

Annex 7: Case study – Recommended response to the hypothetical scenario of *Aedes aegypti* introduction in a Southern European airport

This annex presents recommended actions to be taken in response to a hypothetical scenario of *Aedes aegypti* introduction in an airport located in Southern Europe.

Once detection of *Ae. aegypti* has been confirmed, the competent authority immediately informs the crisis unit and involves specialists to strengthen mosquito surveillance, extend the area under surveillance, and immediately apply appropriate control measures aimed at species elimination.

Surveillance is conducted using traps (Ovitraps, BG-GAT and BG-Sentinel are recommended) labelled by an identification code, georeferenced by portable GPS and positioned in favourable stations (indoor and outdoor). This is done by highly skilled technicians, following SOPs (see Annex 1 and 2 in the scientific publication titled "Practical management plan for invasive mosquito species in Europe: I. Asian tiger mosquito (*Aedes albopictus*)")[8].

At a first stage, a minimum of 20 traps are positioned in an area of approximately 50 hectares. Traps are checked at least weekly (or more intensively in case of available resources), and the area under surveillance is progressively enlarged in case of positive findings. To assure homogeneous covering, the planning of ovitraps' distribution will be supported by Geographic Information Systems (GIS) software.

A series of maps of different scales of the area will be prepared (including the site of first detection), including the position of the traps at a progressive distance from the detection site, considering the more favourable ecological condition. In the meantime, an accurate investigation on possible indoor and outdoor breeding sites should be conducted. In case of larval detection, samples must be checked and identified by a specialist.

Breeding sites with EMS findings are mapped and will be treated with larvicide/pupicide product (see paragraph 6.3). Any objects that could potentially hold water will be removed from the airport area. Water containers such as tanks or underpots in the garden will be positioned upside down.

In case the entomological surveillance reveals that *Ae. aegypti* is already established in the residential area nearby the airport, a door-to-door campaign must be started immediately, by inspecting all properties and reporting to the local authority in case of refusal to collaborate.

Together with local authorities, the crisis unit will analyse the possible pattern of introduction and informed other entities of the risk. In case the entomological surveillance identified *Ae. aegypti* eggs, the number of ovitraps must be increased accordingly, by adding new ovitraps. Newly added ovitraps will be positioned at a distance range of 100-200 or 300-400 meters from the nearby positive ovitraps.

The positive ovitraps are kept fixed for the entire monitoring season, while the negative ovitraps are conveniently re-positioned in possibly more favourable stations. Due to the fact that other *Aedes* species can be present in the area and may lay eggs in ovitraps, species identification is necessary. This can be conducted by egg hatching and larval rearing, or by other methods such as MALDI-TOF, egg scanning or PCR.

The operations will be continued until no further detections of any eggs, larvae or adults are obtained for a whole season.

Annex 8: Case study – A case of dengue fever among seaport staff

This annex describes the response measures to **a hypothetical scenario**, where during August one imported case of dengue fever was identified among staff working at a seaport in Southern Europe, where *Ae. albopictus* mosquitoes have been established. The response measures are based on the "Standard Operating Procedures for emergence vector control operations in case of Dengue, Chikungunya and Zika cases detection" which were produced in the framework of the "LIFE – CONOPS PROJECT".

Emergence control measures in response to an imported case of dengue fever in areas with *Aedes albopictus* are summarized as follows:

1. Determination of area to be treated

1.1 Single case detection

- The area to be disinfested corresponds to a buffer with a radius of at least 100 meters around the place/s where the case has recently spent most of his/her daytime.

1.2 Cluster cases detection

- When a cluster of two or more cases is identified by the responsible authorities, the area to be submitted to mosquito control is advised to be extended to a 300 metres buffer from the peripheral cases of the outbreak, as well as inside the entire outbreak area.

2. Mosquito control activities

2.1 One turn of adult and larval treatment and source removal in private areas (door-to-door)

- By a team of two operators, one equipped with a portable atomizer producing aerosol droplets (size 50-100 μm) or hot fogging atomizer producing more effective droplets (size 5-30 μm) using authorised adulticides; the other equipped with a portable manual pump performing larviciding and source removal.

2.2 Up to three adulticide treatments in public areas in consecutive days

- By truck mounted atomizer 30-60 HP, producing cold aerosol with droplets (size 50-80 μm), using authorised adulticides, during early morning hours.

2.3 One larvicide treatment in public areas

- By portable manual pump using larvicide with immediate acting mechanism (i.e. surface layer).

3. Biocidal products

3.1 Adulticide and larvicide

- It is essential to use only biocides with marketing authorization in the country, in accordance with the instructions of use that provides efficacy already demonstrated and proven in local conditions.

4. Precautions

4.1 Adulticide: Before starting the adulticide treatments, the resident should be advised in order to take adequate precautions (e.g. dissemination of flyers or letters in the target area describing the details on timing). It is advised that the treatments in public and private areas be applied in the foliage (bushes, fences) up to approximately 3-4 metres height. In addition, for the treatments which are applied to the roads, it advised to ensure that the biocide is spread consistently on both sides. Special attention should be given in cases of adulticide treatment in the vicinity of apiaries and surface waters. In accordance with certain national legislations, note that the presence of the synergist piperonyl butoxide in pyrethrin-based formulations is prohibited on organic crops, and a 50 m no-treatment zone (reduced to 25 m for foot treatment) must be respected. It is advised that operators be protected to avoid being bitten by mosquitoes.

Mosquito control execution: mosquito control activities include three actions to be conducted in a synergistic way: treatments with adulticide(s), with larvicide(s) and larval breeding sites removal.

The optimal sequence in which these processes are advised to be conducted is:

- adult treatments in public areas during the early morning hours;
- adult and larval treatments and source removal in private areas (door-to-door);
- contextual larval treatment in public catch basins.

Products: pyrethroids are particularly suitable for adulticide interventions. For larvicide interventions, diflubenzuron, *Bacillus thuringiensis* var. *israelensis* and liquid surface films (e.g. Aquatain™) may be chosen.

Equipment: depending on the accessibility of areas to be treated, atomizer for vehicle-mounted spraying (when inaccessible) or handheld sprayer (when accessible) can be used.

Places to treat: treatment must be directed to the vegetation (hedges, bushes, shrubs) on public and private areas, up to 3-4 metres height. In the case of treatments performed on roads, it is advised that the treatment on both the left and right side, possibly with the double pass is carefully conducted.

Repetitions: depending on authority's capacity, two different scenarios could be implemented: a) adulticide treatments on public roads are advised to be repeated every ten days. The minimum number of repetitions is two (2), and the maximum depends on vector presence/density (surveillance data), as well as on the label and any regulatory constraints; b) adulticide treatments on public roads are advised to be repeated for three (3) consecutive early mornings. In any scenario, the adulticide management must be conducted again in case of heavy rain after treatment.

Warnings: the treatment must be performed in the absence of people and animals. During a storm or breeze with the wind of more than 2-3 m/sec, treatments are advised to be suspended until suitable weather conditions occur. Fog or rain of low intensity do not compromise the outcome of the intervention.

The treatments are conducted guaranteeing the absence of people, therefore, on public areas preferably in the early morning hours. Inhabitants of target areas are advised to be forewarned on the day and time of the intervention; this allows inhabitants to take measures to reduce exposure to insecticides, and facilitate the entrance of operators onto their properties.

In case a municipality does not have any operational service in place, it is advised legislation for hiring pest control operators under emergency be included in the bill of the administrative unit/region/country as soon as possible, before the need for vector control is evident, and preferably, before an outbreak.

In case the situation regarding the local density of *Ae. albopictus* is unknown, mosquito surveillance using BG traps baited with attractant and CO_2 can be conducted immediately. Results will inform the need for activating mosquito control treatments. It is advised that mosquito samples be submitted to analysis for arboviruses.

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