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Original article

Resistance to insecticides in populations of Aedes aegypti in the province of Manabí, Ecuador

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Abstract

The frequent and inappropriate use of insecticides has been one of the factors leading to the development of resistant mosquito populations. In Ecuador, studies on insecticide resistance have been limited, and a loss of susceptibility to the insecticides deltamethrin and temephos has been identified. The aim of this research was to describe the state of resistance in Ae. aegypti, to the main insecticides used for vector control, in the province of Manabí. A descriptive study was conducted in Ae. aegypti populations from 13 municipalities in the province during the year 2020. Individuals collected were evaluated using diagnostic dose bioassays with the insecticide's malathion and deltamethrin, as well as serial doses of temephos. Lethal concentrations were determined for samples collected in the field and compared with the susceptible reference strain ROCK MRA-734. Mosquitoes from 13 localities exhibited widespread resistance to deltamethrin, one locality showed resistance to the insecticide malathion, and four localities displayed resistance to temephos. These findings may be influenced by the lack of control of Aedes aegypti populations due to the development of insecticide resistance and the increasing incidence of arbovirus cases in the province. Therefore, it is considered important to have accessible information for the development of prevention and control strategies at both national and local levels that can efficiently contribute to the use of insecticides.

Keywords: Insecticides, Insecticide Resistance, Vector control, Aedes aegypti, Dengue.

Introduction

Arboviral diseases are acute febrile illnesses prevalent in tropical and subtropical regions, causing morbidity and mortality on a global scale. According to the World Health Organization (WHO), at least 2.5 billion people are at risk of contracting these diseases, making them a significant public health concern.

Aedes aegypti is the primary vector for various arboviral diseases such as dengue, yellow fever, Zika, chikungunya, and Rift Valley fever, leading to outbreaks in both urban and rural populations. Its anthropophilic adaptation and widespread global distribution, driven by population growth, international trade, and adaptation to new ecosystems due to sociodemographic factors, have expanded its vector capacity to regions where it was not previously considered a risk.

Dengue is the most infectious disease among all arboviral diseases due to its high prevalence, rapid spread, and high mortality rates, resulting in approximately 390 million infections annually.

In Ecuador, dengue is one of the primary mosquito-transmitted febrile diseases, with active transmission in around 80% of the country's provinces, especially those with warm, humid, and tropical climates.

As of 2021, up to epidemiological week 52, a total of 20,592 dengue cases have been reported nationally, with a high incidence in the provinces of Guayas, Manabí, Esmeraldas, and Santo Domingo de los Tsáchilas.

Currently, in the absence of specific treatments and vaccines for arboviral diseases, vector control remains the primary strategy to reduce, prevent, and manage epidemic outbreaks. Public health vector control strategies are based on active community participation, health education programs, environmental management, physical control of breeding sites, and the use of insecticides.

In Ecuador, control methods employed by the Ministry of Health include community involvement in eliminating mosquito breeding sites, residual spraying with deltamethrin, spatial spraying with malathion, and targeted control of mosquito breeding sites using the insecticide temephos. All these strategies have contributed to the control of Aedes aegypti populations during outbreak notifications and preventive activities.

Chemical vector control, considered one of the primary strategies since the 19th century, has exerted selective pressures on mosquito populations, reducing the effectiveness of vector control programs. Insecticide resistance has become one of the primary challenges for vector control, developed through hereditary genetic mechanisms within populations and detoxification mechanisms that diminish the target site of insecticides.

In Latin America, resistance to insecticides such as deltamethrin, malathion, temephos, DDT, and cypermethrin has been reported in countries including Brazil, Peru, Paraguay, Colombia, El Salvador, Ecuador, and Mexico.

In Ecuador, insecticide resistance is a relatively understudied phenomenon and poses a challenge to control programs due to limited information. According to available reports, resistance to deltamethrin has been identified in the provinces of Machala, Santo Domingo de los Tsáchilas, Esmeraldas, Manabí, Guayas, and Sucumbíos. However, susceptibility to malathion and temephos has been determined in the same provinces.

Studies on resistance mechanisms have shown that populations in Machala have developed KDR resistance genes, which are linked to resistance to pyrethroids.

Based on the studies of insecticide resistance conducted in the country, there remains a lack of information to guide vector control program decisions. A more specific analysis of the behavior of Ae. aegypti populations regarding insecticide resistance will enable operational vector control programs to develop new strategies in collaboration with government institutions, municipalities, and communities. This collaboration, supported by scientific information, will lead to improved investment in operational costs and the implementation of policies for the proper use of insecticides.

The objective of the present study was to characterize the resistance status to the insecticides deltamethrin, malathion, and temephos in 13 cantons of the province of Manabí. This information aims to contribute to understanding the resistance status and assist in the development of strategies for managing insecticide resistance and integrating it into vector management.

Materials and Methods

Study Area

The study was conducted in 13 cantons within the province of Manabí. According to epidemiological reports, the province of Manabí exhibits a high incidence of dengue cases, which is associated with socio-economic, cultural, entomological, and environmental factors that contribute to the disease's prevalence.

Methodology

Entomological sampling was conducted throughout the year 2020, involving the active search for Ae. aegypti larval stage individuals within households. A minimum of 30 houses was sampled in each locality.

The mosquito larval stages were transported to the entomology laboratory at the Manta District 13D02 for rearing, maintenance, and morphological identification.

The larvae were reared in containers with 500 individuals per liter of water, maintaining a controlled average temperature of 27°C and a 12-hour photoperiod. When the adult mosquitoes emerged, they were kept in breeding cages and fed with a 10% sucrose solution before exposure to insecticide tests.

Insecticide bioassays were conducted using technical grade deltamethrin with a purity of 99% (Dr. Ehrenstorfert, Augsburg, Germany) and malathion with a purity of 98.5% (Dr. Ehrenstorfer, Augsburg, Germany).

Mosquitoes of the F1 generation for each locality (n=100) were exposed to diagnostic doses of deltamethrin (10.46 ppm) and malathion (55 ppm), following the protocol of the Centers for Disease Control and Prevention (CDC).

Mortality of mosquitoes exposed to insecticides was observed every 15 minutes for a total of 120 minutes. A control group consisting of a bottle impregnated with ethanol was used.

Bioassays for mosquito larvae with the insecticide temephos were conducted following WHO protocols. Third and early fourth-stage larvae were used. A series of concentrations (0.005, 0.025, 0.125, and 0.625 ppm) were employed to obtain the lethal concentration value (LC50). Control groups were included, containing ethanol. Mortality observations were recorded after 24 hours of exposure to the insecticide. Four replicates of 25 larvae were tested for each locality and concentration (n = 400).

For deltamethrin and malathion, mortality criteria were based on guidelines provided by the Centers for Disease Control and Prevention (CDC), which defined: mortality >98% as a susceptible population, mortality between 90% and 97% as a population with potential resistance, and mortality below 90% as a resistant population.

Mortality criteria for the insecticide temephos were established based on resistance ratios (RR50 and RR95), calculated as the ratio of LC50 for field strains divided by the LC50 of the Rockefeller susceptible strain. The RR50 scale for the population was defined as RR50 of 0 to 5 for susceptibility, RR50 of 5 to 10 for moderate resistance, and RR50 greater than 10 for confirmed resistance.

Conclusión

Thirteen bioassays were conducted on adult mosquitoes using the insecticide deltamethrin in the cantons of Pedernales, Jama, Rocafuerte, Flavio Alfaro, Portoviejo, Junín, Calceta, Tosagua, Manta, Sucre, San Vicente, Montecristi, and 24 de Mayo. All the analyzed localities exhibited resistance to the deltamethrin insecticide, as they showed mortality percentages below 98% (Figure 1).

For the analysis with the malathion insecticide, the cantons of Pedernales, Jama, Rocafuerte, Flavio Alfaro, Portoviejo, Tosagua, Manta, Sucre, Montecristi, and 24 de Mayo were evaluated. All the examined localities demonstrated susceptibility to the insecticide, with 100% mortality. However, the locality of Jama presented a 96% mortality rate, indicating a potential resistance (Figure 2).

With the temephos insecticide, populations from 12 cantons were evaluated, of which eight cantons exhibited susceptibility to the insecticide (RR50 0.65 to 4.12). The cantons of Portoviejo, Manta, Sucre, and Montecristi showed resistance to the insecticide, with RR50 values ranging from 5.10 to 6.17 (Figure 3).

The distribution of insecticide resistance in the 13 cantons was analyzed. In Figure 4, the overall distribution of resistance to the deltamethrin insecticide, susceptibility to the deltamethrin insecticide, and resistance to temephos in the cantons of Manta, Montecristi, Portoviejo, and Sucre can be observed.

Discussion

Due to the absence of suitable treatments and specific vaccines for arboviral diseases, vector control is considered the only effective means to control and prevent these diseases. The development of vaccines for arboviral diseases such as dengue has taken 30 years of experimentation, resulting in safe prototypes. However, only one candidate, CYD-TDV by Sanofi Pasteur, Lyon, France, corresponds to a DNA-inactivated vaccine, which has successfully passed phase III trials and remains under testing.

The limitation in vaccine development is linked to a lack of understanding of the correlation and interaction between the four serotypes, preventing a tetravalent immune response. Other innovative strategies for Aedes aegypti control, such as the sterile insect technique, the release of insects with dominant lethality, and mosquito infection with Wolbachia, offer additional options for arboviral control. However, their implementation requires commitment from national and local authorities, local entomological research, and the ongoing availability of human and financial resources.

Currently, the use of chemical insecticides is the most effective alternative for many low-resource countries, where the implementation of new control strategies is limited. For this reason, to maintain the effectiveness of control programs, monitoring insecticide resistance represents a fundamental tool for local programs, providing the basis for evidence-based decision-making. In this study, resistance to the deltamethrin insecticide was demonstrated in the 13 cantons analyzed and its distribution in all observed cantons in the province of Manabí. The continued pressure exerted by these insecticides on mosquito populations has led to the development of various cross-resistance mechanisms between DDT and pyrethroid insecticides like deltamethrin.

In Ecuador, DDT was used in mosquito vector control programs for malaria and dengue from 1957 until 2002 when it was replaced by the deltamethrin insecticide. Due to this intensive use of insecticide molecules and the shared target action, the distribution of deltamethrin resistance has been reported in several provinces in Ecuador. In Latin America, resistance to deltamethrin is a widespread issue in several countries like Peru, Guatemala, Brazil, Venezuela, and Cuba, leading to the exploration of alternative control methods.

Organophosphate insecticides such as malathion and temephos have been widely used in the region for Aedes aegypti control. Malathion susceptibility has been generally reported in countries like Colombia and Venezuela, despite its intensive application in vector control programs. In the province of Manabí, susceptibility to the insecticide was similarly noted, except for the Jama locality, which exhibited 96% mortality. This may be linked to the development of carboxylesterase enzyme-related resistance. In Ecuador, the vector control program uses malathion solely for disease outbreak control, reducing its pressure and combining it with other insecticides like deltamethrin. The insecticide temephos was approved by the World Health Organization for use in drinking water, and thus, it was the first-choice insecticide for vector control programs due to its effectiveness and extended residual activity. With the intensive use of this insecticide molecule and selective pressure, various levels of resistance have been reported in Aedes aegypti populations in Cuba, Venezuela, Costa Rica, Panama, Nicaragua, Jamaica, and Brazil.

This study reports temephos resistance with RR50 values ranging from (5.26x to 6.16x) in four of the analyzed cantons, which can be associated with detoxification by carboxylesterases. In Brazil, because of temephos resistance, new control strategies were adopted, including the use of biological insecticides like Bacillus thuringiensis var. israelensis (Bti) and insect growth regulators (IGR). The aim of this strategy was to preserve the proper use of the insecticide, primarily to reduce selective pressure.

Insecticide resistance can potentially compromise vector control measures and result in unnecessary operational costs with the acquisition of ineffective insecticides. Having information about insecticide resistance contributes to the development of management strategies or the incorporation of authorized alternative molecules for application. A vector control program focused on operational measures applicable to its reality will contribute to reducing the incidence of arboviral diseases.

Conclusion

Vector control remains the primary strategy for preventing and controlling the transmission of arboviral diseases due to the absence of specific treatments or vaccines. The use of deltamethrin, malathion, and temephos in Ecuador has been a fundamental pillar in the chemical control strategy. However, the selective pressure from the continuous use of these insecticide molecules has led to resistance in these populations, reducing the impact of control program strategies.

Preliminary studies in Ecuador indicate that insecticide resistance has been associated with various genetic and enzymatic mechanisms, maintaining its continuous development in populations.

A more focused analysis of the dynamics of insecticide resistance distribution will enable the timely development and targeting of strategies by national and local entities, optimizing intervention costs and achieving integrated management.

Currently, the National Vector Reference Center of INSPI-LIP and the National Network of Entomology Laboratories monitor insecticide resistance in the country. Technical and operational strengthening of personnel has contributed to maintaining and expanding surveillance at the national level.

Conflict of Interest: The authors declare that they have no conflicts of interest, and the content of the manuscript has not been previously published.

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Figure 1: Results of the evaluation of resistance to the insecticide deltamethrin in the Province of Manabí.

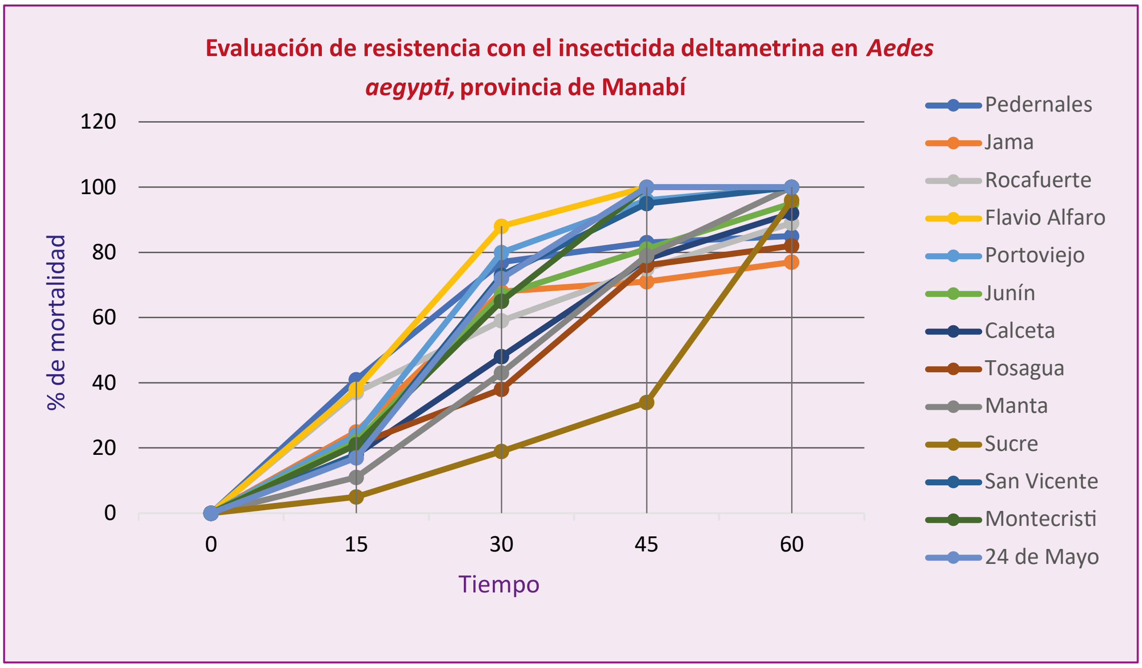

Figure 2: Results of the evaluation of resistance to the insecticide malathion in the Province of Manabí.

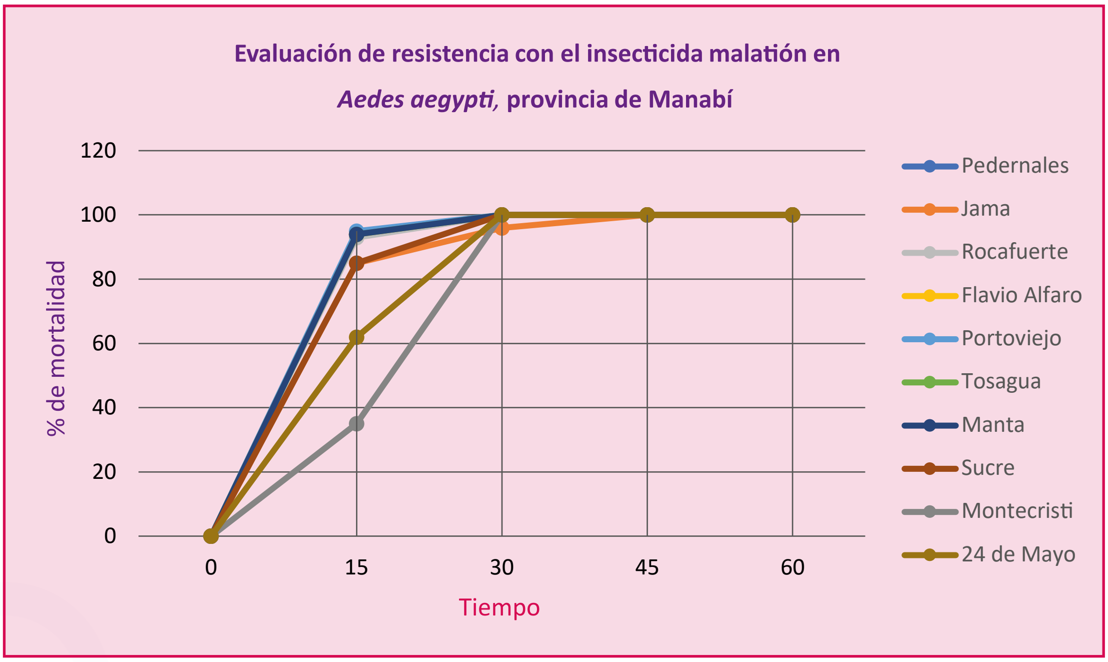

Figure 3: Results of the evaluation of resistance to temephos insecticide in the Province of Manabí.

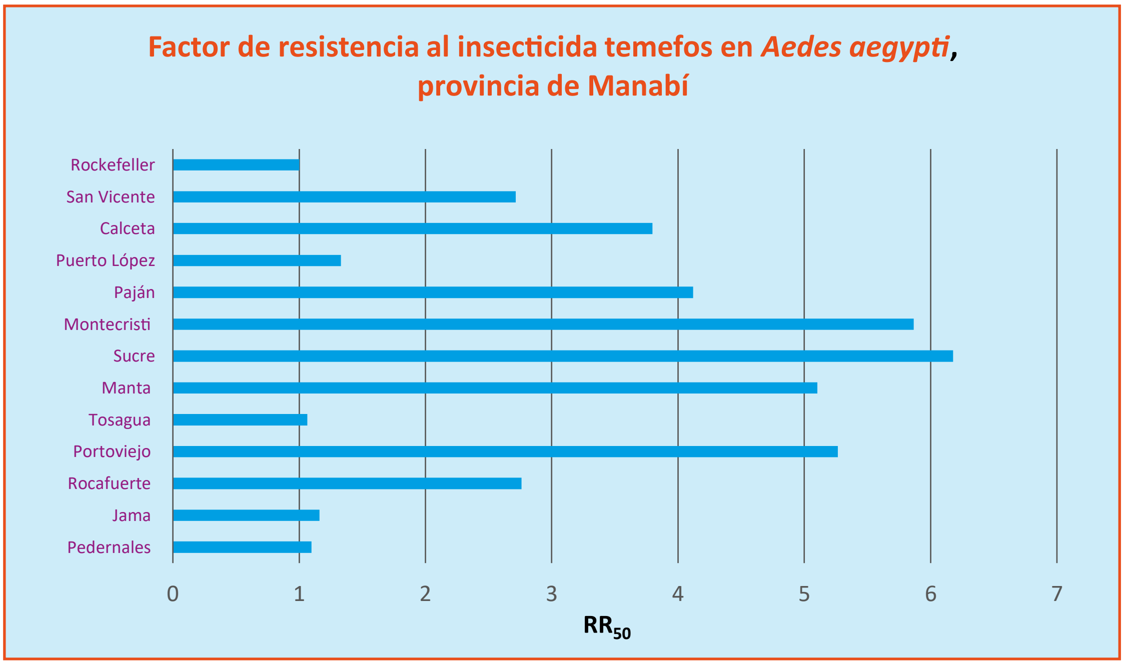

Figure 4: Distribution of insecticide resistance in the Province of Manabí