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LARVAE COLLECTION BY SCHOOL CHILDREN BEFORE AND AFTER AN EDUCATIONAL INTERVENTION ON *Aedes aegypti* Linnaeus 1762 (Diptera: CULICIDAE)

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Abstract: Mosquitoes are a public health problem in the world. In Mexico, *Aedes aegypti* is being studied because of its medical importance. Strategies are sought to control breeding sites that do not contaminate and are economical, and health education in school children is explored. The impact of an entomological inspection carried out by school children before and after an educational intervention on the *A. aegypti* mosquito was evaluated. During the educational intervention, they carried out the inspection in the school to train them. At the end of the workshop, they carried out the first inspection in their homes and the second one a month later. Knowledge of mosquito morphology and the viral origin of Dengue, Chikungunya and Zika was significantly modified. No larvae were found in schools. In the first inspection there are 599 containers and 67 in the second (tanks, drums, cans and buckets). There were 464 containers with larvae in the first search and 55 in the second (tanks, drums, cans and buckets). Four species were found, *A. aegypti, A. epactius*, *Culex coronator* and *Culex quinquefasciatus*. The preferred breeding sites for *A. aegypti* were tanks and drums. The educational workshop had a favorable impact on schoolchildren's knowledge and was reflected in the reduction of breeding sites.
Keywords: vector, physical control of breeding sites, knowledge, health education.

INTRODUCTION

Mosquitoes represent a public health problem due to the transmission of protozoa, arboviruses and microfilariae that makes them the most clinically important arthropods (Benelli, 2018) . In Mexico, most studies are of the family *Culicidae* (subfamily Anophelinar and Culicinae) due to the surveillance of medically important species such as *Anopheles albimanus* (Wiedemann,1820), *A. pseudopunctipennis* (Theobald,1901) and *Aedes aegypti* (Linnaeus,1762), (Espinoza-Gómez *et al.,* 2013).

Culicidae have a complete metamorphosis (egg, larva, pupa, adult). The immature stages always develop in association with free water in lentic bodies in different areas (SSA, 2014). *Aedes aegypti* is responsible for the transmission of serious diseases such as Dengue, Chikungunya and Zika. These cause millions of deaths per year and put approximately three billion people in endemic areas worldwide at risk of infection (Barreto, 2017). There is also evidence of transmission of dengue virus between generations of mosquitoes from the development of infected eggs by vertical transmission in vectors (transovarial transmission). If during viremia the mosquito bites this person, he/she becomes infected. After a period necessary for the development of viral infection in the mosquito (extrinsic incubation period), it will remain infectious for the rest of its life and with the capacity to infect susceptible individuals (SSA, 2014; PAHO/WHO, 2018).

Control methods can be environmental, mechanical, biological or chemical. Environmental control requires environmental redevelopment (improvement of housing, collection of waste and other materials, and improvement of sewage systems), drinking water supply and urban planning. Biological control involves the application of natural enemies, biological larvicides and entomopathogenic fungi. Chemical control aims to reduce density, increase vector mortality and human-vector contact. New technologies aim to release mosquitoes with endosymbiont bacteria, transgenic and irradiated mosquitoes. Mechanical-physical control aims to reduce density or increase vector mortality with washing or covering of containers as well as human-vector contact with the use of mosquito nets, door and window screens and the use of appropriate clothing (PAHO, 2019; PAHO/WHO, 2018).

Education is the capacity to develop or transmit knowledge, it seeks to promote personal responsibility, incorporating knowledge, attitudes and healthy habits and to develop the critical capacity to make decisions that facilitate the care of one's own health and that of others. Integrating health education in the educational environment, is to favor the harmonious growth of the students' personality, developing an educational process (*development of competencies*), socio-cultural (participation in social initiatives) and health promotion (*living experiences*) (Rodríguez-Torres *et al.,* 2017). The objective of this work was to evaluate the impact of an entomological inspection conducted by 4th and 5th grade schoolchildren from Zumpango del Río, Municipality of Eduardo Neri, Gro. before and after an educational intervention on the *Aedes aegypti* mosquito.

MATERIALS AND METHODS

Schoolchildren from four elementary schools participated, with the prior authorization of teachers and parents. The duration of the work was from September to November 2019. We began with the baseline measurement of knowledge with a self-administered survey and reading of each of the questions, then continued with the educational intervention providing information about the mosquito *A. aegypti mosquito* (morphological characteristics, life cycle and habits), arbovirosis it transmits (Dengue, Chikungunya and Zika), prevention measures and the procedure to perform the entomological inspection in each of the schools to identify breeding sites and collect larvae, with the purpose of performing it in each of their homes and finally the second survey was applied to identify the change in knowledge after one month. After the educational intervention, each one was provided with a Pasteur pipette, eppendorf tubes with a capacity of five milliliters, 2 x 3 cm labels (to collect data such as street, house number, date of collection, hatchery and name of the collector), latex rubber bands and a printed message addressed to the parents describing the work and the indications for larvae collection. They were told that the collection at home would be carried out under the supervision of an adult who would go around the house and yard looking for containers with water and mosquito larvae to collect them. This collected material was collected in the classroom the following day to be transported to the Health and Environment Research Laboratory of the School of Chemical and Biological Sciences for identification with the help of a stereoscopic magnifying glass, an optical microscope and the guide "Key for the Identification of Common Mosquito Larvae in Urban and Suburban Areas of the Mexican Republic (Diptera: Culicidae)" by Ibañez and Martínez (1994), the results were given to the students. $X^2$of Mac Nemar's test was used to evaluate the modification of knowledge and with the Microsoft Office Excel program the capture and analysis of the entomological inspections.

RESULTS AND DISCUSSION

A total of 422 4th and 5th graders from four elementary schools participated (Figure 1). By school grade, 5th graders predominated and the most frequent gender was female; the age range was between 9 and 12 years with an average of 9.99 (Table 1).

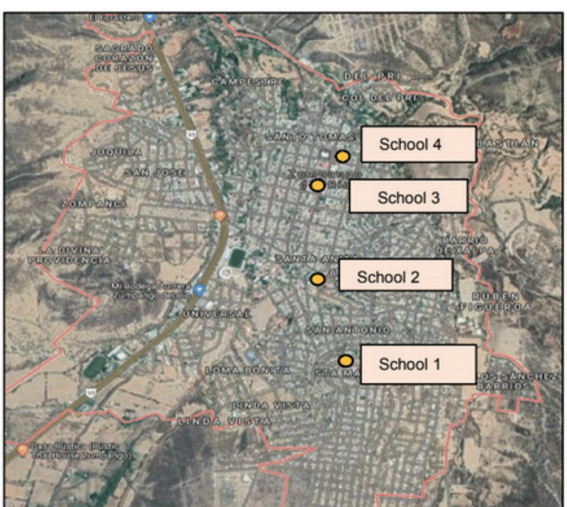

Figure. 1 Location of elementary schools in Zumpango del Río, municipality of Eduardo Neri, Guerrero.

Knowledge of school children. Knowledge of mosquito morphology (size: AI (Before Intervention): 15.6 %, DI (After Intervention): 81.3 %; X^2: 258.2); color: AI: 20.1 %, DI: 88.2 %; X^2: 268.18) and life cycle: AI: 21.1 %, DI: 81.3 %; X^2: 228.6); viral origin of arboviruses (Dengue: AI: 14.7 %, DI: 89.6 %; X^2: 298.8; Chikungunya: AI: 17.3 %, DI: 88.2 %; X^2: 280.1 and Zika: AI: 13 %, DI: 89.8 %; X^2: 314.2) and which are transmitted by mosquitoes (Dengue: AI: 63.5 %, DI: 95 %; X^2: 115.3; Chikungunya: AI: 57.8 %, DI: 95.3 %; X^2: 136.9; and Zika: AI: 34.8 %, DI: 93.8 %; X^2: 232.09). They perceive the risk of getting sick (Dengue: AI: 48.8 %, DI: 76.1 %; X^2: 69.6; Chikungunya: AI: 50.2 %, DI: 76.1 %; X^2: 63; and Zika: AI: 30.3 %, DI: 70.1 %; X^2: 125.6) or die (Dengue: AI: 33.9 %, DI: 90.5 %; X^2: 213.7; Chikungunya: AI: 26 %, DI: 29.4 %; X^2: 109.4) and Zika: AI: 40.5 %, DI: 34.6 %; X^2: 142.4).

Entomological inspection. In the schools, no larvae were found in the containers with water. Of the two that they carried out in their homes, the first was from September 04 to October 15, 2019, finding larvae in 279 homes and the second from October 21 to November 26, 2019, they found larvae in 41 homes. The first search, reported a total of 599 containers and 67 in the second, the most abundant were tanks and drums (1st: 47.7 % [286/599], 2nd: 43.3 % [29/67]) followed by jars and buckets (1st: 27 % [162/599], 2nd: 16.4 % [11/67]) and tubs (1st: 7 % [42/599], 2nd: 13.4 % [9/67]), similar to what was described by Balanzar *et al,* (2014), having inspected containers with water in the Costa Grande Region of Guerrero, (drums: 4,986 and piles: 3,321), but different from what was reported by Vences *et al.,* (2016a) who checked mainly various boys (32.8 %), pots (27.2 %), jars and buckets (16.6 %) and tanks and drums (5.9 %) in Tecoanapa, Gro. However, Vences *et al.,* (2018) in their study in Huitziltepec, Eduardo Neri, Gro., rural locality, also match tanks and drums (AI: 68 %, DI: 41 %), although this work was conducted with the adult population.

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Grade	Genre	School 1		School 2		School 3		School 4		Total	
		n	%	n	%	n	%	n	%	n	%
4°	Children	10	3.1	15	9.9	34	5.2	37	5.1	96	40.5
	Girls	7	2.1	27	17.8	36	5.5	43	7.3	113	47.6
Subtotal 4		17	5.2	42	27.7	70	10.8	80	13.6	209	88.1
5°	Children	10	3.1	7	4.6	37	5.7	40	6.8	94	39.6
	Girls	4	1.2	17	11.2	48	7.4	50	8.5	119	50.2
Subtotal 5		14	4.3	24	15.8	85	13.1	90	15.3	213	89.8
Total		31	7.3	66	15.6	155	36.7	170	40.3	422	100

Table 1. Distribution of schoolchildren by school, grade and gender.

Type of container	First inspection				Second inspection			
	Container				Container			
	Inspected		With larvae		Inspected		With larvae	
	n	%	n	%	n	%	n	%
Tanks and drums	286	47.7	235	50.6	29	43.3	23	41.8
Tires	13	2.2	11	2.4	3	4.5	2	3.6
Batteries	12	2.0	10	2.2	1	1.5	1	1.8
Water tanks	24	4.0	19	4.1	4	6.0	3	5.5
Cans and buckets	162	27	107	23.1	11	16.4	11	20
wells	1	0.2	1	0.2	1	1.5	1	1.8
Cistern	10	1.7	5	1.1	1	1.5	1	1.8
Pots	3	0.5	3	0.6	-	-	-	-
Vases and aquatic plants	5	0.8	5	1.1	-	-	-	-
Bathtubs	42	7.0	36	7.8	9	13.4	7	12.7
Toilets	1	0.2	1	0.2	-	-	-	-
Animal drinking troughs	3	0.5	2	0.4	-	-	-	-
Different boys	29	4.8	24	5.2	7	10.4	5	9.1
Various large	8	1.3	5	1.1	1	1.5	1	1.8
Total	599	100	464	77.5	67	100	55	82.1

Number and type of containers with larvae before and after the educational intervention.

Type of container	*A. aegypti*				*A. epactius*				*C. coronator*				*Cx. quinquefasciatus*		*A. Aegypti* + *A. epactius*		*A. aegypti* + 2 other species	
	1ª		2ª		1ª		2ª		1ª		2ª		1ª		1ª		1ª	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Tanks and drums	147	52.1	12	35.3	60	46.2	10	52.6	5	55.6	1	50	2	100	15	53.6	6	46.2
Tires	8	2.8	2	5.9	2	1.5	-	-	-	-	-	-	-	-	-	-	1	7.7
Batteries	7	2.5	1	2.9	3	2.3	-	-	-	-	-	-	-	-	-	-	-	-
Water tanks	6	2.1	1	2.9	10	7.7	2	10.5	-	-	-	-	-	-	1	3.6	2	15.4
Cans and buckets	68	24.1	9	26.5	28	21.5	2	10.5	3	33.3	-	-	-	-	5	17.9	3	23.1
wells	-	-	1	2.9	-	-	-	-	-	-	-	-	-	-	-	-	1	7.7
Cistern	3	1.1	1	2.9	1	0.8	-	-	1	11.1	-	-	-	-	-	-	-	-
Pots and planters	3	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vases and aquatic plants	5	1.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bathtubs	23	8.2	3	8.8	9	6.9	3	15.8	-	-	1	50	-	-	4	14.3	-	-
Toilets	-	-	-	-	1	0.8	-	-	-	-	-	-	-	-	-	-	-	-
Animal drinking troughs	1	0.4	-	-	1	0.8	-	-	-	-	-	-	-	-	-	-	-	-
Diverse boys	10	3.5	3	8.8	11	8.5	2	10.5	-	-	-	-	-	-	3	10.7	-	-
Various large	1	0.4	1	2.9	4	3.1	-	-	-	-	-	-	-	-	-	-	-	-
Total	282	61	34	61.8	130	28.0	19	34.5	9	1.9	2	3.6	2	0.4	28	6.0	13	2.8

Table 3. Type of larvae-positive containers, distributed by species in the first and second entomological inspection.

After the review of the collected materials, 464 containers with larvae were recorded in the first search and 55 in the second. Those with larvae were tanks and drums (1st: 50.6 % [235/464], 2nd: 41.8 % [23/67]), followed by jars and buckets (1st: 23.1 % [107/464], 2nd: 20 % [11/67]) (**Table 2**)**.** Only one work is available by Vences *et al.,* (2016b) in which children from two elementary schools in Chilpancingo, Gro, conducted a search for breeding sites in their homes to identify the presence of *A. aegypti* mosquitoes, before the intervention they collected larvae from tanks and drums (97/252, 38.5 %), tinacos (31/252, 12.3 %), pilas (22/252, 9 %), buckets (20/252, 8 %) and tubs (18/252, 7.1 %) and after the intervention the most frequent were tanks and drums (33/115, 28.6 %), vases and aquatic plants (19/115, 16.4 %), various large (15/115, 13 %) and tires (14/115, 12.1 %) and in another work by Vences *et al.,* (2019) in Coyuca de Benítez, Gro., the most abundant were pots and flowerpots (AI: 41.20 % [908/2,204], DI: 18.25 % [207/1134]) when inspecting dwellings.

Species identification and preferred breeding sites. After species identification, four species were recognized, *A. aegypti* larvae (61 %, 282/464) *A. epactius* (28 %,130/464), *A. aegypti*+ *A. epactius* (6 %, 28/464), *A. aegypti* + two species (2.8 %, (13/464), *Culex coronator* (1.9 %, 9/464) and *Culex quinquefasciatus* (0.4 %, 2/464). In the second review, 61.8 % (34/55) had only *A. aegypti* larvae, 34.5 % (19/55) *A. epactius* and 3.6 % (2/55) *Culex coronator*. With respect to the preferred breeding site, tanks and drums coincided for the four species (*A. aegypti* 1ª: 52.1 % [147/282], 2ª: 35.3 % [12/34]; *A. epactius* 1st: 46.2 % [60/130], 2nd: 52.6 % [10/19]; *Culex coronator* 1st: 55.6 % [5/9], 2nd: 50 % [1/2] and *Culex quinquefasciatus* 1st: 100 % [2/2]) (**Table 3**) (**Table 3**)**.** In contrast to the study of Balanzar *et al,* (2014), who reported different breeding sites (piles (8.9 %); various boys (8.1 %) and buckets (3.9 %)) and Torres *et al.* (2014) after making rounds in school yards to identify and note the number and types of positive containers for larvae of mosquitoes *A. aegypti mosquito* larvae, before and after giving talks to school children in *Tapachula, Chiapas, Mexico*, (plastic bottles, glass bottles, and buckets mainly), but the same as that entered by school children in the work of Vences *et al,* (2016a), (AI: tanks and drums (21.4 %, 54/135), tubs (6 %, 15/135), tinacos (5.5 %, 14/135), vases and aquatic plants (5 %, 12/135); DI: tanks and drums (12.3 %, 14/59), flower pots and aquatic plants (10.5 %, 12/59) and tires (8 %, 9/59) and those performed in the population by Vences *et al.* (2016b) (tanks and drums (20.7 %) and piles (19.8 %); Vences *et al.,* (2018) (tanks and drums (AI: 68 %, DI: 41 %) and the most recent by Vences *et al.*, (2019) in Coyuca de Benítez, Gro, (tanks and drums (AI: 30 % [15/50], DI: 45.4 % [10/22]) and piles (AI: 24 % [12/50], DI: 9.1 % [2/22]).

CONCLUSIONS

The educational workshop had a positive impact on the schoolchildren's knowledge of the *A. aegypti* mosquito and its control. The most frequently inspected containers with the presence of larvae were tanks and drums, and the number of breeding sites was reduced by approximately ninety percent after receiving the educational intervention.

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