Travel Medicine and Infectious Disease 35 (2020) 101691

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Practical management plan for invasive mosquito species in Europe: I. Asian tiger mosquito (*Aedes albopictus*)

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A B S T R A C T

Aedes albopictus, also known as the "Asian Tiger Mosquito", is an invasive mosquito species to Europe causing high concern in public health due to its severe nuisance and its vectorial capacity for pathogens such as dengue, chikungunya, yellow fever and Zika. Consequently, the responsible authorities implement management activities to reduce its population density, possibly to below noxious and epidemiological thresholds. In urban areas, these aims are difficult to achieve because of the species' ability to develop in a wide range of artificial breeding sites, mainly private properties. This document (Management Plan) has been structured to serve as a comprehensive practical and technical guide for stakeholders in organizing the vector control activities in the best possible way. The current plan includes co-ordinated actions such as standardized control measures and quality control activities, monitoring protocols, activities for stakeholders and local communities, and an emergency vector control plan to reduce the risk of an epidemic.

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https://doi.org/10.1016/j.tmaid.2020.101691
Received 10 March 2019; Received in revised form 19 February 2020; Accepted 19 April 2020
Available online 22 April 2020
1477-8939/

1. Introduction

Globalization of trade and travel facilitate the spread of exotic species across the world. A considerable proportion of these species become established and cause severe environmental, economic and human health impacts. These species are referred to as invasive. As a long-time center for trade, Europe has seen the introduction and subsequent establishment of more than 11,000 non-native species, at least 15% of which are considered invasive and detrimental. Among the non-native terrestrial invertebrates in Europe, insects are the dominant group: of 1,522 established species, 1,306 (86%) are insects [1].

Regarding mosquitoes, several invasive mosquito species (IMS) have been inadvertently introduced into Europe, where they often find favorable environmental and climatic conditions, enhanced by climate change, for the establishment of permanent populations. These IMS, such as *Aedes albopictus*, *Aedes aegypti*, *Aedes atropalpus*, *Aedes koreicus*, *Aedes japonicus* and *Aedes triseriatus* have become an issue of primary importance in Europe as evidenced by the increasing number of detections in different countries and because of the public health risk related to the vectorial capacity of some of these mosquito species [2].

These IMS pose a considerable threat to both human and animal health because of their capacity to transmit diseases. In Europe, *Aedes (Stegomyia) albopictus* (Skuse 1894) (Diptera: Culicidae) has been responsible for the outbreak of chikungunya virus (CHIKV) in Northern Italy in 2007, in which about 250 people were infected [3,4], and in local transmissions of dengue virus (DENV), which were detected in Southern France and Croatia in 2010 and in Southern France in 2014 [5]. In 2017, two CHIKV outbreaks happened in Europe, one in Italy where four clusters displayed 298 autochthonous cases [6,7] and one in France [8] where a cluster of four confirmed autochthonous cases was detected.

There is growing concern now as both vectors and pathogens are reappearing in Europe after a long absence. For example, after decades of absence, *Ae. aegypti* occurs again along the Black Sea coast of Southern Russia, Georgia and Turkey [9]. Moreover, it has been the vector of the DENV type 1 epidemic in Madeira (Portugal) in 2012–2013, with about 2,000 recorded cases [10], and in 2017 it was found established in Fuerteventura (Canary islands, Spain) [11]. This species is an extremely efficient vector of DENV, being responsible for the most severe documented dengue epidemic in Europe which occurred from 1927 to 1928, when about 90% of the population of Athens were infected and more than 1,000 persons died [12]. In 2015–2016, there was an outbreak of Zika virus (ZIKV), which is spread primarily through the bite of **Ae.aegypti** or **Ae. albopictus**, in many regions of South America [13]. In Europe in 2019, the first autochthonous ZIKV case was recorded [14]. There is currently a dengue outbreak in the French Outermost Region of Réunion, sustained by *Ae. albopictus* [15].

Current models estimate some risks of mosquito-borne disease (MBD) outbreaks, with increasing risk linked to climate change impact [16–20]. Countries of the Mediterranean Basin are particularly exposed to MBD due to the high mosquito population densities and the extended seasonal period of mosquito activity [21,22].

Aedes albopictus is an invasive mosquito species which originated from the East Asian regions and has black and white stripes on its body and legs (Fig. 1). *Aedes albopictus* has spread across the world in the last few decades due to its ecological plasticity and human-mediated transport. Since its first detection in Albania in 1979 [23], *Ae. albopictus* has been found in many other European countries (see Fig. 2 for more details and references), as well as in the east and south of the Mediterranean Basin, in the Thrace region of Turkey, and on the eastern Black Sea coast [9,24–27].

2. Management plan components

This management plan to control *Ae. albopictus* in areas where the species is well established includes several activities which may be modulated according to local resource availability and cost-benefit evaluation.

The management plan primary component activities are as follows (and displayed in Fig. 3):

- Public health risk assessment
- Monitoring by ovitraps
- Standard control measures in public and private areas
- Community participation
- Door-to-door control measures in private areas
- Emergency control measures in response to the detection of dengue, chikungunya or Zika imported cases
- Quality control of treatment efficacy
- Prevention of resistance to insecticide

Each component is explained in detail to provide a comprehensive practical and technical guidance to local authorities in organizing the vector control activities in the best possible way. Together with the routine actions aimed at reducing the population density of *Ae. albopictus*, the management plan includes an emergency vector control plan to reduce the risk of an epidemic in case of detection of infected persons.

2.1. Public health risk assessment

The local epidemic risk mainly depends on the vector mosquito density and the number of imported human cases. While the number of imported human cases depends mostly on socio-economic parameters and may be estimated from the historical series, a quantitative standardized monitoring system for *Ae. albopictus* based on mean egg density provides reliable information on the adult mosquito density and can be used to develop prevention models for *Ae. albopictus* transmitted disease epidemics.

The reader may refer to previously published papers to obtain more precise directions on how to perform quantitative monitoring and risk assessment analysis [28–30].

2.2. Monitoring by ovitraps

Monitoring of *Ae. albopictus* is best conducted using ovitraps that attract females to lay their eggs on the provided oviposition substrate.

The ovitraps are black plastic bucket with about 1 L capacity. The operator places the oviposition substrate in the bucket with the corrugated side exposed to facilitate female egg laying. After positioning, the ovitrap is filled with tap water up to the overflow hole (approximately 2/3 of the pot volume). In case of biweekly inspection (every 14 days), a larvicide (e.g. *Bacillus thuringiensis* var. *israelensis-B.t.i.*) should be added to the water to prevent the development of larvae [31]. The ovitrap density can be adjusted according to the aimed data precision level. Knowing, by preliminary investigation using the same ovitraps, the mean egg density range in the study area, the number of oviposition traps can be calculated using the Taylor equation [32].

$$N = m^{b-2} \text{ x } a \text{ x } [Z_{\alpha/2} / D]^{2}$$

where m is the known average egg density value; b and *a* are coefficients defined by Taylor's power law [32–34] (*a* is a constant that depends on the size of the monitoring network, i.e. number of ovitraps, and environmental conditions, while b measures data aggregation); Z is the standard normal distribution value for a given probability [35]; D is the degree of accuracy expected by the monitoring, which can be set as a percentage of the mean [36].

The first positioning of the ovitraps should be done by highly skilled entomologists in well-shaded sites which cannot be easily accessed by humans and animals, preferably on the ground.

The position of each ovitrap must be kept fixed during the season

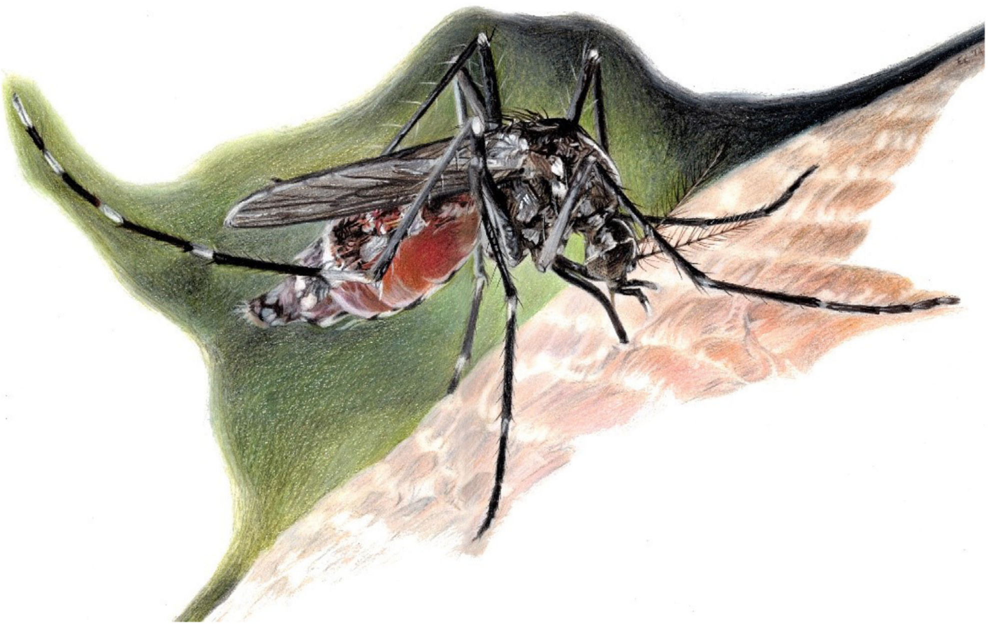

Fig. 1. Female Asian tiger mosquito during blood meal (drawing by Elisa Canaglia).

and over the years. The precise description of each position (sampling station) together with the exact geographical coordinates must be registered in a dedicated database.

Each ovitrap should have a unique code (indicating the selected site) written on the outside label of the ovitrap (for double-checking during the replacement of the old with a new oviposition substrate) with a permanent marker as well as on the top of the oviposition substrate.

The ovitraps must be placed precisely in the same places on each occasion to avoid any displacement throughout the sampling period. If an ovitrap is lost or damaged several times, a new suitable site should be chosen not far from the previous one for continued monitoring.

The oviposition substrate should be collected using disposable plastic gloves, wrapped with gauze or paper, and stored in a plastic bag

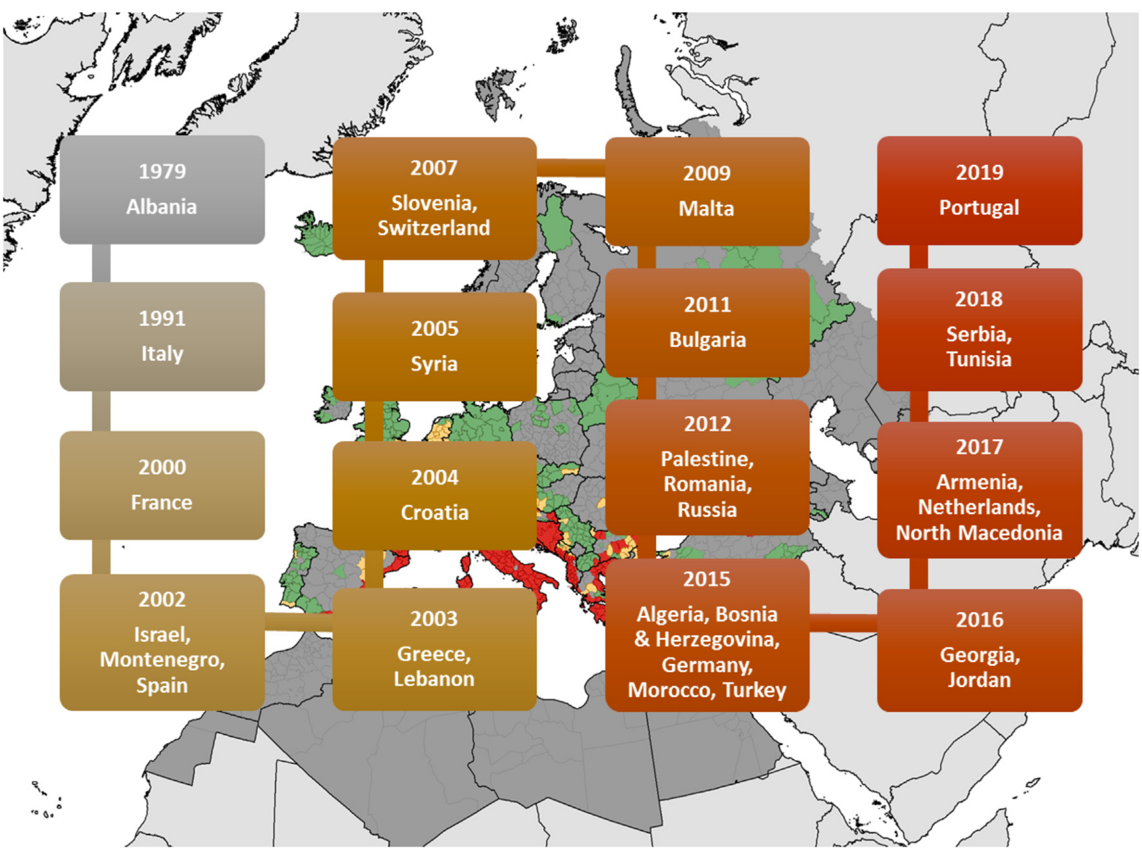

Fig. 2. Timeline of invasion of *Aedes albopictus* in EU and neighbouring countries. Dates indicate the first observed established population in the respective country. Background map: adapted from ECDC Mosquito maps, August 2019 (www.ecdc.europa.eu).

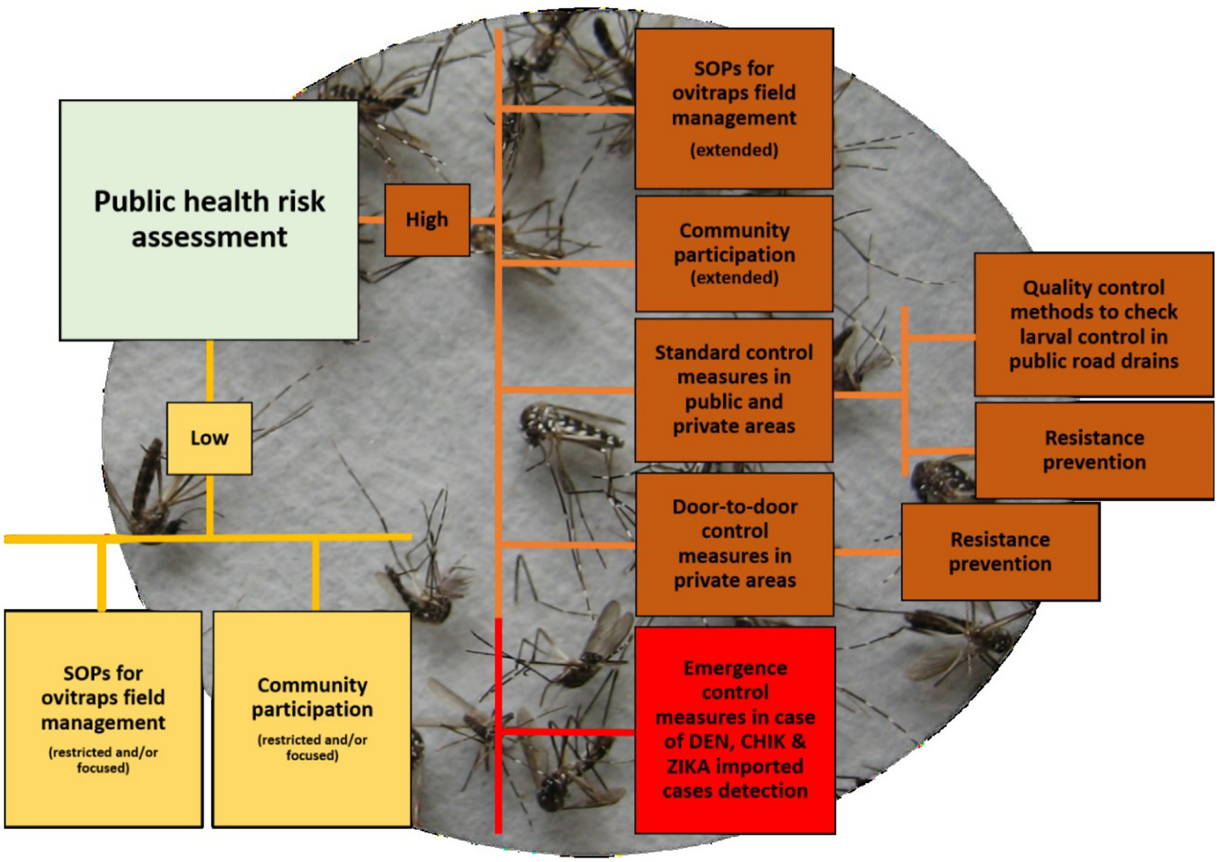

Fig. 3. Components of the management plan to control *Ae. albopictus* (see text for more details). Photo by A. Michaelakis.

(to avoid dehydration of the eggs during transportation). The date of sampling should be marked on the sampling bag. The samples should be kept at room temperature and shipped as soon as possible (1–2 days after collection) to the laboratory responsible for egg counting.

The inner walls of the ovitrap must be cleaned thoroughly with water and a soft sponge or directly by a gloved hand, rinsed with clean water, and refilled with new water (added with B.t.i.).

Ovitraps may be managed weekly, biweekly (adding B.t.i. to the water) or, in case of limited resources, once per month (discontinuous monitoring). The last option is less suitable for population density estimation but might allow better coverage of the region in a case where only presence/absence data are needed. For more information see also "Annex 1: Standard Operational Procedures for ovitraps field management" (https://doi.org/10.13140/RG.2.2.24106.57281), "Annex 2: Standard Operational Procedures for eggs counting" (https://doi.org/10.13140/RG.2.2.25784.29441) and "Annex 3: Standard Operational Procedures for quality control of the *Aedes albopictus* monitoring" (https://doi.org/10.13140/RG.2.2.35850.62403).

In case of large monitoring network involving many operators, it may become necessary to establish a quality control procedure to validate the data before uploading to the database [37].

2.3. Standard control measures in public and private areas

In areas where *Ae. albopictus* is well established and its population density has attained epidemiological importance, and/or where the species causes significant nuisance, control measures should be adopted according to the national/regional regulations.

In areas where the species is not well established because the colonization process is still at the early stages and/or because the environmental conditions are less conducive, the elimination approach could be considered, using all available methods (these are not described and discussed in the present document).

Before and during control operations, all habitats suitable for potential larval breeding should be inspected, registered for larval presence/absence, and mapped. A digital database on GIS software (e.g. QGIS 2.x) is suggested to assist in the planning and management of mosquito control measures. This database may also include the list and locations of the "most sensitive sites", where high vector densities can make particular impact. Examples of such sites include nurseries, kindergartens, clinics for the elderly, and hospitals.

In public areas, removal of breeding sites and larval control should be accurately executed by targeting all available breeding sites. Source removal, having a permanent effect, is highly advantageous from the cost-benefit point of view, and can be applied in public areas by cleaning abandoned sites filled with discarded materials that can hold rainwater, by clearing vegetation harboring abandoned containers, and by disposing of the containers [2,38–44].

Larvicides should be applied in all breeding sites that cannot be removed, as typically is the case for catch basins or road drains. Catch basins collect and maintain water as well as many urban human by-products, which make them highly polluted micro-environments and, at the same time, highly suitable for mosquito larvae growth. In this type of breeding site, the *Ae. albopictus* larval density may become significant, with up to 500 larvae per litre (RB personal observation). Considering seasonality of the mosquito development, which varies depending on local climate conditions, continuous larvicide treatments should be organized accordingly, possibly starting at the beginning of the seasonal development. Larvicidal products should be chosen from the available registered biocides for mosquito control, which forms a monomolecular surface layer that acts by creating a physical barrier and preventing the larvae from breathing on the surface, utilized at the maximum dose indicated on the label (due to the harsh condition in road drains). For the same active ingredient, liquid formulations are usually preferable to tablet or granule formulations in terms of cost-effectiveness, because tablet/granules do not typically guarantee long-lasting activity and because they are more prone to be blocked above the water surface by floating materials or adsorbed by mud accumulating on the road drain bottom. Larval treatment periodicity must be well planned. Estimates of the duration of the larvicide efficacy should be derived from the specialized literature since information regarding product efficacy, reported on the product's label, is usually imprecise [45–47].

When using liquid formulation, the operator must pause at each catch basin for the appropriate time to spray the desired volume of solution. To ensure proper dispersion of the insecticide, it is advisable to distribute per catch basin not less than 30 cc of solution containing the appropriate concentration of the active compound. It is also essential that the spray wand with the nozzle is always inserted into the grid of the drain cover, and the nozzle is adjusted to cover the whole surface of the water. If only a part of the required amount of the product ends up in the water, it is as good as wasted and would be less effective. In recent years in Italy, it was verified that one operator walking or using a bicycle could treat 80–90 catch basins per hour.

Rotation of products with different mechanisms of action should also be considered to prevent the rise of resistance (see Insecticide resistance prevention).

The operational units conducting road drain treatments can be equipped with a GPS device to aid real-time control of their tracks as well as the correct timing of spray on each road drain. The urban area to be treated should be divided into sectors with extensions proportional to the number of workers simultaneously involved in the treatment. The size of these sectors should allow the completion of treatment in no more than two consecutive working days.

The overlaying of the GPS-produced maps on the available road drain mapping may serve as a quality control method. A list of technical specification requirements to be considered while preparing the tender for the delivery of the road drain treatment service is presented in the "Annex 9: Standard Operational Procedures –Public tender template for the selection of Pest Control Operators" (https://doi.org/10.13140/RG.2.2.11838.23361). In private areas, removal of breeding sites and larvicide treatment are mainly the responsibility of the owners (see Community participation), unless the door-to-door campaign is implemented by public bodies (see Door-to-door control measures in private areas).

2.4. Community participation

Many *Ae. albopictus* larval breeding sites are in private properties. The management of these larval breeding sites are usually considered the responsibility of the owners. It is, therefore, necessary to inform and raise awareness in the local community on how to prevent and control *Ae. albopictus* in private areas, and in the meantime, communicate what measures the public bodies have put in place to reduce the problem. Specific actions to improve the community participation are summarized in Table 4.1 in Annex 4: "Standard Operational Procedures – Template for mayor ordinance" (https://doi.org/10.13140/RG.2.2.28300.87684).

Usually, community participation campaign does not produce satisfactory results, as only a small part of the citizens actively manage their property.

Municipal or regional ordinances requiring specific attention and management actions in private areas have proven to be useful when enforced by regular control of public representatives such as municipal police, environmental services and public health officers, with the possibility of fines for non-compliance. An example of Municipal regulation is reported in "Annex 4: Standard Operational Procedures – Template for mayor ordinance" (https://doi.org/10.13140/RG.2.2.28300.87684).

2.5. Door-to-door control measures in private areas

In case the standard control measures do not achieve satisfactory reduction of the *Ae. albopictus* population density, additional control strategy might be implemented such as the door-to-door (DtD).

DtD operators deal with both public and private areas:

- regular DtD actions performed during the mosquito breeding season in all private properties. These activities should include source removal or modification, larval treatment of permanent and temporary breeding sites, and direct information to inhabitants of the properties (a minimum access threshold can be fixed, i.e. ≥95% of premises);
- introduction of indigenous copepod species, such as *Macrocyclops albidus* or *Mesocyclops leukarti* – predators of mosquito larvae – in permanent water containers (e.g. drums in gardens);
- reporting any individual who refuses the treatment and critical cases to the authorities;
- quality controls by an independent organization/group on the efficacy of interventions in the public and private areas;
- additional monitoring with human landing collection or adult traps performed by the quality control organization to find out the residual population density in the treated areas [48].
- management of an information channel open to the public via the Internet and the local press;
- management of a website providing additional information on why the action should be performed and explaining the absence of risks to humans and pets when larvicides are used;
- making a direct telephone number available to individuals requesting specific assistance.

A database of detailed information for each premise, such as contact information in case of owner absence and number and exact location of permanent breeding sites, should be created and updated to assist and optimize the DtD campaign. Properties that do not show any potential breeding site can be surveyed less frequently, whereas properties that are critically affected should be visited more frequently.

2.6. Emergency control measures in response to detection of imported cases of dengue, chikungunya or Zika

In areas and in seasonal periods when the *Ae. albopictus* density is high enough to sustain transmission of DENV, CHIKV or ZIKV, it is appropriate that the public health system is prepared for the early detection of possible imported cases. The risk that an epidemic may start from an infected traveler is real as proven by several outbreaks of dengue and chikungunya reported in some Southern European countries in recent years [7].

In areas where the *Ae. albopictus* density is not known, adult surveillance should be conducted immediately using adult traps (e.g. BG-Sentinel®: Biogents, Regensburg, Germany), with attractant (e.g. BG-Lure®) and CO_2 if possible. Results will be used to evaluate the need for emergency control measures and mosquito samples submitted for virus analysis.

In case the public health service detects CHIKV, DENV or ZIKV imported cases (suspected or confirmed), it is necessary to implement an immediate and capable mosquito control activity. Procedures for emergency vector control operations in case of dengue, chikungunya or Zika detection are reported in "Annex 5: Standard Operational Procedures for emergency vector control operations in case of dengue, chikungunya and Zika detection" (https://doi.org/10.13140/RG.2.2.13201.38242), while Table 5.1 presents useful actions/activities that should be commenced within 24 h of reporting the case.

2.7. Quality control on treatment efficacy

2.7.1. Quality control on routine larval treatments of public road drains and other important breeding sites

In urbanized areas, catch basins are often the most productive mosquito breeding site categories [28]. Together with *Ae. albopictus*, other mosquito species may colonize catch basins, such as *Culex pipiens* and, more rarely, *Culiseta spp*. It is fundamental to adopt a standardized protocol for the quality control of larval treatment of public catch basins.

Because of the obvious conflict of interest, the quality control activity should be the responsibility of a body that is independent from the operator performing mosquito control.

In case of treatments made by a pest control company (PCO) identified by a public tender, the quality control activity should be well described in the tender in order to make the PCO aware of its duty and possible penalties for negligence. In case of treatments made by public employees (e.g. municipality or mosquito abatement districts), quality control should be the responsibility of a different body (e.g. university, public health agency, research institute).

In any case, operator's treatment application units need to be equipped with a suitable GPS device to allow real-time remote detection of the units during the work. The GPS equipment can provide the track of the unit as well as the validation of appropriate volume of insecticide solution distributed in each drain.

However, the remote control is not enough to assess the quality of larval treatments, and post-treatment sampling of drains is necessary.

For effective quality control on larval treatments of road drains, the following parameters should be considered:

- Number of catch basins needed for quality control
- Location of drains and timing of quality control
- Sampling techniques
- Outcome of the quality control
- Safety aspects and accident prevention

For more details please check "Annex 6: Standard Operational Procedures for quality control on larval treatments performed in road drains" (https://doi.org/10.13140/RG.2.2.19912.26880).

2.7.2. Quality control on emergency treatments

In case of detection of a traveler infected (or suspected to be infected) by CHIKV, DENV or ZIKV, immediate mosquito control action, including adulticiding and larviciding, should be taken to prevent the start of an epidemic. It is suggested that an independent institution may conduct quality control following these treatments. Quality control should be conducted in a time window of about 1–3 days from the end of the treatments, considering both public and private areas. A technical report must be prepared on the set of data collected.

For effective quality control on the efficacy of emergency treatments, the following activities should considered:

- Quality control on larvicidal treatments
- Quality control on adulticide treatments
- Safety aspects and accident prevention

For more details please check "Annex 7: Standard Operational Procedures for quality control on emergency treatments" (https://doi.org/10.13140/RG.2.2.29978.59845):

2.7.3. Prevention of resistance to insecticide

The strong selective pressure exerted in case of intensive control programs covering both public and private areas, together with the restricted spectrum of active ingredients authorized as biocide products in the EU market, may give rise to the risk of development of resistance. The choice of the insecticide/s to be used for larval control is currently restricted to a few products, including the IGR category (diflubenzuron, methoprene, pyriproxyfen), the microbial category (B.t.i. and *Lysinibacillus sphaericus*) and surface layer films (following decision 2015/655 of the EU commission of the 23 April 2015, surface layer films do not refer to the Biocidal Products EU Regulation No. 528/2012). Adulticide products mainly refer to the category of pyrethroids, which in the case of *Ae. albopictus* control are suggested only in specific cases and not as a method to be used on a large scale.

In order to prevent the onset of resistance to biocides, it is essential to regularly monitor the sensitivity of the target species to the applied active ingredients. This can be performed by laboratory bioassays conducted according to the WHO protocols [49,50]. Standard Operational Procedure for bioassay is reported in "Annex 8: Standard Operational Procedures for bioassays" (https://doi.org/10.13140/RG.2.2.36689.48480).

In case of long-term control programs, rotation of available insecticides should be adopted, as resistance has already been detected in some European populations following insecticide-based mosquito management programs. Grigoraki et al. [51] warned about *Cx. pipiens'* high resistance to Diflubenzuron, as a consequence of repeated Diflubenzuron treatments of *Ae. albopictus* in Northern Italian urban areas; while Pichler et al. [52] detected possible resistance of *Ae. albopictus* to permethrin following continuous adulticide treatments of *Ae. caspius* in touristic areas of Northern Italy. The rotation of larvicides should be planned by considering products with different mechanisms of action.

3. Conclusions

Since the last decades, *Ae. albopictus* has invaded parts of many European countries, such as Albania, Bosnia and Herzegovina, Bulgaria, Croatia, France, Greece, Hungary, Italy, Macedonia, Malta, Montenegro, Romania, Slovenia, Spain, and Switzerland. Some other countries are considered as being at risk of invasion, such as Austria, Belgium, Czech Republic, Cyprus, Germany, Portugal, Serbia, Slovakia, the Netherlands, Turkey and the UK.

While the species is well known for its anthropophylic behavior, aggressiveness and vectorial capacity, its impact on human societies largely depends on its population density. Despite that a spatial analysis of the population density has never been attempted, it is known that gradients exist, mostly regulated by the seasonal weather dynamic and by the local carrying capacity.

The *Ae. albopictus* management plan we developed and field-tested during the LIFE CONOPS project was ameliorated by integrating the experiences of the many mosquito control agencies and institutions actively engaged in the field. It has been conceived as a practical manual detailing all the main activities considered useful to bring the problem under control in areas where the species is well established. Suggested methodologies and technologies have been defined following careful field testing in a cost-benefit evaluation approach.

The plan does not consider situations where the species is not established yet and, therefore, other approaches, such as preventative surveillance and elimination programs, could be implemented. Nor does it consider situations where ongoing outbreaks have been detected and specific vector control activities should be implemented to stop the epidemic.

Local/regional authorities, responsible for mosquito management, should take advantage from this manual and adopt the actions that they consider more appropriate to their specific condition, after a cost-benefit evaluation.

Because of the large variability in *Ae. albopictus* population density, it will not be surprising for the authors if in some circumstances the local authority may decide not to adopt any of the activities, while in other situations the whole management plan should be implemented.

Acknowledgments

This plan has been prepared in the frame of the LIFE CONOPS project, “Development & demonstration of management plans against - the climate change enhanced - invasive mosquitoes in S. Europe” (www.conops.gr, LIFE12 ENV/GR/000466), co-founded by the EU Environmental Funding Programme LIFE + Environment Policy and Governance. This publication is based upon work from COST Action AIM-COST CA17108 (https://www.aedescost.eu), supported by COST (European Cooperation in Science and Technology). www.cost.eu. The founding sponsors had no role in the writing, preparation, or submission of the manuscript.

References

[1] Keller RP, Geist J, Jeschke JM, Kühn L. Invasive species in Europe: ecology, status, and policy. Environ Sci Eur 2011;23:8–11.
[2] European Centre for Disease Prevention and Control. Gudelines for the surveillance of invasive mosquitoes in Europe. 2012. Stockholm.
[3] Angelini R, Finarelli AC, Angelini P, Po C, Petropulacos K, Macini P, et al. An outbreak of chikungunya fever in the province of Ravenna, Italy. Euro Surveill 2007;12. 7–9:281.
[4] Angelini R, Finarelli AC, Angelini P, Po C, Petropulacos K, Macini P, et al. Chikungunya in north-eastern Italy: a summing up of the outbreak. Euro Surveill 2007;12. E071122-2.
[5] Medlock JM, Hansford KM, Schaffner F, Versteirt V, Hendrickx G, Zeller H, et al. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector Borne Zoonotic Dis 2012;12:435–47.
[6] Venturi G, Di Luca M, Fortuna C, Remoli ME, Flavia R, Severini F, et al. Detection of a chikungunya outbreak in central Italy, August to September 2017. Euro Surveill 2017;vol. 22.
[7] European Centre for Disease Prevention and Control. Clusters of autochthonous chikungunya cases in Italy. Stockholm: first update; 2017.
[8] European Centre for Disease Prevention and Control. Cluster of autochthonous chikungunya cases in France. 2017. Stockholm.
[9] Akiner MM, Demirci B, Babuadze G, Robert V, Schaffner F. Spread of the invasive mosquitoes Aedes aegypti and Aedes albopictus in the Black Sea region increases risk of chikungunya, dengue, and Zika outbreaks in Europe. PLoS Neglected Trop Dis 2016;10:1–5.
[10] Rezza G. Dengue and chikungunya: long-distance spread and outbreaks in naïve areas. Pathog Glob Health 2014;108:349–55.
[11] European Centre for Disease Prevention and Control. New settlements of Aedes aegypti raising concerns for continental EU n.d. 2020https://ecdc.europa.eu/en/news-events/new-settlements-aedes-aegypti-raising-concerns-continental-eu.
[12] Louis C. Daily newspaper view of dengue. Emerg Infect Dis 2012;18:1927–31.
[13] Hajra A, Bandyopadhyay D, Hajra SK. Zika virus: a global threat to humanity: a comprehensive review and current developments. N Am J Med Sci 2016;8:123–8.
[14] European Centre for Disease Prevention and Control. Disease data from ECDC surveillance atlas - Zika virus disease n.d. 2020https://ecdc.europa.eu/en/zika-virus-infection/surveillance-and-disease-data/disease-data.
[15] European Centre for Disease Prevention and Control. Dengue outbreak in reunion. France; 2018.
[16] Reiter P. Climate change and mosquito-borne disease. Environ Health Perspect 2001;109:141–61.
[17] Gould EA, Higgs S. Impact of climate change and other factors on emerging arbovirus diseases. Trans R Soc Trop Med Hyg 2009;103:109–21.
[18] Medlock JM, Leach SA. Review Effect of climate change on vector-borne disease risk in the UK. 2015. p. 3099.
[19] Ebi KL, Nealon J. Dengue in a changing climate. Environ Res 2016;151:115–23. https://doi.org/10.1016/j.envres.2016.07.026.
[20] Tagaris E, Sotiropoulou REP, Sotiropoulos A, Spanos I, Milonas P, Michaelakis A. Climate change impact on the establishment of the invasive mosquito species (IMS). Perspect. Atmos. Sci. Springer International Publishing; 2017. p. 689–94. https://doi.org/10.1007/978-3-319-35095-0.
[21] Vazeille M, Jeannin C, Martin E, Schaffner F, Failloux AB. Chikungunya: a risk for Mediterranean countries? Acta Trop 2008;105:200–2. https://doi.org/10.1016/j.actatropica.2007.09.009.
[22] Gardner LM, Chen N, Sarkar S. Global risk of Zika virus depends critically on vector status of Aedes albopictus. Lancet Infect Dis 2016;16:522–3. https://doi.org/10.1016/S1473-3099(16)00176-6.
[23] Adhami J, Murati N. The presence of the mosquito Aedes albopictus in Albania. Rev Mjekesore 1987;1:13–6.
[24] Pener H, Wilamowski A, Schnur H, Orshan L, Shalom U, Bear A. Aedes albopictus in Israel. Eur Mosq Bull 2003;14:32.
[25] Haddad N, Harbach RE, Chamat S, Bouharoun-Tayoun H. Presence of Aedes albopictus in Lebanon and Syria. J Am Mosq Contr Assoc 2007;23:226–8.
[26] Oter K, Gunay F, Tuzer E, Linton YM, Bellini R, Alten B. First record of Stegomyia albopicta in Turkey determined by active ovitrap surveillance and DNA barcoding. Vector Borne Zoonotic Dis 2013;13:753–61.
[27] European Centre for Disease Prevention and Control. Aedes albopictus – current known distribution in Europe, June 2018. 2018https://ecdc.europa.eu/en/publications-data/aedes-albopictus-current-known-distribution-june-2018.
[28] Carrieri M, Angelini P, Venturelli C, Maccagnani B, Bellini R. Aedes albopictus (Diptera: Culicidae) population size survey in the 2007 chikungunya outbreak area in Italy. I. Characterization of breeding sites and evaluation of sampling methodologies M. J Med Entomol 2011;48:1214–25.
[29] Carrieri M, Angelini P, Venturelli C, Maccagnani B, Bellini R. Aedes albopictus (Diptera: Culicidae) population size survey in the 2007 chikungunya outbreak area in Italy. II: estimating epidemic thresholds. J Med Entomol 2012;49:388–99. https://doi.org/10.1603/ME10259.
[30] Bellini R, Bonilauri P, Puggioli A. Chikungunya and dengue risk assessment in Greece. Vector Biol J 2016;1.
[31] Carrieri M, Masetti A, Albieri A, Maccagnani B, Bellini R. Larvicidal activity and influence of Bacillus thuringiensis var. israelensis on Aedes albopictus oviposition in ovitraps during a two-week check interval protocol. J Am Mosq Contr Assoc 2009;25:149–55.
[32] Taylor LR. Aggregation, variance and the mean. Nature 1961;189:732–5.
[33] Taylor LR. Assessing and interpreting the spatial distributions of insect populations. Annu Rev Entomol 1984;29:321–57.
[34] Kuno E. Sampling and analysis of insect populations. Annu Rev Entomol 1991;36:285–304.
[35] Buntin GD. Developing a primary sampling program. In: Pedigo LP, Buntin GD, editors. Handb. Sampl. methods arthropods Agric. Boca Raton, FL: CRC Press; 1994. p. 99–115.
[36] Southwood TRE, Henderson PA. third ed. Ecological methods vol. 278. 2000. https://doi.org/10.1038/278674a0.
[37] Carrieri M, Albieri A, Urbanelli S, Angelini P, Venturelli C, Matrangolo C, et al. Quality control and data validation procedure in large-scale quantitative monitoring of mosquito density: the case of Aedes albopictus in Emilia-Romagna region. Italy. Pathog Glob Health 2017;111:83–90. https://doi.org/10.1080/20477724.2017.1292992.
[38] Henk van den B, Velayudhan R, Ejov M. Regional framework for surveillance and control of invasive mosquito vectors and re-emerging vector-borne diseases 2014-2020 vol. 18. 2013. https://doi.org/10.1016/j.jecp.2012.05.001.
[39] Becker N, Schön S, Klein A-M, Ferstl I, Kizgin A, Tannich E, et al. First mass development of Aedes albopictus (Diptera: Culicidae) —its surveillance and control in Germany. Parasitol Res 2017;116:847–58.
[40] European Centre for Disease Prevention and Control. Guidelines for the surveillance of native mosquitoes in Europe. 2012. https://doi.org/10.1186/1756-3305-6-209.
[41] Scherrer AM, Motti R, Weckerle CS. Traditional plant use in the areas of monte vesole and ascea, cilento national park (campania, southern Italy). J Ethnopharmacol 2005;97:129–43. https://doi.org/10.1016/j.jep.2004.11.002.
[42] Richards SL, Ghosh SK, Zeichner BC, Apperson CS. Impact of source reduction on the spatial distribution of larvae and pupae of Aedes albopictus (Diptera: Culicidae) in suburban neighborhoods of a piedmont community in North Carolina. J Med Entomol 2008;45:617–28. https://doi.org/10.1038/jid.2014.371.
[43] Unlu I, Farajollahi A, Strickman D, Fonseca DM. Crouching tiger, hidden trouble: urban sources of Aedes albopictus (Diptera: Culicidae) refractory to source-reduction. PloS One 2013;8:1–12. https://doi.org/10.1371/journal.pone.0077999.
[44] Stefopoulou A, Balatsos G, Petraki A, Ladeau SL, Papachristos D, Michaelakis A. Reducing Aedes albopictus breeding sites through education : a study in urban area. PloS One 2018;13:e0202451.
[45] Bellini R, Albieri A, Carrieri M, Colonna R, Donati L, Magnani M, et al. Efficacy and lasting activity of four IGRs formulations against mosquitoes in catch basins of northern Italy. Eur Mosq Bull 2009;27:33–46.
[46] Anderson JF, Ferrandino FJ, Dingman DW, Main AJ, Andreadis TG, Becnel JJ. Control of mosquitoes in catch basins in Connecticut with Bacillus thuringiensis israelensis, Bacillus sphaericus, and spinosad. J Am Mosq Contr Assoc 2011;27:45–55. https://doi.org/10.2987/10-6079.1.
[47] Harbison Layden J, Xamplas C, Zazra D, Henry M, O'Hara Ruiz M. Observed loss and ineffectiveness of mosquito larvicides applied to catch basins in the northern suburbs of chicago IL, 2014. Environ Health Insights 2015:1. https://doi.org/10.4137/EHI.S24311.
[48] Medlock J, Balenghien T, Alten B, Versteirt V, Schaffner F. Field sampling methods for mosquitoes, sandflies, biting midges and ticks vol. 15. 2018. https://doi.org/10.2903/sp.efsa.2018.en-1435.
[49] World Health Organization. Techniques to detect insecticide resistance mechanism (Field and laboratory manual). 1998. Geneva https://apps.who.int/iris/handle/10665/83780.
[50] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. 2005. Geneva https://apps.who.int/iris/handle/10665/69101.
[51] Grigoraki L, Puggioli A, Mavridis K, Douris V, Montanari M, Bellini R, et al. Striking diflubenzuron resistance in Culex pipiens, the prime vector of West Nile Virus. Sci Rep 2017;7:1–8. https://doi.org/10.1038/s41598-017-12103-1.
[52] Pichler V, Bellini R, Veronesi R, Arnoldi D, Rizzoli A, Paolo Lia R, et al. First evidence of resistance to pyrethroid insecticides in Italian Aedes albopictus populations 26 years after invasion. Pest Manag Sci 2018;74:1319–27.