<u>Action B.5: Design of management plans to control IMS</u>
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1. Introduction

The current report presents the detailed design of management plan to control IMS (Invasive Mosquito Species) part of LIFE CONOPS project. It is focused on Aedes albopictus as the IMS already well established both in Greece and in Italy, to which the terms “control” therefore apply as the only option we currently have to reduce the density of the species
Others IMS not present yet in the two countries or present in a limited areas may deserve specific and different approaches.
The document has been structured as a comprehensive practical technical guideline to assist local authorities in organizing the field activities in the best possible way.
The LIFE CONOPS scientific team which is involved in Action B.5 and contributed to the development of the current report includes:
- CAA as the coordinating beneficiary;
- all partners.

2. Aedes albopictus

Aedes albopictus (Skuse) is an invasive mosquito species originating from the East Asian regions, which due to its ecological plasticity and because of the human-mediated transport, it has spread in the last few decades across the world (Hawley 1988; Benedict et al. 2007;

Porretta et al. 2012). This is a species of public health concern because vector of arboviruses such as chikungunya (CHIKV) and dengue (DENV) (Gratz 2004; Rezza 2012). In Europe Ae. albopictus has been responsible in the outbreak of CHIKV which took place in Northern Italy in 2007 (Rezza et al. 2007) and in local transmissions of DENV which were detected in Southern France and Croatia in 2010 (La Ruche et al. 2010; Gjenero-Margan et al. 2010; Schmidt-Chanasit et al. 2010) and in Southern France in 2014. In the Emilia-Romagna region Ae. albopictus has been detected for the first time in 1994 (data not published) and since then, despite its low active dispersal ability, it rapidly colonized all the urban areas in the plain and low hill part of the region, reaching high population densities (Urbanelli et al. 2000; Romi et al. 2008; Bellini et al. 2010).

3. Aedes albopictus management plan components

The management plan to control Ae. albopictus is a complex system of components that includes:

- Standardized quantitative monitoring by specific ovitraps
- Public health risk assessment
- Community participation
- Standard control measures in public areas
- Standard control measures in private areas
- Emergence control measures
- Pilot doo-to-door control measures in private areas
- Efficacy & Quality control methods
- Resistance prevention

Each component will be explained in detail in the following chapters.

3.1 Standardized quantitative monitoring

Historically, the monitoring of species belonging to the subgenus Stegomyia (Ae. aegypti and Ae. albopictus) was achieved through the use of ovitraps (Focks 2003). This method provides several advantages over other methods, such as high sensitivity (it can detect the presence of the insect even at low densities), ease of field management, achievable even by unskilled staff, and low material costs (Bellini et al. 1996).

However, it may be influenced by micro-environmental changes such as the cutting of vegetation close to the ovitrap, the removal of breeding sites close to the ovitrap, the local adulticide treatments, the displacement of ovitrap even minimal.

The main aims of the quantitative monitoring by ovitraps are the following:

- to estimate the vector density level during the favorable season;
- to support the estimation of the sanitary risk for vector borne diseases (VBD);
- to evaluate the impact of vector control measures on the vector density;
- to evaluate the possible population dynamic fluctuation in long term.

A precision level has to be prefixed for the estimation of the vector density and its spatial significance (e.g. d=0.25) using the Taylor power law (Taylor 1961, 1984, Kuno 1991), which has been largely used to quantify the aggregation degree and the statistically significant sample size for insect monitoring.

s2=a *mb

where b is a constant for the species and measures data aggregation similar the Variance Mean Ratio (VMR), when b is greater than 1 it indicates that data are aggregated; a is a constant depending on environmental conditions; m is the mean eggs density value.
In the urban localities with surface ≥600 ha the ovitrap density can be determined using the Taylor equation(1) (Carrieri et al. 2011, Albieri et al. 2010), while in the localities with surface <600 ha the number of ovitraps can be kept in the range 1–20 depending on the available financial resources.

$N = [Z_{□/2}/D]^2 * a * m^{b-2}$ (1)

Where Z is the Standard Normal Distribution Value for a defined probability level (Buntin, 1994), D is the desired precision level, a and b the Taylor's coefficients and m the average.
In the inhabited areas < 600 ha that conduct for the first time the Ae. albopictus monitoring, from 10 to 20 ovitraps can be used (it depends on the financial availability of the municipality); in inhabited areas ≥600 ha, 50 ovitraps every 1000 ha of urban surface can be used. After the first monitoring year, Taylor's equation to optimize the monitoring network have to be used.
To assure homogeneity the planning of the ovitraps distribution may be supported by Geographic Information Systems (GIS) software dividing each area into a number of quadrants equal to the number of ovitraps needed (Albieri et al. 2011).
Each ovitrap have to be georeferenced by portable GPS and labeled by an identification code.
The stations have to be kept fixed for the whole monitoring season.
Within each quadrant, the ovitrap have to be placed in a green, shaded, and easily accessible area.
In order to reduce the management cost it results convenient to use an ovitrap allowing the sampling every two weeks. To guarantee water permanence during the summer months in the Mediterranean region a large ovitrap must be employed (Fig. 1). A plastic container with a total volume of 1.4 liter containing 800 mL of Bacillus thuringiensis var. israelensis solution (1 ml of Vectobac 12AS /ovitrap) (Valent BioSciences, Sumitomo) and a strip of masonite (15 × 2.5 cm) as the egg deposition substrate, has been tested satisfactory (Carrieri et al. 2009).

Other points to be included in the quantitative standardized monitoring are the following:

- first ovitrap positioning must be performed by highly skilled technicians;
- it needs to be written a Standard Operational Procedure (SOP) and performed formation for the field operators managing ovitraps (Annex 1);
- SOP and formation for the operators that are assigned to eggs counting (Annex 2);
- quality control procedure to the monitoring system have to be applied regularly (Annex 3);
- data obtained from monitoring system have to be processed and disseminated using on-line platform (es. www.zanzaratigreonline.it) .

3.2 Risk assessment

Quantitative standardized monitoring system for Ae. albopictus based on mean egg density provides detailed and reliable information on the biting females population, and can be used to develop prevention models in the view of Ae. albopictus transmitted disease epidemics.
The model developed is based on R0 (Reproduction Number) that is defined as the number of secondary cases that originate from the primary case (Anderson and May 1991, Roberts and

Heesterbeek 2003, Bolle et al. 2008). If the R0 value is 1, only accidental and isolated cases can occur, whereas for an epidemic, R0 has to be >1.
The Ae. albopictus egg number thresholds calculated in the Northern Italy condition with the ovitrap described above are reported in the following table (Carrieri et al., 20119.
The highest risk refers to the mutated CHIKV strain (E1-Ala226Val) followed by the non mutated CHIKV strain and by DENV.

3.3 Community participation

To inform the local community on how to prevent and control Ae. albopictus in private areas and communicate what the Government has put in place to contain the problem is necessary to conduct information campaigns using the most appropriate channels and methods.
Tools of disclosure:

- brochure on the biology of the tiger mosquito and methods of control;
- posters and flyers to be posted in the public health units, pharmacies, garden shops, in the waiting rooms of public places, bus stops, etc.;
- specific web page within the websites of public administrations involved;
- TV spots to be transmitted on local stations themed public meetings held by experts.

From the strategic point of view it is important to provide regularly a press release on the performance of the mosquito control campaign to be conveyed in the local press. Dissemination activities are also useful in schools, addressed to the classes and teachers.
Interventions could be conducted in primary schools of first and second degree for the impact that the knowledge of the problem has on children and the positive effect of amplification in the family.
It also recommended training meetings for teachers to provide them with an update on the cutting scientific issues of tiger mosquito and suggestions to treat in the classroom.
At local level, the tools available are:

- Major ordinance (Annex 4)
- Municipal Regulations of Hygiene and Public Health

These regulations should include the following points:

- remediation of micro dumps in suburban and peripheral areas;
- the deletion, emptying water and indoor storage of containers and handworks of potential risk;
- Indoor storage of unused tires (alternatively the cover with sheets without creating depressions that can maintain rainy water);
- the treatment of cavities in the trunks;
- avoid where possible the use of saucers;
- hermetic coverage (with mosquito netting, with caps or lids) of drums, bins, tanks used in orchards and gardens;
- the weekly emptying and cleaning the bottom of the troughs for birds and pets;
- larval treatment of the catch basins of the courtyard areas.

3.4 Standard control measures in public and private areas

Larval control is the main activity to be organized accurately by targeting as much as possible all the breeding sites. To achieve this aim it is fundamental to develop and continuously update the breeding sites mapping. Breeding sites in public areas must be censed and reported in a database, preferably in digital form with a GIS software (es. QGIS 2.x). Another data

base including the "most sensible sites" must also be organized and updated continuously. "Most sensible sites" are locations where the presence of high vector densities can create particular impact: examples are nurseries, kindergartens, nursing homes for the elderly and hospitals.
Most larval breeding sites of Ae. albopictus in the urban environment are concentrated in the private properties. These larval breeding sites should be considered under the responsibility of the owners (see community participation).
Larvicide treatments are necessary in breeding sites that can’t be removed, as typically is the case of catch basins. In these breeding sites five larval treatments per season (from April until September) with liquid formulation based on Diflubenzuron (minimum threshold guaranteed: ≥95% of treated catch basins) should be indicatively conducted and submitted to quality control check.

3.5 Emergence control measures in case of DEN, CHIK & ZIKA imported cases detection

In areas where CHIKV or DENV imported cases (suspected or confirmed) are detected by the public health system it is necessary to implement an immediate and capillary mosquito control activity, beginning within 24 hours from the case reporting.
For single case detection the area to be disinfested correspond to a buffer with a radius of 100 meters from the residence of the suspected subject.
Department of Public Health (DPH), based on epidemiological investigation, can give indications about any other areas to be submitted to mosquito control assessed above all in relation to the movement of the subject. The DPH also has the task of providing the subject behavior rules to avoid the mosquito bites.
In case of cluster cases (two or more cases) identified and defined by the DPH, the area to be submitted to mosquito control will be extended up to 300 meters buffer from the more peripheral cases of the outbreak itself, as well as of interest to the entire outbreak area.
Mosquito control activities are divided into three stages that must be conducted in a synergistic way: adulticide treatments, larvicide treatments and larval breeding sites removal.
The optimal sequence in which these processes should be conducted is:
- adults treatments in public areas during the night;
- adults and larval treatments and source removal in private areas (door-to-door);
- contextual larval treatment in public catch basins.

3.6 Door-to-door control measures in private areas

The door-to-door control strategy against Ae. albopictus involves the private areas in addition to the public area, and includes the following actions:
- five door-to-door larval treatments during the season in all private properties with treatment of permanent and occasional breeding sites and direct information of the citizen (minimum access threshold: ≥95% of premises);
- reporting to the authority of any citizens who refuse the treatment and management of critical cases;
- quality controls on the efficacy of interventions in the public and private areas;
- monitoring with ovitraps for measuring the lowering of population density in the treated areas;
- adulticide treatments targeted at specific sites and only in case of real necessity;
- introduction of copepods, predators of mosquito larvae, collected in permanent water (eg. cans of vegetable gardens);

- release of sterile males of Ae. albopictus in inaccessible areas;
- management of an information channel open to the public via Internet and local press;
- direct telephone number for to citizens who request assistance.

3.7 Quality control methods to check larval control in public road drains

Catch basins are one of the main larval breeding site typology in public areas colonized by Ae. albopictus.

In a good management plan it is fundamental to define a standardized protocol for the quality control of treatment of public catch basins (Annex 6).

The larvicidal product, equipment, methods of intervention, timing are defined case by case in the tender documents that should be read carefully.

In the case of the use of the liquid formulation, the operator must operate necessarily stationed at each catch basin for an appropriate time to the spraying volume. To ensure a good dispersion of the active principle is proposed to distribute not less than 30 cc of the mixture/catch basin dosing the larvicidal depending on the concentration of active compound. It's also essential that the dipstick with the nozzle is always inserted into the grid.

The same criterion will also adopted operating using larvicides with formulation in tablet or granules.

If only part of the product ends up in the water, it will produce waste and lower effectiveness.

In recent years in Italy it was verified that is able to treat 80-90 catch basins / hour.

3.8 Resistance prevention

Due to the shortage of biocidal products availability on the EU market registered in the last years the choice of the insecticide/s to be use for larval control is restricted to few products pertaining to the Insect Growth Regulators (IGR) category and to the microbial category (Bacillus thuringiensis israelensis and Bacillus sphaericus). From the trial conducted in Italy the most cost-effective insecticide against Ae. albopictus in the road drain system is Diflubenzuron.

To evaluate the sensibility status of the target population it is necessary to collect about 2,000 eggs in each locality to be checked, hatch the eggs and rear the larvae in standard condition and conduct standard bioassays for LD calculation in parallel on field and on reference lab strain (see Annex 7).

4 Annex list

- Annex 1. Standard Operational Procedures for ovitraps field managements
- Annex 2. Standard Operational Procedures for eggs counting
- Annex 3. Quality control procedure for the Aedes albopictus monitoring
- Annex 4. Mayor ordinance scheme
- Annex 5. Standard Operational Procedures for emergence vector control operations in case of Dengue, Chikungunya and Zika cases detection
- Annex 6. Quality control procedure for larval treatments efficacy in road drains
- Annex 7. Standard Operational Procedures for bioassays on insecticide sensibility
- Annex 8. Public tender for PCO

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6 Summary

Aedes albopictus is the IMS already well established both in Greece and in Italy, causing high concern in public health for its vectorial capacity of pathogens causing human diseases such as Dengue, Chikungunya and Zika viruses. It is therefore necessary that the responsible authorities implement specific vector control plan aimed at the reduction of the mosquito population density, possibly below the epidemiological and the noxious thresholds. This objective results difficult to achieve because of the capacity of the species to develop in urban areas exploiting a number of artificial breeding sites expecially present in private properties. Therefore this document has been structured as a comprehensive practical technical guideline to assist local authorities in organizing the vector control activities in the best possible way.

Results

The management plan to control Aedes albopictus is a complex system that includes coordinated actions to adequately face the most important aspects involved into the problem such as:

- standardized quantitative monitoring by specific ovitraps to obtain regular information on the mosquito population density dynamic;
- the mosquito population density data will also serve the risk assessment for arboviruses like Dengue, Chikungunya and Zika causing serious threat in public health;
- the local community should be involved in the control campaign in private areas where mostly of the Aedes albopictus breeding sites result available and simple actions adopted by citizen may have a significant impact on the mosquito density;
- standard control measures in public areas should be organized regularly using larvicides in the road drains to cover the whole breeding season. It results also necessary to conduct independent quality control operations on the larval treatment in order to assure high efficacy and promptly adopt possible corrective actions (e.g. emergence of resistance);
- an emergence vector control plan should be prepared and responsibilities clearly assigned to the stakeholders to face the epidemic risk in case of importation of infected persons;
- attention is also devoted in a pilot doo-to-door control strategy to be adopted locally in case the regular control campaign does not achieve sufficient results. Specific annexes to practical organize the activities such as: standard operational procedures for ovitraps field managements; standard operational procedures for eggs counting; quality control procedure for the Aedes albopictus monitoring; mayor ordinance scheme; standard operational procedures for emergence vector control operations in case of Dengue, Chikungunya and Zika

cases detection; quality control procedure for larval treatments efficacy in road drains; standard operational procedures for bioassays on insecticide sensibility; template for public tender for PCO, are also provided.

Conclusions

In this document all the information required to perform an adequate response to the problems caused by Aedes albopictus in the Mediterranean basin are organized in a simple and practical way to assist the technical responsible. Attention is also devoted to communication aspects and actions to be adopted in case of emergency.

General instructions

- Describe what has been done regarding each sub-action. Avoid describing the objectives and targets as such. The description should include the status of the activities (started, completed, in progress).
- Indicate what problems you have had, how you have solved them or plan to solve them, what delays (if any) you have and how this will (or will not) impact on the other actions of the project whose implementation depend on this action, and how you plan to catch up.
- Compare the progress made with the established time schedule. Indicate a timetable showing how you plan to continue this action during the next reporting period. Please provide a clear and comprehensive milestone table.
- For each of the objectives of the action, indicate whether you estimate you will achieve them. Where these objectives are quantitative, indicate the target, what you have achieved so far and what you think you will achieve by the end of the project.
- Attach completed deliverables as annexes (with a clear reference in the text of the report).