Harmonised surveillance of *Aedes* Invasive Mosquitoes (AIMs) throughout season 2020-2022. https://www.aedescost.eu/aimsurv

FIELD PROTOCOL – #AIMSurv

1. Introduction

The main aim of *Aedes* Invasive Mosquito (AIM) COST Action is to establish a transboundary network of partners and institutions across Europe to cost effectively address the management of the risk of introduction and spread of Exotic Invasive *Aedes* Mosquito-Borne Viruses (EIAMBVs). The project is organised in different Working Groups (see for more details https://www.aedescost.eu) where the WG1 deals with the Monitoring/Surveillance of AIMs.

One of the objectives of the WG1 is to promote data sharing and harmonisation to assure comparable outputs in terms of AIM surveillance (https://www.aedescost.eu/aimsurv) . For this, AIM-COST organised a training course on harmonising AIM surveillance across Europe in, Akrotiri, Cyprus on January 2020. Here, we provide the field sampling protocol (**AIMSurv**) to be applied from 2020 to 2022 in order to harmonise surveillance among the participants. Contribution to **AIMSurv** is open to any participant in Europe and beyond (Near East and North Africa). This will be the first applied attempt of harmonised mosquito surveillance throughout Europe.

2. Objective of AIMSurv

The main objective of **AIMSurv** is to provide longitudinal data enabling comparison of seasonality and abundance across the continent and also to compare field data with reports obtained by citizen science. The outcome will be a harmonised continental overview of the AIMs species in Europe and the Mediterranean Basin, by combining results from different geographical locations for drawing distribution, abundance and seasonality maps including by modelling.

In that perspective we encourage the use of harmonised methods in terms of equipment, time and frequency of sampling, as well as in collecting data and reporting of results. Both presence and absence results of AIMs species are equally important to improve our knowledge at continental level.

We are combining trapping-based methods (i.e. by means of ovitraps and adult traps) with Citizen Science methods (i.e. by use of Mosquito Alert App). We suggest the use of the VECMAP® system to collect and report the data. The VECMAP® App is made available for free by Avia-GIS to all participants for the duration of the project.

There are a series of minimum requirements to fulfil the harmonisation of the surveillance. In addition, teams having sufficient resources are encouraged to apply the recommended requirements, with increased period of sampling and number of samples to collect.

3. Methodology

Summary

Minimum requirements:
- Survey of 3 sites with 5 ovitraps per site, biweekly, over 3 months that must include the populations' peak

Recommended:
- Survey more sites sharing similar or different environment, with 5 ovitraps on each, weekly, over the whole season (including start, peak and end of the mosquito season)
- Add also one adult trap BG-Sentinel™ trap baited with BG-Lure™ and $C0_2$ per site, for one trap/night per week
- Complement with a Citizen Science survey
- Use of VECMAP® to report the data

Hereafter are listed the general requirements. Practical details are given in Annex 1.

3.1 Minimum requirements

- **Traps**
- Density of 5 ovitraps per location with 15 to 100 m of distance between each of the traps, on a minimum of 3 locations, distant by 10 Km or (better) more; The sampling locations should share similar environment, e.g. garden of single family houses in residential urban/peri-urban areas, public parks near residential areas, recreational areas.
- As a substrate for AIM spp. female oviposition, a wood tongue depressor (1.7*15 cm) should be used.
- **Period of sampling**
- A minimum of 3 months sampling is required, making sure that the population peak of the targeted species is included within that period (e.g. Spain: from September to November).
- This applies to ovitraps and Mosquito Alert.
- **Frequency of sampling**
- Conduct sampling every two weeks during the three minimum months of sampling.
- **Parameters to record**
- Geolocation: latitude and longitude of the position of each trap; Use the decimal system (i.e. 46.759463 N, 3.568237 E) and not the degree, minutes, seconds system.
- Name of municipality/county/district (according to the country) and locality (see format in VECMAP® guidelines).
- Start and End date refer to the trapping event for which the data are reported (e.g. a period of 14 days / 2 weeks for ovitraps), in order to get, for the final analysis, numbers per trap/night.
- Land use category (see VECMAP® guidelines page 13 for possible options of the land use field).
- Trap status: report technical issues that could have influenced the trapping result (negative or not), e.g. in case of trap missing or broken, oviposition support missing, battery out, etc.
- When/where no AIM eggs are sampled or adults are caught, 0 (absences) have to be reported.

3.2 Recommended

More locations and/or prolonged sampling period and/or more frequent sampling and/or associating adult sampling.

- **Locations**
- Same density of ovitraps (5 per site) but conducted at more than 3 locations, distant as much as possible from each other to cover a wide area, at locations either sharing similar environments or showing different conditions (e.g. urban areas, rural areas, high altitude areas...).
- Adult traps can be also used, BG-Sentinel™ trap baited with BG-Lure™ and CO_2 is recommended as the standard; One trap-night per site per week is recommended.
- **Period of sampling**
- Whole mosquito season; The period of sampling is suggested to be increased in order to ensure to record the start, the peak and the end of the population activity in each site of sampling (e.g. May – November in Central Europe).
- This applies to ovitraps, adult traps, and Mosquito Alert.
- **Frequency of sampling**
- Weekly sampling during the three minimum months of sampling.
- Weekly sampling during the whole season (start-peak-end) for adults and/or eggs.
- **Parameters to record**
- Same as for minimum requirement, plus:
- Daily or weekly record of meteorological parameter (maximum, minimum, average temperature) per site, collected by using data loggers or local weather stations.
- A map showing the sampling locations (numbered) and countries' administrative units can be provided.

3.3 Processing of the samples

- Collected eggs of AIMs (or native spp., e.g. *Ae. geniculatus*) on oviposition support are counted.
- For every location, a sub-sample of eggs should be (a) reared to confirm species by larva/adult morphology or (b) identified by MALDI-TOF MS or other molecular method.
- A sub-sample consists of 2 out of 5 tongue depressors from one locality that could be submitted to hatching or analysis (to be done for every sampling location and sampling date).
- Ratio "Number of *Aedes albopictus* specimens/total number of hatched specimens" (same for all other species sampled), should be then applied for every ovitrap sample per site per date.
- Adults (BGS trap) of AIMs species to be identified by morphology, sexed and counted. [*Suggested identification keys: ECDC Guidelines (2012) Annex 4 and MosKeyTool V2.1 (2018)*].
- Samples to be preserved (e.g. reared or caught adults frozen) for confirmation if needed (e.g. via molecular tools).

3.4 Data reporting

- Number of *Ae. albopictus* eggs (same for other species) should be reported applying the corresponding ratio after hatching or molecular identification (see section 3.3).
- A reporting form will be provided on the VECMAP® App, with data automatically uploaded to a centralised formatted data base (see section 5).
- In the case contributors cannot use the VECMAP®App, an Excel™ sheet will be provided to all participants to report the data; If reported via Excel™ sheet, the data can be entered straight

into the VECMAP® database via the Web component https://admin.VecMap.com ; We will provide a login for each country to be able to access this platform and a manual on how to do this.

4. Mosquito Alert App

- The Mosquito Alert App can downloaded on the smartphone from Google Apps platform.
- General information about the app can be checked at: http://www.mosquitoalert.com/.
- The protocol for using Mosquito Alert in the framework of **AIMSurv** can be downloaded here: https://www.aedescost.eu/ProtocolCitizenScienceAIM-COST.

5. VECMAP®

- The VECMAP® App can be downloaded for free from Google Play for Android devices version 8.0 and higher and from the App Store for iOS devices version 11.4 and higher. The Web component can be found at url: https://admin.VecMap.com.
- Participants will be provided with the required login credentials to use the platforms.
- Each country will get access to its own project on a shared VECMAP® Workspace. The project structure, settings and digital forms will be the same for the projects of the different countries. This ensures a consistent system for data gathering, while keeping the data separated on country level. Since the databases of the different projects have the same structure, they are easily merged afterwards by an administrator of the Workspace (who has access to all projects in the Workspace).
- General information about the software can be checked at https://www.avia-gis.com/VecMap.
- Guidelines for using VECMAP® in the framework of **AIMSurv** can be downloaded here: https://www.aedescost.eu/VECMAPAIM-COSTGuidelines.

6. Contacts

- If any doubt or comment, please contact any of the following persons:

Name	Affiliation	E-mail	Role
Frederic Bartumeus (Mosquito Alert)	Centre for Advanced Studies of Blanes, SP	fbartu@ceab.csic.es	Mosquito Alert App
Damian Robinson	AVIA-GIS, BE	drobinson@avia-gis.com	VECMAP®
Miguel A. Miranda	University Balearic Islands, SP	ma.miranda@uib.es	Field surveillance
Dusan Petrić	Faculty of Agriculture, Novi Sad, SR	dusanp@polj.uns.ac.rs	Field surveillance
Francis Schaffner	FSConsultancy, CH	fschaffner.consult@gmail.com	Field surveillance
Filiz Gunay	Hacettepe University, Ankara, TR	gunayf@gmail.com	Communication

7. Enrolling for the AIMSurv: We need you!

We need you to enhance our knowledge about presence/absence, abundance and seasonal dynamics of the AIM vector species in Europe. If you are willing to contribute, please register here (if you do not have access to, please send a request for access to fschaffner.consult@gmail.com and ma.miranda@uib.es).

Disclaimer:

Every participant (or institution) is owning the data he collects in the frame of that programme but agrees to share them for the AIM-COST project. AIM-COST will not use the data obtained from the different participants during the AIM Surveillance for any scientific publication without the permit of the owners. By participating in the AIM Surveillance you accept that the data provided by each participant will be part of the public database developed by the project AIM-COST in the web portal for improving networking in Europe about AIM species as one of the main objectives of the project.

ANNEX 1. Practical details for the implementation of AIMSurv protocol.

A. OVITRAPS

1. Ovitraps features and equipment

Ovitraps do attract adults which lay their eggs on the provided oviposition support.
Ovitraps consist of:

- A black plastic container with a total volume of approximately 1 litre with an overflow hole, filled with water to two thirds (Fig. 1).
- An oviposition support: a standard tongue depressor (1.7*15 cm) should be used; before use it can to be scratched with fish descaling knife (Fig. 1).

For the management of ovitraps in the field, you might also need:

- ☐ A sharp object for the scratching of one side of a tongue depressor (e.g. fish descaling knife)
- ☐ Permanent marker for (1) writing the ovitraps code and start/positioning date behind the oviposition support (on the smooth side of a tongue depressor) and (2) writing the end/removal date on the sample zip bag
- ☐ White labels to record the code on the ovitrap
- ☐ Screwdriver to screw the overflow hole on the ovitrap (diameter about 5mm) in upper 1/3 of the container
- ☐ Thin wire or a big paperclip in order to fix the tongue depressor to the inner wall of the ovitrap
- ☐ A warning waterproof label which will also have the necessary information about the project or the institution as well as contact information (e.g. phone number); Suggested dimensions of the label are as follows: a length of 15.5 cm and a width of 7 cm
- ☐ Container/canister for holding the water that will be used in the filling of the ovitraps during placement in the selected sites
- ☐ White plastic dish to check the presence of the larvae in the water remaining in the trap at the time of inspection
- ☐ Sample vial to take the larvae to the laboratory
- ☐ Disposable plastic gloves for the operator
- ☐ Plastic bags to put the tongue depressors collected; Particularly suitable are the “zip it” bags with white marking surface
- ☐ Wrappers for the tongue depressors (e.g. kitchen towel)
- ☐ Android phone or IPhone to use the VECMAP app and record the geographical coordinates (in decimal format)
- ☐ Kitchen sponge (paper towels) for cleaning of the ovitraps

Figure 1. Ovitrap components and tool; left: black container and tongue depressor; right: fish descaling knife to scratch the tongue depressor.

Figure 2. The final set up of the ovitrap ready for placement.

You can also check for videos about ovitraps set up here: https://www.aedescost.eu/aimsurv

2. Preparation

In the internal cavity of containers, the operator will fix the tongue depressor with the scratched (rough) side facing inside, using a small piece of wire or a big paperclip (Fig. 2). The tongue depressors are scratched to facilitate the adhesion of the eggs on its surface. The last step is filling with the ovitrap with tap water up to the overflow hole (approximately 2/3).

3. Placement

Ovitraps should be placed in a shady and safe position (protected from easy access by humans and animals); on the ground or at the height of no more than 50 cm from the ground. Place traps near breeding sites (water collections, tyres…) or resting places (bushes, trees…) to be easily detected by the gravid females of AIM, which are attracted by the dark color of the ovitrap and the wooden support, and lay eggs on the latter. The position of the ovitraps should be kept fixed at same place along the season (and over the years).

In the selected area each ovitrap should be placed distant by 15 to 100m from each other in order to cover a wide area (Fig. 3-5). Each ovitrap should be marked with a unique code identifier on both the outside of the container (label) and the smooth top side of the tongue depressor (Fig. 6-7), with a permanent marker.

Once the final positions are selected, the ovitraps should be placed each time exactly at the same place and not moved during the sampling period, except for particular reason (e.g. frequent disturbance by people or animals, first misplacement at the sun-exposed place…). If ovitraps are lost systematically at a selected site, a new and more hidden site should be chosen in order to continue monitoring.

Ovitraps should be checked (remaining water checked for the presence of the larvae, traps emptied and cleaned inside, tongue depressors replaced, water refilled), in biweekly or weekly intervals (see main protocol). Small variations of 1 or 2 days are accepted, given the real dates (start/positioning and end/removal) are reported.

Figure 3. An appropriate site for ovitrap placing (under a bush)

Figure 4. Wrongly chosen, sun-exposed position

Figure 5. An appropriate site for ovitrap placing (between walls)

Figure 6. Wooden stick with ovitrap code

Figure 7. Ovitrap with a label

4. Replacement of tongue depressors (and ovitraps)

At the end of one sampling period (7/14 days after the placement), the tongue depressor should be collected, wrapped with a slightly wet paper kitchen towel (to avoid dehydration of the eggs) and stored in a plastic "zip it" bags (Fig. 8). It is highly recommended to mark the "zip it" bags, with the code for the site with 5 ovitraps and date of sampling. These samples should be kept at room conditions and processed as soon as possible (1-2 days after collection) for hatching or PCR or MALDI-TOF MS analysis.

In case of possession of two sets of traps, the first set should be replaced by the clean and dry ones brought from the laboratory. After return to the lab, inner walls of the ovitraps used in previous period must be cleaned thoroughly with soap and water using a sponge, rinsed with clean water and allowed to dry until the next sampling (Fig. 9). This step is also essential because there is a high risk to have remaining eggs in the ovitrap which in the next sampling will hatch (when flooded with water) and create a new breeding site.

IMPORTANT: Do not forget to fill the VECMAP App sheet with data at every sampling site.

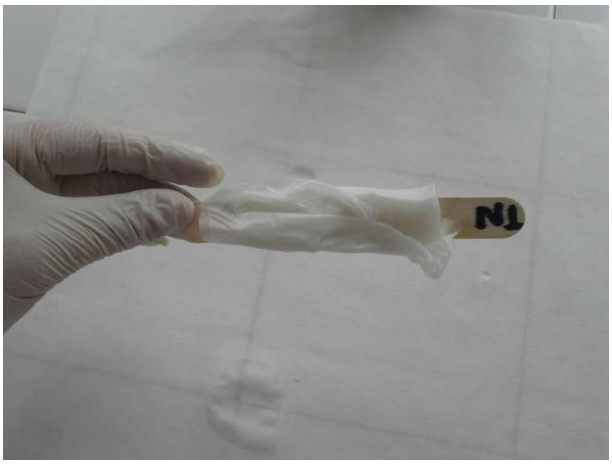

Fig 7. Wooden stick wrapped in slightly wet paper

Fig 8. Ovitrap cleaning

*This ovitrap protocol is based on "Practical management plan for invasive mosquito species in Europe: I. Asian tiger mosquito (*Aedes albopictus*)" [doi: 10.1016/j.tmaid.2020.101691] and its "ANNEX 1: Standard Operational Procedures for ovitrap field management" [doi: 10.13140/RG.2.2.24106.57281]. For egg counting, see also the "ANNEX 2: Standard Operational Procedures for egg counting" [doi: 10.13140/RG.2.2.25784.29441]. Life Conops project: LIFE CONOPS (LIFE12 ENV/GR/000466).*

B. BG-Sentinel traps

A BG-Sentinel™ trap baited with BG-Lure™ and $C0_2$ is recommended to be used, for one trap-night per site every week. The trap should be run at a central place considering the position of the 5 ovitraps.

For the practical use of BS-Sentinel 2 trap, please refer to Biogents protocol at: https://eu.biogents.com/wp-content/uploads/BG-Sentinel-2-Manual-EN-web.pdf

More information on AIM surveillance can be found in: ECDC Guidelines (2012) Annex 3.