Roadmap toward the harmonization of entomological surveillance systems in the Mediterranean area regarding the risks of mosquito borne virus transmission
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Executive summary

Vector borne diseases (VBDs) are a major threat to human and animal health. The epidemiology of these diseases is changing on local, regional, and global scales due to several determinants (e.g., travel and trade globalization, changes in land use and cover, pathogen evolution, changing lifestyles, urbanization, climate change, etc.). Surveillance is a cornerstone for risk management of VBDs and should be considered as an integrated and a holistic approach from a One Health perspective. In this regard, entomological surveillance is a major component of every surveillance system dedicated to VBDs and is complementary to human, animal and environment surveillance.

The purpose of this roadmap is to facilitate the definition of entomological surveillance systems in order to improve preparedness and response activities to VBDs. It first recognizes the impossibility to develop a unique surveillance system that would respond to all geographical and epidemiological situations. As a consequence, the purpose of harmonization is rather to propose evidence-based standards to identify the most relevant surveillance activities, to improve data sharing process, and to optimize the use of financial and human resources. This roadmap is intended to policymakers for decision support in implementing entomological surveillance programs. It might also be useful to health professional involved in other area of surveillance to share a common background on the possibilities and limitations of the entomological surveillance. Medical entomologists and vector control professionals can also rely on it to advocate for adapted entomological surveillance systems.

Every surveillance system should be designed to answer to well-defined objectives. Considering entomological surveillance, different main objectives can be listed: (1) to identify the vector species involved in transmission of infectious agents, (2) to identify the circulating strain pathogens, (3) to perform risk assessment of VBD transmission and set public health priorities, (4) to provide an early warning system tool to detect public health threats and guide public health actions, (5) to optimize and guide implementation of vector control in time and space, (6) to detect the development of insecticide resistance, and (7) to evaluate the efficacy of implemented vector control measures. The design of entomological surveillance system must consider the entomological and epidemiological contexts in order to be adapted to ongoing situation.

Beyond these aspects, more concrete guidance is proposed for arboviruses transmitted by recently established populations of invasive mosquitoes (e.g. *Aedes aegypti*, *Ae. albopictus*) and main emerging mosquito borne pathogens. Some basic knowledge on mosquito fauna is always useful prior to disease emergence. Regardless of the risk, some basic knowledge on the mosquito fauna is always needed. This basic background consists of the identification of the main vectors that are present in the area of interest as well as the most likely invasive species to be introduced and established in the region. Population density, seasonal
dynamics and spatial distribution should also be considered as relevant data to anticipate possible invasion risks of both vectors and VBDs.

Then, a state of knowledge in the area of interest is proposed for the different arboviruses and their potential vectors, leading to risk-based surveillance proposals. The first group of mosquito-borne arboviruses is dengue, chikungunya, Zika, and yellow fever; the second one includes West Nile fever; and the third comprises Rift Valley fever. The main objectives which can be pursued in the Mediterranean area are highlighted depending on the entomological and epidemiological contexts.

Finally, major priorities are discussed to implement entomological surveillance in a comprehensive framework. The interest to adopt a “One Health” approach is undoubtedly relevant given the specific nature of vector borne diseases at the interface between humans, animals, and the environment. Particular emphasis is given to the implementation programs, policies, and operational research within an integrated and holistic framework. In this regard, two key essential dimensions for progress are detailed: (1) the integration of surveillance capacities by interdisciplinary expertise and cooperation, and (2) an integrated approach at the different geographic scales: local, regional and global. The relevance of this approach is now widely acknowledged. However, advocacy and collaboration opportunities are needed for the implementation and support of sustainable surveillance systems aiming to prevent and control VBDs.
1. Purpose of the roadmap

The incidence and the geographical distribution of arboviruses, particularly those transmitted by mosquitoes, are globally increasing and spread across the world. Different factors concur with this dramatic global spread of arboviruses. These factors include travel, trade, urbanization, tourism, climate change and land management.

MediLabSecure is a European project aiming to create a framework for collaboration to improve surveillance and monitoring of emerging vector borne viral diseases (arboviruses), to provide training for public health experts in participating countries to increase the communicable disease control in the Mediterranean and Black Sea regions and to promote knowledge and transfer of best laboratory practices and biosafety measures. In this framework, guidance is proposed for the harmonization of entomological surveillance around the Mediterranean area. The purpose is to develop entomological surveillance systems on an effective and sustainable basis to improve preparedness and response activities. When dealing with VBDs, entomological surveillance is a key source of data used for public health surveillance.

However, simple observation makes clear that there is no universal surveillance system. The purpose of harmonization is therefore not to advocate the establishment of strictly identical surveillance systems in all the countries of the Mediterranean zone. It rather aims to define common and evidence-based standards to promote best practices and most relevant surveillance activities, to improve data sharing, and to optimize the use of financial and human resources.

From a global perspective, harmonization is however an achievable objective in terms of definition, promotion and implementation of a global framework with the stakes of vector borne diseases. Such strategy is encompassed by the One Health concept defined by the One Health Initiative (http://www.onehealthinitiative.com) as “a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of healthcare for human, animal and the environment”.

This roadmap is intended to the policymakers and diverse stakeholders to be used as a framework during implementation of entomological surveillance programs. It might also be useful for health professionals involved in other areas of disease surveillance to share a common background on the possibilities and limitations of entomological surveillance.

Medical entomologists and vector control professionals will be able to rely on this report to advocate for adapted and tailored entomological surveillance strategies.

The present report targets mosquito vectors and mosquito-borne viruses. However, it may be expanded to other vectors and vector-borne diseases with specific adjustments.
2. What is entomological surveillance?

Surveillance of vector borne diseases (VBDs) is generally based on an integrated surveillance system relying on the different relevant sources of data to deal with these risks. Ideally, the surveillance compiles data provided by human surveillance (human cases), veterinary surveillance (animal hosts), entomological surveillance (arthropod vectors) and environmental surveillance (environmental risk factors) from a One Health perspective (Dente et al., 2016).

General principles of public health surveillance apply to entomological surveillance. As defined by World Health Organization (WHO), it must be considered as a three steps process: (1) continuous, systematic collection of data, (2) analysis and interpretation of these data, and (3) dissemination of analyses results to guide the planning, implementation, and evaluation of public health practice. Thus, entomological surveillance in a public health setting is a tool to guide definition and implementation of prevention and response activities.

Based on considerations of efficiency and effectiveness, every surveillance system should be designed to answer well-defined objectives.

As with every health system, entomological surveillance should be implemented through an adaptive process based on surveillance results, evaluation of the system and changes in the entomological and epidemiological situation (Figure 1).
Figure 1. Process for the implementation and updating of entomological surveillance system. * The step consisting to take actions is not part of the surveillance system process but is based on surveillance results and is the basis for evaluation of surveillance activities.
3. What are the objectives of entomological surveillance?

The general objective of entomological surveillance is to contribute to prevent and control pathogens threatening public health and transmitted by an arthropod vector.

In the first phase of implementation of entomological surveillance, the following questions should be addressed:

1. Which is/are the target vector-borne pathogen/s?
2. Which vector species should be targeted by the surveillance program?
3. Which is the specific objective of the surveillance program?
4. Which are the relevant area and time to implement the surveillance?
5. Which are the most appropriate methods and tools to conduct the surveillance?

Depending on the situation (entomological and epidemiological context), several specific objectives may be listed:

1. To identify the vector species involved in transmission in case of emerging situations,
2. To identify and characterize the circulating pathogens,
3. To perform and update risk assessment of vector borne diseases transmission and set public health priorities,
4. To serve as an early warning system tool to detect public health threats and guide public health actions,
5. To optimize the implementation of vector control in time and space,
6. To guide larval source reduction campaign,
7. To detect the development of insecticide resistance,
8. To evaluate the efficacy of vector control.

3.1. Risk assessment: prioritization of public health threats

The purpose of risk assessment is to qualitatively or quantitatively determine the likelihood and impact of an identified threat (or hazard) to the environment, individuals or populations. In the infectious diseases perspective, risk assessment can be described as a successive process based on the following steps: (1) identification of the hazard(s) posed by pathogens, (2) probability of pathogen introduction into a specific area by any possible route, (3) probability of transmission in the at-risk zone (implying the presence of competent vectors, susceptible vertebrate hosts, as well as biotic and abiotic conditions suitable for transmission), (4) probability of establishment, spread and persistence of the disease, and (5) impacts on health and economy.
Risk assessment requires by nature an interdisciplinary approach and entomology provides key inputs to assess any VBDs. Moreover, risk assessment is an iterative process and must be updated when additional information becomes available, including information obtained from entomological surveillance.

De Vos et al. (2011) proposed an interesting framework for risk assessment allowing perceiving the global added values of entomological field (Figure 2). Although dedicated to animal diseases, it can be easily transferred to human health.

![Figure 2. General framework for risk assessment of vector borne diseases (de Vos et al., 2011)](image)

Entomological surveillance has mainly an added value for the hazard identification step and the estimation of the probability of transmission. The hazard identification will consider the presence of competent native vector species and the invasive vector species most likely to be introduced. The evaluation of the probability of transmission will be based on the distribution of the vector and the estimate of vectorial capacity, which are two major entomological parameters for the assessment of both actual and potential transmission rates. Key parameters required to calculate vectorial capacity of a particular mosquito species are obtained by entomological surveillance: vector abundance, seasonality, longevity, biting rate and behaviour, probability of transmission of infection from vector to host, and from host to vector, extrinsic incubation period (Petrić et al., 2014). Moreover, the likelihood of persistence of a specific vector borne pathogen will depend on the vector infection rate,
survival of infected adult vectors during winter, the vertical transmission of the pathogen in the vector and the number of reservoir hosts.

Some of these data can be retrieved from the literature, but in most cases, estimation of risk is much precise when it is based on studies performed on local vector population at the same settings of ongoing situation.

3.2. Early warning system

In some cases, entomological surveillance can be used for the early detection of viral circulation, before the emergence of the index case in human and/or animal and thus allows the implementation of response measures including: surveillance reinforcement, mosquito control, implementation of public education campaigns, and vaccination and treatment measures (van den Hurk et al., 2012; Bellini et al., 2014). Early warning systems should display critical features: sensitivity to detect outbreaks, specificity to avoid the detection of false negatives, and timely response to implement early interventions (Runge-Ranzinger et al., 2014). The usefulness and relevance of mosquito-based arbovirus surveillance as a tool for early warning system is situation dependent. This type of early warning system seems relevant, particularly for endemic enzootic diseases, when viral amplification in wild hosts and enzootic vectors precedes human cases. Surveillance should thus focus on areas known for sylvatic or synanthropic transmission cycles, constituting hotspots for transmission, rather than targeting an extensive coverage (Gu et al., 2008). Novel approaches have recently been developed and mitigate some previous limitations (Ramírez et al., 2018).

3.3. Identification of the vector species involved in a transmission event

Two situations justify the implementation of entomological surveillance to identify the species involved as a vector in a specific transmission event. First, when several potential vectors are present and established, the respective role of each species in the transmission needs to be clarified in order to adapt strategies of control. As an example, in the event that both Aedes albopictus and Ae. aegypti are present in an area, the transmission of the pathogen could be ensured by one of them or both and can depend on the viral genotype (Sivan et al., 2016; Tsetsarkin et al., 2014). Even if they are closely related species, their behaviour differs (Ae. albopictus being mostly exophagous, whereas Ae. aegypti presents endophagous habits) and this can have an impact on the different strategies of vector control. Consequently, there is an operational (and scientific) interest for identifying infected vectors to guide actions. Second, in the event of viral emergence in a new territory (e.g., Zika virus in Americas), it is essential to identify the involved vector or vectors to optimize the response strategy. The possibility of virus transmission by unknown vectors for a dedicated pathogen should not be neglected (Ayres, 2016).

3.4. Identification of the circulating pathogen strain

In some cases, viral detection in the vectors is an effective way to identify the circulating virus or strain. This can be particularly useful for viruses that are very difficult to detect in the blood of the vertebrate hosts due to the very short viremia induced (e.g., West Nile virus). Such identification contributes to a better understanding of the vector-host-pathogen system, in particular the virus-
vector interactions (vector competence, duration of the extrinsic incubation period, etc.). The detection of West Nile virus lineage 2 for the first time in pools of *Culex pipiens* in Northern Italy (Savini et al., 2012) showed the contribution of entomological surveillance in this perspective.

The value of this goal is relative for viruses such as dengue or chikungunya, as it is generally easier to obtain viral material in humans.

### 3.5. Optimization of vector control in time and space

Knowledge of the spatial and temporal dynamics of mosquito populations provides critical information for the characterization of areas and seasons that are most likely at disease transmission risk (Diallo et al., 2014; Lacour et al., 2015; Samy et al., 2016). Such information is essential to design human or animal surveillance programs and the vector control strategies such as larval source reduction.

Population dynamics of certain mosquitoes can exhibit strong variation over diverse regions and years under the pressure of different factors including climate, land cover, agricultural practices and water management. For urban species, strong heterogeneity in density is observed between sectors at the city level.

The characterization of the factors affecting vector abundance and dynamics can facilitate the development of models to prioritize measures for mosquito surveillance and control.

### 3.6. Guidance for source reduction campaigns

In addition to the point mentioned above, the characterization of breeding sites will enhance source reduction campaigns either by mosquito control operators through larval control or by communities following social mobilization actions, aiming to stimulate larval control by community itself especially towards domestic and peri-domestic breeding sites (which are most often of anthropogenic origin).

In this perspective, a typology of breeding sites can be built in order to focus on the most common and productive breeding sites (Manrique-Saide et al., 2011). Local specificity can exist and the private and public domains must be considered. For example, in Italy, Carri et al. (2011) estimated that catch basins in Central and Northern Italy, both in public and private areas, produced 97% of the pupae.

Larval control through environmental management, larvicides, biological control and social mobilization is a key component of proactive and sustainable vector control strategy and must be pursued in case of a health threat event. In the case of viruses transmitted by *Stegomyia*, coverage of the intervention is critical and source reduction campaigns should strongly rely on community mobilization (Bowman et al., 2016; Andersson et al., 2015).

### 3.7. Insecticide resistance monitoring

Most of the vector control programs depend to a large extent on chemical insecticides and monitoring of vector susceptibility to the commonly used active substances should be considered as a key component of entomological surveillance and an integral part of these programs (WHO,
Knowledge of status, changing trends and distribution of resistance in vectors is a basic prerequisite to guide policy and operational decisions by choosing appropriate insecticides and implementing comprehensive resistance management strategies. Decision-making needs to be based on reliable vector susceptibility data calling for standardized monitoring. The information concerning insecticide resistance is highly important as to identify areas where resistance may jeopardize vector borne diseases management (Corbel et al., 2016).

3.8. Evaluation of the efficacy of vector control

Health authorities and local medical entomology laboratories can implement entomological surveillance in order to evaluate the efficacy of control measures. In this case, the monitoring will focus on species and developmental stage targeted by the control actions. However, the evaluation of control programs will be different when considering source reduction versus adult control actions. Indeed, source reduction is a preventive measure aiming to reduce larval population by biological, chemical control or elimination of mosquito’s breeding sites. Such actions are planned in advance, enabling to implement proper evaluation measures based on larval and/or pupal indices (Alvarado-Castro et al., 2017).

Adult mosquito monitoring is more challenging as sampling results are less reproducible, and actions are most often implemented in reaction to an unexpected event. It is therefore more difficult to conduct proper evaluation studies on a routine basis. In this case, monitoring will be mostly considered as operational research dedicated to assess the effectiveness of vector control method, requiring standardized studies of high quality and optimally based on entomological and epidemiological metrics (Bowman et al., 2016).
4. Entomological and epidemiological scenarios

Once the risk assessment is performed, public health actions (and entomological surveillance) can be tailored to the entomological and epidemiological situation.

Different conditions are necessary for the transmission of a pathogen by a vector:

- Presence and established population of a competent and capable vector,
- Presence or introduction of the pathogen (most of the time by a viremic vertebrate),
- Presence of a susceptible host (especially in zoonotic diseases),
- Presence of an immunologically naive population.

The last condition is ordinarily fulfilled in the absence of an endemic situation. The vaccination of a large proportion of human (e.g. yellow fever) or animal hosts (e.g. WNV, and RVFV) provides herd immunity and prevents the spread of the disease.

Thus, the risk of spread of a given vector-borne pathogen can be graduated in a first step reflecting an entomological risk (introduction of a competent vector) then in a second step transferring into an epidemiological risk (risk of transmission) when a vector is present. In order to differentiate between entomological risk and epidemiological risk, different scenarios have been proposed to distinguish the surveillance of invasive species and the surveillance of native species (ECDC, 2012, 2014). However, once an invasive vector is deeply established, the situation is similar to the situation with a native vector.

Tailored and graduated actions of vector surveillance and control arise from this differentiation of scenarios (cf. table 1).
**Scenario A: surveillance of invasive vectors**

A1 Surveillance of introduction and establishment of invasive species

A2 Surveillance of locally established invasive species

**Scenario B: surveillance of established vectors**

B1 Absence of the pathogen with possible risk of introduction (risk of emergence)
- Main introduction route: animal
- Main introduction route: human

B2 Autochthonous circulation of the pathogen
- Introduction of infected livestock (epizootic/rural cycle of diseases for which domestic animals are the amplifying hosts)
- Introduction of infected human (urban cycle of diseases for which humans are the main source of infection due to high viremia)
- Pathogen circulation in the wildlife fauna.

B3 Epidemic situations

**Box 1.** Different scenarios based on entomological and epidemiological contexts.
### Table 1. Objectives of surveillance and possible public health actions based on entomological and epidemiological scenarios.

Adapted from ECDC (2012) and ECDC (2014).
5. Knowledge of the entomological fauna: basic knowledge

Basic knowledge of the mosquito fauna is needed to perform risk assessment and to implement targeted surveillance or vector control actions that are tailored and proportionate to the risks.

For native species, this basic knowledge is most often available in the literature. This is by definition less obvious in the case of invasive species, especially if the invasion process is in its early stages.

Values of parameters for risk assessment purpose can be found in the literature for most of the major vectors and invasive species. However, these parameters vary according to the population and the local environmental conditions. It is thus recommended to implement field and laboratory work to obtain values specific to the local context.

5.1. Inventory of species and potential vectors

Inventory of species is needed to perform risk assessment. A checklist of the different arthropod species present in the considered area is useful to identify the species that are known as putative vectors.

Most often, such inventories are conducted for a group of vectors of interest (e.g. ticks, mosquitoes, sandflies, etc.). The establishment of a distribution chart of mosquito species for the whole occidental Palearctic area (Europe up to Ural Mountain, countries bordering Mediterranean and Black Seas) provides valuable information in this respect (Robert et al., in preparation).

Once a list of species is established, it is then necessary to identify the vectors. Distinction should be made between potential and proven vectors.

It seems worth recalling here the different criteria enabling to identify a species as a vector (Barnett, 1962 In Eldridge and Edman, 2000):

- Demonstration that members of the suspected arthropod population commonly feed upon vertebrate hosts of the pathogen, or otherwise make effective contact with the hosts under natural conditions,
- Demonstration of a convincing biological association in time and space between the suspected vectors and clinical or subclinical infections in vertebrate hosts,
- Repeated demonstration that the suspected vectors, collected under natural conditions, harbour the identifiable, infective stage of the pathogen,
- Demonstration of efficient transmission of the identifiable pathogen by the suspected vectors under controlled experimental conditions.

Thus, it is necessary to gather relevant data regarding the potential role of each arthropod species as a vector:
- Involvement of the species in the transmission of a pathogen worldwide,
- Involvement of the species in the transmission of a pathogen in the target area,
- Detection of the pathogen in the species in specimens collected *in natura*,
- Data on vector competence of the species in experimental conditions.

The knowledge of the entomo-fauna must then be confronted with the epidemiological situation and in particular with the mode of pathogen circulation that is most likely to occur.

Captures in areas and periods of transmission enable not only to draw up a specific inventory for these areas at risk in terms of presence/absence but also to list the most abundant species. This inventory of native (and established) species should be completed by the identification of invasive species that are most likely to be introduced.

5.2. **Entomological population parameters with an impact on virus transmission**

To perform risk assessment, it is helpful to provide reliable information on the different entomological parameters having an impact on virus transmission.

Again, data on local population may be favourably provided: blood-feeding behaviour (host preference, multiple blood feeding), longevity, extrinsic incubation period for relevant pathogens, density, dispersal, and productivity. Petrić et al. (2014) propose a list of relevant parameters.

5.3. **Seasonal dynamics**

The knowledge of the seasonal dynamics of vectors provides useful information for risk assessment and risk management activities. The seasonal activity can be more or less pronounced according to the species.

5.4. **Spatial distribution**

The knowledge of the presence of a potential vector is a data of primary importance but far from sufficient. In particular, it is necessary to specify the spatial distribution of these species as well as the importance of occurrence frequency to distinguish rare species from common species.

5.5. **Monitoring insecticide resistance**

The emergence and spread of resistance to the most commonly used insecticide groups is a major challenge for the control of VBDs. Hence, monitoring and management of insecticide resistance in field mosquito populations is essential to maintain the efficacy and sustainability of vector control (cf. § 3.7).
6. Dengue, chikungunya, Zika, and yellow fever

In the Mediterranean region, the risk of dengue, chikungunya, Zika, and yellow fever transmission is embodied by the presence of *Ae. albopictus* and *Ae. aegypti* which are invasive species in the area (Medlock et al., 2012). The Mediterranean native mosquito species are considered as non-vector of these arboviruses.

Surveillance of *Ae. albopictus* and *Ae. aegypti* aims to identify the most productive breeding sites, to gain better knowledge on vectors distribution, to estimate vector abundance and to improve understanding of environmental parameters influencing mosquito population dynamics. On this basis, vector control actions can be prioritized and timely implemented.

The design of surveillance will depend on the level of colonization by one or several invasive species within the country and in the neighbouring countries as well as on the objectives being pursued by surveillance (cf. Table 1 and box 1).

6.1. Surveillance of introduction and establishment of *Ae. albopictus* and *Ae. aegypti*

The main objective of entomological surveillance in this context is to detect the introduction of invasive species. Surveillance will focus on main routes of introduction.

Two main modes of dispersal exist for invasive *Aedes* species. On an intercontinental scale, dispersion of mosquitoes mainly occurs in their egg stage. This is associated with the trade of specific goods that are susceptible to introduce a sufficient number of individuals that might survive and subsequently reproduce. The main incriminated activities are the international trade of used tyres and, to a lesser extent, of lucky bamboo (Medlock et al., 2012). On an intracontinental scale, the species expand step by step along major communication routes in association with the traffic especially when the mosquito harbours natural ability to enter in the vehicles, which is common for *Ae. albopictus* (Eritja et al., 2017).

The situation for *Ae. aegypti* introduction could be different as the species was broadly spread over the Mediterranean basin until the 1950s (Schaffner and Mathis, 2014). Some recent genetic evidences suggest the persistence of remnant populations around the Black Sea region (Kotsakiozi et al., 2018) with a recent spread in Northern Turkey (Akiner et al., 2016).

Owing to the key routes of invasion, a typology of high-risk sites for invasive species introduction can be drawn up. These include in particular points of entries (ports, airports, and ground crossings), parking areas and resting places along main communication axes, road-train platforms, railway nodes, storage sites of used tyres, greenhouses dedicated to importation of exotic plants, and green urban spaces. These different places should thus be selected for targeted surveillance.

Prioritization of trapping locations can be assessed by considering traffic data, volume of imported goods and the vicinity of the colonized area (Flacio et al., 2015; ECDC, 2012). Ovitrap is the main tool dedicated to the surveillance of invasive mosquito introduction. Recent guidelines (ECDC, 2012; ECDC & EFSA, 2018) provide useful technical information in the respect of trapping procedures and modalities (type, frequency, density, period of trapping). Ovitraps are less sensitive at certain locations such as storage sites of used tyres or greenhouses, because a large number of available
oviposition sites are competing with the traps. In these contexts, entomological surveys on a regular basis should be preferred with repeats between two and four times a year.

Modelling approaches can contribute to specify the most suitable areas for invasive species establishment, mostly based on abiotic (climate, photoperiod) and landscape (urbanization) variables. Different methods have been used to conduct this type of risk assessment for *Ae. albopictus* and *Ae. aegypti* (e.g. ECDC, 2009b; Kraemer et al., 2015; Ducheyne et al., 2018).

Establishment of the species can however occur anywhere. Introduction and establishment of *Ae. albopictus* is likely to occur in urbanized areas owing to its affinity to human environment. In this kind of environment, a plethora of artificial breeding sites compete with ovitraps revealing a sensitivity issue. Therefore, early detection of introduction in risky areas such as urban or peri-urban setting is challenging. Passive surveillance is particularly promising and economically profitable to address the shortcoming of “random” introduction in urbanized areas or at a considerable distance from the colonized area (Palmer et al., 2017). Passive surveillance is related to citizen science or participatory science and involves general public for the detection, the reporting and then the surveillance of invasive species. Reporting can be done by emailing photos or mailing specimens to entomological experts. The widespread use of smartphone facilitates the mobilization of the population in this respect. Data gained through the recent projects ‘Mueckenatlas’ in Germany and ‘Mosquito Alert’ in Spain bring the demonstration of the value of such surveillance (Walther and Kampen, 2017).

In many situations, identification of mosquitoes and especially *Stegomyia* is feasible from a mere photo. The main challenges are (1) to organize and formalize the reporting system, (2) to communicate and disseminate widely the information regarding its occurrence, and (3) to confirm and assess the level of infestation by field investigation. Development effort of the reporting system could be usefully shared at a regional level. Indeed, a unique platform (web-portal) could automatically return the public reports to the territorially competent authority. It will also enable the development of a regional dynamic through cooperation and data sharing which is a critical point in the surveillance process of invasive species.

### 6.2. Surveillance in areas with locally established population of *Ae. albopictus* or *Ae. aegypti*

Once an invasive species is established, different objectives can be targeted by entomological surveillance.

**Monitoring of the dispersal**

The precise knowledge of the vector distribution is an issue to support the implementation of epidemiological surveillance and the decision to trigger vector control programs. Monitoring the distribution of the species in areas with locally established population aims to rationalize vector control around imported arboviral cases, mostly by viremic travellers coming back from endemic or epidemic countries. For this, surveillance should be concentrated in the most densely populated areas and in major tourist locations, where imported cases are most likely to occur.

The monitoring of the dispersal will be similar to the system used for surveillance of introduction. It will be mainly based on a network of ovitraps deployed according to the criteria indicated above, with a trapping effort in proximity to the colonized area.
The use of passive surveillance will take its full meaning in this context considering the difficulties previously underlined.

There is usually a latitudinal and altitudinal limit above which species can no longer able to establish its population. For example, the threshold of 0°C for the average temperature in January is usually considered for the survival of *Ae. albopictus* diapausing eggs (Knudsen et al., 1996). However, this threshold is to be taken with caution in urban areas, due to the presence of microclimate with higher temperatures. Thus, in Trento, Italy, the species was present while minimum temperatures drop to -10°C and an average temperature in January of -5°C was observed (Roiz et al., 2011). Another approach to estimate altitudinal limits is by monitoring species establishment along altitudinal transects.

**Estimation of vector abundance**

The estimation of the vector density (per person, per trap, per surface unit...) is a major element to provide guidance for vector control. However, estimation of adult densities on a routine basis is challenging. The sampling of immature stages (larval or pupal index such as Breteau, container, house, pupae per person, pupae per hectare) continue to be widely used to estimate vector density (Focks, 2003). These container-based indices exhibit a weak correlation with density of biting mosquitoes per human and constitute a poor risk indicator for arbovirus transmission (Bowman et al., 2014). The value of ovitraps for this purpose is also discussed and undoubtedly depends on the context, especially the presence of numerous competing breeding sites given the skip-oviposition behaviour of *Stegomyia* (Fonseca et al., 2013).

For these reasons, adult sampling remains the method of choice to estimate adult abundance. Considering the various traps, the BG-Sentinel trap with lures (BG trap; Biogents, Regensburg, Germany) is currently the gold standard for monitoring adult populations of *Aedes spp.* (Farajollahi et al., 2009; Maciel-de-Freitas et al., 2006). It is nonetheless unrealistic to deploy BG traps on a large scale, due to the cost of the traps and the workload needed to collect them. Gravid *Aedes* Trap (GAT) could be an alternative but more studies are needed to confirm their potential use for routine surveillance purpose (Johnson et al., 2017).

A pragmatic approach to estimate adult density would be to develop fine-scale models based on land cover, land use, meteorological, and socio-demographic data (review in Sallam et al., 2017) in order to target interventions towards locations with high density of mosquito populations (Manica et al., 2016).

**Seasonal dynamics**

The good knowledge of the seasonal dynamics provides useful information for risk assessment and risk management purposes. In particular, it allows to determine a surveillance period for human surveillance and to identify the months with the highest entomological risks.

The seasonal dynamic in a particular region can be assessed with a dense network of ovitraps (Lacour et al., 2015; ECDC, 2012).

The seasonal dynamic could vary according to the climate. The photoperiod and hence the latitude are key factors for the diapause of *Ae. albopictus*. Within a country, it can be relevant to deploy such a dense network in all climatic regions.
Guidance for vector control actions

Once an invasive species is established, eradication is an almost unattainable objective. A control strategy only may be implemented. It will mainly rely on routine larval control through source reduction, chemical larvicides, and biological control.

Entomological surveillance actions will contribute to support larval source reduction and social mobilization actions. Larval surveys retain interest in this context as they can be implemented simultaneously. A typology of breeding sites could be drawn up from larval surveys carried out in the private and public domains (Carrieri et al., 2011). Such surveys will identify different breeding sites that are most often encountered and most productive. On this basis, public action and key messages for the mobilization of the community will focus on the most relevant breeding sites in order to achieve the greatest impact on the adult population (Focks, 2003). Larval surveys should as far as possible take into account the contribution of cryptic larval breeding sites (Chandel et al., 2016). This kind of investigation can then contribute to identify local specificities concerning aquatic sites and thus to implement targeted actions (Boo, 2001; Montgomery et al., 2002; Unlu et al., 2014).

Surveillance efforts will focus on areas and locations that present the greatest risk for public health (history of high densities, public nuisance complaints, hospitals, automotive recycling yard, storage of tires, neighbourhoods with green areas favouring shade and presence of containers, significant presence of water storage...).

Insecticide resistance

Once established, the susceptibility of the mosquito population to the commonly used insecticides has to be evaluated. It will constitute the collection of baseline data regarding resistance and thus will facilitate the implementation of effective control as well as the monitoring of eventual changes in insecticide resistance over time.

6.3. Surveillance of widely established population of *Ae. albopictus* or *Ae. aegypti*

When populations of invasive mosquitoes are widely established, most of the activities implemented in the previous scenario (locally established) are carried out (monitoring of the dispersal, surveillance for abundance and seasonal dynamics, and characterization of breeding sites).

The main supplementary activities will deal with guidance and evaluation of vector control activities implemented around arboviral cases (imported or autochthonous).

As the colonized area expands, the probability of introduction of viremic cases increases. Control measures to prevent local transmission or to limit an eventual autochthonous transmission will become more frequent. Effectiveness of vector control measures can be assessed by trapping adults in the intervention area before and after the treatment. Trapping can also take place at the same time in non-treated areas presenting a similar environment as a control (Bowman et al., 2016).
6.4 Evidence of autochthonous transmission of virus

The general objectives of entomological surveillance remain substantially the same as for the previous scenario but modalities of surveillance will have to be adapted to control actions. Indeed, in the event of viral circulation, vector control will systematically target adult populations in addition to source reduction.

In case of autochthonous transmission, mosquitoes can be captured for virus screening. It is particularly relevant when both *Ae. albopictus* and *Ae. aegypti* are present in order to identify the main vector(s) involved in the transmission event and to adapt the strategy of vector control. Due to the low virus prevalence in mosquitoes, the screening of virus has to be performed on a large number of mosquitoes timely collected in the direct vicinity of arboviral cases (Thomas et al., 2015). Mosquito-based surveillance does not seem relevant for early warning system of *Stegomyia*-borne virus introduction based on the previous consideration and the random nature of the geographical distribution of imported cases. It is more efficient to rely on human surveillance in this context.

Anyhow, mosquito surveillance of all stages should be initiated immediately and with the dense network of sampling station in order to allow comparison of entomological data (species density around outbreak area) to epidemiological data. Such data are not existing or rare for some vector species in a transmission setting but are of crucial importance for epidemiological risk assessment and modelling.
7. West Nile fever

West Nile virus (WNV) is considered as the most widely distributed Flavivirus and the major cause of arboviral human encephalitis worldwide (Winkelmann et al., 2016). It is a zoonotic arbovirus maintained in nature in an enzootic cycle involving birds, mostly passerines, and ornithophilic mosquitoes of the genus *Culex* (Figure 3). Birds are considered as amplifying hosts of the virus and contribute to its dispersal. The disease can also affect humans and horses but they are considered as dead-end hosts, meaning that while they become infected, they do not develop sufficient viremia to infect mosquitoes and support the disease spread. The virus is mainly transmitted by species of the *Cx. pipiens* complex, although experimental transmission has been demonstrated and the virus was detected in mosquitoes from other genera (Engler et al., 2013; Vogels et al., 2017).

First isolated in 1937 from a febrile case in Uganda, the first isolation from mosquitoes was realized from *Culex spp.* in 1952 in Egypt (Smithburn et al., 1940; Taylor and Hurlbut, 1953). Since then, the circulation of the virus has been reported in Africa, Asia, Americas, Oceania, Middle East, and Europe (Chancey et al., 2015). WNV exhibits genetically and geographically diverse lineages, for which the total number could be up to nine (Rizzoli et al. 2015a). Historically, lineage 1 (clade 1a) was the most widespread lineage around the Mediterranean basin. However, the disease pattern changed over the last two decades with the emergence in 2004 of lineage 2 in Eastern Europe (Hungary), becoming endemic throughout Eastern, Central and Southern Europe since then. Lineage 2 is henceforth the source of the majority of human and equine neurologic cases reported in Europe, causing in particular the important outbreaks in Greece (2010) and Serbia (2013) as well as an unexpected avian mortality. Other lineages were isolated from mosquitoes collected in the Mediterranean area but are not associated so far with human or animal diseases, i.e. lineage 3 (Czech Republic) and lineage 4 (Southern Spain, Austria) (Chancey et al., 2015; Rizzoli et al., 2015a; Zehender et al., 2017). Extensive data of WNV circulation in the Mediterranean area are available (e.g. Hubálek & Halouzka, 1999; Murgue et al., 2001; Chancey et al., 2015; Failloux et al., 2017; Shaibi et al., 2017). Furthermore, co-circulation of different lineages across Mediterranean countries constitutes a suitable environment for genetic reassortments and emergence of new strains (Di Sabatino et al., 2014).

Environmental factors for a risk-based approach

The ecological aspects of WNV are complex. The environment has major influences on the intensity of interactions between the protagonists of the vector system (i.e. the virus, the vectors and the hosts). Many factors have been introduced to explain WNV spread. Temperature is one of the most important drivers in WNV transmission, and above-average summer temperatures have been associated with human and equine cases in Europe (Paz et al., 2013; Tran et al., 2014). The role of precipitation is a moot question and depends on the ecology of vectors species that are involved as well as the type of ecosystem (urban and semi-urban biotopes provides breeding sites in the absence of rainfall) and the soil composition. Considering early detection purposes, wetlands with a great avian diversity located along major flyways of migratory birds, constitute interesting potential hotspots to monitor enzootic activity (Jourdain et al., 2007).

Different other environmental risk factors have been assessed, including the Normalized difference vegetation index (NDVI), avian biodiversity, land use and landscape composition. A review of these different factors in the Mediterranean basin was provided by Chevalier et al. (2013).
Predictive models have in particular been proposed for the Mediterranean area (Conte et al., 2015; Paz et al., 2013; Tran et al., 2014), Morocco (Calistri et al., 2013) and Tunisia (Bargouï et al., 2013; Ben Hassine et al., 2017).

Surveillance

A single solution for West Nile surveillance does not exist. Integrated approach should be promoted considering the enzootic, epizootic, and epidemic transmission cycles of the virus. However, the proportionate design of a surveillance system is country-dependent and needs to consider the environmental conditions and local epidemiology of the disease (Chevalier et al., 2011). Several Mediterranean countries have implemented WNV integrated surveillance systems (ECDC, 2009a; Gossner et al., 2017). The entomological component of most European surveillance systems was reviewed by Engler and collaborators (2013).

Entomological surveillance can be implemented as an early warning system given that human and equine cases appear as the result of spill over from the enzootic cycle. Vector surveillance can thus anticipate a viral circulation before the onset of symptoms in humans.
In Italy, entomological surveillance was set up for such purpose in the framework of an integrated surveillance system along with human, equine and avian surveillance. At several occasions, mosquito-based surveillance anticipated human infections between one and six weeks (Bellini et al., 2014; Calzolari et al., 2013). In Serbia, mosquitoes indicated the virus circulation in six out of ten occasions from 2013 to 2015. When it happened, it was between one and nine weeks prior to the detection of first human cases (Petric et al., 2017). Similarly, WNV was detected in pools of Cx. pipiens in Greece one month before the onset of first human cases in 2013 (Papa et al., 2014).

Entomological surveillance can contribute to the sensitivity, early detection and spatial specificity of WNV surveillance systems (Bellini et al., 2014) and gives support to implement risk management measures such as raising awareness among clinicians for diagnosis, public education for appropriate personal protection against mosquito bites, and blood screening for reducing virus transmission by transfusion.

This latter point deserves emphasis as early detection is particularly challenging for securing blood and organ donations, given that approximately 80% of WNV infections in humans are asymptomatic (Pisani et al., 2016). Entomological surveillance – together with animal surveillance – can be a trigger criteria for the introduction of targeted WNV screening in blood donors in order to reduce risks posed to recipients of blood, tissue and organ transplantations. Thus, in Italy for example, such strategy is implemented as soon as positive mosquito pools are reported (Velati et al., 2017). According to the same scheme, deferral of blood donation can be decided for any person having stayed or travelled in geographic areas with virus circulation. Entomological surveillance together with ornithological surveillance has been assessed as a cost-effective strategy for blood and organs safety compared with a systematic screening in provinces where infected humans or equids have been detected in the previous year (Bellini et al., 2014). In conclusion, such strategy appears efficient regarding risk management of organs transplantation and blood transfusion in endemic settings.

An additional advantage of mosquito surveillance is the possibility to identify new viral strains/lineages or other arboviruses – such as Usutu virus (USUV) – at little over-cost, especially when using Flavivirus-genus PCR (Vázquez et al., 2010; Calzolari et al., 2012; Bellini et al., 2014). Such integration clearly provides added values in terms of anticipation, preparedness, and knowledge building as new WNV strains or pathogens like USUV have emerged in different European countries causing outbreaks in wild birds and also human cases of encephalitis and therefore require an adequate surveillance (Vázquez et al., 2011; Ashraf et al., 2015; Calzolari et al., 2017).

Mosquito-based WNV surveillance presents some limitations (ECDC, 2009a; Alba et al., 2014; Papa et al., 2014). It is costly and labour intensive as capacity for early detection requires a significant sampling and testing efforts. The number of mosquito pools submitted for virus detection appears to be critical for sensitivity of such surveillance system. Moreover, results have to be delivered in a short time in order to be timely integrated in the risk management process. In particular, entomological surveillance as an early warning system calls for rapid mosquito identification and virus screening. Due to these constraints, early warning surveillance based on mosquitoes was considered of little value in countries such as France, Spain and Israel (ECDC, 2009a).

To overcome these disadvantages, the definition of entomological surveillance must evaluate the environmental and epidemiological context. It should be (1) seasonally based on the knowledge of vector population dynamics, (2) implemented/intensified when risk is increasing (virus circulation on the previous year or in neighbouring countries/areas), and (3) focused on high-risk areas that are identified according to biotic and abiotic factors as mentioned above.
Entomological surveillance can however pursue other objectives. Monitoring mosquito population builds basic knowledge on the cycles of the disease (Muñoz et al., 2012) and improves significantly spatiotemporal risk assessment process and preparedness (Engler et al., 2013). Mosquito collection can be implemented either in areas at risk or in areas of known viral circulation to improve knowledge on the species involved in the transmission and specify their breeding ecology, abundance, distribution and trophic preferences (Muñoz et al., 2012; Rizzoli et al., 2015b; Wagner et al., 2018).

Adult stages are most often targeted, but larval sampling can usefully complete entomological monitoring (Engler et al., 2013). Most often, samplings are performed on a monthly basis during the period of vector activity. However, weekly to biweekly periods for adult sampling seems to be more appropriate to have sensitive early warning system (Bellini et al., 2014; Papa et al., 2014; Petrić et al., 2017).
8. Rift Valley fever

Context

Rift Valley fever virus (RVFV) emergence may have a considerable impact on both humans and livestock. It is a complex vector-borne disease as several vertebrate hosts can be affected, different transmission routes are possible, numerous vectors can be involved and pathways of virus introduction are also multiple and highly dependent on the local context (Figure 4).

The disease in livestock is characterized by spontaneous abortions and severe illness of pregnant animals and new-borns. Human infections are generally subclinical although the virus can cause a severe febrile illness and jaundice. Less than 5% develop the three major complications: encephalitis, retinitis and other ocular lesions, and haemorrhagic fever. The main transmission route in humans is direct or indirect contact with infected animals and their organs, but the virus can also be transmitted by mosquito bites (Khan and Smith, 2016).

RVFV constitutes an exception in the sense it is readily transmitted through a broad range of mosquito genera including Aedes, Anopheles, Culex, Eretmapodites, Coquillettidia and Mansonia, and by other vectors including sand flies and ticks (Linthicum et al., 2016). Mosquito species of the Culex and Aedes genus are considered as the most competent vectors. The role of the following species in RVFV transmission was discussed and considered relevant for the Mediterranean area: Ae. vexans, Ae. caspius, Ae. detritus, Cx. pipiens, Cx. theileri, Cx. perexiguus, Cx. antennatus, Cx. tritaeniorhynchus and Ae. albopictus (EFSA, 2013). The presence of Ae. aegypti henceforth observed in Egypt and along the Black Sea in Russia, Georgia and Turkey (Abozeid et al., 2018; Akiner et al., 2016) justify to include this latter species in the list.

Global potential of RVF emergence in many parts of the world is threatening due to the presence of susceptible hosts, increase of animal trade and presence of potential vectors (Linthicum et al., 2016).

Historically, RVFV was geographically limited to sub-Saharan Eastern Africa, especially the Rift Valley of Kenya and Tanzania. In the past decades, the geographic range of the disease expanded, including more Northern part of Mauritania (El Mamy et al., 2011; EFSA, 2013), South Africa, the Nile Valley from Sudan to the Egyptian delta and the Arabian peninsula (Weaver and Reisen, 2010). In fact, regular incursions of the virus have been observed along the Nile River in Egypt with epidemics in 1977-1978, 1993-1994, 1996-1997, and 2003. Serological investigations in humans and animals indicate a viral circulation apart from these epidemic events (Kamal, 2011; Mroz et al., 2017). Other serological studies suggest RVFV circulation in areas of Algeria, Morocco, Tunisia and Libya (Arsevska et al., 2016a; Failloux et al., 2017).

Livestock movements are the main factor for introduction of the virus into disease-free areas, especially camels crossing the border from Sudan to Egypt, and sheep trade between sub-Saharan Africa and Northern Africa (Chevalier et al., 2010). The dissemination of the disease to Europe is however controlled as livestock trade from Middle East and Northern Africa to Europe is forbidden, even though illegal importations cannot be excluded particularly in Central and Southern Europe (EFSA, 2005). An increasing risk of RVFV introduction can be anticipated for the future. Such observation justifies the implementation of dedicated surveillance system and knowledge development on bionomics of RVFV vectors in the Mediterranean area (EFSA, 2005).
Risk-based approach to be promoted

In the interests of efficiency and efficacy, risk-based surveillance should be promoted to focus on potential hotspots and periods of disease introduction and transmission (Stärk et al., 2006). In its scientific opinion on “The Risk of a Rift Valley Fever Incursion and its Persistence within the Community” (EFSA, 2005), the European Food Safety Authority (EFSA) recommended to develop early-warning systems for EU countries based on epidemic intelligence and predictive models. Such models have been developed, based on rainfall, sea surface temperature and NDVI. However, their area of validity is restricted to East Africa. The geographical extension of this approach to other areas (including Mediterranean basin) is limited due to differences in climate and environmental drivers, and in the epidemiology of the disease (Chevalier et al., 2010). For the Mediterranean basin, areas at higher risk for RVF can be identified by considering animal trade data, and distributions of competent vectors and susceptible hosts.

Modelling the distribution of RVF vectors around the Mediterranean basin has been realized by EFSA (2013). Risk maps for the identification of suitable areas for RVF emergence have also been provided for the Maghreb region (Arsevska et al., 2016b), Egypt (Napp et al., 2018), Spain (Sánchez-Vizcaino et al., 2013) and Italy (Tran et al., 2013).

As a conclusion, “northern areas of Maghreb are moderately suitable for RVF enzootics and highly suitable for RVF epizootics. More specifically, at-risk areas extend along the coasts and in the Atlas

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**Figure 4.** Epidemiological cycle of Rift Valley fever virus.
Mountains in Morocco, Algeria and Tunisia, but are restricted to two distinct areas in Northern Libya” (Arsevska et al., 2016b). In Northern Africa, potential hotspots were also identified (Arsevska et al., 2016b). These hotspots include northern farming areas and desert oases especially when located close to water bodies and animal facilities. In Egypt, the risk of virus introduction stems from camels importation from Sudan whereas the risk of transmission is heightened in animal markets and slaughterhouses most commonly located in crowded areas (Napp et al., 2018). In Europe, the probability of the introduction of RVFV was assessed as very low (EFSA, 2005).

Proposals for surveillance

RVFV is a typical example of a zoonotic arbovirus located at the intersection of animal and human health and vector ecology supporting an integrated approach within the One Health framework (Bird and McElroy, 2016). One Health approach can indeed yield to better use of scientific and resource capacities, with cost-saving, especially when this approach is promoted upstream of surveillance activities (Rostal et al., 2018).

In terms of surveillance, emphasis should be given to animal and human surveillance, including passive surveillance of animal abortions, sentinel herds around the Mediterranean basin and during high risk periods and human laboratory-based surveillance (EFSA, 2005; Arsevska et al., 2016b).

In addition to animal and human surveillance, efforts in entomological surveillance should be focused on the areas at the highest risk of virus introduction and transmission. Spatially, entomological surveillance will be preferentially implemented in the vicinity of the previously mentioned areas at higher risk for RVFV transmission (quarantines, slaughterhouses and animal markets). Moreover, illegal movements of animals call to strengthen surveillance in animal facilities along borders with endemic countries. In terms of time, entomological surveillance could be reinforced in specific countries in three cases 1) viral circulation in other neighbouring countries, 2) during weeks preceding the Islamic feast Eid al-Adha as a result of increasing animal trade, and 3) occurrence of heavy rains (especially in natural biotopes).

Targeted entomological surveillance will guide mosquito source reduction, larval control and raising awareness of residents and professionals from the livestock sector in areas the most at-risk.

With more specific reference to entomological scientific issues, the seasonal dynamics of main vectors should be assessed in different areas vulnerable to the risk of virus introduction and transmission. Additional studies can be conveniently devoted to determine vector competence and host preferences of the different potential vectors (EFSA, 2013).

Given the borderless nature of RVFV risk, risk management strategies will take advantage of regional approach and cooperation based on information exchange and pooling of resources (Arsevska et al., 2016b; Chevalier et al., 2010; EFSA, 2005).
9. Surveillance of the Point of Entries according to the International Health Regulation

The International Health Regulations (IHR) have been revised in 2005 under the impetus of the World Health Assembly, because the previous regulations did not meet the public health challenges the world was facing (WHO, 2016b). The efficacy of IHR to tackle the international spread of diseases was questioned since a long time (Dorolle, 1968). The major criticisms mostly concern its application to a narrow scope of diseases, the lack of political will of some States to report events and the absence of global response strategy. The development and the retention of core surveillance and response capacities was a fundamental step forward to deal with these issues. In this framework, IHR 2005 makes explicit reference for vector surveillance and control at points of entry and up to at least a 400 meters perimeter around them.

A handbook was published by WHO to provide guidance for the implementation of vector surveillance and control in the framework of IHR (WHO, 2016c). Both invasive and native species are concerned as the main purpose of IHR is “to prevent, protect against, control and provide a public health response to the international spread of disease in ways that are commensurate with and restricted to public health risks”. This handbook provides technical advice for implementing a comprehensive program for surveillance and control of vectors, by setting out the required procedures and capacities. Regarding more specifically the definition of entomological surveillance, special emphasis was given to the description of the global baseline condition of Points of Entries (description of the natural and urban environment of PoE and surroundings, the local entomological situation, and the epidemiological context) to implement adapted and proportionate surveillance.
10. The present situation in the Mediterranean area

Different mosquito borne viruses of medical interest have been inventoried in the last two centuries as circulating in the Mediterranean basin and the Black Sea area. Main viruses and potential vectors are presented in the table 2.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Virus</th>
<th>Amplifying hosts</th>
<th>Geographic distribution</th>
<th>Known or suspected vectors in the area</th>
</tr>
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<tbody>
<tr>
<td>Bunyaviridae</td>
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<td>Cosmotropical</td>
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<td>Africa, Asia,</td>
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<td>Yellow fever</td>
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<td>Africa, South</td>
<td></td>
<td>Ae. aegypti</td>
</tr>
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<td></td>
<td>humans</td>
<td>America</td>
<td></td>
<td>Ae. albopictus</td>
</tr>
</tbody>
</table>
Table 2. Known and suspected arbovirus vectors in the Mediterranean area. Important vectors are indicated in bold; (°): transmission was reported for Ae. vexans arabiensis; *: considered as a main vector of Sindbis virus in South Africa. Source: EFSA, 2013; ECDC, 2014; Failloux et al., 2017; Muñoz et al., 2012; Hubálek, 2008; Chevalier et al., 2013.

The main invasive species have been identified by previous review for Europe (Medlock et al., 2012) and are relevant for the whole Mediterranean area. The main invasive species are Ae. albopictus, Ae. aegypti, Ae. japonicus, Ae. koreicus, Ae. atropalpus. Aedes aegypti and to a lesser extent Ae. albopictus are considered as major vectors regarding their ability to transmit a wide variety of arboviruses (e.g., dengue, chikungunya, Zika, and yellow fever).

These species are subject to an intensive surveillance effort in the Mediterranean area (Di Luca et al., 2017) and distribution maps are regularly updated (Figures 5 and 6).
Figure 5. Distribution of *Aedes albopictus*. Source: European Center for Disease Control (www.ecdc.europa.eu). Map does not show the presence of the species in Armenia, Morocco and Romania (Günay et al., 2018; Bennouna et al., 2017; Prioteasa et al. 2015).
Figure 6. Distribution of *Aedes aegypti*. Map does not show the presence of the species in Canary Islands. Source: European Center for Disease Control (www.ecdc.europa.eu).
11. Inventory of the current situation

A consultation of the different laboratories involved in medical entomology activities of MediLabSecure (Figure 7) resulted in an inventory of the surveillance and awareness of mosquito-borne viruses around the Mediterranean and Black Sea region. The list of representatives of the laboratories who replied (n=18) is proposed in Annex 1 and corresponds to 17 countries (two Moroccan institutes are part of the network; their responses were largely similar and grouped hereafter).

West Nile virus

100% (n=18) of respondents consider that there is a risk of WNV transmission in their country. At risk areas are known for 71% of responding countries (12/17) and mapped for 25% (4/16). 94% of respondents (17/18) believe that entomological surveillance is useful regarding risks of WNV transmission. However this surveillance is only implemented in 35% (6/17) of the countries, systematically with at least one other sector of surveillance: human (n=6), equine (n=4), avian (n=1). When implemented, entomological surveillance of WNV vectors is part of a national plan for preparedness and response for 83% (5/6) of the countries.

Four countries consider that knowledge of the seasonal dynamics of WNV vectors is well characterized. Knowledge of vector biology is considered good in 3 countries, moderate in 2 countries and low in one country (11 countries did not respond).

Four countries report vector competence data on their vector populations: Algeria, Lebanon, Morocco, and Tunisia (Amraoui et al., 2012; Zakhia et al., 2018).

Rift Valley Fever virus

72% of respondents believe that there is a risk of RVF and 78% think that a dedicated entomological surveillance should be implemented.

An entomological surveillance is effective in 2 countries: Egypt and Algeria. An epidemiological and an animal surveillance are implemented in 2 (Egypt and Montenegro) and 3 countries (Egypt, Morocco, Kosovo), respectively.

The identification of risk areas for RVF was carried out in 5 countries (Algeria, Egypt, Libya, Morocco, Tunisia), which bring together all the countries of North Africa.
Figure 7. Map of the countries involved in MediLabSecure and identification of the laboratories constituting the medical entomology network
Invasive species

83% of respondents (14/17) set up a surveillance of invasive species. Such entomological surveillance is not implemented in 3 countries (Former Yugoslav Republic of Macedonia, Moldova and Palestine). Epidemiological surveillance is in place in 6 countries (Tunisia, Montenegro, Egypt, Armenia, Algeria, Albania) for which an entomological component is also implemented. Seasonal activity of invasive species is monitored in 7 countries where such species are present.

The species at risk of introduction that are monitored are *Ae. albopictus* (13/14), *Ae. aegypti* (7/14) *Ae. japonicus* (3/14), *Ae. koreicus* (2/14) and *Ae. vexans* (1/14). The monitoring sites are distributed according to the table 3.

<table>
<thead>
<tr>
<th>Type of site being monitored</th>
<th>Number of responses (on 18 responses)</th>
</tr>
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<tbody>
<tr>
<td>Main urban centres</td>
<td>8</td>
</tr>
<tr>
<td>Vicinity of areas known as colonized</td>
<td>7</td>
</tr>
<tr>
<td>Used tyres storage sites</td>
<td>6</td>
</tr>
<tr>
<td>Borders with neighbouring countries</td>
<td>8</td>
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<tr>
<td>Highways and main communication routes</td>
<td>7</td>
</tr>
<tr>
<td>Greenhouses</td>
<td>4</td>
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<tr>
<td>Airports</td>
<td>5</td>
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<tr>
<td>Ports</td>
<td>5</td>
</tr>
<tr>
<td>Car scrapyards or garages</td>
<td>3</td>
</tr>
<tr>
<td>Main tourist sites</td>
<td>3</td>
</tr>
</tbody>
</table>

*Table 3.* Type of sites being monitored for the introduction of invasive mosquitoes.

For 71% of the countries involved in the surveillance of invasive species (10/14), there is an involvement of health authorities.
12. Discussion and conclusion
Towards a global and integrated surveillance framework

Number of factors impede the implantation of entomological surveillance systems: limited investment, lack of human resources, limited capacities in entomology. The extension of surveillance at a regional level raises some difficulties, such as the need of standardization in data collection, concern of economic impact on tourism sector or issues on privacy of health data of individuals (Fernandes et al., 2018).

These different challenges may partly be addressed through implementation of global and integrated surveillance framework in the prism of the One Health strategy. It requires therefore to promote surveillance systems in which the different sectors concerned by animal and human health interact and work together to improve public health at all levels: locally, nationally and globally. It necessitates in particular broadening the expertise and skills to ecological and environmental sciences and to join different disciplines that are concerned in health policy implementation.

This interdisciplinary approach should go beyond the surveillance area, by considering the impacts of control strategies not only on vertebrate hosts of vector-borne pathogens but also their consequences on the environment and ecosystems. The development of multidisciplinary networks, such as MediLabSecure, provides means to address these challenges and more specifically to promote integrated surveillance, and effective knowledge and information sharing.

Integrated surveillance for a better use of resources. Vector-borne diseases are complex systems and their surveillance involves different disciplines (public health, virologists, entomologists, veterinarians, risks analysts, ornithologists, modellers) and call for coordination at the national level. This issue is mainly addressed by dealing within the framework of integrated vector management. A common framework does not entail uniform surveillance systems. Indeed, there is no one size fits all surveillance systems. Surveillance strategies should be designed to specific entomological and epidemiological situations. Thus, situation analysis and the proposed scenario may assist to define priorities and surveillance objectives.

The rational process for surveillance described above (Figure 1) would be supported by key elements of any integrated vector management strategy (WHO, 2012):

- **Advocacy.** Political and financial commitment is critical to support and maintain expertise and technical resources over time to enable the availability of preparedness and response capacities. Moreover, advocacy with health decision makers may usefully insist on the economic benefit of VBD surveillance compared to delayed outbreak response (Vazquez-Prokopec et al., 2010) and consequently move activities towards a more proactive agenda.

- **Capacity building.** Entomological surveillance requires skilled human resources and adequate infrastructures (entomological laboratories and insectaries). Medical entomologists need a variety of field and laboratory skills such as morphological and biomolecular identification, monitoring of insecticide resistance, trapping methods, use of GIS, data analysis, etc. Moreover, sustainability calls for attractive career structure and opportunities for professional evolution. These challenges can be met with proper training adapted to the different career profiles (Almeida et al., 2017). Networks such as MediLabSecure aim to provide major contribution in this area (Escadafal et al., 2016).
- **Integrated approach.** Integrated surveillance activities aim to share a common understanding of health threats between public health professionals, animal health specialists and entomologists involved in vector surveillance and control by the inclusion of knowledge from human, hosts, vectors, pathogen and environmental components. These issues call for the coordination of the different surveillance activities and their integration in a unique surveillance system in order to strengthen the reliability of the whole system and improve the efficiency of control. The implementation of a formal multidisciplinary committee at country-level facilitates the exchange of information, the coordination of activities and, *in fine*, the integration of surveillance activities. Integration of activities should begin upstream from the surveillance process, at the early stage of data collection and not only for dissemination purposes (Dente et al., 2016; Gossner et al., 2017). Moreover, integrated surveillance systems that are developed for a specific disease may offer synergies and capacities for surveillance of other diseases.

- **Collaboration within the health sector and with other sectors.** The definition and implementation of dedicated policies call for coordination between different levels of administration and public institutions according to national organization as well as collaboration between countries at the regional scale. Other sectors such as urban planning, water management, wetlands management can facilitate the mapping and monitoring of sites at risk. Inter- and intra-sectoral collaborations will have special relevance at the transition point to the vector control stage.

- **Evidence-based decision making.** Every public health action, including entomological surveillance, has to be monitored and evaluated on a regular basis in a perspective of a process of continuous improvement. Data provided by core activities of entomological surveillance are collected and analyzed to generate the evidence-base for implementation and adaptation of the most appropriate surveillance strategy. Hence, monitoring and evaluation is central to the overall approach and requires to be based on the definition of relevant indicators (WHO, 2006) which are critical for efficient monitoring and evaluation of surveillance systems. Different types of indicators can be identified: input indicators (resources needed: personnel, budget, forms for surveillance...), process indicators (tools and activities for implementation: guidelines, training, mosquito identification resources...), output indicators (carried out activities: progress reports, proportion of staff trained, number of surveys implemented, number of controlled larval sites...), outcome indicators (measure of surveillance objectives achievement: use of surveillance data in vector control or community mobilization, implementation of prescriptions based on surveillance...) and impact indicators (measure of overall system objectives achievement) (WHO, 2006). When implementing a surveillance system, monitoring should focus in particular on the input and process indicators. As systems tend to level off over time, efforts are being directed to outcome indicators and impact indicators.

Dente and collaborators (2018) proposed a conceptual framework providing criteria to assess the integration of surveillance systems for arboviral diseases at the following levels: policy and institutional, data collection and analysis, and results dissemination. This framework could be usefully used to evaluate actual surveillance systems and to improve preparedness and response activities towards the arboviruses identified as being of paramount concern.

**Global surveillance to face global emergences.** The situation regarding mosquito-borne viruses evolved in the last decades. New lineages of West Nile virus emerged and modified the global epidemiology of the disease. Other diseases such as dengue or chikungunya are not anymore
geographically restricted to the tropics and exhibit a strong urban component because of urbanization, global trade and travel. Viruses such as dengue, chikungunya and Zika have spread worldwide to an unprecedented degree (Mayer et al., 2017). Other candidates for emergence have been proposed such as Mayaro and Oropouche viruses (Rodríguez-Morales et al., 2017). These general observations claim for reinforcement and coordination of surveillance on a global scale (Fernandes et al., 2018).

Global approach calls for the definition of a common framework for surveillance enabling the exchange, integration and use of data surveillance collected on a regional scale in order to improve the detection and the response to public health events. Regional coordination and knowledge sharing is proposed as a desirable objective in a variety of operational and scientific areas, including trapping and molecular analysis protocols, positive controls of entomological material, mosquito dynamics monitoring, tools for mosquito identification, etc. To go further, surveillance data should be collected according to a standardized way and recorded and retained in a format for ease of access, use and sharing (Corbel et al., 2016; Engler et al., 2013).

Control measures are not part of surveillance process. Both are however closely linked. There is, therefore, a real challenge to develop, the response capacities, in particular vector control, in parallel with the surveillance systems. Such investment is also a precondition for the sustainability of surveillance systems.
Roadmap toward the harmonization of entomological surveillance systems in the Mediterranean area regarding the risks of mosquito borne virus transmission

Glossary

**Arbovirus**: is an acronym for arthropod-borne-virus and refers to the viruses transmitted by an arthropod vector.

**Autochthonous case**: human case locally acquired, without recent travel history.

**Breteau Index**: number of positive breeding sites per 100 houses.

**Capacity**: see vectorial capacity.

**Competence**: see vector competence.

**Container Index**: percentage of water-holding containers with the presence of larvae and/or pupae.

**Established**: a species is considered as established as soon as there is evidence of reproduction and overwintering of a population of the species at least in one municipality.

**House Index**: percentage of houses with at least one positive breeding site.

**Imported case**: human case with recent travel history from a region where the disease is endemic or epidemic.

**Integrated surveillance**: is defined as a surveillance system based on the integration of the different surveillance data from all the components involved in an infectious system. In the field of vector borne diseases are considered all the surveillance modalities of the vector (entomological surveillance), the vertebrate hosts (human and animal surveillance) as well as the characterization of the environmental determinants of disease transmission (environmental surveillance).

**Introduced**: a species is considered as introduced when its presence is reported but without any evidence regarding its establishment (see established).

**Locally established**: refers to an arthropod population considered as established but for which the ecological niche is not yet fully occupied.

**Surveillance**: ongoing systematic collection, collation, analysis and interpretation of data and the dissemination of information to those who need to know in order that action may be taken.

**Vector**: a hematophagous arthropod, responsible for the active transmission of a pathogenic agent from one vertebrate to another.

**Vectorial capacity**: the combined effect of all of the physiological, ecological, and environmental factors relating vector, host and pathogen that ultimately determine the ability of a given arthropod population to efficiently transmit a particular pathogenic agent. It is influenced by parameters such as vector density and longevity, vector-host contact as well as vector competence.

**Vector competence**: the intrinsic (genetic) permissiveness of an arthropod vector for infection, dissemination, and transmission of a pathogen.
Acknowledgments

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Katia GRUCKER (IRD) for the production of illustrations.
Cover picture: © IRD - N. Rahola / Aedes albopictus
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Roadmap toward the harmonization of entomological surveillance systems in the Mediterranean area regarding the risks of mosquito borne virus transmission


EFSA. Opinion of the scientific panel on animal health and welfare (AHAW) on a request from the Commission related to “Rift Valley fever”. EFSA J. 2013. 11(4):3180.


Roadmap toward the harmonization of entomological surveillance systems in the Mediterranean area regarding the risks of mosquito borne virus transmission


Roadmap toward the harmonization of entomological surveillance systems in the Mediterranean area regarding the risks of mosquito borne virus transmission


ANNEX 1 – List of the respondents to the consultation on mosquito-borne virus risks and entomological surveillance

<table>
<thead>
<tr>
<th>Country</th>
<th>Laboratories represented</th>
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<tbody>
<tr>
<td>Albania</td>
<td>Institute of Public Health, Entomology Laboratory</td>
</tr>
<tr>
<td>Algeria</td>
<td>Institut Pasteur of Algeria, Eco-épidemiologie Parasitaire et Génétique des Populations</td>
</tr>
<tr>
<td>Armenia</td>
<td>National Center for Disease Control, Reference Laboratory Center</td>
</tr>
<tr>
<td>Bosnia and Herzegovina</td>
<td>Public Veterinary Institute of the Republic of Srpska</td>
</tr>
<tr>
<td>Egypt</td>
<td>Ain Shams University, Entomology Department</td>
</tr>
<tr>
<td>Georgia</td>
<td>National Center for Disease Control and Public Health</td>
</tr>
<tr>
<td>Jordan</td>
<td>Ministry of Health, Parasitic and Zoonotic Diseases Department</td>
</tr>
<tr>
<td>Kosovo</td>
<td>University of Prishtina, Faculty of Agriculture and Veterinary Sciences</td>
</tr>
<tr>
<td>Lebanon</td>
<td>Lebanese University, Laboratory of Immunology</td>
</tr>
<tr>
<td>Libya</td>
<td>National Center for Disease Control, Laboratory of parasitology and vector borne diseases</td>
</tr>
<tr>
<td>Moldova</td>
<td>Institute of Zoology, Laboratory of Systematics and Molecular Phylogeny</td>
</tr>
<tr>
<td>Montenegro</td>
<td>Biotechnical faculty, Laboratory for applied zoology</td>
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<tr>
<td>Morocco</td>
<td>Institut Pasteur du Maroc, Laboratoire des Maladies Vectorielles</td>
</tr>
<tr>
<td>Morocco</td>
<td>Institut National d’Hygiène, Laboratoire d’Entomologie Médicale</td>
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<tr>
<td>Palestine</td>
<td>Ministry of Health, Central public health laboratory</td>
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<tr>
<td>Serbia</td>
<td>Faculty of Agriculture, University of Novi Sad, Laboratory for Medical and Veterinary Entomology</td>
</tr>
<tr>
<td>The Former Yugoslav Republic of Macedonia</td>
<td>Institute of Public Health, Laboratory for Virology and molecular diagnostics</td>
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<td>Tunisia</td>
<td>Institut Pasteur of Tunis, Medical Entomology Laboratory</td>
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<tr>
<td>Turkey</td>
<td>Hacettepe University, Ecological Sciences Research Laboratories-Vector Ecology Research Group</td>
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</tbody>
</table>
MediLabSecure is a European project that started in 2014, which aims at reinforcing a network of laboratories and public health institutions to provide a collective response to vector-borne diseases.

Participating countries
Albania
Algeria
Armenia
Bosnia & Herzegovina
Egypt
FYR of Macedonia
Georgia
Jordan
Kosovo
Lebanon
Libya
Moldova
Montenegro
Morocco
Palestine
Serbia
Tunisia
Turkey
Ukraine

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