The possible role of entomological surveillance in mosquito-borne disease prevention

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Summary - Current GIS technologies and laboratory diagnostic capacities are strongly improving the potentiality of vector monitoring and vector-borne disease surveillance programs. When the entomological/veterinary surveillance activities are organized and implemented with the support of a strong baseline of bio-environmental data set, they are showing to be of high usefulness for the early detection of invasive exotic mosquito species and the definition of the colonized area; the risk assessment of vector borne diseases such as Dengue and CHIK throughout vector density estimation at local scale; the surveillance of arboviruses activity in large areas; and the support to epidemiological understanding of vector borne diseases. The efficiency of the surveillance program may be optimized and related costs reduced, by the progressive introduction of GIS satellite supported technologies, by the progressive understanding of the role played by environmental determinants, and by the introduction of more efficient methods of sampling.

Key words: *Aedes albopictus*, invasive mosquito, dengue, Chikungunya, West Nile

INTRODUCTION

Vector borne diseases are threatening a large part of the world human population and seem to make roots in temperate regions previously considered at low risk. Evidences indicate that factors driving this new phase are currently more related to globalization of human activities than to climate change (even if the two aspects can not be clearly distinguished for certain parts), including movement of people (travelers, workers and refugees), animals, and gods (i.e. used tires and ornamental plants).

Environmental changes related to new agricultural practices and wetland restoration, influence the wildlife species as well as the vector species compositions opening the way to new epidemiological scenarios.

Improvements in diagnostic tests and surveillance capacity play of course a relevant role in evidencing the situation, especially in the case of rare or mild diseases, which were probably much less diagnosed in the past.

Human movements through the globe have initiated the spread of invasive mosquito species and related vector-borne diseases centuries ago and the ongoing globalization is just accelerating the phenomenon (Reiter, 1998).

Several mosquito species have been introduced recently into Europe, such as *Aedes albopictus*, *Ae. aegypti*, *Ae. japonicus, Ae. atropalpus*, *Ae. triseriatus* and *Ae. koreicus*, all sharing the ability to develop in artificial containers and to rely on dry resistant eggs.

Of these species *Ae. albopictus* has proven its capacity to act as a vector in temperate region, being responsible of the Chikungunya virus epidemic in northern Italy in 2007 (Angelini *et al.,* 2007; Bonilauri *et al.,* 2008), and of Dengue virus transmission in France and Croatia in 2010 (La Ruche *et al.,* 2010; Gjenero-Margan *et al.,* 2011).

Ae.albopictus is already established in Albania, Bosnia and Herzegovina, Croatia, France, Greece, Italy, Montenegro, Spain, and Switzerland, whit the potential to expand its distribution in Europe. The key of its successful adaptation to temperate climates is the egg diapausing mechanism enabling the permanent establishment in temperate regions, despite the high mortality during the winter period. Evidences indicate that international dispersal mainly exploits trade of used tires and of ornamental plants to a less extent, while local dispersal is easily obtained by ground transport vehicles. *Ae.albopictus* mainly exploits man made containers thus showing distribution mostly related to inhabited areas, while very low presence is observed in rural and natural areas. This distribution has important implication on the vector related sanitary risks.

Thus it becomes necessary to develop capacities to promptly detect introduction of new mosquito species and pathogens, as well as to keep under surveillance the sanitary risk related to the known vector-pathogen systems. This should be done at the country/regional level in a comprehensive EU coordinated frame.

The entomological surveillance, when supported by strong baseline data, may offer the capability for early detection of arbovirus circulation in a specific area.

The case of West Nile virus (WNV) is a good example of this possibility. WNV is one of the most extensively distributed flavivirus worldwide. A large number of wild and domestic bird species may serve as reservoirs of WNV, while transmission is mainly performed by *Culex* species. Mammalian species, including humans and horses, are terminal hosts, unsuitable as virus reservoirs because of low viral titers they develop.

Several outbreaks have been registered in Europe, the largest were in southeastern Romania in 1996 and in Central Macedonia (Greece) in 2010.

The epidemiological picture of WNV is complex being influenced by a number of environmental, climatic and faunistic parameters, often obscuring the role of vector density. The recent evidences obtained in Europe confirm the primary vector role of *Cx. pipiens* (a complex including *Cx. pipiens pipiens*, *Cx. pipiens molestus* and intermediated hybrids) in the amplification as well as in the human infection. The virus is able to overwinter in temperate regions developing a certain level of endemicity.

Cost benefit analysis of concurrent surveillance methodology/systems may be conducted on case-by-case basis providing that the optimization of methods and procedures may progressively be achieved.

It is of relevance that the European Centre for Disease Prevention and Control (ECDC) has just launched an initiative to produce guidelines for implementing surveillance/monitoring of invasive mosquitoes (ECDC, 2011) in order to assist EU Member States and EEA/EFTA countries.

MATERIALS AND METHODS

Invasive mosquitoes

Thorough assessments are necessary to actively prevent and/or control the introduction and establishment of new mosquito species in previously free territories. One of the key issues to be carefully analyzed is the selection of sites of possible introduction through the creation of a priority check list.

This issue requires a thorough knowledge of the region under surveillance to efficiently consider the sites with a high risk of importation from far away regions (i.e. used tire facilities, plant import companies, harbors, airports, freight containers, container terminals) and from neighboring already infested countries/regions (i.e. border crossings, rest areas and petrol stations along traffic paths, and facilities for local transport and trade). An adequate balance/compromise between risk and effort must be progressively developed on the basis of a continuous long term managed effort.

Other key issues to be considered are the collection/trapping methods tailored not only to species biology/behavior but also (Tab. 1) according to analyses of cost efficacy adapted to local conditions; the possible engagement of municipality/local people in the monitoring activities; and the level of information to the public.

An emergency plan should be prepared with clear and complete organization of the surveillance and control activities and attribution of responsibilities, to be adopted in case of detection of invasive species.

In Emilia-Romagna, since 2007, it has been established a working group coordinated by the General Direction for Health and Social Policies composed by physicians, veterinarians and entomologists with the aim of creating a regional surveillance and risk

Table 1 - Scheme of relative usefulness of some mosquito collection methods related to newly introduced species

	CO_2 trap	Light trap	BG sentinel	Ovitrap	Gravid Trap	Sticky trap	HLC	Larval inspection
Aedes albopictus	+	-	++	++	-	+	+++	++
Aedes aegypti	+	-	++	++	-	++	+++	++
Aedes japonicus	+/-	-	-/+	-/+	+	+	-	++
Aedes atropalpus	+	-	?	+	-	?	++	++
Aedes koreicus	?	?	?	+	-	?	?	++
Aedes triseriatus	++	?	++	+	?	**?**	+++	++

Legend: - low efficacy/efficiency; + fair efficacy/efficiency in some situation; ++ good efficacy/efficiency; +++ excellent performances; ? not known assessment system based on multidisciplinary network with the capability of collecting data about both vector populations dynamics and possible presence of pathogens in vectors, men and animals. Referring to the entomological surveillance on invasive mosquitoes the work of the group is dedicated to keep under control sites potentially involved in the introduction of new species, such as Ravenna seaport and companies importing used tyres. Near these sites some traps are located and entomologists conduct periodical inspections to collect larvae in potential breeding sites.

Established mosquitoes

In case of sanitary surveillance focused on already widespread species (autochthonous and exotic) the entomological surveillance may result highly efficient in producing reliable information (Tab. 2).

It may be useful to distinguish between entomological surveillance aimed at estimating the population density distribution for the direct implication it has on the epidemiological risk (i.e. the *Ae. albopictus*-Chikungunya or *Ae. albopictus*-Dengue binomials), and entomological surveillance aimed at collecting mosquito samples to be directly screened for pathogens.

In any case it is important to standardize trapping techniques and to organize a network sensitive enough to detect temporal and geographical variation of population density at the local scale and/or to collect sufficiently significant samples to be screened for pathogens.

The "classic indices" used to evaluate *Stegomyia* population densities such as the House Index (HI: percentage of houses with at least one active breeding site), the Container Index (CI: percentage of containers with larvae), and the Breteau Index (BI: number of active breeding sites per 100 premises), still widely used in tropical countries are of limited value in Europe because of the high requirement of man power and the contribution of important breeding sites in public areas (i.e. road drains) on the productivity per unit area. Other indices such as the PPI (number of pupae/premise), the PHI (number of pupae/hectare), the PDS (Pupal Demographic Survey), and the API (adult productivity index), which defines the mosquito density per unit area, considering both public and private domains, are well correlated with ovitraps data but more expensive to perform and standardize (Carrieri *et al.,* 2011a). As a consequence in Emilia-Romagna the surveillance of *Ae. albopictus* in urban contexts, aimed to assess the epidemiological risk of Chikungunya and Dengue transmission, is based on the use of ovitraps as a tool for mosquito population density estimation. During the favorable season (May-October), about 2,800 ovitraps are activated in the urban areas of 242 municipalities according to standard criteria and checked bi-weekly.

Referring to the surveillance of mosquitoes in rural contexts, aimed to assess the circulation of viral pathogens, in Emilia-Romagna the WNV surveillance system is based on the regular (bi-weekly) collection of mosquitoes in the period June-October. Mosquito collections is conducted using 90-100 CO_2 baited traps positioned in fixed stations in a grid of 10x10 km, to cover the surveillance area The collected mosquitoes are managed in a cold chain, pooled (max 200 individuals) by species, date and site of collection and examined by RT-PCR pan-Flavivirus, and in case of positivity to species specific RT-PCR. When virus activity is low a super-pool strategy is applied to reduce the number of analysis (Calzolari *et al.,* 2010).

Geographic information systems

In recent years, the use of the Geographic Information Systems (GIS) is providing important practical contributions to the investigation and understanding of the spatial component of the epidemiology of vector-borne diseases such as malaria, trypanosomiasis, rickettsiasis and a range of arboviral diseases. The collection and thorough management of georeferenced epidemiological data is useful in the investigation of possible environmental/climate explanatory parameters.

Global and local indicators of spatial association like Moran I or Getis-Ord statistics may assist in the measure of data clustering level.

Geostatistical techniques are also used to produce prediction surfaces and level of uncertainty for these surfaces, which provides an indication of how good the predictions are.

The mapping of the vector population density geographic distribution may provide information both on the environmental variables that drive

Table 2 - Scheme of relative usefulness of some mosquito collection methods for established mosquitoes of sanitary importance

	CO_2 trap	Light trap	BG sentinel	Ovitrap	Gravid Trap	Sticky trap	HLC	Larval inspection
Aedes albopictus	+/-	-	++	++	-	+	+++	++
Culex pipiens	+++	+	+	-	+++	-	-	-
Aedes japonicus	+/-	-	-/+	-/+	+	+	-	++
Anopheles	+	+	?	-	-	?	+	-

Legend: - low efficacy/efficiency; + fair efficacy/efficiency in some situation; ++ good efficacy/efficiency; +++ excellent performances; ? not known species development, and on the epidemic diseases risk level, which are essential to developing effective disease prevention programs, particularly for Chikungunya and Dengue.

In the Emilia-Romagna *Ae. albopictus* surveillance system, the universal kriging interpolation is used to estimate the seasonal abundance of the species at unsampled locations, while the spatial cluster analysis is used to identify particular areas that had statistically significant high or low mosquito density.

Model parameters obtained by variogram analysis were used in an ArcGIS Geostatistical Analysis to obtain prediction maps, the quality of which may be examined by creating a prediction of standard error. The predicted standard errors quantify the degree of uncertainty for each location on the surface.

The extrapolation and interpolation of data need to be conducted with caution, and the production of computer-generated maps that appear to be more informative than the data upon which they are based, should be avoided. Bearing this in mind, contour smoothed maps obtained from geostatistical analyses and cluster maps obtained from cluster detection can be overlaid on other smoothed informative layers to identify environmental variables such as elevation, rainfall distribution, mean air temperature and relative humidity, that could influence seasonal mosquito population densities in the region. These maps can also be overlaid on epidemiological data to identify health risks.

RESULTS

Invasive mosquitoes

The experience matured in Europe in the case of *Ae. albopictus* invasion (VBORNET vector maps: http://ecdc.europa.eu) indicates that, where attempts to early detect and eliminate the invasive species when still confined to a limited area at the beginning of the colonization process have been implemented locally, without a country level of coordination and support (in Italy, Switzerland, Spain) only limited temporarily successes were achieved. Even in Southern France where a more organized plan was deployed the species is rapidly spreading. This is probably because the large number of vehicles coming from infested Italian areas bringing inadvertently mosquitoes on board directly into the towns, where the species is difficult to locate in the initial colonization phase. Huge efforts and investments are deemed necessary to organize a preventative program in the case the invasive species has already achieved a wide distribution range in the continent, with high population density pushing continuously for expansion.

Ae. japonicus has also been introduced, probably via the used tire or the ornamental plants trade (the way of introduction has been hypothesized), from the original Asian area to USA and Central Europe (Austria, Belgium, France-eradicated, Slovenia, Switzerland and Germany) where it is spreading (Schaffner *et al.,* 2009; Becker *et al.,* 2011; Schneider, 2011). This species has been tested a lab competent vector for several arboviruses (i.e. West Nile and Japanese encephalitis), and found positive in the field (i.e. West Nile in the US), but its real medical importance remains to be clarified.

Looking at the distribution the species has achieved in the US (see at http://www.rci.rutgers.edu/~insects /ojdist.htm), where the invasion started about 10 years before than in Europe, it seems that the species has the potential to colonize large part of Europe.

Aedes aegypti originated in Africa has progressively colonized tropical and subtropical areas around the world. It is highly anthropophagic and synanthropic and notorious as the vector of the yellow fever, chikungunya and dengue viruses. In the first half of last century it was present in southern Europe and involved in deleterious dengue epidemics in Athens in the 1927-28 (Theiler *et al.,* 1960), but disappeared afterwards. Nowadays it is spreading along the Black See cost (since 2004), was introduced to Madeira (2004) and in the Netherlands (2010). In the Netherlands, *Ae. aegypti* was found at a company that imports used tires and presumably imported by a tire shipment from Miami together with *Ae. albopictus*. It is currently intolerant to cold temperatures (no diapausing eggs) that will limit possible northerly spread in Europe.

Another exotic species that recently showed up in Europe is *Ae. koreicus*, a poorly known species with Asian distribution, recently detected in Belgium during a research study (Modirisk, 2009) and in northeastern Italy (Capelli *et al.,* 2011). The relevance of this species in terms of possible sanitary impact is largely unknown.

The one Nearctic species invasive to Europe, *Ae. atropalpus*, was first observed in Italy in 1996 (Romi *et al.,* 1997), at used tires storage company, and was most probably collaterally eradicated by the treatments against *Ae. albopictus*. Since that, it is observed in France (2003) and Netherlands (2009, 2010). The species is native to Central and North America, up to southeastern Canada, and probably would have high potential for establishing itself in Europe. In United States is mainly considered a nuisance species that readily bites humans. There, it is found positive for WNV in nature but vector status of this species is still unclear.

One more temperate climate, Neartic species native to North America (Southern Canada and the eastern United States, south to the Florida keys and west to Utah and Idaho) that has potential to invade Europe is *Ae. triseriatus*. This species is the most widely distributed tree hole-breeding mosquito in North America. The larvae develop occasionally in artificial containers such as wooden tubs, barrels, and watering troughs. Females are included in second tier of mosquitoes causing nuisance in the USA (McKnight, 2005). *Ae. triseriatus* is vector of La

Crosse virus and potential vector of West Nile virus in North America. In Europe, it is intercepted only once, in France (2004) in used tyres imported from USA, but is potentially hazardous species having the diapause in egg stage.
From the available evidences it is clear that container breeding species are the most favorites to be passively introduced and established in new regions. This pose a number of questions related on the possible regulation of the international trade of goods that may be exploited by these species, which appears to be the best strategy to develop in order to reduce the risk of new invasions.
It may also be underlined that surveillance programs based on ovitraps are of limited usefulness in term of detection of possible new species because of the difficulties in discriminating the species at the egg stage, and the amount of labor required in egg hatching and larval development to achieve older stages (larva or adult).

Established species
In the case of Dengue and Chikungunya viruses, both strictly connected to the binomial *Ae.albopictus*/human, the vector population density play a key role in the epidemiological equation (Fine, 1981; Reisen, 1989).

$$R_0 = \frac{m\ b\ Sm\ V\ Sv\ p\ i}{(-\log_e p)}$$

where:
m is the mean number of bites per human per day;
b corresponds to 1/GC, were GC is the duration of the gonotrophic cycle;
Sm is the species vector competence;
V is the period during which the infected host has a sufficient viremia to infect the mosquito vector;
Sv is the proportion of the human population sensitive to the infection;
p is the female mosquito daily survival rate;
i is the duration of the extrinsic incubation period of the virus in the vector.
Of course the number of bites/human/day depends on several factors, some of which relate to the human socio-economic condition determining the level of exposure to mosquito bites, including the vector population density.
We may directly influence vector density through mosquito control campaigns.
In this context it is important to know if the vector density in a certain area may be able to sustain an epidemic in case the introduction of the virus through a viremic person. Thus an adequate system of quantitative monitoring has been implemented in the Emilia-Romagna region to provide real time data at a fine geographic scale through the use of ovitraps positioned in a statistical sound network using the Taylor model (Albieri *et al.,* 2010; Carrieri *et al.,* 2011b).
The number of ovitraps to produce values of Relative variation in the range $0.2 < D < 0.3$ has been considered sufficient (about 2,800 ovitraps). This method provides several advantages over other methods, including high sensitivity, ease of field management, and low management costs. Ovitrap data reliability, in terms of quantitative estimation of adult population densities, is controversial and questionable in the tropics while it seems sufficiently sensitive and precise in temperate regions (Tab. 3) (Carrieri *et al.,* 2011a, 2011c).
This system has also some disadvantages such as the strict selectivity for container breeding species, the unavailability of adults for virus screening, the difficulty in determining species at the egg stage (so in case a new *Aedes* species is introduced the system may not be able to discriminate the eggs).
Standard ovitraps currently in use allow a biweekly inspection requiring 10-11 checks per season, producing data that are processed with GIS geostatistical analysis to obtain maps, as the one reported in Figure 1, with information which are regularly publicized on a dedicated website (www.zanzaratigreonline.it).
In the case of WNV surveillance plan in Emilia-Romagna, the entomological and veterinary surveillance, when properly organized, have proven useful to early detect the virus circulation 3-4 weeks before the appearance of human cases (Fig. 2). When validated for a sufficient number of years the surveillance system may allow for the adoption of public health measures only in case they are really needed.

Table 3 - Pearson product moment correlations (R) between mosquito population indices and the mean number of eggs/ovitraps/week collected the week before, the week of and the week after the inspection (from Carrieri *et al.* 2011a)

Population Indices	Mean number of eggs/week/ovitrap		
	Previous week	Inspection week	Week after inspection
HI - House Index	0.0867	-0.1117	-0.3778
CI - Container Index	0.3194	0.0482	-0.4175
BI - Breteau Index	0.0623	-0.1465	-0.4313
PPI – Pupae/premise	-0.0289	-0.2553	-0.5118
PHI - Pupae/ha	0.1703	0.3396	0.8622*

*P < 0.01. HI: percent of houses with at least one positive container. CI: percent of infested containers. BI: Number of positive containers/100 houses. PPI: number of pupae per premise. PHI: number of pupae per hectare.

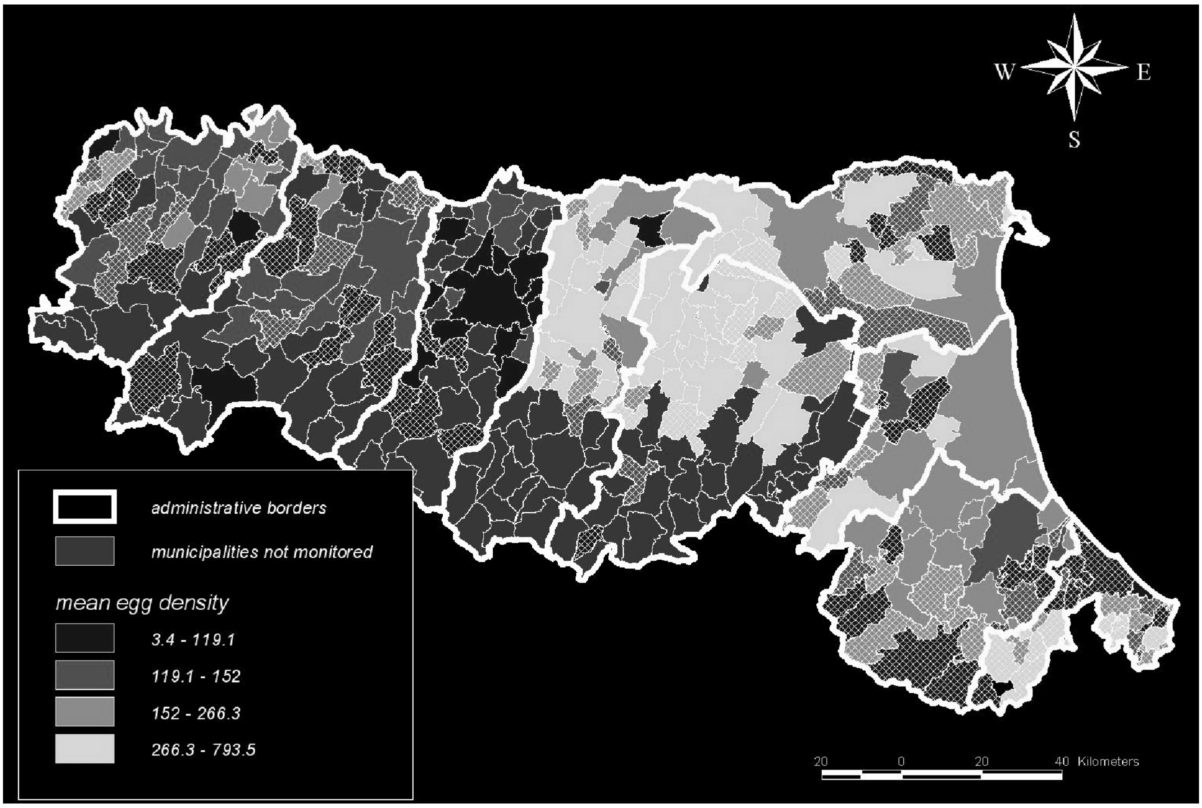

Figure 1 - Choropleth map of *Aedes albopictus* mean egg density (number of eggs/ovitrap/week) during the season 2011 in the Emilia-Romagna Region (Italy) calculated for 10 be-weekly data. Legend values are subdivided into quartiles; wired polygons represent municipalities with sampling designs that were not statistically efficient for measuring true population densities for RV ≤ 0.3.

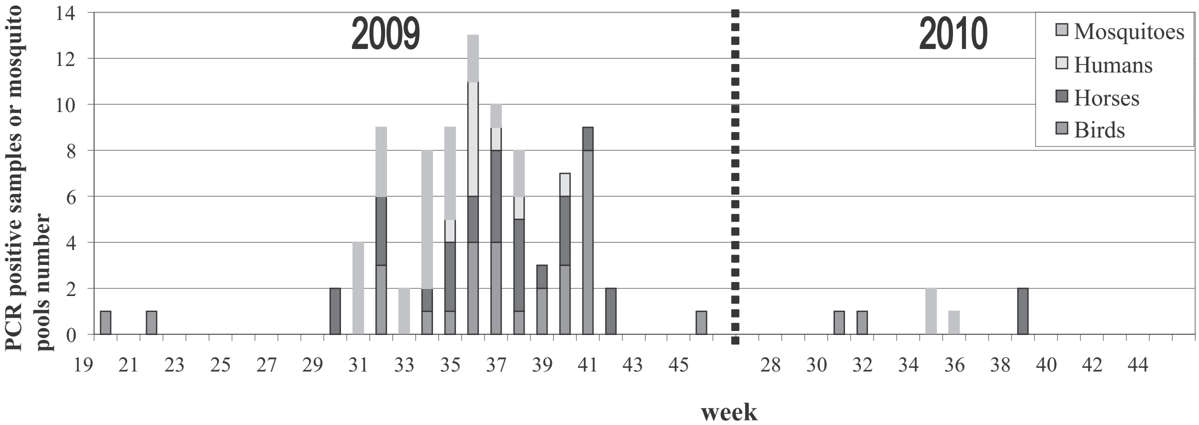

Figure 2 - Seasonal dynamic of WNV positivity obtained during the 2009 and 2010 seasons.

These measures include the information campaign regarding personal protection precautions to be adopted by citizen in risky areas, eventually vector control operations, policy on blood and organs donations.

The WNV surveillance plan in the Emilia-Romagna region detect another arbovirus which resulted highly active in the last three years (2009-2011): Usutu virus (USUV). This virus seems to co-circulate with the WNV, using *Cx. pipiens* as the main vector, and several birds species as hosts. Its epidemiology needs to be investigated to clarify the role of non-bird hosts and of *Ae. albopictus* as possible secondary vector (Weissenböck *et al*., 2003, Tamba *et al*., 2011).

CONCLUSIONS

Vector monitoring and vector-borne disease surveillance programs when applied in a properly organized way, are showing to be of high usefulness for the: (i) early detection of invasive exotic mosquito species and relative infested area definition; (ii) risk assessment of vector borne diseases such as Dengue and CHIK throughout vector density estimation at local scale; (iii) surveillance of arboviruses activity in large areas; (iv) support to epidemiological understanding of vector borne diseases.

The entomological surveillance may be conveniently integrated with medical and veterinary surveillance activities to produce a more comprehensive understanding of the situation and to better planning the use of resources.

When integrated with meteorology, environmental spatial techniques and informatics/statistics the entomological surveillance may be able to produce output of information that go to a fine scale, allowing explanation or hypothesis to understand observed phenomena.

Modeling may produce the best outcome in term of explanation and/or prediction when monitoring/surveillance are well planned on statistical bases and on baseline knowledge of biology of the involved species and ecology of the interested territory.

The capacity to early detect the presence of invasive species and define the size of the colonized area is fundamental to increase the chances of elimination of invaders at the beginning of the colonization process with much less efforts than it would need in case of wide infested areas. A number of eliminations of new populations of *Ae. albopictus* have been achieved in Italy, France and Serbia when the species was confined to a used tire company and surrounding area or border crossing.

It has been demonstrated on several occasions within different countries and environmental conditions that it is possible, and perhaps highly convenient in term of cost-benefit balance, to eliminate an invading mosquito species by promptly applying intensive suppression methods if the colonized area is still well delimited.

A country level specific evaluation of the most probable way/sites of entry of the species could be conducted to assist a focused surveillance plan aimed at the prompt detection and elimination of the species. Cost comparative analysis of this approach compared with the control cost (including the sanitary cost and risk) in case of wide colonization of the country may produce an indication of the financial support to be invested in the preventative measures.

The main concern the southern Europe countries are facing is named *Ae. aegypti*, tropical widely present species, which recently implanted a bridgehead in Madeira island and Abkhazia (Almeida *et al.,* 2007). Frequent and rapid connections from these areas to climate prone regions pose a clear risk of introduction and establishment in the continent. It may be of significance to recall that this species was quite commonly found in the Mediterranean ports until the beginning of the 19th century, and still is one of the major vector of Dengue worldwide. A coordinate international effort aimed at the elimination of *Ae. aegypti* from Madeira and Abkhazia must be evaluated as a preventative measure to protect southern Europe.

The ability of European countries to obtain data on the presence and abundance of invasive species and to develop efficient control programs and tools for their evaluation needs to be rapidly and consistently improved in order to increase the chances for early detection and elimination of invaders at the beginning of the colonization process.

Where the invading species is established on a large area (large town or region), monitoring of population abundance is needed with standardized methods on a long term basis to perform a risk assessment of arbovirus transmission such as dengue and Chikungunya, obtain data about the evolution of the vector density and guiding the planning of information/control campaigns.

On the basis of the current available technology the possibilities to develop well focused and cost benefit surveillance programs are certainly increasing with important benefit for the prevention of vector borne diseases.

WNV surveillance based on mosquito collection coupled with the rapid laboratory screening for arbovirus presence has proven reliable in terms of sensitiveness and precociousness in the detection of virus circulation. This is particularly useful in the case of periodical incursion of the virus in a geographic area, with silent periods sometimes lasting many years. By adopting an active entomological surveillance it is possible to assist the public health authorities in the target adoption of preventative measures only in case of real need.

The efficiency of the surveillance program may be optimized and related costs reduced, by the introduction of GIS satellite supported technologies, by the progressive understanding of the role played by environmental determinants, and by the introduction of more efficient methods of sampling.

Depending from the collection methods utilized, the entomological surveillance may provide information on the possible activity of other arboviruses. In the case of CO_2 baited traps, considering their efficacy in collecting several haematophagous species, it is possible to maintain under surveillance not only mosquito borne viruses, but sand fly, biting midge and black fly borne pathogens as well.

ECDC is engaged in stimulating the awareness and the capacities of European countries to organize and conduct surveillance and control of mosquito vector-borne diseases. The European Spatial Agency (ESA) is also on the ground by promoting better exploitation of remote sensing satellite capacities in the field of vector surveillance and by supporting the VECMAP initiative inside the Integrated Applications Promotion (IAP) ESA ESTEC, an integrated spatial tool and service for modeling the distribution of mosquito vectors of disease.

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