Acta Tropica 128 (2013) 680–685

Contents lists available at ScienceDirect

Acta Tropica

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

Imperviousness as a predictor for infestation levels of container-breeding mosquitoes in a focus of dengue and Saint Louis encephalitis in Argentina

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ARTICLE INFO

Article history:
Received 1 July 2013
Received in revised form
12 September 2013
Accepted 14 September 2013
Available online 23 September 2013

Keywords:
Aedes aegypti
Culex pipiens
Urbanization
Vector-borne diseases
Generalized linear models

ABSTRACT

Dengue and Saint Louis encephalitis virus are among the most important emerging viruses transmitted by mosquitoes at the global scale, and from 2009 onward both diseases have reached temperate Argentina. To test whether the urbanization level can be used as a predictor for the infestation levels of container-breeding mosquito vectors, we searched for *Aedes aegypti* and *Culex pipiens* in 8400 water-filled containers from 14 cemeteries of Buenos Aires Province and we used generalized linear models to relate positive containers with the impervious area quantified inside (internal PIA) and outside (external PIA) cemeteries. The best model for *Ae. aegypti* explained 91% of the variability and included the season, the internal PIA and the external PIA at 1 km as a quadratic function, showing a parabolic response peaking in ~75%. Regarding the infestation levels of *Cx. pipiens*, the final model explained 75% of the variability and included only the season. In view of these results, the percentage of impervious area efficiently predicted the infestation levels of *Ae. aegypti* but not of *Cx. pipiens*. Considering the worldwide relevance of the former in dengue transmission, the simple quantification of imperviousness proposed herein provides a helpful basis for vector surveillance and control in urbanized areas.

1. Introduction

Dengue and Saint Louis encephalitis virus (SLEV) are among the most important emerging virus transmitted by mosquitoes at the global scale (Solomon and Mallewa, 2001). The incidence of dengue has grown dramatically around the world in recent decades, with over 40% of the world's population at risk and around 50–100 million dengue infections every year (WHO, 2012). On the contrary, SLEV cases are usually detected in small numbers; in the USA, since the outbreak of >2500 cases in 1975–1977, approximately 55 cases are reported per year (Ciota et al., 2011).

In Argentina, dengue reemergence occurred in 1997 transmitted by the mosquito *Aedes aegypti*. During the following decade, at least 4700 cases were registered (Vezzani and Carbajo, 2008) and in the 2009 epidemic more than 25,000 cases were recorded (MSN, 2009). Regarding SLEV, human cases have been sporadically reported since the first detection in 1964 (Spinsanti et al., 2003) until a large human encephalitis outbreak including nine deaths was recorded in 2005 in Cordoba Province (Spinsanti et al., 2008). Argentine strains of the *Culex pipiens* complex were demonstrated to be an efficient vector of SLEV in experimental studies (Mitchell et al., 1980) and were also found infected with the virus in nature (Mitchell et al., 1985). More recently, two genotypes of SLEV were isolated from members of the complex in the center of the country (Díaz et al., 2006).

During the last years, the transmission of dengue and SLEV has extended toward the south in South America. In the summer of 2009, the first dengue outbreak occurred in the Federal District of Argentina (latitude 34°36′ S) and its surrounding municipalities in Buenos Aires Province, accounting for 105 autochthonous confirmed cases (Seijo et al., 2009). Also during 2009–2010, the first autochthonous cases of human SLE were confirmed in the area (López et al., 2010). The vectors of these viruses, *Ae. aegypti* and *Cx. pipiens* complex, have been recorded as the most abundant mosquito species in urbanized areas of the region, both in surveys of immatures from different types of containers (Rubio et al., 2011; Vezzani and Albicócco, 2009) and in adult collections (Vezzani et al., 2006).

Human activities and their impact on local ecology have been highlighted as one of the main drivers of the prevalence or

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http://dx.doi.org/10.1016/j.actatropica.2013.09.015

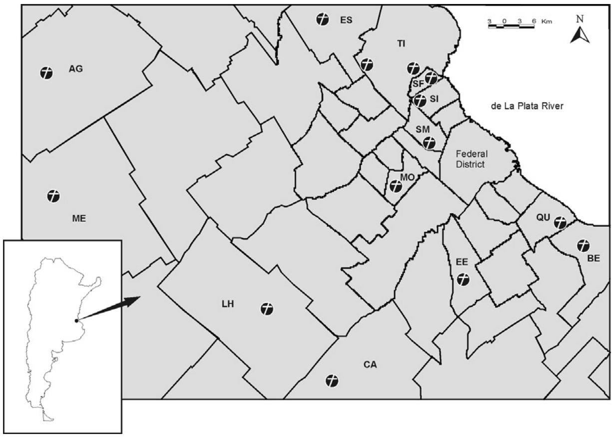

Fig. 1. Geographic location of the studied cemeteries in Buenos Aires Province (Argentina). The districts involved in the study and their total populations (INDEC, 2010) were AG (San Andrés de Giles, 22,257 inh), ME (Mercedes, 62,807), LH (Las Heras, 14,889), CA (Cañuelas, 50,526), EE (Esteban Echeverría, 298,814), ES (Escobar, 210,084), TI (Tigre, 380,709), SF (San Fernando, 163,462), SI (San Isidro, 291,608), SM (San Martín, 422,830), MO (Morón, 319,934), QU (Quilmes, 580,829), and BE (Berazategui, 320,224).

distribution range of dengue and other mosquito-borne diseases (Gubler, 2010; Mackenzie et al., 2004; Wilder-Smith and Gubler, 2008). Imperviousness is one of the primary characteristics of the urbanization process, affecting urban climate and landscape patterns. The percentage of impervious area, represented by paved roads and concrete structures, has been widely used to quantify imperviousness in urban landscape gradients (McDonnell and Hahs, 2008). Impervious areas within cities could be unsuitable for mosquitoes due to the scarcity of vegetation, high temperature and low humidity. In contrast, intermediate levels of imperviousness could be related with a high density of water-filled containers, promoting the proliferation of many urban mosquito species. Although there is no consensus about the effect of different degrees or types of urbanization on dengue transmission [e.g. Barbazan et al. (2000) versus Honório et al. (2009)], the effects of urbanization on the distribution and abundance of dengue vectors have been previously recognized (e.g. Braks et al., 2003; Rubio et al., 2011). Here, we investigated whether the urbanization level can be used as a predictor for the infestation levels of *Ae. aegypti* and *Cx. pipiens* immatures. With this aim, we used generalized linear models to assess the association between flower vases harboring mosquitoes in cemeteries and the impervious area quantified inside and up to 3 km surrounding these urban patches.

2. Materials and methods

2.1. Study area

Buenos Aires Province has a temperate climate with annual mean temperature averaging 14–17 °C and annual precipitation ranging from 600 to 1200 mm. The study area embraces the greatest megalopolis of Argentina, namely Greater Buenos Aires (GBA), and four neighboring rural districts located approximately at 100 km, namely Cañuelas (CA), San Andres de Giles (AG), General Las Heras (LH) and Mercedes (ME) (Fig. 1). The GBA covers 3827 km^2 and has the greater population density of Argentina (335 inh/ha); the four rural districts have less than 1 inhabitant per hectare (INDEC, 2010).

The surveys were conducted in 14 municipal cemeteries with a surface between 3.8 and 25.8 ha (mean 10), located at least 3-km apart (Fig. 1). Internally, cemeteries have two main patch types related to burial traditions, i.e. graves and mausoleums. The former are located in open and vegetated areas whereas the latter are characterized by high coverage of impervious surface and scarce or no vegetation cover (Vezzani, 2007).

2.2. Data collection

Mosquito samples were collected during spring (October 2007), summer (January 2008) and autumn (April 2008). In each cemetery and sampling period, 200 water-filled flower vases were inspected (8400 in total). The number of containers surveyed in grave and mausoleum areas in each cemetery was proportional to the area occupied by each burial type. Containers random selection was based on aleatory points over a grid map of each cemetery and the inspection of up to ten contiguous water-filled flower vases from each point. To collect all immature mosquitoes present in each container, water was filtered with a fine mesh strainer. Larvae were fixed in 70% ethanol and pupae were reared until adult emergence. Third and fourth instar larvae and adults were identified using the dichotomical key of mosquitoes from Buenos Aires Province (Rossi et al., 2002). Two members of the *Cx. pipiens* complex, *Cx. pipiens s.s.* and *Cx. quinquefasciatus*, are sympatric in Buenos Aires (Diez et al., 2012); these species were not distinguished from one another and are here referred to as *Cx. pipiens*.

2.3. Data analysis

Infestation levels of *Ae. aegypti* and *Cx. pipiens* were characterized by means of the number of water-filled containers harboring third and fourth instar larvae and/or pupae. As the sampling effort was equal in each cemetery and month, these values are representative of the Container Index, one of the most widely used measurements in *Ae. aegypti* infestation surveys (Silver, 2008). To verify whether the infestation level reflected mosquito abundance per cemetery, the Spearman rank correlation coefficient between the number of containers with mosquitoes and the number of immatures collected was calculated. Such coefficient makes no assumptions about linearity in the relationship between the two variables (Daniel, 1990).

The percentage of impervious area (PIA) was estimated within cemeteries (internal: I-PIA) and in their surroundings (external: E-PIA). The I-PIA was quantified in each cemetery using Google Earth software 4.3 and further checked by ground proofing. All areas occupied by mausoleums and other edifications (e.g. administrative areas) were considered impervious areas. The E-PIA of each cemetery was quantified using a Landsat 5 TM satellite image (30 m × 30 m resolution) captured in January 22, 2010. A non-supervised classification was performed to identify impervious areas (paved roads and concrete structures) using Erdas Imagine 8.4 software. The E-PIA was calculated in a circle of 1, 2, and 3 km radius (E-PIA$_1$, E-PIA$_2$, and E-PIA$_3$, respectively) around the geometric center of each cemetery using GIS-ArcView 3.2. We assumed that the surface occupied by each cemetery within this circle did not affect the estimation of PIA because it was lower than 8.5% in all cases. In addition, the size of each cemetery and the total population of each district (log transformed, $\log_{pop}$) (INDEC, 2010) were included in the analysis.

Generalized linear mixed models (GLMM) were used to model the infestation levels of both mosquito species. These models allow the use of error distributions other than normal, and the inclusion of random terms (grouping variables) to control for correlations that arise from grouped observations (Paterson and Lello, 2003). In order to model the occurrence of *Ae. aegypti* and *Cx. pipiens* separately, we first tested independency between the presence of both species per cemetery by means of a Chi-squared test and the C_8 coefficient of interspecific association (Hurlbert, 1969). Both analyses showed no association between mosquito species (Yates corrected $X^2 = 0.016$, $p = 0.9$; $C_8 = 0.54$).