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Entomological Impact and Current Perceptions of Novaluron and Temephos against the *Aedes Aegypti* (Skuse) Vector of Dengue, Chikungunya and Zika Arboviruses in a Coastal Town in Ecuador

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Abstract

Objective: Two larvicides, temephos (Abate®) and novaluron (Mosquiron®) were compared in a field trial in Colonche, Ecuador against *Aedes aegypti* (Skuse). Community perceptions of dengue fever along with acceptance of new methods were evaluated.

Methods: Homes were inspected for water storage containers and the evaluation of two larvicides was conducted post-application to 189L drums citywide. The city was split in half using the main street to assign treatment areas: the northern part of the city received temephos in 43 drums, while the southern side of the city received novaluron applications in 66 drums. *Aedes* population density was assessed by eggs counts. Eggs were collected from oviposition traps every 7days for 56 days. A survey was administered to the community to determine dengue related knowledge and risk perception.

Results: Eggs were reduced from an average of 37 and 15 to 0 at day 7 post-treatment for the temephos and novaluron sites, respectively. Significant reductions were found only at days 7 and 14 for both treatment sites. Most of the respondents thought that protection against mosquito bites was important, however only 50% of them knew about either the locations of larval habitats or source reduction practices. About 16% reported they did not want to use a different larvicide than temephos. Yet, some participants reported concerns about temephos applications.

Conclusion: Results showed that fewer eggs were collected at pre and post-treatment but were apparently lower in the novaluron site. Both interventions were effective up to 15 days post-application. Short effect could be explained by the lack of knowledge in the community about dengue prevention practices. Interventions need to be tailored to the local ecology and social conditions so they can be effective, thus such should follow the principles of integrated vector management.

Keywords

Novaluron; Temephos; Integrated vector management; Community perceptions

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Introduction

The public health consequences of dengue fever (DF), chikungunya (CHIK), and Zika include deaths, arthritis complications, and possibly microcephaly in newborns [1-3]. *Aedes aegypti* is the mosquito vector of these arboviruses [4]. The world health organization (WHO) stated that proximity of this mosquito breeding sites to human quarters poses a significant risk of disease transmission [5]. Thus, in absence of vaccine, control of epidemics relies on integrated vector management (IVM) [6]. Findings in Ecuador propose to target control in containers that supports the development of *Ae. aegypti* near or within household premises [7].

Many coastal cities of Ecuador are at risk of vector-borne arbovirus transmission due to their proximity to major highways that connect individuals, pathogens, and mosquitoes across the country [8]. Human migration, urbanization, climate change, viral incubation, and distribution of dengue virus and its vectors, are all factors that worsen driven epidemics [9]. *Ae aegypti* thrives in newly urbanized areas as they start to acquire basic infrastructures such as water access or sewage services [10]. Given the irregularity of water services (i.e. water supply interruptions), residents store their water in 50 gallons drums, which has become an idiosyncratic behavior in urban and rural areas [11,12]. multi-country studies have implicated these drums as the main production site of *Ae. aegypti* mosquito larvae in five urban settings in Latin America (LA) including Ecuador [12].

Routinely, city residents allow inspectors to treat their drums with chemicals against larvae [11]. Susceptibility studies to temephos, an organophosphate (OP) used against mosquito larvae, have been done in selected communities in Ecuador [13]. Findings show a resistance factor RR50 causing only 50% mortality in specific larval strains found in the main port of the country [13]. Such situation reflects upon the general LA environment where, in larval bioassays, temephos resistance was found to be high in larval strains from Cuba, Costa Rica, Jamaica, and Peru, and moderate in larvae from Nicaragua and Venezuela [14]. To reduce resistance to OPs, it is recommended to rotate the application of insecticides classes with different modes of action, one of the main themes of IVM [15]. This may not be possible in specific countries where vector control specialists may only have one larvicide available.

As new agents become available, public health specialists need to incorporate social-ecological sciences in a successful IVM implementation plan. Locally, investigators found associations of increased presence of *Ae. aegypti* pupae and social variables including water storage practices, water infrastructure, housing conditions, and risk perceptions [16]. Therefore, including certain components, like improved infrastructures, and not others, such as education and local adaptation, can affect the outcomes of vector control interventions [17,18]. Novel insecticide implementation based on IVM strategies necessitates from adequate tailoring to each community [19].

For these reasons, our research monitors the entomological impact of two larvicides against *Ae. aegypti* mosquitoes in a field trial. This report also presents local perceptions in reference to dengue in a coastal town of Ecuador.

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Methods

Ethical considerations

Ethical considerations for this trial were discussed with the director and board members of the Ecuadorian former *Servicio Nacional de la Erradicación de la Malaria y otras Enfermedades transmitidas por Vectores Artrópodos* (SNEM). SNEM technicians conducted the application of the treatments, facilitated entomological surveillance, and supervised the distribution of surveys in the community of Colonche. Household members that participated in the study granted consent to the SNEM workers to perform larvicide applications and surveillance visits.

Study site

The study was conducted in Colonche, Ecuador (2˚1'21" S, 80˚39'59" W). The town is located at the margins of the Pacific Ocean, roughly 560 km southwest of the capital, Quito, Ecuador. Similar to other coastal towns in Ecuador, Colonche experiences two seasons. The rainy and humid season starts in December and ends in May while the rest of the year is dry and cool. Temperatures in the coastal region fluctuate between 26˚C to 30˚C [20].

There are two paved streets in the city of Colonche and four adjacent dirt roads. The town is semi-urbanized (Figure 1) and homes consist of one-story houses built with cement blocks. A few homes on the outskirts of the city are made of wood. Home flooring consisted of ceramic, paved cement, or dirt. Most of the homes have a dirt patio where the residents keep water containers, cultivate plants, and keep animals (dogs, goats, sheep, and chickens). Drums and containers were covered or uncovered and located inside or outside of the household. The continuity between indoors and outdoors is one characteristic of this coastal town. Physical barriers between the inside and outside home environment such as windows screens are rare.

Field trial

Treatment assignment: The trial started in May 2014 and ended in July 2014 in the middle of the dry season. As a reference, we considered the main street as the dividing line that separates the town of Colonche into a northern and southern area. Colonche consisted of roughly 278 homes separated in 43 blocks at the time of the trial. There were 159 homes on the northern portion of Colonche and 119 homes on the southern half of the town. Both sides were inspected for containers that held water and were positive for the presence of Aedes larvae. We randomly assigned treatment applications. The northern portion of the town received temephos applications while the southern part of the community received novaluron applications. We were not able to assign a control area due to ethical issues.

Treatment application: containers and treatment: At the inspected homes, the containers suitable for larval habitats were classified as drums, elevated drums, tires, cisterns, barrels, and tins. Other small containers were classified as others and included spoons, bottle caps, or sliced plastic bottles.

SNEM inspectors treated containers and larval habitats with the government standard treatment, which is the OP larvicide temephos, (Abate®, Clarke Mosquito Control Products Inc., Roselle, IL). OPs impair nerve signal transmission by inhibiting the enzyme acetyl cholinesterase [21]. Government workers also facilitated the application of novaluron (Mosquiron® 0.12 CRD Controlled Release Formulation, Makhteshim Chemical Works Ltd., Hamilton, Canada). Novaluron inhibits formation of the insect cuticle by inhibiting chitin synthesis (CSI), a major constituent of the insect cuticle. CSIs, applied to control the larval stage of the mosquito, impede the development from larva to adult annulling the opportunity of the mosquito to bite an infected individual and transmit a given virus [22]. The WHO and the Environmental Protection Agency (EPA) in the US and the Pest Management Regulatory Agency (PMRA) in Canada have approved temephos and novaluron agents to treat domestic water sources against *Aedes* mosquitoes [23-25].

Larvicides were applied to the larval habitats by hand. Temephos was applied following the WHO guidelines of 20 grams of granular product per 189 L and rates did not exceeded more than 1 mg/L [26]. The novaluron application was done to water-holding containers following the label instructions of Mosquiron 0.12 CRD. The application rate was done at one briquette of the commercial presentation to each 50-gallon drum (application rate 0.035 – 0.070 Lbs per 50 gallons) [24]. The briquettes that were introduced to the

Figure 1: Aerial view of Colonche, Ecuador where 12 oviposition traps were placed to track adult *Aedes aegypti* egg-laying behavior before and after larvicide treatments.

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drums had the appearance of personal soap bars. They disintegrated into a powder mass at the bottom of the containers becoming less visible as weeks passed.

Entomological monitoring: A classic measure of *Ae. aegypti* population density are egg counts from oviposition traps [27]. Such are often used to measure the effectiveness of treatments against *Aedes* mosquitoes in the field [28]. Traps, made of dark green plastic cemetery vases (Figure 2) Factory Direct Craft Supply®, Springboro, OH), were placed in the two experimental sites. The placement remained constant throughout the trial. Figure 1 illustrates the site location of the 12 ovipostion traps; 6 traps were placed in the temephos treatment site and 6 in the novaluron treatment site. A sheet of oviposition paper was placed around the inside wall of the trap. Traps were 3/4 filled with local piped water. Oviposition paper was replaced weekly. Egg collections started 7 days prior treatment. Eggs were collected and counted using a magnifying glass to observe such in the oviposition paper. This process was conducted for a period of 42 days. Time wise, we set up the ovitraps 7 days prior treatment

Figure 2: Placement of 12 oviposition traps in the temephos (T) and novaluron (N) application sites in the coastal city of Colonche.

so we could collect the first oviposition paper and egg records on the treatment day or day -7 (as seen on Table 1) and continue this process for 42 days.

Measures of dengue knowledge in the community: A survey was developed based on the research of Nazareth and colleagues in 2014 to assess dengue risk perception and knowledge [29]. As part of the survey, we collected demographic data such as gender, education level attained, occupation, and age to understand the composition of the community. We measured dengue perceptions through five parameters: knowledge of dengue (identification of viral disease transmitted by mosquitoes), knowledge of breeding sites (being able to identify at least three larval habitats), knowledge of preventative measures (identify what measures they can use to protect themselves against mosquito bites), and acceptance of new larvicides (questioned if they were open to trying a new larvicide instead of temephos).

We surveyed every third home starting from the first house at the northeast corner. We used a community-based map that was provided by the local authorities of Colonche. The self-reported head of household was selected to complete the survey after verbal consent. Households that were assigned to host oviposition traps and homes selected for the entomological surveillance were excluded.

Statistical analysis

Summary statistics provided information about number of containers and positive containers. To assess the impact of the larvicides application on the adult mosquito population, number of eggs collected through ovitraps were counted 7 days prior treatment (day -7) and after treatment (day 0 through day 42) every 7th day. A Poison distribution was used to obtain the mean number of eggs collected in each treatment site per time. A negative binomial analysis was carried out to compare larvicide treatment effect per time. Egg counts represented the outcome variable. Dependent variables used were site, treatment type (temephos or novaluron), and time in days. For the community survey we used frequency procedures to obtain percentages. The final sample size was made of 75 household respondents (in 75 houses) and 100% response rate. All analyses were carried using the SAS statistical software version 8.01 (SAS Institute 2001).

Results

Field trial

Treatment application: Inspectors performed applications to 262 wet containers and identified 876 larval habitats in the community. While wet containers were treated, other smaller and diverse breeding sites were destroyed. Our treatment application efforts were guided towards all identified drums, which made up 12% of the total number of breeding sites (n=876). There were 109 drums in the town of Colonche, from which 25.7% were positive for the presence of *Ae. aegypti* larvae. We applied temephos to 43 drums and novaluron to 66 drums.

Entomological monitoring: Relative to mean collection, Figure 3 shows the average number of eggs collected through oviposition traps. At pretreatment, day -7, the mean number of eggs per ovitrap for the temephos site were 37.5 (SE ± 8.45 DF=70) while for the novaluron site, mean collections of eggs were of 15.6 (SE ± 3.7 DF=70). Seven days post-treatment, the two sites, temephos and novaluron, showed an equally substantial reduction of eggs with mean collections of 0.9 (SE ± 0.4 DF=70) and 0.3 (SE ± 0.2 DF=70).

Table 1 also portrays that treatments stayed low at day 0 and 7 post-treatment with no significant difference between treatment sites (p=0.28, p=0.9). By days 14, 21, and 28 post-treatment egg counts

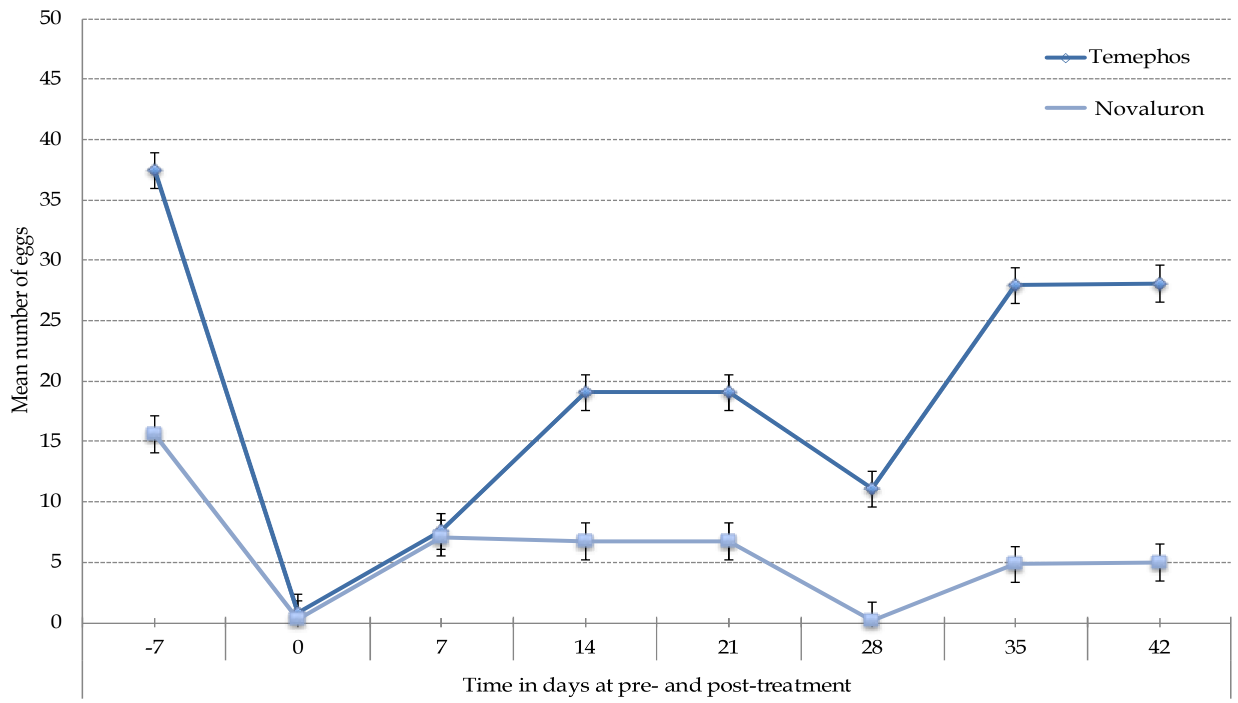

Figure 3: Average number of eggs collected through ovitposition traps 7 days before the intervention at pre-treatment day 0 and at 49 days post-treatment at the novaluron and temephos treated sites.

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Table 1: Simple effect comparisons of temephos and novaluron treatments least squares means by time, along with standard errors (SE), p values, and degrees of freedom (DF).

Time in days at pre- and post-treatment	Estimate	SE	p-value	DF
-7	0.874	0.33	0.009	70
0	0.991	0.873	0.260	70
7	0.075	0.368	0.839	70
14	1.044	0.354	0.004	70
21	1.044	0.354	0.004	70
28	4.262	1.052	<0.001	70
35	1.752	0.364	<0.001	70
42	1.725	0.362	<0.001	70

remained low. However, by day 35 and 42, eggs counts increased to numbers that were similar to pre-treatment numbers. At day 42, the mean number of eggs collected was 28.1 (SE ± 6.4 DF=70) at the temephos site, and 5.0 (SE±1.4 DF=70) at the novaluron site. There were differences in treatment application by time.

Dengue knowledge in the community: Demographic information of the population is found in Table 2. Respondents were mostly females (69.3%) and worked taking care of the household. Almost 70% had completed primary school but not finished high school. About 60% of the respondents were between the ages of 18 and 45 years old. Nearly 10% of the respondents were over the age of 75.

On the dengue relevance section of the survey the majority were able to define dengue and how it was transmitted. The majority acknowledged the importance of protecting themselves against mosquito bites.

In the larval habitat identification section, about 55% of respondents were able to identify at least 3 breeding sites. When assessing the knowledge of how to reduce Aedes larval habitats, 40% of the respondents stated that they performed larviciding and they also used commercial brands of insecticides to spray inside their homes. Fifty five percent stated that they practiced source reduction.

When asked about their knowledge of immediate protection practices, over 60% responded they did not know about protection practices. Others responded that they use protective clothing and adulticiding. Less than 15% of the participants responded that they used repellants and bed nets to avoid being bitten by mosquitoes. Results of the introduction of new treatments to control larvae showed that the 69% of surveyed individuals will accept a new larvicide different than temephos; about 16% will not and 15% are not sure.

Discussion

Although this trial was done in the dry season, *Ae. aegypti* breeds year-round in coastal Ecuador. This indicates IVM and control strategies should be encouraged not only in the wet season but extended to periods with little to no rain. We highlight three main discussion points.

First, the novaluron treatment application site had fewer eggs throughout the trial when compared to the temephos treatment application site, at at pre- and post-treatment. Our findings are consistent with semi-field trials in Mexico that demonstrated that larval populations are reduced significantly in treated containers, and that novaluron residual effect lasts longer than temephos [30]. This suggests that slow release agents may work for longer periods of time regardless of environmental conditions and water storage practices. In our trial, treatments were subject to the influence of human behavior relative to water storage, such as restitution rates. Similar to our findings, studies describe the use of an insect growth regulator, diflubenzuron, in Brazil [31]. Accounting for a 20% daily water replacement in tanks diflubenzuron emergence inhibition was 98% in the first 30 days, and 80% at day 46 [31]. Laboratory evaluations have also proved a larval emergence inhibition of 96% and 80% at high and low concentrations of novaluron providing adequate control of 90 to 190 days [32].

A second important implication is feasibility relative to the local environment. Feasibility is an important aspect when implementing novel agents. When we compared novaluron against the standard treatment, fewer eggs were found in the novaluron treatment site. Both sites showed a significant reduction in egg counts within the first 14 days post treatment application, compared with pretreatment numbers. Yet, at day 14 and thereafter, eggs counts increased. Findings suggest that the intervention with both agents was feasible as they had similar application methods and similar results in reduction of eggs. However, treatments were successful for a short period of time. They both require either reapplications and/or more visits.

Possible explanations to the limited control can be found in our survey results and behavioral characteristics. Regarding survey results, less than 54% of our respondents stated that they performed any type of source reduction, larviciding or adulticiding at home. Control interventions may only work if the community is engaged to sustain the effects of interventions. This includes not only applying new agents but also identifying and reducing the number of available larval habitats near household premises. These findings mirror the research results done in a different coastal town of Ecuador in 2014 where researchers unveiled that residents wrongfully thought mosquitoes did not breed inside their houses or in clean water [33]. Regarding behavior, although all treatable water containers were treated and all larval habitats were destroyed, residents would still keep some containers around homes post-inspections or fill up drums that were coded as empty at the time of inspection. Such behavior highlights again the need of IVM approaches incorporating social sciences and improving infrastructure such as water access and trash collection services.

Our third key finding was that 16% of the survey participants would not accept a new larvicide and almost 15% were unsure about trying new methods. Although it was not a formal question, some respondents stated their concern about a larvicide being applied to their water, which may be used for domestic purposes (bathing, watering plants). Also, we observed the majority of the households rely on bottle water for drinking purposes. Individuals also stated concerns about domestic water and temephos. Although the EPA, the PMRA, and the WHO approve novaluron and temephos in domestic water [23], the ultimate permission for application has to be given by community. Aedes control can be more effective and feasible if interventions are based in the community acceptance, integrated, ecologically tailored [34]. We argued that the educational campaigns and interventions should start by obtaining baseline information to identify the gaps in knowledge. This can help addressing the main concerns of the communities where interventions will take place.

Our study had limitations; the design was ecological and we were not able to control for outside factors like human behavior or climate conditions. Manpower, government resources, and ethical considerations to support a control arm limited our study. Budget also constrained the period of evaluation. For instance, at the time of this trial in the dry season, there were a high number of dengue cases in the neighboring

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Table 2: Community profile and survey results presented as percentage (%) of respondents in Colonche, Ecuador

Community profile based on respondents		%
Gender	Male	30.6
	Female	69.3
Occupation*	Homemaker	62.7
	Agricultural worker	8.0
	Retired	8.0
	Unemployed	5.3
Education level completed	< Grade school	4.0
	Completed grade school	68.0
	Completed high school	22.7
	> High school	5.3
Age categories (years)	18-25	21.3
	26-45	40.0
	46-65	28.0
	>65	10.7
Survey results		
a) Dengue relevance	Correctly defined dengue	86.2
	Thought that protecting themselves against the bite of the *Aedes* mosquito was important	98.7
b) Larval habitat knowledge	Were able to cite at least 3 larval habitats	54.7
c) Protection practices	Use any type of protection	64.0
	Use adulticide at home	57.3
	Use protective clothing	16.0
	Use bednets	8.0
	Use repellents	4.0
d) Accept new larvicide	Yes	69.3
	No	16.0
	Don't know	14.7

**Categories such as business owners, construction workers, firefighter, teachers, and technical workers represented less than 4% each*

towns that needed control efforts. This limited the help provided by government workers and our ability to hire technicians. Comprehensive assessments should include year-round monitoring.

This study provides information on novaluron and temephos entomological impact in a coastal town in Ecuador. Both agents significantly reduced egg counts at day through day 14 but the effect was not sustainable. This research highlights the feasibility introducing a new agent, like novaluron, and the entomological monitoring through ovitraps. We also assessed the community current knowledge of dengue fever and related practices. Cost effectiveness of novaluron is yet to be explored, but we could suggest that the agent may need fewer applications. Although, novel agents, like novaluron, require lower dosages and provide longer residual effects, evaluation of other IVM components is important. These should include the integration of different larvicides and adulticides, seasonal assessments, and community evaluations. In addition, control programs can explore how to incorporate the community to better sustain interventions, regardless of the insecticide selection. The introduction of novel field-tested agents that enhance the current strategies is an example of an alternative tool, from an IVM perspective.

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Competing Interests

The authors declare they have no competing interests.

Author's Contributions

All authors participated equally in the design, implementation, and write up of this research project and met the criteria for authorship according to the Vector Biology Journal.

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