# The largest dengue outbreak in Argentina and spatial analyses of dengue cases in relation to a control program in a district with sylvan and urban environments

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## ARTICLE INFO

## ABSTRACT

**Objective:** To analyze the largest outbreak of dengue in Argentina in the municipality of Tigre during 2016, through detailed spatial analyses of the occurrence of cases in relation to demographic factors and vector control actions. **Methods:** Detailed and georreferenced data on dengue cases with laboratory results (NS1 or IgM) were analyzed. The occurrences of imported and autochthonous cases by census tract were modeled using demographic variables (population by age class, proportion of foreigners, proportion with university grade, proportion of males), dwelling variables (number of homes, proportion of dwellings with latrine, number of dwellings, proportion of houses, proportion of flats, proportion of slums) and census tract area as explanatory variables. The probability of occurrence of autochthonous and imported cases was modeled separately. The spatio-temporal occurrence of cases was studied in relation to focal and perifocal control actions (involving education campaigns, removal of *Aedes aegypti* breeding sites and exhaustive insecticide spraying) to assess the efficiency in stopping autochthonous cases spreading. **Results:** All autochthonous cases occurred in the urban environment with no sylvan cases. The majority of the imported cases registered came from Paraguay and Northeastern Argentina. The age structure of imported and autochthonous cases did not differ from the age structure of the municipality, while that of the negative cases did. When studied spatially, the occurrence of imported cases by census tract was mildly associated with a higher proportion of foreign population and more people at active age, while occurrence of autochthonous cases was not significantly associated with any of the studied variables. For census tracts with laboratory confirmed results, the models showed higher probability of autochthonous cases related to higher population density and population age structure. The clustering of autochthonous cases was generally mild, with prevailing isolated cases and a weak spread inside the municipality. The biggest outbreak focus was associated with a delay in the focal vector control. **Conclusions:** Results confirmed the virus pressure coming from neighboring countries and related to population movement by workers. All autochthonous cases occurred in the urban environment with no sylvan cases. The susceptibility of residents to dengue may be similar among age classes and the laboratory tests were performed more frequently in the younger. Autochthonous cases incidence was low and spatio-temporal clustering of cases weak, suggesting that control measures were effective when no delay occurred in their application.

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# 1. Introduction

Both the incidence and the geographic spread of dengue are growing worldwide[1]. This mosquito-borne viral disease, whose main vector is *Aedes aegypti* (*Ae. aegypti*) but can also be transmitted by *Aedes albopictus*, is present in 128 countries and regions where 3.97 billion people live at risk[2]. During the last decades, the pattern of epidemics with long intervals, typical of the Americas, has shifted to persistent outbreaks and the occurrence of hyperendemicity in most countries[3]. Health status in this region has become alarming since the recent introduction of chikungunya[4] and Zika[5] and the increasing outbreaks of yellow fever[6], all of which are transmitted by the same vectors[7]. This casts doubts on the efficiency of the usual strategies of vector control implemented so far.

In Argentina, dengue is non endemic, but is maintained by annual outbreaks of varying magnitude and geographic location[8]. During the first decade since the re-emergency of dengue, in 1998, 3 541 autochthonous confirmed cases of dengue caused by serotypes 1, 2 and 3 were registered in outbreaks circumscribed to the northern provinces in the frontier with Bolivia, Brazil and Paraguay[9]. In the last seven years, dengue expanded toward southernmost provinces, reaching the metropolitan area of Buenos Aires, and accounting for 74 549 autochthonous confirmed cases of the four serotypes; the two major outbreaks occurred in 2009 and 2016 with 26 923 and 41 207 cases respectively[10]. Immigrants and tourists are the key carriers of dengue into nearby northern provinces[11] and large urban areas in Argentina[8,12-14]. In the latter, a strong association between high incidence of dengue and large influx of travelers and high population density has been demonstrated[11,13]. Previous studies have shown age dependent population susceptibility to dengue. Age classes that travel more for occupational or leisure activities present higher risks of contracting dengue than children or older adults[15,16]. The study of the spatial association of demographic factors with the autochthonous and imported cases is crucial to understanding the epidemiology of dengue in non-endemic areas and is an indispensable tool for focusing on the prevention and control of outbreaks in critical areas[17].

A promising dengue vaccine has been recently licensed in several endemic countries in Asia and Latin America[18,19]. Nevertheless, its efficacy trials have reported partial protection levels and therefore control efforts against dengue transmission are still targeted on the elimination of immature or adult mosquito vectors or interrupting the human-vector contact[3]. In situations of active dengue transmission, when social mobilization, breeding site reductions and other primary control approaches are insufficient or have failed to reduce the vector population below critical thresholds, chemical control is implemented through perifocal spraying in areas of high transmission risk where dengue cases are detected[20,21]. This methodology employs both larvicide and adulticide in and around the residence of the patient, up to 400 m from it[20]. A quick response whenever a new case is detected is essential to stop the spread to wider areas due to both vector dispersal and people movements.

Evidence on efficacy of spraying in reducing *Aedes* populations has been summarized by Esu *et al*[21], Pilger *et al*[22] and Bonds[23], who found that there is very poor empirical support for recommending this method as an effective control intervention when not applied in the framework of an integrated control strategy. Although significant reductions in entomological indices (*e.g.* ovipositioning rate, House-Index, Breteau-Index, Container-Index) were achieved, this was insufficient to assume a decrease in disease transmission[22]. Direct effects of vector control against disease transmission have been poorly documented, thus the association between control actions and dengue outcomes is an area of active investigation[14,24].

The objective of this study was to analyze the dengue outbreak that took place in Tigre municipality during 2016 in relation to the ongoing control program and demographic factors. This is one of the southernmost municipalities with dengue cases in Argentina and part of the most densely populated area of the country. Routine control interventions were carried out in this area and registered along with the location of dengue cases in a geographic information system, which allow us to conduct a detailed spatio-temporal study of dengue cases.

# 2. Materials and methods

Tigre municipality has a population of 381 000 inhabitants and is located 32 km north of Buenos Aires City (Figure 1). It comprises 148 km$^2$ of mainland and 220 km$^2$ of island belonging to the lower delta of the Paraná River. The delta sector is a complex environment of islands and streams where original vegetation has been almost totally replaced by human plantations[25]. Although population density is less than one person per hectare, the delta is a recreational area highly visited during weekends[26]. The mainland area is the forced passage for those tourists and is part of a continuous urbanized matrix surrounding the capital city of the country, with around 12 million inhabitants[27].
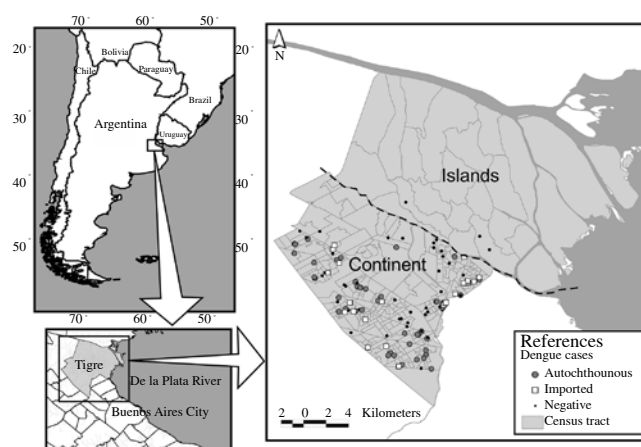


**Figure 1**. Study sites and dengue cases location.

Since 2009, the Secretaría de Política Sanitaria y Desarrollo

Humano of Tigre municipality has implemented a control program based on the early detection of dengue suspected cases reported by private and public health institutions, medical professionals and individuals, either personally or by telephone. In 2013, a special outpost at the Tigre bus station was set and was open during weekdays. The outpost consisted on a stand with a thermal scanner for detecting febrile passengers, plus a team of specialists who provided information about dengue and personal care kits. A suspected case was defined as any person with febrile illness without upper airways affection who had traveled to an area with transmission of dengue within 14 d previous to the onset of symptoms. A suspected case was also any person showing headache and at least two of the following symptoms: retro-ocular pain, muscle and joint pain, rash and minor bleeding phenomena, anorexia, nausea, leukopenia, thrombocytopenia, diarrhea and vomiting. All suspected cases with residence in the municipality, detected by the local control program or by other municipalities, were reported to the Secretaría de Política Sanitaria y Desarrollo Humano through a notification file with information of the patient and a request for serologic analyses. The reports were received by e-mail, telephone or physically and included basic information like name, address, symptoms, onset of fever and, recent travels. They were recorded in a geographic information system at the same day of report and subsequently updated with laboratory results and information related to the evolution of the patient. The suspected cases were contacted by phone and, if they had not received medical treatment already, were appointed for a blood sample in a medical center as soon as possible or medical staff was sent for a home visit. In this latter case the staff decided if laboratory blood diagnosis and hospitalization were necessary. A personal care kit with repellent, bed net and information on prevention was given. Blood samples were sent for laboratory analyses to the provincial reference laboratory (Región Sanitaria V, Ministerio de Salud de la Provincia de Buenos Aires). Samples taken up to 4 d before the onset of symptoms were tested by NS1 and from day 5 onwards by IgM. A second sample was regularly required to all patients. Results of the laboratory analyses were communicated to patients by telephone or through the corresponding health structure that had reported the case. As a second blood sample result was rarely obtained, cases with at least one laboratory result were considered as confirmed. Positive cases who had traveled during the 14 d before the onset of symptoms to endemic regions or areas with transmission going on were classified as imported and the rest as autochthonous.

Focal and perifocal control treatments were instructed centered in the suspected case residence the day after the report without waiting for laboratory results. They were planned according to the number and location of other cases, the day of onset of symptoms and the date of the last focal and perifocal control treatments in the area. If the weather was rainy, or no person could be present at the case house, the treatment was postponed. The focal and perifocal control treatments was fulfilled by private company personnel accompanied by municipality personnel. It included the exhaustive spraying with a solution of 20 mL *cis*-Permethrin DEPE ® EC 10%

a.i. (Chemotecnica S.A., Argentina), 30 mL polyethylenglycol and 1 000 mL water. The suspected case residence and several premises around it up to 100 m (as allowed by the neighbors) were sprayed with a backpack machine. All breeding places for *Ae. aegypti* were removed when possible. The inhabitants were queried about other febrile people and given repellent and information about *Ae. aegypti*. Neighboring houses up to 300 m of the suspected case were treated from the street with vehicle mounted ultra low volume motor sprayer. The information recorded during the focal and perifocal control treatments included date of treatment, addresses visited, streets sprayed and new suspected cases. Data used in the present study about the focal and perifocal control treatments included address, date of application, ancillary addresses treated and streets sprayed. Data about patients included address, age, date of onset of symptoms, travel destination during the last 14 d to areas with transmission in progress and dengue laboratory results: positive (imported or autochthonous) and negative. Until 2015 all cases were imported, therefore we chose to analyze 2016 data, which represented the first epidemic in the municipality. This study assumes that the vector abundance necessary for autochthonous transmission of dengue was given. The first and last imported cases occurred on January 4 and March 29, while the autochthonous cases were from January 12 to April 22, January to March is the period with highest adult activity of *Ae. aegypti* in the study area[28].

Cases were tabulated by origin of travel and positivity. The frequency of imported, autochthonous and negative cases by age was compared to the population age structure with *Chi* square tests of frequencies.

## 2.1. Clusters of dengue cases

The chronological occurrence of cases was studied in relation to the application of focal and perifocal control treatments. Individual cases were studied by date of onset of symptoms and location to evaluate the efficiency of focal and perifocal control treatments. Each focal and perifocal control treatment was mapped in the geographic information system with a polygon around the treated houses and lines along sprayed streets. Each positive case was followed chronologically to check if autochthonous cases occurred afterwards up to 150 m away. Taking into account the squared urbanization of the area with 100 m × 100 m blocks, this distance was chosen to comprise cases in the block of the original case plus the 8 blocks around it. The focal and perifocal control treatments were also followed chronologically to register the later occurrence of autochthonous cases inside of them, in which case they were considered unsuccessful. The laboratory results were taken into account, to assess whether the focal and perifocal control treatments were carried out over negative or positive cases and if there were autochthonous cases after the treatment or not. All the cases that were included in the focal and perifocal control treatments area were considered treated cases. We did also identify untreated positive cases. This procedure also helped to visualize different clusters of cases beyond the 150 m.

To characterize the spreading of the autochthonous cases and determine the presence of transmission foci, each positive imported or autochthonous case was identified in each cluster and considered an origin. Then, the distance and time in days until the following autochthonous cases in the cluster were recorded. Initially cases closer than 150 m and between 12 and 28 d were considered to have spread from the corresponding origin. Every subsequent imported or autochthonous case was considered a new potential origin provided that a minimum period of 12 d had passed from the previous origin. We used 12 d as the minimum possible time required for spreading, because the estimated duration of the extrinsic incubation period in the area in January is at least 10 d, assuming the mosquito bites just before the beginning of symptoms and adding 2 d as a very permissive minimum time required for the next infected person to develop symptoms. On the other hand, the maximum of 28 d corresponded to an infected person theoretically bitten at the 6th day of viremia, 20 d of extrinsic incubation period plus mosquito survival and a delay of 2 more days for the onset of symptoms in the next infected person. The parameters were selected in a conservative way because the area is close to the southern limit of transmission, and weak transmission conditions are expected. After an initial calculation, the time and distance intervals were broadened to assess the sensibility of the procedure (*i.e.* check if changing the intervals considerably affects the number of transmission foci identified). In this way we obtained the explicit autochthonous cases that could have been derived from other nearby cases and the clusters where this happened.

## 2.2. Models for dengue cases

To model the occurrence of cases we used demographic information in digital format by census tract, the minimum available spatial unit[29]. It included inhabitant and dwelling related variables. Inhabitants related variables: population by age class (4 years old, 5-14, 15-44, 45-69, 70 and more), proportion of foreigners, proportion with university grade, proportion of males. Dwelling related variables: number of homes (families), proportion of dwellings with latrine, number of dwellings, proportion of houses, proportion of flats, proportion of slums. All these variables plus the census tract area were used as explanatory variables to separately model the occurrence of imported and autochthonous cases. Generalized linear models were fitted using a maximum likelihood method[30], assuming a binomial distribution of errors and applying the logistic function as a link between the response variable and the linear predictor. The response variable presence-absence of cases was modeled for imported and autochthonous cases considering all the municipality census tracts. Separately, the probability of autochthonous and imported cases (No. cases / total population) was modeled for the subset of census tracts with laboratory confirmed results. The goodness-of-fit was evaluated in terms of the Akaike's information criterion (AIC)[31]; the model that yielded the lowest AIC was selected from all possible models[32]. Models with $\triangle AIC \leqslant 2$ were considered equivalent. We performed a manual stepwise forward procedure to select those explanatory variables significantly associated with each of the response variables. Centered explanatory variables were added one by one, and kept if the AIC was reduced more than 2[32]. Squared variables and two way interactions (when appropriate) were also tested. To discard collinear explanatory variables, the variance inflation factors[33] were calculated after each step, and the variable removed if its variance inflation factor value was higher than 5[32]. The procedure was stopped when no further step could significantly reduce the AIC, obtaining a full model without redundancy among explanatory variables. For binomial models, output variables (predicted values) lie between 0 and 1. Instead of using a threshold probability of 0.5 (*i.e.* a fixed cut-off of $P$=0.5) for assigning presence or absence, we tried all possible cut-off points from $P$=0.01 to $P$=0.99 to select an alternative cut-off point that maximized the classification effectiveness of the model. This was evaluated using the Kappa index to assess improvement of classification of the model over chance[34]. For the probability models, the percentage of explained deviance was used as an index of explained variance. All analyses were performed using the open-source software R 3.2.3 with lme4 and car packages[35].

## 2.3. Ethical approval and informed consent

The study was approved by the Departamento de Medicina Preventiva of the Secretaría de Política Sanitaria y Desarrollo Humano, Tigre municipality, Buenos Aires, Argentina. Health workers carried out a door-to-door visit trough the village to inform about the objectives of the project and control activities; heads of households were consulted for authorization to carry out the control actions.

## 3. Results

Out of a total of 132 dengue cases with at least one laboratory result in Tigre municipality during the 2016 outbreak, 83 (62.9%) were positive and 49 (37.1%) negative. Of the positive cases 36 were autochthonous and 47 imported (27.3% and 35.6% of the total cases respectively). All positive cases occurred in the continental part of the district, and there were only 3 negative cases in the islands. There were more positive than negative cases from Paraguay and the northeastern provinces of Argentina, which border that country (Table 1). On the contrary there were more negative than positive cases from Brazil and the northwestern provinces bordering with Bolivia. The frequency of negative cases by age class did not fit the municipality age structure, but autochthonous and imported cases did (Table 2). There were more negative cases than expected in the 0-14 years old interval.

**Table 1**

Suspected dengue cases by travel destination and laboratory result.

| Regions | Negative | Positive |
|---|---|---|
| Paraguay | 1 | 26 |
| Brazil | 6 | 3 |
| Northeastern Argentina | 3 | 18 |
| Northwestern Argentina | 1 | 0 |
| Central Argentina | 2 | 0 |
| No travel | 36 | 36 |
| Total | 49 | 83 |

Dengue cases frequency and total population by age class [$n(\%)$].

| Age (years old) | Autochthonous ($n$=36) | Imported ($n$=47) | Negative[***] ($n$=49) | Total population ($n$=376 381) |
|---|---|---|---|---|
| 0-14 | 12 (33) | 8 (17) | 26 (53) | 103 334 (27) |
| 15-44 | 18 (50) | 28 (60) | 20 (41) | 173 476 (46) |
| 45+ | 6 (17) | 11 (23) | 3 (6) | 99 571 (26) |

[***] $P<0.001$ *Chi* square test against total district population.

### 3.1. Clusters of dengue cases

A total of 47 focal and perifocal control treatments were implemented around suspected dengue cases. Afterwards, laboratory tests confirmed that 10 interventions were applied on negative cases and 37 on positive dengue cases. Of the latter, two presented autochthonous cases after the treatment, while 35 did not (Table 3). It is noteworthy that while the median delay from the onset of symptoms to the focal treatment was 6 d, the delay for the two unsuccessful treatments was 7 and 16 d (Table 3). Other 16 suspected cases that were untreated did not result in subsequent autochthonous cases nearby.

**Table 3**

Delay in the focal and perifocal control treatments application since the onset of symptoms of each suspected case. Potential spread of autochthonous cases inside each cluster under three different intervals of time and distance between cases.

| Cluster | FPCTs | Delay in days+ | | Autochthonous cases ($n$) | | |
|---|---|---|---|---|---|---|
| | | Positive case ($n$) | Negative case ($n$) | 12-28 d 150 m | 8-28 d 150 m | 12-38 d 320 m |
| 1 | 7 | 3, 5, 6, 8, 9, *7,16* | - | 10 | 11 | 11 |
| 2 | 6 | 2, 2, 6, 8 | 1, 11 | 0 | 0 | 2 |
| 3 | 4 | 1, 2, 8 | 9 | 0 | 0 | 0 |
| 4 | 3 | 2, 4 | 9 | 0 | 0 | 0 |
| 5 | 3 | 5 | 5, 6 | 0 | 0 | 0 |
| 6 | 3 | 5, 9, 11 | - | 0 | 0 | 0 |
| 7 | 2 | 2, 11 | - | 0 | 1 | 1 |
| 8 | 2 | 2, 6 | - | 0 | 0 | 1 |
| 9 | 2 | 6 | 3 | 0 | 0 | 0 |
| 10 | 2 | 9, 10 | - | 0 | 0 | 0 |
| 11 | 2 | 10, 10 | - | 0 | 0 | 0 |
| 12 | 1 | 3 | - | 0 | 0 | 1 |
| 13 | 1 | 3 | - | 0 | 0 | 1 |
| 14 | 1 | 10 | - | 0 | 1 | 1 |
| 15 | 1 | 1 | - | 0 | 0 | 0 |
| 16 | 1 | 2 | - | 0 | 0 | 0 |
| 17 | 1 | 4 | - | 0 | 0 | 0 |
| 18 | 1 | 7 | - | 0 | 0 | 0 |
| 19 | 1 | 18 | - | 0 | 0 | 0 |
| 20 | 1 | - | 6 | 0 | 0 | 0 |
| 21 | 1 | - | 7 | 0 | 0 | 0 |
| 22 | 1 | - | 23 | 0 | 0 | 0 |

FPCT: focal and perifocal control treatments; + Each number separated by a colon indicates the delay in days of one FPCT. They are separated in two columns according to the laboratory result (positive or negative) of the suspected case that originated the treatment. Italics indicate the FPCTs whereautochthonous cases occurred after the treatment.

Thirty clusters of positive cases were identified with distances between cases up to 700 m from each other. While 8 clusters resulted untreated 22 received between 1 and 7 focal and perifocal control treatments (Table 3). Among the 36 autochthonous cases two lacked address information so that they could not be included. The most important cluster presented 11 autochthonous cases, followed by a cluster with 3 cases and 5 with 2 cases each. The remaining 8 cases were isolated. Considering an interval of up to 150 m and 12 to 28 d among cases, the number of autochthonous cases that could have been transmitted successively inside a cluster forming a transmission focus would be 10, and in one unique focus (Table 3). If the temporal interval is extended to 8-28 d, 3 more autochthonous cases are added, one to the previous focus and the rest isolated. If the interval is up to 320 m and 12-38 d, 18 cases appear as possible, 1 focus with 11 cases, another with 2 and 5 isolated cases. It was in the focus with 11 cases where autochthonous cases occurred inside focal and perifocal control treatments after their application.

### 3.2. Models for dengue cases

From the 320 census tracts of Tigre municipality, 56 presented positive laboratory results and 32 negative while the remaining 232 did not present laboratory confirmed cases. There were 16 census tracts with only autochthonous cases, 34 with only imported cases and 6 with both types of positive cases. There were no positive records in the 22 tracts from the islands, and as they are very sparsely inhabited (5 500 inhabitants), they were excluded from the modeling. The models give insight on which variables are associated with the presence of positive cases in a tract. Models for imported cases per census tract showed significant variables related to age classes and foreign population (Table 4). Although the models were satisfactory, the classification only improved a random classification by 30% (Kappa index=0.3). No model for autochthonous cases presence was significant.

**Table 4**

Generalized lineal model for the occurrence of imported cases for all census tracts ($n$=298).

| Model | AIC | d.f. | K |
|---|---|---|---|
| Null | 237.03 | 1 | - |
| + Foreign inhabitants | 214.96 | 2 | 0.30 |
| – Proportion of Argentines + Inhabitants 15 to 44 years old | 213.38 | 3 | 0.29 |

Only significant explanatory variables are given. The sign before each explanatory variable indicates the effect on the linear predictor of the response variable (+ positive or –negative). d.f. = degrees of freedom, K = Kappa index.

When only the census tracts with laboratory confirmed results were considered (88 tracts), the models give insight on the variables related to the intensity of transmission. Two models were selected for the probability of a census tract presenting autochthonous cases, explaining between 37% and 49% of the variability in the data. Both included age related variables (less inhabitants of 45 to 69 years old)

and population density indicators (population density or tract area, which are inversely correlated) and no interaction was significant (Table 5). No model for the probability of imported cases was satisfactory.

**Table 5**
Generalized lineal model for the probability of autochthonous cases for census tracts with laboratory confirmed results (*n*=88).

| Model | AIC | d.f. | E.D. |
|---|---|---|---|
| Null | 164.5 | 1 | - |
| – Census tract area – Inhabitants 45 to 69 years old | 131.6 | 3 | 0.37 |
| + Population density – Inhabitants 45 to 69 years old + Inhabitants >70 or older – Interaction (Population density & Inhabitants 70 or older) | 121.4 | 5 | 0.49 |

Only significant explanatory variables are given. The sign before each explanatory variable indicates the effect on the linear predictor of the response variable (+ positive or –negative). d.f. = degrees of freedom. E.D. = proportion of explained deviance.

## 4. Discussion

The dramatic increase in the occurrence of dengue in the last years forces to question the effectiveness of the control strategies taken to avoid its transmission. This research examined dengue cases detected in Tigre municipality during 2016, more severe outbreak occurred in Argentina, focusing on control actions and demographic factors as determinants of transmission dynamics.

As in other regions where dengue is not an endemic disease, neighboring countries of Argentina play a key role in virus introduction and outbreaks occurrence[36]. Generally, the sources of dengue virus have been Bolivia, Brazil and Paraguay[12,14]. Most imported cases registered in this study came mainly from Paraguay and Northeastern Argentina, in accordance with the epidemics occurring in the neighboring country which later spread to bordering Argentina provinces. In Tigre municipality, where there are no airports, arrival of suspected cases of dengue from neighboring countries is monitored with a thermal scanner in the bus terminal for detecting feverish passengers. This is not a barrier to transmission itself, and although it does not detect cases that move in other aquatic or terrestrial vehicles, the importance of this type of epidemiological monitoring is sustained by the results of the models for dengue cases.

The distribution of cases by age is an important feature in the epidemiological profile of dengue disease[31]. The frequency of negative cases did differ among age classes. The younger age classes (<14 years old) were more frequent than expected according to total population. This result suggests a higher propensity to perform laboratory analysis in younger age classes. This measure, whether protocolized or not, is consistent with the evidence supporting increments in cases in the pediatric population of Latin America and the Caribbean[3,15]. The frequency distribution of positive cases by age class, whether imported or autochthonous, did not differ from the population age class distribution. The result of the imported cases suggests a virus pressure proportional to population density, and not related to traveling age classes. The result of the autochthonous

cases suggests a similar susceptibility to dengue among age classes. However, when the location of cases is taken into account a new pattern comes out.

The occurrence of imported cases by census tract showed a weak but significant association with higher proportion of foreign population and more people of 15 to 44 years old. This is an interesting result because the raw data showed 36% of imported cases, very similar to the province 32%[10], and no relation to population age structure. Data on the whole districts (*e.g.* country, province) can be diluted by areas where transmission is not possible due to vector absence or concentrated by the more risky zones. For example, the country rate of cases per 100 000 inhabitants is 108, but includes districts without transmission, and others like Buenos Aires City with 156 or Misiones Province with 1 784[10]. Buenos Aires Province showed a value close to Tigre (23 *vs.* 22 respectively). But in a similar way to the country, it included municipalities without transmission (*i.e.* outside the distribution of the vector) and others with many more cases. The analyses taking into account the location of cases allowed us to identify the areas where virus pressure was higher and their demographic characteristics. This information is essential to identify areas with higher risk of virus introduction and focus future control actions.

Unifying the three factors identified by the models (adult-active people, foreign origin and infected dengue outside our country), a recurrent migration pattern in Argentina clearly emerges, related with the periodic visits made by foreign workers to their countries of origin[37]. This is the case of most workers in neighboring countries such as Paraguay and Bolivia, two countries with dengue epidemics closely related to the outbreaks detected in Argentina in recent years. There are no sufficiently disaggregated data to analyze the nationality of the foreign population to verify its relation to the neighboring countries with ongoing transmission, but from an integrative perspective of the results of this work, it is possible to argue that virus pressure of bordering countries is conditioned by working more than tourist activities. There are no positive cases of dengue in the islands even though the vector *Ae. aegypti* has been recently found in the area[38]. The population and urbanization in this area are very low, with more houses than inhabitants (6 500 houses), because premises are mainly used as weekend residences.

Autochthonous cases occurrence by zone was not significantly associated with any of the studied variables. This suggests no pattern about the transmission risk. The first question that one asks is if so few cases (36) or so few census tracts with cases (22) might be diluted in 298 tracts, so that statistics do not detect a significant effect. The other question is the under diagnosis of cases and asymptomatic cases[39]. So we could not be sure if tracts without cases are truly negative. The model for only the tracts with laboratory results allowed us to select the best quality data available. When only areas with laboratory confirmed cases were considered, the models showed more probability of autochthonous cases related to higher population density and less people of the 45-69 age class. The relation to higher population densities may be straightforward. The age related association is not very clear, and maybe indicating particular neighborhood infrastructure or inhabitants behavior that favor vector breeding and transmission, or simply a higher sensitivity of detection of these cases.

In the analysis of cases by clusters, there was a surprising weak spatial association among dengue cases, evidenced by the occurrence of only one, maybe two transmission foci and high numbers of isolated 2nd cases. This suggests that there were not many high transmission foci in the municipality, and probably many of the cases classified as autochthonous were infected in other places where transmission was going on outside of the municipality. This could have been any nearby municipality, including the country's capital, to which plenty of people travel daily for work, study or entertainment, and where almost 5 000 autochthonous cases occurred[10]. It is a common procedure to query suspected patients about travels to the northern provinces or neighboring countries, but not about local and routine movements, to verify if the antecedent of trip is compatible with the clinical picture of the patient. We suggest that more information should be gathered onwards, such as neighborhoods or municipalities visited daily or regularly, to help in the comprehension of the local epidemiology of dengue beyond municipal boundaries. Although many cases might have passed undetected, and it is usually considered that only a tenth of the cases are confirmed, we think it is unlikely that a big spreading focus might have passed undetected due to the detailed assessment carried out in the municipality. The analysis to detect transmission foci with the most conservative parameters showed only 1 valid focus. When the criteria was broadened in terms of time and distance some foci were added. But these had very few cases in large areas (*e.g.* 3 cases separated 700 m from each other). We do not think this might reflect a transmission focus, as this spans a 4 month period.

Regarding the efficacy of focal and perifocal control treatments on dengue control, there is feeble evidence to make an unequivocal decision. Even though the 2016 epidemic was the most important outbreak up to date, the number of autochthonous cases in Tigre municipality was low (36 cases in 381 000 inhabitants) giving few replicates of local transmission foci to test the efficacy of treatments. Moreover, not all autochthonous cases might have happened locally, so there are elements to suspect that transmission within the municipality was weak. It was not possible to determine if the occurrence of the foci among all confirmed cases treated with focal and perifocal control treatments was lower than expected if they were not treated, because none of the untreated cases or clusters resulted in a focus of transmission. The best approximation we can make is about the cluster where 11 autochthonous cases occurred. Five of seven focal and perifocal control treatments were not followed by further autochthonous cases, (71% efficiency) but we have no replicates for this figure.

It is not straightforward to conclude about the efficiency of the vigilance carried on in the municipality. The proportion of imported cases (36%) exceeded those of the national, provincial and capital records (28%, 32% and 27% respectively)[10], suggesting a higher virus pressure and therefore that autochthonous transmission was somewhat controlled. But this may have been due to vector abundances below the required for transmission or to infection during daily trips to work or recreation in other municipalities. The comparison with neighboring districts was not possible given that they had different dengue case detection, epidemiology status reporting and control programs. In general, data on epidemics are kept unavailable for months and the national data do not include the spatial detail required.

Other issues are remarkable to discuss. First, it is evident that interventions on suspected cases, without laboratory confirmation,

results in control treatments where there is no risk of transmission due to lack of viraemic. In the case of Tigre municipality, not all suspected cases could be treated, so there was a clear loss due to slow laboratory analyses. This implies a waste of inputs, equipment and human resources that would be avoided provided that laboratory analyses were faster. However, in the absence of laboratory confirmation it is a good strategy, since it was also observed that one of the determining factors in the occurrence of the two foci of transmission in Tigre municipality could have been the delay in the implementation of the focal and perifocal control treatments (7-16 d since the beginning of symptoms). Second, it was impossible to compare the detailed assessment carried out here with any other similar or near district. The recording of spatially and temporarily disaggregated data would help to conduct more complex analyses with information from independent municipalities, to take more efficient prevention and control measures. Third, we could not complement the data with infestation information, as no vector monitoring was carried out. However, even if the impact of the intervention on vector populations is demonstrable, this does not guarantee a resulting reduction in the transmission of dengue[40]. We assume the efficacy of the integrated approaches applied during focal and perifocal control treatments interventions in reducing the vector population[21-23] as a starting point for studying the effectiveness of the intervention on dengue transmission, an area of knowledge in which information is scarce[41].

Although dengue transmission in this area is circumscribed, this is not a result that can be fully attributed to the control measures adopted. Nevertheless, the results strongly suggest that understanding the demographic factors and processes that determine the epidemiology of dengue at the local level should serve as a guideline for future research aiming at improving the success of dengue transmission disruption.

## Conflict of interest statement

The authors declare that there is no conflict of interests.

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## References

[1] Guzman MG, Harris E. Dengue. *Lancet* 2015; **385**(9966): 453-465.

[2] Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl Trop Dis* 2012; **6**(8): e1760.

[3] Lopez-Gatell H, Hernandez-Avila M, Hernandez JE, Alpuche-Aranda CM. Dengue in Latin America: a persistent and growing public health challenge. In: Franco-Paredes C, Santos-Preciado J. (eds.) *Neglected*

*tropical diseases–Latin America and the Caribbean*. Vienna: Springer; 2015, p. 140-147.

[4] Weaver CW, Forrester LF. Chikungunya: evolutionary history and recent epidemic spread. *Antiviral Res* 2015; **120**: 32-39.

[5] Fauci AS, Morens DM. Zika virus in the Americas-yet another arbovirus threat. *N Engl J Med* 2016; **374**: 601-604.

[6] PAHO and WHO. Epidemiological alert: yellow fever. [Online]. Available from: http://www.paho.org/hq/index.php?option=com_topics&view=read all&cid=2194&Itemid=40784&lang=en [Accessed on 11 Dec 2017].

[7] Patterson J, Sammon M, Garg M. Dengue, Zika and chikungunya: emerging arboviruses in the New World. *West J Emerg Med* 2016; **17**(6): 671-679.

[8] Carbajo AE, Cardo MV, Vezzani D. Is temperature the main cause of dengue rise in non endemic countries? The case of Argentina. *Int J Health Geogr* 2012; **11**: 26.

[9] Tittarelli E, Mistchenko AS, Barrero PR. Dengue virus 1 in Buenos Aires from 1999 to 2010: towards local spread. *PLoS One* 2014; **9**(10): e111017.

[10] Ministry of Health. Bulletin of integrated vigilance No. 322-SE 32. [Online]. Available from: http://msal.gob.ar/index.php/home/boletin-integrado-de-vigilancia?start=60 [Accessed on 11 Dec 2017].

[11] Seijo A. Dengue 2009: Cronology of epidemics. *Arch argent pediatr* 2009; **107**(5): 387-389.

[12] Seijo A, Romer Y, Espinosa M, Monroig J, Giamperetti S, Ameri S, et al. Autochthonous dengue outbreak in the Buenos Aires metropolitan area. *Medicina* 2009; **69**(6): 593-600.

[13] Estallo EL, Carbajo AE, Grech MG, Frias-Cespedes M, Lopez L, Lanfri MM, et al. Spatio-temporal dynamics of dengue 2009 outbreak in Cordoba City, Argentina. *Acta Trop* 2014; **136**: 129-136.

[14] Gil JF, Palacios M, Krolewiecki AJ, Cortada P, Flores R, Jaime C, et al. Spatial spread of dengue in a non-endemic city in northern Argentina. *Acta Trop* 2016; **158**: 24-31.

[15] Tantawichien T. Dengue fever and dengue haemorrhagic fever in adolescents and adults. *Paediatr Int Child Health* 2012; **32**(s1): 22-27.

[16] Yung CF, Chan SP, Thein TL, Chai SC, Leo YS. Epidemiological risk factors for adult dengue in Singapore: an 8-year nested test negative case control study. *BMC Infect Dis* 2016; **16**: 323.

[17] Duncombe J, Clements A, Hu W, Weinstein P, Ritchie S, Espino FE. Geographical information systems for dengue surveillance. *Am J Trop Med Hyg* 2012; **86**(5): 753-755.

[18] Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, Chua MN, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 2014; **384**: 1358-1365.

[19] Villar L, Dayan GH, Arredondo-Garcia JL, Rivera DM, Cunha R, Deseda C, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med* 2015; **372**(2): 113-123.

[20] WHO. Dengue guidelines for diagnosis, treatment, prevention and control. [Online]. Available from: http://www.who.int/rpc/guidelines/9789241547871/en/. [Accessed on 4 Sep 2014].

[21] Esu E, Lenhart A, Smith L, Horstick O. Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. *Trop Med Int Health* 2010; **15**: 619-631.

[22] Pilger D, De Maesschalck M, Horstick O, San Martín J. Dengue outbreak response: documented effective interventions and evidence gaps. *Tropika* 2010; **1**(1): 0-0.

[23] Bonds JAS. Ultra-low volume space sprays in mosquito control: a critical review. *Med Vet Entomol* 2012; **26**: 121-130.

[24] Seidahmed OME, Siam HAM, Soghaier MA, Abubakr M, Osman HA,

Abd Elrhman LS, et al. Dengue vector control and surveillance during a major outbreak in a coastal Red Sea area in Sudan. *East Mediterr Health J* 2012; **18**(12): 1217-1224.

[25] Kandus P, Malvárez AI. Vegetation patterns and change analysis in the lower Delta Island of the Paraná River (Argentina). *Wetlands* 2004; **24**: 620-632.

[26] Kandus P, Quintana RD y Bó R. *Landscape patterns and biodiversity of the lower delta of the Paraná River. Environments maps.* Buenos Aires: Pablo Casamajor Ed; 2006.

[27] Loetti V, Burroni N, Vezzani D. Seasonal and daily activity patterns of human-biting mosquitoes in a wetland system in Argentina. *J Vector Ecol* 2007; **32**(2): 358-365.

[28] Carbajo AE, Vezzani D. Waiting for chikungunya fever in Argentina: spatio-temporal risk maps. *Mem Inst Oswaldo Cruz* 2015; **110**(2): 259-262.

[29] National institute of statistics and census. Population and dwellings national census. [Online]. Available at: https://www.indec.gob.ar/bases-de-datos.asp?solapa=5 [Accessed on 8 Dec 2017].

[30] McCullagh P, Nelder JA. *Generalized linear models.* London: Chapman and Hall; 1989.

[31] Akaike H. A new look at the statistical model identification. *IEEE Trans Automatic Control* 1974; **19**: 716-723.

[32] Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. *Mixed effects models and extensions in ecology with R.* New York: Springer; 2009.

[33] Davis CE, Hyde JE, Bangdiwala SI, Nelson JJ. An example of dependencies among variables in a conditional logistic regression. In: Moolgavkar SH, Prentice RL, (eds.) *Modern statistical methods in chronic disease epidemiology.* New York: Wiley; 1986, p. 140-147.

[34] Fielding AH, Bell JF. A review of the methods for the assessment of prediction errors in conservation presence/absence models. *Environ Conserv* 1997; **24**: 38-49.

[35] R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [Online]. 2014 [cited 2011 Apr 6]. Available from: http://www.R-project.org. [Accessed on 6 Dec 2017].

[36] Shang CS, Fang CT, Liu CM, Wen TH, Tsai KH, King CC. The role of imported cases and favorable meteorological conditions in the onset of dengue epidemics. *PLoS Negl Trop Dis* 2010; **4**(8): e775.

[37] OIM. Migratory profile of Argentina. Buenos Aires City, Argentina. [Online]. Available from: http://www.argentina.iom.int/co/perfil-migratorio-de-la-argentina [Accessed 11 Dec 2017].

[38] Cardo MV, Rosínc P, Carbajo AE, Vezzani D. Artificial container mosquitoes and first record of *Aedes aegypti* in theislands of the Paraná Lower Delta, Argentina. *J Asia–Pacific Ent* 2015; **18**: 727-733.

[39] Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis* 2016; **16**(6): 712-723.

[40] Bowman LR, Runge-Ranzinger S, McCall PJ. Assessing the relationship between vector indices and dengue transmission: a systematic review of the evidence. *PLoS Negl Trop Dis* 2014; **8**(5): e2848.

[41] Bowman LR, Donegan S, Mccall PJ. Is dengue vector control deficient in effectiveness or evidence? Systematic review and meta-analysis. *PLoS Negl Trop Dis* 2016; **10**(3): e0004551.