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TECHNICAL REPORT

Guidelines for the surveillance of native mosquitoes in Europe

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Abbreviations

AMCA	American Mosquito Control Association
CDC	US Centers for Disease Control and Prevention
EDEN	FP6 research programme 'Emerging diseases in a changing European environment'
EDENext	FP7 research programme 'Biology and control of vector-borne infections in Europe'
EEA	European Economic Area
EFSA	European Food Safety Authority
EMCA	European Mosquito Control Association
ENIVD	European Network for Diagnostics of 'Imported' Viral Diseases
IHR	International Health Regulations
IMS	Invasive mosquito species
IPCC	International panel on climate change
KOM	Kick-off meeting
MBD	Mosquito-borne disease
MIR	Minimum infection rate
MoH	Ministry of health
NMS	Native mosquito species
NUTS	Nomenclature of Units for Territorial Statistics
PH	Public health
PoE	Point of entry
RA	Risk assessment
SR	Systematic (literature) review
SOVE	Society for Vector Ecology
VBD	Vector-borne disease
VBORNET	European Network for Arthropod Vector Surveillance for Human Public Health
VECTORNET	European network for sharing data on the geographical distribution of arthropod vectors, transmitting human and animal disease agents (successor to VBORNET)
WHO	World Health Organization

Glossary

An exotic plant or animal species (syn: alien, foreign, non-indigenous, non-native)
is a species or subspecies that is not native to an ecosystem, and if present, has been introduced.

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 harm, or harm to human health.

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).

Monitoring
consists of procedures implemented for temporary or continuous observation (e.g. of species dynamics) and is not followed by any additional activities.

Surveillance
consists of procedures developed in response to a risk and carried out to support subsequent actions.

Active surveillance
consists of procedures specifically developed and applied to collect the targeted species.

Passive surveillance
refers to the gathering of data through monitoring programmes or reports from different sources.

Targeted surveillance
is based on sampling methods carefully selected and applied to a specific aim and context.

The status of a mosquito species
is a rough estimate of its presence in a defined area (e.g. rare/patchy/ubiquitous).

A species complex
is a group of related species where the exact demarcation between species is often unclear or cryptic; such a group can be denominated with the representative species name with the addition 'sensu lato' (s.l.).

Executive summary

During the latter part of the 20th century, the role of mosquitoes as vectors of diseases of public health concern was generally considered to be limited to the tropics. The disappearance and eradication of dengue and malaria in Europe by the 1950s saw a European landscape with minimal or no mosquito-borne disease. However, at the start of the 21st century, the picture is different. A world increasingly connected through travel, trade and tourism means that Europe sees regular transmission of mosquito-borne disease, a trend confirmed by advances in pathogen detection. West Nile virus is now known to be transmitted to humans by native mosquitoes in a number of European and neighbouring countries each year (Figure 1). In recent years Europe has witnessed the return of malaria, the emergence and persistence of Usutu virus, as well as the ongoing transmission of Sindbis virus, all transmitted by native European mosquitoes. On the borders of Europe, Rift Valley fever virus is expanding its geographical range, and several of the potential vectors already occur in Europe. This is not to mention, of course, the risks posed by the introduction and establishment of invasive mosquitoes (many of which could arguably be termed part of our native fauna) and the associated outbreaks of dengue and chikungunya viruses. The changes in mosquito-borne disease epidemiology in Europe over recent years cannot be ignored, and risks to public health cannot be understated.

This changing landscape of pathogen transmission comes at a time of environmental change, with large-scale initiatives for landscape change which include the creation of wetlands, landscape-scale flood mitigation initiatives, enhanced sustainable urban drainage, and 'green- and blue-space infrastructure'. Coupled to this are the predicted climatic changes with an increased incidence of extreme weather (i.e. flooding and drought). All these factors are likely to influence not only the aquatic habitats for mosquitoes, but also the rate of mosquito development and human exposure.

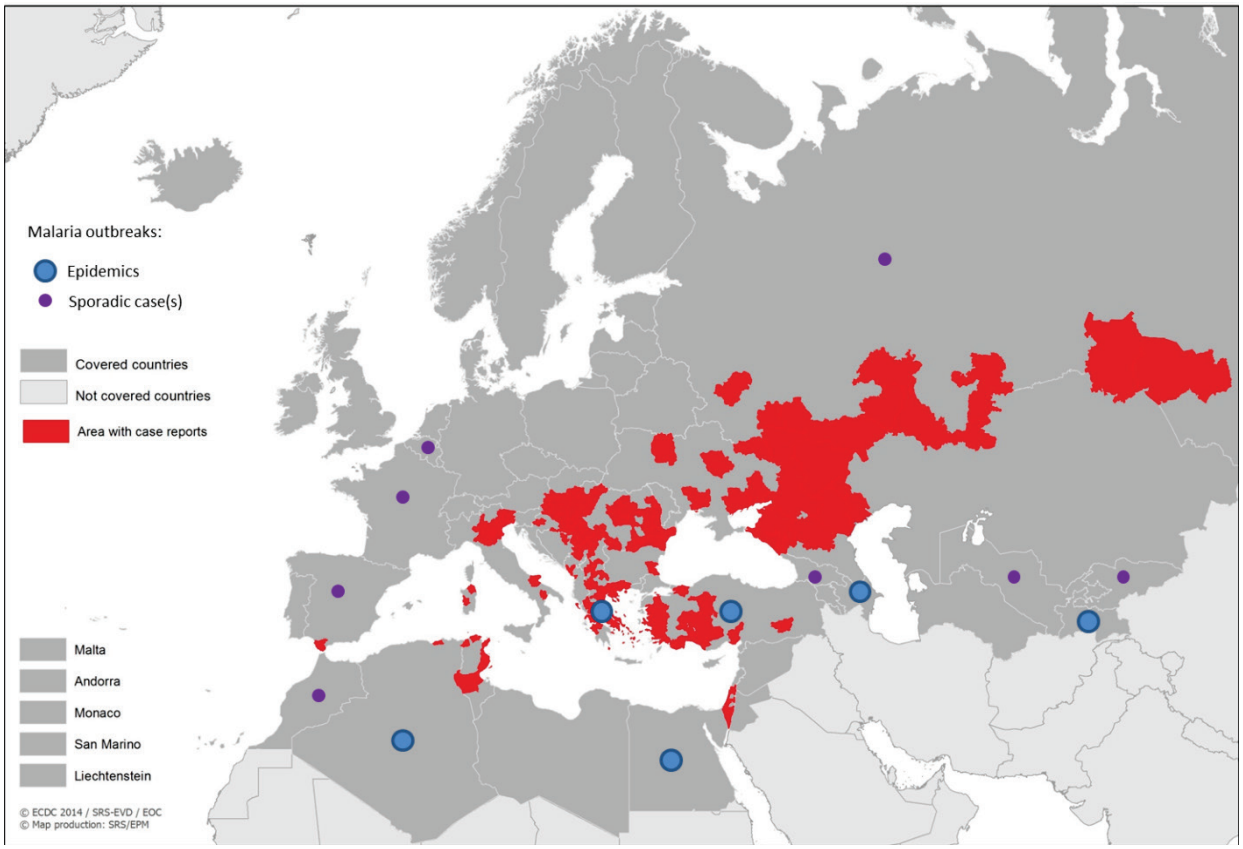
Vector-borne diseases, including those transmitted by native mosquitoes, should therefore be a high priority for public and veterinary health authorities across all of Europe. In some countries, mosquito surveillance is limited, and where surveillance does occur, strategies vary from one country to another. There is a definite need to collate existing knowledge on the surveillance of native mosquitoes in Europe and provide guidelines, not only for entomologists but also for public and veterinary health authorities in order to ensure that appropriate surveillance is conducted, making the best use of scarce economic resources.

ECDC's Programme on Emerging and Vector-Borne Diseases develops timely and topical assessments of the risks posed by vector-borne diseases to European citizens and aims to provide tools and accurate information to support public health decision-making. In line with these objectives, ECDC identified the need for guidance on customised surveillance methods that encourage European countries to collect data on mosquito vector species. As a first step, ECDC produced a guideline document for surveying invasive mosquito species (2012), followed by this document on native mosquito species. Both guidelines follow the same format and ensure a comprehensive coverage of surveillance activities within Europe, and help to provide comparable and timely information about both native and invasive mosquito vectors. This guideline document on the surveillance of native mosquitoes offers technical guidance on how to conduct surveillance during the pre-disease and disease stages (outbreak) and supplies all information needed to produce risk assessments on mosquito-borne diseases.

European countries are required to conduct rapid risk assessments on emerging infections, including those transmitted by vectors, on an iterative basis. The ability to conduct these rapid risk assessments is contingent on available data, in this case data on vector diversity, biology, ecology and phenology. As such data are either difficult to gather or not up-to-date, these guidelines demonstrate how surveillance strategies can be implemented to generate data for rapid risk assessments and how these strategies can be scaled up when countries move from the pre-disease to the disease stage. Depending upon the mosquito-borne disease of concern, different native mosquito species will have to be surveyed. These guidelines provide details on the role of each species as potential vectors, best practice sampling techniques for surveying mosquitoes in all life stages, as well as quantitative entomological indices for measuring mosquito density (and environmental parameters) and the impact of control measures. The costs of surveillance and appropriate evaluation strategies are detailed. In addition, this document offers guidance on mosquito identification, pathogen screening, and data management.

These guidelines are aimed at public health decision- and policymakers, stakeholders in public health, professionals involved in implementing mosquito surveillance, and mosquito control experts. The presented methods may be applicable to the whole of the geographical region of Europe including EU Outermost Regions, but not Associated Overseas Countries and Territories.

Figure 1. Reported autochthonous cases of malaria and West Nile fever in Europe and neighbouring countries, 2010–2013



Source: ECDC (West Nile fever) and WHO (malaria). Mapping units used for West Nile fever are NUTS 3.

Introduction

Mosquitoes and mosquito-borne diseases in Europe: a changing picture

While the number of mosquito-borne diseases (MBD) and their outbreak risk in Europe is currently quite low, there is an increasing trend in their global incidence and geographical distribution. Anthropogenic environmental changes, together with globalisation, create suitable conditions for the (re-)emergence of MBDs across Europe. It is estimated that over the last two decades nearly a third of all recorded events related to emerging infectious diseases were vector-borne. Among these, mosquito transmitted pathogens are a prime concern. Moreover, increasingly favourable conditions in Europe will promote the spread of mosquitoes into new areas where ecological factors may facilitate the transmission of some of these pathogens. The recent autochthonous European cases of dengue fever and chikungunya virus infection and outbreaks of malaria, and the now frequent and consistent case reports of West Nile fever in Europe and neighbouring countries (Figure 1) provide firm evidence of the vulnerability of the region to the transmission of these pathogens in areas where mosquito vector species are present and active.

Why survey native mosquitoes?

A key factor in the increased susceptibility of Europe to MBD is globalisation. Economic and demographic factors, coupled with the speed and pervasion of modern transport networks, global trade, transport and tourism, are pivotal in the spread of vectors and the pathogens they transmit. Invasive and native mosquitoes are putative vectors of pathogens with human and veterinary relevance and their surveillance contributes to interdisciplinary collaboration and communication in healthcare for humans and animals within the 'One Health' concept (see: http://www.onehealthinitiative.com).

The presence of a competent vector does not necessarily result in the transmission of vector-borne pathogens: it is merely the basic requirement for transmission to occur. Therefore the knowledge on the presence and distribution of mosquitoes needs to be regularly updated by implementing systematic and harmonised surveillance procedures across Europe.

In 2012, ECDC released guidelines for the surveillance of invasive mosquitoes in Europe and subsequently launched a second project on native mosquitoes. A systematic literature review (SR) confirmed that some countries released guidelines on specific mosquitoes and mosquito-borne pathogens, but that no transnational guidelines existed on native mosquitoes in Europe (see Annex 1A). Moreover, there is no uniform active surveillance of mosquito vectors in Europe, and surveillance is often only regional and sporadic. European countries with continuous active surveillance and control programmes include some Mediterranean countries, e.g. France and Italy.

European-scale surveillance programmes for mosquito vector species are needed and highly recommended. The ECDC/EFSA-initiated VECTORNET network (the successor of the VBORNET project) combines information on European invasive and native mosquitoes provided by various sources (e.g. medical entomologists and public health experts dealing with vector-borne diseases) with data collected for different objectives, using a range of different methods (http://ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/vector-maps.aspx).

It is crucial to share information on mosquito distribution and biology, as well as the results of control efforts. Efficient risk assessment (RA) and risk management depends on high-quality data on the presence and abundance of mosquito vector species. For that reason, surveillance programmes should incorporate data collection routines, with the key objective of providing adequate information for the production of RAs which can then inform decision-makers and guide interventions. The capacity of European countries in this area should be rapidly and consistently expanded in order to 1) inform timely RAs on pathogen transmission, and 2) support vector control measures.

Table 1. Important mosquito-borne pathogens that cause disease in humans

Pathogens or diseases	Transmission in Europe	Important vectors to human
Arboviruses		
Chikungunya fever virus	Italy 2007; France 2010	*Ae. aegypti*, *Ae. albopictus*
Dengue virus (DENV 1–4)	Until early 20th century; Croatia and France 2010, Madeira 2012, France 2013	*Ae. aegypti*, *Ae. albopictus*
Eastern equine encephalitis virus, La Crosse encephalitis virus, Rift Valley fever virus	No disease transmission to date	*Aedes* species, *Culex* species, *Culiseta melanoura*
Sindbis virus	Endemic in northern Europe	*Aedes cinereus*, *Cx. torrentium*
Japanese encephalitis virus, Murray Valley encephalitis virus, St. Louis encephalitis virus, Ross River fever virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus	No disease transmission to date	*Culex* species
West Nile fever virus	Endemic in southern/central Europe	*Cx.* species, *Cx. pipiens*, *Cx. modestus*
Yellow fever virus	Until 19th century, in ports and occasionally inland	*Ae. aegypti*, *Ae. africanus*, *Haemagogus* species
Filarial worms		
Wuchereria bancrofti	Not to date	*Aedes* species, *Anopheles* species, *Culex* species
Dirofilaria spp.	Endemic in southern Europe, spreading	*Ae. albopictus*, *Ae. caspius*, *Cx. pipiens*
***Plasmodium* protozoa**		
Malaria	Widely endemic until mid-20th century; resurging epidemics in the 1990s in far eastern countries; remains endemic in Azerbaijan and Turkey, while sporadic cases occur elsewhere; resurging epidemics in Greece 2011–2013	*Anopheles* species

Note: More information on transmission of MBD pathogens and the biology of native mosquitoes is given in Annex 2.

Guidelines on harmonised surveillance and the monitoring of native mosquitoes are likely to:

- improve the integration of monitoring measures for indigenous vector mosquito species and MBD;
- accelerate the establishment of sustainable surveillance and monitoring programmes in risk areas;
- support the development of a unified database on native mosquito species, which will help to detect faunal changes and provide a baseline for adaptation and mitigation measures;
- increase and update knowledge on the presence, spatial distribution, and abundance of native (vector) species;
- promote partnerships and collaborations between human and animal health government bodies as well as research, training and development units;
- improve the knowledge of native mosquito species and MBDs and identify gaps in monitoring techniques – as further research topics;
- accelerate the development of enhanced control programmes and adapt sustainable and efficient control tools; and
- support the collection of European-wide longitudinal datasets to better assess the impact of environment and climate change.

Figure 2. Native mosquito species

Anopheles maculipennis *s.l.; left: larvae; right: female*
Photos: F. Schaffner

Left: Aedes geniculatus*, female; right:* Culex pipiens*, male*
Photos: F. Schaffner (left), G. Hendrickx (right)

Rationale and scope

Vector-borne diseases are a (re-)emerging threat to Europe, and the collection of information and data on vectors of public health significance is of crucial importance to the understanding of the levels of risk that affected countries face. In addition, these data define the actions that need to be taken to control these threats to public health.

These guidelines aim to support the implementation of tailored surveillance for native mosquito species (NMS) of public health relevance. They provide evidence-based guidance and technical support for focused field data collection, proposing adaptations dictated by the epidemiological situation and taking into account estimated costs. They may also contribute to harmonising surveillance methods and information records at the European level so that data from different countries/areas can be compared over time. They intend to provide support to decision/policymakers, stakeholders in public health and professionals involved in implementing NMS surveillance or control, as well as to non-specialists of mosquito surveillance.

Pathogens considered of public health importance and a risk for Europe are those causing severe disease in humans and circulating in Europe or showing high risk of introduction into Europe owing to the occurrence of large outbreaks and/or evidence of spread in other regions of the World. Thus these guidelines will address the risk for Eastern equine encephalitis, Japanese encephalitis, Rift Valley fever, St. Louis encephalitis, Sindbis fever, Usutu fever, Venezuelan equine encephalitis, West Nile fever, human intraocular filariasis (caused by *Dirofilaria* spp. nematodes), and human malaria (*Plasmodium* spp.) (see also Table A2-A, Annex 2). Other arboviruses such as Batai, Inkoo, Lednice, and Tahyna have also been shown to circulate in Europe, but these pathogens are not considered a priority for surveillance.

The targeted mosquito species are:

- species with significant vector potential (= putative vectors) for pathogens of public health importance according to the list given above (Figure 3 and Table 2): *Ae. caspius, Ae. cinereus* s.l. (*Ae. cinereus* and *Ae. geminus*), *Ae. communis, Ae. dorsalis, Ae. excrucians* s.l., *Ae. vexans, Anopheles atroparvus, An. claviger, An. cinereus, An. hyrcanus, An. labranchiae, An. maculipennis* s.s., *An. melanoon, An. messeae, An. multicolor, An. plumbeus, An. sacharovi, An. sergentii sergentii, An. superpictus, Coquillettidia richiardii, Culex modestus, Cx. perexiguus, Cx. pipiens* s.l., *Cx. theileri, Cx. torrentium, Cx. tritaeniorhynchus, Culiseta morsitans.*

Main pest species are also considered (Table 2), as they can generate severe nuisance:

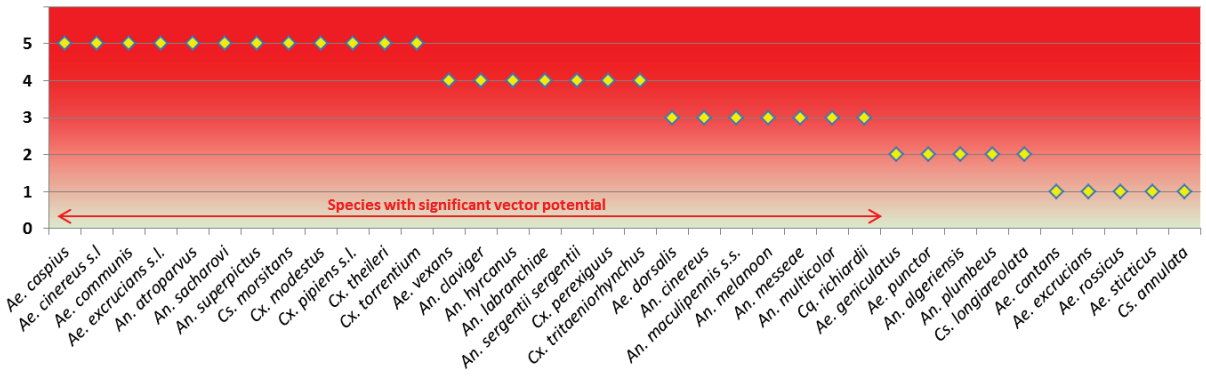
- *An. plumbeus*, *Ae. caspius*, *Ae. cinereus/geminus*, *Ae. detritus/coluzzii*, *Ae. sticticus*, *Ae. vexans*, *Cq. richiardii*, *Cx. pipiens* s.l.

In addition, invasive mosquitoes may also play a role in the transmission of the selected pathogen, but their surveillance has been addressed in a 2012 guideline document[1].

More information on these pathogens and their transmission is given in Annex 2.

[1] European Centre for Disease Prevention and Control. Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm: ECDC; 2012. Available from: http://www.ecdc.europa.eu/en/publications/publications/ter-mosquito-surveillance-guidelines.pdf

Figure 3. Potential vector role of European mosquito species for eight arboviruses (Eastern equine encephalitis virus, Japanese encephalitis virus, Rift Valley fever virus, St. Louis encephalitis virus, Sindbis virus, Usutu virus, Venezuelan equine encephalitis virus, and West Nile virus) and *Dirofilaria* spp. and *Plasmodium* spp.



5 = Species known as past/present vector in Europe; 4 = Species known as vector outside Europe; 3 = Species infected in nature and competent, for the same pathogen or for different pathogens, or, for malaria, secondary vector only; 2 = Species competent in the laboratory only (at low, moderate or high level); 1 = Species infected in nature only; 0 = Species not implicated in any pathogen transmission or absence of information on their possible role (applies to approximately 60 additional species; not shown but listed in Annex 2). Invasive mosquitoes could play a role but are not shown on the chart.

Table 2. Overview of native mosquito species showing significant vector potential for pathogens of public health importance and/or causing severe nuisance

Mosquito species	Eastern equine encephalitis virus	Japanese encephalitis virus	Rift Valley fever virus	Saint Louis encephalitis virus	Sindbis virus	Usutu virus	Venezuelan equine encephalitis	West Nile virus	*Dirofilaria* species	*Plasmodium* species (human malaria)	Can generate severe nuisance
Aedes (Aedes) cinereus/geminus [1]	1				**5**			1			X
Aedes (Aedimorphus) vexans	1		**4**		0			**3**	**3**		X
Aedes (Ochlerotatus) caspius			**4**[2]					1	**5**		X
Aedes (Och.) communis					**5**						
Aedes (Och.) detritus/coluzzii [1]						1					X
Aedes (Och.) dorsalis								**3**			
Aedes (Och.) excrucians s.l.					**5**			1			
Aedes (Och.) sticticus								1			X
Anopheles (Anopheles) atroparvus										**5**	
Anopheles (Ano.) claviger									1	**4**	
Anopheles (Ano.) hyrcanus					**4**				**3**	2	
Anopheles (Ano.) labranchiae										**4**	
Anopheles (Ano.) maculipennis s.s.								1[3]	**3**	**3**	
Anopheles (Ano.) melanoon										**3**	
Anopheles (Ano.) messeae										**3**	
Anopheles (Ano.) plumbeus								2		2	X
Anopheles (Ano.) sacharovi										**5**	
Anopheles (Cellia) cinereus										**3**	
Anopheles (Cel.) sergentii sergentii										**4**	

Mosquito species	Eastern equine encephalitis virus	Japanese encephalitis virus	Rift Valley fever virus	Saint Louis encephalitis virus	Sindbis virus	Usutu virus	Venezuelan equine encephalitis	West Nile virus	*Dirofilaria* species	*Plasmodium* species (human malaria)	Can generate severe nuisance
Anopheles (Cel.) multicolor										**3**	
Anopheles (Cel.) superpictus									1	**5**	
Coquillettidia (Coquillettidia) richiardii								1	**3**		X
Culex (Barraudius) modestus								**5**	**5**		
Culex (Culex) perexiguus			**4**		**3**	1		1			
Culex (Cux.) pipiens s.l.			**4**		**5**	1		**5**	**5**		X
Culex (Cux.) theileri					**3**			1	**5**		
Culex (Cux.) torrentium					**5**						
Culex (Cux.) tritaeniorhynchus		**3**	**4**		1			**3**			
Culiseta (Culicella) morsitans					**5**			1	**3**		

Empty cell = Absence of information for the species
Arboviruses:
0 = Refractory to infection in the laboratory, 1 = Species infected in nature only, 2 = Species competent in the laboratory only (at low, moderate or high level), 3 = Species infected in nature and competent, 4 = Species known as vectors in regions and countries outside Europe only; 5 = Species known as vectors in Europe; human malaria: 2 = Species competent in the laboratory that could be an occasional vector, 3 = Secondary vector only, 4 = Primary vector outside Europe only, 5 = Primary vector in part of Europe

[1] *Adult females of these sibling species cannot be sorted;* [2] *Reported for* Ae. vexans arabiensis*;* [3] *Reported for* An. maculipennis *s.l.*

Note: Numbers in bold indicate significant vector potential.

The surveillance strategies and methods described in these guidelines can be applied to other pathogens of public health or veterinary importance and their native mosquito vectors species, provided that they are carefully customised and adapted to the particular context. The proposed methods are applicable to the EU/EEA, including EU Outermost Regions but not associate Overseas Countries and Territories.

The information and recommendations presented in this document are based on systematic reviews of methods for (i) the collection and (ii) identification of NMS (see Annex 1B and 1C, respectively) and have been supplemented by materials from the authors and input from two major European networks (VBORNET and EMCA). This document has been reviewed by entomologists, public health experts and readers from several European countries. Updates are planned every three years or whenever a major change in vector fauna or MBD risk occurs.

These surveillance guidelines were produced to provide European countries with a comprehensive and practical document on mosquito surveillance and data collection. They were primarily designed to be used to develop MBD risk assessments, surveillance plans, and response measures to putative mosquito vectors.

This document specifically mentions the 'surveillance' of mosquitoes in its title – as opposed to 'mosquito monitoring' – as this implies a set of procedures developed in response to a recognised risk and carried out to support subsequent actions (see Glossary).

The publication of this document completes a toolset on efficient risk control and assessment with regard to mosquito vector species (Box 1 and Figure 4). Surveillance of mosquito vectors contributes significantly to the assessment of risk from MBD and the management of MBD (Box 2 and Figure 5).

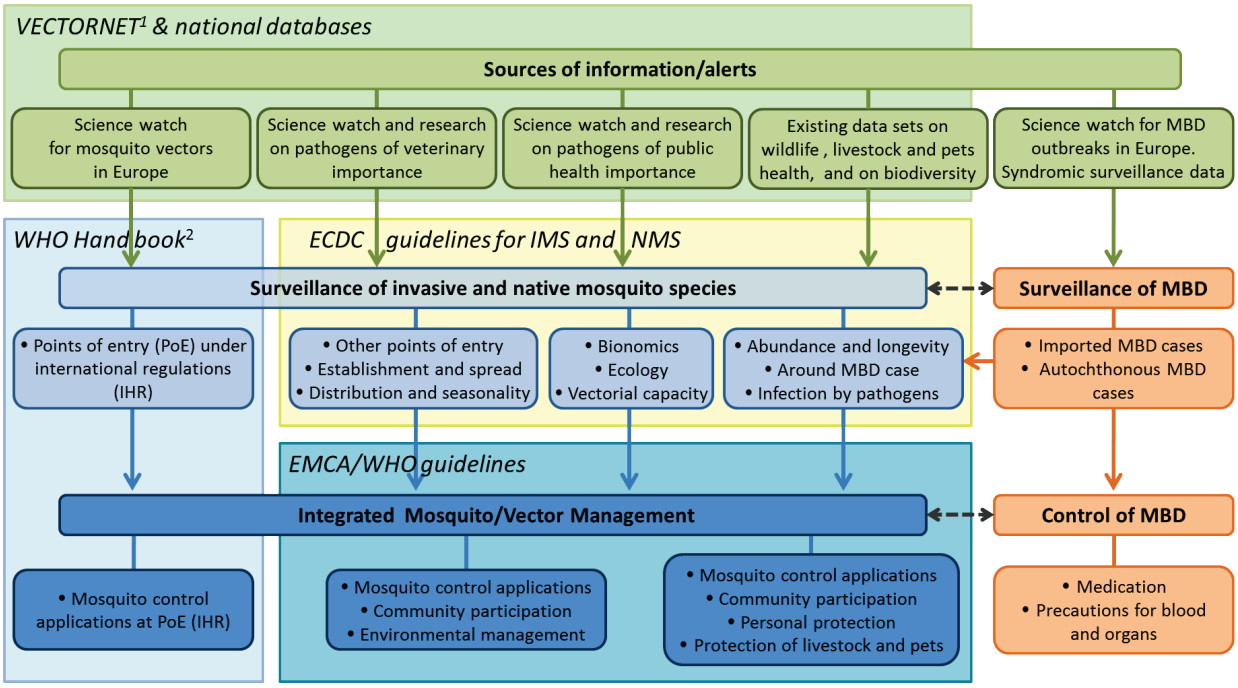
The surveillance procedures presented in this document are intended to provide information required to implement response activities and improve preparedness activities aimed at emerging MBDs. Ideally, these procedures will eventually lead to a comprehensive, harmonised European surveillance coverage with reliable, comparable and timely information about native mosquitoes of public health importance. This publication and the entire toolset is part of a more general effort by ECDC towards the prevention and control of emerging and vector-borne diseases.

Box 1: Global framework for managing the risk of mosquito-borne diseases of public health importance in Europe; toolset for assessing and controlling the risk posed by native mosquitoes

These guidelines, together with earlier guidelines, cover what appears as a yellow rectangle in Figure 4. The left part of the figure has already been addressed by the WHO guidelines (light blue rectangle), whereas the lower section is addressed by the EMCA/WHO initiative on 'Guidelines for the control of invasive mosquitoes and associated vector-borne diseases on the European continent' (dark blue rectangle).

Blue rounded rectangles show procedures for the surveillance (light blue) and control (dark blue) of mosquito vectors. Orange rounded rectangles show procedures which are addressed by risk plans other than mosquito surveillance and control. Green rounded rectangles show sources of information and risks alerts for vectors and MBD pathogens (upper light green rectangle); information at the international level is gathered by VECTORNET, and dedicated databases may exist at the national (or sub-national) level.

Figure 4. Flowchart of the procedures and main issues of mosquito vectors and MBD surveillance



[1] Successor to VBORNET; [2] Addresses only invasive vector species; [3] Other national/regional data bases may exist

WHO guidelines (www.who.int/ihr/en)

In the context of the application of the International Health Regulations (IHR 2005), WHO aims to strengthen national capacities by developing and updating guidelines and tools on vector surveillance and control. Thus, a Web-based global point of entry (PoE) vector identification platform is under development, as well as a 'Handbook on vector surveillance and control at points of entry'. This handbook focuses on actions that can be performed at PoE and on conveyances, containers, cargo, postal parcels and baggage. It considers all vector species (including mosquitoes) relevant to major vector-borne diseases.

EMCA/WHO guidelines (www.emca-online.eu)

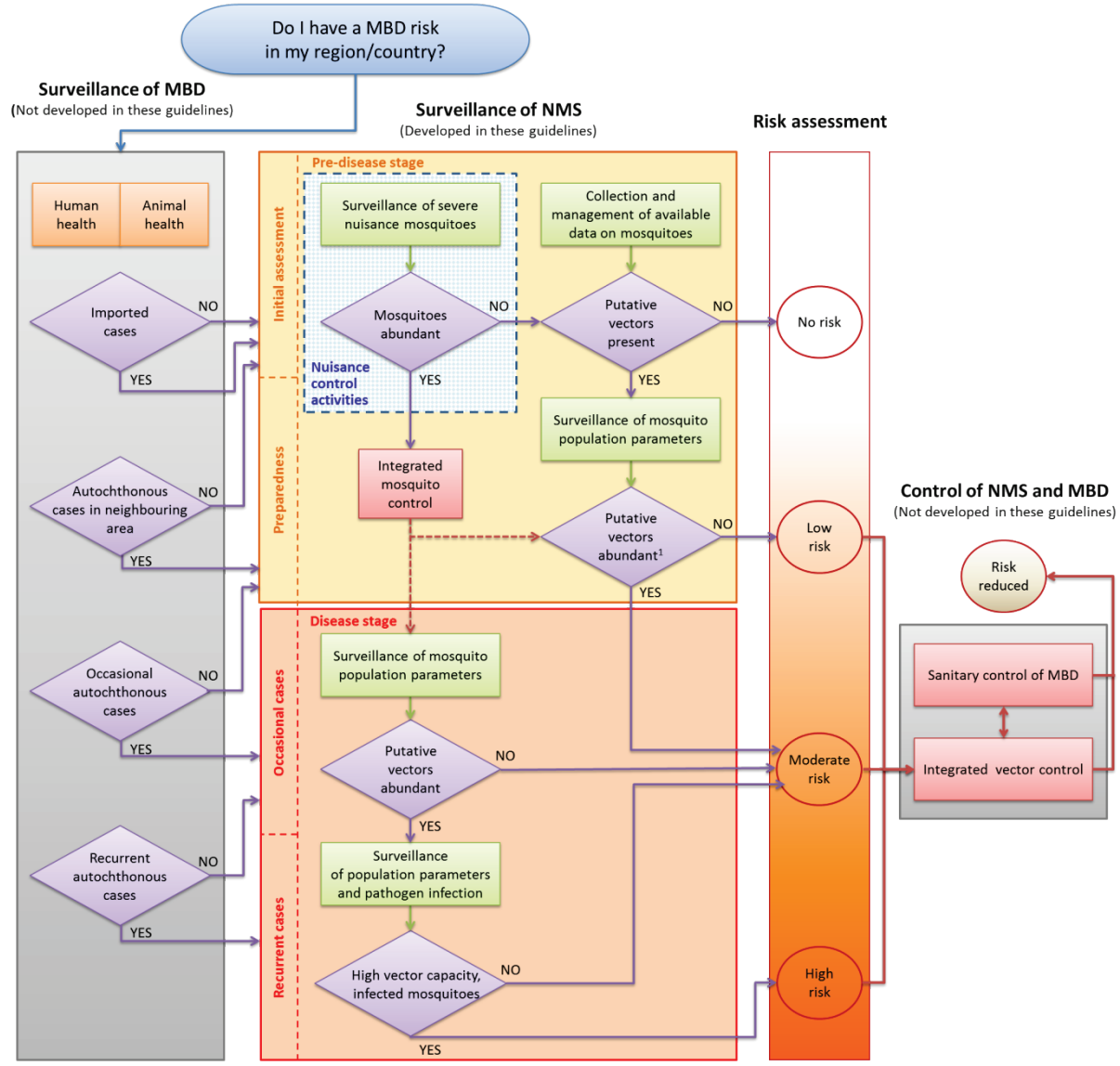
EMCA and WHO have recently launched an initiative to develop 'Guidelines for the control of invasive mosquitoes and associated vector-borne diseases on the European continent', based on pan-European consultations. The first deliverable is a strategic document with special emphasis on control issues.

Box 2: Surveillance of native mosquito species, in relation to MBD risk assessment and management

Mosquito surveillance is part of the global response to MBD. Mosquito risk assessment and management of threats to human or animal health (which includes activities such as disease surveillance for humans and other vertebrate hosts) and the surveillance and control of the vector are crucial elements of this response strategy. In the epidemiology of an MBD, the vector may be the critical link so that removing the vector prevents new cases.

The large outlined orange and red rectangles (Figure 5) below show activities and decisions related to NMS surveillance that are covered by the two scenarios 'pre-disease stage' and 'disease stage' (and their two intensity levels) for defining the surveillance strategy developed in these guidelines (see Chapter 1.2). Grey rectangles show activities and decisions to be implemented in parallel to NMS surveillance (as covered by MBD risk plans), including surveillance of MBD and control of NMS and MBD. Depending on the MBD, invasive mosquitoes may also be targeted. Presence of risk related to NMS strengthens the need for surveying MBD, and vice-versa. A number of surveillance outputs, e.g. vectorial capacity parameters, contribute directly to the assessment of risk from MBD. Nuisance mosquito surveillance may provide preliminary data, and nuisance mosquito control may impact vector abundance.

Figure 5. Decision diagram for the implementation of surveillance of NMS, in relation to the assessment of risk from mosquito-borne disease



[1] *To further assess the risk level, additional surveillance of pathogens may be performed; infected mosquitoes indicate a high risk, which necessitates enhanced surveillance of MBD.*

Document structure

These guidelines cover all procedures relevant to the surveillance of NMS (Figure 6).

Chapter 1: Strategic issues and options for stakeholders during the decision-making process: aim and scope of the surveillance (1.1), surveillance objectives and strategies for two scenarios: pre-disease stage and disease stage (1.2), entomological rapid risk assessments (1.3), and organisation and management of the surveillance programme (1.4).

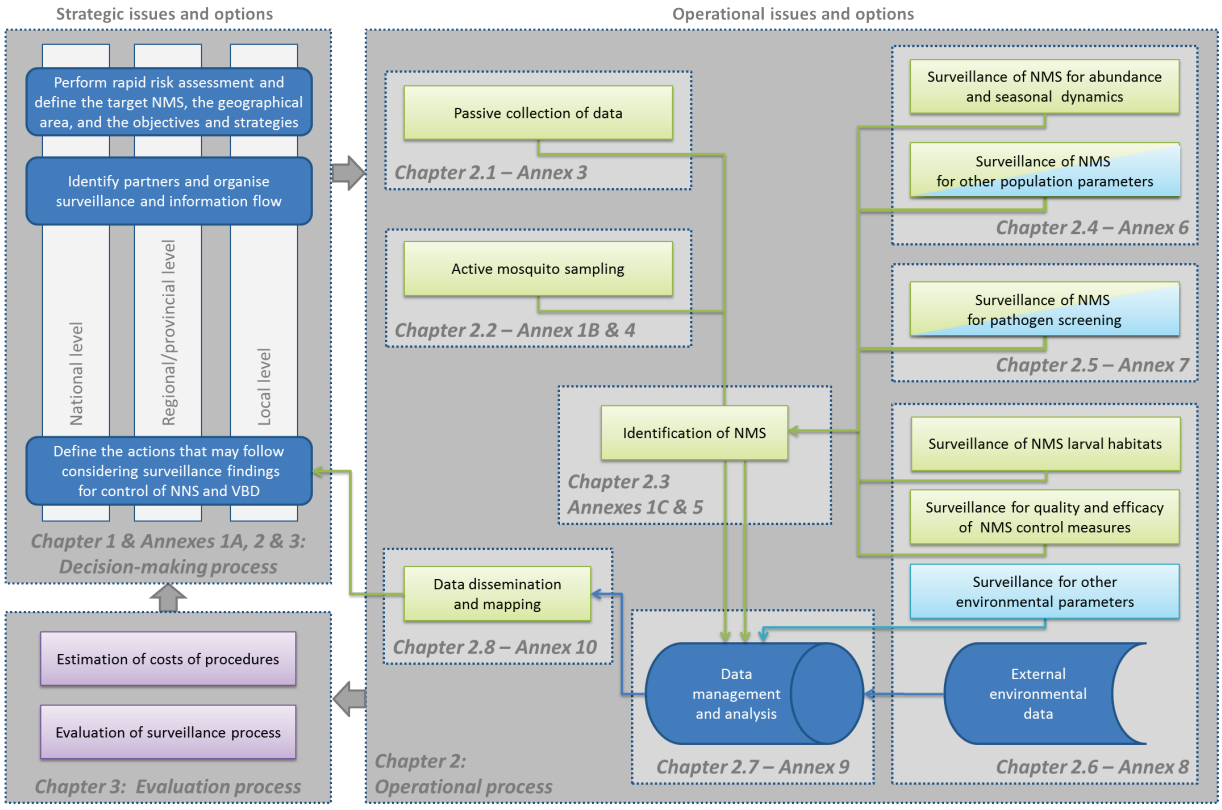
Chapter 2: Operational issues and options to be implemented by professionals involved in the operational process: methods for compiling existing data and collecting new information (2.1), methods for active mosquito collection (2.2); methods of NMS identification (2.3); key and optional procedures for field collection of population parameters (2.4); pathogen screening and identification (2.5); collection of environmental parameters (2.6); data management and analysis (2.7); strategies for data dissemination and mapping (2.8).

Chapter 3: Evaluation of the defined surveillance programme, including guidance for estimating costs of surveillance activities (3.1); evaluation of the operational surveillance process (3.2).

Annexes: Additional information

A preliminary evaluation of the situation will allow the definition of appropriate strategic and operational issues. Processes to be applied include key procedures (green rectangles) and optional procedures (blue rectangles).

Figure 6. Flow chart summary of the procedures to be developed for the surveillance of NMS



1 Decision-making process and rapid risk assessment

1.1 Definition of aim and scope

In a first step, the scope of the surveillance has to be determined, i.e. 1) the aims of the surveillance programme and the subsequent actions that should result from surveillance findings, 2) the targeted native mosquito species and MBD (one or more), and 3) the geographical area.

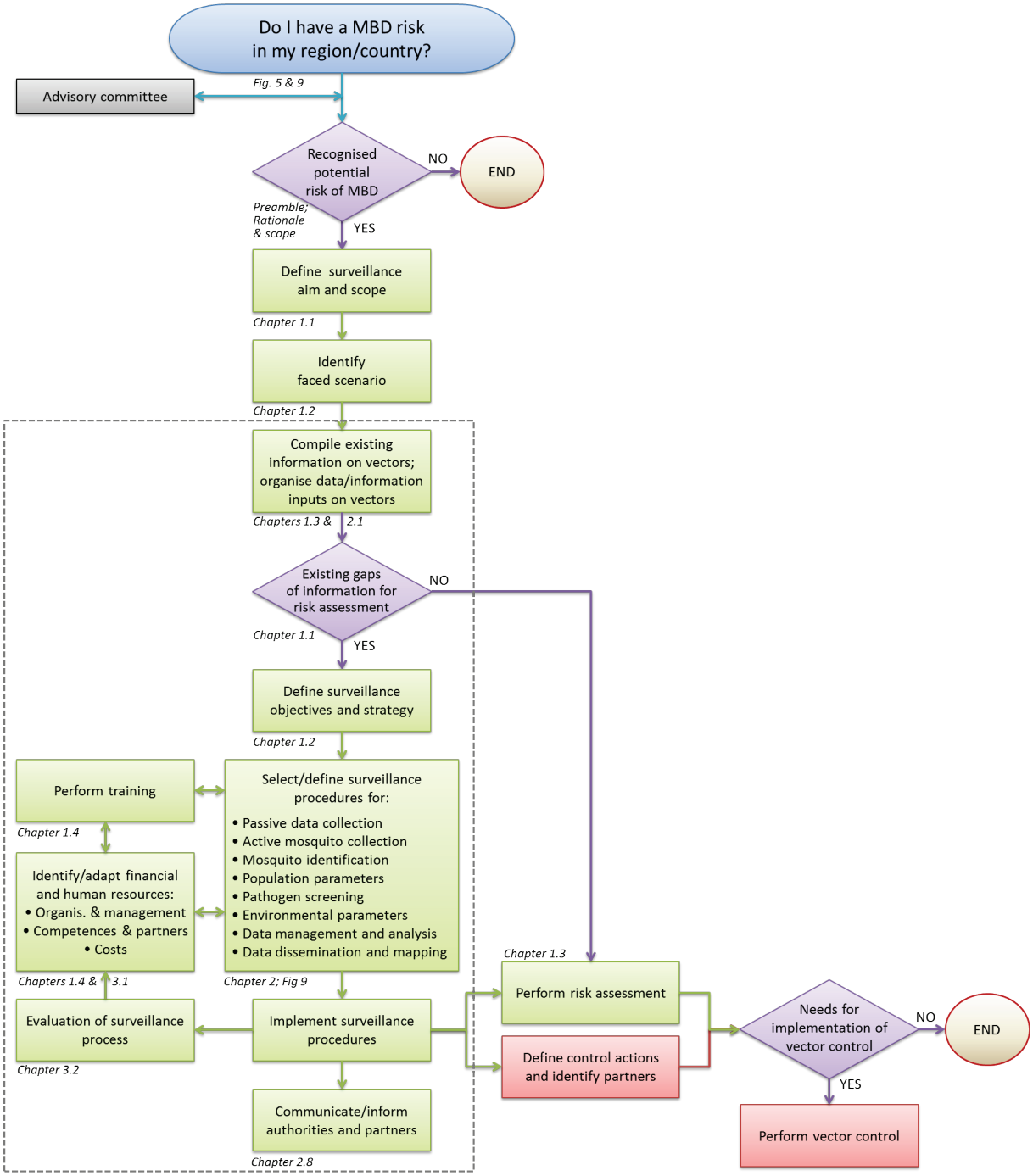
a. Assessment of nuisance and disease risks to human and animal health related to native mosquito species

In areas with putative NMS vectors, surveillance activities should support the entomological assessment (entomological RA; see Chapter 1.3) of the risk to human and animal health, including impact of nuisance (hypersensitivity to bites, mosquito phobia, etc.) and mosquito-borne diseases. All surveillance efforts should provide decision-making bodies with evidence to evaluate both the nuisance and the disease risk and thus enable them to plan adequate control measures (nuisance control; reduction of MBD pathogen transmission risk). Measures can be extended to address the possible impact on biodiversity, e.g. the balance/interaction between native mosquito species and other non-human species.

b. Implementation of mosquito control measures and assessment of their efficacy

In areas where native mosquito species are abundant, the surveillance network should provide details on vector density and longevity in order to ensure the optimal implementation of mosquito control measures. The surveillance network should also provide all information necessary to measure the cost-effectiveness of all short-, medium- and long-term control measures (including pre- and post-campaign measures).

Figure 7. Decision diagram of the decision-making process for the assessment of risk from mosquito-borne diseases



This document provides guidance for MBD risk assessments and MBD management (global diagram). Guidelines are provided for designing surveillance activities (dashed frame), which can be customised to country-specific needs. Vector control options (red rectangles) are not covered.

1.2 Surveillance objectives and strategies

Surveillance procedures are designed to achieve certain surveillance objectives. Depending on the local situation, surveillance procedures (as well as the strategies they are based upon) must be customised to local contexts and needs.

The following paragraphs describe two likely scenarios and threat levels. Scenarios are based on the presence/absence of locally acquired (autochthonous) cases of MBD in animal and/or humans. A further distinguishing factor is the occurrence of MBD cases in the country/region or in a neigbouring region. Possible

objectives and expected outcomes of surveillance strategies (Table 3) vary according to the global aim (see above), the scenarios, and the threat levels, but surveillance strategies and procedural flows (Chapter 2) may be similar for both scenarios.

If autochthonous animal or human cases of MBD or infection of competent vectors are detected, surveillance activities may have to be extended and/or strengthened in accordance with the guidelines for the surveillance and control of vectors and MBD (see Figure 4). Surveillance plans can cover a range of geographical areas and can be scaled from the regional to the provincial or national level. Efficient and rapid customisation of plans and measures usually requires centralised implementation at the regional/provincial or national level (see Chapter 1.4).

Scenario 1: Pre-disease stage (no autochthonous cases detected in animals or humans)

There is no evidence of local transmission of MBD pathogens. The pre-disease stage is characterised as follows:

- There is a latent risk of transmission of MBD pathogens.
- Insufficient or out-of-date data make it difficult to estimate the risk of transmission of MBD pathogens and to produce an entomological RA.
- Validated models show that climatic and environmental factors match the transmission-specific ecological requirements of mosquito-borne diseases.

In this scenario, two different threat levels can be distinguished by the presence/absence of specific triggers.

Threat level 1A: Initial assessment (pro-active surveillance)

An intial assessment is based on a pre-emptive rapid RA which takes into account the global context of mosquito-borne diseases, surveillance of native mosquito species is implemented to assess the risk of pathogen transmission. Available data, for example from passive surveillance, are compiled and analysed within the local context.

Threat level 1B: Preparedness (significant risk of MBD emergence)

The preparedness stage also relies on a pre-emptive RA, but alerts from surveillance systems and/or surrounding regions suggest a significant risk of MBD emergence (e.g. rising number of imported MBD cases; report of autochthonous MBD cases in neighbouring regions) and thus the need for the rapid implementation of a surveillance system. Strategies for surveillance include more active or intensified procedures (e.g. in level 1A passive surveillance procedures are more prevalent than in level 1B) and rely more on active procedures and a focused approach (e.g. a clear focus on high-risk areas near regions that report local transmission). At this stage, pathogen screening in vectors may become relevant.

Scenario 2: Disease stage (evidence of autochthonous cases in animals or humans)

- Case(s) of MBD which meet the EU/national case definitions have been detected in animals and/or humans. This constitutes evidence of local transmission of an MBD pathogen in the area, and, in the absence of evidence of alternative routes of pathogen transmission (e.g. blood transfusion), the presence of a competent vector population is demonstrated.

In this scenario, two different threat levels can be distinguished, both characterised by specific triggers which necessitate the implementation of reactive surveillance.

Threat level 2A: Occasional cases (novel or occasional local MBD pathogen transmission)

Reports of occasional autochthonous cases require the implementation of vector surveillance in a targeted, investigative manner that include confirmation of the local transmission of cases, determination of the area at risk, and assessment of the transmission risk. Surveillance procedures may be implemented to confirm the transmission through native mosquito species, which would provide the data needed to initiate vector control measures to prevent further transmission.

Threat level 2B: Recurrent cases (repeated MBD pathogen local transmission)

Frequent reports of autochthonous cases are usually a clear sign that a mosquito-borne disease is becoming 'endemic'. This requires broad-scale vector surveillance to facilitate predictive assessments (e.g. start/end of the transmission period). Surveillance data also support public health actions aimed at limiting transmission. At threat level 2B, the vector species has often already been determined, and surveillance procedures can focus on vectorial capacity (e.g. breeding season, geographical spread).

Table 3. Objectives of surveillance strategies in accordance with global surveillance aims, scenarios, and threat levels

Scenarios and threat levels		Assessment of disease risk	Assessment of mosquito control
Scenario 1: Pre-disease stage	1A. Initial assessment	• Determine presence of putative vectors • Determine distribution and status of putative vectors • Assess vector competence of abundant mosquito species and vectorial capacity of putative vector populations	• Identify larval and adult habitats of putative vectors
	1B. Preparedness	For putative vectors, determine: • active/non-active season • abundance • feeding habits	• Define mosquito control strategies • Define strategies to avoid mosquito bites
Scenario 2: Disease stage	2A. Occasional cases	• Detect presence of pathogens in mosquitoes • Assess vector competence of putative vector populations • Determine weather conditions that would permit the transmission of pathogens • Determine environmental changes that would permit the transmission of pathogens	• Assess dispersal rates of potential vectors
	2B. Recurrent cases	• Determine start/end date of pathogen transmission • Determine if weather conditions will influence the persistence and evolution of transmission • Determine if environmental changes could influcence persistence and evolution of transmission	• Assess susceptibility of targeted mosquito populations to insecticides

Scenarios and threat levels support the characterisation of the current situation, for which surveillance objectives have to be determined. All surveillance strategies and procedures should be adapted to the local context. For each scenario, a series of key surveillance measures should be implemented (see Box 3). Optional surveillance measures can be initiated to gain additional information. Key and optional surveillance measures are described in Chapter 2 and Annexes 4 to 8.

Box 3: Checklist for the two scenarios used for the development of surveillance strategies

Scenario 1: Pre-disease stage

There is a risk of local transmission of one or several MBDs, but so far no cases have been reported in animals or humans. Surveillance aims to detect the presence of native mosquito species and/or assess the entomological risk of severe nuisance and/or the transmission of MBD pathogens.

- Data and information are shared to assist the entomological risk assessment (RA).
- If relevant, data collection through passive surveillance is implemented, in accordance with the outputs of the entomological RA.
- Active surveillance of putative vectors is implemented in accordance with the entomological RA.
- Native mosquito species populations which cause severe nuisance are surveyed in order to assess the efficiency of control measures.
- Prepare a plan for the activation of enhanced surveillance of native mosquito species populations identified as putative vectors of MBD.
- Prepare a plan for the activation of surveillance to assess the efficiency of control measures if MBD cases are reported.
- Prepare a communication plan.

Scenario 2: Disease stage

MBD cases have been reported in animals and/or humans. Surveillance aims to assess the current entomological risk of severe nuisance and/or transmission of MBD pathogens and supports the decision-making process for the control of MBD.

- Surveillance is implemented and focuses on MBD cases.
- Surveillance of all at-risk areas is intensified to assess abundance and seasonal dynamics.
- Surveillance area is extended.
- Mosquito control plans are initiated for all areas and events.
- Surveillance is conducted to assess quality/efficiency of control measures.
- Surveillance is carried out to detect pathogens in field-collected mosquitoes.
- Communication plan is activated.

1.3 Rapid risk assessment

Preparing a rapid risk assessment: questions about vector-borne diseases

Step 1: Defining the presence of entomological risk

1. Have we identified the vector species? Which native mosquito species are present and may be implicated?

2. In which part of the country was the presence of native mosquito species detected? How common are these putative mosquito vector species? Do we know their distribution (local/national) and status (rare/patchy/ubiquitous)?

If native mosquito species have already been identified as a potential vector for an MBD, the first task when preparing an MBD risk assessment (RA) is to determine whether the mosquito species responsible for transmission of a pathogen actually occur in the host country/region. Surveillance may then focus on major pest species and on putative vector species. Species lists by country are useful, but can be misleading – particularly in large countries – without further data on the distribution and status (rare, localised, ubiquitous) of each species, as well as their relative roles as vectors of non-zoonotic pathogens of humans (human – vector – human) or as putative enzootic (animal – vector – animal) and bridge vectors (animal – vector – human). Another important consideration should be the role of non-native species (technically classified as invasive). Where established, non-native species should be considered along with the native species.

Step 2: Determining the spatio-temporal occurrence of entomological risk

3. When do the pest and putative vectors bite during the year? When are they active? Determine the non-active adult season; how do these species overwinter?

4. Do we have a lot of mosquitoes? How abundant are the pest and putative vector species?

5. Who do the putative vectors bite and are they a pest? What do the putative vectors feed on? Do they bite humans/animals? When, where? Are they a pest?

Having established that the native vector population poses a potential risk for transmission of pathogens to humans, the authors of the RA have to determine the transmission period and ascertain which mosquito species are currently causing a biting nuisance and/or could be a vector. Surveillance can then focus exclusively on putative vector species, which may include some major pest species because (1) their abundance and biting behaviour make them efficient vectors, and (2) the nuisance they cause may alone be reason enough to initiate control measures. Knowledge of when the key mosquito species are abundant will help to determine the active transmission period and direct efforts to reduce human exposure through public health communication.

Step 3: Assessing vector control options

6. Where do the putative vectors develop and rest? What are the larval/adult habitats of the putative vectors? Where are they located?

7. How do the putative vectors disperse? How can the putative vector mosquito species disperse? How far do they disperse?

8. How can these vector species be controlled (environmental management, insecticides, other strategies)?

9. Are the insecticides efficient? Are the mosquito populations susceptible to the insecticides?

10. Can we avoid bites? How can we avoid bites (personal protection, physical barriers)?

Once the temporal and spatial risk of MBD is determined, authors need to identify options for vector control or strategies to reduce public/animal exposure to vectors. This will require specific information relating to the aquatic habitats of the key putative vectors, e.g. rural/urban, freshwater/coastal, and permanent/temporary. Information is also needed on the mosquitoes' capacity/range for dispersal in order to assess the possible dispersal of vectors from key habitats to human habitats (e.g. urban areas). This will direct options for vector control such as insecticidal and/or environmental management.

Step 4: Assessing entomological drivers of pathogen transmission

11. Does the weather influence the vectors? Are there climatic/weather conditions which exert an influence on the activity/distribution of putative vector species? What are the effects of heat waves, floods, coastal flooding and climate change have on the vectors, hosts and pathogens?

12. Does the environment impact the vectors? Are there environmental change issues and agricultural/industrial issues that might affect putative vectors and transmission?

13. Have any species been found infected? Have the putative vectors been found infected?

14. Is the identified mosquito species an efficient transmitter of the pathogen? What is the vector competence of the local populations?

15. Is it warm enough for the infection of the vector? Are the local temperature conditions suitable for the development of the pathogen in native vectors?

16. When does the transmission period start/end? When are the local temperature conditions suitable for seasonal transmission?

Concern should also be given to whether mosquito populations respond to weather and environmental changes. Risk assessments on emerging infections are largely driven by the risk from pathogens. However, pathogen detection in vectors requires large numbers of specimens collected so as to increase the chances to detect the pathogen DNA/RNA.

The detection of a pathogen does not indicate vector competence, and therefore insectary and laboratory investigations are often required. Additionally, the absence of pathogens in collected vectors does not exclude the presence of pathogens in the vector population. Finally, local weather conditions may limit the development of the pathogen in vectors and therefore its transmission.

Step 5: Assessing zoonotic and other non-entomological drivers of pathogen transmission

17. Are wild animals affected? If yes, which wild animals are involved in the transmission cycle?

18. Are domestic animals affected? If yes, which domestic animals are involved in the transmission cycle? What is their distribution? Which population dynamics have to be taken into account, and which factors control the abundance of domestic animals?

19. Where/when are these hosts involved? What is the distribution of wild and domestic animal hosts? Which population dynamics have to be taken into account, and which factors control the abundance of non-human hosts?

20. Are there other possible drivers of transmission? What else could be driving transmission (e.g. travel history, human behaviour)? Are there possible unknown interactions?

The RA should explore the role of wild and domestic animals as reservoirs or amplifying hosts and how their population dynamics might impact transmission to humans through mosquito bites. Finally, additional consideration should be given to any external drivers such as trade (e.g. animal hosts) and travel and tourism.

At least 16 of the 20 questions above require entomological surveillance data. However it is important to note that different types of data on native mosquitoes are required to answer these questions. Data on status, geographical distribution, abundance, pest status, non-vector seasons, and pathogen detection surveillance all require a slightly different approach.

Gap analysis as a basis for defining surveillance objectives

The 20 questions above should be explored by experts (possibly in a multidisciplinary advisory committee). If relevant information is missing, the experts need to discuss possibilities on how to acquire the missing information (e.g. through literature/expert data gathering or specific surveillance).

The 'pre-disease' and a 'disease' stages differ in the surveillance strategies used and the triggers that necessitate mosquito surveillance. Other differences include the available budget, the approach to surveillance, and the production of RA.

Prioritising mosquito surveillance during the pre-disease stage

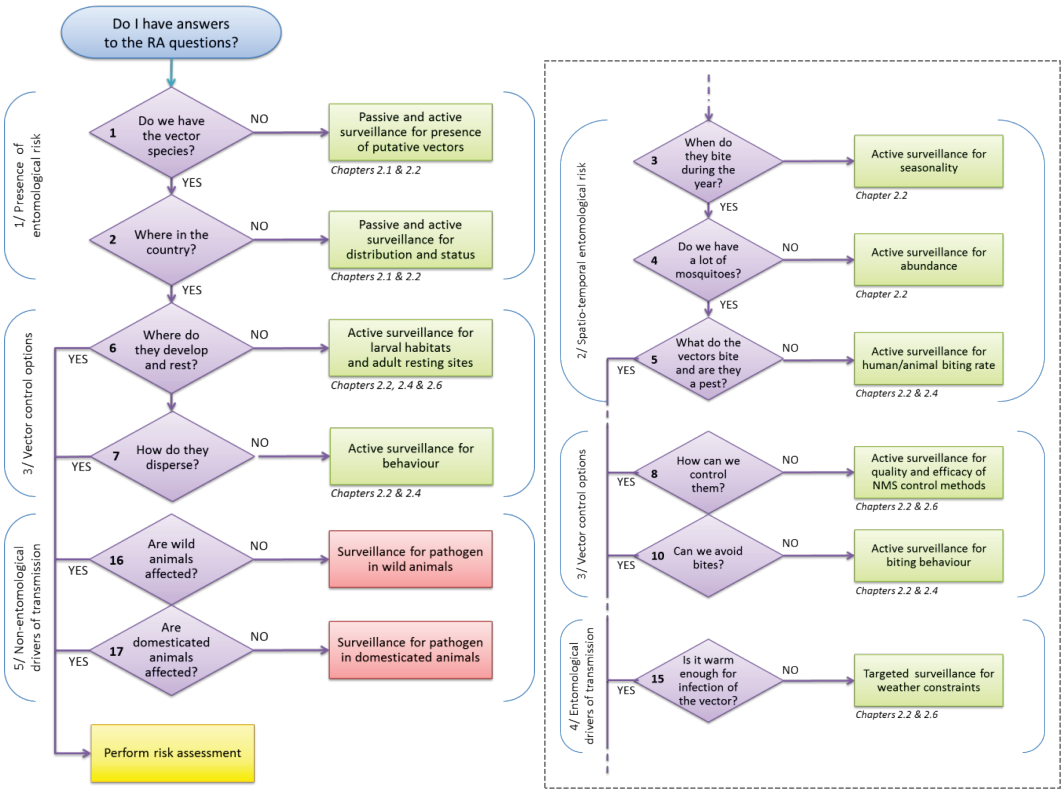
Pre-disease stage, threat level 'initial assessment'. Questions should first address the entomological risk (questions 1–2). If putative vector species are present, the location and dispersal of vectors should be determined (questions 6–7). These data can then be used for the application of vector control measures. A complementary step would be to investigate the non-entomological drivers of vector-borne disease transmission (questions 16–17).

The following threat level ('preparedness', which is initiated after the entomological risk is defined) addresses the spatio-temporal aspects of the entomological risk (questions 3–5). Vector control options can be investigated further by identifying suitable control methods (question 8) and ways to avoid mosquito bites (question 10). As to non-entomological drivers of transmission, the suitability of weather conditions for the infection of mosquitoes should be assessed (question 15).

Prioritising mosquito surveillance during the disease stage

The shift from the pre-disease stage to the disease stage happens when cases are reported in animals or humans. At this stage, the 20 questions above first address the presence and spatio-temporal occurrence of entomological risk (questions 1–5), followed by vector control options (questions 6–8, 10). Next are the entomological drivers of transmission, including weather conditions which determine the start/end date of pathogen transmission (questions 11–15). As for non-entomological drivers, further assessment is needed to determine the distribution, population dynamics, and abundance of non-human hosts, if involved in the transmission cycle (question 17–18). Finally, human-related drivers may be assessed (question 20). During threat level 2B ('recurrent cases'), several additional assessments are requested. Insecticide efficiency (i.e. susceptibility of the mosquito population) should be investigated (question 9). Entomological drivers of transmission, for example weather conditions which determine the start/end date of pathogen transmission, should be assessed (question 16). Finally, it should be investigated how/when non-human hosts are involved (question 19) (non-entomological drivers of transmission).

Figure 8a. Algorithm diagrams for risk assessments with corresponding surveillance procedures; Scenario 1, pre-disease stage: threat levels 1A 'initial assessment' (left) and 1B 'preparedness' (right, dashed frame)



Questions and procedures addressed in threat level 1A can also be discussed in threat level 1B but are not shown on the diagram. Vector control and non-entomological options (red rectangles) are not addressed in these guidelines.

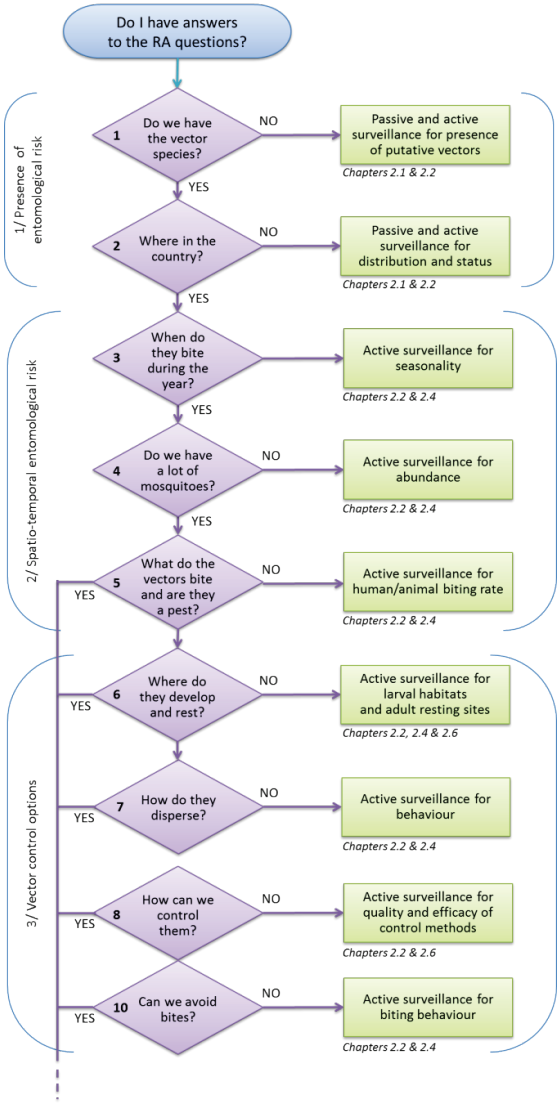
Options for passive surveillance

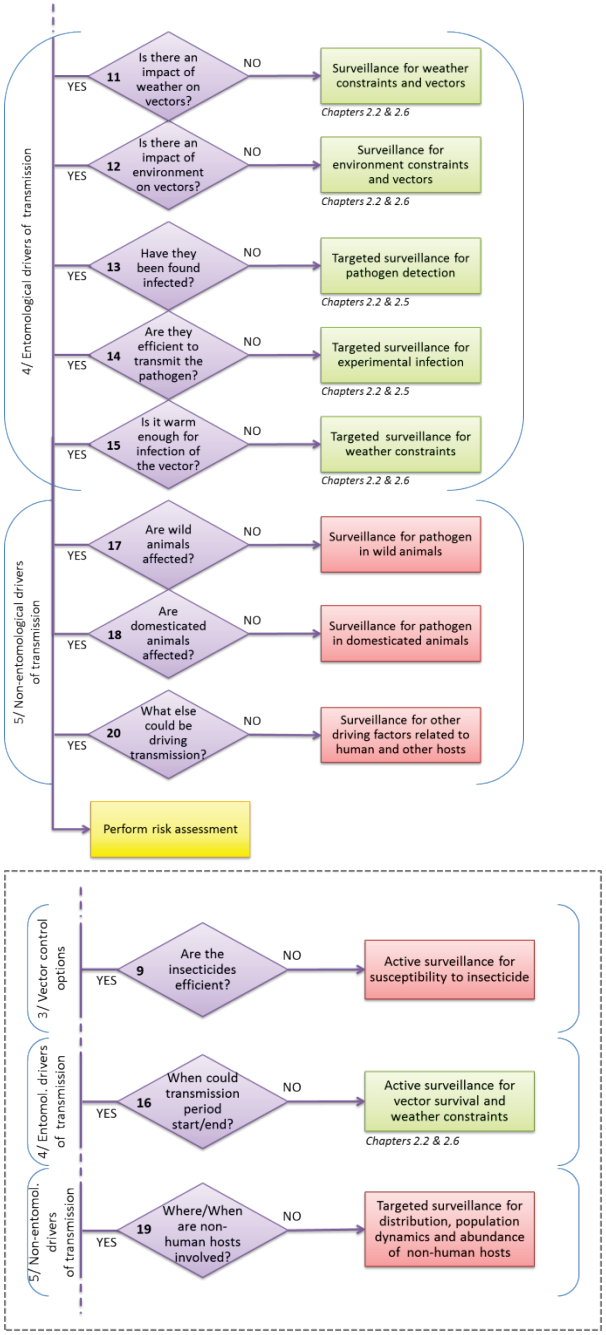
Data on the distribution of native mosquitoes can be sought from a variety of resources. Reviewing published data or historical atlases on mosquito distribution provides some historical perspective on mosquito distributions. These data can be made available as an online resource which then encourages individuals, amateurs and professionals to supplement the database with their own records (data submitted online should be validated by an identification service). Providing a forum or a data submission tool for data/specimens should be encouraged. Passive surveillance data can hugely improve RAs where no data previously exist.

Still, data gaps and geographical bias in reporting are impossible to avoid. Passive data provide very little information on species abundance or seasonal activity, nor do they provide a sufficient number of samples for pathogen detection.

Examples of passive surveillance programmes are given in Annex 3.

Figure 8b. Algorithm diagrams for risk assessments with corresponding surveillance procedures; Scenario 2, disease stage: threat levels 2A 'occasional cases' (top) and 2B 'recurrent cases' (bottom, dashed frame)





Questions and procedures addressed in threat level 2A can also be discussed in threat level 2B but are not shown on the diagram. Vector control and non-entomological options (red rectangles) are not adressed in these guidelines.

Options for active surveillance

Available resources for the active surveillance of native mosquitoes should be directed at key pest or vector species, which should get identified during the first phase of the RA production process. Data procured during the passive surveillance process should be utilised to direct active surveillance to locations with a full range of potential vector species.

With regard to active surveillance it may be preferable to run a large number of traps for only a few nights at sites across the entire country. This approach aids future geo-spatial modelling and has been very successful in detecting non-native species. The disadvantage of this approach is that the intensity of surveying is too low to understand abundance and seasonal activity; also, the number of samples from specific locations is too low to detect pathogens or determine pathogen infection rates in the vectors.

Active surveillance may also be employed to gather data for specific requirements, such as longitudinal data for climate and weather modelling, mosquito abundance data at environmental change sites (e.g. new wetlands in urban, rural and coastal sites) or data on mosquito nuisance or the impacts of subsequent mosquito control efforts.

Finally, some contexts call for pathogen detection, e.g. transmission episodes. Pathogen detection may not always be logistically or economically viable. Some degree of testing should be considered in the RA process; however, authors should keep in mind that a proper assessment of pathogen presence requires a large number of mosquitoes to be tested (see also Chapter 2.5).

Further guidance on how to prioritise mosquito surveillance (passive and active) as part of the RA process is given in Annex 3.

1.4 Organisation and management

Three organisational levels can be conveniently identified, 1) national, 2) regional/provincial and 3) local. Particular actions and tasks may best be assigned to one or more of these levels, as befits the risk and the national context (see Tables 4, 5).

Once the aim of the surveillance is defined, the second step will be to identify available information and organise data/information inputs from diverse sources in order to carry out an entomological RA. Options to implement passive surveillance should be explored. For the implementation of specific tasks related to NMS surveillance, and in order to ensure sufficient capacity, potential partners have to be identified and the various tasks have to be allocated (see Table 5). If specific capacities are missing, external providers (e.g. universities, consultancies) can be recruited; alternatively, a capacity building process can be initiated.

In addition to the various measures (see Box 1 and Figure 3), the surveillance programme should be part of a transnational plan. National regulations usually stipulate the identification and nomination of partners and define their responsibilities and roles. Field datasets should be linked to GIS applications so that spatio-temporal distribution maps of native mosquito species (see Chapter 2.8) can be produced.

Feedback procedures should be added to the surveillance programme to assess any side effects of the control measures, i.e. impact on non-target fauna, impact on human health, and insecticide resistance management (see EMCA/WHO guidelines, Box 1).

Table 4. Actions to be taken at the different administrative levels

National	Regional/provincial	Local
Developing national risk assessment and management plans for NMS and MBD: • ensuring NMS and MBD are surveyed according to global and local context; • defining NMS surveillance strategy and sampling/trapping methods; • determining NMS and pathogen screening capacities; • developing a national NMS and MBD control plan.	Developing regional plans for NMS and MBD surveillance: • organising and implementing surveillance activities in locations at risk for MBD pathogen transmission; • collecting biological parameters of NMS; • implementing the NMS and MBD control plan when necessary.	• Providing resources and ensuring cooperation for the application of surveillance and control measures in locations at risk for MBD pathogen transmission. • Cooperating in the dissemination information for residents to promote community participation and avoid local conflicts.

Box 4: Key issues and recommendations: surveillance on native mosquito species

- Is the aim of the surveillance well defined?
- Are the targeted species defined with respect to the pathogen threat?
- Are there sufficient competent experts to implement procedures and methods? The following fields are particularly important:
  - Study design
  - Field implementation
  - Laboratory work
  - Data management and analysis
- Are partners identified according to the necessary competences?
- Are training and skill-sharing activities scheduled?

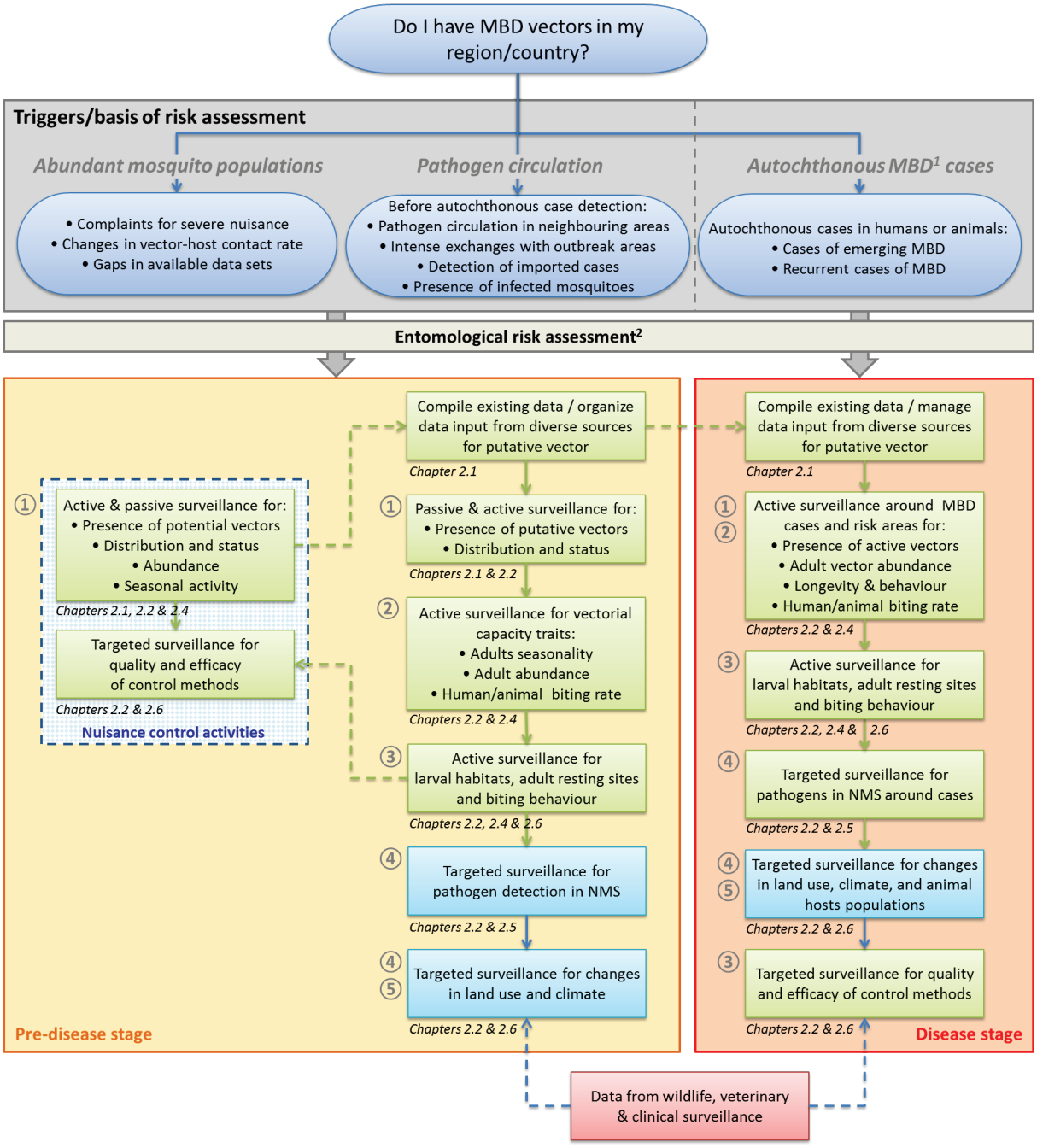
Table 5. Ideal allocation of responsibilities and tasks to potential partners in surveillance activities

Ministry in charge of human health (state) and ministry in charge of animal health (state)	Co-development and coordination of entomological RA and management plans for NMS and MBD, including identification of capacities, training activities, and allotment of responsibilities.
Ministry in charge of environment (state)	Contribution to the coordination of entomological RA and management plans for biodiversity; assistance in the assessment of side effects of mosquito control measures and inclusion of the vectors, non-human hosts and pathogens in the biodiversity analysis.
Public health services (state, regional/provincial, local)	Contribution to the surveillance of NMS and MBD (i.e. mosquito pathogen screening, human cases) and the assessment of the impact of control measures on human health.
Veterinarian services (state, regional/provincial, local)	Contribution to the surveillance of NMS and MBD (i.e. cases among wildlife[1], livestock and pets; mosquito pathogen screening) and the assessment of the impact of control measures on animal health and biodiversity.
Regional/Provincial government (depending on the degree of autonomy)	Definition and implementation of regional/provincial entomological RA and management plans for NMS and MBD.
Research institutions	• Contribution to the surveillance of NMS. • Support for risk assessment/management activities, the efficacy/quality assessment of mosquito control applications and their side effects (impact on non-target fauna, impact on dispersal of mosquitoes, impact on human health), pesticide resistance management. • Support for the collection of data on bionomics of NMS in specific contexts, determining spread, nuisance and vector potential. • Contribution to the training of field/lab workers.
Municipalities	Participation in implementation of surveillance and mosquito control measures.
Mosquito abatement agencies (public or private)	Management of surveillance and mosquito control applications[2].

[1] The Ministry in charge of environment may contribute to wildlife surveys. [2] Mosquito control measures can also be conducted by pest control companies if previously trained in mosquito control applications and subject to external quality control.

2 Operational processes

Figure 9. Diagram of surveillance procedures for native mosquito species, according to the identified risk and the two defined diseases stages



[1] Suspected to be transmitted by NMS; [2] To be performed within the decision-making process, to identify the faced risk and the corresponding scenario; Numbers in circles correspond to RA topics.

Key procedures (green rectangles) and optional procedures (blue rectangles) should be implemented according to the estimated risk and information gathered during the preparation phase. The process must be adapted in real time and take into account surveillance results as well as additional external information. An optional procedure during the pre-disease stage may become a key procedure during the disease stage.

Ideally, mosquito surveillance/collection strategies are determined by the vector species targeted by the surveillance (see Table 2) and the specific environmental characteristics of the sites to be surveyed (see Table 8). Native mosquitoes can be easily collected at different stages of their life cycle, as larvae or adult females (see information on the mosquito life cycle and biology in Annex 2). Logistic and cost factors may also affect the choice of surveillance methods.

Active surveillance refers to sampling methods specifically selected and applied to collect the targeted species at the sites where they may occur, according to available data or scientific knowledge on larval ecology (i.e. specific larval habitats requirements) or adult behaviour (i.e. specific female host seeking or resting behaviour).

By contrast, passive surveillance encompasses data gathering through existing monitoring programmes or submission of reports from non-specialised field work and/or from the general public, for example citizen science programmes.

Targeted surveillance is based on sampling methods carefully selected and applied to a specific aim and context.

Data collected through mosquito nuisance control activities can provide relevant information and are a solid basis for the further implementation of active and targeted surveillance.

2.1 Methods for compiling existing data and collecting new information

Prior to implementing any surveillance of putative vector mosquitoes, it is worthwhile to gather existing data from 1) existing mosquito nuisance control activities (usually data on presence, distribution, status, abundance, and seasonal activity of pest mosquitoes) and 2) monitoring studies (published articles or grey literature). These data can then be imported into a central database, which would also guarantee long-term availability (see Chapter 2.7). Passive surveillance can be used to supplement active sampling (Table 6) and should be organised at the regional or national level. It is essential that partners or the general public are well informed about the surveillance goals, and that practical tools are provided so that relevant data can be gathered. Examples of passive surveillance programmes are given in Annex 3.

Table 6. Possible contribution of passive surveillance methods

Data gathering methods	Required information and entomological datasets									Contributors					
	Presence/absence	Distribution	Presence status	Abundance and seasonality	Longevity	Flight range & dispersal	Biting behaviour	Adult resting behaviour	Larval habitats[1]	General public	Children/Schools	Pest control companies	public health Officers	Entomologists/Biologists[2]	Research teams
Hotline[3]	x	x	x				x	x		x	x			x	
Smartphone app	x	x	x				x	x		x		x	x	x	
Sampling[4]	x	x	x				x	x	x	x	x	x	x	x	x
Trapping[5]	x	x	x	x						x		x		x	x
Museum collections[6]	x	x												x	x
Literature[7]	x	x	x	x	X	x	x	x						x	x
Databases[8]	x	x	x	x	X	x	x	x	x				x		x

[1] Localisation and characteristics in local context; [2] Mainly non-professionals; [3] Centralised collection of data via phone hotline, web interface (online), or ground mail; [4] Field data collection except trapping; [5] Traps already used by contributors for other purpose (e.g. protection of private ground, pest control activities) or specifically allocated (e.g. to volunteers); [6] Historical data only; [7] Mainly national and historical data; [8] Data collected by various studies.

2.2 Methods of active mosquito collection

The prerequisite for active mosquito monitoring and surveillance is an efficient mosquito sampling scheme, including accurate trapping techniques, adapted to the objectives of the study. Most studies collect adult mosquitoes because they are usually easier to survey, collect, and identify compared to immature insects. However, the presence of adult insects does not provide information on the breeding sites, and although habitat preferences for a species can overlap, oviposition site selection differs strongly between species. It is therefore essential to decide, as a first step, which development stage should be surveyed (immature vs. adult). Factors which influence this decision include the objectives of the study, funding, staff, equipment, and the size of the surveillance area.

Active surveillance methods are listed in Table 7.

Table 7. Datasets and active surveillance methods according to RA topics and surveillance procedures addressing the vectors

RA topics and surveillance procedures	Required information and datasets	Surveillance method
Defining the presence of entomological risk		
Presence of putative vector species	Presence or absence of defined species	Larval or adult sampling 1) at defined sites or 2) at randomly selected sites over a wide area
Distribution and status	• Distribution data and maps • Presence status (rare, common, ubiquitous, etc.)	Snapshot larval or adult sampling 1) at defined sites or 2) at randomly selected sites over a wide area
Defining the spatio-temporal occurrence of entomological risk (vectorial capacity traits)		
Seasonal activity of putative vectors	Presence/abundance of adults over the season	Longitudinal adult sampling at defined places
Vector abundance	Abundance data over periods of time or by comparing locations	Longitudinal adult sampling at defined places
Vector longevity	Mosquito adult daily survival rate	Mark–Release–Recapture trials in the field; laboratory experiments
Human and animal biting rate	Mosquito adult daily biting rate for human and selected animal hosts	Field/laboratory experiments on host choice, human and host-baited catches or trapping
Assessing vector control options		
Defining specific vector larval habitats	Presence and locality of larval habitats	Field larval collection and larval habitat mapping
Vector adult resting sites	Presence and locality of adult resting sites	Field adult catching and resting sites mapping
Vector dispersal	Flight range and dispersal of adult females according to local environmental conditions	Field Mark–Release–Recapture trials
Quality and efficacy of NMS control	Abundance of larvae or adult mos-quitoes before and after application of treatments; Susceptibility of vectors to insecticides	Targeted field larval sampling and adult trapping; Laboratory susceptibility tests of local vector populations
Vector biting behaviour	Adult biting behaviour in local conditions	Targeted field adult sampling
Assessing entomological drivers of pathogen transmission		
Changes in climate	Impact of weather conditions on vector populations	Laboratory experiments (and modelling)
Changes in environment	Impact of changes in land use on vector populations	Field surveys on mosquitoes and landscape before/after changes
Pathogen screening in NMS	Presence and prevalence of pathogens in mosquitoes	Targeted field adult trapping
Vector competence	Vector competence rate of local vector populations	Laboratory experimental infection of local vector populations
Field transmission	Weather suitability for transmission, start/end of the season	Field temperature data

Surveillance of larval (juvenile) stages is of key relevance for monitoring native mosquito species because it allows targeted and rapid sampling, focusing on specific larval habitats of the targeted mosquito species. Mosquito larvae can be collected by netting, dipping, or sucking, depending on the size of the larval habitat being sampled. Another advantage of this method is its optimal cost–benefit ratio.

Surveillance of adult females can be carried out by using aspirators which capture adult mosquitoes sitting in resting sites or on their hosts and/or by sweep-netting vegetation. Human landing collection (HLC) is a viable alternative, provided that the ethical issues involved are fully taken into account. Adult surveillance is, however, most commonly carried out using traps that attract the female insects. The most efficient traps for native mosquito species are suction traps baited with carbon dioxide and possibly chemical lures which attract host-seeking females; other traps attract ovipositing females. Trap choice depends on surveillance objectives, the targeted mosquito species, the environmental conditions at the selected trap sites, the availability of resources (skilled technicians, hosts, dry ice, power supply, budget, etc.), and the logistical limitations of site access.

Detailed information on sampling methods is given in Annex 4.

Chosing a collection method

Recommendations for the choice of collection methods for native mosquito species are given in Table 8. Table 9 gives an overview of the trapping efficiency of the most common types of mosquito traps and sampling methods for native mosquito species putative vector species (as defined in the introduction, see Table 2), and Table 10 provides a summary of the main larval habitats and sampling periods during the year, according to these mosquito species or group of species. These tables should be considered together, in order to select/adapt the appropriate trapping/sampling strategy according 1) to the aim of the surveillance and its expected outcomes, and, because the response of species to trapping attractants is not well understood and may vary considerably, and 2) to the targeted native mosquito species. Surveillance strategies should also take a number of additional factors into account related to the local context and constraints e.g. survey area size, site accessibility, and the numbers of traps needed for each type of environment present.

A number of methods are available to collect native mosquito species population parameters (see Annex 6, Table A). Adult trapping for pathogen screening is best conducted using gravid traps that attract egg-laying females (which have already taken a blood meal and thus may be infected). However, this is efficient only for container-inhabiting species (mainly *Culex* species), and thus an association with CO_2-baited traps will enlarge the trapping radius (but capturing host-seeking females, including numerous young females that had never taken any blood meal). Indeed detection of native mosquito species should be performed with a wide spectrum of traps and methods (including larval search and HLC) to ensure the largest sampling radius. Long-term surveillance may be based on the most efficient traps for human-biting mosquitoes, as CO_2-baited suction traps. Additional factors determining trap choice are the combination of parameters required from a single trapping programme, the availability of material already acquired, the availability of a CO_2 source, and the available funding. Surveillance costs can be reduced if the traps are run by local operatives who send the samples to a central processing centre.

Several complementary sampling/trapping methods can be performed simultaneously (e.g. larval dipping and adult traps); Required frequencies are indicative and must be adapted to the risk level, the local climate, and logistic possibilities; Traps are run for 24h, 48h, or one week, depending on the available power source; human landing collection (HLC) can be performed occasionally or in specific cases; Trapping periods are given for central Europe and must be adapted to local climate; Indicative number of trappings according to surface is discussed in Chapter 3.1.

Table 8. Recommended mosquito collection methods with trapping frequency and trapping period, by required information/data

Required information and datasets	Methods and traps/tools	Frequency of trapping	Period of trapping
Presence, distribution, and status of mosquito species	Larval search (dipping) and any adult trapping or catching method; Intuitive sampling in suitable landscape, or randomly selected sites throughout land use units in the surveyed area	Once or twice a month	Apr–Nov
Adult mosquito abundance	CO_2-baited traps	Twice a month	Apr–Nov[1]
Seasonal activity	CO_2-baited traps Larval search (dipping) Ovitraps[2]	Twice a month	Apr–Nov
Adult mosquito resting behaviour and resting sites	Site visual inspection and aspirating Resting boxes	Twice a month Daily[2]	Jan–Dec Apr–Nov

Required information and datasets	Methods and traps/tools	Frequency of trapping	Period of trapping
Human and animal biting rate	Host-baited traps or host landing collection HLC	Weekly	Jun–Oct[3]
Larval habitats locality	Larval search	Monthly	Mar–Nov[4]
Quality and efficacy of control measures	CO_2-baited traps Ovitraps[5]	Before and after treatments	Jun–Sep
Pathogen screening in NMS	Gravid traps and CO_2-baited traps[6]	Weekly	During and after outbreaks

[1] It is recommended that trapping is performed also during the winter time to define the adult activity-free winter period if not known; [2] Mosquitoes are resting during daytime, thus they might be collected as frequently as possible; [3] Or during specific MBD-transmission risk periods; [4] Can also be performed during winter without ice and snow cover for some species; [5] For container-inhabiting species only; [6] And by larval sampling if suspecting trans-ovarial pathogen transmission.

Table 9. Efficacy of collection methods for native adult mosquitoes, by mosquito species

Targeted species	Host-seeking females				Ovipositing females	Resting females
Trap type	CO_2 traps	HLC	Light traps	MM (CO_2)	Gravid traps	Aspirating
Ae. cinereus/geminus *Ae. communis* *Ae. caspius* *Ae. detritus/coluzzii* *Ae. dorsalis* *Ae. excrucians s.l.* *Ae. vexans* *Ae. sticticus*	+++	+++	-	+++	-	+ (vegetation)
An. claviger *An. plumbeus*	++	+++	+	++	-	-
An. hyrcanus	++	+++	+	++	-	++
An. atroparvus *An. labranchiae* *An. sacharovi*	+	++[1]	++	+	+?	++
An. maculipennis s.s. *An. melanoon* *An. messeae*	+	-	++	+	+	++
An. cinereus *An. sergentii sergentii* *An. multicolor* *An. superpictus*	?	++[1]	?	?	?	?
Cq. richiardii	+++	+++	-	+++	-	++
Cx. modestus	++	+++	?	+++	-	-
Cx. perexiguus	?	+[1]	?	?	+++	++
Cx. pipiens s.l.	+++	+[1]	?	++	+++	++
Cx. theileri	++	+[1]	?	++	++?	?
Cx. torrentium	-	-	?	-	?	++
Cx. tritaeniorhynchus	++	+[1]	?	++	+++	++
Cs. morsitans	-	-	-	-	-	-

CO_2 traps = CO_2-baited suction traps (e.g. CDC light trap [with light on or off], EVS trap, BG Sentinel); HLC = human landing collection; light traps = light-baited suction traps; MM = MosquitoMagnet CO_2-baited suction traps with chemical attractant; gravid traps = infusion-baited suction traps; – = low efficacy; + = fair efficacy in some situations; ++ = good efficacy; +++ = excellent performance; ? = unknown

[1] = at night

Table 10. Main types of larval habitats to be surveyed and period of year, by mosquito species

	Main types of larval habitats	Sampling period
Ae. cinereus/geminus *Ae. vexans* *Ae. sticticus*	Flooded meadows and ditches (temporary fresh water pools)	May–Sep
Ae. communis	Snow-melting ponds, peat bogs, and ditches (in forests)	Mar–Sep
Ae. caspius	Temporarily flooded coastal and inland salt marshes, rice fields	May–Aug
Ae. detritus/coluzzii *Ae. dorsalis*	Temporary flooded coastal and inland brackish marshes	Feb–Oct
Ae. excrucians s.l.	Temporary fresh water marshes	Mar–Jun
An. claviger	Fresh water marshes, ponds, ditches, springs, wells	Jan–Dec
An. hyrcanus	Rice fields, marshes	Jun–Aug
An. atroparvus *An. labranchiae* *An. maculipennis s.s. An. melanoon* *An. messeae* *An. sacharovi*	Semi-permanent water bodies with vegetation, edges of slow-running water, ponds, fountains	Jun–Sep
An. plumbeus	Tree holes, containers with water and dead leaves, abandoned cesspits	Jan–Dec
An. cinereus *An. sergentii sergentii An. multicolor*	River banks, ponds, springs, wells	May–Sep
An. superpictus	River banks, small streams	May–Sep
Cq. richiardii	Permanent water bodies with tall emergent vegetation[1]	Oct–May
Cx. modestus	Reed beds, irrigated rice fields, ditches, ponds	Jun–Sep
Cx. perexiguus *Cx. theileri* *Cx. torrentium* *Cx. tritaeniorhynchus*	Containers, ponds, wells, road puddles	May–Oct
Cx. pipiens s.l.	Containers, ponds, marshes, flooded meadows, catch basins, flooded basements, cesspits	May–Nov[2]
Cs. morsitans	Ponds, ditches, marshes	Oct–May[3]

[1] Larvae are very difficult to collect as they do not breathe on the water surface; [2] Also in winter for populations breeding underground; [3] Cohorts of larvae may be found also in July but in limited numbers.

The results of a systematic literature review on sampling/trapping methods are given in Annex 1B; complementary information is given in Annex 4, including an overview of the strengths and weaknesses of the various collection methods, examples of trap positioning, and illustrations of trap models and larval habitats.

Box 5: Key issues and recommendations for mosquito collection

- Are the objectives of the surveillance well defined?
- Are the surveillance methods adapted to the targeted native mosquito species?
- Are the resources sufficient to implement the defined procedures and obtain solid data?
- Will the expected data adequately support decision-making?

2.3 Methods of identification of native mosquitoes

Traditionally, species identification is done based on morphological characteristics. Although there are several easily recognisable features, they may not be informative enough to distinguish between so-called 'sibling species' within 'species complexes' (e.g. *An. maculipennis* complex, *Cx. pipiens* complex).

A systematic literature review on identification methods (see Annex 1C) showed that a number of morphological identification keys are available, often limited to some genera and/or limited to a geographical region. For Europe, efforts have been made to develop identification keys for the entire mosquito fauna of Europe, either as dichotomic (Becker et al. 2010) or computer-aided keys (Schaffner et al. 2001); these keys address both larvae and adults. More information on species morphology and identification keys can be found in specific (often doctoral) studies which often describe only one genus or species.

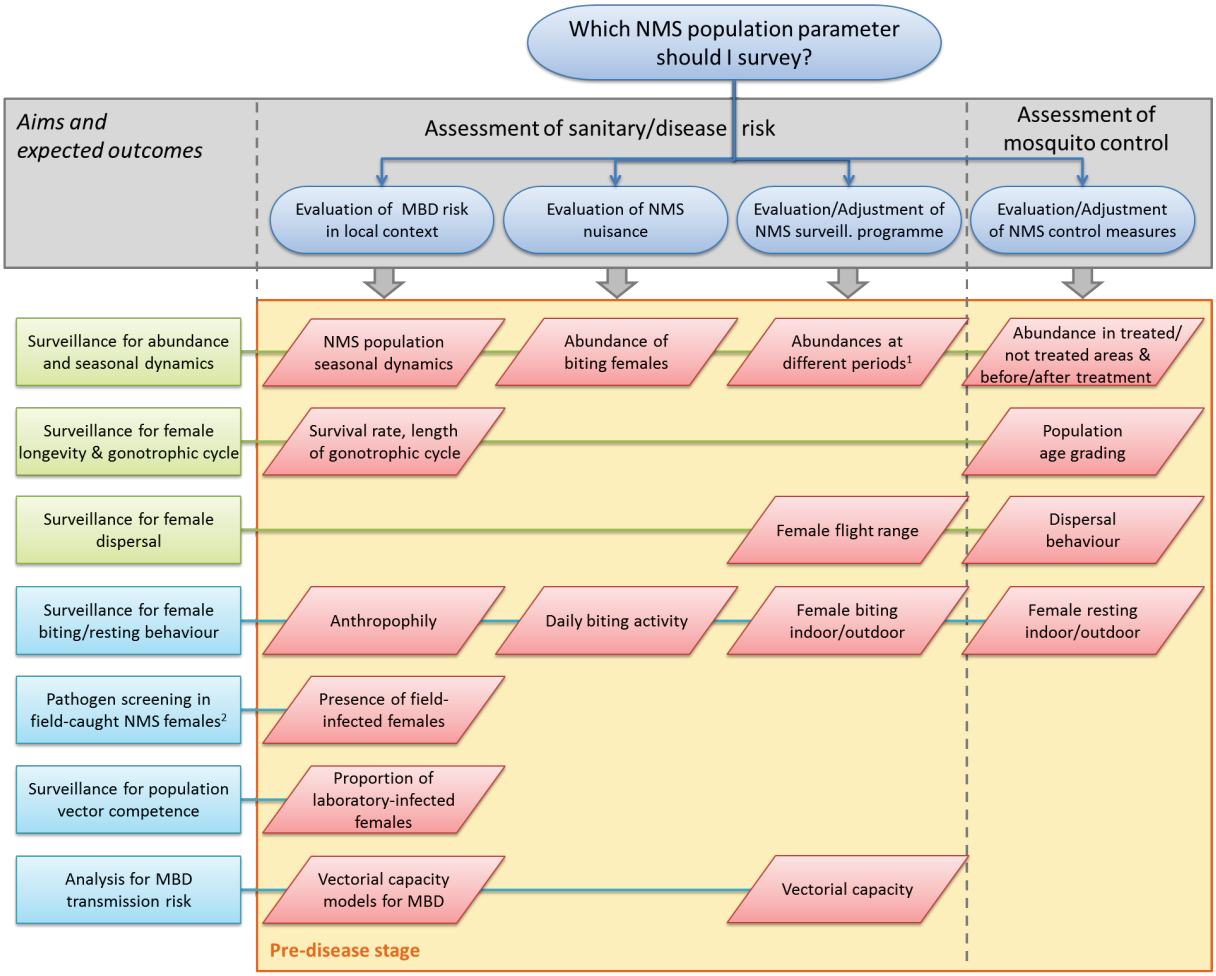
The morphological identification process requires a high level of expertise, and experts do not always succeed to accurately identify a specimen to species level. Morphological keys are not always sufficiently accurate or are missing for some or all life stages. For example, the surveillance of container-inhabiting mosquitoes (such as *Ae. geniculatus*) is usually carried out by using ovitraps (see Chapter 2.2), i.e. by collecting eggs. However, the morphological identification of eggs is highly time-consuming, requires a high level of expertise, and specialised microscopy equipment. Identification is further complicated by the fact that not all species are well characterised. In addition, female mosquitoes collected by trapping have often lost their scales or are damaged, and thus some essential morphological features are no longer discernable. Therefore, alternative (molecular) identification methods have been developed to allow the identification of sibling species, which were subsequently extended to cover a wide range of species, whichs improved the identification process, especially during routine operation. Molecular techniques – analysing gene sequences or protein profiles – allow the rapid identification of species and can be carried out by non-taxonomists. Molecular genetic analysis offers the added advantage of processing several samples (regardless of life stage) simultaneously. Currently, a vast range of molecular techniques and markers are employed to study mosquito taxonomy, systematics, population structure and dynamics. An overview is given in Annex 5 and serves as a brief introduction into the main molecular tools for mosquito surveillance and research.

Molecular techniques require a well-equipped laboratory and skilled personnel, which is rather costly, particularly in large-scale studies/operations, yet molecular genetic analysis remains one of the easiest methods to determine the presence of a species. A major disadvantage, however, is the fact that protocols and primers are often species specific (see Annex 5). Genetic information (of different loci) is already available for a large number of species. Polymerase chain reaction (PCR), combined with sequencing of the amplicon, can identify a specimen, provided that corresponding sequence data are available in open-access databases such as GenBank and BOLD Systems. For some groups of species (e.g. invasive mosquitoes, malaria vector species complexes), specific conventional real-time PCR assays have been developed for rapid molecular identifications. Finally, protein profiling by mass spectrometry starts to become a method of choice for identifying mosquitoes as egg, larvae, or adult stages.

2.4 Procedures for determination of mosquito population parameters

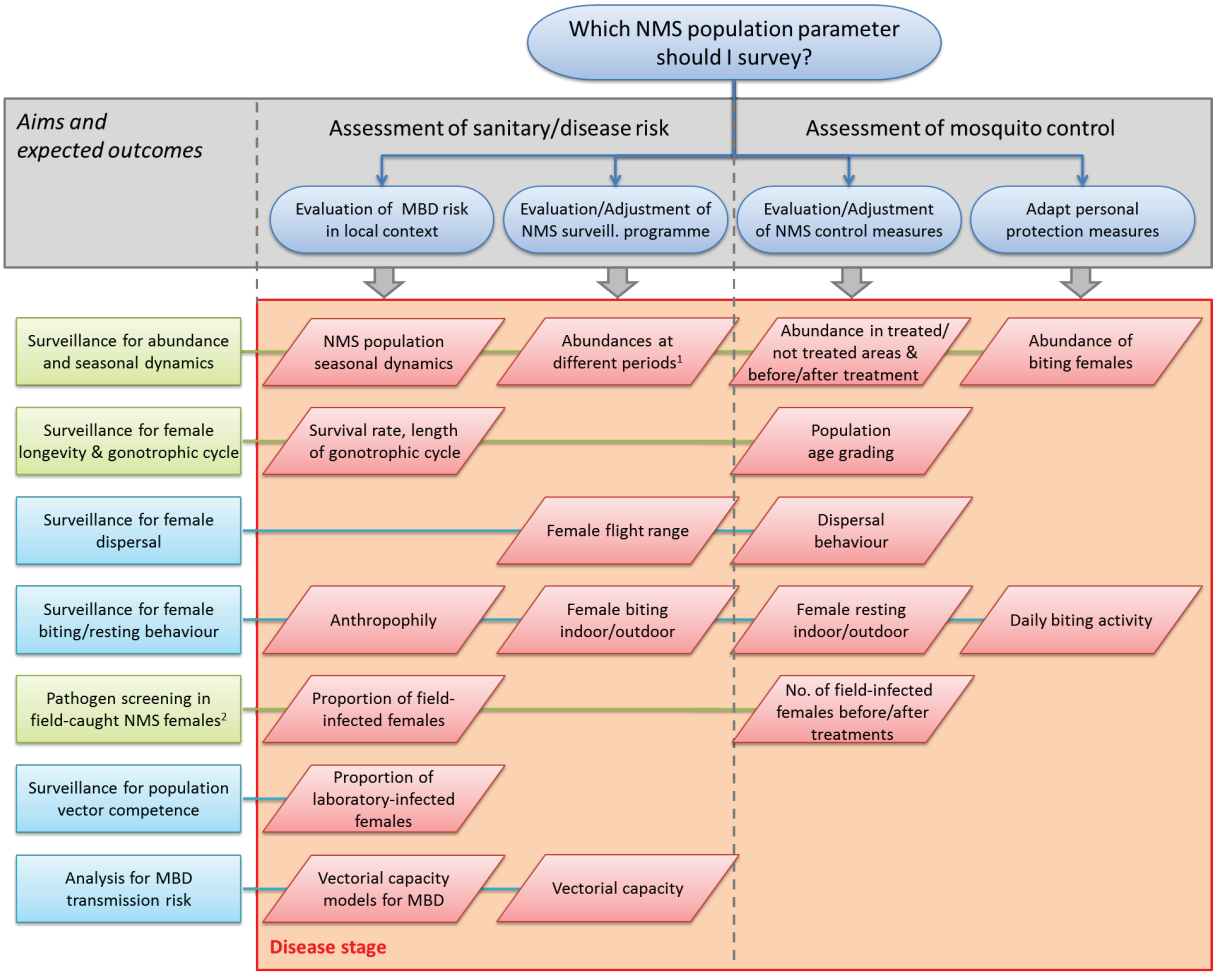
The collection of datasets (red parallelograms, Figure 10 below), obtained from key (green rectangles) and optional procedures (blue rectangles), is based on required outputs.

Figure 10a. Collecting population parameters of native mosquito species: desired output and related key and optional procedures; pre-disease stage



[1] Can be collected as part of nuisance control activities; [2] Discussed in Chapter 2.5

Figure 10b. Collecting population parameters of native mosquito species: desired output and related key and optional procedures; disease stage



In order to assess the risk of pathogen transmission, data on the distribution of native mosquito species are needed, in addition to population and life history parameters. Without this information, the vectorial capacity of the native mosquito species population cannot be evaluated at the local level, and planning and conducting vector control operations becomes inefficient. Data on distribution and population parameters can be collected before there is any evidence of local MBD pathogen transmission, but also during outbreaks.

Population parameters are numerous and their values are affected by the characteristics of the environment. These parameters include: abundance, longevity (including duration of growth of different mosquito life stages – egg, larva, pupa, adult – and their survival rates), capacity for dispersal, host preference, the number of gonotrophic cycles (duration of time between two ovipositions) per lifetime, length of gonotrophic cycle, mosquito fertility/fecundity, mosquito mortality, the intrinsic rate of increase, the net reproductive (replacement) rate, birth rate, death rate, and generation time (for definition of terms see Annex 6).

These parameters are all species specific, i.e. their values depend on the mosquito species, which adapts to its new environment, whereas environmental parameters (see Chapter 2.6) are determined by environmental and climatic conditions, which are more or less favourable to the native mosquito species.

The key population parameters to be considered are: 1) population abundance and dynamic during the season, 2) female longevity, 3) female biting behaviour, and 4) dispersal capacity. These key parameters combined with vector competence may help to determine the vectorial capacity and basic disease reproduction number (R_0) to provide the foundation for risk assessments of mosquito-borne diseases (see Box 6).

Table 11 summarises the strengths and weaknesses of mosquito population key parameters as well as procedures and methods for estimating vector competence and disease transmission risk. Disease transmission risk is arrived at by combining several population parameters with vectorial capacity (or R_0), indicating the ability of a mosquito population to maintain MBD transmission (see Box 6). Complementary information is given in Annex 6.

When planning NMS surveillance, stakeholders should contact research institutions dealing with native mosquito species to provide support during the preparation and implementation phases of pre-disease and disease stages.

Interdisciplinary research teams (e.g. medical entomology, public health, meteorology, GIS) might be involved in investigating population parameters and estimating the risk of MBD pathogen transmission. Based on these results, decision-makers from ministries responsible for human and animal health, agriculture and environment may be able to apply tailored control measures which can efficiently combat outbreaks.

Box 5: Key issues and recommendations for mosquito population parameters

- The main parameters are 1) abundance and population dynamics, 2) longevity, 3) biting behaviour, and 4) dispersal. Estimates of these parameters are available in the literature. However, it is recommended that they are assessed for each local mosquito population as they might vary due to the species and environmental factors.
- These four parameters are important for determining the epidemiological status of the vector population, for assessing the risk of MBD pathogen transmission, and for developing/evaluating effective control programmes.

Table 11. Main characteristics of mosquito population key parameters

Parameters	Information provided	Strengths	Weaknesses	Data collection methods, equipment
Population abundance and seasonal dynamics	Quantitative estimates of NMS adult population; seasonal dynamics; comparative analysis over the years; nuisance and MBD RA	Supports the evaluation of nuisance threshold definition and specific RA and control efforts	Requires good organisation and quality control	• Larval habitat or adult surveys • Adequate field material
Female longevity, gonotrophic cycle and dispersal	Life history traits required to evaluate MBD risk	Valuable data to feed epidemiological equation	Requires high-tech laboratory; large spatio-temporal variability; needs replication; expensive	• Mark–release–recapture • Laboratory experiments • Rearing facilities and specialist equipment
Female biting behaviour	Life history trait required to evaluate MBD risk, nuisance protection, nuisance threshold	Valuable data to feed epidemiological equation; inform citizens	Requires high tech laboratory; extensive field work; expensive	• Field and laboratory experiments • Traps and laboratory equipments
Female resting behaviour	Life history trait required to evaluate MBD risk; nuisance protection; vector control	Informs citizen; defines adult control measures	Extensive field work; medium costs	• Natural resting site surveys • Resting traps
Population vector competence	Life history trait required to evaluate MBD risk for main pathogens	Essential data to feed epidemiological equation	Requires BSL3 laboratory; expensive	• Laboratory infections • BSL3 rearing facilities and equipment
Transmission risk	Synthetic estimate of the MBD risk for main pathogens in local context and during the season	Evidence-based support for public health policies	Requires skilled entomologists, epidemiologists, statisticians; high tech laboratory; expensive	• Gathering field and laboratory data • Analysis • Modelling • Mapping equipment

Box 6: Introduction to MBD transmission models

Many transmission models have been developed for MBD (i.e. primarily for malaria), e.g. human blood index (HBI), entomological inoculation rate (EIR), vectorial capacity (C), and human biting rate (HBR). The complexity of the vector-borne diseases cycles implies that mathematical models have to include a large number of parameters. A discussion of the basic reproductive number for MBD and its implications for MBD control can be found in Smith & McKenzie 2004.

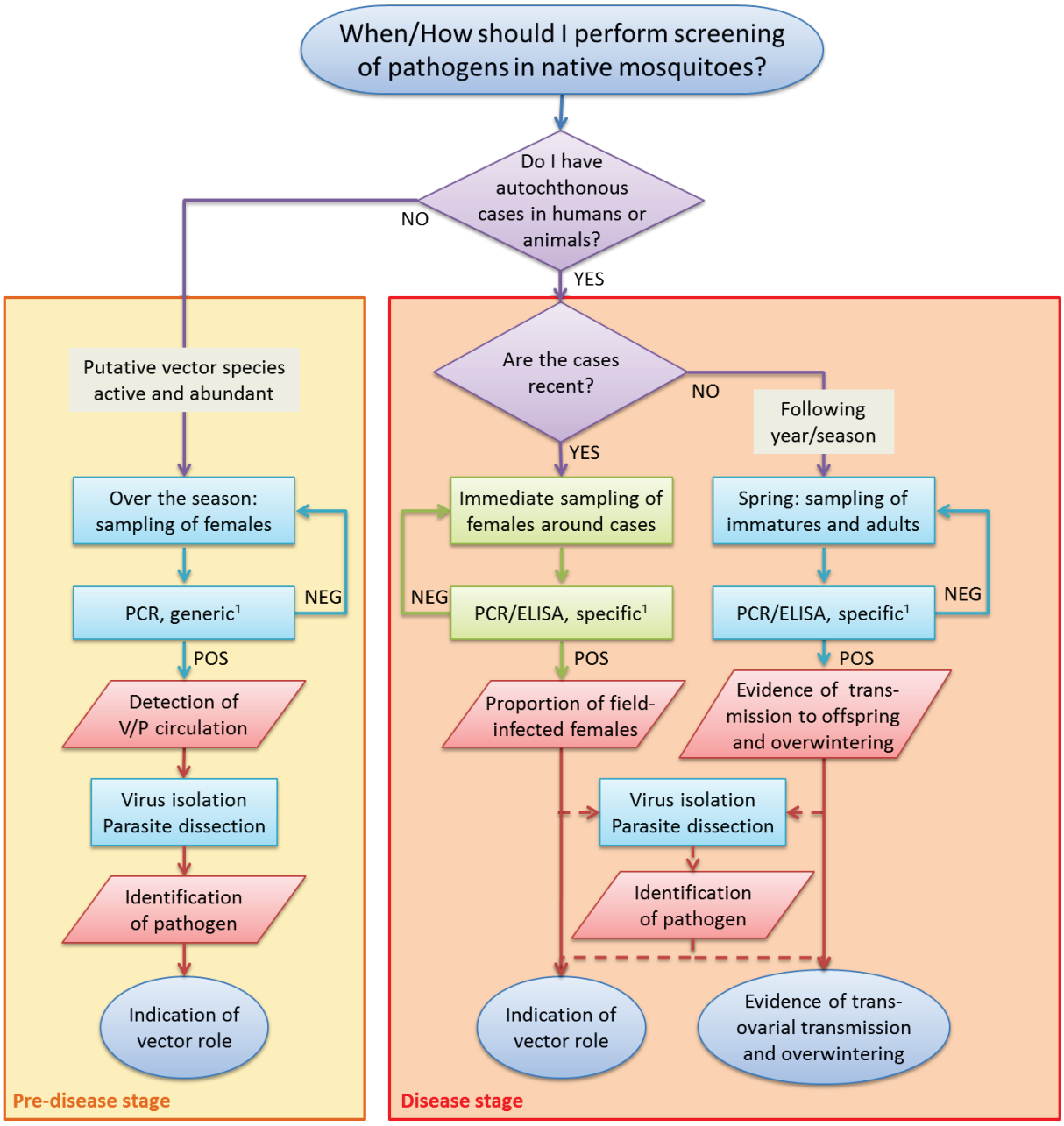
The commonly used basic reproduction number R_0 (sometimes incorrectly called 'basic reproductive rate'; R_0 is a dimensionless number and not a rate, which would have units of time) is defined as the expected number of secondary cases produced by a single (typical) infection in a completely susceptible population. It is the product of transmissibility (i.e. probability of infective contact between an infected and a susceptible individual), the average rate of contact between infected and susceptible individuals, and the duration of infectiousness. When $R_0 < 1$, each infected individual produces, on average, less than one new infected individual, and therefore the infection will eventually disappear from the population. If $R_0 > 1$, the number of infections will increase and the disease will spread further within the susceptible population.

Vectorial capacity (a mosquito parameter component of R_0) is a measure which is essentially independent of the prevalence of pathogen infection. It represents the transmission potential of a local mosquito population and is very similar to R_0 because it represents the expected number of humans infected per infected human per day (assuming perfect transmission) in a completely susceptible human population. Adding transmissibility and the duration of infectiousness, produces a measure directly analogous to R_0. (See Annex 6 for references).

2.5 Procedures for mosquito pathogen screening and identification

The collection of datasets (red parallelograms, Figure 11 below), obtained from key (green rectangles) and optional procedures (blue rectangles), is based on required outputs (blue ovals at the bottom).

Figure 11. Decision tree for screening of virus/parasite (V/P) pathogens in native mosquito species, showing procedures, datasets, and their expected outcomes (pre-disease and disease stages)



[1] *If results are negative, representativeness and sample quality have to be reconsidered.*

Why and when screen pathogens in mosquitoes?

In the case of an MBD outbreak or when the presence of a MBD pathogen is reported in, or close, to an area under surveillance, or if a significant risk of MBD pathogen introduction exists, the risk for transmission of the MBD pathogen should be assessed, following the established RA procedures. If preventative measures are already in place, they should be strengthened. Risk management decisions (including vector control measures) can be restrictive and costly, and need to be based on reliable evidence obtained through an RA process (see Chapter 1.3). When the implicated native mosquito is present in wide areas, a survey should assess the abundance of the population and its seasonal dynamics (throughout the year). These data, together with other mosquito population parameters (e.g. daily survival rate, biting rate) will help to evaluate the vectorial capacity of the mosquito population in the local context. Knowledge of when putative mosquito vectors are active is critical at this point and needs to be linked to other data in order to exclude other sources for potential outbreaks, e.g. the import of infected animals, or people who have travelled to an affected area from a third country.

The RA process is lengthy and complex, and reliable indicators may be more rapidly obtained by active screening of pathogens in mosquito females collected initially around reported MBD cases. However such screening provides limited information as 1) negative results do not prove the absence of pathogen circulation in the considered species, 2) positive results do not necessarily prove an active vector role for that species. But if efficient vector competence and vectorial capacity can be confirmed from scientific literature, it is likely that the species is involved in the transmission process, which helps to focus the risk management measures. When vector species are well known, there is no need to further investigate mosquito infection for the considered pathogen, except if a change in transmission patterns is observed. Triggers of such change can be the involvement of a mosquito species usually not known as a vector, or a genetic mutation/adaptation of a pathogen to a vector species. Investigating these possibilities will contribute to understanding the transmission picture. At the end of the mosquito season, all surveillance results and other mosquito population parameters should be analysed in order to evaluate more accurately the specific vectorial capacity of a species/population in a given local context.

Active screening of pathogens in larvae or freshly emerged female and male mosquitoes should be performed during and after every MDB outbreak in order to identify possible trans-ovarial pathogen transmission. A positive result indicates that the pathogens may be able to overwinter in mosquitoes, which means that supplementary control measures may be required to prevent/limit further transmission, persistence, or spread of the MBD.
A negative result, while not conclusive, indicates that the risk of overwintering is negligible, provided the sampling was sufficiently extensive.

Active screening of pathogens in female native mosquitoes can be carried out even if there is no evidence of MBD pathogen activity in order to rule out the possibility of introduction. Here again, negative results do not prove the absence of the pathogen with certainty, but they can be used to reassure the general public and counter media speculation about MBD threats. Also, screening female native mosquitoes contributes to the global screening for circulating pathogens.

Screening of pathogens

Pathogen screening in mosquitoes helps to evaluate the infection rate for single species or species groups collected over a defined time period and geographical area; several calculation programmes exist for this purpose (Annex 6). Apart from providing the basis for the rapid implementation of risk management measures, pathogen screening also provides 1) the option of genome sequencing, which can confirm the preliminary identification of the pathogen species and strain, and may also give an indication of its geographical origin, its pathogenicity, and evidence of genetic mutation/adaptation; and 2) the opportunity to detect other pathogens not expected to be present in the area.

Mosquito/pathogen surveillance can be implemented without any reports of MBD cases as a tool for detecting a pathogen before there is evidence of human cases. Thus, mosquitoes are used as 'sentinels'; alternatively, mosquito/pathogen surveillance can also detect non-target pathogens.

Research programmes, rather than routine surveillance schemes, would be the natural choice when conducting pathogen surveillance. Whether an economic argument can be made for the introduction of routine pathogen surveillance, depends on a convincing cost-benefit analysis, available financial resources, and the support from policymakers.

Pathogen detection

Screening for pathogens in mosquitoes can be expensive, particularly if extensive mosquito sampling has to be carried out. To bring down costs, collected mosquitoes can be used for both standard surveillance and pathogen detection, provided that trapping and all subsequent activities are organised appropriately. When aiming at detecting parasites, mosquito samples can be stored in alcohol or dried. In order to optimise detection capacity, mosquitoes need to be collected alive (trap catches collected within 24 hours), transported alive (or stored in dry ice during transport) to the laboratory, rapidly identified using refrigerated plates (chill table), and pooled by species. Pooled samples should be labelled and documented (time, day, and place of collection; see Chapter 2.7). Samples should be stored at −20° C (short term) or at −80° C or below (long term) and should at no stage be thawed and refrozen.

If freezing is not possible, and the target of surveillance contains a RNA genome, using RNA-stabilising reagent (e.g. RNA*later*) for storage at room temperature is a proven alternative, but this may prevent successful virus isolation because of protein inactivation. Samples (individual mosquitoes, or, more likely, pools) need to be homogenised, ideally in a medium suitable for virus isolation, and nucleic acid needs to be extracted. This can be done manually but bead-beating devices and robotic extraction platforms are commonly used to increase throughput. Extraction methods for RNA and DNA vary, and a decision on which one to use depends on the pathogen(s) under investigation. In order to efficiently use resources and maximise the range of detected pathogens, screening should be a two-stage process, first using generic molecular detection methods (e.g. polymerase chain reaction) on all samples, followed by specific tests on those samples that test positive.

An aliquot of the sample homogenates investigated by molecular screening should be retained so that the aliquot of the samples that tested positive with molecular methods can be tested for specific pathogens.

Native mosquitoes can be tested for arboviruses (RNA genome) or for parasites (DNA genome), such as *Plasmodium* spp. or filarial nematode worms, by adapting existing methods. Very few commercial kits are available for analysing samples for arboviruses. However, molecular detection methods are improving quickly, both in terms of efficiency and cost reduction, and laboratories should be aware of these developments. Optimal handling, preparation and storage of the sampled mosquitoes are essential to obtaining good results, and protocols should be defined in collaboration with the laboratory that will perform the analysis. Most European countries have laboratories that are able to provide advice on (or screening services for) the infection status of sampled mosquitoes and can identify pathogen species and strain. Depending on the equipment and skill of the laboratory, the vector susceptibility and competence of mosquito populations can also be investigated. These tests must be performed by laboratories with appropriate biosafety levels.

Once a surveillance programme has been selected, it is recommended that networks such as the 'European Network for Diagnostics of 'Imported' Viral Diseases' (ENIVD; www.enivd.org) are informed. Surveillance networks can facilitate the search for appropriate laboratories which boast the facilities required for pathogen identification and at the same time meet biosafety requirements. Since there are no diagnostic laboratories focusing only on diagnostics of arboviruses, networks and laboratories in other European countries may be asked for support. In addition, EC-funded projects dealing with vector-borne diseases, e.g. VBORNET (www.vbornet.eu) or EDENext (www.edenext.eu), may be able to assist in identifying the laboratory best able to provide advice and/or support for collaborative studies.

Complementary information on methods, analysis and presentation of data from mosquito pathogen screening is given in Annex 7.

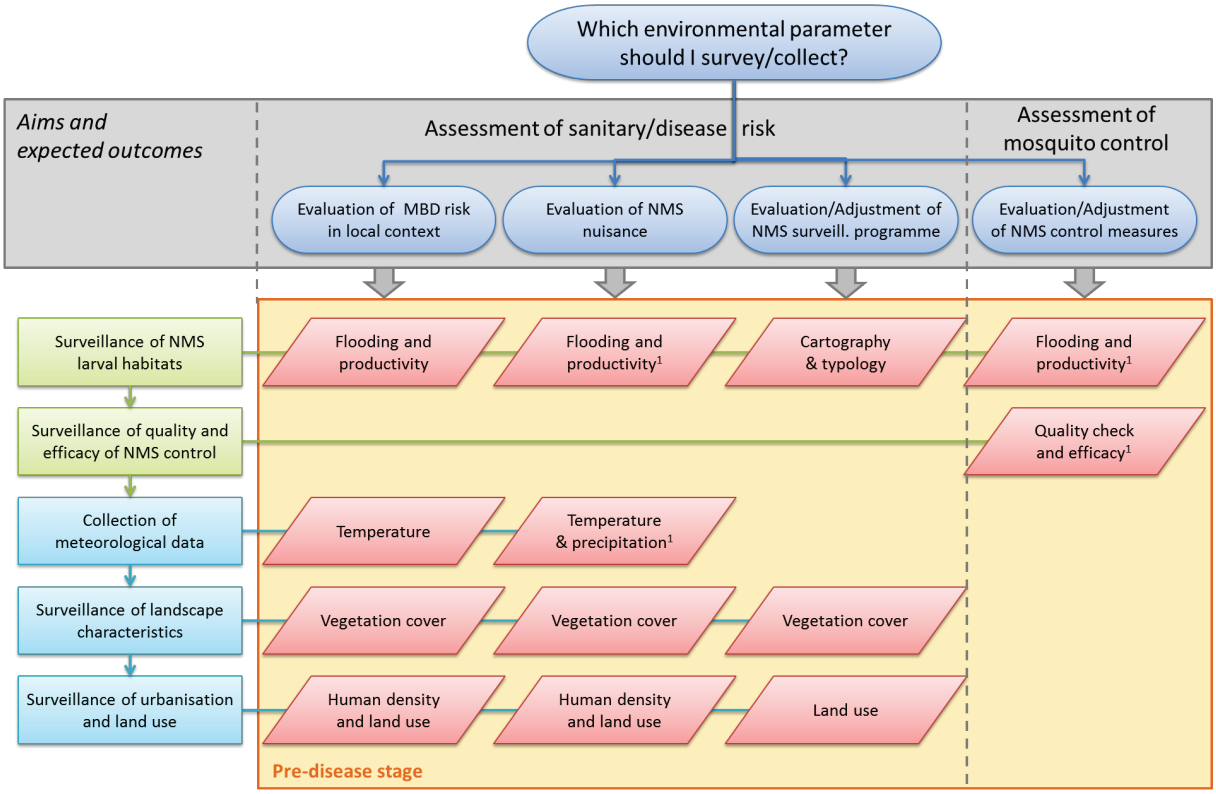
Box 7: Key issues and recommendations for pathogen screening

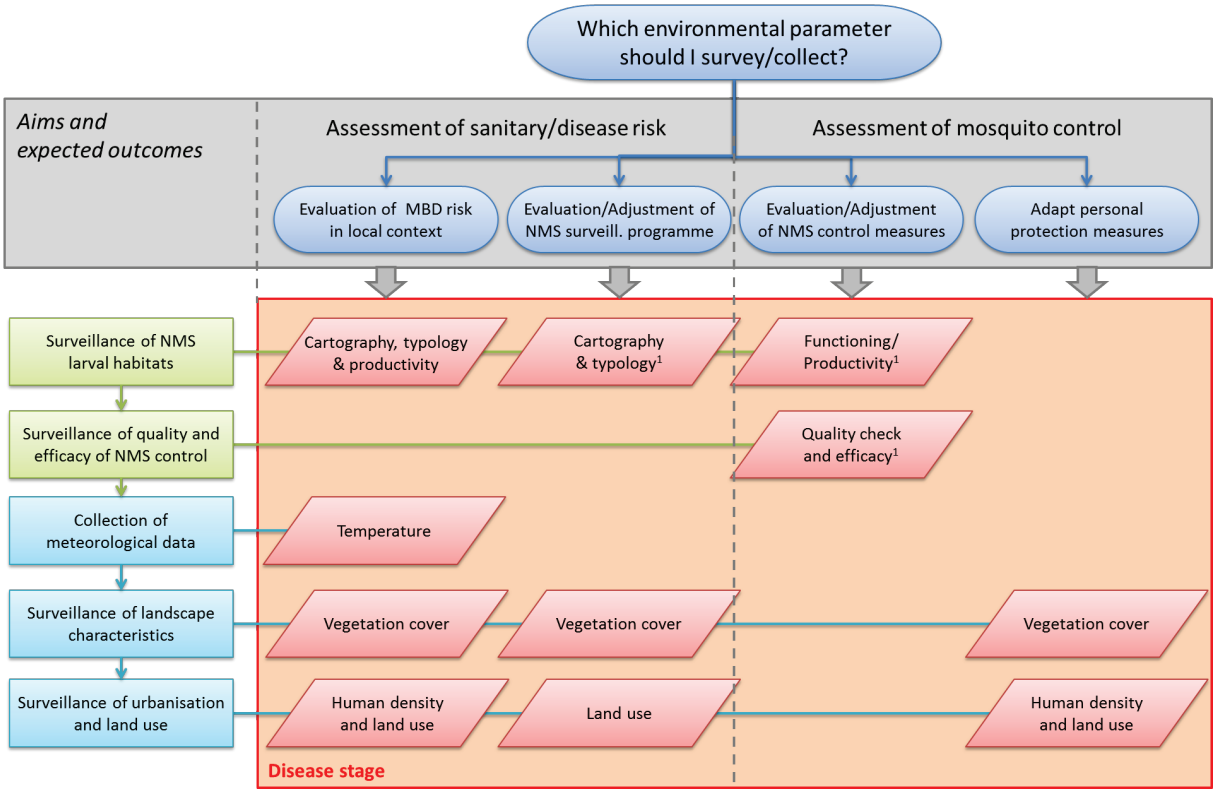
- Determine the aim of the surveillance for pathogen screening in surveyed mosquitoes based on the context.
- Identify a competent and properly equipped laboratory and decide on a method of analysis; adapt the protocol for sample collection and handling in accordance with the analysis method chosen.
- Keep an aliquot of the sample submitted to molecular detection for further pathogen isolation/identification on positive samples.

2.6 Procedures for the collection of environmental parameters

The collection of datasets (red parallelograms, Figure 12 below), obtained from key (green rectangles) and optional procedures (blue rectangles), is based on required outputs.

Figure 12. Surveillance procedures and data collection for environmental parameters and their related datasets; pre-disease (top) and disease stages (bottom)





[1] *Can be collected as part of nuisance control activities.*

In addition to the biological factors specific to particular mosquito species, environmental factors play an important role in determining the mosquito population size, the vectorial capacity of native mosquito species, and consequently the pathogen transmission risk. Environmental factors include 1) type and size of available larval habitats, 2) climatic conditions (in particular precipitation and groundwater levels), 3) environmental change, 4) human population density, 5) increased human travel, 6) changes in living and agricultural and veterinary habits (land use), and 7) reduction of resources in the life cycle of mosquitoes by interventions (e.g. source reduction of aquatic habitats).

Crucial environmental parameters to be considered are the distribution, typology, functions (flooding rhythm), and productivity of larval habitats. They provide key information needed to calculate population abundance and assess the risk of pathogen transmission.

A thorough knowledge of the most productive larval habitats is needed to establish which type of larval habitat should be sampled to provide the best indicators of mosquito population abundance. Control programmes may also benefit directly from the identification of which larval habitat would be most effective to target. Reporting the precise location (cartography) of larval habitats is crucial. Finally, collecting information on control measures may be worthwhile in order to assess the quality and efficiency of control activities. This information might prove to be helpful when cost effectiveness has to be evaluated or a control campaign needs to be justified.

Table 12 summarises the main characteristics of environmental parameters to be considered for native mosquito species surveillance. Additional information on environmental parameters and their acquisition methods are given in Annex 8.

Box 8: Key issues and recommendations for environmental data

- Key parameters to collect are 1) larval habitat availability, typology, functioning, and productivity (for adapting native mosquito species control) and 2) quality and efficacy of native mosquito species control measures.
- Other parameters may be prioritised according to scenarios, aims and expected outcomes.
- The main meteorological factors include 1) temperature, 2) rainfall and humidity, and 3) wind; these data are usually monitored locally, with data maintained nationally; portable meteorological stations can provide measurements at places where national meteorological institutes maintain no monitoring points.
- In addition, monitoring environment changes (land use, climate) is particularly relevant for the planning of mitigation measures.

Table 12. Main characteristics of environmental parameters to be considered for native mosquito species surveillance

Parameters	Information provided	Strengths	Weaknesses	Data collection methods and equipment
Larval habitat typology, functions and productivity	Breeding locations for a species; relative productivity of breeding site types; distribution of sites; time/season of mosquito production	Good support in the ecological understanding of NMS; identification of targets for NMS control	Requires skilled technicians; high cost	• GIS and field data collection
Temperature geo-distribution and trend over the year	Indicates the suitable period for activation of surveillance; informs the model for MBD RA; correlates with NMS longevity and vectorial capacity; explains vector behavioural changes	Data usually available in high definition	Site-specific weather data cannot always be obtained from local weather stations	• Data from weather stations usually available locally • Field-collected data based on portable weather stations
Precipitation distribution	Correlates with the NMS population density; informs the population estimation models	Data usually available	Large local variability difficult to define	• Field-collected weather data
Human density and movements	Informs the model for MBD RA	Data usually available with good local definition	Human behaviour influences the usefulness	• Socio-statistical data
Landscape characteristics/vege-tation covering	Suitability of the area for colonisation and dispersal	Data usually available in good local definition (e.g. CORINE dataset)	Requires skilled GIS technicians	• Remote sensing data • Satellite imagery
Urbanisation and land use in relation to water-storage habits	Type of water bodies and land cover to be characterised in terms of potential larval habitat availability and energy resources	Data usually available for public areas, but to be correlated with specific NMS requirements	Private areas difficult to assess; requires time-intensive research study	• Remote sensing data • Satellite imagery • GIS field data collection
Quality and efficacy of NMS control measures	Informs the models for estimation of cost effectiveness; evaluation of control methods including community participation; resistance management	Maintain independent quality control on NMS control programmes	Requires independent, objective and science-based evaluation, and skilled technicians	• Internal evaluation • External evaluation

2.7 Methods for data management and analysis

The agencies funding NMS surveillance may consider themselves sole owners of the collected data, and may therefore be reluctant to share them. However, it is important that NMS surveillance data, including pathogen detection data, are made freely available to the public health authorities. Data exchange between competent authorities should be promoted, both between local and regional/provincial authorities, as well as between national and international authorities. This is even more crucial if important changes are detected (e.g. pathogen transmission or MBD outbreak). Comparing and interpreting shared data will be made much easier if data are collected and stored based on a set of harmonised procedures.

Basic data management features for storage and analysis

Data collection involves a number of steps including a) trapping, b) sample handling, c) labelling, d) transportation, e) diagnostics, f) database population, and g) preparation of acquired data for analysis. All these steps have associated errors which may include inaccurate recording of sample locations (GPS coordinates can reduce the error rates); samples that get lost, handled, or stored in a way that prevents further processing and analysis of specimens or pathogens; labelling errors or samples not labelled at all; diagnostic mistakes, mixing-up samples during the identification process ('Did this mosquito come from this vial or that vial?'); mistakes during data entry; mixing-up rows/columns in a spreadsheet. Each of these mistakes will affect the final analysis. Generally speaking, the more steps, the greater the chance that mistakes and errors will be made. A combination of well thought-through methods and data management, combined with an awareness of mistakes in all steps can help prevent most of them.

Box 9: Field sample labelling

Small-scale surveillance studies with relatively few samples can probably be conducted with hand-written labels, but as soon as large numbers of samples are expected, labelling needs to be more rigorous. Each label must record a range of information, such as collection date, location (as precise as possible), and type of sampling. This information can be put on the label, with the identification result added later, but since space is limited it has to be decided early which information to include in the label.

The preferred method is to use 'unique computer-generated label numbers'. When a sample is collected in the field (e.g. a vial with mosquito larvae in alcohol), the strip is placed in a sealed bag (only one sample per bag) and immediately labelled with a unique identification number on an irremovable sticker. Basic sample data (type of surveillance, date, location) are filled in on either a) a paper form marked with a sticker with the unique identification number to prevent errors, or b) are entered electronically in a tailored software programme on a smartphone which connects to the data-management system on a server (e.g. Modirisk, http://www.modirisk.be/; VecMap, http://iap.esa.int/vecmap). In the laboratory, further information on the sample can be entered into the data management system. This way, all additional information on individual mosquito samples remains connected to its unique identification number.

Data analysis is the end point of a chain of events which includes several steps.

- Step 1. Defining the type of outputs needed (e.g. presence/absence maps of a native mosquito species in a defined geographical area and time period.
- Step 2. Defining the type of data required to produce the desired output, e.g. geo-referenced presence/absence data on a native mosquito species in a defined area and time period. In most cases of NMS surveillance, presence/absence data of a certain native mosquito species in a defined geographical area and time period is the type of basic data needed.
- Step 3. Defining the type of surveillance necessary to obtain the required data (see scenarios in Chapter 1.2).
- Step 4 marks the actual start of data management.

Table 13 lists the minimal datasets required for key (and some optional) procedures. It is suggested that European countries use these standardised datasets to facilitate collation of data at the European level.

Table 13. Suggested basic set of variables to be included in databases of surveillance of native mosquito species; key procedures (top) and optional procedures (bottom)

Data label	Format
Level 1 – per sampling	
Type of surveillance	Name
Type of sampling or trap	Method or trap model ID
Date	DD/MM/YYYY
Country	Name
NUTS	Code
Geo-referenced latitude[1]	DD.NNNNN
Geo-referenced longitude[1]	DD.NNNNN
Altitude	N
Data entering	Name (person)
Level 2 – per mosquito species	
Mosquito species	Species name or ID
Presence/absence	1/0
Female	N
Male	N
Pupa	N
Larva	N
Egg	N
Identificator	Name (person)
Method of identification[2]	Name (from a list)
Validator	Name (person)

[1] For the sampling point, UTM WGS 84 system, decimal degrees units; [2] Morphology/molecular (gene)/MALDI-TOF

Data label	Format
Level 1 – per sampling	
Temperature	N
Relative humidity	N
No. of mosquitoes tested	N

Data label	Format
Data entry	Name (person)
Level 2 – per mosquito species	
Physiological status	Blood fed/unfed/gravid/nulliparous
No. of mosquitoes tested	N
No. of pools tested	N
Pathogen name, positive pools	N

N = numeric field

Database

Most researchers are comfortable with spreadsheet software such as Microsoft *Excel*. For easy data mining (extracting data from a large database), such software is useful, but for more complex analysis and for mapping, it is recommended that data be stored in database management system with networking capabilities. Examples of frequently used relational database management systems (RDBMS) are Microsoft *Access*, Microsoft *SQL Server*, IBM *DB2*, and Oracle *Database*. By using a RDBMS, data integrity can be ensured, e.g. a properly defined rule can ensure that entered data conform to a valid format. Another advantage is that the amount of data administered by an RDBMS is virtually unlimited. System administrators can assign various levels of access rights, which makes it possible, for example, that the analyst cannot modify surveillance data. (See Annex 9, Figure A, for a sample database.)

For GIS analysis or modelling, data can then be imported into statistical software packages or dedicated geographical information systems software such as ArcGIS, GRASS, or QGIS. The mobile GIS solution Sentinel GIS lets users handle field surveillance data and mosquito control data, but requires Esri *ArcPad*, a mobile field mapping and data collection software. Currently, a new system under development ('VECMAP') promises an integrated approach which covers the entire process from data collection to the production of vector and risk maps. VECMAP will offer safe data storage and provide data analysis and statistical distribution modelling based on weather data and satellite imagery. The development of this system, which received initial funding by the European Space Agency, is in its demonstration phase[2].

Groups that carry out NMS surveillance often develop customised approaches to surveillance, which makes data exchange difficult. It is therefore advisable to standardise data management and processing by using one centralised database for all surveillance data. Access should be online and managed by dedicated system administrators. The advantages of a centralised database are that 1) there is no need to extract data from different databases in order to compare separate studies, 2) data loss is less likely, 3) training needs are low when only one database system has to be taught, and, perhaps most importantly, 4) data integrity is ensured (e.g. automatic removal of 'wrong doubles').

Additional information on data management and analysis is given in Annex 9.

Box 10: Key issues and recommendations for data management and analysis

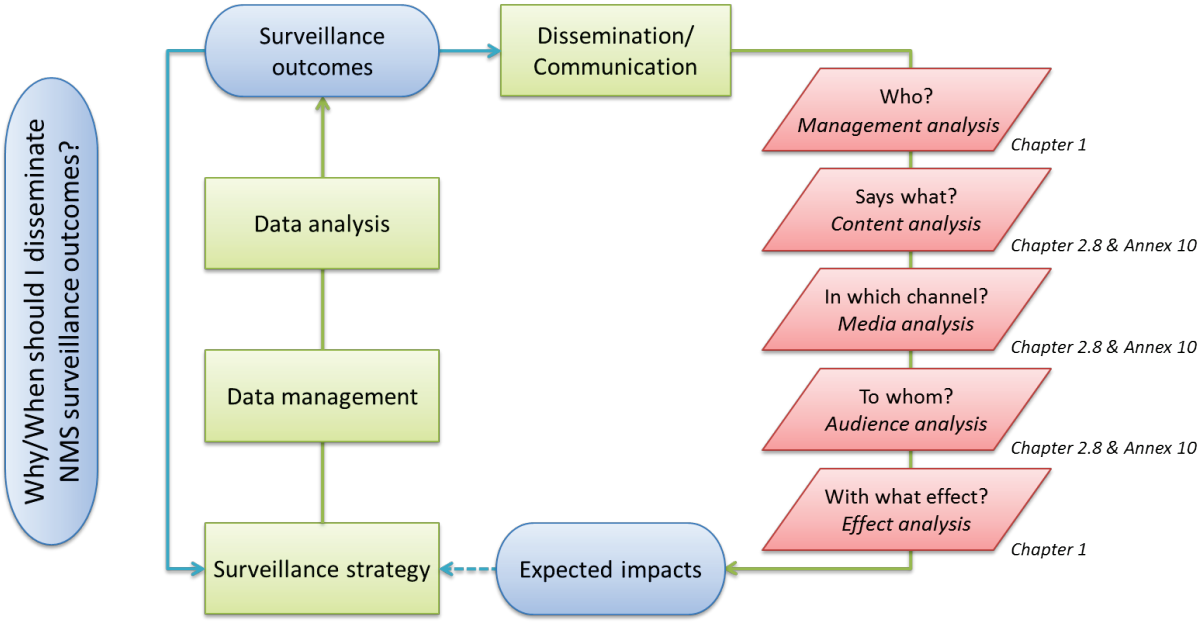
- Harmonise data management through standardisation.
- Ensure correct labelling of samples at each step.
- Ensure long-term storage and continuity of datasets on surveillance activities.

[2] European Space Agency. Mapping the deadly mosquito. [Internet]. 2013 [cited 2014 Oct 20]. Available from: http://www.esa.int/Our_Activities/Telecommunications_Integrated_Applications/Mapping_the_deadly_mosquito

2.8 Strategies for data dissemination and mapping

The red parallelogram chain corresponds to Lasswell's communication model.

Figure 13. Native mosquito species surveillance: dissemination/communication of outcomes and effects



Surveillance of native mosquito species produces a wide range of data that need to be disseminated to the different stakeholders. The method of dissemination depends on a) the communicator; b) the message and thus the type of data and level of detail; c) the selected presentation medium (charts, maps, plain text); d) the recipients, e.g. end-users or non-technical audiences; and e) the desired social, political or scientific impact and any feedback envisaged (see Figure 13, Lasswell's model). Both the timing (e.g. during an MBD outbreak or not) and the costs incurred by the chosen dissemination method also have to be considered.

Dissemination pathways should be carefully developed and adapted to stakeholders, end-users and other target audiences (general public, NGOs, etc.), depending on the sensitivity of the information. The choice of data type, level of detail and presentation style (charts, maps, plain text, etc.) should be tailored to the target group and desired impact. The language used may depend on the scale of the dissemination campaign. The timing of a campaign should be adjusted according to the response required from the target audience (i.e. before mosquito breeding season if a preventative response is expected, and/or during the season when the general public is already aware of the nuisance caused by mosquitoes).

Scope and audience

Suggestions of the major types of target audience and prioritisation of data dissemination for each scenario for NMS surveillance are set out in Table 14. It is important that the local context is carefully assessed when defining the communication strategy, in particular the mechanisms of collaboration between agencies and the required level of community participation.

Table 14. Data dissemination: main audiences and prioritisation of dissemination, by scenario and threat level

Main surveillance outcomes	National authorities	Regional/provincial authorities	Local authorities	Mosquito control units	Health units	Environment agencies	Scientific community	Media	General public
Scenario 1: Pre-disease stage									
1a: Initial assessment									
• Presence of competent vectors	1	1	1	1	2	2	2		
1b: Preparedness									
• Active and abundant vectors	1	1	1	1	1	2	2	2	2
• Implementation of control programmes	1	1	1	1	2	2	2	2	2
Scenario 2: Disease stage									
2a: Occasional cases									
• Detection of pathogens in mosquitoes	1	1	1	1	1	1	1	1	1
• Reports about competent vector populations	1	1	1	1	1	2	1	2	2
• Reports about environmental and weather conditions suitable for transmission	1	1	1	1	1	1	1	1	1
• Implementation of control programmes and evaluation of their effectiveness	1	1	1	1	1	1	1	1	1
2b: Recurrent cases									
• Start/end of period of transmission	1	1	1	1	1	1	2	1	1
• Reports about suitable environmental and weather conditions which could lead to changes in transmission patterns	1	1	1	1	1	1	2	1	1

1 = priority; 2 = secondary. Audiences listed as '1' should be sent the surveillance information as soon as possible, whereas audiences listed as '2' may be informed later. Priorities must be re-assessed/adapted according to the local context. Outcomes are listed when first included in a threat level but are not repeated in higher threat levels.

The scope and methodologies of NMS surveillance differ greatly between areas, local situations, and scenarios. Consequently, data dissemination and communication should be adapted to the local context, and steps should be taken to avoid misinterpretations of surveillance results or the level of risk.

Reports on putative vector NMS populations (presence, abundance, adult activity, infection by pathogens, etc.) should be immediately reported to all national and local authorities so that risk plans and customised control measures can be amended accordingly. Data exchange with neighbouring countries and regions, in particular for data that indicate infected vector populations, should be established.

If, by chance, infection is detected in a putative vector (by passive or active surveillance), the scientific community should be informed because they can conduct a study even before specific surveillance measures are implemented. If they detect evidence of a vector role, it is advisable to inform the scientific community – preferably EMCA through their EMCA national directors. Healthcare professionals in cities/areas of concern should be rapidly informed about the MBD risk (and MBD symptoms). This is particularly important when changes in the transmission pattern are detected (e.g. evidence of circulation of pathogens, adult mosquito activity period, and suitable environmental and weather conditions).

Since some native mosquito species are strongly connected to urban environments (e.g. *Cx. pipiens*), larval control is most efficient if the community participates. In some countries, gaining entrance to private properties for mosquito surveillance and/or control purposes is difficult, which may stymie larval habitat treatment and the removal of breeding containers (see Annex 10, Box A). Community participation can be improved through active dissemination of information at various levels (municipality, province, local or national public health institutes), educational efforts aimed at schools, appearances on TV and radio, internet activities, door-to-door leaflets, etc.

Dissemination methods

The dissemination of results should be tailored to the intended target audience, the intended impact, and the type of information to be disseminated. Among the most suitable methods for sharing information is a dedicated website which presents programme details, progress, and results. Maps which show distribution changes in a defined area (see below) are particularly instructive.

More information on this topic can be found in Annex 10.

Mapping strategy

The bulk of surveillance data will be geo-referenced information on the presence/absence of native mosquito species at a defined time period (day, week, month, year). It should be noted that the absence of native mosquito species from one location does not prove that native mosquito species are not present at or near the sample location. Mosquitoes may not be detected because of unfavourable weather conditions, ineffective traps (bait is often species-specific), or low mosquito density (i.e. below the detection threshold). However, continued sampling throughout the season will increase the data accuracy, making it possible to interpret negative results as true absences.

Surveillance data can be visualised as maps which depict 1) the presence/absence of native mosquito species for both scenarios, 2) abundance levels of a native mosquito species in scenario 2, and 3) show the presence of MBD pathogens in field-collected mosquitoes. Such data can be extrapolated by modelling possible NMS distributions or using known NMS vectorial capacity within an R_0 model of pathogen transmission risk for a defined period.

3 Cost and evaluation

3.1 Estimating cost of surveillance activities

In order to help with cost control, the following section provides the costs of all major field sampling/trapping activities. Table 15 lists procedures and sampling methods with estimates of the related costs in working days (wd, first line) and of additional costs (in euros, second line: travel, consumables, etc.) for a whole year of surveillance.

Table 15. Cost estimate (per year of surveillance), active field surveillance procedures

Pre-disease stage	Disease stage	Procedures and sampling methods	Parameters	Field investigations[i] (EUR)	Laboratory investigations (EUR)	Data processing[ii] (EUR)	Communication/dissemination (EUR)	Total (EUR)
x	x	**Presence and distribution of mosquito species**						**212 wd 8 630**
		Larval search (dipping)	Twice a month Apr–Nov 2 500 km^2 1 sampling-trapping site/100 km^2	50 wd 2 100	25 wd 200	5 wd 5	5 wd 10	85 wd 2 315
		Adult trapping		67 wd 5 900	50 wd 400	5 wd 5	5 wd 10	127 wd 6 315
x	x	**Adult mosquito abundance**						**127 wd 6 315**
		CO_2-baited traps	Twice a month Apr–Nov 2 500 km^2 1 trapping site/100 km^2	67 wd 5 900	50 wd 400	5 wd 5	5 wd 10	127 wd 6 315
x	x	**Seasonal activity**						**212 wd 8 630**
		CO_2-baited traps	Twice a month Apr–Nov 2 500 km^2 1 trapping site/100 km^2	67 wd 5 900	50 wd 400	5 wd 5	5 wd 10	127 wd 6 315
		Larval search (dipping)		50 wd 2 100	25 wd 200	5 wd 5	5 wd 10	85 wd 2 315
x	x	**Adult mosquito resting behaviour and resting sites**						**191 wd 5 530**
		Site visual inspection and aspirating	Twice a month Jan–Dec 2 500 km^2 1 sampling site/100 km^2	75 wd 3 000	38 wd 200	5 wd 5	5 wd 10	123 wd 3 215
		Resting boxes	Twice a month Apr–Nov 2 500 km^2 1 trapping site/100 km^2	33 wd 2 100	25 wd 200	5 wd 5	5 wd 10	68 wd 2 315
x	x	**Human and animal biting rate**						**332 wd 5 380**
		Host-baited traps or host landing collection	Weekly Jun–Oct 2 500 km^2 1 trapping site/100 km^2	125 wd 2 550	31 wd 200	5 wd 5	5 wd 10	166 wd 2 765
		HLC[iii]		125 wd 2 400	31 wd 200	5 wd 5	5 wd 10	166 wd 2 615
x	x	**Larval habitats locality**						**1 070 wd 3 950**
		Larval search	Monthly Mar–Nov 2 500 km^2 1 sampling site/10 km^2	703 wd 3 130	352 wd 800	10 wd 10	5 wd 10	1 070 wd 3 950
	x	**Quality and efficacy of control measures**						**34 wd 2 240**
		CO_2-baited traps	Before and after treatment (Jun–Sep) 2 500 km^2	20 wd 2 180	10 wd 50	2 wd 5	2 wd 5	34 wd 2 240

Pre-disease stage	Disease stage	Procedures and sampling methods	Parameters	Field investigations[i] (EUR)	Laboratory investigations (EUR)	Data processing[ii] (EUR)	Communication/dissemination (EUR)	Total (EUR)
			1 trapping site/250 km^2					
	x	**Pathogen screening in NMS**						**19 wd 3 730**
		Gravid traps and CO_2-baited traps	Weekly During and after outbreaks (May–Oct) 2 500 km^2 1 trapping site/250 km^2	5 wd 720	10 wd 3 000	2 wd 5	2 wd 5	19 wd 3 730

[i] *Including field work, training, travel;* [ii] *Including management, analysis, dissemination, and mapping; wd = working day;* [iii] *Human-landing collection*

In order to reduce the costs for a single procedure it is recommended that only one sampling method is used. The costs are listed for an area of 2 500 km^2 and are based on mean costs in the EU. As a general rule, all values should be adapted to the local situation to accurately calculate working days and other costs. Detailed calculations of cost estimates are shown in Table 16. The size of the surveillance area is chosen arbitrarily; the number of trapping sites per surface area is a realistic proposal but there is room for variations: if a large panel of native mosquito species is targeted (with little information available), the recommended 25 trapping sites can be distributed randomly throughout the area; if only few native mosquito species are targeted, the 25 traps should be placed in environments favoured by the species. The number of traps should be adapted in accordance with the resources and the study design: cross-sectional random sampling requires at least one trapping site per 300 km^2 to gather sufficient data for vector distribution modelling (as suggested by the Belgian MoDiRisk programme) and a tool (e.g. the VECMAP system) to determine the number of sites and their location.

Additional costs to be considered are initial investments in equipment (e.g. traps and batteries, animal bait, other field equipment, computers, software, vehicles, laboratory equipment), as well as human resources for the preparation phase (e.g. identification of sites, securing access agreements), training, management, quality checks, and evaluation costs. Purchase costs for traps are approximately EUR 150 for a CO2-baited trap (CDC or EVS), a light trap or a BG-Sentinel trap; EUR 700 for an MM trap; EUR 180 for a gravid trap; EUR 3 for a sticky trap; and EUR 1 for an ovitrap. All these costs should be adapted according to the local context (i.e. number of sites, size of surveillance area, local price levels, and specific constraints).

The number of traps and visits should be in accordance with the recommendations given in Table 15. The following formulae were used:

- Travel costs = total distance x cost rate per km travelled x number of visits
- Working days = number of sites * number of visits / number of sites per day

Adult trapping necessitates two visits per trapping period (setting and retrieving the trap), but retrieved traps can be moved to different locations on the same day. Visits for 'control measures' and 'pathogen' are calculated for four treatments and one outbreak, respectively. Laboratory working days are calculated for one sample/site/visit, 30 min/sample for morphology, and two hours for morphology and pathogen screening.

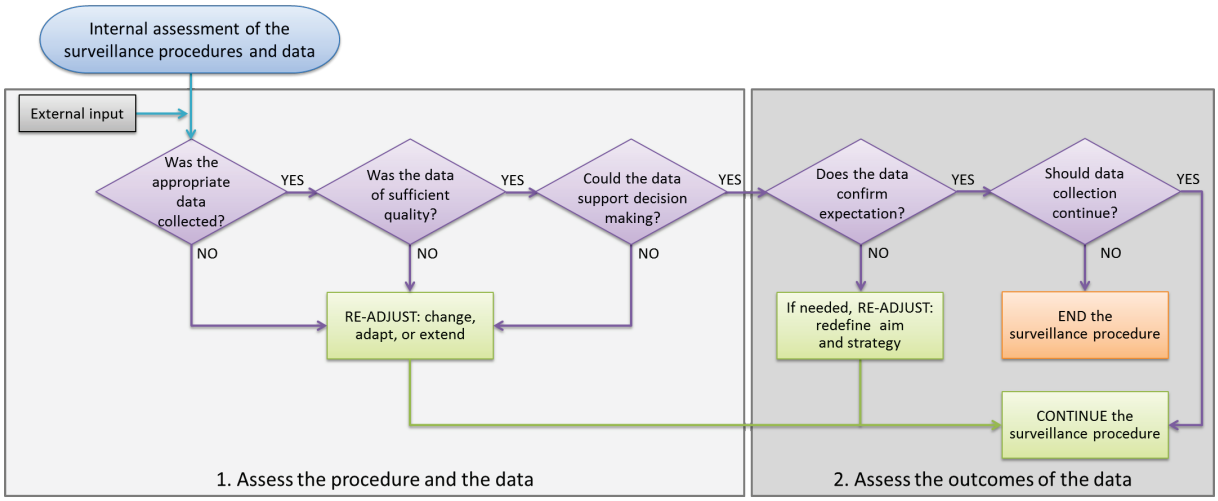
Table 16. Detailed calculations of cost estimates for field investigations according to the procedure and sampling methods, for a surveyed area of 2 500 km^2

	Km²	No. sites	Distance[i]	Total distance[ii]	Km rate	No. visits	Travel costs	No. sites/day	No. field working days	Consumables[iii]	No. of laboratory working days
Presence Larval search (dipping)	2 500	25	15	375	EUR 0.3	16	**EUR 1 800**	8	**50**	**EUR 300**	**25**
Presence Adult trapping	2 500	25	15	375	EUR 0.3	32	**EUR 3 600**	12	**67**	**EUR 2 300**	**50**
Abundance CO_2-baited traps	2 500	25	15	375	EUR 0.3	32	**EUR 3 600**	12	**67**	**EUR 2 300**	**50**
Seasonality CO_2-baited traps	2 500	25	15	375	EUR 0.3	32	**EUR 3 600**	12	**67**	**EUR 2 300**	**50**
Seasonality Larval search (dipping)	2 500	25	15	375	EUR 0.3	16	**EUR 1 800**	8	**50**	**EUR 300**	**25**
Resting Resting sites catches	2 500	25	15	375	EUR 0.3	24	**EUR 2 700**	8	**75**	**EUR 300**	**38**
Resting Resting boxes	2 500	25	15	375	EUR 0.3	16	**EUR 1 800**	12	**33**	**EUR 300**	**25**
Biting rate - Host-baited traps/catches	2 500	25	15	375	EUR 0.3	20	**EUR 2 250**	4	**125**	**EUR 300**	**31**
Biting rate HLC	2 500	25	15	375	EUR 0.3	20	**EUR 2 250**	4	**125**	**EUR 150**	**31**
Larval habitat Larval search	2 500	625	1.5	938	EUR 0.3	9	**EUR 2 530**	8	**703**	**EUR 600**	**352**
Control measures CO_2-baited traps	2 500	10	35	350	EUR 0.3	16	**EUR 1 680**	8	**20**	**EUR 500**	**10**
Pathogen Gravid and CO_2-baited traps	2 500	10	35	350	EUR 0.3	4	**EUR 420**	8	**5**	**EUR 300**	**10**

[i] Mean distance between sites; [ii] Total distance to be covered if visiting all sites within an area (starting point located in the same area); [iii] Trap lures, food for animal bait, vials and alcohol, labels

3.2 Evaluating the operational surveillance process

Figure 14. Flowchart for the assessment of surveillance procedures and data



Assessment of the surveillance procedures and data

As an initial step, the surveillance procedure and the significance and quality of the collected data have to be assessed based on the defined surveillance objectives: Were the collected data compatible with the objectives of the surveillance procedure? Were the data of sufficient quality? Can the data support decision-making?

In a second step, the outcome of the data is assessed: Do the data confirm the original assessments of the native mosquito species, the pathogen, and the site/landscape selection, or does the surveillance strategy need amending? Finally, is the expected outcome met and should data collection continue?

This assessment – carried out internally by a team member – will allow adjustments with regard to the reallocation of funding and/or other resources to other surveillance procedures within the same programme or to a different health programme. Nominating a trusted team member to conduct an assessment has the advantage that he or she has a good understanding of the surveillance objectives/strategy (as well as related problems), but it may be difficult for him/her to objectively assess the work carried out because of the close involvement in the project. If an internal assessment fails to deliver innovative solutions to recognised problems, an external expert should carry out the assessment. This assessment may be conducted annually.

Evaluation of the global surveillance process

The purpose of this evaluation is: i) to assess the structure and management of global activities, ii) to critically examine achievements and constraints, iii) to analyse cost effectiveness, and iv) to provide recommendations for possible improvements. The evaluation of the programme should focus on the overall management, the internal coherence and clarity of the objectives, the coordination (sharing of responsibilities), and the internal and external communication procedures. Attention should be paid to the sustainability of financial, logistical and human resource support and commitment given/offered by national/regional/local governments and stakeholders. The same criteria should be applied to networking and partnership activities with other relevant entities.

A global evaluation is preferably carried out by an external evaluator, i.e. by someone who is/was not directly involved in the development or operation of the surveillance system, is objective, has no vested interests, and can provide a fresh perspective. Disadvantages of tasking an external evaluator are related to a lack of involvement in project-related decisions. Documenting the different surveillance steps will facilitate such an evaluation. It is recommended that this assessment is repeated every three or five years.

Annex 1: Systematic reviews on existing surveillance guidelines and sampling and identification methods for native mosquito species

The use of systematic reviews (SR), as a standardised method, allows the identification, selection and critical appraisal of the currently available information, i.e. guidelines, sampling and identification methods for (native) mosquito species. Therefore, three SR were performed separately: i) on the existence of guidelines for the surveillance of native mosquitoes, ii) on the sampling methods used for native mosquitoes, and iii) on the different identification methods currently available for native mosquitoes.

Systematic review on guidelines for the surveillance of mosquitoes of public health importance in Europe

Research questions

- Are there any guidelines on the surveillance of mosquitoes of public health importance that could be used to harmonise surveillance in Europe?
- What are the recommendations for the surveillance of mosquitoes of public health importance in Europe?
- Which recommendations apply to native mosquito species?

Search string

'(mosquito OR mosquitoes OR Culicidae OR culicides OR Aedes OR Aedini OR Ochlerotatus OR Culex OR Coquillettidia OR Anopheles OR Anophelinae OR Culiseta OR vector) AND (surveillance OR survey OR plan OR monitoring OR study OR studies) AND (guidelines OR handbook OR template)'

Record exclusion criteria:

- not on mosquitoes
- clinical cases/management
- disease control
- abstract of meetings
- only abstracts available

Note: No restrictions based on time, geographical location, or language; search performed for all fields or, if number of records was too large, on title only.

Results

Table A1-A. Results of SR searches by database and first round of selection by screening title and abstract; SR A (existing guidelines), SR B (sampling methods), and SR C (identification of native mosquito species)

Literature databases	SR A			SR B			SR C		
	Collected	Included	Excluded	Collected	Included	Excluded	Collected	Included	Excluded
TRIP	385	3	382						
ArXiv	198	0	198						
US NGC	6	0	6						
Clinical DBS	6	0	6						
CDC	339	1	338						
MMWR CDC	198	2	196						
Vectorbase	0	0	0						
Cairns.info	140	0	140						
Cochrane review				1	0	1	2	0	2
PROSPERO				0	0	0	0	0	0
Ovid Medline	62	10	52	92	52	40	659	56	603
Embase Elsevier	0	0	0	0	0	0	0	0	0
Science Direct	1	1	0						
Web of Knowledge	5	4	1						
Web of Science				1,132	413	719	625	210	415

Literature databases	SR A			SR B			SR C		
	Collected	Included	Excluded	Collected	Included	Excluded	Collected	Included	Excluded
HAL.archives-ouvertes.fr	0	0	0	0	0	0	0	0	0
Open Grey	291	1	290	19	3	16	26	4	22
SUDOC	1	1	0	27	2	25	22	8	14
Thesis (FR, DE)							0	0	0
Total	**1 632**	**23**	**1 609**	**1 271**	**470**	**801**	**1 334**	**278**	**1 056**

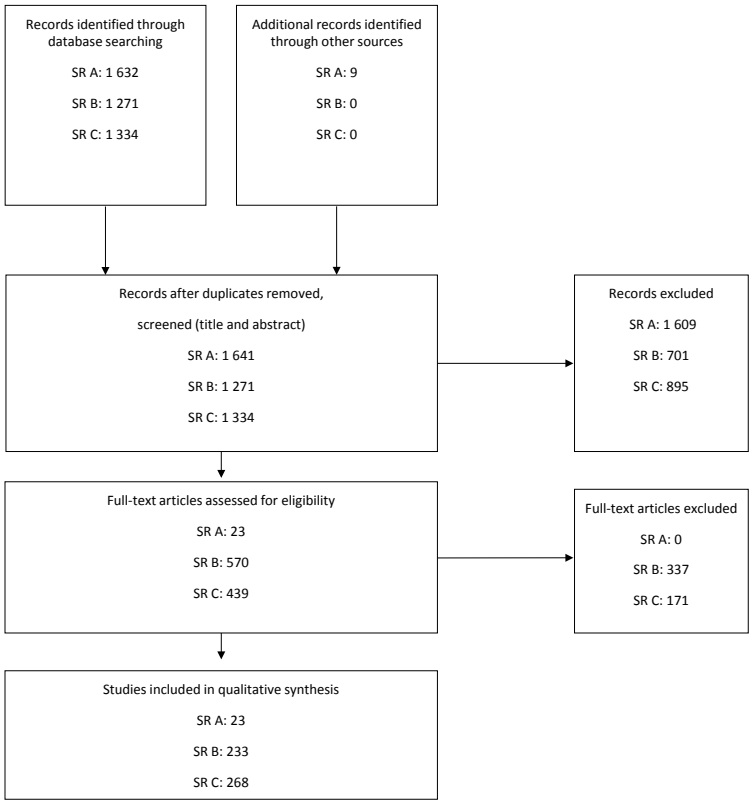
Because of the low number of hits when searching titles, the search was extended to all fields of the databases. Even then, only a few guideline documents (n = 23, see Table A and Figure A) on mosquito surveillance could be retrieved.

Most information came from sources in the USA where mosquito surveillance is implemented as part of national and/or regional arbovirus surveys (in particular for West Nile virus). Most US surveys are targeted towards the rapid detection of, and response to, arboviral activity in specific regions and monitor risk factors.

The most relevant document for Europe refers to guidelines for the surveillance of invasive mosquitoes.

Some European countries have national/regional risk management plans for vector-borne diseases in place. Most of these plans focus on invasive mosquitoes and the diseases they can transmit (primarily dengue and chikungunya). Some plans focus on the prevention of West Nile fever through disease surveillance while other documents focus on mosquito control. Surveillance plans aimed at controlling vector populations commonly underline the necessity of a thorough understanding of vector distribution and density. The applied sampling schemes (duration, frequency and trap choice) depend highly on the target species (e.g. for dengue surveillance, ovitraps and BG Sentinel traps are the traps of choice).

Figure A1-A. Prisma flow chart: paper selection and inclusion/exclusion process for SR A (existing guidelines), SR B (sampling methods), and SR C (native mosquito species identification)*



** Specific information on sampling methods and surveillance strategies was extracted from these guidelines.*

Systematic review on sampling methods used in mosquito (surveillance) studies

Research question

What are the sampling methods used for mosquitoes of public health importance in Europe?

Search string

In a first step, the SR used the following search string:

'(Systematic Review) AND (sampling OR trap OR resting OR netting OR bait OR collect*) AND (mosquito* OR Culicid* OR Aedes OR Aedini OR Ochlerotatus OR Culex OR Coquillettidia OR Anophel* OR Culiseta)'

This search produced very few hits (e.g. only two records in Web of Science), and we quickly proceeded to the second step of the SR as this allowed a broader search on mosquito sampling methods.

Therefore the SR was performed using following search string:

'(sampling OR trap OR resting OR netting OR bait OR collect*) AND (mosquito* OR Culicid* OR Aedes OR Aedini OR Ochlerotatus OR Culex OR Coquillettidia OR Anophel* OR Culiseta)'

Records exclusion criteria:

- not on mosquitoes
- not on mosquitoes
- not on sampling methods
- only methods specific to the study area
- on mosquito species not of interest for this review
- abstract of meetings
- only abstracts available

Remarks: There were no geographical and language limits for the first screening; during the full text screening, publications in languages other than English, French, Dutch, German and Spanish were eliminated; due to difficulties in finding literature prior to 1997, only full text published from 1990 to 2014 were screened, despite the fact that the SR covered the period from 1980 to 2014.

Results

A total of 1 271 publications were examined during the first screening (mainly on title and key words) on the currently available methods (see Table A and Figure A). Of these, 570 publications were kept for the full text screening. A total of eight papers, mainly in Portuguese, were excluded because of their publication in a language other than English, French, Dutch, Spanish and German.

During the full text screening, 337 papers/reports were excluded. This step was carried out with the objective to gather reliable information on sampling methods and strategies for surveying native mosquito species. A major problem was that a large number of older papers (published before 1997) were not available online. Papers published between 1990 and 1997 could sometimes be found online as scans. For the remaining publications, the main author was contacted, but only few authors replied to our request. No full text could be found online for 167 references, and 170 other references were excluded. A total of 233 papers were retained after both screening rounds.

Most of the papers (95%) address adult mosquito surveillance. Several types of traps are used for adult surveillance because mosquitoes are attracted to different trap types depending on species, sex, and physiological condition. The most common traps use attractants to lure mosquitoes, i.e. light, carbon dioxide, chemical bait (mimicking animal/human odours), infused water, and resting area substitutes. The optimal trap choice will thus depend on the target species' behaviour (seeking for host, oviposition, or resting), the available resources, and trap specificities (e.g. total operating time). The most commonly used/encountered trap was the CDC light trap (John Hock Co., mostly baited with CO_2).

Systematic review on identification methods for native mosquitoes

Research question

What are the available methods for the identification of mosquitoes of public health importance in Europe?

Search string

In a first step, the SR used the following search string:

'(Systematic Review) AND (identification OR key OR identification method) AND (mosquito* OR Culicid* OR Aedes OR Aedini OR Ochlerotatus OR Culex OR Coquillettidia OR Anophel* OR Culiseta)'

This search produced very few hits (e.g. only two records in Web of Science), and we quickly proceeded to the second step of the SR which allowed a broader search on mosquito identification methods.

The following search string was used:

'(identification OR key OR identification method) AND (mosquito* OR Culicid* OR Aedes OR Aedini OR Ochlerotatus OR Culex OR Coquillettidia OR Anophel* OR Culiseta)'

Record exclusion criteria:

- not on mosquitoes
- not on identification methods
- only methods specific to the study area
- on mosquito species not of interest to this review
- abstract of meetings
- only abstracts available

Remarks: There were no geographical and language limits for the first screening; during the full text screening, publications in languages other than English, French, Dutch, German and Spanish were eliminated; due to difficulties in finding literature prior to 1997, only full texts from 1990 to 2014 were screened, despite the fact that the SR covered the period from 1980 to 2014.

Results

A total of 1 334 publications were examined during the first screening (mainly on title and key words) for the currently available methods for identifying mosquitoes (see Table A and Figure A). Among them, and according to the predefined exclusion criteria, 433 publications were kept for the second screening. A total of eight papers, mainly in Portuguese and Russian, were excluded as they were published in a language other than English, French, Dutch, Spanish and German.

After the full text screening, 171 papers/reports were excluded. This step was performed to gather reliable information on the existence and availability of identification methods for native mosquito species. A major problem was that a large number of older papers (published before 1997) were not available online. Papers published between 1990 and 1997 could sometimes be found online as scans. For the remaining publications, the main author was contacted, but only few authors replied to our request. No full text could be found online for 102 references, and 69 other references were excluded. It was very difficult to find academic theses or grey literature on this topic. A total of 268 papers were retained after both screening rounds.

Molecular identification tools have gained ground during the last decades as a major identification tool, especially for vector species and species complexes. Several techniques are available depending on the species and/or the development stage, although the most commonly used are polymerase chain reaction (PCR) of both the mitochondrial and ribosomal DNA regions. Many identification keys based on morphology cannot be freely accessed or date back to the pre-digital era, making them difficult to consult. For Europe, two main references exist: Schaffner et al. (2001) and Becker et al. (2010); additional regional identification keys exists.

Annex 2: Basic information on the biology of mosquitoes and transmission of MBD pathogens

Box A: Mosquito species names

'Major generic changes in the tribe Aedini were recently published (Reinert 2000; Reinert et al. 2004, 2006, 2008), leading to a scientific debate and two or more names being simultaneously used for a single taxon.' (Medlock et al., 2012).
Editors of several scientific journals suggest that the traditional names (JME Editors 2005) should be kept until there is a consensus on this major nomenclature change. In this guideline document we use the traditional names, with alternate names shown here:

Aedes vittatus, also known as Fredwardsius vittatus sensu Reinert et al. 2004

All aedine species from subgenera Finlaya, Ochlerotatus, and Rusticoidus are suggested to belong to the genus Ochlerotatus (e.g. *Aedes annulipes* also known as *Ochlerotatus annulipes* sensu Reinert et al. 2000), except:

Aedes echinus, also known as *Ochlerotatus echinus* sensu Reinert et al. 2000, and *Dahliana echinus* sensu Reinert et al. 2006

Aedes geniculatus, also known as *Ochlerotatus geniculatus* sensu Reinert et al. 2000, and *Dahliana geniculata* sensu Reinert et al. 2006

Aedes gilcolladoi, also known as *Ochlerotatus gilcolladoi* sensu Reinert et al. 2000, and *Dahliana gilcolladoi* sensu Reinert et al. 2006

The biology of mosquitoes

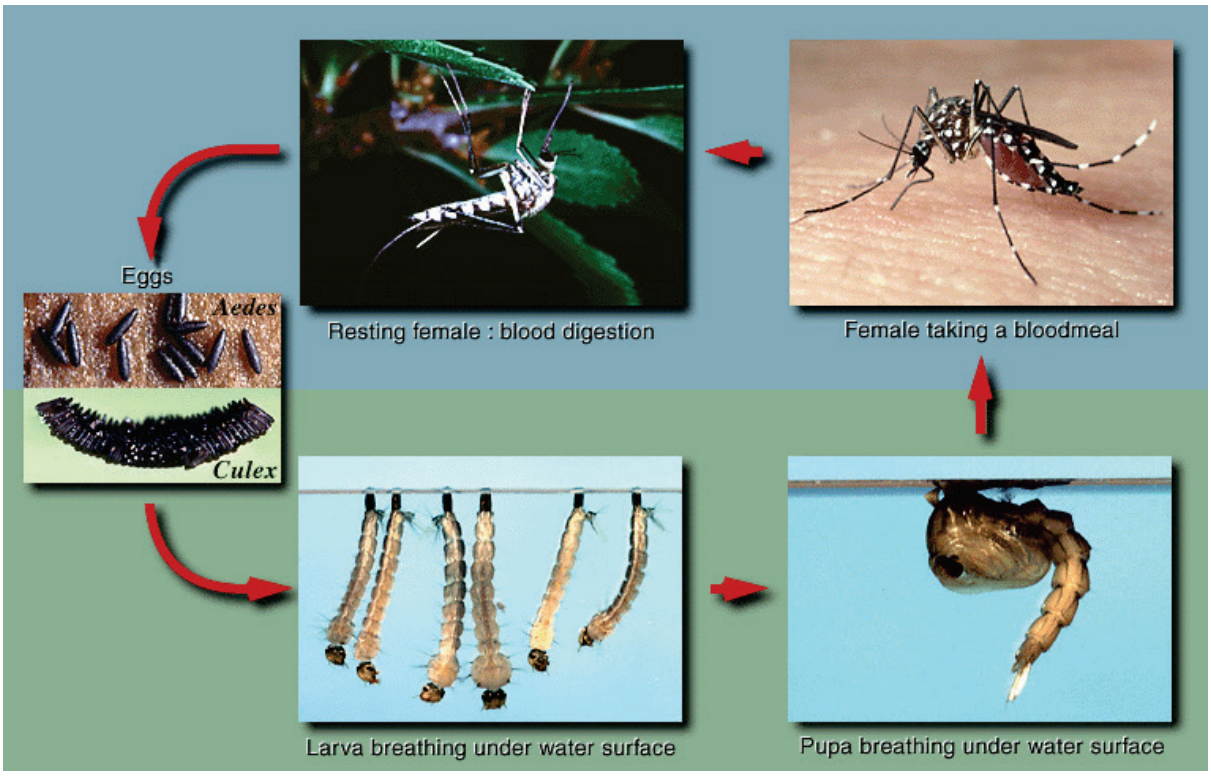
Mosquitoes belong to the family Culicidae (order Diptera, suborder Nematocera). A total of 3 537 species of Culicidae are currently recognised worldwide (Harbach 2014, Mosquito Taxonomic Inventory, http://mosquito-taxonomic-inventory.info, accessed 20 February 2014). Mosquitoes are ecologically beneficial for their contribution to biodiversity, food chains, pollination, and only some species are sources of threat to human and animal health because of their role as vectors of disease pathogen. About 20 invasive species are known to have significantly extended their distribution range in recent years.

The mosquito biological cycle comprises larva (four instars), pupa, adult (male and female), and egg (Figure A).

Eggs are laid by the female, either individually (e.g. *Aedes*) or grouped in egg rafts (e.g. *Culex*), on the water surface (e.g. *Anopheles*, *Culex*), on the edge of water bodies, on wet ground (most *Aedes*), or in a few cases on the edge of water bodies (a few *Aedes*). Eggs laid on the water surface generally hatch soon after deposition, while those not laid directly in water bodies hatch after flooding. *Aedes* eggs laid on the ground will remain quiescent for up to a year (univoltine species) or even several years if not flooded. Some species overwinter as mated females (e.g. *Anopheles*, *Culex*) or as eggs which are highly resistant to desiccation and low temperatures (e.g. *Aedes*).

From the egg a young larva hatches directly into the water, where it feeds by filtering particles suspended in the water or laying on the bottom (organic matter, algae, bacteria, fungi). It grows in stages of four instars and moults, during which its size increases from about 2 mm (L_1) to about 10 mm length (L_4). The final larval moult produces the pupa, which does not feed. During the pupal phase a complete transformation known as metamorphosis takes place, which creates the final adult stage. The four larval instars and the pupa are aquatic, but need air to breathe, and so need to come to the water surface, which means they can survive in polluted water with low oxygen content. However a few species are specialised to exploit oxygen from aquatic plant tissues (i.e. *Coquillettidia*). In the most favourable conditions of food availability and water temperature (20–25 °C), the aquatic phase is completed in less than a week.

Figure A2-A: Biological cycle of a mosquito (subfamily Culicinae)



Source: Schaffner F, Angel G, Geoffrey B, Hervey JP, Rhaiem A, Brunhes J. The mosquitoes of Europe/Les moustiques d'Europe. An identification and training prpgramme Programme d'identification et d'enseignement (CD ROM). Montpellier: IRD Éditions & EID Méditerranée; 2001.

The adult, male or female, emerges from the pupa by shedding the pupal skin in water, then rests a short time on the surface and eventually flies away. Mating occurs during flight, with males swarming to attract conspecific females. Monogamy is considered the rule for females, while a male remains sexually active after mating. Adults take sugar meals to obtain energy from plant fluids (i.e. nectars, fluids, decaying fruits). Females also need to take additional blood meals from animals (mammals, birds, amphibians, and reptiles) to provide the proteins needed to mature their eggs. It is this behaviour which makes mosquitoes so important to public health because during blood meals the female may ingest pathogens from an infected host. The female injects saliva into the host when feeding to stop blood coagulation, and a number of pathogens have evolved to accumulate in the salivary glands to exploit this route to parasitise their host. The female may therefore be able to infect a new host during a subsequent blood meal. We therefore call the mosquito a 'vector' (transmitter) due to their capacity to spread viruses, protozoa, filarial worms, or bacteria, causing human and animal diseases which may seriously affect the lives of millions of people. *Aedes* females bite and rest mostly outdoors, whereas *Culex pipiens* (the house mosquito) bites and rests mostly indoors. Females may take several blood meals during their lifespan, which is usually three to four weeks, depending on weather conditions and predation.

Characteristics of native mosquito genera of public health importance

Mosquitoes of the genus *Anopheles*

Anopheles species develop mainly in permanent standing water.

Biological characteristics. The boat-shaped eggs of *Anopheles* are provided with lateral floats and are laid singly onto the water surface. Larvae lie parallel to the water surface (see Figure 3) while all other *Culicine* larvae have only the abdominal extremity (i.e. their respiratory siphon) at the water surface, with the rest of their body and head hanging almost vertically from the water surface. Unless larval sites dry out in prolonged dry periods, successive ovipositions maintain and augment population sizes until the start of winter. Overwintering may occur by environmentally conditioned fertilised adult females or in the larval stage. Depending on the species, European *Anopheles* mosquitoes may bite mammals or birds, indoors or outdoors. Main malaria vectors (members of the *Maculipennis* complex, see Figure 3) bite indoors during the night and also rest indoors during blood digestion. Adults seldom move far from their breeding sites (no more than two or three km).

Larval sites can be found in permanent water in ponds, marshes and ditches, but also along the shores of lakes or river banks if marginal vegetation provides shelter from predators and the current. Some species oviposit in fresh water, but others prefer brackish oviposition sites. Mosquitoes have occasionally been found in containers, in particular the tree-hole breeder *An. plumbeus*.

Mosquitoes of the genus *Aedes*

Aedes species (including *Dahliana, Fredwardsius*, and *Ochlerotatus*, see Box A) form the most diverse species group in Europe.

Biological characteristics. *Aedes* mosquitoes lay drought-resistant eggs in temporarily dried-out locations. The eggs can withstand freezing as well as desiccation, and most temperate climate species overwinter in the egg stage. Eggs withstand long periods of desiccation. Exploitation of temporary water collections has enabled these mosquitoes to colonise harsh environments, even in the Arctic, where they are notorious pests. Many temperate climate species hatch with the thaw and develop in snow-melt pools, giving rise to pest infestations early in the year. Some species are univoltine (producing a single annual generation) but other, multivoltine, species cause long-lasting biting problems.

Eggs are deposited by the female on the ground or on the sides of dried-out potential breeding sites where they lie dormant until flooded several weeks, or months, or even years later. Some eggs require several episodes of alternating drying and wetting before they will hatch. Hatching occurs one to two days after flooding, and in large-sized sites will give rise to numerous cohorts. Immature densities can be high, particularly in the absence of predators and competitors, which is frequent in recently flooded habitats. Females rarely enter dwellings and bite during the day (see Figure 3), most species exhibiting a crepuscular peak of activity. They can fly long distances (several kilometres) in search of a host, generally a mammal, including humans.

Larval sites. *Aedes* mosquitoes develop in transiently flooded natural situations such as flood-prone ditches, ponds, meadows, forests, woodland depressions and both salt and freshwater marshes, and in semi-natural sites created by humans. Some species choose smaller sites such as tree holes, rock holes, livestock footprints, and artificial sites, including human artefacts such as discarded tins, jars or tyres. Water in these habitats is often fresh though brackish habitats exploited by halophilic species are common.

Mosquitoes of the genus *Culex*

Culex oviposit directly onto a water surface and arrange their eggs into a raft. Of the dozen or so species recorded in Europe, few are of more than local interest, and of these, *Cx. pipiens* is of greatest epidemiological importance.

Biological characteristics. The most frequently encountered species is *Cx. pipiens* (see Figure 3), commonly known as the 'house mosquito', which embraces two morphologically indistinguishable, but biologically distinct biotypes: the nominate biotype (*pipiens*), which cannot lay any eggs without a blood meal, requires large open spaces for mating, bites outdoors and mainly birds; and the *molestus* biotype, which can lay a first batch of eggs without any blood meal, can reproduce in a small cage, bites indoors and mainly mammals. However, these delineations are not always clear, in particular in southern Europe. Both biotypes may be involved in arbovirus transmission cycles, the nominate biotype in avian cycles, and *molestus* in periodic outbreaks, which occur in European towns and cities.

Larval breeding sites. *Culex pipiens* larval sites are of two kinds, each favoured by one of the two biotypes. The nominate biotype has been reported from a wide range of surface waters, ponds, banks of lakes, flooded gravel pits, ditches and other depressions, and from container habitats such as troughs, water butts, discarded tyres, rock pools, tree holes and dew pools on plastic sheeting, often in rural or suburban situations. The *molestus* biotype is almost entirely confined to cloistered situations in urban, usually domestic situations, in flooded cellars, sub-surface tanks, foul seepages from broken, underground drains, flooded wells of lift shafts, sewage treatment plants, and underground railway systems. In southern Europe it may also breed to a certain extent in some enclosed surface water collections, and hybrids of these biotypes are reported. Most *Culex* larvae develop in a broad range of artificial or natural environments, including permanent natural environments, such as ponds, ditches, tree holes, rock holes and meanders of slow flowing rivers if significantly immersed or semi-immersed vegetation is present to provide protection against predators.

Distribution of native mosquito species on the European continent

The distribution of mosquito species in Europe is detailed in Snow & Ramsdale 1999, Schaffner et al. 2001, and Becker et al. 2010. More detailed and updated distribution maps (NUTS 3 level) exist for the main vector species and are available from: http://ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx.

Transmission of mosquito-borne disease pathogens

Table A2-A. Important mosquito-borne pathogens that cause disease in humans

Pathogen	Disease	Case fatality rate (%)	Important vectors to human[1]
***Bunyaviridae* arboviruses**			
La Crosse encephalitis	Encephalitis	<1	*Ae. triseriatus*
Rift Valley fever	Febrile illness	0	*Aedes* species, *Cx. pipiens*
***Flaviviridae* arboviruses**			
Dengue 1–4	Febrile to haemorrhagic	3–12	*Ae. aegypti, Ae. albopictus*
West Nile	Febrile to encephalitis	3–15	*Culex* species (*Cx. pipiens, Cx. modestus*)
Japanese encephalitis	Encephalitis	30–40	*Cx. tritaeniorhynchus*
Murray Valley encephalitis	Encephalitis	20–70	*Cx. annulirostris*
St. Louis encephalitis	Encephalitis	4–20	*Cx. pipiens, Cx. nigripalpus*
Yellow fever	Haemorrhagic	5–20	*Ae. aegypti, Ae. africanus, Haemagogus* species
***Togaviridae* arboviruses**			
Chikungunya	Febrile to severe illness	Very low	*Ae. aegypti, Ae. albopictus*
Eastern equine encephalitis	Encephalitis	50–75	*Coquillettidia perturbans, Ae. vexans*
Ross River	Febrile illness	0	*Culex annulirostris*
Sindbis	Febrile illness	0	*Ae. cinereus, Cx. pipiens*
Venezuelan equine encephalitis	Encephalitis	0.1–20	*Cx. Pipiens*
Western equine encephalitis	Encephalitis	5–10	*Cx. Tarsalis*
Filarial worms			
Wuchereria bancrofti	Lymphatic filariasis	0	*Culex* species, *Aedes* species, *Anopheles* species
Dirofilaria species	Subcutaneous nodules, conjunctiva	0	*Culex* species, *Aedes* species, *Anopheles* species
***Plasmodium* protozoa**			
Malaria	Febrile to renal failure	1–7 (< 5 years)	*Anopheles* species

[1] *Worldwide*
Source: Beaty & Marquardt 1996; Schaffner 2003

In the presence of capable mosquito vector species, a suitable climate, and availability of infection sources, the likelihood of mosquito-borne infections increases with vector abundance. Transmission may be also possible with low or moderate vector densities if the vector population has a high competence (% of infectious females).

Changes in climate and environment trigger putative vectors and, with them, pathogens can spread and emerge in new areas. Such spreading may not only be caused by active vector migration but can be due to their passive transport, such as by vehicles, trains and aircraft. Perhaps more importantly, the increase and globalisation of international and intercontinental travel and trade therefore increases the risk of the accidental import of vectors and vertebrate reservoir hosts (i.e. carrying a pathogen). Control programmes may therefore need to shift from a pest control strategy to a vector control strategy, adapting and improving the efficiency of control methods (e.g. by supplementing or replacing larviciding with adulticiding for better and quicker control).

Table A2-B. Mosquito species of Europe that are not directly addressed by these guidelines*

Mosquito species	Mosquito species	Mosquito species
Aedes (Aedes) rossicus (1)	*Aedes (Fredwardsius) vittatus*	*Aedes (Fin.) eatoni*
Aedes (Fin.) echinus	*Aedes (Fin.) geniculatus* (2)	*Aedes (Fin.) gilcolladoi*
Aedes (Fin.) j. japonicus (3)	***Aedes (Fin.) koreicus***	*Aedes (Ochlerotatus) annulipes*
Aedes (Och.) atropalpus (3)	*Aedes (Och.) behningi*	*Aedes (Och.) berlandi*
Aedes (Och.) cantans (1)	*Aedes (Och.) cataphylla*	*Aedes (Och.) cyprius*
Aedes (Och.) diantaeus	*Aedes (Och.) euedes*	*Aedes (Och.) flavescens*
Aedes (Och.) hexodontus	*Aedes (Och.) hungaricus*	*Aedes (Och.) impiger*
Aedes (Och.) intrudens	*Aedes (Och.) leucomelas*	*Aedes (Och.) mercurator*
Aedes (Och.) mariae	*Aedes (Och.) nigrinus*	*Aedes (Och.) nigripes*
Aedes (Och.) nigrocanus	*Aedes (Och.) phoeniciae*	*Aedes (Och.) pionips*
Aedes (Och.) pulcritarsis	*Aedes (Och.) pullatus*	*Aedes (Och.) punctodes*
Aedes (Och.) punctor (2)	*Aedes (Och.) riparius*	*Aedes (Och.) surcoufi*
Aedes (Och.) zammitii	*Aedes (Rusticoidus) lepidonotus*	*Aedes (Rus.) quasirusticus*
Aedes (Rus.) refiki	*Aedes (Rus.) rusticus*	*Aedes (Rus.) subdiversus*
Aedes (Stegomyia) aegypti (5)	***Aedes (Stg.) albopictus*** (5)	*Aedes (Stg.) cretinus*
Anopheles (Ano.) algeriensis (2)	*An. (Ano.) beklemishevi*	*An. (Ano.) marteri*
An. (Ano.) petragnani	*An. (Ano.) pseudopictus*	*An. (Cel.) pulcherrimus*

Mosquito species	Mosquito species	Mosquito species
Coquillettidia (Coq.) buxtoni	*Culex (Barraudius) pusillus*	*Cx. (Culex) brumpti*
Cx. (Cux.) laticinctus	*Cx. (Cux.) mimeticus*	*Culex (Cux.) vagans*
Culex (Maillottia) arbieeni	*Cx. (Mai.) deserticola*	*Cx. (Mai.) hortensis hortensis*
Cx. (Mai.) hortensis maderensis	*Cx. (Neoculex) impudicus*	*Cx. (Ncx.) martinii*
Cx. (Ncx.) territans	*Cs. (Allotheobaldia) longiareolata* (2)	*Culiseta (Culicella) fumipennis*
Cs. (Cuc.) litorea	*Cs. (Cuc.) ochroptera*	*Culiseta (Culiseta) alaskaensis*
Cs. (Cus.) annulata (1)	*Cs. (Cus.) atlantica*	*Cs. (Cus.) bergrothi*
Cs. (Cus.) subochrea	*Cs. (Cus.) glaphyroptera*	*Orthopodomyia pulcripalpis*
Uranotaenia (Pseudoficalbia) unguiculata		

** Invasive species (in bold) and native species without significant known vector potential (ranking < 3) for the pathogens considered for these guidelines, i.e. eight arboviruses (Eastern equine encephalitis virus, Japanese encephalitis virus, Rift Valley fever virus, St. Louis encephalitis virus, Sindbis virus, Usutu virus, Venezuelan equine encephalitis virus, and West Nile virus) and Dirofilaria spp. and* Plasmodium *spp., Dirofilaria species and human malaria* Plasmodium *spp. parasites*

Ranking: 5 = Species known as past/present vector in Europe; 4 = Species known as vector outside Europe; 3 = Species infected in nature and competent, for the same pathogen or for different pathogens, or, for Malaria, secondary vector only; 2 = Species competent in the laboratory only (at low, moderate or high level); 1 = Species infected in nature only; 0 = Species not implicated in any pathogen transmission or absence of information on their possible role. Only ranking > 0 is shown, in brackets.

Native mosquito species that can, or are suspected to, contribute to the transmission of the pathogens addressed by these guidelines (see Figure 2) are listed in Table 2. In addition, around 60 species (Table B) cannot be considered as putative vectors of any of these pathogens because they have shown to be refractory to infection in the laboratory or there is no available information on their possible role. However, this status may change upon new laboratory or field findings, when/if the European mosquito fauna is exposed to a newly introduced exotic pathogen. At the opposite, a number of invasive mosquitoes could play a role (not shown on the chart), but their surveillance is addressed in specific guidelines [http://ecdc.europa.eu/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf].

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Annex 3: Prioritisation levels for the surveillance of native mosquitoes

Mosquito surveillance during the pre-disease stage

Strategies for establishing mosquito surveillance differ between a 'pre-disease stage' and a 'disease stage'. For example, the financial means provided by the central government are most likely to be different during the two stages, as is the approach to surveillance and risk assessment (RA).

Pertinent questions during the pre-disease stage are:

- Are the vectors (and, eventually, the pathogens in the vectors) present?
- What is the public health risk and is its emergence possible?
- Which areas should initiate surveillance of vectors/pathogens?
- Which areas should initiate control measures?

The amount of funding available for mosquito surveillance will determine which surveillance strategies can be implemented. The absence of a dedicated budget massively hinders the ability to properly assess disease risks. However, there are some relatively inexpensive options for passive surveillance, and these ought to be explored, provided there is a suitably trained entomologist available (to identify specimens). It should be noted that this approach will only provide limited information and is generally insufficient; a combination of passive surveillance and active surveillance would be ideal. The scale of the active surveillance depends on the available resources: if more locations are surveyed, more data are available to inform RAs and preparedness/response measures.

During the pre-disease stage provisions should be made to collect mosquitoes for pathogen detection. This increases costs, but provides relevant information, even at such an early stage.

Mosquito surveillance during the disease stage

When cases are reported in animals or humans, the disease stage is initiated. At this stage, the public health questions relating to entomology are:

- Where are the known positive cases?
- Which mosquito species is involved in human-human vector cycles or, in case of zoonoses, in enzootic and bridge vector cycles?
- In which habitats are these mosquitoes found?
- What is the extent of the putative mosquito distribution?
- What is the extent of the infected mosquito distribution?
- Does the weather or habitat management have an influence on mosquito density and hence the number of cases? What advice can public health authorities give to pest control managers, land managers, and the general public?

At this point, additional funds should be made available and attention should focus on answering the above public health questions within the target 'disease' zone. All available mosquito trapping resources and field teams should be deployed to the target zone. Maximum vector surveillance should be instigated, with teams working radially from the locations where mosquitoes were detected.

One of the key public health tasks related to vectors will be to identify the mosquito species involved in transmission. This can be achieved by: a) surveillance of mosquitoes in the disease zone to identify activity patterns and relative abundance of species, b) surveillance of nuisance species, and c) analysis of mosquito samples for pathogen detection. For the latter, a protocol needs to be established to ensure that samples collected in the field are transported to the laboratory and tested for pathogens in a timely fashion. This needs to be properly organised, and funds have to be available for consumables.

In conjunction with adult mosquito surveillance, pre-imaginal surveillance in and around the disease zone will assist with targeting control strategies and identifying locations with disease transmission. This approach will vary between urban and rural areas, and collected data will improve predictions of adult emergence and forecasts of mosquito density.

All data should be stored in a GIS system for inspection and national planning. A handheld GPS recording system, particularly for pre-imaginal surveillance, is recommended. Surveillance is also needed to monitor abundance (adult and pre-imaginal stages), determine seasonality and non-vector season as well as the impact of any control measures.

Options for passive surveillance

Data on the distribution of native mosquitoes can be sought from a variety of resources. Reviewing published data or historical atlases on mosquito distribution provides some historical perspective on mosquito distribution. ECDC has produced distribution maps for a number of native species. Although data are presented at NUTS3, a finer spatial scale for a number of countries is available upon request. In the United Kingdom, historical data were procured from the Biological Records Centre, the British Mosquito Recording Scheme and the Natural History Museum and collated into one national surveillance scheme (see other section below). These data have been made available as an online resource through the UK's National Biodiversity Network, which encourages individuals, amateurs and professionals to supplement the database with their own records. The scheme provides an identification service and ensures validation of data.

Generating maps and making them publically available promotes national record keeping. Data may also be gathered from interested amateurs, professional entomologists and those exposed to nuisance biting. The submission of data and/or specimens should be encouraged. In Germany, media campaigns have been very successful in 'harnessing citizen science' to map native and non-native mosquito species. (Examples of citizen science projects are given below.) This option can also be extended to local municipality pest control officers, thus providing a forum to submit specimens of, and gather data on, species known to have caused a nuisance, which in turn makes it easier to identify pest species and produce RAs.

Passive surveillance data can improve RA in areas for which previously no data were available. Nevertheless, data gaps and geographical bias will be substantial, and passive surveillance data provide very little information on species abundance and seasonal activity. Another drawback of this method is the insufficient number of samples for pathogen detection.

Options for active surveillance

Resources for active surveillance of native mosquitoes should be directed to cover key pest or vector species. These species are usually identified during the first phase of the RA process. Data procured during the passive surveillance process should be utilised to direct active surveillance to locations where the full range of putative vector species may occur. It may be preferable to run a large number of traps for only a few nights across the country, as has proved successful in Belgium and the Netherlands. This aids future geo-spatial modelling and has been very successful in detecting non-native species. However this approach is not sufficient to understand abundance and seasonal activity, nor does it produce a sufficient number of samples from specific locations to detect pathogens or determine pathogen infection rates in the vectors.

If RA questions focus on defining the temporal risks from transmission (species abundance, non-vector seasons etc.), then selecting a smaller number of locations (across a range of habitats) and surveying every two weeks (for a number of nights) from spring to autumn should provide the data needed for a national RA. However, by reducing the number of locations, the accuracy of species detection is reduced accordingly.

The costs and logistics of running an active surveillance scheme can be forbidding, and options to exploit existing species recording schemes should be explored. Often, nature reserve wardens are willing to run traps and send samples for identification. In Sweden, members of the public were asked to supply records and samples from their mosquito traps, thus maximising records and minimising costs. However, samples may not always be preserved appropriately if not handled professionally.

Information on previously unrecorded or rare species can range from interesting entomological findings to the discovery of a hitherto unknown putative vector species which can change a country's RA. Examples of this include the discovery of European species like *Culex modestus* (UK) or certain *Anopheles* spp. (Belgium), which both led to an amendment of national public health RAs. Publishing new or unusual findings raises awareness of ongoing surveillance and sustains interest.

Active surveillance may also be employed to provide data for specific requirements, such as longitudinal data for climate and weather modelling, mosquito abundance data at environmental change sites (e.g. new wetlands in urban, rural and coastal areas) or data on mosquito nuisance or the impact of mosquito control efforts.

Pathogen detection

Ideally, all samples should be preserved and tested for pathogens; however this may not always be logistically or economically viable. Some degree of testing should be considered during the RA process; it should be kept in mind, however, that a large number of mosquitoes are needed to establish the presence or absence of a pathogen.

Examples of passive surveillance in Europe

The following section describes tools for passive surveillance. Some are developed to survey primarily invasive mosquitoes, but tools and methods can easily be adapted to native mosquitoes.

Community participation in surveying *Aedes albopictus* in France, with the tool 'i Moustique'

Source: S. Chouin, EID Atlantique, Rochefort, France; www.eidatlantique.eu

In the year 2000, France implemented a plan aimed at the early detection of *Aedes albopictus* in the non-colonised areas to prevent the mosquito's establishment and outbreaks of MBD. Before the year 2000, entomological surveillance of invasive mosquitoes was essentially based on a network of traps located at high-risk locations. In 2012, EID Atlantique made it possible for smartphone users to report mosquitoes through an online app. During that season, approximately 400 reports were received, only a third related to mosquitoes, while the rest referred to other insects. In 2013, an additional phone application was created (i Moustique, Figure A3-A) that allowed users to upload photographs of mosquitoes. The app also provided 1) a way to differentiate a mosquito from other insects, 2) an information sheet on common mosquito species, and 3) recommendations for preventing mosquito proliferation (e.g. source reduction, minimising breeding sites).

In 2013, more than 600 datasets were received. A third of them (34%) were reported via i Moustique, and nearly 90% of these concerned mosquitoes. 87% of the data related to native mosquito species (15 species). In addition, reports confirmed the establishment of *Aedes albopictus* in seven cities.

Entomological monitoring is a challenge, particularly when considering the rapid spread of *Aedes albopictus* throughout France. The objective of *i Moustique* is to enhance community participation, conduct mosquito surveys and contribute to a better knowledge of the local distribution of native species. This approach strengthens mosquito monitoring networks and enhances vector risk awareness.

'Mückenatlas': a citizen science project to monitor mosquitoes in Germany

Source: D. Werner, Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany; H. Kampen, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald – Insel Riems, Germany; www.mueckenatlas.de

The 'Mückenatlas' (mosquito atlas; Figure A3-A) was launched in early 2012 as part of a German national mosquito monitoring programme which had originally been planned to rely exclusively on mosquito trapping. However, after the detections of the first adult *Ae. albopictus* specimens in Germany in 2011, people started to send in mosquitoes for identification. The willingness of the community to assist was tapped by launching the 'Mückenatlas' project, soliciting the submission of mosquito specimens through the media. This citizen science project has now matured into an accepted tool for passive mosquito monitoring and the collected data on the German mosquito fauna are used to supplement trapping data. Although these single collection site yield only a few specimens at best (as opposed to trapping, which can produce very high numbers), the added value of the 'Mückenatlas' is the possibility to sample a large number of collection sites, including numerous areas where it would be difficult and time-consuming to place traps.

Figure A3-A. Flyer for the 'i Moustique' project, France (left); website for 'Mückenatlas', Germany (right)

Every person submitting a record/sample to the 'Mückenatlas' receives written response with information on the species and its biological characteristics. Users can also have their names or alias published on the project's homepage. As soon as a significant number of mosquitoes have been identified, the map of collection sites will be replaced by a species distribution map.

Contrary to initial expectations, submissions to the 'Mückenatlas' also yielded some rare mosquito species which have not been recorded in Germany for decades. To date, a total of 38 species (out of 50 presumably indigenous to the Germany) have been submitted. Most prominently, the 'Mückenatlas' led to the detection of two unknown populations of the invasive Asian bush mosquito *Ae. j. japonicus* in western and northern Germany in 2012 and 2013.

Community participation in surveying *Aedes albopictus* in Spain, project 'AtrapaelTigre.com'

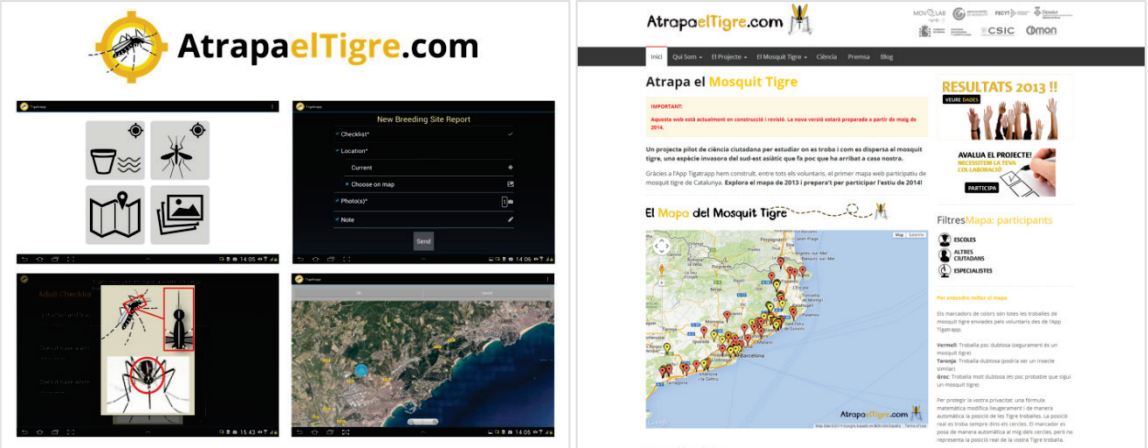
Source: F. Bartumeus, ICREA-Movement Ecology Lab (CEAB-CSIC), Blanes, Girona, Spain; www.atrapaeltigre.com

Atrapa*el*Tigre.com ('Trapping the tiger', Figure A3-B) is a citizen science project aiming at exploring alternatives to expensive and time-consuming traditional surveillance and control programmes for the invasive Asian tiger mosquito (*Aedes albopictus*). It is led by a research group on movement ecology (ICREA-Movement Ecology Laboratory, CEAB-CSIC) and funded primarily by the Spanish Foundation for Science and Technology and supported by an increasing number of public and private institutions. In the long term, a citizen-driven alert system for invasive species is planned.

'Atrapa*el*Tigre' was first initiated as a pilot project in 2013 in NE Spain. The project consists of three elements: 1) workshops for training and raising awareness, 2) online communication through the project website (www.atrapaeltigre.com, Figure A3-B), and 3) a smartphone application. Through the app, the main participatory element also used in the workshops, citizens collect and share data on adult tiger mosquito sightings and potential breeding sites in public spaces. Reports of tiger mosquitoes and breeding sites include a standard description of the environment, the location, and pictures and/or complementary notes. Data are automatically published on the project website in a web map. Data are encrypted, ensuring the privacy of participants. Data are not individually validated, but partially confirmed by training workshops, taxonomic questionnaires and pictures. Citizens can also

share daily mobility data through the app, raising awareness of the potential role of humans as disease vectors. A citizen-based mobility dataset can help modelling sources/sinks and spreading routes of tiger mosquitoes in Spain. Moreover, the project is testing other means of automated validation through 'crowdcrafting' tasks and dynamic and geolocation-driven tasks.

Figure A3-B. Project Atrapa*e*/Tigre.com, Spain: Tigatrapp App screenshots (left), web site screenshot (right)



Source: R. Eritja (left), AtrapaelTigre.com

'Muggenradar': a citizen science approach to collect biting mosquitoes in the Netherlands

Source: C. J. M. Koenraadt and A. J. H. van Vliet, Laboratory of Entomology, Wageningen University, Wageningen, the Netherlands; www.muggenradar.nl

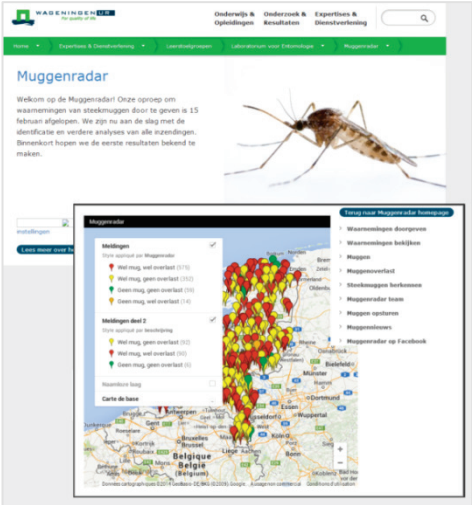
Because most mosquito survey and research activities take place during the development season in spring and summer, little information is available about the overwintering strategies of mosquitoes. When investigators received complaints from concerned Dutch residents about biting nuisance during the winter months, the question arose whether some species were still blood feeding in winter. Therefore, 'Muggenradar' ('mosquito radar'; Figure A3-C) was launched, addressed to the Dutch general public. The launch was accompanied by a press release and a website which includes mapping functionality, general background information on the biology of mosquitoes, and an online questionnaire. Information obtained through the questionnaire confirmed the off-season biting nuisance, its location (up to postal code level) and time span.

'Muggenradar' contributors can also indicate whether they wish to submit a mosquito specimen. If so, users receive clear and standardised packaging instructions and a unique code which has to be put on the back of the envelope.

Investigators encouraged all people to report to 'Muggenradar', even if they did not experience any biting nuisance. From a scientific perspective, observations on the absence of biting activity are equally important as observations on the true presence of a biting mosquito.

All in all, 'Muggenradar' was considered a background measurement and thus no statement was made about the total number of samples that was sent in and the impact of the relatively mild winter of 2013–2014 on mosquito feeding patterns. The campaign ran from 10 January 2014 to 15 February 2014.

Figure A3-C. Project 'Muggenradar', the Netherlands: website screenshot (left); project 'Mosquito Recording Scheme', UK: reporting form (right)



Mosquito Recording Scheme - data form

Please complete the following form and send this along with any mosquitoes collected to the address provided.

Person submitting data	
Name of person submitting mosquito(es)	
Company name	
Address	
Telephone number	
Email address	
Location of collection	
Date of collection	
Specific location of collection (grid reference if possible)	
General location of collection (nearest village/town/city)	
Local habitat (deciduous woodland, coniferous woodland, pasture)	
Any further information/notes	

Scientific and educational goals of 'Muggenradar':

- To gain insights into mosquito biting activity during a period when most mosquito species are expected to have entered diapause and are thus inactive
- To identify mosquitoes (by molecular discrimination) that are submitted for further investigation to species or biotype level
- To inform the Dutch general public about the biology of mosquitoes and the role mosquitoes have in the ecosystem

'Mosquito Recording Scheme' and 'Mosquito Watch' in the UK: a medical entomology resource

Source: J. Medlock, Medical Entomology and Zoonoses Ecology, Public Health England, Emergency Response Department, Porton Down, UK;
http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Mosquitoes/MosquitoRecordingScheme/

In 2005, Public Health England (then the Health Protection Agency) and the Biological Records Centre set up the Mosquito Recording Scheme (MRS; Figure C). In the same way as for other species groups, the scheme would provide a national focus for Culicidae data in the United Kingdom, and data would be made publically accessible via the National Biodiversity Network Gateway: a free-to-access online mapping system for all species. The MRS built upon the previous mosquito group database published and held by the University of East London. In addition, the MRS receives datasets from entomologists (amateurs and professionals), museums and universities, and also lets the general public submit mosquitoes for identification that may be causing a biting nuisance. The samples are sent to Public Health England for identification by medical entomologists who respond with information about the species and its habitats. Since 2005, the mosquito recording scheme has received approximately 3 500 submissions in addition to the 7 000 records (totalling >10 000 species records) from historical datasets.

Also, in 2005, the Medical Entomology Group of Public Health England established a 'Mosquito Watch' (MW) scheme with the Chartered Institute of Environmental Health (CIEH) and Killgerm Ltd. to provide a forum for environmental health officers (EHOs) to submit records of mosquitoes for identification. In the UK, EHOs are often required to respond to nuisance biting issues raised by the public. From 2005 to 2012, there were 116 submissions to the project.

Following the collaboration with CIEH, Public Health England conducted a questionnaire-based survey of local authorities in 2009, in continuation of surveys published since the 1960s. A total of 221 local pest control units supplied information (64% response rate), with 57 (25%) indicating mosquito nuisance biting in the last ten years, and 29 (13.7%) in the last 12 month. Eleven local authorities reported having conducted mosquito control within the last ten years. MRS and MW are important and affordable tools that provide a medical entomology resource for the UK. They offer a better response to nuisance biting issues, serve as an early warning system for invasive mosquitoes, and provide a repository for records collected by a range of people which can be shared with the public, pest controllers, government officials, and academics. The system can also be used to detect unusual and rare species, e.g. non-native species.

Further information can be found in Kampen et al.: Approaches to passive mosquito surveillance in the EU [manuscript in preparation].

Annex 4: Collecting native mosquitoes

Methods for collecting native mosquito species

Larval sampling

Why collect larvae?

Surveillance by larval survey is of key importance as all mosquito species rely on water bodies for their development, thus allowing a focused and rapid inspection (sampling and identification can be performed in the same day) and offering an optimal cost-benefit ratio. Larval sampling is used to determine the exact breeding location as well as the species and population density of a pest or vector species. Routine larval surveillance thus provides more complete and accurate information on mosquito breeding sites, thereby providing the necessary documentation of native mosquito species (NMS) production as a basis for mosquito control. Furthermore, larval surveillance facilitates a clear understanding of species distribution, density and seasonal occurrence and can thus enhance the knowledge obtained from adult NMS surveillance.

Where to collect?

Immatures occupy a broad spectrum of aquatic habitats: from highly polluted to clean, and from small to large water bodies. They are commonly found in temporary or permanent ground water bodies, but can also be observed in tree holes and rock pools, bamboo stems and leaf axils, pitcher plants, bromeliads as well as in a vast number of artificial containers (such as flower pots, tyres, waste water basins and even septic tanks). It is therefore important to organise known breeding sites into several classes (which can then be stratified and used in a standardised surveillance operation). Overall, four main classes can be distinguished 1) (semi-)stagnant water (ditches and ponds with vegetation, forest ditch, fen, flooded meadows or forest), 2) running water (rivers, streams, ditches, drains), 3) temporary water bodies (puddles, tyre tracks, etc.) and 4) natural containers (tree holes, rock pools) and human-made containers (e.g. pots, pits) (Bates 1949). These can, in turn, be further subdivided depending on the targeted species.

In natural areas, mosquito larvae can be found in puddles and road tracks, swamp areas, drains and ditches, irrigated croplands, streams and riverbeds, ponds, tree holes, etc. Human-made containers can also often be detected in natural areas such as drinking troughs, or discarded waste containers such as tyres, tarpaulins, etc.

In urbanised areas, larval sampling must focus on the available human-made water bodies found in both public and private environments, below and above ground level, such as: discharged containers of different shapes and volumes, flower vases and flower pot dishes in gardens and cemeteries, used tyres left outdoors, rain water barrels, road drains, pits, etc.

When to collect?

The best season for larval detection is species-dependent and will shift depending on latitude, altitude, and mosquito species. In general, mosquito larvae develop when water temperature is above 10 °C and have an optimal development at water temperatures in the range of 25 to 30 °C. In spring, the blossoming of dandelions indicates the start of mosquito activity season, and the first frost of autumn may indicate its end. The precise start and end of surveillance, however, needs to be adapted to the particular native mosquito species, considering its specific tolerances (temperature, diapause).

Some tips

- When sampling larvae it is suggested that one should wait a few minutes between two dips to allow the re-surfacing of larvae disturbed by the operation.
- Field teams should record the size of the breeding site and the colour of the water (indicates presence of organic material) in which the larvae were collected.
- A breeding site is considered to be negative after several dips (10 to 20 dips, depending on site size).

Methods for sampling

The most commonly used trapping device is the dipper. The term 'standard pint dipper' is used in the scientific literature, but, in practice, there is no standard dipper or standardised dipping techniques (Service 1993). The dipper generally consists of a white plastic cup, 400ml in volume, with a two-to-five-foot handle to give it an extended reach. The dipper can be used as a survey tool by taking several dipper samples from designated areas in the habitat of interest and then counting the larvae captured in each dip. Thus, it is well adapted to determine abundance of larvae, whereas the netting, which allows to sample over larger parts of the larval habitats, is more appropriate to determine presence/absence. The netting/dipping methods will vary with water depth, presence of aquatic vegetation or other debris, and water clarity.

In general, mosquito juveniles (larvae and pupae) can be collected from potential larval breeding sites by netting, dipping, or aspiration, depending on the size of the container inspected. Large containers (surface > 0.5 m²) can be checked with an aquatic net (maximum diameter 20 cm, 500µ mesh), a classical dipper or a 1 litre-plastic tray. Smaller containers can be checked with a smaller net (aquarium water nets are usually fine), a kitchen ladle or a small white plastic cup. Very small containers can be emptied into a plastic tray; if not removable (like tree holes), water can be sucked out with a silicon tube or a mucous vacuum.

Several dipping techniques exist, each with certain advantages (Table A4-A). For routine immature monitoring of all species (for generic comparisons of aquatic habitats), complete or partial submersion is recommended, depending on the depth of the water.

Collected water samples can be better inspected for juvenile presence when decanted in a white plastic tray (1 litre). Larvae are then easily collected with a plastic pipette and put in a fully labelled vial with water (for rearing the larvae to 4th instar (L4) or to adults) or directly in 70% ethanol for later species determination. Rearing larvae to L4 is recommended for more reliable identification.

Table A4-A. Seven dipping techniques, modified from O'Malley (1995)

Dipping method	Targeted mosquito genera	Method details	Notes
Shallow skim	*Anopheles*	The leading edge of the dipper is submerged at approximately 45 ° and about 2.5 cm below the water surface. The dipper is drawn along the water surface and filled at the end of the stroke.	This method is analogous to the slow dipping technique of Collins and Resh (1989). It works better for sampling *Anopheles* larvae that remain at the water surface comparatively longer than do other mosquito larvae after the dipper enters the water. A good technique for sampling when submerged macrophytes have leaves just below the water surface.
Complete submersion	*Aedes, Ochlerotatus, (Culex, Culiseta)*	The dipper is submerged quickly in open water, usually in floodwater habitats. The dipper is brought up to water surface through the submerging larvae that have reacted to the disturbance created by submerging the dipper.	This method is used primarily to sample mosquitoes whose larvae respond rapidly to the dipper entering the water, but are visible. (This technique also is appropriate for sampling larvae adjacent to vegetation. The dipper is brought to the water surface while contacting the emergent vegetation.)
Partial submersion	*Anopheles, Culex, Culiseta*	The dipper is submerged at approximately 45 ° along the emergent vegetation. Water flows rapidly into the dipper. The dipper is not moved horizontally. The dipper can be moved vertically to scrape along the edge of emergent vegetation.	This method works well when sampling in robust emergent vegetation such as cattail and bulrush. The suction created by water flow into the dipper and scraping also collects small insect predators and herbivores associated with mosquito larvae on or near the vegetation.
Flow-in	*Aedes, Ochlerotatus, (Culex)*	This technique is used in shallow water that has a depth < height of the ladle on the dipper. The bottom of the dipper is pushed into the substrate and the water, along with the larvae and debris, flows into the dipper.	This method works well in shallow habitats, root masses and other habitats that are shallower than the dipper's profile.
Scraping	*Coquillettidia*	The dipper is scraped against the underside of floating vegetation to dislodge attached larvae. The scraping action is usually a vigorous back-and-forth motion.	Used to sample larvae that reside under, and usually attached to, the underside of floating vegetation or the roots of floating plants. Because a vigorous back-and-forth motion is used with the dipper completely submerged, this technique works best with dippers having a screened bottom.
Simple scoop	*Culex*	A quick flip of the wrist is used to submerge the dipper directly below the water surface. The technique is similar to taking water to drink.	Not a preferred method, especially if the sample is not taken adjacent to a mosquito microhabitat. Might be closest to the quick dip technique of Collins and Resh (1989). This technique would be adequate in hypereutrophic situations where the abundance of larvae often approaches 1000/dip.
Background	*Aedes, Ochlerotatus*	The dipper is used to provide a light background against which darker coloured immature mosquitoes are more easily seen. After mosquitoes are found, they are collected by quickly pulling the dipper through the water surface.	A technique used primarily to identify mosquitoes inhabiting woodland ponds and pools.

The larvae should be first transferred with a dropper to a small cup or bowl with fresh clean water as a washing procedure. If much debris or sediment is still present, additional serial transfers should be made until it is eliminated. Remove as much water as possible from the cup or bowl using a fine pipette. In the lab, you can heat a beaker or pan of water to about 60 ºC and pour the hot water into the cup or bowl. In the field, the larvae can first be transferred to a vial using the pipette after which pure ethanol can be added to this vial. As soon as the larvae float up to the surface, the water is removed with a fine pipette and replaced with a quantity of 70–80% ethanol. After five minutes the larvae are transferred with a lifter (do not use forceps) to a vial with 70–80% ethanol. Completely fill the vial with 70–80% ethanol to remove all air and cap it tightly. Keep larvae from the same species and same sampling together. However, no more than 20 larvae should be placed into a single vial as the water contained in the bodies of the larvae will significantly dilute the concentration of a small quantity of ethanol and jeopardise preservation.

Figure A4-A. Larval sampling: dipping (top left); netting (top right); sampling with a 1l plastic tray (bottom left), and with a small net and a tray (bottom right)

Photos: F. Schaffner

Adult sampling/trapping

Methods for collecting

A wide range of trapping techniques have are used to collect adult mosquitoes (Service 1993, Kline 2006), differing in design and varying greatly in effectiveness and usefulness (Campbell 2003). Adult mosquito females can be caught by aspirating from resting sites (indoor in shelters, or outdoor in the vegetation) or on hosts (animal or human), and by netting in the vegetation (successful for dense mosquito populations).

Human landing collection (HLC) can be performed for estimating 'landing rates'. It is sometimes the fastest way for estimating a nuisance and can be done in 15 minutes when visiting a site while also checking for larvae or adult traps. On the other hand, it raises the ethical question of potential risk for collectors that are subjected to mosquito biting and their exposure to various pathogens. It can thus only be used if there is no evident risk of transmission. Results depend on collectors' skills and on the attraction this person exerts on mosquitoes. Several alternative sampling methods have been developed: use of various traps developed to collect mosquito species using their preferences in odour cues, colours, light, host characteristics, or behaviour. Surveillance by adult collection may be best organised by exploiting the efficacy of traps for the specific native mosquito species.

Where and when to collect

The choice of trap depends on the mosquito species most likely to be encountered, the environmental conditions where the trap is operated, and the availability of resources (skilled technicians, laboratory/domestic animals, dry ice). Traps should be positioned in the sites under surveillance and operated periodically or continuously to cover the whole sampling season, with regular inspections to collect the samples. As local climate can influence capture rates, trap placement needs to be carefully planned and monitored. Most traps yield more species when they are not placed in direct sunlight and away from dense vegetation.

Some tips

Overall, good trap sites are:

- within a fairly open space with some bushes and trees, but not where trees are too dense, preferably between shrubs/trees and open areas;
- safe from interference by the public either in an enclosure (e.g. a garden), or out of normal public view and at least 10m away from buildings;
- away from competing light sources such as street or garden lights;
- in a sheltered, wind-protected location and away from dust or other pollutants;
- easily accessible for fast collection.

Egg sampling/trapping

Aedes/Ochlerotatus usually deposit their eggs on the insides of containers, just above the water level. They can be collected with strong white paper tape applied to surfaces or with polystyrene substrates placed on the water surface. Surveillance of container-inhabiting NMS presence and activity can efficiently be based on ovitraps that attract adults laying their eggs on oviposition supports provided. If enough traps are employed to cover the area, indirect data on adult population can be inferred.

Ovitraps are cheap and easy to handle, but identification of eggs is difficult and time-consuming (see Chapter 2.3) and the correlation of egg number and female density is not always clear, as females do not lay all their eggs in a single location.

Methods for collecting adult native mosquitoes: strengths and weaknesses

Mosquitoes use a complex set of cues (short, middle and long range) to detect a host or food source. Mosquitoes usually rest in the vegetation or other hidden places (e.g. crevices, tree holes, caves, basements) and start to fly when they receive host-recognition cues. This particular behaviour, together with the odours enticing the mosquito into flight, was taken into account for the development of the various trap types or collection techniques.

Human landing collection (HLC)

This is probably the oldest and simplest method of collecting host-seeking mosquito females. HLC is used to catch anthropophilic species most efficiently when performed during the daily peaks of activity in shaded environments. Disadvantages include high cost (manpower, particularly if HLC is performed after hours, e.g. at dusk) and there is a risk of becoming infected if there is pathogen circulation, even though it is recommended to collect the females before they bite. For standard comparisons, a sampling duration of 15 minutes is recommended.

Animal-baited traps

As mosquito host preferences differ between species, they are attracted to different cues from a number of hosts. In general, *Culex* spp. prefer to feed on birds, while *Aedes/Ochlerotatus* spp. generally feed on mammals and *Anopheles* spp. on humans. These general rules do not apply to all species as, for example, *Cx. territans* feeds preferably on amphibians; *Cx. pipiens* is known to feed on birds, other animals, and humans; and some *Anopheles* species prefer to feed on mammals. Therefore bait is varied and includes cattle, horses, goats, pigs, rodents and various birds. Mosquitoes are removed from the tethered animal using an aspirator or collected from the walls of the animal shelter. Animal bait is often used in West Nile virus surveillance studies, but are not in large-scale surveillance schemes.

Resting and blood-fed mosquitoes in vegetation and natural crevices and shelters are collected with aspirators. They are commonly used for collecting malaria vectors and very helpful when collecting in heavy vegetation around homes.

CO_2-baited suction traps

Suction traps baited with CO_2 are the most commonly used mosquito traps and are operated with dry ice (producing carbon dioxide through sublimation) or a carbon dioxide tank. CO_2 is a highly effective attractant for some species and their host-seeking females. Suction traps may run on battery or AC power. These traps may run with or without a light source and have the advantage of not being significantly influenced by background the lights in urbanised areas. The main disadvantage is the need to have access to dry ice or carbon dioxide. CO_2 traps also collect non-mosquito haematophagous insects such as Phlebotominae (sand flies), Simuliidae (black flies), and haematophagous Ceratopogonidae (biting midges). Operating time is limited by the CO_2 supply (one trapping day/night with dry ice) or battery capacity (12V, 10A, maximum 48h).

Mosquito Magnet (MM) traps

Mosquito Magnet traps are CO_2-baited suction traps that produce carbon dioxide by burning butane. They can thus be used in locations without power supply for up to three weeks. Chemical attractants such as Lurex (L-lactic acid) and octenol (1-octen-3-ol) can be added. Several studies confirmed the utility of traps such as the Mosquito Magnet Liberty Plus for broad non-specific sampling. This trap outperformed other trapping systems, both in number of specimens and number of species and genera collected. Major disadvantages of these traps are that they are large, heavy and relatively expensive, which can be a major obstacle to their use in large-scale studies.

Light traps

Light traps are most often used in malaria surveillance, although they have also been used in other arboviral surveillance. Light traps operate at night and are most efficient when there are no other lights nearby. The advantages of the light trap are that they are portable and powered by a standard 6-volt lantern battery. The disadvantages are that they may only be marginally attractive to diurnally active mosquitoes. However, carbon dioxide can be added in order to attract more mosquito species. The trap can be baited with dry ice (about 1 kg for one night) in routine monitoring programmes. The traps are best installed during the late afternoon and removed the next morning. Live-trapped females can be counted and tested for mosquito-borne arboviruses. As the trap can only operate for one night (especially when baited), this can pose a major drawback to its use in large areas and/or when a high number of traps are needed for monitoring. They also have a large by-catch of other insect species.

BG-Sentinel trap and Biogents Mosquitaire

These traps have been designed to attract *Ae. aegypti* and *Ae. albopictus* and are baited with a specific chemical lure (BG-Lure or Sweetscent). Their effectiveness can be increased by a potential host (e.g. a mouse in a cage) or a carbon dioxide source (Biogents device). With carbon dioxide, the trap attracts a wide range of mosquito species. Traps can be operated continuously when a power supply is available, but also run on 12V batteries. They can be used in urbanised as well as rural and natural areas. The traps have also been reported to catch male, gravid and blood-fed *Ae. albopictus*, and were successfully used to catch *Cx. pipiens* and *An. plumbeus.*

Gravid traps

These traps use standing water in a black bucket and are designed to collect gravid females as the mosquitoes search for oviposition sites. Gravid traps are used in arbovirus surveys because the probability of collecting blood-fed mosquitoes is higher, which in turn increases the likelihood of detecting viral infections in the mosquito population. Gravid (blood-fed) females are more effective indicators of pathogen activity than host-seeking females (which may have not taken a blood meal). Gravid traps are not considered to be particularly attractive to *Aedes* mosquitoes, but their attractiveness can be improved by using infusions of dead oak leaves or grass (bluegrass, *Poa* spp.).

Sticky traps

Like ovitraps and gravid traps, sticky traps attract gravid egg-laying females which land on internal surfaces and stick to the trap surface. It is therefore possible to determine the species and use the caught specimens for pathogen screening. When used in high numbers, these traps also reduce adult populations, especially when they are placed low enough and few alternative breeding vessels are available. They are thought to be effective for all container-breeding species, although this not yet been confirmed for all species.

Resting boxes

Most species are either nocturnal or crepuscular and remain mostly inactive during daylight hours. When inactive, mosquitoes can be found resting in dense vegetation, animal burrows, caves, tree holes, stables, basements, etc. It is therefore often more efficient to collect mosquitoes during their period of inactivity. Since the early days of malaria control, artificial resting structures have been designed to sample various mosquito species. The standard

resting box is a cubic wooden black painted box (often plywood) that is open on one end; the interior should be painted red or rust brown. A small hole should be drilled into one of the side panels allowing easy access into the box for collecting and should be covered with a window screen to prevent mosquitoes escaping from the box. Resting boxes should always be placed in shaded areas and not in open fields, gardens, marshes and any other non-shaded sites. The highest collections are made in dark forested sites with a high canopy. A clear bias towards *Culiseta* and *Anopheles* has been found in several studies. This makes the trap only suitable for some species in some sites.

Ovitraps

Ovitraps are very simple and are considered effective for container-inhabiting *Aedes* species. They consist of a small black plastic bucket (0.3 to 1.0 l, 2/3 filled with water and with a hole preventing overflowing and flooding the eggs) and an oviposition support (usually germination paper, a wooden stick, or a piece of polystyrene). The size of the bucket has to be adapted to the frequency of trap checking and the local rainfall (frequency and intensity) in order to prevent the trap drying out completely. If checking intervals exceed eight days, it is recommended that some long-lasting insecticide be added to the water (bio-pesticide or insect growth regulator) to stop the trap becoming a breeding vessel. Ovitraps can be positioned close to or under vegetation or near buildings and should be labelled, for example: 'Scientific study. Please do not remove. Please contact...' (Figure A4-B).

Though ovitraps are cheap and easy to use, identification of eggs is difficult and time-consuming, and the correlation between the number of eggs and female density is not always clear because females do not lay all their eggs in one location, and many other containers may be used if available. Increasing the number of traps will make such calculations easier.

If many breeding sites are available, ovitrap efficiency is reduced, but trap attractiveness can be improved by using oak leaves or grass infusion (see above: gravid traps). Ovitraps do not need to be inspected often and are thus a useful tool for surveying remote locations (control intervals of several weeks to several months are sufficient). In high-risk areas traps should be checked more frequently; checks should be combined with active larval searches in other containers. In some areas (e.g. in malaria-endemic regions), clay pots have been used as a replacement for ovitraps, with varying degrees of success.

Figure A4-B: Adult sampling/trapping; backpack aspirator (top left), bird-baited trap (top right); dog- and chicken-baited traps (bottom left); horse-baited trap (bottom right)

Photos: F. Schaffner (top), A. Schoenenberger/IPZ (bottom)

Table A4-B. Commonly used traps for adult mosquitoes

Trap model	Attractants	Targeted mosquito	Main species attracted	Strengths	Weaknesses	Remarks
BG-Sentinel	• Lure (mixture of human skin odours: BG-Lure, Sweetscent) • Carbon dioxide (optional)	Host-seeking females	• With lure only: *Ae. aegypti, Ae. albopictus* • With carbon dioxide: almost all mammophilic spp.	• Relatively small and light • Easy to handle in the field • Efficient lures	• 12V battery or power source needed • Easily damaged	Has been shown to also catch male and gravid or blood-fed *Ae. albopictus* females.
Miniature CDC (without light)	Carbon dioxide (dry ice)	Host-seeking females	Almost all mammophilic spp.	• Small, easy to manage • Light can be disconnected • Some models have a light-sensitive switch	Needs a source of carbon dioxide	When using light, many other insects are trapped (e.g. moths), mixing up the sample; better to switch off light.
Mosquito Magnet	• Carbon dioxide (from propane) • Lure (octenol)	Host-seeking females	Almost all mammophilic spp.	No power supply needed, operates for three weeks	Heavy, large, and expensive	High costs
Gravid trap	Fresh grass infusion	Oviposition-seeking females	Mainly *Culex*	Used for pathogen screening in mosquitoes, as these traps attract females that have taken at least one blood meal (more chances to be infected)	Capture range limited	Method of preparing infusion can influence attractiveness. Gravid mosquitoes are often used in surveillance of arboviruses.
	Oak leaves or grass infusion		Container-inhabiting *Aedes*		Capture range enhanced compared to standard but still limited	
Sticky trap	Infusion, water, lure (AtrAedes)	Oviposition-seeking females	Container-inhabiting *Aedes*	No power supply needed, relatively easy identification of species	Low catch rates	Handling the glue and sticky sheets can be challenging.

BG-Sentinel trap

CDC trap with dry-ice container

Mosquito Magnet trap

Gravid trap

Sticky trap

Photos: A. Rose/Biogents (left), F. Schaffner/IPZ (others)

Detailed procedures for active native mosquito species site surveillance

In order to optimise resources and increase effectiveness of surveillance activities, surveillance sites for each identified area should be identified and prioritised. Maps of risk areas which show the proliferation of mosquito vector populations can help with this task, but local teams, who can adapt regional strategies to the local context, should determine the final location of surveillance sites. It is therefore essential that stakeholders at the national level should identify local entomological, public health, and mosquito control expertise before initiating mosquito surveillance. If no national experts can be identified, the experts mentioned in these guidelines can be contacted. National/international experts in entomology, surveillance, pesticide science or pest control can also provide useful input and advice, and can help provide any necessary training. At least one expert in medical entomology should be involved at the very beginning of the surveillance planning process.

Generally speaking, there are three types of surveillance programmes:

- Baseline surveys are conducted to determine the types of vectors and pests occurring in the area of operation, their respective breeding sites or source habitat, and seasonal activity patterns. This type of surveillance can be performed during the 'pre-disease stage'.
- Operational survey: data collected in an operational survey are used specifically to aid pest management personnel in making decisions on when to start or stop control measures. The decision to start control or management activities is based on the data collected from this type of survey when it is determined that the vectors/pests are occurring in population densities greater than that observed in baseline surveys. This type of surveillance can be performed during the 'disease stage'.
- Specific surveys are conducted when a specific vector or pest species is targeted for specific surveillance beyond the scope of the baseline or operational survey (for example a survey in only one site).

The first step in the organisation of any efficient surveillance measure is to define and describe the objectives of the study. After stating the objectives (and ascertaining available funding and other resources), the size of the area under surveillance, the trap type, and the trapping frequency has to be determined. If several traps are used, it is important to place all traps that they do not interfere (e.g. broadest range of CO_2 detectability; range of chemical lures, animal/human bait).

Survey design can be defined as cross-sectional versus longitudinal.

- Cross-sectional surveys collect data to make inferences about a population of interest at one point in time. Cross-sectional surveys have been described as snapshots of the populations on which they gather data. Cross-sectional surveys may be repeated periodically; however, if a cross-sectional survey is repeated, sites sampled at one point in time are not sampled again, but it is possible that a survey site is randomly selected to be sampled again.
- Longitudinal surveys are research studies that involve repeated sampling of the same sites over long periods of time. These surveys are used to assess population dynamics (e.g. seasonality, density peaks, number of generations, oviposition strategy) of one or more species in a specific site. This information can then be used in pest RAs.

It is important to obtain a map of the area of interest, including adjacent areas, which shows land use classes. Basic ecological and meteorological knowledge of the geographical location will add immeasurably to successful surveillance efforts. However, if some of the above information is missing, the surveillance campaign should still be conducted. State-of-the art geographical information systems and integrated software components and other tools will help the team to efficiently plan all surveillance activities. Surveys based on these tools can be planned fast and efficiently and increase the overall success rate.

If several teams are in charge of the operational aspects of the surveillance campaign, a protocol needs to be used. This will ensure the standardisation of all activities and provides a basis to make comparisons between teams. A field form can be drafted to assist in the standardised collection of field data; this can be done on paper or with mobile apps.

Data obtained from mosquito surveys should be properly compiled and correlated to provide meaningful information. All data need to be stored in a (centralised) database which records trapping and identification results as well as all necessary environmental and/or climatological information.

Determination of surveillance period in relation to climatic conditions

The climatic conditions of the region under surveillance must be taken into account when determining the active surveillance period/seasonality. In more temperate regions, the activity period will be shorter (i.e. May–September) than in more Mediterranean climatic regions (i.e. March–October). In spring, the blossoming of dandelions indicates the start of mosquito activity season, and the first frost of autumn may indicate its end. Prior to any study, reports from neighbouring countries can be consulted to determine the exact time period in which the activities should be planned and executed.

For some mosquito species, severe climatological conditions (such as heavy rains) could trigger development. This is particularly rlevant for floodwater species, which can exhibit mass development after heavy rainfall and flooding.

Choice of places for trapping and sampling

Placement of traps

Some behavioural characteristics of native mosquitoes can help determine suitable sites to place mosquito traps:

- For resting, mosquitoes prefer shaded, wind-protected areas, e.g. bushes and shrubs.
- For laying eggs, mosquitoes will look for potentially flooded ground areas or small and dark water containers in shaded areas.
- Flying mosquitoes prefer to fly relatively close to the ground, below 2m (except for bird-baiting species that can be caught in the canopy as well).

- Mosquitoes prefer to fly through bushes and shrubs and try to avoid open terrain like meadows; they prefer to use of 'shrub-corridors' like hedges to fly from one place to another rather than crossing open terrain.
- Some trap types should be placed on smooth, flat-soil surfaces (e.g. MM traps).

Areas to be avoided:

- Areas near other sources of artificial light.
- Areas exposed to strong winds.
- Places near buildings or industrial sites.

Traps should be placed out of sight and where children cannot reach them, and should be labelled 'Ongoing scientific study/surveillance', with additional information on ownership, purpose of the study and further contact details (e.g. organisation/coordinator). If the trap is placed on a private propriety, it may be necessary to inform municipalities and gardening staff to obtain permission of the landowners. This is particularly important for expensive traps (i.e. BG Sentinel, MM).

Ideally, one or more traps should be located in the area of interest, especially when estimating abundances/density because a single trap cannot yield a representative sample. If a trap fails to catch the expected number of mosquitoes in a specific site, the trap should be relocated. A shift of a few metres can already make a considerable difference in the number of mosquitoes attracted. If several traps are simultaneously operated, traps should be placed in such a manner that they do not interfere or that interference is reduced to the minimum. To obtain the least interference between traps, a Latin-square design can be applied where traps are placed at least 70m apart from each other.

Tips for fieldwork:

- Each site or trap can be associated to a data logger to measure onsite temperature and humidity.
- The GPS position of each trap should be recorded.
- On every visit, spare parts (or material to repair the traps) as well as extra traps should be brought along.

Figure A4-C. Habitats unsuitable for native mosquito species; adults (top) and larvae (bottom)

Photos: F. Schaffner

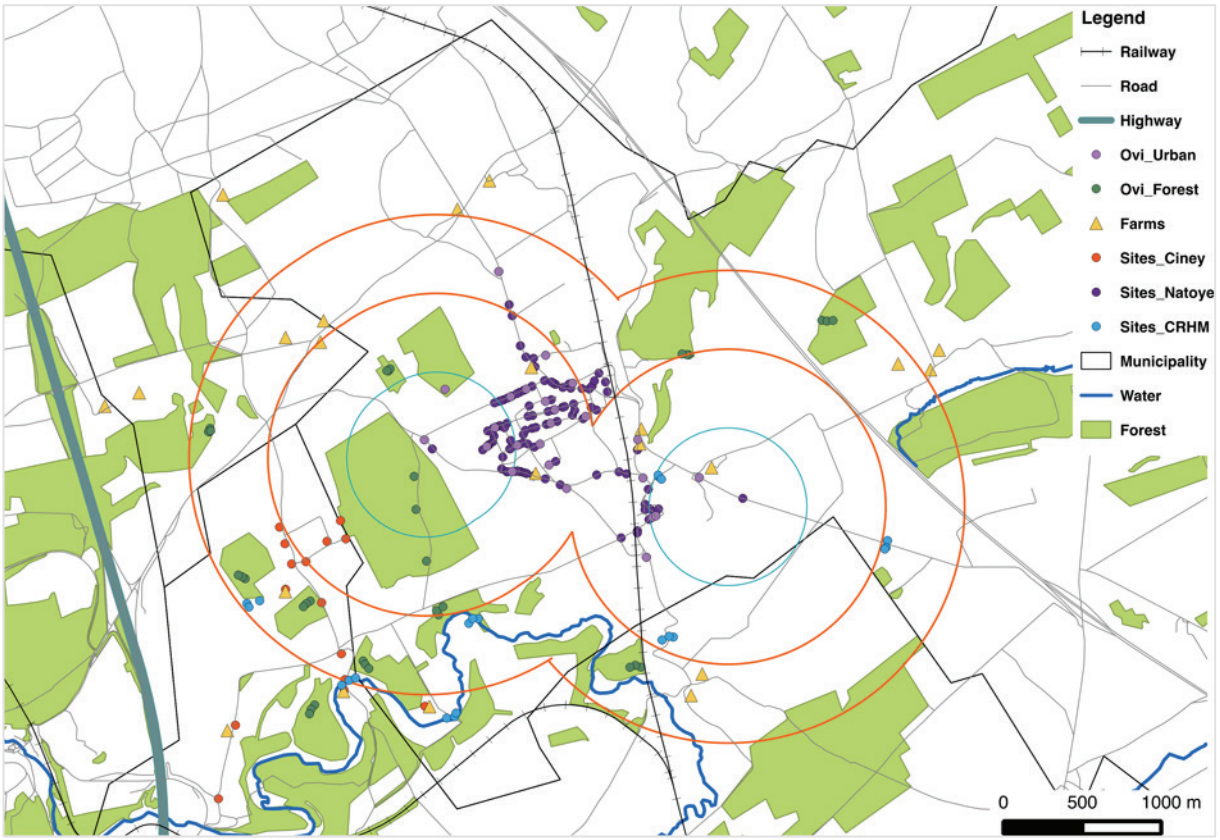
Figure A4-D. Proper placement of mosquito traps. Top: CO_2-baited CDC miniature light trap in a wetland; bottom left: CO_2-baited CDC miniature light trap (next to a black-light trap for biting midges); bottom right: gravid trap

Photos: F. Schaffner

Figure A4-E: Habitats suitable for native mosquito species

Photos: F. Schaffner.

Figure A4-F. Example of an operational surveillance plan



Map A (top): Three zones are delineated to prioritise the surveillance of container-breeding mosquitoes: blue = very important (inner circles, up to 500m), orange = important (centre circles, up to 1000m), orange red = less important (outside circles, up to 1500m). Green areas = forests; cyan dots = all urban sites/units within the target municipality that could be sampled; yellow triangles = rural sites/units.
Map B (bottom): Some urban sites were randomly selected (dark and light purple dots for municipality X, orange dots for municipality Y); most of the sites were sampled (light purple and orange dots); traps were placed in forested sites (green dots) and along rivers (blue dots).

Source: Avia-GIS, Belgium

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Annex 5: Methods for identification of native mosquitoes

A. Morphological identification

Although some species can be identified in the field, morphological identification of adults and larvae usually needs to be done in the laboratory. Critical characteristics of adult mosquitoes can be distinguished with a stereo microscope. Sufficient lighting and positioning of adult or larval specimens under the stereo microscope (at least 60x magnification is recommended) are important. Adult specimens may be pinned (double mounted method) or glued on a card point. The specimen needs to be examined at its dorsal, ventral, lateral and caudal sides, so sufficient space for rotating the pinned specimens need to be foreseen. A stage manipulator may be used to avoid multiple touching of the specimen (which would increase the risk of damaging the specimen). Adult specimens that are not pinned may be identified under a stereo microscope using a petri dish with a white surface underneath the petri dish, manipulating specimens with a pair of thin forceps or mounted pins. Larval specimens (4th instar) can be observed under a stereo microscope while they are submerged under ethyl alcohol or water. However, some specimens need to be mounted on slides either temporarily or permanently to observe extremely small structures (e.g. setae, pecten, comb scales) for diagnosis under a compound microscope.

Detailed procedures on how to mount mosquito adults, immature exuviae and whole larvae can be found on the website of the Walter Reed Biosystematics Unit at http://wrbu.si.edu/wrbu.html.

Morphological identification records should be stored in a (centralised) database, preferably linked to eco-climatic information (habitat class, sampling period for the trapping site, etc.).

Morphological identification keys

Two identification keys based on morphology exist for the European mosquito fauna: a computer-aided key (Schaffner et al. 2001) and a printed, illustrated dichotomic key (Becker et al. 2010). Other keys exist but only cover certain geographical areas and/or species or genera.

B. Molecular identification methods

Molecular identifications can only be performed in adequately equipped laboratories with trained staff. Table A gives an overview of the required material. Pending DNA extraction, field-collected mosquitoes need to be stored at a minimum temperature of -20 °C; preferably each specimen should be stored in a separate vial to avoid contamination. Although DNA can also be extracted from dried mosquitoes, better results are obtained with frozen specimens.

PCR gives an exponential amplification of the target DNA (can also be applied to RNA). This occurs by a cyclic reaction of different steps with changing temperatures. The temperatures are specific for each step and for each PCR reaction (mainly depending on the nucleotide sequence of the primers):

- Denaturation step: making DNA single stranded (mostly 94 °C)
- Annealing step: annealing of the primers (mostly 50–70 °C)
- Elongation step: extension of the complementary DNA chain (mostly 72 °C)

PCR also demands the use of correct buffer conditions, presence of target DNA, dNTP and an enzyme, mostly *Taq* polymerase, a thermostable enzyme originating from *Thermus aquaticus*. A simple visual detection of the amplicons can be done by ectrophoresis on an agarose gel followed by an ethidium bromide staining. Amplified products can afterwards be sequenced or they can be processed further by adding restriction enzymes (PCR-RFLP) to the amplified product. This latter technique is often used to rapidly distinguish between closely related species (e.g. *Anopheles maculipennis* s.l. complex).

Table A5-A. Required material and chemicals needed for DNA extraction

Laboratory equipment	DNA extraction protocol
	Lab notebook
	Lab coat
	Gloves
	Heat block
	Eppendorf tubes
	Tube rack
	Ice bucket filled with ice or ice block
	Permanent marker
	Micropippetes (P20, P200, P1000)
	Tips
	Sterile forceps
	Rinse beaker
	Pestle
	Vortex
	Timer
	(Micro)centrifuge
Chemicals	Sterile water
	Javel
	Ethanol (95%, 100%)
	Buffer
	Proteinase K
	AE and/or AL buffer
	dNTP

Definitions and abbreviations

- Amplicon: final product of a PCR reaction (amplified DNA)
- DNA: deoxynucleic acid
- rDNA: ribosomal DNA
- dNTP: deoxynucleotide triphosphate, basic elements needed for the extension of a DNA chain
- PCR: Polymerase chain reaction
- Primers initiators: short nucleotide sequences (mostly 10–25bp)
- Complementary to the endings of the target DNA
- RNA: ribosomal nucleic acid
- Target DNA: limited region of the (chromosomal) DNA that one wants to amplify with the aid of specific primers

Allozyme analysis

Isoenzymes assayed by gel electrophoresis have long been used to study molecular taxonomy and population structure in several mosquito groups. Most work has been done on the three main genera *Aedes*, *Anopheles* and *Culex*. Species identification keys based on isoenzyme profiles were designed for *Annopheles* complex species from the 1970s, and enzyme electromorphs have been used to unravel taxonomic problems in *Anopheles* and *Culex* complexes, including complexes occurring in Europe (Bullini and Coluzzi 1982; Urbanelli et al. 1985; Lopatin 1993; Chevillon et al. 1995; Byrne & Nichols 1999; Schaffner et al. 2000; Schaffner et al. 2003; Weitzel et al. 2009). Also, some *Aedes* species groups were investigated, including the *Ae. annulipes*, *Ae. caspius*, *Ae. detritus*, and *Ae. communis* groups (Pasteur et al. 1977; Lambert et al. 1990; Brust & Munstermann 1992; Gad et al. 1992; Schutz & Eldridge 1993).

Enzyme gene loci offer suitable characteristics due their presumptive selective neutrality (Kimura 1983), which enables the quantification and comparison of genetic differentiation and the extent of genetic flow between and within genetic entities and dispersed populations. Since the discovery of hyper-variable microsatellite DNA sequences (see below), isoenzyme analyses are less used, especially in population genetic studies.

DNA barcoding

DNA barcoding is a technique based on standardised short DNA sequences (ca. 500 bp) that can be universally used to identify species. Mitchell (2008) states in his overview that there were four main novel factors to DNA barcoding in general: 'standardization on a particular gene region, the large scale of operation, compulsory vouchering of specimens and active curation of the resulting databases'. In summary, DNA barcodes provide a unique 'tag' for each of the studied species. For this purpose, a fragment of the cytochrome oxidase subunit 1 (COI) in the mitochondrial genome is selected because of several advantages such as maternal lineage, lack of recombination, lack of 'indels' and higher mutation rates (Saccone et al. 1999). The COI region is also the most conserved gene among other amino acid coding genes (Knowlton & Weight 1998), aiding resolution of deeper taxonomic affinities and primer design. One of the main advantages for worldwide research is comparability. The only major drawbacks associated with the marker arise from illegitimate amplification of mitochondrial genes that have inserted into the nuclear genome (so called numts) in some species (Bensasson et al. 2001).

The complete or partial COI gene is increasingly used in phylogenetic, systematic and population genetic studies. So far, this region has hardly been targeted in 'basic' mosquito barcoding research in Europe. In northern Africa, DNA barcodes were applied to 'DNA taxonomy' (Laboudi et al. 2011), although there is growing concern about the use of a single gene to solve taxonomic problems. In such cases, supplemental analyses combined with other traits, such as nuclear genes, are required (Hebert et al. 2003; Baker et al. 2009). Besides being used as a target genome in DNA barcoding, mitochondrial DNA has been used in a wide range of different molecular studies. Main advantages include high copy numbers, the availability of conserved primers and PCR protocols (Loaiza et al. 2012). Several studies indicate that results obtained in mtDNA studies generally match those of others carried out with different markers (Vicente et al. 2011) although there are also exceptions (Di Luca et al. 2004). These can probably be linked to contemporary hybridisation between previously isolated entities and introgression of sister taxa. The main limitations in the use of mitochondrial genes include the fact that they are inherited as a single block and may therefore provide limited information and the strong AT bias, which can rapidly lead to loss of phylogenetic signal due to homoplasy (see Loaiza et al. 2012).

Other mitochondrial regions that are often used for mosquito species identifications are ND4, COII and D2 (Versteirt 2012). Several primers have already been developed for these regions and can be found in the literature, as is the protocol most often used to amplify the extracted DNA. A selection of positive PCR products (5–10 per species) is sequenced in the laboratory or by an external company. Obtained sequence data are aligned (several free alignment tools are available) and have been compared with data available in BOLD (www.barcodinglife.org) or GenBank (BLAST, ww.ncbi.nlm.nih.gov/guide/sequence-analysis).

As for ribosomal DNA (rDNA), the most commonly used region, especially in *Anopheles* studies, is the non-coding internal transcribed spacer two (ITS2) (Linton et al. 2001, 2003). Due to length differences and fixed substitutions among ITS2 sequences of different species, rapid distinction is possible. Species specific primers and PCR protocols are developed for a wide range of species including *Anopheles spp.* (Proft et al. 1999; Kampen et al. 2003) and *Culex spp*. (Vinogradova & Shaikevich 2007; Talbalaghi & Shaikevich 2011). This marker seemed promising as a phylogenetic informative marker (Djadid et al. 2007) but failure to identify cryptic species has been reported (Di Luca et al. 2004; Bargues et al. 2006). Possible explanations include intra-individual variation, sampling gaps and an evolutionary mechanism which is not fully understood. The ITS2 region is, to a lesser extent, also explored in population structure studies (Loaiza et al. 2012). Overall, this region has proved its utility by solving taxonomic and systematic problems, most often in (cryptic) *Anopheles* species studies. Loaiza et al. (2012) state that 'patterns of genetic divergence by the ITS2 seem to be consistent with the geographical origin of populations, and this may indicate that concerted evolution is acting intraspecifically regardless of significant intra-individual variation', which is supported by a large number of studies (Patsoula et al. 2007; Bezzhonova & Goryacheva 2008). Moreover, its evolutionary cohesion was recently supported (Alquezar et al. 2010), underscoring its importance in taxonomic studies.

Other rDNA regions that are often used are ITS1 and D2/3. Several primers have been developed and there is a protocol used to amplify the extracted DNA. A selection of positive PCR products (5–10 per species) is sequenced in the laboratory or by an external company. Obtained sequence data are aligned (free alignment tools are available) and have been compared with data available in BOLD System (www.barcodinglife.org) or GenBank (BLAST, ww.ncbi.nlm.nih.gov/guide/sequence-analysis/).

Microsatellite DNA

Microsatellite DNA is composed of short tandem repeating sequences, mostly 2–6 base pairs of DNA which are repeated for a certain number of times (5–40). Di-, tri- and tetranucleotide repeats are the most commonly chosen sites (Selkoe & Toonen, 2006). The DNA sequences surrounding a microsatellite are called a flanking region, which are generally conserved among individuals of the same and sometimes different species. Microsatellites are co-dominant, polymorphic and are now mostly used in population genetic and molecular ecology studies. Due to the presence of variable repeats that differ between alleles, microsatellites are a measure for inter- and intraspecific polymorphism. They provide data on patterns of paternity and kinship in natural populations and are used to detect hybrids in natural populations (Selkoe & Toonen 2006). Due to their exceptionally high mutation rate, they

are conducive to the analysis of the relationships among closely related species and sub-populations of single species. Primers have been developed for microsatellite analyses of major vector species and were used to study the population structures of *Anopheles* (Weill et al. 2003); *Aedes* (Fonseca et al. 2010; Widdel et al. 2005; Kamgang et al. 2011) and *Culex* species (almost 33 microsatellite markers have been developed for *Cx. pipiens* complex, e.g. Keyghobadi et al. 2004; Smith et al. 2005; Chambers et al. 2007; Edillo et al. 2007; Hickner et al. 2010). Despite the many advantages, there are also several drawbacks such as species-specific marker development, amplification problems and homoplasy (Selkoe & Toonen 2006).

MALDI-TOF mass spectrometry

A newly developed technique in mosquito egg identification is protein profiling using MALDI-TOF MS (matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry). It has been widely used in routine identification of microorganisms in clinical microbiology laboratories, but also seems to be suited for robust, low-cost and high-throughput identification of mosquito vectors in surveillance programmes (Schaffner et al. 2014). This technique is especially useful in routine operations because large samples of mixed species can be screened at various stages (eggs, larvae, adults). When pooling eggs, all species need to account for at least 30% of the collected eggs, making this technique less valuable for the detection of low-abundant species.

Proteomic analyses

The next step in the development of molecular tools is the use of protein instead of genetic fingerprinting. If we see DNA as a chemical instruction manual for 'building' a plant or an animal, proteins are the dynamic components. DNA or mRNA sequences cannot sufficiently describe the structure, function and cellular location of proteins. The term 'proteome' thus constitutes the total of proteins expressed by the genome. In the last ten years, technical progress has led to the development of better proteomic analyses and enabled the quantitative analysis of protein expression inside cells. This technique, however, is usually only used to study parasite-vector interactions and not for the mere identification of the vector (Chaerkady et al. 2011).

Used as a complementary tool alongside current morphological identification systems, molecular techniques are a valuable asset to improve the speed and accuracy of mosquito identifications (Table B). Molecular identification techniques enable the rapid detection of biological invasion (Armstrong & Ball 2005) and population changes in terms of location and possible speciation in response to environmental stimuli (Podivinsky 2003) and have the potential to detect changes before they become apparent in morphological features.

Table A5-B. Frequently used molecular identification techniques

Technique	Target species	Frequently used (Y/N)
Isoenzyme analysis	All species	N
DNA barcoding (mtDNA)	All species	Y
ITS2, rDNA	*Anopheles*	Y
Microsatellites	*Culex, Aedes*	Y
MALDI-TOF MS	Container-inhabiting (eggs) and other mosquitoes	N
Proteonomics	Vector species (*Anopheles*)	N

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Annex 6: Principles and detailed methodologies for determination of mosquito population parameters

Mosquito life history parameters: a glossary

Abundance

Strictly applicable to quantity only; number of specimens of a certain species (absolute, relative, or index).

Basic reproduction number R_0

The average number of secondary cases of disease arising from each primary infection in a certain population of susceptible humans/hosts. A disease spreads if $R_0>1$ and decrease if $R_0<1$.

Birth rate

The ratio of the number of live births in a period of time in a given area/larval habitat in relation to a given portion of the population in that area/larval habitat.

Biting behaviour

Usually related to host finding (foraging) behaviour of the species. Most often related to the part of the day (diurnal, crepuscular, nocturnal bating behaviour) when most of the specimens of a particular mosquito species forage for the blood meal.

Blood-feeding behaviour

Haematophagy is the practice of mosquitoes to feed on blood. In relation to host preference, mosquitoes can be opportune (specialised) or catholic (unspecialised) feeders. If females tend to feed repeatedly (from the same or different hosts) to complete one blood meal, this is referred to as 'multiple feeding'.

Death rate

The frequency of death; the proportion of deaths in a specified number of the population (mortality rate).

Density

The number of individuals of the same species that live in a given unit area.

Dispersal

The outward extension of the range of the species, usually resulting from the chance event; ability of mosquitoes to spread around/from the breeding site.

Fecundity

The innate capacity of an organism to form reproductive elements such as ova or sperm; the potential capacity for reproduction.

Fertility

The natural capability to produce offspring; as a measure, 'fertility rate' is the number of offspring born per mating pair, individual or population.

Generation time

The doubling time of a species under the influence of certain ecological conditions, or the time elapsed from one egg laying to the next.

Gonotrophic cycle

The duration of time between two ovipositions, i.e. the time females spend for host-seeking, blood feeding, resting (digestion and egg maturation time), and oviposition (seeking the site and laying the eggs) in nature, or from blood meal to egg laying in the laboratory.

Intrinsic rate of increase

A population's growth rate, derived by subtracting the instantaneous death rate from the instantaneous birth rate (innate rate of increase).

Longevity

The duration of life of an individual (lifespan).

Net reproductive (replacement) rate

The total amount of offspring that a newly born female can expect to bear during a lifetime.

Survival rate

The rate of specimens remaining alive in a given period of time (e.g. daily), especially under adverse conditions.

Vectorial capacity

A mathematical expression of the probability of disease transmission by a specific vector species. The average number of inoculations from a single case of disease in a day, from vector population to man, assuming that all mosquitoes that bite an infected person get infected. This is mosquito component of the basic reproduction number R_0.

Vector competence

Ability of mosquito species to transmit a specific disease expressed in relative number of females infective (usually head or salivary glands are checked) with the pathogen.

Source: Petric et al. 2014

Table A6-A. Methods, tool, indices and formulas for the determination of mosquito population key parameters and procedures

Parameters and procedures	Methods	Tools	Indices	Formulas
Population abundance and seasonal dynamic	Larval habitat surveys: inspection of immature habitats	Dipper, water net	Larval density	Number of larvae per litre, extrapolated to number per hectare
	Adult trapping with adequate trap	Adult trap	Adult mosquito per trap/period	No. of females per trap/period
		Resting box/trap, aspirator etc.	Human blood index	Mosquito no. fed on human/total no.
	Anthropophilic adult catching	HLC, mouth aspirator	Human landing rate	No. of females/human/15min
			Nuisance threshold	
Female longevity, gonotrophic cycle and dispersal	Mark–Release–Recapture	Rearing facilities, marking equipment, adult traps or aspirators	Mean distance travelled	Mean distance in km
			Maximum distance travelled	Maximum distance in km
			Flight range	The maximum distance reached by certain part of population (e.g. 90% of the marked adults)
	Laboratory experiments	Rearing facilities, flight mills	Flight performance	Mean/Maximum distance in km
	Laboratory experiments	Rearing facilities under specific climatic conditions	Adult longevity; gonotrophic cycle	Weibull model; Time period between two consecutive blood meals
	Field experiments	Host-seeking adult traps; Mark–Release–Recapture	Parity rate; daily survival rate	No. of parous females/total no.; daily probability of survival
Female biting and resting behaviour	Daily biting activity	Baited trap or HLC	Circadian activity	Biting activity per 15min (HLC) or per hour (trap) over 24 hours
	Blood fed females trapping/collection	Blood meal analysis, traps, aspirators	Host feeding indices, anthropophily	Mosquito no. fed on host or human /total no.
	Endophagy/Exophagy comparison	Baited trap or HLC	Endophagy rate	Percent of females biting indoor
	Endophily/Exophily comparison	Resting catches/traps	Endophily rate	Percent of females resting indoor after the blood meal
	Indoor experiments for host preferences and repellence	Olfactometers or cages, hosts	Host feeding patterns	Percent of females feeding on different hosts or treated/untreated skin
	Outdoor experiments for host preferences	Host-baited traps, odour-baited traps	Host feeding patterns	Percent of females per different host

Parameters and procedures	Methods	Tools	Indices	Formulas
Population vector competence	Vector susceptibility/ competence studies	Rearing and infection facilities in BSL3	Infection rate	Percent of females with infected head
			Dissemination rate	Percent of females with infected saliva
Transmission risk	Gathering field and laboratory data on abundance, longevity, biting behaviour and dispersal	Mathematical/ statistical analysis, modelling, mapping	Gonotrophic cycle	The time elapsing between females finding a host to laying eggs in nature or from blood meal to egg laying in laboratory
			Vectorial capacity (mosquito component of the basic reproduction number R_0)	The average number of inoculations from a single case of disease in a day, from vector population to humans, assuming that all mosquitoes that bite an infected person get infected
			Basic reproduction number R_0 [the disease can spread if $R_0>1$ and decreases if $R_0<1$]	The average number of secondary cases of disease arising from each primary infection in a certain population of susceptible humans/hosts.

Information about native mosquito species mosquito population parameter values from specific national/regional context can be found in literature or obtained through exchanges with research teams and experts.

Principles

Mosquito population parameters and vectorial capacity

The life cycle of female mosquitoes requires searching for physiological needs like sugar, mates, blood, resting places, and oviposition sites. The success of these foraging activities will determine the parameter values affecting a species' vectorial capacity in local conditions, i.e. population estimates like longevity, dispersal, density, and pathogen transmission. In places where a species is a putative vector of a disease pathogen, these parameters are important in determining the epidemiological status of the population.

Population estimates of mosquitoes are numerous and usually describe developmental and life history parameters like: longevity (including duration of growth of different mosquito life stages – egg, larva, pupa, adult – and their survival rates); the number of gonotrophic cycles per lifetime and their length, fertility, fecundity, mortality, the intrinsic rate of increase, net reproductive (replacement) rate, birth rate, death rate, generation time, host preference, capacity for dispersal, and size of population. Differences in body size of adult mosquitoes can, for example, be important from an epidemiological standpoint: large females may have higher human host attack rates and obtain multiple blood meals (from multiple hosts) more frequently than small females, thus spreading the disease more efficiently. Body size may also affect mosquito survival and longevity under natural conditions. The ability to determine the age structure and the survival rate of female mosquitoes is of paramount ecological importance because longevity affects net reproduction rates and dispersal distance (Service 1999). Knowledge of feeding behaviour is also important: Does a mosquito prefer to take the blood meal from human or from animal hosts? Does it feed from different/multiple hosts before being completely fed? When does it search for a blood meal?

Other aspects of the life history of pest insect species are of crucial importance to estimating its vectorial capacity (potential for transmitting the disease) and for assessing the risk of MBD transmission: Is a mosquito population which can transmit a certain disease present in the country? How numerous is this population? How efficiently can it transmit the disease in a human population? What are the pathways for disease introduction from abroad? This knowledge also supports the development of effective control programmes and the evaluation of its impact. It also has a direct bearing on the establishment of efficient mass rearing facilities for the sterile insect technique (SIT, used to successfully eradicate agricultural and veterinary pests from isolated areas), the evaluation of sterile insect release impact, the interpretation of trap data, and the modelling of potential outbreaks.

Mosquito population parameters are monitored for a variety of purposes and by using different approaches. The most important issue in planning the collection of information is to clearly define the objectives of a study at the beginning of the planning process because this will largely determine the methods to be used. It is also important to consider the type of statistical analysis that will be applied and therefore to ensure that data are collected, managed and stored (see Chapter 2.7) in the most appropriate manner.

Evidence is accumulating that changes in these parameters may occur in fluctuating mosquito populations, and the population parameters of different cohorts of a species may be quite different. In some cases, fluctuation of the vector populations might be linked to the adaptation of population parameters to abiotic factors (see Chapter 2.4). These parameters need to be determined under controlled conditions to better understand the dynamics of vector populations and their vectorial capacity, especially in countries with a wide range of temperatures (different climates and a broad altitude range). Therefore, laboratory (e.g. simulating the influence of different temperatures) and field measurements of, for example, fertility, longevity, or dispersal should be continually checked for local populations.

Longevity

In order to estimate a mosquito population's longevity, a collection of absolute data or the conversion of relative values is needed. This type of research can be done both in the laboratory, by rearing the target species, and in the field, typically by deriving the data from mark–release–recapture (MRR) trials. Experiments in semi-natural conditions can combine the advantages of controlled and natural environments.

Within a given population of mosquitoes, for example, vectorial capacity and the extent to which the potential fecundity is realised are influenced by the longevity of the females. The influence of temperature and other environmental factors (studied in the laboratory) can be incorporated into the regression equation. Observations of Richards and Waloff (1954, in: Southwood 2000) show that it may be justified to apply data from the laboratory to populations in the field. Such equations cannot, however, be applied outside the populations from which they were derived.

Blood feeding/host preference

Investigations of mosquito blood feeding and resting behaviour are of crucial importance for areas where epidemics occur and usually comprise investigating host-seeking and feeding behaviours on several vertebrate species, measuring endophagous/exophagous biting behaviour, endophilic/exophilic resting behaviour and the mosquito's daily (over 24h) biting activity. Mosquitoes can be opportunist, feeding on a wide range of cold and warm-blooded hosts, and thus can be involved in various vertebrate-virus transmission cycles, acting as bridge vectors for zoonotic viruses (circulating in animals and transmitted to humans). Preference for humans, so-called anthropophily, combined with multiple blood feeds during completion of one meal, increases the risks of spreading arboviruses to the human population.

Blood-feeding behaviour can influence vector potential depending on the vertebrate host groups with which the mosquito has contact. If reservoir and amplifier hosts (where pathogens multiply) are the primary focus of vector blood feeding, the likelihood of pathogen acquisition by the vector increases. A mosquito species that can feed on a wide range of hosts is a potentially dangerous bridge vector of zoonotic pathogens to humans (e.g. West Nile virus), but also less efficient as an epidemic vector of pathogens restricted to humans (e.g. human malaria *Plasmodium*). In addition, the blood-feeding behaviour of a vector may influence the spatial distribution of a disease. The spatial distribution of a population of blood-sucking insects among its available vertebrate hosts has important epidemiological consequences for the transmission of vector-borne diseases. In fact, after daily survival rate, the vectorial capacity is most sensitive to changes in host preference (Dye & Hasibeder 1986). Therefore, knowledge of the biological parameters that lead to host choice can be highly relevant for the planning of vector and disease control measures (McCall & Kelly 2002).

Population size, mosquito density

When sufficient data are obtained, both relative estimates and population indices can sometimes be related to absolute population (if this is measured at the same time) by regression analysis.

Dispersal

Flight, flight ranges and dispersal are parameters which indicate the distance that a mosquito travels (actively, by itself, or passively, by human transportation), e.g. from its breeding place in its search for sugar meals, mates, blood meals, resting and oviposition sites. Dispersal to seek a host is epidemiologically important as it is the means by which female mosquitoes acquire and disseminate pathogens. Dispersal for oviposition is also relevant to pathogen transmission as it increases dispersal of the progeny which can be infected. Knowledge about the movements of adult mosquito vectors in endemic or epidemic areas in Europe is needed to understand disease dynamics and to determine the area to implement control for reducing/interrupting pathogen transmission.

The flight of mosquitoes is influenced by factors such as species-specific traits, blood source and oviposition site availabilities, weather (e.g. wind, humidity, temperature, rainfall), terrain, vegetation and housing characteristics (in urban environments) (Petrić 1989; Petrić et al. 1995). If wind speed is higher than their flight speed (on average 1 m/s), most mosquitoes will not take off, and if airborne, will attempt to land. Estimations of active mosquito dispersal are most frequently carried out by means of mark–release–recapture (MRR) studies, the effectiveness of which is strongly affected by the quantity of marked specimens released and the ability to carry

out recapture over a large study area (Service 1999). Moreover, the availability of an effective recapture method may represent a serious limitation in MRR studies. Results obtained from MRR experiments cannot be generalised because they depend greatly on the ecological characteristics of the study sites. Moreover, the ecological factors affecting dispersal vary depending on the objectives of the mosquito dispersion (i.e. host seeking, resting or oviposition site seeking), which, in turn, implies different recapture approaches. Preferably surveys should be conducted for all host seeking, resting and ovipositing females and also for males (if SIT is going to be implemented).

Detailed methodologies

Types of mosquito population measurements and statistical considerations

Generally, studies can be divided into extensive and intensive (Morris, 1960, in: Southwood 2000). Extensive studies are carried out over larger areas than intensive studies. They are frequently used to provide information on distribution and abundance for surveillance or management programmes. Current advances in remote sensing capability and geographical information system (GIS) software have provided support to many studies in recent years. In extensive surveys, an area is sampled once (or a few times at the most) per study period. Timing, which is of critical importance, is tailored to the life cycle of the mosquito species. Intensive studies involve the frequent observation of the mosquito population. Usually, information is acquired on the population size of successive developmental stages so that longevity, nuisance and/or vectorial capacity can be estimated. Intensive studies focus on a rather limited set of objectives, e.g. the determination of the level of larval habitat infestation, the dispersal, or the host preference of the species.

Population estimates can be further subdivided into three major types:

1. Absolute estimates (comparisons in numbers)

Population estimates can be expressed as absolute or relative and in the form of population indices. For most animals (except the large ones that are easily observed and have small, countable populations, e.g. elephants, whales, or some birds, for which the overall population size may be given as a total number of individuals), numbers of absolute estimates are expressed as a density per unit area or volume (absolute population) or density per unit of the habitat e.g. per water container or per host (population intensity) (Southwood 2000). Such estimates are given by census and distance sampling; mark, release and recapture; sampling of a known fraction of the habitat (e.g. transect and suction trapping, sampling from vertebrate hosts, dry sieving and flotation of floodwater mosquito eggs); and removal sampling (use of different traps e.g. baited traps, and converting the estimates to absolute terms, using specific correction factors) (Southwood 2000). As the size of a species' population is related to breeding site and host availability, measuring population intensity often yields the first relevant data and is more meaningful than an estimate of absolute density.

2. Relative estimates (comparisons in space or time)

In relative estimates the sampled numbers cannot be interpreted as data on density or intensity per area or habitat unit, and can only be used to compare data in space or time. They are especially useful in assessing the species-relative density, dispersal, distributions and host preference and are helpful for monitoring environmental changes, MBD surveillance, and the evaluation of the efficacy of control measures. The methods employed are usually oriented towards various types of trapping, in which the number of individuals caught depends on a number of factors besides population density (Service 1999; Southwood 2000). Consequently, the implementation of such methods should be done only by highly skilled technicians; data analysis needs to be conducted by specialists.

3. Population indices

If mosquitoes are not counted, and instead their incidence in breeding sites is recorded, the resulting estimate is a population index. There is no clear-cut division between relative and absolute methods of sampling. Absolute methods are rarely 100% efficient, and relative methods can occasionally be corrected to give absolute density estimates. Relative methods are important in areas which already have NMS and MBD surveillance programmes, where most of the information available may be derived from egg, larvae and pupae sampling, and adult trapping. In mosquito surveillance, density is often difficult to calculate from sampling statistics (except when a comprehensive surveillance programme is implemented) because of changes in trapping intensity and/or sampling equipment following changes in financial support.

Despite the almost exclusive use of relative estimates in mosquito surveillance programmes, absolute values are of crucial importance for estimating longevity or studying survival rate and mortality factors, therefore laboratory studies are highly recommended, as are relative population estimates which were corrected to absolute figures.

The statistical errors of obtained values are usually referred to as the fiducial limits (the estimate (x) being expressed as $x \pm y$, where y = fiducial limits). The fiducial limits are normally calculated for a probability level of 0.05, which means that there are only five chances in 100 that the range given by the fiducial limits does not include the true value. If more samples are taken, the limits will be narrower, but the estimate may not move closer to the actual value. Entomologist are often worried that some of the assumptions about sampling efficiency

is incorrect ('Does the survey for the breeding sites really find all habitats in the district?'). Most of them, quite correctly, believe that acquired estimates should be compared with another method that has different assumptions. If the estimates are of the same scale, then the researcher can have much greater confidence in the reliability of the result of this study. It is therefore sound practice to estimate the population parameters with more than one method. In the long run, more knowledge of the ecology of the animal may be gained by studying other areas, by making other estimates, or by taking further samples than by struggling for a very high level of accuracy in each operation (Southwood 2000). Laughlin (1976, in: Southwood 2000) has suggested that the entomologist may be satisfied with a higher probability level (e.g. 0.2) and thus narrower fiducial limits for estimates based on more than one method because such estimates have a qualitative, biological assurance that the true mean lies close to the estimate, in addition to improved data consistency.

Mark–Release–Recapture method

The MRR method is frequently used to estimate longevity and growth, population size and dispersal. If a sample from a population is marked, returned to the original population, and then, after complete mixing, re-sampled, the number of marked individuals in the second sample will have the same ratio to the total numbers in the second sample as the total of marked individuals originally released have to the total population. A basic prerequisite for the use of these methods is a technique for marking the animals so that they can be released unharmed and unaffected into the wild and recognised again on recapture (Southwood 2000). Fluorescent dyes have been most widely used in mosquito MRR experiments but recently methods using mosquito strains whose natural infection with *Wolbachia* had been removed have been employed as well.

Longevity

The most frequently investigated factors when estimating the longevity of adult mosquitoes in the laboratory are food availability (water, blood meal, sugar solution) and temperature. It is worthwhile mentioning that larval density directly affects size and longevity (Miller & Thomas 1958, in: Southwood 2000). Rising temperatures can speed up development but reduce the body size of juveniles, influencing longevity.

Delatte et al. (2009) used the Weibull model as a classical nonlinear parametric model to describe the relation between death rate and time. This model is generally applied, with a mortality rate increasing roughly exponentially with increasing age at senescence. Löwenberg Neto & Navarro-Silva (2004) analysed data obtained in the laboratory by means of ANOVA for longevity and chi-squared test for survivorship linearity.

Blood feeding and resting behaviour

Host preference and blood feeding behaviour can be assayed in the laboratory using an olfactometer or cages of various construction and various hosts, or even outdoors. Using humans as a host is very important in the study of mosquito attractants, repellents, and host preference. However, mosquito bites cause potential medical problems because of hypersensitivity and perhaps secondary bacterial infection, even when using laboratory mosquitoes. Moreover, once a normal female mosquito bites and feeds on human blood, it cannot be used in subsequent probing tests. Shirai et al. (2000) offered a solution to these problems by the introduction of a proboscis (mosquito mouth part) amputation technique.

Outdoors, experiments on host preference are based on the use of host-baited traps of various design (Service 1999; Jaenson 1985; Becker et al. 1995), odour-baited traps (Costantini et al. 1993), or on blood meal analysis (Apperson et al. 2004; Molaei & Andreadis 2006; Richards et al. 2006). Hosts of blood-fed mosquitoes can be identified with an indirect enzyme-linked immunosorbent assay by using antisera made in rabbits for sera of animals that would commonly occur in particular habitats. Blood meals taken from birds can be identified to species by a polymerase chain reaction-heteroduplex assay (PCR-HDA) (Lee et al. 2002), and from humans (including multiple blood meals taken from more than one human) by STR/PCR-DNA profiling technique that involves amplification of three short tandem repeats loci (Chow-Shaffer et al. 2000; De Benedictis et al. 2003; Richards et al. 2006). Richards et al. (2006) also presented valuable methods for data processing, host feeding patterns and host feeding indices calculation.

Assessing the nuisance thresholds for dominant mosquito species is of a great value for the evaluation of conventional control measures (Petrić et al. 1999; Becker et al. 2010) but estimating a pathogen transmission threshold needs intensive sampling and experienced data processing. This usually involves deciding which seasonal estimates to use, what temperature to use, and what value for overall seroprevalence of virus antibody to use.

Gonotrophic cycle (the time females spent from finding a host to laying the eggs in nature or from blood meal to egg laying in the laboratory) is another population parameter connected with both host finding and blood feeding but also with resting, digestion of blood and oocyte maturation and oviposition. Its duration determines how many hosts a female will be feeding on during its lifetime, hence greatly influencing chances of finding an infectious host and transmitting a pathogen. Gu et al. (2006) divided the length of the gonotrophic cycle in natural conditions into three parts: (a) the time spent for host seeking, i.e. starting with a blood meal in the laboratory (Clements 1999); (b) resting as digesting and egg maturation time; and (c) oviposition time (seeking the site). The frequency of mosquitoes biting humans is estimated as the ratio of the human blood index (HBI) to the length of the gonotrophic cycle by Gu et al. (2006).

Dispersal

While searching for a blood meal, some species of mosquitoes may fly close to the ground while others do not; this peculiarity of a species should be taken into account when choosing a trap type for adult sampling in MRR studies. Gu et al. (2006) assumed that the 'poor searcher' mosquito has a searching flight path of around 250m per day, whereas the 'good searcher' should be capable of searching up to 500m per day. This assumption can be incorporated in the estimation of the basic reproduction number (R_0, the expected number of newly infected humans that will occur if one infected human is introduced into a totally susceptible human population), the most important measure of transmission dynamics.

If endophilic species are investigated, marked mosquitoes can be efficiently recaptured by active aspiration in houses during their indoor resting phase (Harrington et al. 2005), but this approach is much less efficient for collecting exophilic mosquitoes resting outdoors (Facchinelli et al. 2008). Mouse-baited traps were used to assess the longevity and dispersal of male and female *Ae. albopictus* by MRR (Lacroix et al. 2009). Bellini et al. (2010) investigated the dispersal of male *Ae. albopictus* mosquitoes in urban areas in northern Italy by MRR techniques, recapturing the males on human hosts while swarming. Traditionally, CDC backpack aspirators are used for recapturing resting females (Maciel de Freitas et al. 2008; David et al. 2009).

Early dispersal studies used fluorescent pigments and applied them to a number of mosquito species in different habitats and with different dispersal behaviour. Recently, methods using mosquito strains whose natural infection with *Wolbachia* had been removed (Bellini et al. 2010) have been employed. The mean distance travelled for *Wolbachia*-free males was significantly higher than for males marked with fluorescent powder. In the same paper, authors characterised the dispersal pattern by the mean distance travelled (MDT), the maximum distance travelled (MAX), and the flight range (FR) and presented useful procedures for data processing.

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Annex 7: Methods for pathogen screening in field-collected mosquitoes

Table A7-A. Methods for handling, preparing and storing mosquito samples, by pathogen identification method (viruses)

Pathogen identification method	Strengths	Weaknesses	Mosquito handling and preparation	Sample storage
Pan-flavivirus PCR	Sensitive, optimal cost/work rate	Not specific and liable to detect non-pathogenic mosquito-only flaviviruses	Transport alive or in dry ice; rapid identification on chill table	Store at low temperatures or use RNA-stabilising buffer
West Nile real time RT-PCR	Sensitive, optimal cost/work rate	90% specific, may amplify Usutu virus	Transport samples in dry ice and rapid identification on chill table	Store at low temperatures or use RNA-stabilising buffer
Usutu real time RT-PCR	Sensitive, optimal cost/work rate		Transport samples in dry ice and rapid identification on chill table	Store at low temperatures or use RNA-stabilising buffer
Pan-alphaviruses,	Sensitive, optimal cost/work rate	Not specific	Transport alive or in dry ice; rapid identification on chill table	Store at low temperatures or use RNA-stabilising buffer
Pan-orthobunyaviruses	Sensitive, optimal cost/work rate	Not specific	Transport alive or in dry ice; rapid identification on chill table	Store at low temperatures or use RNA-stabilising buffer
Discriminating PCR analysis	Highly sensitive, highly specific	Narrow target range, potentially to single virus strain	Transport alive or in dry ice; rapid identification on chill table	Store at low temperatures or use RNA-stabilising buffer
VecTest, RAMP	Easy and fast	Sensitivity and specificity not optimal	Transport alive or in dry ice; rapid identification on chill table	Store at low temperatures
Virus isolation using mammalian or insect cells: Vero, BHK-21, RK-13, C6/36	Virus availability for full genome description	Time-consuming, expensive. Used for isolation and culture of virus	Transport alive or in dry ice; rapid identification on chill table	Store at low temperature
Microarray	Maximum pathogen coverage	Poor sensitivity, expensive and limited availability	Transport alive or in dry ice; rapid identification on chill table	Store at low temperatures or use RNA-stabilising buffer
RT-LAMP (loop-mediated isothermal amplification)	Highly sensitive and specific, no need for thermal cycler	Narrow pathogen detection range	Transport alive or in dry ice; rapid identification on chill table	Store at low temperatures or use RNA-stabilising buffer

Table A7-B. Methods for handling, preparing and storing mosquito samples, by pathogen identification method (parasites)

Pathogen identification method	Strengths	Weaknesses	Mosquito handling and preparation	Sample storage
Dirofilaria dissection	Accurate, viable larvae in fresh specimens	Time consuming	Dissection of head for worms	Fresh, frozen, or in alcohol
Dirofilaria PCR or RT-FRET PCR	Practical, highly sensitive and specific, simultaneous detection of different species	No information on the viability of larvae	Transport alive; kept refrigerated until rapid identification	Frozen
Malaria classical dissection and identification	Accurate	Time consuming	Dissection of salivary glands (for sporozoites) and midgut (for oocysts)	Fresh or frozen
Malaria parasite ELISA	Practical and specific	Needs confirmation by PCR	Transport alive; kept refrigerated until rapid identification	Fresh, frozen, or dried mosquitoes
Malaria parasite PCR	Practical, highly sensitive and specific		Transport alive; kept refrigerated until rapid identification	Frozen, dried or in alcohol

Pathogen screening in mosquitoes

Guidelines for invasive mosquitoes should be consulted regarding testing invasive species for dengue (flavivirus) and chikungunya (alphavirus). Native mosquitoes should be tested for EU-endemic viruses, e.g. West Nile virus (Flaviviridae; *Flavivirus*), Usutu virus (Flaviviridae; *Flavivirus*), Sindbis virus (Togaviridae; *Alphavirus*), Tahyna virus (Bunyaviridae; *Bunyavirus*); Inkoo virus (Bunyaviridae; *Bunyavirus*), Batai virus (Bunyaviridae; *Bunyavirus*), Lednice virus (Bunyaviridae; *Bunyavirus*). Native mosquitoes may also be tested for two European endemic parasites: *Dirofilaria* sp. (cause of dirofilariasis in humans and dogs) and *Plasmodium* spp. (cause of malaria in humans). Additionally, native mosquitoes may be tested for non-European viruses including: Japanese encephalitis virus (Flaviviridae; *Flavivirus*), Rift Valley fever virus (Bunyaviridae; *Bunyavirus*), Western equine encephalitis virus (Togaviridae; *Alphavirus*), Venezuelan equine encephalitis virus (Togaviridae; *Alphavirus*), and Eastern equine encephalitis virus (Togaviridae; *Alphavirus*).

Detection of malaria parasites (*Plasmodium* spp.) in infected mosquitoes is nowadays commonly performed by semi-nested multiplex PCR or real-time PCR techniques on single whole mosquitoes or on pools of up to 100 mosquitoes. These assays will detect stages of *Plasmodium* spp. in the blood meal/gut of the mosquito; therefore, to ensure only sporozoites within the salivary glands are detected, DNA should be extracted from head-thorax only and the abdomen removed prior to extraction. Field-collected mosquitoes can also be screened by enzyme-linked immunosorbent assays (ELISAs) to detect human *Plasmodium* spp. circumsporozoite proteins in mosquitoes. However, an ELISA-positive mosquito does not establish that species as a vector, as it does not prove salivary gland infection. Finally, dissection of the midgut to observe oocysts or dissection of the salivary glands to detect sporozoites are appropriate techniques, but delicate and time-consuming, and thus mainly performed during infection experiments in the laboratory rather than for field surveys (for further information on dissection techniques and the morphology of the protozoan stages, see: http://www.jove.com/video/228/dissection-of-midgut-and-salivary-glands-from-ae-aegypti-mosquitoes). Obtained data can be useful to: 1) estimate (by mosquito pooling) *Plasmodium* prevalence in *Anopheles* populations in low-prevalence areas where large numbers of individual mosquitoes would need to be processed to obtain a reliable estimate; 2) incriminate a specific *Anopheles* species as malaria vector; 3) identify in one go all *Plasmodium* spp. in vectors circulating in an area; 4) detect mixed infections in mosquitoes; and 5) detect mosquitoes with low-level parasite infections. More information can be found at http://www.mr4.org.

Filarial worms can be detected as larval stage worms or microfilaria by dissection of head, thorax, and abdomen (keep separated because this will provide different information on mosquito infection); this also indicates infection levels in mosquito populations. However, dissection becomes increasingly costly and is laborious, so it is not recommended in areas where mosquito infection prevalence is low; PCR methods tend to be adequate for testing large numbers of mosquitoes. Multiplex Rt-PCR can simultaneously identify different species of filariae, as *Dirofilaria immitis* and *D. repens*.

Sample collection and storage

Mosquito trapping systems are described in Annex 4.

When aiming at detecting parasites, mosquito samples can be used fresh, or stored in alcohol or dried out. For the screening of arboviruses, collected mosquitoes should be brought to the laboratory within 24h, alive or frozen (i.e. using dry ice) and then be sorted, identified and pooled by species (using refrigerated tables if frozen). Pooled samples should be labelled with time, day and collection site. Samples should be stored at least at -80 °C for long-term storage, or -20 °C for shorter periods, avoiding thawing and re-freezing. If freezing is not possible, using RNA-stabilising buffer (RNA-later tissue storage solution) to store at room temperature is a proven alternative. Generic PCR-positive samples may be checked for virus isolation, which requires splitting the samples before further processing. Storage in RNA-stabilising buffer will hinder successful virus isolation.

Pool size depends on the collection method, the population density, the expected infection rate, and the sensitivity of the screening method, but 10–200 individuals are usually sufficient. Females should be specifically targeted to reduce numbers. When the infection rate is expected to be very low, larger pools might be considered to reduce costs.

Data presentation for infected mosquitoes

Data on arboviruses in field-collected mosquitoes are useful to track virus activity and provide an index of vectorial capacity for specific arboviruses. The standard measure of mosquito-based arbovirus surveillance (as used by the ArboNET system [US Centers for Disease Control and Prevention (CDC); available from: http://www.cdc.gov/ncidod/dvbid/westnile/index.htm) is the number of positive mosquito pools found in collections of a particular mosquito species from a defined period and area.

While the absolute number of positive pools provides valuable information, it does not provide an index of virus prevalence in the vector population. The proportion of the mosquito population carrying the virus should ideally be

expressed as the infection rate (IR). At a regional/provincial level, weekly tracking of mosquito IR can provide an important predictive indicator of transmission activity associated with elevated disease risk to human. At the European level, the comparison of indices between outbreak areas may help to understand arbovirus transmission patterns.

Estimates of the IR are usually presented as the number of infected mosquitoes per 1 000 tested. The simplest estimate for a defined period and surveillance area, the minimum infection rate (MIR), is calculated as: ([number of positive pools/total specimens tested] x 1000). The MIR assumes that a positive pool contains only one infected mosquito, which may be invalid when infection rates are high. When infection rates are high or sample sizes are small, a more accurate estimate of IR may be obtained by using a maximum likelihood estimate of the infection rate.

A user-friendly programme has been developed at CDC for calculating IR (i.e. infection prevalence) estimates from mosquito pool data using methods that do not require the assumptions used in the MIR calculation. This programme also includes calculation of confidence intervals which reflect, in part, the sample sizes used in the calculations. The confidence intervals provided (or any other uncertainty measure) are needed to interpret the precision of the IR estimate. This programme is written as a Microsoft Excel add-in and can be downloaded from http://www.cdc.gov/ncidod/dvbid/westnile/software.htm. It computes point and confidence interval estimates of IRs using data from pooled samples, where pool sizes may differ. Bias-corrected likelihood methods are used to estimate infection rate, and a skew-corrected score confidence interval is computed by default. The documentation provided describes traditional methods for calculating the MIR for comparison.

Source: www.cdc.gov

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Annex 8: Measuring environmental parameters

Environmental parameters: a glossary

Typology of larval habitats

Describes in its broadest sense the type of aquatic habitat that pre-imaginal mosquitoes are found in. These can be classified for example as temporary floodland (woodland or grassland), coastal area, pond, river bed, rock pool, tree hole, man-made container, or underground.

Functions of larval habitats

Habitats of the same type serve different functions depending on a series of external factors, for example: numbers of wet and dry cycles, the seasonality of wet cycles (winter/spring flooding, or summer flooding), abundance and diversity of predators (particularly important for determining mosquito abundance), the degree of nutrient input, the scale and intensity of vegetation and habitat management (such as vegetation cutting and managing aquatic habitats like ditches), the seasonality and scope of water-level management, the impact of precipitation and extreme weather (sensitivity to drought or floods), water sources (rain, ground water, flooding, or artificial irrigation), and the degree of salinity.

Productivity of larval habitats

The type and functions of a larval habitat will determine its productivity in relation to the diversity and density of mosquitoes. This can in turn determine the diversity and seasonality of immature mosquitoes in an aquatic habitat and the voltinism (number of generations in a season) of different species.

Quality of control activities

Control activities may be applied to an area where mosquitoes are a nuisance or vector problem. Measures may be applied to the immature or adult stages and may involve the use of larvicides, adulticides or aspects of water and vegetation management. The quality of control is defined by the appropriateness of measures directed at the species of concern and the geographical coverage of the control measures. The quality of control activities is compromised if preparatory steps are skipped, e.g. larval surveying, assessment of the nuisance, and determination of the vector species. The quality of control activities is also negatively affected if adulticides/larvicides are improperly dosed because the manufacturers' guidelines are disregarded.

Efficacy of control

Prior to conducting any control measures it is advisable to conduct a baseline survey of mosquito density. The efficacy of this control will then be determined by the comparison with the post-intervention mosquito density. This will then allow the quantification of the proportional impact (efficacy) of any control measure.

Vegetation cover

Using a broad-scale approach (GIS), the vegetation cover can be used to characterise (and later identify) habitats that offer favourable conditions for specific mosquito species or groups. For some species, the degree of vegetation cover of an aquatic habitat can be important. This may be classified simply as the presence or absence of vegetation in an aquatic habitat; classification can also be more precise by describing the presence/absence (and proportional coverage) of floating, submerged, marginal and emergent vegetation.

Landscape characteristics

Landscape is a complex product of many factors. The term can refer to a visible habitat, but this habitat is impacted by a range of interconnected factors; these include, for example, a landscape's bedrock, the soil type, soil permeability, fluctuations of the ground water (which in turn is impacted by geological strata and the surface soil structure), whether a wetland is a ground (minerotropic) or rain-fed (ombrotropic) habitat, and its salinity. Furthermore, the structure of a landscape is often determined by human impact, and in the case of aquatic habitats, this is likely to include habitat and water management.

Land use

Aquatic habitats are often managed by and for humans. Aquatic habitats can, for example, include irrigation systems that respond to non-weather related fluctuations; they can be part of a nature reserve wetland ecosystem; or they can be subject to minimal or intensive management. In urban areas, aquatic

habitats can be managed for flood control, sustainable urban drainage, or sewage treatment. Container aquatic habitats, particularly in urban areas, may be related to personal and community water storage systems, be impacted by the intensity of personal and commercial horticulture and urban wildness. More broadly, aquatic habitats may be part of unprotected habitats such as river flood zones, semi-natural aquatic sylvatic habitats, or recreational wetlands.

Urbanisation

Not all urban areas are homogenous, and capturing this heterogeneity can be an important factor in understanding the role of urban areas as aquatic habitats for mosquitoes. Human population density can be a useful indicator, but how this population varies vertically will have an impact on exposure to mosquitoes or available aquatic habitats. Measuring the proportion and accessibility of urban green and blue space, the proximity of natural habitats, the size and structure of gardens and parklands, and the degree of container habitat suitability will impact on the suitability of urban spaces for mosquitoes.

Measuring environmental parameters

Environmental factors play an important role in shaping the population size and the pathogen transmission risk. These parameters are all associated with the environment, i.e. parameter values are first and foremost dependant on environmental and climatic conditions, and mosquitoes must adapt to these (e.g. temperature, blood and nectar availability, breeding sites availability, etc.), whereas mosquito population parameters (see Chapter 2.4 and Annex 6) are first and foremost determined by the NMS population itself, i.e. mosquito-borne or internal/intrinsic (longevity, survival rates, host finding, host preference, dispersal etc.).

Larval habitat productivity, typology, and functions

The frequency and quality of larval habitats are the primary function of the landscape (natural) and human population (cultural) characteristics in a particular environment (natural, semirural, rural, urban). Larval habitats may be identified and classified according to their typology and their productivity for a defined species or a group of native mosquito species. Their productivity is directly related to the flooding, in terms of mode of flooding (i.e. rain water, ground water, river or lake overflowing) and rhythm. These habitats can be mapped, and plant communities can be characterised as indicators of flooding frequency and mosquito communities (Rioux et al. 1968). This can be performed by inspection of larval habitats and collection of mosquitoes (with deeper or water net: see Chapter 2.2 and Annex 4), estimating the density of immature mosquitoes (Carron et al. 2003). Despite the rather simple equipment, this task needs to involve highly trained and skilled professionals with a profound knowledge of both targeted environment and NMS behaviour (adult oviposition habits, larval and pupal defensive behaviour, etc.). This requires a high level of manpower but is indispensable for the proper application of control measures.

Vector control quality assessment and efficacy

Mosquito control methods consist of rendering the environment unsuitable for mosquito development or apply other control measures (biological, chemical, physical). Methods for evaluating native mosquito species control quality and efficacy are available to assess the reduction of larvae/pupae per treated breeding site and measure the reduction of adult mosquitoes (to measure efficacy of larval and adult control). Reduction of juveniles can be assessed based on the same methods as described above except in those instances when insect growth regulators are used (targeting container-inhabiting species); larvae should then be brought to laboratory and the adult emergency rate should be recorded. Presence and reduction of adult mosquitoes can be estimated by comparing the number of mosquitoes that are sampled with an adult trap (number of females/trap/night) or with human landing collection (number of females/person/15min) before and after the treatment. For a reliable assessment of reduction levels, untreated plots with mosquito abundance similar to the treated area should be selected and the same method of sampling/trapping should be applied. Mosquito abundance is best monitored three days before and three days after the treatment because of likely variations in adult mosquito numbers. Oviposition traps can also be used for container-inhabiting mosquitoes. In addition to assessing the efficacy of the control measures, a quality check of the control method and procedure may be performed, preferably by an independent external team, in order to review the quality of the performance of the control measures (choices of treatment sites and methods, quality of the performance itself, resistance management, prevention of environmental and health impact, etc.).

Climate and meteorological parameters

Growing international concerns regarding climate change, which have been expressed in the national communication reports of most European countries within the United Nations Framework Convention on Climate Change (UNFCCC), emphasise a need for the development of climate change mitigation and adaptation strategies. In the area of infectious disease, a key adaptation strategy will be the improved surveillance of vector-borne diseases. However, improvements in surveillance, monitoring and research on whether and how various vector-

borne diseases are influenced by meteorological patterns and climate change are also needed, especially interdisciplinary research that considers interactions with other risk factors.

The drivers of meteorological and climate change are also of growing international and European interest (Semenza & Menne 2009; ECDC 2009). Projected increases in air temperatures are predicted to have an impact on poikilotherm species (for which body temperature depends on environmental temperature), including insects that pose a threat to human health. The responses of native mosquito species to these changes (in addition to physiological changes such as the potential for increased vector competence) could lead to an expansion of colonised areas and the invasion of new sites. Warmer cities could favour mosquito development and, along with higher air temperatures, shorten extrinsic incubation periods. Data on *Culex* spp. mosquitoes, vectors of West Nile virus (WNV), and meteorological factors indicate that higher mosquito populations in a given month may be associated with higher air temperatures and precipitation in the preceding month (Paz et al. 2013). Similarly, a study that examined the emergence of WNV in British Colombia, Canada, after a spread westward across the continent (Roth et al. 2010) suggests that higher average air temperatures, low snow cover and consequently reduced stream flows may have caused the observed increase in *Cx. tarsalis* populations, which facilitated viral amplification and spillover into human and equine populations. The overall pattern of the current studies on MBD suggests expanded ranges for disease incidence. Meteorological and climate change factors were identified as drivers for some of these patterns, but it is clear that many other factors are involved and may be more important. It is likely that similar scenarios could result in new geographical redistributions of other transmissible diseases and their insect vectors, which will be shaped by the ability of the insects to adapt to environmental changes caused by various factors.

Furthermore, climate change is predicted to increase the incidence of extreme weather events, which means that drought and flooding events are likely to become more common.

Urbanisation and land use

The adoption of urban lifestyles in rural regions and likewise rural activities such as farming in urban areas has driven the growth of Urban-Peri-Urban agriculture, merging the agri-market channels of both settings. Urban agriculture is increasingly being recognised by public health professionals, urban planners, community organisations, and policymakers as a valuable tool for economic development, preservation or production of green space, and improvement of food security (Brown & Jameton, 2000). The benefits are many in the context of climate adaptation, economic alleviation and self-sustenance but urban agriculture presents also challenges for human and animal health including the increase of NMS development sites (mainly containers) and hazard of zoonotic diseases.

Abandonment of farms encourages a tree hole-breeder mosquito (*An. plumbeus*, potential vector of malaria) to invade new habitats and breed in abandoned manure tanks, thus becoming a man-made container-inhabiting mosquito. Increased irrigation to combat drought may provide new breeding sites for some mosquito species (*Cx. pipiens* in particular). On the other hand the reduced availability of aquatic habitats can significantly affect population dynamics and the vectorial capacity of mosquitoes. The global economic crisis is also fostering development of some mosquito species. Many houses are being left unoccupied as the owners default on their mortgages, which generates more mosquito habitats.

Other anthropogenic changes may be surveyed as well. Any activity that modifies water storage or flooding events can generate changes in NMS distribution and abundance. In particular hunting and agricultural practices that use irrigation are of prime importance. For example, rice cultivation has changed over the last decades in Europe. Thus, an intertwined influence of political context, environmental constraints, technical improvements, and social factors led to changes in mosquito abundance in southern France (Ponçon et al. 2007).

Changes in flooding events (due to climate or land use changes) have a direct impact of mosquito populations in that an unnatural drying then re-wetting cycle can promote mosquito colonisation, and in the absence of regulatory predators and competitors, lead to high densities of native mosquito species. Furthermore, extensive flooding can provide additional habitats for native mosquito species. In an attempt to mitigate these risks, and in turn adapt to climate change, policymakers are devising strategies to ensure that these effects are managed. This might include the development of new coastal wetlands to mitigate storm surges and sea level rises. It may include the restoration and expansion of wetlands, either as an additional provision for wildlife, or as a store for floodwaters. In urban areas, floodwater management becomes increasingly more important, particularly where urban development is expanded. Strategies for sustainable urban drainage, and the need to introduce 'green- and blue-space infrastructure' and habitat connectivity presents additional opportunities for native mosquito species, particularly where wetlands are designed to deal and process waste. Nutrient-rich aquatic habitats can provide suitable habitats for native mosquito species in the absence of an assemblage of invertebrate and vertebrate predators.

Globalisation

Drivers for the emergence of infectious diseases include human demographics (e.g. the growth of megacities), international movements of people (travellers and refugees), the smuggling of wildlife, the trade of animals, inter-continental transport of disease vectors via specific trades (e.g. used tyres and some ornamental plants) and various other aspects of globalisation.

Increased trade and travel promote the transport of invasive mosquito species eggs in goods (used tyres, Lucky bamboos), and adults in vehicles (cars, trucks, boats, aeroplanes), as well as pathogens in infected travellers. Human movement (from neighbourhood to international) is a critical behavioural factor underlying observed patterns of MBD spread, because movement determines exposure to vectors, i.e. bites from infected mosquito and transmission of pathogens. Stoddard et al. (2009) estimated a dengue reproduction rate (R_0) of 1.3 when exposure is assumed to occur only in the home, as opposed to 3.75 when exposure occurs at multiple locations, e.g. during visits to markets and friends. Interestingly, their model predicted little correspondence between vector abundance and estimated R_0 when movement is taken into account. The authors illustrated the importance of human movement for understanding and predicting the dynamics of MBD and encouraged investigation of human movement and disease. They also reviewed methods for studying human movement and proposed key parameters for designing such a study.

Monitoring of the meteorological parameters

A mosquito's flight is influenced by many factors, including the availability of blood sources and oviposition sites, weather (e.g. wind, humidity, temperature, rainfall), terrain, vegetation and housing characteristics (Honorio et al. 2003).

Bellini et al. (2010) demonstrated that low relative humidity, high temperatures, and intense solar radiation negatively influenced the mean flying distance and reduced the dispersion homogeneity. In hot and dry summer conditions, male mosquitoes showed reduced dispersal and sought shade.

The dispersal ability of a given species depends on the weather conditions during the searching period and the characteristics of the study locality. In urban areas, important factors include the vegetation type, its abundance and distribution; and the shape and position of buildings, squares, and main roads (Beier et al. 1982; Muir & Kay 1998; Reisen et al. 2003; Russell et al. 2005).

Temperature (seasonal averages, altitudinal variation) and precipitation (quantity, seasonal distribution pattern, water management habits of the human population) are crucial factors influencing the risk of establishment of NMS in an area and should be included in any risk modelling process.

Larval density directly affects mosquito size and longevity (Miller & Thomas 1958, in: Southwood 2000), as does an increase in temperature which may speed up development (but also reduces the juvenile body size of the mosquito, which affects adult longevity). Evidence is accumulating that fluctuations in the weather affect the size of the mosquito population; it is therefore important to include meteorological parameters in any analysis of fertility, longevity, dispersal and vector capacity of local populations.

Meteorological parameters should be continually monitored for local populations, especially in countries with a wide range of temperatures (with different regional climates and a broad altitudinal range). In such countries, monitoring and recording meteorological parameters are of crucial importance to understand spread and other aspects of NMS vector populations.

Historical records of temperature and other meteorological data are available for many locations. These databases should be extensively used for the analysis of the IMS populations. However, portable meteorological stations are useful for more precise measurements at locations that are far from the main monitoring points of national meteorological institutions. Medium resolution satellite imagery may also provide valuable meteorological data.

Monitoring changes in aquatic habitats

Mapping of larval habitats in a defined environment and their characterisation is very useful for both entomological and epidemiological surveys. Mapping can be done at very high spatial resolution of up to 1m using satellite data. Several satellites carry sensors that have dedicated electro-magnetic channels which can be used to characterise urban habitats. The relevance of these data to the implementation of control measures and efficiency assessment could be crucial. Gu & Novak (2009) used an agent-based model to track the status and movement of individual mosquitoes; they show that the elimination of habitats within 100 m, 200 m and 300 m of surrounding houses resulted in reductions of 13%, 91% and 94% in malaria incidence, compared with -3%, -19% and -44% for the corresponding conventional interventions. These findings indicate that source reduction might not, as previously thought, require coverage of extensive areas and that the distance to human homes can be used for habitat targeting. The authors also emphasise the importance of acquiring and archiving of data about breeding sites/container types used by different mosquitoes.

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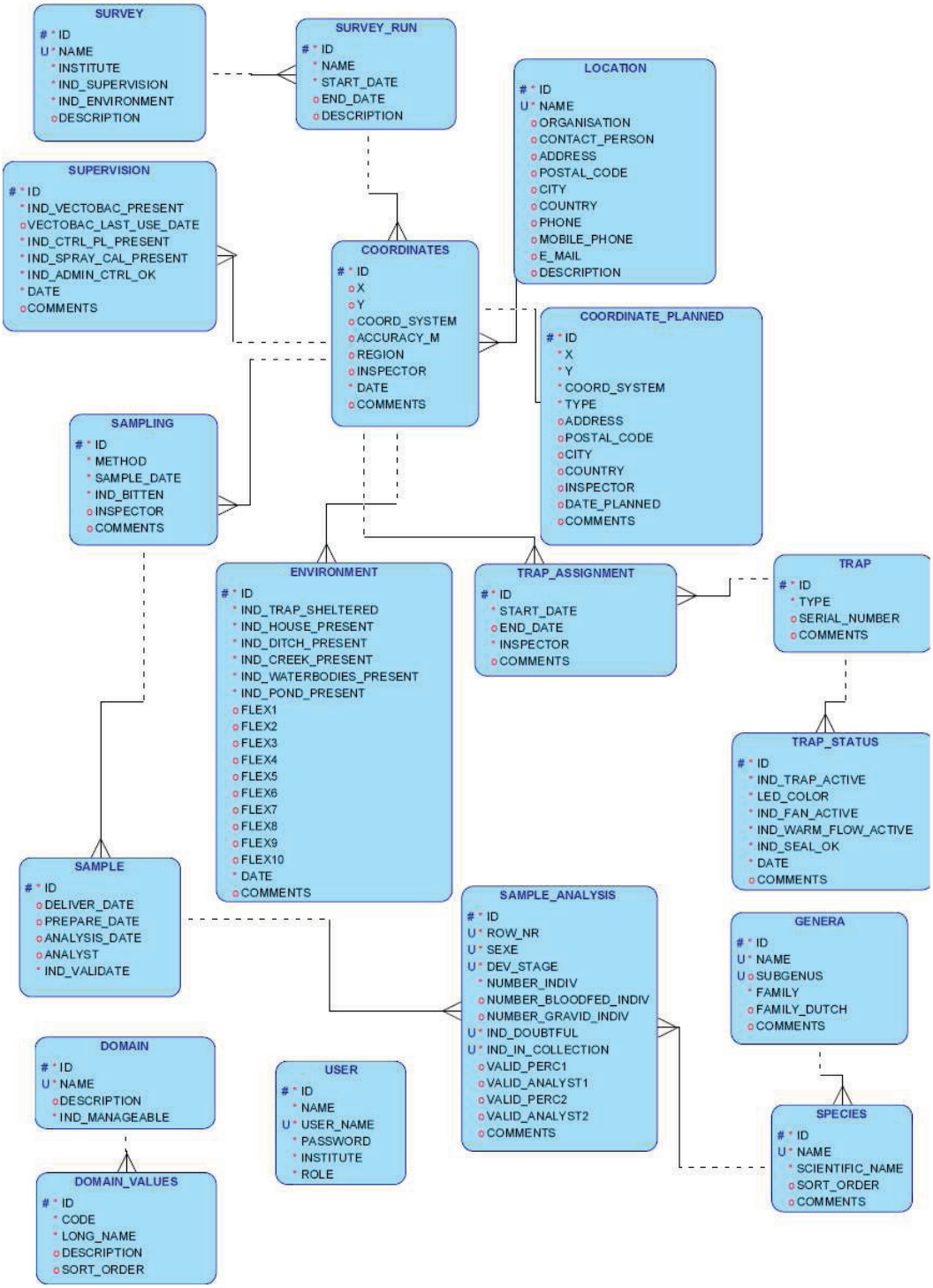
Annex 9: Data management and analysis

Figure A9-A. ERD (entity relation diagram) of a database specifically designed for vector surveillance



'VecBase' is a vector-surveillance database used in the Netherlands. Tables that are not connected to the main database contain complementary information, i.e. on database users, or drop-down lists for domains. Coordinates planned are those suggested by computerised random selection, while other coordinates are the ones used in the field. IDs are specific to each table.
Source: Dutch National Centre for Monitoring of Vectors (CMV), NVWA

Description of a logical data model (see Figure A):

In this example, the entity at top level is SURVEYS (campaigns for collecting vectors). SURVEYS can have one or more SURVEY_RUNS, which have of one or more SURVEY_RUNS COORDINATES.

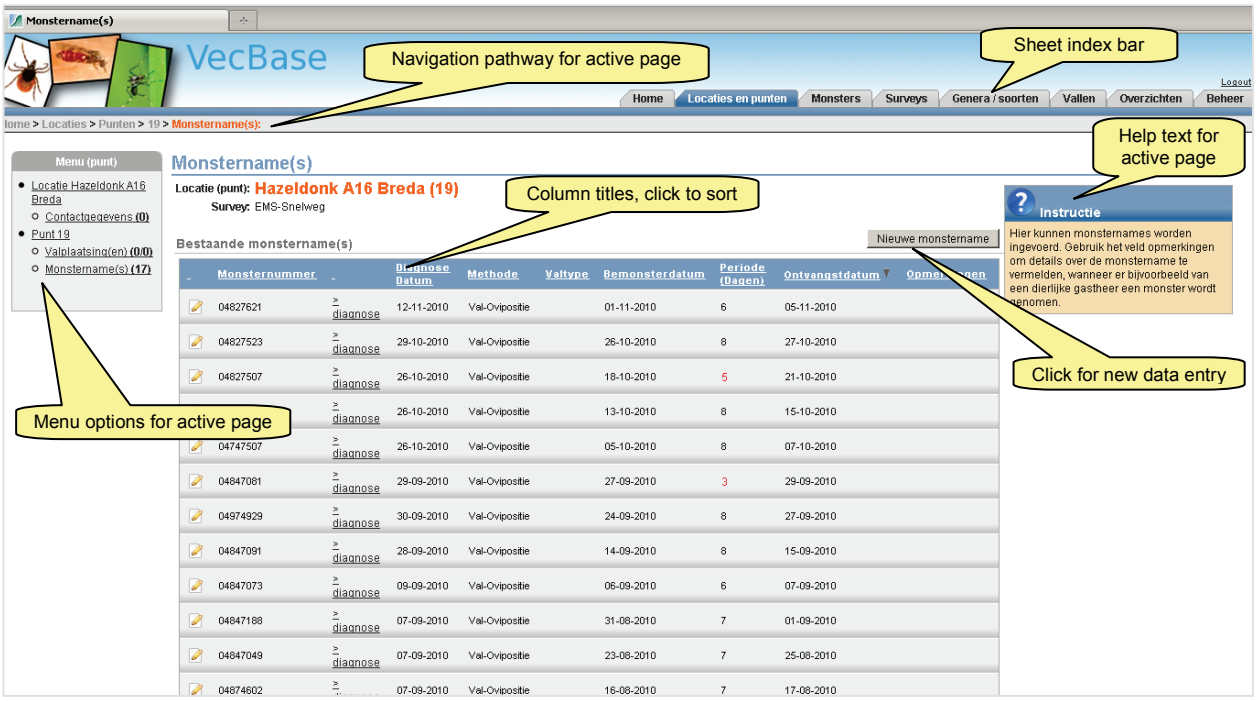
The central entity in the data model is the entity COORDINATES. Multiple COORDINATES can be grouped under one LOCATION. COORDINATES can also have planned versions: COORDINATES _PLANNED and can have ENVIRONMENT data appended to them. One or more TRAPS can be included in a COORDINATE (TRAP_ASSIGNMENTS). TRAPS can have one or more TRAP_STATUS.

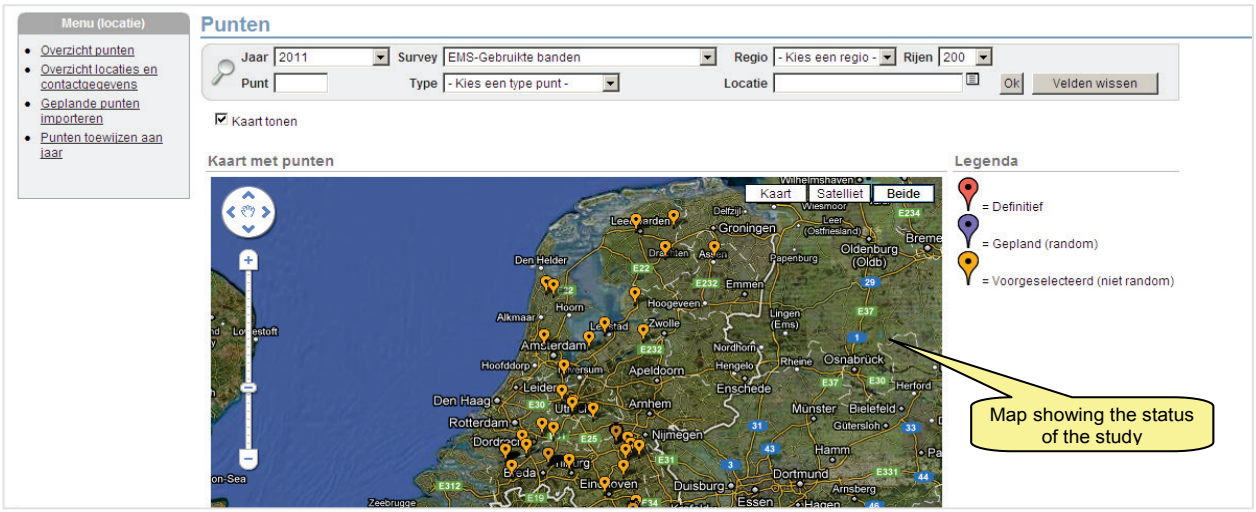
A COORDINATE may also have one or more SAMPLINGS. A SAMPLING may consist of one or more SAMPLES. A SAMPLE may have only one SAMPLE_ANALYSIS. This SAMPLE_ANALYSIS consists of the intersection of SPECIES and SAMPLES. SPECIES are grouped under GENERA.

The entity DOMAIN VALUES contains one or more values associated with a DOMAIN. The entity USER contains the user names and roles of the database users.

In order to track changes and provide information for auditors, all additions and changes of data in the database tables are automatically logged (user, date and time).

Figure A9-B. Web interface (screenshots) of a database designed for vector surveillance

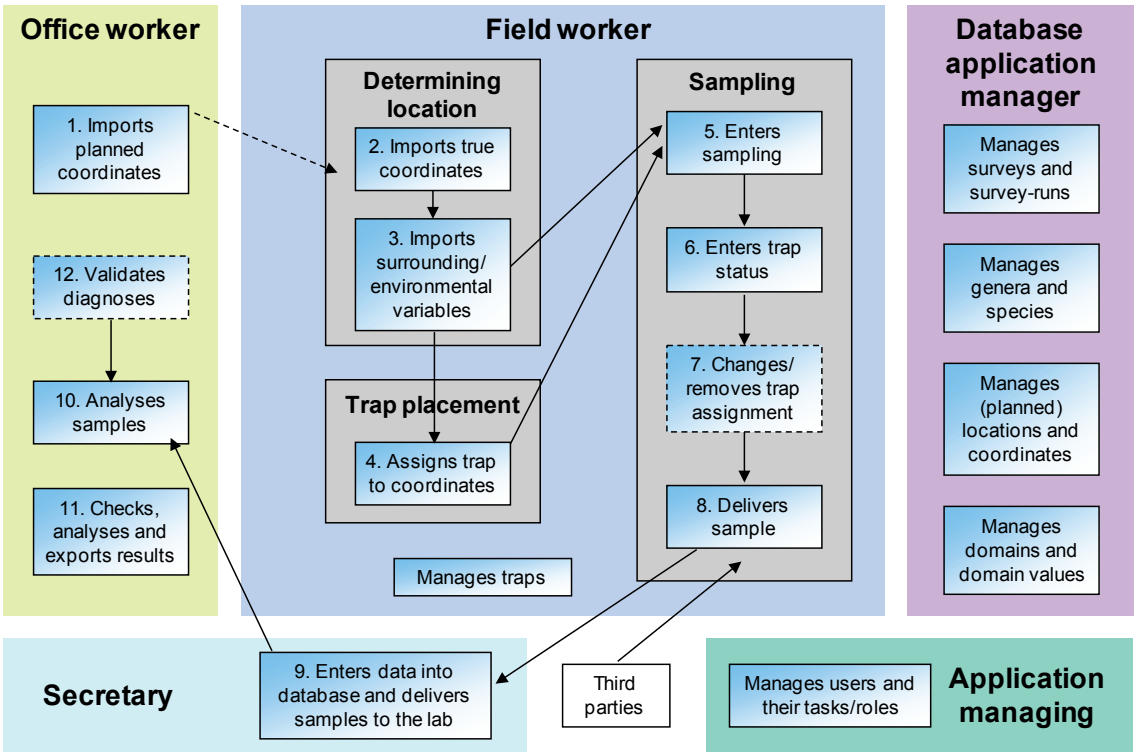




Above: Screenshots of a database user interface specifically designed for vector surveillance ('VecBase', used in the Netherlands)

Source: Dutch National Centre for Monitoring of Vectors (CMV), NVWA

Figure A9-C. Example of process flow within a vector surveillance programme



Source: 'VecBase', the Netherlands

Continuity of datasets

In order to make decisions on NMS control programmes, policymaking/legislation regarding native mosquito species, communication to stakeholders, assessment of control effectiveness, and to investigate trends in proliferation of native mosquito species in a defined area, it is crucial to have access to long-term surveillance data for the area of interest. Surveillance activity levels depend on the financial, political, institutional, and other factors (e.g. changes or lack of change in abundance of native mosquito species, pathogen transmission). Major objectives, however, should include the loss of datasets (which can happen when surveillance is interrupted and/or changes in partnerships occur) and data gaps, and to produce a continuous data flow for all surveillance activities.

Data harmonisation through standardisation

Data management is part of mosquito surveillance, and a well-developed data management system is a prerequisite for efficient data sharing and analysis. Its development may require investment in terms of time, energy, and money, but will definitely prove its usefulness especially where large datasets are involved. Data analysis/mapping on a European scale may require data sources that originate from various countries and areas. To avoid countless databases and surveillance organisations that may be difficult or even impossible to compare, it is important to harmonise not only surveillance design, but also data collection management. Only harmonised standards of data collection and data management make it possible to meaningfully compare temporal and spatial data.

Annex 10: Methods of dissemination of surveillance results

Table A10-A. Most suitable dissemination method according to the type of surveillance data and information relevant for dissemination

Type of data and information to be disseminated		Most suitable dissemination method								
		Report	Newsletter	E-mail	Website	Press release	Local media & TV	Door-to-door leaflet	Scientific publication	Telephone
Surveillance data results	Description/text	✓	✓	✓	✓	✓			✓	
	Tables/figures/maps	✓	✓		✓				✓	
	New finding			✓	✓	✓	✓			✓
Background information	Why surveying NMS?	✓	✓		✓	✓	✓	✓	✓	
	NMS biology	✓	✓		✓				✓	
	NMS & public health	✓	✓	✓	✓				✓	
	Why controlling NMS?				✓	✓	✓	✓	✓	
What can the reader do?			✓		✓	✓	✓	✓		✓

Table A10-B. Format of data, efficiency and main audiences according to the type of dissemination method

Dissemination method	Format of data				Speed to reach audience	Comprehensiveness	Main audiences					
	Text	Figures	Tables	Maps			Policymakers	Researchers	Technicians[1]	Local authorities	Public in affected areas	General public
Report	✓	✓	✓	✓	Low	High	✓	✓	✓	✓		
Newsletter	✓	✓	✓	✓	Low	Medium	✓		✓	✓	✓	
Dedicated website	✓	✓	✓	✓	High	High	✓		✓	✓	✓	✓
E-mail	✓				High	Low	✓		✓			
Press release (national)	✓				High	Low						✓
Local media & TV	✓				High	Low				✓	✓	
Door-to-door leaflet	✓	✓		✓	Medium	Medium			✓	✓	✓	
Scientific publication	✓	✓	✓	✓	Low	High	✓	✓	✓			
Telephone	✓				Very high	Low	✓		✓			

[1] *In charge of control measures or part of the surveillance team*

Methods of dissemination: strengths and weaknesses

Reports

Considered 'grey literature', reports are nonetheless very useful for stakeholders, policymakers, mosquito control and public health units, but are largely inaccessible to journalists, scientists and the general public. Reports can hold vast amounts of data, thus providing precise and detailed information.
However, writing a report requires considerable resources and production times are long, as the reported

surveillance data usually cover relatively long periods. Moreover, reports are static, cannot be easily corrected, and multiple reports may be needed to cover one single area.

Newsletter

A newsletter is a regularly distributed publication, generally about one main topic that is of interest to its subscribers. Newsletters can be distributed as hard copies, or electronically via email (e-newsletters) and websites. Newsletters include news and upcoming events, as well as contact information for general inquiries. Newsletters on native mosquito species may be distributed by, for example, NMS-affected municipalities, control agencies, surveillance groups and/or public health institutes. One of the advantages of a newsletter is that native mosquito species trends can be identified by screening subsequent issues. Also, since newsletters are distributed to a selected group of people that have at least some interest in NMS-related issues, it is probable that recipients have a certain predisposition to the main messages.
Newsletters are usually not specific to NMS-related issues, which may obscure the message. Also, newsletters are static and cannot be easily corrected.

Websites

Surveillance units may develop and launch specific websites that cover surveillance activities, objectives, and results. Websites are flexible, easily updated, link to other relevant websites and literature, can handle virtually unlimited amounts of information, and are accessible to everybody who has access to the internet.
As similar topics are discussed on other websites, which are not always backed by scientific data and research, some readers may get confused.

E-mails

E-mails are useful for rapidly communicating new information or data to a selected audience, mostly people involved in surveillance or stakeholders involved in complementary programmes (mosquito control, disease control, etc.).
As e-mails are not a formal data dissemination method, the accuracy and correctness of the data may seem less to some readers, particularly when compared to other formats (e.g. reports and scientific publications).

Press release

National press releases are a useful medium for informing the press and subsequently the general public. If used properly, press releases will greatly enhance transparency, improve involvement of the general public, and demonstrate the usefulness of the implemented surveillance programme. Moreover, press releases can inspire journalists to publish complimentary or more detailed information.
As always, there is a risk that information is misused or misinterpreted, or that the message is exaggerated, alarming rather than informing the public.

Local media

Local media provide coverage of events in a local context. Usually this includes local newspapers, radio and/or TV stations, which inform the local population on issues regarding a defined and relatively small area (mostly ranging from municipality to region/province level). Communication/dissemination via local media does usually not cover detailed information, but may increase in frequency and level of detail if the situation so requires (e.g. presence of native mosquito species coincides with vector-borne diseases transmission). Local media often depend on information acquired from involved locals, which may bias the message. Often, native mosquito species issues occur in the summer, a slow time for news, and local journalists may allocate a disproportionate amount of time to NMS events, which may be an advantage, but may also garner unwarranted attention from the local population. To inform the local population on NMS-related issues, avoid misinterpretation of facts, and avoid being accused of withholding information that affects the local population, it is advisable to provide a 'press release' (see above) with contact details for well-informed, media-savvy personnel.

Door-to-door leaflets

Door-to-door leaflets are distributed to people living in an NMS-affected area. This dissemination method is effective in reaching the people that are most likely to be affected. In addition to disseminating NMS surveillance results, door-to-door leaflets are arguably among the best methods of involving people in an NMS-affected area in preventive and/or control actions that need public involvement. The strength of this approach is that the accuracy of data and advice are assured, and that a lot of information can be provided indirectly by adding website addresses or other internet links.
A weakness is the relatively high cost (editing, printing, and distribution), and the fact that leaflets may not include the most up-to-date information on NMS dispersal, especially if the native mosquito species is new to the area.

Scientific publications

Scientific information on NMS surveillance data is mostly published in scientific journals or as research monographs in book form. Manuscripts published in peer-reviewed journals are considered validated. Scientific publications are authored and co-authored by experts and researchers, all of whom must agree to their content before publication. Published literature is permanently available (and thus useful for trend analysis), and the analysis of surveillance data is assured.

One drawback is the long process from data collection to analysis, writing, and publication, so that often the most recent information cannot be included. Also, the terminology and language used in scientific publications is very technical and may not be readily understood by the general public. Access to some published manuscripts can be restricted or costly.

Telephone

With the advent of the smartphone, the concept of 'telephone' began to change. Voice-only communication with one person is still useful when sharing selected surveillance data and/or recent developments, or when trying to resolve a specific situation. Also, personal contact by phone is helpful but unfortunately also prone to misunderstandings and misinterpretations, especially in international teams with different native languages. Moreover, no records of shared information are kept, which may be an advantage or a disadvantage.

Modern smartphones provide multimedia capabilities and fast online access. Often, smartphones are used to quickly communicate with office and laboratory personnel: transferred images, for example, can help to assess a complicated situation in the field. It should be noted that smartphones are increasingly used as data communication tools and GPS transmitters to connect to servers in advanced vector-surveillance programmes (e.g. VecMap and GIS tools).

Box: Access to private property

Access to private property is essential for the efficient control of NMS in breeding containers. Owners usually grant access, but access can also be denied or simply not possible if the owners cannot be reached. In tourist areas, many properties are used only part of the year (vacation homes). Abandoned properties are less numerous but even more problematic in terms of accessibility and mosquito proliferation (they usually offer many breeding choices).

Several European countries have already adopted legislation that forces private owners to allow the work of mosquito abatement or health-unit personnel to be carried out on their properties, e.g. in Germany, where prevention measures are included in federal laws. However, this is rarely enforced because of the difficulty and length of the application process. Countries should consider legislation which allows rapid public health intervention methods on abandoned properties.

Surveillance and control personnel should have an official mandate and corresponding identification documents; local authorities should provide advance information to communities in order to prevent hostility from owners.