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Seasonal distribution of Phlebotomine sandfly in a vulnerable area for tegumentary leishmaniasis transmission in Córdoba, Argentina

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ABSTRACT

Thirty-seven sandfly species are listed for Argentina distributed in 14 provinces and Leishmaniasis cases extend from the north of the country to Unquillo City (Córdoba Province), but potential vectors are found further to the south. This is the first study on diversity, spatial and temporal distribution of sandflies on the outskirts of the temperate Córdoba City, and the factors that influence their presence. *Migonemyia migonei*, record here for Córdoba City for the first time, and the *Evandromyia cortelezzii-sallesi* Complex was found, also *Ev. cortelezzii* males were captured for the first time, these sandflies being more abundant during the warm months due to meteorological factors and the presence of blood meal sources. At least the eastern outskirts of Córdoba City, the second most important city of the country, are at risk of Leishmaniasis transmission if *Leishmania* spp. enters into the area due to the presence of competent vectors and adequate vertebrate hosts, in a favorable socio-economic context.

1. Introduction

Leishmaniasis is widely distributed in America and human cases of Tegumentary Leishmaniasis (TL) have been reported from 35° N (United States) (Aronson et al., 2016) to 31° S in Unquillo, Córdoba, Argentina, since the notification of the first autochthonous case of TL in 2014 (SIVILA, 2015). Thirty-seven sandfly species are listed for Argentina, distributed in 14 provinces (Szelag et al., 2016; Fuenzalida and Quintana, 2017), including Córdoba Province since 2004 (Salomón et al., 2008b; Visintin et al., 2016)

In general, sites with a high abundance of sandflies correspond to areas with many human and canine Leishmaniasis cases (Margonari et al., 2006). Therefore, the distribution and abundance of sandflies are considered as indicators in space and time of the probability of transmission of the parasite (Salomón et al., 2008b, 2011). The report of the first TL autochthonous case in Unquillo locality, just 20 km from Córdoba City, where potential vectors of Leishmaniasis agents have recently been found, which increases the necessity to carry out eco-epidemiological studies of sandfly distribution and abundance. The objective of this study was to determine the diversity and spatial and temporal distribution of sandflies in the Bajo Grande area, in the east of Córdoba City, and factors that influence their presence.

2. Materials and methods

2.1. Study area

The province of Córdoba is located in the geographical center of Argentina. This bio-region belongs to the Chaco domain and includes the biogeographical provinces of the dry Chaco, Espinal and Pampeana (Cabrera, 1976). The study was carried out in the Bajo Grande area (31°23′38"S; 64°04′36"W), in the eastern sector of Córdoba City (Espinal)(Fig. 1), a suburban area with a few isolated houses, with removal of floors for construction and land dedicated to vegetable cultivation and fruit trees. The Bajo Grande sewage treatment plant of Córdoba Municipality is located in the area which is characterized by a temperate climate, with rainfall between 500 and 800 mm annually, and the rainiest months being December-February. November-March is the warmest period, with average temperature of 17.7 °C, and maximum and minimum averages in summer of 31 °C and 17 °C, and in winter of

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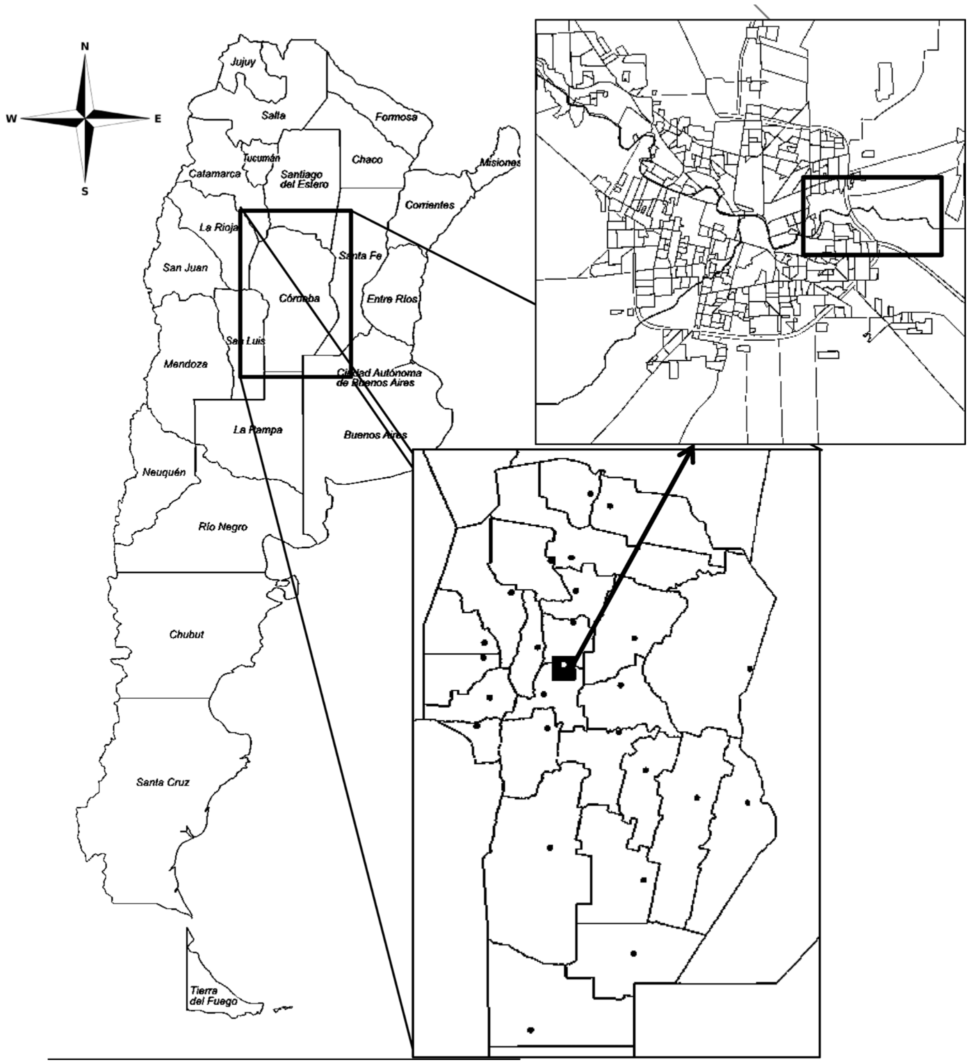

Fig. 1. Map of Argentina. Map of the Province of Córdoba. Map of the city of the capital, Córdoba. Study area.

19 °C and 4 °C, respectively (National Weather Service, 2016).

2.2. Capture and processing of adult sandflies

Samples were collected at 8 sites, in five peridomiciles of inhabited dwellings with the presence of domestic animals and 3 sites in the Bajo Grande plant considered wild. All sites presented the conditions of an epidemiological scenario where the sandfly captures were probable, characterized by shade, moist soil, organic debris, proximity to patches of dense vegetation and accessibility to blood sources (barnyard animals, kennels, etc.), without external light interference (Correa Antonialli et al., 2007). At each site a CDC (Centers for Disease Control and Prevention) light trap (Sudia and Chamberlain, 1962) was placed at 1.5 m above ground level, remaining active from 6pm to 8am of the following day. Captures were taken forthnightly between November 2015 and June 2016, covering the spring, summer and autumn seasons. Phlebotomine sandflies were identified using the Galati's (2003) key.

2.3. Data analysis

The abundance of sandflies collected in different climatic seasons, months and environments was compared by means of Kruskal Wallis test and multiple comparisons. In addition, the absolute abundance of sandfly correlated with meteorological variables: mean temperature, relative humidity and accumulated rainfall, obtained from the Meteorological Station of Barrio Empalme (31°26′26″S; 64°07′36″O), as it is the closest to the study area. Considering sandfly development time, time lags from one week to two months were analyzed, on the basis of weekly and beweekly periods. Multiple linear regressions were also performed to predict variations in the abundance of sandflies explained by meteorological variables. The model was developed for the analysis of the most abundant sandfly. The selection of the subset of regressor variables was obtained by the Stepwise method. The best model was selected using the Akaike (AIC) and Bayesian (BIC) information criteria.

Analyses were performed using INFOSTAT ® computer program (Di Rienzo et al., 2016).

3. Results

Phlebotominae were captured at all the sampling sites to a total of 97 individuals. *Migonemyia migonei* 18 (9 females and 9 males), 65 females of *Evandromyia cortelezzi-sallesi* Complex, since females of these species can not be identified morphologically, and 14 *Evandromyia cortelezzii* males.

Evandromyiacortelezzi-sallesi abundance was significantly higher in summer (H = 4.19, df = 2, p = 0.023), when the highest number of positive samples was recorded (n = 6) (Fig. 2), totaling 41 captured specimens, while in autumn only 2 samples were positive, the second sampling being the most abundant (April), totaling 37 specimens. *Migonemyia migonei* abundance was higher in autumn (n = 12 individuals) than in summer (n = 6) there being 3 positive samples in

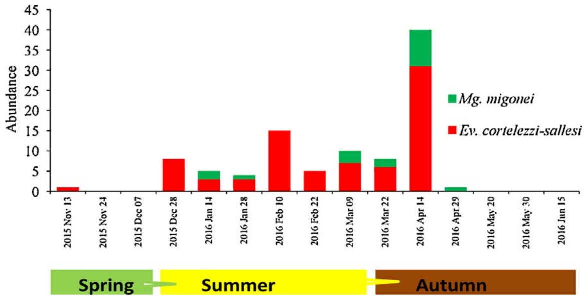

Fig. 2. Sandflies collected by sampling at each weather station from Nov 2015 to Jun 2016 in the area of Bajo Grande, Córdoba.

both seasons, though difference was not significant (H = 0.69, df = 2, p = 0.085) (Fig. 2).

The total abundance by month (*Ev. cortelezzii-sallesi* + *Mg. migonei*) was higher in April 2016 (n = 41), although there were no significant differences (H = 3.14, df = 4, p = 0.33). *Evandromyia cortelezzii-sallesi* was captured between November 2015 and April 2016, being more abundant in April (n = 31), followed by February (n = 20), although there were no significant differences (H = 3.61, df = 4, p = 0.211). *Migonemyia migonei* (males) were only captured in January March and April, and, more abundant in April (n = 10), though there was no significant difference between the months (H = 1.32, df = 4, p = 0.238).

Sandfly monthly abundance by sex varied according to the species/complex. *Evandromyia cortelezzii-sallesi* female were more abundant in April (n = 28), and males during February (n = 5), but no significant differences were detected (H = 5.02, df = 4, p = 0.068, H = 1.75, df = 4, p = 0.257, respectively). *Migonemyia migonei* males (n = 4) and females (n = 6) were more abundant in April, but these differences were not significant (H = 0.72, df = 4, p = 0.39, H = 0.89, df = 4, p = 0.18, respectively).

Average sandfly abundance was higher in the peridomicile (14.4 specimens) than at the wild sites (8.33), although the difference was not significant (H = 0.05, df = 1, p = 0.77). *Evandromyia cortelezzii-sallesi* was more abundant in the peridomicile during April with an average of six specimens, and at the wild sites during February (3.66), thus showing that it was present in both environments on most sampling dates. *Migonemyia migonei* was found in the peridomicile during January, March and April, with respective averages of 0.6, 1 and 1.8 specimens, whereas at the wild sites the specimens (average of 0.33) were collected only in April.

Evandromyia cortelezzii-sallesi abundance correlated positively the mean temperature on the catch day (r = 0.54, p = 0.04). As regards cumulative rainfall, the highest correlation was obtained between 15 and 21 days prior to sampling (r = 0.71, p = 0.003), being also positively and significantly correlated during periods ranging from 1 to 7 days (r = 0.54, p = 0.04), 15 days (r = 0.57, p = 0.03), 30 days (r = 0.59, p = 0.02), and 45 days (r = 0.54, p = 0.04) prior to sampling. The abundance was also positively correlated with mean relative humidity (r = 0.53, p = 0.04) between 1 and 7 days prior to sampling.

Migonemyia migonei abundance was positively correlated with cumulative rainfall between 15 and 21 days prior to sampling (r = 0.65, p = 0.01) and also during the 30 days (r = 0.57, p = 0.03) and 45 days (r = 0.55, p = 0.03) prior to sampling, but no correlation was found with mean temperature or relative humidity.

The multiple linear regression model best fitted to the observed *Ev. cortelezzii-sallesi* abundance ($R^2_{Aj} = 0.746$, $p < 0.005$), was:

$$Y = -49.54 + 0.82^*X_1 + 0.45^*X_2 + 0.15^*X_3$$

Analyzing the regression coefficients, average temperature on the sampling day (X_1) had great influence on *Ev. cortelezzii-sallesi* abundance, followed in decreasing order by Relative Humidity between 29 and 35 days prior to the sampling date (X_2) and Cumulative Rainfall between 15 and 21 days prior to the sampling date (X_3). The correlation between data observed and expected by the model was r = 0.89 ($p < 0.01$) (Fig. 3).

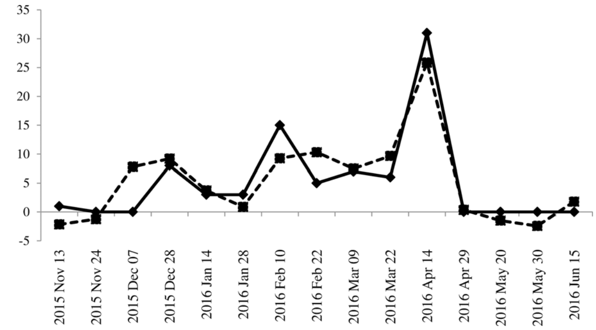

Fig. 3. Abundance of *Ev. cortelezzii-sallesi* from Nov 2015 to Jun 2016 in the area of Bajo Grande, Córdoba. In continuous line the observed values and in dash line the values expected from the model generated.

4. Discussion

Finding 97 sandfly specimens on the eastern outskirt of Córdoba city, added to records of their presence in previous years, indicate established population of *Mg. migonei* and *Ev. cortelezzii-sallesi*, or at least *Ev. cortelezzii* since males of this species were found, although both species of the complex have been found associated in the same habitats (Galati et al., 1989), as occurred in Donadeu, Santiago del Estero Province, Argentina (Salomón et al., 2008b). This would indicate a southward since expansion of the risk zone of transmission of Leishmaniasis, since the sandflies mentioned in this study are potentially vectors. *Evandromyia cortelezzii-sallesi* was found naturally infected by *Le. braziliensis* in Chaco Province (Rosa et al., 2012), and *Mg. migonei* is considered a putative vector of visceral leishmaniasis agent in Santiago del Estero, associated with periurban-rural transition environments and with domestic animals (Salomón et al., 2010).

Our results would also indicate that sandflies are distributed at least along the transect covering the study area (3.63 km between the furthest sites), since they were collected at all sampling sites, even when sandflies have limited dispersion capacity covering 200–300 m/day (Cáceres, 1995).

The greatest likelihood of finding *Ev. cortelezzii-sallesi* during the warm season, shows the importance of weather conditions for the abundance and distribution of the species, since it was recorded in all the samples summer. On the other hand, *Mg. migonei* was more meaning not clear in the temperate zone. For this reason, further study of the factors that favor the presence of the species in the area are necessary.

Sandfly abundance variations are due to changes in community composition throughout the study period. *Evandromyia cortelezzii-sallesi* was dominant, differing from the findings of Salomón et al. (2008a) for the Chaco Seco eco-region, where it was always recorded in a lower proportion than *Mg. migonei*. In the temperate Córdoba Province, *Mg. migonei* abundance would be lower, in coincidence with records by Salomón et al. (2008b) in Altos de Chipión (Córdoba Province). Therefore, *Ev. cortelezzii-sallesi* would tolerate a wider range of meteorological conditions than *Mg. migonei*.

The differences recorded in this study for sandfly abundance according to the sex suggest females increase towards the end of the season, which could be a strategy to ensure the next generation. Although *Ev. cortelezzii-sallesi* female abundance was always higher than males, the tendence was to increase monthly, while males maintained low abundance, thereby males and females would respond differently to trap attraction as was mentioned for *Phlebotomus longipes* in Ethiopia (Foster, 1972). The greater abundance of females could also be related to the proximity to sites where they breed and seek shelter. On the other hand, *Mg. migonei* males were recorded earlier (January, March) may be due they emerge before females (Forattini, 1973; Chaniotis, 1967).

The sandfly abundance at peridomiciliary was probably due to the presence of domestic animals as blood source, organic matter and permanent shelters. The results also suggest to this environment as preferential site to feed and shelter during the lower colder months. *Migonemyia migonei* was found more associated to the peridomiciliary environment, in coincidence with results presented by Barretto (1943) in Brazil and Fernández (2012) in Misiones, Argentina. On the contrary, *Ev. cortelezzii-sallesi,* that was present in both, was more abundant in the wild sites during February and the relation is reversed towards April, when the greater abundance was found in the peridomicile, indicating this species has tendency to be in wild sites when the weather conditions are favorable, but would find shelter in the peridomicile during months when the weather conditions are not favorables.

Temperature and humidity effect on the activity and abundance of sandflies varies according to the species (Salomón et al., 2012). In this work, the association found among *Ev. cortelezzii-sallesi* abundance and temperature, during the capture day, would be related with the temperature at which they are active, and females can bite (15–28 °C) (Lucientes et al., 2005). The association with cumulative precipitation and relative humidity during the previous week of sampling is due to the importance of moisture for adult and immature sandfly survival (Rangel and Lainson, 2003). Rainfall had a positive correlation during the 45 days prior to sampling, which would be related to soil moisture and conditions necessary for larval survival, since the delay found is consistent with the larval period described for sandflies (Forattini, 1973). This would explain the abundance variation between months, therefore, alterations in rainfall would have a direct effect on the regulation of *Ev. cortelezzii-sallesi* population. We did not find significant relation with the average temperature of the capture day for *Mg. migonei*, which is not coincident with the findings of Fernández (2012), in Misiones Province, with a subtropical climate. This could be due, as discussed above, to the low abundance recorded for this species, or the presence and abundance of this species in a temperate zone responds to other factors. The relation of *Mg. migonei* abundance with the accumulated precipitation, as for *Ev. cortelezzii-sallesi*, would indicate the importance of soil moisture for immature stages. The close association of the vectors with the meteorological variables would explain the exposure and establishment of these species in the periphery of the city of Córdoba possibly as a result of the climatic change.

According to the linear regression models obtained in the present study, the abundance of *Ev. cortelezzii-sallesi* on the outskirts of the city of Córdoba can be predicted from temperature, relative humidity and accumulated precipitation, which would influence different stages of development and sandfly survival.

Finally, based on the results obtained in the eastern periphery of Córdoba City there are at least two sandfly populations already established, which were recorded during the warm months, finding them in greater abundance and richness in peridomiciles. *Evandromyia cortelezzii-sallesi* was more abundant, but the apparently secondary role that *Mg. migonei* (lower abundance) can eventually change from one season to another, if weather conditions were favorable. *Migonemyia migonei* was mainly found in peridomiciles, whereas *Ev. cortelezzii-sallesi* was present in both environments studied, changing the odds of finding it in one or the other depending on weather conditions. According to our results, at least the eastern periphery of Córdoba City is at risk of Leishmaniasis transmission if *Leishmania* spp. will enter into the zone, since the distribution and abundance of sandflies are considered as indicators in space and time of the probability of transmission of the parasite (Salomón et al., 2008b, 2011; Peterson, 2006), if that did not already happened but was not detected yet.

Conflict of interests

The authors state that they have no conflict of interest.

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