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ORIGINAL CONTRIBUTION

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Field comparison of oviposition substrates used in ovitraps for *Aedes aegypti* surveillance in Salta, Argentina

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Abstract

Aedes aegypti L. (Diptera: Culicidae) is a mosquito broadly found in tropical and temperate areas of the world. It is the main vector of dengue, chikungunya, zika and yellow fever (urban cycle), among other viruses. *Aedes aegypti* immatures develop in water holding containers, and frequently use artificial containers in urban settings as larval habitat. Ovitraps are artificial oviposition sites, a tool developed for mosquito population surveillance and to assess effectiveness of control measures. The preference for different oviposition substrate materials was assessed in the field, in two localities of Salta province, northern Argentina, where dengue outbreaks are frequent. The proportion of positive traps did not differ between oviposition substrates. However, higher numbers of eggs were laid in cotton fabric and velour paper, which were better than wooden paddle and blotting paper if the aim was to maximize the numbers of eggs collected. The results also evidenced that substrate preference for oviposition did not differ between geographic regions.

KEYWORDS
Culicidae, dengue, eggs, monitoring, mosquitoes, zika

1 | INTRODUCTION

Aedes aegypti L. (Diptera: Culicidae) is a mosquito broadly found in tropical and subtropical areas of the world, and in temperate areas with maximum monthly temperatures exceeding 14°C (Brady et al., 2014). Together with *Aedes albopictus* (Skuse), it is the main vector of dengue, yellow fever (urban cycle), chikungunya and zika, among other viruses they transmit (Halstead, 2015; Pereira-Lima, Goulart, & RolimNeto, 2015; WHO (World Health Organization), 2015). These viruses circulate in the Americas (Marcondes, Contigiani, & Gleiser, 2017). Dengue and dengue haemorrhagic fever have become important health issues worldwide. Annually, it is estimated that 3.97 billion people from 128 countries are at risk of dengue (Brady et al., 2012), with an estimated burden of 96 million cases in 2010 (Bhatt et al., 2013). In the Americas, cases are reported from most countries, with 27,290 cases reported in 2016 (PAHO (Pan American Health Organization/World Health Organization), 2016). In Argentina, the dengue epidemic of 1998 affected 19 people in Tartagal, in Salta in the northwest of the country, followed by relatively small outbreaks in 2004 (Rotela et al., 2007). Towards the end of 2008 and beginning of 2009 one of the largest dengue epidemics in Argentina was recorded and extended over several provinces including Buenos Aires (Gil et al., 2016; Seijo, 2009); more than 26,000 cases were officially reported, there were six deceased and two congenital cases in San Ramón de la Nueva Orán city, Salta (Bernardini, 2011). During the epidemic of 2016, 42.3% more cases were informed compared to 2009, totalling 41,207 clinical plus laboratory confirmed cases countrywide (Ministerio de Salud de la Nación Argentina [Internet], 2016), and approximately 1,000 confirmed cases in Salta. The first autochthonous cases of chikungunya in the Americas were detected in 2013, in the French island of Saint Martin (Leparc-Goffart, Nougairede, Cassadou, Prat, & de Lamballerie, 2014) from where it spread through the Caribbean. In September 2014 the first cases were reported in Brazil and later the same year, the virus reached Paraguay (Maguiña Vargas & Pitsfil, 2015; Ministerio de Salud Pública y Bienestar Social de Paraguay [Text article], 2015). Since then, it has spread southward reaching Bolivia (Ministerio de Salud de Bolivia [Internet], 2016). In Argentina, the first autochthonous cases of chikungunya and zika were detected in 2016 (WHO (World Health Organization), 2016).

Aedes aegypti immatures develop in water holding containers, and frequently use artificial containers in urban settings as larval habitat, laying their eggs in the container walls above the water line. An individual female of this vector may distribute the eggs among multiple larval sites within the same gonotrophic cycle, a behaviour known as "skip oviposition" (Abreu, Morais, Ribeiro, & Eiras, 2015). In fact, laboratory and semi-field tests have shown that most *A. aegypti* females spread their eggs in multiple larval habitats whenever available (Abreu et al., 2015). A variety of environmental factors influence the oviposition behaviour of *A. aegypti* (e.g. Harrington, Ponlawat, Edman, Scott, & Vermeylen, 2008; O'Gower, 1963; Rodríguez-Tovar, Badii, Olson, & Flores-Suárez, 2000; Wong, Astete, Morrison, & Scott, 2011; Wong, Stoddard, Astete, Morrison, & Scott, 2011). In laboratory experiments, when *A. aegypti* var. *queenslandis* Theobald were given choices between two oviposition sites, the females deposited their total egg batch in one clutch in sites with rough-surfaces while ovipositing was spread in more batches on smooth surfaces (O'Gower, 1963). When combining chemotactile, humidity, visual, olfactory and tactile stimuli, visual had the highest influence on mosquito activity, while olfactory had the least (O'Gower, 1963). Also, in laboratory settings, besides rough-surfaces, darker colors were preferred (Fay & Perry, 1965). However, in Cairns, Australia, neither the rate of positive ovitraps nor the mean number of eggs differed between mansonite paddle, wooden tongue depressor and seed germination paper oviposition substrates (Ritchie, 2001).

Ovitraps are artificial oviposition sites, a tool developed for monitoring mosquito populations and to assess the control measures (Rose, 2001), that have even shown to be more sensitive and effective than the traditional larvae surveys (deMelo, Scherrer, & Eiras, 2012). Ovitraps provide several advantages over other surveillance methods because of their low cost, high sensitivity and ease of field management, which allow operation by unqualified personnel (Bellini, Carrieri, Burgio, & Bacchi, 1996; Carrieri et al., 2011). The standard model consists of a black plastic container with water holding a relatively rough oviposition substrate surface (wooden tongue depressor or fine sandpaper) (Hoel et al., 2011). Other studies have used blotting paper (Steinly, Novak, & Webb, 1991) and velour paper (Campos & Macia, 1996). Trap modifications have mostly focused on the addition of attractants or alternatively ovicides and/or insecticides, changing the function from a passive monitoring system to a means of population control (Abad-Franch, Zamora-Perea, Ferraz, Padilla-Torres, & Luz, 2015; Gopalakrishnan, Das, Baruah, Veer, & Dutta, 2012; Harburguer, Licastro, Masuh, & Zerba, 2016). Also, ovitraps are used to collect eggs for further studies of insecticide resistance (Maciel-de-Freitas et al., 2014) or of population dynamics, for example to assess rates of egg predation or egg winter survival (Byttebier, de Majo, & Fischer, 2014; Fischer, Alem, De Majo, & Campos, 2011), among others.

Aedes aegypti populations have shown variations in several traits including larval site choice and oviposition behaviour (Powell & Tabachnick, 2013). Preference for oviposition substrate may be influenced by environmental humidity and differ between populations from different climate conditions (Madeira, Macharelli, & Carvalho, 2002). In the present study we compared the relative preference for different oviposition substrate materials in the field, in two different biogeographical regions in Salta province, Argentina.

2 | MATERIALS AND METHODS

2.1 | Study sites

The field work was done in two localities of Salta province, northern Argentina (Figure 1): Hipólito Yrigoyen (23°14′45″ S, 64°16′26″ W) and Salta city (24°44′02.09″ S, 65°24′19.46″W). Hipólito Yrigoyen is in the Yungas ecoregion, characterized by a warm humid to

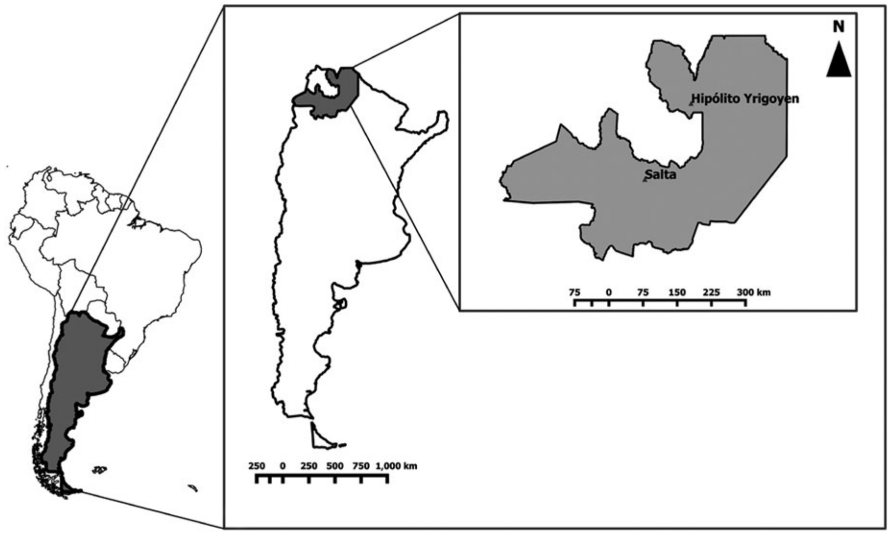

FIGURE 1 Map of South America showing the location of Argentina and the province of Salta with the two sampling sites

subhumid climate (average summer temperature 27.0°C and winter temperature of 15.8°C), with annual rainfall of 900-1,000 mm, occurring mainly in the summer. The climate of Salta city is Chaco subtropical-dry, with 500-700 mm rainfall and wide temperature range (average summer and winter temperatures are 20.7°C and 10.7°C, respectively) (Brooks, 2018; Cabrera, 1971).

Ovitraps consisting of 500 ml black plastic cups, 10 cm high, were used. A hole was drilled in each cup 4 cm below the cup edge for excess rainwater to drain. Ovitraps were filled 2/3 of their volume with dechlorinated tap water and one of four different substrates were placed inside the trap: red velour paper, white blotting paper, light cream coloured cotton fabric or light-coloured wooden paddle, held in place by a paper clip. Substrates were 4 cm wide × 22 cm long, except the wooden paddle (2 cm × 15 cm). Within each city, four traps were installed per site, one trap per treatment (maximum distance between traps 10 cm; treatments assigned randomly to traps) to assess female preference for a substrate. One week later, ovitraps were collected and transferred to the laboratory for counting of eggs under a stereoscopic microscope. The traps were established in 35 sites in Salta city in March of 2014 and 34 sites in Hipólito Yrigoyen locality in March of 2017, outdoors in gardens or backyards in private residences. Sites were placed at a minimum distance of approximately 30 m (with buildings between sites) in Salta; and distanced 70–300 m in Hipólito Yrigoyen.

At some sites, one or more traps were lost or vandalized; for consistency of comparisons and to reduce bias due to potential interference between traps, only traps from sites where all ovitraps were recovered, with at least one having counts different from zero, were considered for data analysis. Thus, there were data for 68 ovitraps from Salta (from 17 sites) and 96 ovitraps from Hipólito Yrigoyen (from 24 sites). Previous and ongoing studies on tree hole and container mosquitos in Salta province (e.g., Espinosa et al., 2016; Mangudo, Aparicio, & Gleiser, 2011; Mangudo, Aparicio, Rossi, & Gleiser, 2018; José F. Gil pers. obs.) have not shown the presence of *A. albopictus*. Regardless, following recommendations of WHO (Reiter & Nathan, 2001), some substrates were submerged for eggs to hatch, and larvae raised to forth stage to discard the presence of *A. albopictus* in the study locations.

2.2 | Data analysis

The percentage of positive ovitraps was compared between substrates and localities using binary logistic regression (software R; R Core Team, 2017). Dependent variable was the presence (positive) or absence (negative) of eggs in a trap, fixed effects were substrate (velour paper, cotton fabric, blotting paper, wooden paddle) and locality (Hipólito Yrigoyen and Salta city). The effect of locality and substrate type on the number of *A. aegypti* eggs deposited on a trap (abundance) per ovitrap were assessed using generalized lineal mixed models with negative binomial link function (GLMM; Infostat). Dependent variable was the number of eggs per ovitrap, fixed effects were substrate (velour paper, cotton fabric, blotting paper, wooden paddle) and locality (Hipólito Yrigoyen and Salta city), and random effect was site, nested within locality.

3 | RESULTS

Except for blotting paper, the proportions of positive ovitraps were high in both localities. In Hipólito Yrigoyen we recorded a higher proportion of positive ($p < 0.0002$), ranging between 0.79 and 0.87 (20/24 ≈ 0.83 in cotton fabric, 21/24 ≈ 0.87 in wooden paddle, 19/24 ≈ 0.79 in velour paper, 21/24 ≈ 0.87 in blotting paper), while we observed more variability (but in a smaller sample) in Salta city (11/17 ≈ 0.65 in cotton fabric, 8/17 ≈ 0.47 in wooden paddle, 9/17 ≈ 0.53 in velour paper, 8/17 ≈ 0.47 in blotting paper). However, differences in positivity between substrates were not significative ($p = 0.94$).

As expected, significantly more eggs per ovitrap were collected in Hipólito Yrigoyen than in Salta city ($p = 0.0001$). For both localities there were significant differences among substrates ($p = 0.004$)

(Figure 2). The highest numbers of eggs were laid in velour paper and the lowest in blotting paper. Cotton fabric received slightly less eggs than velour paper, but differences were not significative. Cotton fabric collected significantly more eggs than wooden paddle in both localities.

4 | DISCUSSION

We did not find significant effects of substrate on the frequency of ovitraps positive for *A. aegypti* eggs. These results are consistent with a study by Lenhart, Walle, Cedillo, and Kroeger (2005) in Tamaulipas, Mexico, where percentage of positive ovitraps was similar in traps placed outdoors with cotton fabric substrate compared to wooden paddle; however, for traps placed indoors, a significantly greater percentage of traps with a cotton fabric substrate were positive for eggs compared with traps containing a wooden paddle substrate.

We detected differences in the number of eggs between localities and substrates. Abreu et al. (2015) observed that even under identical conditions, when several oviposition sites are available, one of the sites usually receives most of the eggs. We observed that in both localities, highest numbers were collected in cotton fabric and velour paper substrates, while lowest numbers were collected from blotting paper. Results indicate that although *A. aegypti* from Salta province will lay their eggs in diverse substrates, they may differ in their substrate preference as suggested by significant differences in the numbers of eggs laid. Field assessments in Mexico comparing oviposition in red velour paper strips and fiberboard paddles within the same trap showed a higher number of eggs were deposited on the velour paper strips, even under contrasting sun exposures of the traps (Rodríguez-Tovar et al., 2000). In field studies in Iquitos, Peru, lining containers with textured brown paper towel increased egg counts in plastic and metal, but not cement containers, suggesting that container material and/or texture influences oviposition (Wong, Astete et al., 2011).

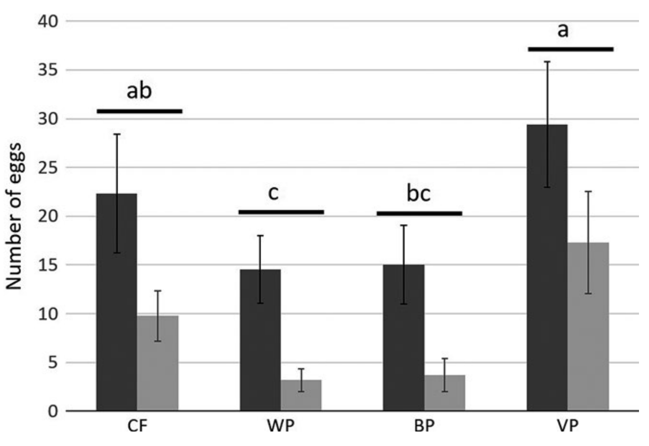

FIGURE 2 Number of eggs per ovitrap (and the corresponding standard errors) for each substrate and for both localities (for each substrate, dark gray columns correspond to Hipólito Yrigoyen and light grey column to Salta City). Significant differences between localities are observed for all substrates. [a-c]Substrates not sharing a letter differ significantly

It is likely that the greater absorbency of cotton fabric and velour paper results in a greater area of moist substrate, compared with the wooden paddle, thus being better oviposition stimulants. O'Gower (1963) showed humidity to be an important factor for oviposition site selection. Although colour may influence oviposition, darker colours preferred (Frank, 1985), still texture may have prevailed since fabric and velour had contrasting shades (cream and red, respectively). In an enclosed field assay in Thailand, no differences were detected between grey, brown or black ovitraps in the proportion of positives or numbers of eggs (Harrington et al., 2008).

Due to the skip oviposition behaviour of female and the diversity of substrates that may be used for depositing eggs, it is possible that clustering the ovitraps together at each site are more reflective of a direct substrate competition than representative of a realistic ovitrap application, exaggerating the effects of substrate type on egg counts. Observed differences in egg abundance among some of the treatments might otherwise have been negligible if ovitraps had been spaced further apart.

If the aim of the study is to detect female activity, then cotton fabric followed by wooden paddle may be the best options due to low cost and easier replacement. Moreover, if the aim is to maximize the number of eggs collected, then cotton fabric would be the best choice because the velour paper is 150% more expensive. We would not recommend blotting paper as substrate because of the lower number of eggs collected. If substrates are replaced infrequently (e.g., on a weekly basis), we would not recommend use of blotting paper or velour paper for practical reasons related to material breaking when wet (these substrates had to be handled with care when recovered to prevent ripping). Results did not provide evidence that substrate preference for oviposition may differ between geographic regions.

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AUTHOR CONTRIBUTION

MC, JFG, JPA and RMG conceived the research. MC, JFG, PC, CM and GNC conducted experiments. MC, JFG, GNC, PC and CM contributed material. MC, JPA and RMG analysed data and conducted statistical analyses. MC and RMG wrote the manuscript. JFG, JPA and RMG secured funding. All authors read and approved the manuscript.

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