Proceedings of the Ninth International Conference on Urban Pests
Matthew P. Davies, Carolin Pfeiffer, and William H Robinson (editors) 2017
Printed by Pureprint Group, Crowson House, Uckfield, East Sussex TN22 1PH UK

SURVEILLANCE AND CONTROL OF *AEDES ALBOPICTUS* IN EPIDEMIOLOGICAL RISK AREAS OF VALENCIA (SPAIN)

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Abstract In Spain Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) are frequently imported by tourists and immigrants infected in endemic countries. The Spanish Ministry of Health has declared DENV, ZIKV and CHIKV as priority notifiable diseases in the country. Research presented here is focused on entomological interventions, surrounding imported cases of DENV, ZIKV and CHIKV, carried out in Valencia, Spain. The objective was to evaluate and control the risk of disease amplification at local scale. Valencia has a population of about 800,000 and has a Mediterranean climate of mild winters and long, hot and dry summers. *Aedes albopictus* was first recorded in the city in July 2015. Since then, the spread of the species has been constant through the municipality although local authorities have implemented a comprehensive control program based on preventive measures, insecticide treatments, vector monitoring, and social awareness. We present the methodology and results of all entomological interventions associated to imported cases of DENV, ZIKV and CHIKV in 2016. The use of Geographic Information Systems techniques, the procedures of inspections in domestic and public risk environments, the treatment of vector breeding and resting sites, the epidemiological information about imported cases, and the results of vector activity inside the influence range of each imported case will be presented.

Key words *Aedes albopictus*, arbovirus, surveillance, mosquito control, vector-borne diseases.

INTRODUCTION

The Asian tiger mosquito, *Aedes albopcitus*, is a potential vector of several arboviruses like Dengue (DEN), Zika (ZIK) or Chikungunya (CHIK) in urban and periurban environments. These viruses are currently emerging worldwide, especially in tropical and temperate regions where vectors are capable of proliferating and reach high population densities. In Spain DEN, ZIK and CHIK are diseases frequently imported by tourists and immigrants infected in endemic countries. This context of increasing incidence and spread of potential local vectors, high infection rates of these arboviruses in tropical countries and globalization, that facilitates quick and continuous human movements all over the world, has motivated to Spanish Ministry of Health to declare DEN, ZIK and CHIK as priority notifiable diseases in our country.

This invasive mosquito was recorded for the first time in Valencia (Eastern Spain) in July 2015 (Bueno and Quero, 2015), during routine samplings associated to a surveillance system against exotic culicids implemented in the city since 2014 and based on different trapping strategies of mosquitoes (eggs, larvae and adults) in some of the most suitable urban points (gardens, cemeteries and bus and train stations). Valencia is the third most populated city of Spain (800,000 inhabitants) and has excellent environmental conditions for *Ae. albopictus* establishment, with a typical Mediterranean climate (hot summers and mild winters) and high density of ideal breeding sites in public areas; there are 67,708 catch basins which are the most important biotopes for larval development in urban areas. Once the

species was detected in the city, during the second half of 2015, many efforts were carried out to know the mosquito range expansion. Press articles and other printed/online material informing about the Asian tiger mosquito findings were published in order to promote new citizen communications of species activity in different urban areas, high number of awareness talks with variable contents were exposed to several targeted groups of population like municipal workers (gardeners, technicians of sewer maintenance), school students (educative projects), sanitary professionals (medicals, pharmaceuticals, veterinaries and biologists) as well as the rest of citizens. Other passive surveillance strategy were related with the implementation of the citizen science platform named Mosquito Alert as an additional system of information input about vector activity. Regarding the "active" measures conducted for the analysis of *Ae. albopictus* spread, a reinforcement of intensive samplings with ovitraps, adult collection devices (BG-Sentinel Trap ®) and larval search in catch basins was carried out in all districts of the city. As a result of this monitoring strategy, 47 isolated hot spots of *Ae. albopictus* distribution were identified in Valencia at the end of 2015. It is important to note that all positive detections of Asian tiger mosquitoes were followed by the subsequent execution of control tasks, either mechanical breeding sites elimination or larvicides/adulticides applications.

With all this information properly analysed and georeferenced, the urban mosquito control campaign was designed in 2016 (first year after *Ae. albopictus* establishment) with 3 main axes:

Periodic surveillance and control of Asian Tiger Mosquito Risk Points (ATMRP). These points correspond to the 47 areas where the species was firstly recorded the previous year plus 2 areas referred to the surroundings of sanitary centres which are the reference hospitals for arbovirus diagnostic and consequently are places where there is a potential racking of patients who can carry the mosquito-borne disease. Consequently, this combination of historical activity data and epidemiological issues, is the basis to conform the 49 ATMRP of 2016. The main identifiable breeding sites of this 49 ATMRP (a total of 4.026 catch basins) are monitored every 4-6 weeks and also treated if mosquito presence is detected between the most suitable activity period (April-November).

Quick citizen complaints attention. This is also a notable source of information about cryptic problems linked to *Ae. albopictus* activity. In this kind of activities control actions are done in public areas, but not in private ones, and citizen awareness is also promoted through the distribution of informative brochures about mosquito prevention in domestic environments. Several traditional communication ways of these citizen complaints have been employed, like phone calls, e-mails or written instances. Moreover, with the aim to promote new computer technology tools to improve communication between citizens and public administration, the citizen science platform called "Mosquito Alert" (Oltra et al., 2016) was also used as an additional entry point of information about vector status in the city.

Vector monitoring and control in areas of high epidemiological risk. Through the implementation of an all-inclusive and agile protocol coordinated between Epidemiology and Mosquito Control Services, entomological surveillance is quickly conducted around imported cases in viremic period of DEN, ZIKA and CHIK diagnosed in the city. The aim of this axis of the mosquito control campaign is to evaluate the possibilities of virus circulation in local vector populations while lessening the probabilities of autochthonous infections. The results and analysis of this last branch of the mosquito control programme will be the main objective of this paper.

MATERIALS AND METHODS

Epidemiological Issues

According to DENV, ZIKV and CHIKV Surveillance Protocols approved by General Direction for Public Health of the Valencian Community (GVA, 2016) we can distinguish 3 types of arbovirus cases depending on the diagnostic phase:

Suspected case. Person who complies with the clinical criteria (difficult due to non-specific symptoms in most cases) and at least one of the next epidemiological criteria: a) travel history to epidemic/endemic areas inside the range of 15 days before the symptoms appearance, b) spatial-temporal

coincidence in a territory where probable/confirmed cases have been diagnosed or c) non-protected sexual relations with people returned from epidemic/endemic countries inside the range of 28 days upon the arrival if there is a lack of symptoms or up to 6 months if there is a confirmed laboratory diagnostic. This criterion is only assumed in the case of ZIKA. For DEN and CHIK suspected cases can be admitted directly with only clinical criteria.

Probable case. In the case of ZIKA and DEN, person who complies with clinical criteria, with or without epidemiological criteria, but with a laboratory criteria of probable case: a) presence of antibodies IgM and b) seroconversion of antibodies IgG specific for the virus or increase of 4 times the title of the samples taken in acute and convalescent phases. For CHIK is enough with clinical criteria and at least one epidemiological criteria (except sexual transmission).

Confirmed case. Person who complies with clinical criteria, with or without epidemiological criteria, and at least one laboratory criteria of confirmed case: a) viral isolation in a clinical sample, b) detection viral nucleic acid in a clinical sample or c) detection of neutralizing antibodies in serum with positive Ig-M samples.

Once there is an evidence of a confirmed case in the city of Valencia, the Epidemiology department of Public Health Center of Valencia (PHCV) informs the Pest Control section of the Health Service of Valencia (HSV) municipality about the major issues related with the case (patient residence, mobility degree during the viremic period, anamnesis about mosquito bites, etc.) in order to configure an integrated plan for vector surveillance and control in epidemiological risk areas.

Entomological Proceedings

After the communication of the confirmed case, a sequence of tasks is immediately accomplished by HSV:

Delimiting the risk areas to study. Entomological prospections are always done at least at domiciliary and peri-domiciliary level. This is because these areas correspond to the zones where theoretically the contact between patients and vectors is more likely. Of course, this assumption is also affected by numerous factors that should be analysed individually for each case (confinement degree of patient at home, type of edification, vector status in surrounding areas, suitable/unsuitable period of the year for mosquito proliferation, etc.). However, the standardization of entomological monitoring in all patients domiciles is a good basis to homogenize the surveillance proceedings. This delimitation of the study area in the domiciles is articulated in two phases: a) on the one hand, through GIS software a map is quickly created with all the relevant information about mosquito breeding and resting sites surrounding the patient residence. This map includes all public areas in a buffer of 150 meters diameter (based on known and common flight range of *Ae. albopictus*) from the patient's home and it's an essential tool for entomologists and mosquito control technicians to increase the precision and speed in their tasks. When other areas, different from peri-domiciliary, are additionally suspected to be at risk due to patient mobility behaviour during the short viremic phase, like working environment, medical centres for symptoms treatment or disease diagnostic or gardens frequented for recreation, etc., the procedure was the same. b) On the other hand, interviews and entomological surveys with patients are arranged in their homes thus to evaluate the activity of vectors at intra-domiciliary scale. Health municipal inspectors take this opportunity of close contact among citizens to promote citizen awareness actions in the neighbourhoods through the distribution of informative brochures about mosquito prevention in domestic environments.

Entomological survey. In all identifiable breeding, mainly catch basins but also occasionally ornamental fountains or other small containers, and resting (catch basins and adequate vegetation masses) sites mosquito samplings are fulfilled. Immature stages are collected by dipping method, while two kinds of entomological aspirators are used for adult captures (the superior heavy duty hand-held aspirators BioQuip® for sampling small and well identifiable resting units, as well as the backpack aspirators BioQuip® for quick collections in big areas like hedges and other vegetation masses of high dimensions). Only if high densities of vectors have been detected or a factor that can mask the real activity

of mosquitoes during the sampling is supposed, then traps are installed in the risk areas to monitor the situation for 48-72 hour period. Those specific traps for urban aedine mosquitoes are the BG-Sentinel® for adult collection and ovitrap (small plastic container with 350 ml of distilled water and a wood tablet partially submerged) for egg catching. This kind of passive monitoring tool is particularly interesting at intra-domiciliary level, where usually the information about vector activity is scanty because of the limits related to identifying breeding and resting sites.

Mosquito control strategies. In order to minimize the potential development of competent vectors surrounding the domiciles of imported DEN, ZIKA and CHIK cases, all small water bodies (mainly catch basins) are treated with mosquito specific larvicides. Although entomological survey will give us the precise information about real transmission risks, we apply a preventive premise to block the mosquito development in all the vulnerable areas for mosquito breeding. After this initial inspection/treatment, weekly entomological reviews are implemented in the risk areas. The case is considered as closed and no more environmental interventions are done once two consecutive negative revisions are accomplished. Consequently surveillance of the risk areas is always maintained at least during 3 weeks (initial prospection plus two further weekly revisions). Two different types of biocides have been employed following doses and other use specifications provided by the manufacturers: Vectomax FG® (bacterial larvicide most commonly employed) and Device TB2® (Insect Growth Regulator based on diflubenzuron specifically indicated for high organic charge of water and/or prevalence of late and non-feeding immature stages like fourth instar larvae and pupae). In exceptional cases, where high densities of *Ae. albopictus* have been detected, localized adulticides treatments are done with Deflow Plus® (combination of deltamethrin and diflubenzuron).

Laboratory tasks. All entomologic material collected was processed in laboratory conditions for mosquito species and sexes identification. The final goal was to separate and preserve the blood-fed females of *Ae. albopictus* collected in the risk areas in order to identify hypothetical arbovirus presence in further analyse with molecular techniques.

RESULTS AND DISCUSSION

A total of 7 confirmed cases of arboviruses (4 ZIKA, 2 DEN and 1 CHIK) have been diagnosed in Valencia during 2016 (Table 1). Asian tiger mosquito activity was only identified in one case, detecting two females of the species resting in the outside structure of the building where the ZIKA viremic patient lived and other three males in surrounding catch basins. Globally, 1,042 catch basins have been prospected during the first environmental interventions related with arbovirus cases, being the activity of mosquitoes evidenced in a 7% of these potential breeding sites. Main species collected were precisely the two most common urban mosquitoes in Mediterranean environments: *Culex pipiens* (vector of other potential urban mosquito-borne diseases like West Nile Virus or Dirofilariosis) and *Culiseta longiareolata* (ornitophylic species). Regarding to *Ae. albopictus*, the species was only detected in the 0.003% of the prospected breeding sites. After first treatment, all subsequent weekly entomological revisions were always negative and consequently the "arbovirus cases" have been closed in the shortest period according to protocol (two weeks). It is important to note that at intra-domiciliary level, fortunately, no evidence of mosquito activity was detected and the vulnerability to mosquito proliferation of those homes was considered very low (flats in raised floors without small stagnant water bodies in terraces or inner courtyards). Other relevant data regarding the municipal Mosquito Control Programme conducted in 2016 is that 98 of 375 (26%) citizen complaints about mosquito presence were related with *Ae. albopictus* activity after the entomological surveys. None of these complaints were spatio-temporal overlapped with areas of epidemiological risk (arbovirus attendance). On another hand, only 14% of the complaints linked to Asian tiger mosquito activity overlapped with ATMRP, where monthly preventive control measures were accomplished in catch basins (Figure 1). This statistic is even more enhanced by the fact that all overlaps were not associated with *Ae. albopictus* development in catch basins and other regularly monitored structures, since all these complaints coinciding with ATMRP were linked to mosquito proliferation in temporary containers, mainly abandoned ones, as well as water filled vegetable cavities or private breeding sites.

Figure 1. Map with confirmed *Ae. albopictus* activity points (positive citizen complaints), ATMRPs and 7 confirmed arbovirus cases diagnosed in Valencia during 2016. *Reference hospitals for arbovirus diagnostic are typified with "H".*

One major concern, that should be emphasized, is that case interventions should not wait until arbovirus verification, because this provokes a notable delay in the entomological risk evaluation. As we can observe in the data of 2016, only two of seven confirmed cases were communicated to HSV inside the viremic phase of patients. Being able to carry out entomological prospections during the viremic period is essential to estimate the risk of disease transmission. According to this, in 2017 the Protocol has been modified and entomological prospection of cases has been introduced even for low level disease confirmation, which is the suspected case. Although this will suppose an increased effort in surveillance and control tasks, we strongly believe that this preventive measure is highly recommendable in order to maximize the possibilities to act inside viremic periods. Following with the review of 2016 data, besides the 7 confirmed cases another 23 cases were notified as probable cases (no case remained as suspected case, so all patients with symptoms and travel history to endemic countries had at least one serologic confirmation regarding to the laboratory criteria of probable case). Finally, at global geo-epidemiological level we can highlight that 6 of 7 imported cases of arboviruses were imported from Latin American countries, where these diseases showed a marked epidemic tendency in 2016. Our country has a major commercial, touristic and cultural entailment with this territory of the American Continent, and subsequently this is one of the most important explanations for the tendencies observed and to some extent a preceding of what can be expect in Spain, as well as other Mediterranean regions, in the framework of vector-borne disease globalization during the next years.

Cases	Virus / origin country	Date	Entomological findings	Control measures	Observations
Case 1*	DEN / Ecuador	Feb	*Cx. pipiens* (L, A) Catch basins analysed: 132 (2% positive).	Larvicides	Notification outside the theoretically favourable period for vector activity (March-November)
Case 2	ZIKA / Bolivia	Apr	*Cx. pipiens* (L, A) and *Cs. longiareolata* (L, A). Catch basins analysed: 206 (5% positive).	Larvicides and removing breeding sites	Additional risk area: school (patient was a student who went to school during viremic period).
Case 3	ZIKA / Bolivia	May	*Cx. pipiens* (L, A) and *Cs. longiareolata* (L, A). Catch basins analysed: 71 (7% positive).	Larvicides	*None particular situation to remark*
Case 4	DEN / Tahiland	Jul	Catch basins analysed: 157 (26% positive).	Larvicides	*No particular situation to remark*
Case 5	ZIKA / Colombia	Aug	*Cx. pipiens* (L, A) and *Cs. longiareolata* (L, A). Catch basins analysed: 101 (18% positive).	Larvicides	*No particular situation to remark*
Case 6	CHIK / Bolivia	Sep	*Cx. pipiens* (L, A) and *Cs. longiareolata* (L, A). Catch basins analysed: 264 (3% positive).	Larvicides	Additional risk areas: 2 hospitals (patient was in the urgencies units of both hospitals during viremic period).
Case 7*	ZIKA / Mexico	Oct	*Cx. pipiens* (A) and *Ae. albopictus* (A**). Catch basins analysed: 111 (3% positive).	Larvides and adulticides (local spraying in catch basins)	Additional risk area: hospital (patient was a sanitary professional who was working in the hospital during viremic period).

Table 1. Confirmed cases of DEN, ZIKA and CHIK diagnosed in Valencia during 2016. * *Cases environmentally attended inside the viremic period.* ** *Two females of Ae. albopictus were collected resting outside patient building coinciding with the viremic period.*

CONCLUSIONS

Main conclusions can be summarized as follows:

1) A strong coordination between epidemiologists from PHCV and entomologist from HSV is essential to improve the quality of vector-borne risk assessment. Quick communication (interventions inside the viremic period) and meticulous anamnesis (identification of other risk areas different from domiciles) are basic.

2) An exhaustive entomological monitoring in the risk areas provides crucial information, not only for assessment regarding disease transmission but also for establishment of an effective and rational Mosquito Control Programme.

3) Beyond the entomological actions quickly carried out after the notification of arbovirus cases, a global strategy of vector surveillance and control based on the identification of the most vulnerable areas for *Ae. albopictus* proliferation should be implemented. These ATMRP are also essential to minimize the potential impact of these diseases, because we strongly believe that these arboviruses are clearly infra-diagnosed due the frequent clinical asymptomatism. Recent researches indicate that people who are infected with arbovirus like DENV without developing detectable clinical symptoms are also infectious to mosquitoes (Duong et al., 2015). Even at a given level of viremia, symptom-free people were more infectious to mosquitoes than clinically symptomatic patients. This conjuncture could change the current paradigm for arbovirus epidemiology and control in non-endemic countries, based on quickly detection of imported cases with apparent illness and entomological actions surrounding the risk affected areas.

4) According to the experience in 2016, several modifications and new ideas will be applied in the surveillance and control programme: a) As has been commented before, the disease verification level to start with the communication circuit should be at the stage of probable case; b) Thus, to complete a real risk assessment, a proceeding of quick viral identification in vectors collected in the risk areas must be accomplished; c) After two years of *Ae. albopictus* establishment and acknowledging that the species is now homogeneously distributed in the whole territory of the municipality (activity evidenced in all urban districts in 2016), the implementation of GIS based spatial analysis will be increased to improve the detection of potential *Ae. albopictus* proliferation hot spots.

The combination of high precision normalized difference vegetation index (NDVI), shade models and humidity-temperature interpolation will help us to identify suitable *Ae. albopictus* resting areas in the city and the analysis of catch basin information layers (typology, degree of water storage, density by square kilometre, etc.) will be useful to determinate the most suitable territories for breeding in public environments. The overlap level of both data sources will be decisive to select the ATMRP in 2017.

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