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Short Communication

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First Report of Adult *Aedes aegypti* (Diptera: Culicidae) Resistance to Pyrethroids in Argentina

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Abstract

Severe human arboviral diseases can be transmitted by the mosquito *Aedes aegypti* (Linnaeus), including dengue, chikungunya, Zika, and yellow fever. Adult control using spatial sprays with adulticides is recommended only when dengue outbreaks occur. In Argentina, mainly pyrethroids, like *cis*-permethrin, have been used as an adulticide, especially since 2008. The evolution and spread of resistance to insecticides is a major concern for vector control. This study reports for the first time pyrethroid resistance in *Ae. aegypti* adults from Argentina, in the city of Salvador Mazza (Salta). WHO discriminating doses of 0.75% were used for permethrin, 0.05% for deltamethrin, and 5% for malathion. Also the discriminating dose for *cis*-permethrin (0.6%) was calculated and evaluated for the first time. We found a resistance ratio 50 (RR_{50}) of 10.3 (9.7–10.4) for *cis*-permethrin, which is considered as high resistance. Our results also indicated resistance to deltamethrin (22.6% mortality) and permethrin (53.6% mortality), and a total susceptibility to malathion (100% mortality). Results from this study highlight the importance of the correct use of insecticides within an Integrated Vector Management (IVM) approach and of early detection of resistance to enable *Ae. aegypti* control in Argentina. More studies are needed to determine the spread of mosquito resistance to pyrethroids.

Key words: *Aedes aegypti*, insecticide resistance, malathion, pyrethroids

Aedes aegypti (L.) control is mostly aimed at the larval stages (removal of breeding sites, larviciding, and community education) to reduce the population of new adults. Also, adult control using spatial sprays with adulticides is recommended when arbovirus outbreaks transmitted by this vector occur (WHO 2009).

By the end of the 1980s, Argentina was re-infested by the mosquito and at present, the vector is found in most of the Argentinean territory (Vezzani and Carbajo 2008). Since 1997, the northwest and northeast parts of Argentina have suffered a series of dengue epidemic outbreaks almost every year (Bulletin from the Ministry of Health Argentina 2020). Due to the significant increase in *Aedes* mosquito populations detected through the monitoring programs (Masuh et al. 2008) and the higher frequency of outbreaks and epidemics, a significant increase in insecticide-based vector control interventions was conducted over the last decade. The insecticides used were the organophosphate temephos for larvicidal treatment in water containers and the pyrethroid *cis*-permethrin as an adulticidal ultra-low volume (ULV) formulation.

Susceptibility levels to insecticides used on *Ae. aegypti* must be constantly screened in different geographical areas in order to develop successful control strategies (WHO 2009). Temporal variation of larval susceptibility to temephos was studied in some cities at the northeast and northwest of Argentina between 2004 and 2009, revealing a weak decrease in susceptibility to the larvicide. The resistance ratio (RR) observed (around 3) indicated a very incipient resistance and no control failures were detected (Seccacini et al. 2008, Albrieu Llinas et al. 2010).

However, the extensive use of organophosphorus compounds resulted in high values of resistance in almost all countries of the world and pyrethroids began to be used as adulticides in a resistance management action (Macoris et al. 2007). This change led to pyrethroid resistance some years later (Vontas et al. 2012, Naqqash et al. 2016). In Argentina, no variations in the susceptibility status data for pyrethoids were found during monitoring evaluations until now (unpublished results). Control failures were detected in Salvador Mazza (Salta, Argentina) in 2013; a new insecticide resistance monitoring program was established for *Ae. aegypti* populations according to the methodology established by WHO (2016).

The *cis* isomer of permethrin (*cis*-permethrin) is not a commonly available pyrethroid but is widely used in Argentina and some other Latin-American countries under the trade name DEPE (more than 80% *cis* isomer), because it was found to be much more effective on *Ae. aegypti* than the isomers mixture (Seccacini et al. 2006). In this study, we also calculated *cis*-permethrin diagnostic dose (DD), obtained the lethal concentration 50% and 90% values (LC_{50} and LC_{90}) for the susceptible strain and the Salvador Mazza's population, and calculated the resistance ratio for *cis*-permethrin for the field collected samples. Additionally, the susceptibility of *Ae. aegypti* adults to permethrin, deltamethrin, and malathion at the DD recommended by WHO (2016) was evaluated.

Materials and Methods

Biological Material

The insecticide susceptible CIPEIN strain of *Ae. aegypti* was used. This strain was originated from the Rockefeller strain from Venezuela and has been reared since 1996 in our insectary, free of exposure to pathogens, insecticides, or repellents. *Aedes aegypti* were reared according to the protocols established in our laboratory and described by Harburguer et al. (2018). Non-blood-fed adult females between 3 and 5 d old were used for assays.

Aedes aegypti eggs were collected in the province of Salta (Argentina), in the urban area of Salvador Mazza (22°03′S 63°42′W), a city with historical cases of dengue and high *Ae. aegypti* entomological indices (Bulletin from the Ministry of Health Argentina 2020). Ovitrap collections of *Ae. aegypti* eggs were carried out by local entomology teams from December 2012 to February 2013. Ovitraps were prepared according to Seccacini et al (2008), using black plastic jars. The number of ovitraps installed was based on the number of buildings (an indirect measure of the population density): ≤60,000 buildings, 100 ovitraps (Lima et al. 2003), as in this study. Eggs collected were sent to CIPEIN's laboratory where they were reared in the same way as the susceptible strain. For this study, 3- to 5-d-old adult females from F1 generation were used and obtained according to the protocols established in our laboratory (Seccacini et al. 2008).

Bioassays

WHO impregnated papers with the DD (permethrin 0.75%, deltamethrin 0.05%, and malathion 5%) were purchased from the University of Sains Malaysia (WHO Collaborating Center). We used the DD for *Anopheles* because the DD for *Aedes* mosquitoes are tentative and are being re-evaluated (WHO 2016). Because the DDs of *Anopheles* are higher than those for *Ae. aegypti*, if the *Ae. aegypti* mosquitoes survive the test, it is even more likely to be resistant to the insecticides evaluated.

To calculate the DD for *cis*-permethrin and obtain the resistance ratios (RR), Whatman filter papers (12 × 15 cm) were impregnated with serial concentrations of the technical grade insecticide diluted in a mixture of acetone with silicone oil Dow Corning 556 (Daltosur S.R.L., Buenos Aires, Argentina) following the same procedure used by the University of Sains, Malaysia (Lee et al. 1997). Technical grade *cis*-permethrin 96% (permethrin *cis*:*trans* ratio of 95:5) was supplied by Chemotecnica S.A., Argentina. Five concentrations of *cis*-permethrin (between 0.0025 and 0.25%) were used and at least five replicates were performed; the papers were left to air dry overnight and then were covered in aluminum foil and kept at 4°C until use.

Papers were inserted into standard exposure tubes as established by the WHO protocol (WHO 2016). Mosquitoes were kept in the exposure tubes for a period of 1 h. Following exposure, mosquitoes were transferred back to the holding tubes and a pad of a cotton-wool soaked in 10% sugar water was placed on the mesh-screen end. Mosquitoes were maintained in the holding tubes for 24 h (the recovery period), and the number of dead mosquitoes was counted and recorded. Percentages of mortality were adjusted by Abbott's (Abbott's (1925) formula.

Statistical Analysis

Dose-mortality data from each *Ae. aegypti* pool of insects were subjected to probit analysis (Litchfield and Wilcoxon 1949). Lethal concentration of 50 and 90% values (LC_{50} and LC_{90}) was obtained by means of PoloPlus 2.0 software (LeOra Software Company, Petaluma, CA). The DD for CIPEIN strain to *cis*-permethrin was calculated as $LC_{99} \times 2$ as proposed by WHO (2016). The resistance ratios (RR_{50} and RR_{90}) were estimated, based on LC_{50} and LC_{90} values, as the quotient between the LC of field population and the LC of the control strain as reference as stated by WHO (2016).

Results and Discussion

In this study, the susceptibility of a field population from Salvador Mazza (Salta, Argentina) to the insecticides permethrin, malathion, and deltamethrin was evaluated using the diagnostic doses established by WHO. We also determined for the first time the DD for the pyrethroid *cis*-permethrin.

There was 22.6% mortality for permethrin, 53.6% mortality for deltamethrin, and 100% mortality for malathion, indicating phenotypic resistance for the two pyrethroid insecticides and susceptibility for the organophoshorus compound (Table 1). Resistance to deltamethrin is a very important fact to consider, since this pyrethroid has never been used for controlling *Ae. aegypti* in our country; therefore, we could be facing a case of cross resistance due to the fact that both insecticides share the same target site. Conversely, in Yacuiva, Bolivia, a city close to Salvador Mazza, resistance to deltamethrin in *Ae. aegypti* was detected (López Rodriguez 2015). The movement of people and merchandise between Yacuiba (Bolivia) and Salvador Mazza (Argentina) is continuous and intense; therefore, there may have been an introduction of the resistance genes of the Bolivian vector population in the Argentine strain, what is currently being studied in our laboratory. Genetic analysis revealed that there are

Table 1. Susceptibility to insecticides of adult *Ae aegypti* CIPEIN and Salvador Mazza strains following WHO protocol bioassay

Strain	Mortality percentage (±SD) to the DD recommended by WHO		
	Permethrin (0.75%)	Deltamethrin (0.05%)	Malathion (5%)
CIPEIN	100 ± 0.0	100 ± 0.0	100 ± 0.0
Salvador Mazza	22.6 ± 8.7	53.6 ± 16.8	100 ± 0.0

DD: Diagnostic Dose. No dead *Ae. aegypti* adults were found in the control groups during bioassays.

Table 2. Lethal concentration 50 and 90% values (LC_{50} and LC_{90}) and the resistance ratios (RR) for the susceptible strain (CIPEIN) and the Salvador Mazza's population against *cis*-permethrin

	$LC_{50} \pm CI_{95}$	$LC_{90} \pm CI_{95}$	Slope (± SD)	$^{*}RR_{50}$	$^{**}RR_{90}$
CIPEIN	0.034% (0.028–0.042)	0.112% (0.084–0.167)	2.48 ± 0.26	—	—
Salvador Mazza	0.351% (0.287–0.409)	0.622% (0.521–0.900)	5.22 ± 0.70	10.3 (9.7–10.4)	5.5 (5.4–6.2)

*RR calculated from LC_{50} values.
**RR from LC_{90} values.

different *Ae. aegypti* lineages in different regions of Argentina, one of which shared two haplotypes between the Salta and Bolivia populations (Albrieu-Llinas and Gardenal 2011). This can give an idea of *Aedes* mosquito movements in both regions and explain the sudden emergence of resistance to *cis*-permethrin. In addition, a study has determined high levels of resistance to deltamethrin linked to deficient chemical control actions in kissing bugs of the species *Triatoma infestans* (Klug) collected in the city of Salvador Mazza (Fronza 2019). The coexistence of vectors, *Ae. aegypti* and *T. infestans*, is recognized in this locality, and chemical control policies for one species may have an indirect impact on the other vector.

Until now the DD for *cis*-permethrin was not known; however, this chemical was the most commonly used pyrethroid in Argentina for controlling *Ae. aegypti* adults in the last 20 yr. We calculated the DD for *cis*-permethrin and the LC_{50} and LC_{90} values for the susceptible strain and the field population evaluated (Table 2). The LC_{50} for the susceptible CIPEIN strain was 0.03%, whereas the LC_{90} was 0.11%. The LC_{99} value was 0.29% and the DD, defined as $LC_{99} \times 2$, was 0.60% slightly below that of permethrin. The LC_{50} for the field population of Salvador Mazza was 0.35%, whereas the LC_{90} was 0.62%. Based on the RR_{50} (10.3) and RR_{90} (5.5) for the population in response to *cis*-permethrin, resistance is classified as high and medium, according to WHO (2016) definitions (Table 2).

Usually, the RR_{50} is the method of choice to determine the resistance status because their CI_{95} is smaller compared with RR_{90}. However, RR_{90} is complementary to RR_{50} when the dose–response curves of the field populations and the reference strain are not parallel, like in this study. A smaller slope of the field population, which leads to RR_{90} greater than RR_{50}, is an indicator that resistance is developing and that the population is heterogeneous, probably with susceptible and resistant individuals (Braga et al. 2004). Conversely, in this study, RR_{90} was lower than RR_{50}, indicating that resistant genes could be setting in the population, but even considering either of the two parameters the population of Salvador Mazza is considered resistant.

This study is the first to demonstrate the existence of resistance to pyrethroids in a population of *Ae. aegypti* in Argentina and the susceptibility of this population to the organophosphate, malathion. It is also the first to calculate the DD following WHO (2016) guidelines for *cis*-permethrin, the most commonly used insecticide to control *Ae. aegypti* in Argentina. These findings are important for decision makers of vector control programs in Argentina in the context of an Integrated Vector Management (IVM) and suggest the urgent need to use a new insecticide with a different mode of action in this area. The geographic extent of resistance is unknown but our laboratory is currently studying it as well as the biochemical and molecular mechanisms involved.

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