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Surveillance of *Aedes aegypti* Using a Reduction Sampling Size for Its Application during the COVID-19 Pandemic in Havana, Cuba

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Keywords: Arbovirus vectors; Pandemic; Surveillance; Cuba.

Abstract

Background: Pan American Health Organization (PAHO) indicated guidelines on the prevention and control of dengue during the COVID-19 pandemic, reinforcing the surveillance and control of its vectors: *Aedes aegypti* and *Aedes albopictus*.

Objective: To evaluate the larval indices and others entomological indicators in high risk blocks in Havana in order to rationalize the efforts in entomological surveillance, speed.

Methods: The study was carried out in four municipalities (Playa, Plaza de la Revolución, Habana Vieja y Arroyo Naranjo) belonging to Havana province in Cuba. In each municipality, the surveillance took one week, visiting 33% of the total number of houses in each neighborhood. The surveillance was conducted from March 5 till April 2, 2019.

Results: A total of 6 647 containers were inspected from 1599 (Houses total sampled in Plaza de la Revolución municipality was 399) houses in the four study areas of which only 500 (7.5 %) were positive for *Ae. aegypti* immature stages. Aedes infestations levels was high with a general Breteau Index (BI) of 31.3 a Container Index (CI) of 7.5 and a House Index (HI) of 18.3 respectively. All municipalities showed mainly presence inside the houses with preference in living room and kitchen.

Conclusions: This type of sampling in the neighborhoods with high risk for *Ae. Aegypti* in Cuba would help the sustainability of the program at a time of COVID-19 when human, material and financial resources are limited since it would focus interventions more towards prevention and control in the event of dengue transmission.

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Introduction

Dengue (VDEN) and other diseases transmitted by Aedes spp. like chikungunya (VCHIK) and Zika (VZIK) affect 129 countries with more than 4 billion people at risk worldwide. The year 2019 was characterized by dengue outbreaks in many countries in the Americas, with more than 3.1 million reported cases, including 28,176 severe ones and 1,535 deaths [1]. Dengue continues on the same trend in 2020 when a total of 2 300 546 cases were reported including 1019 die [2]. On the same time, there is a novel disease, COVID-19, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), first detected in Wuhan, China, in December 2019 [3,4]. Due to its fast spread over the globe and its severity, WHO (World Health Organization) declared it a pandemic on March 11, 2020. While the COVID-19 pandemic is still unfolding and uncertainty remains high, there are strong indications that both COVID-19 and its subsequent control measures have high impact on the global health, economic, political and social situation [5]. Its recent appearance and worldwide distribution; variable and exponential rates of infection and mortality; the high number or proportion of asymptomatic cases; and the clinical and psychological manifestations associated with it, have alerted all health and political systems at the international level [6-9]. The Pan American Health Organization (PAHO), for its part, has emphasized the crucial need to maintain efforts to prevent, detect, and treat vector-borne diseases since the combined impact of COVID-19 and dengue epidemics could have potentially devastating consequences for the population at risk [10].

In view of this situation, PAHO indicated guidelines on the prevention and control of dengue during the COVID-19 pandemic, reinforcing the surveillance and control of its vectors: *Aedes Aegypti* and *Aedes albopictus*, urging countries to make effective use of their human resources, encouraging to the participation of the community in the integrated surveillance of the prevention of COVID-19 and the vector of dengue within their homes, as well as, if necessary, the application of adulticides insecticides, use methods such as indoor fogging or the application of residual indoor spraying, taking into consideration the insecticide susceptibility of the local vectors [10].

Cuba has developed a comprehensive strategy to control COVID-19, respecting the models established by the WHO and incorporating the country's own measures, such as investigations at the population level, isolation of confirmed and suspected cases, and active surveillance of direct contacts, among others, guaranteeing effective containment of the disease. The country has faced three outbreaks of the disease and until end of January 12 of the 2022 a total of 12 090 041 samples were analyzed accumulated 988 789 positive, 8 331 deaths and two foreigners evacuated [11].

Regarding dengue vector control Cuba established the *Ae. Aegypti* eradication campaign in 1981, when the country suffered a huge epidemic of severe dengue. This has been translated into a national disease control programme, currently called the National Program for the Control of *Ae. Aegypti* and *Ae. Albopictus*. It has been considered as one of the most successful in the world, since at that time it managed to minimize *Ae. Aegypti* densities in many parts of the national territory [12-14]. Larval surveillance and control was carried out in one hundred percent of the houses on the island in a top-down implementation strategy, a methodology that is maintained in the current program. In addition, during the years 1981-1990, house inspection and control cycles were established every 11 days, which favored the drastic reduction of mosquito populations during this period. This frequency suffered modifications in the period 2000-2019, where most of the inspection cycles changed to 21 or 30 days, which led to increases in *Ae. Aegypti* densities, seen that this frequency was more adapted for surveillance and less for control [15].

In order to rationalize the efforts put in entomological surveillance, speed up its assessment and also following international trends [16, 17] a modification in the sample size, established by the program for more than 35 years, requires being assessed. For this reason, we developed a study where larval indices and entomological indicators were evaluated in an approach of surveillance coverage of 33% of houses in high-risk block, identified as repeatedly *Ae. Aegypti* presence neighborhoods by the program. At this moment, when COVID19 is circulating and social distancing is important, this method of rapid monitoring of mosquitoes could be a way of protecting human resources of the control program and of having a quick, less intensive surveillance to guide control efforts.

Study area

The surveys took place in Havana, the capital city of Cuba, which is located in the Caribbean area. Havana is located in the 22°58', 23°10' north latitude and 82°30', 82°06 a west longitude and belongs to the western region of Cuba and is characterized by a tropical climate with a rainy season between May and October and a dry season from November to April. The average temperature varies between 18 and 32 °C, and the average monthly rainfall varies between 2 and 222 mm, in the dry and rainy seasons, respectively. Havana covers an area of 726,75 km^2 and has an estimated population of almost 2 million people. The city is administratively subdivided into 15 municipalities in this study; four municipalities were purposively selected with diverse ecological, urbanization, and entomological conditions. Playa municipality it is located northwest of the province of Havana, and has an area of 36.2 km^2. The territory has 12 km of coastline that serve as an appropriate setting for international tourism facilities. In addition, numerous commercial representations, diplomatic headquarters and residences of diplomatic personnel are located. Its main economic and social activities are health, education, tourism, scientific research and the medical-pharmaceutical industry. Its population is 177,773 inhabitants, with a density of 4,910 inhabitants per km^2.Habana Vieja municipality corresponds to the so-called historic center of the capital. It has an extension of 4.32 km^2. Its population is 82,889 inhabitants with a density of 17,554 inhabitants/km^2. It is one of the most tourist areas of Havana due to the restoration of churches, fortresses and other historic buildings. Most of it is bordered on the north by the Bay of Havana. Plaza de la Revolución municipality has an area of 12 km^2. Its population is 144,190 inhabitants with an approximate population density of 12,315 inhabitants /km^2. In this municipality are most of the government institutions of the country, several ministries and a large part of the cultural sites. Arroyo Naranjo municipality is located in the south of the province, it has an area of 83 km^2, which covers 8,225 hectares of land, of which 36% is dedicated to agricultural use, and the rest to other non-agricultural activities, of which 27% they are unsuitable land and 7% is occupied by water Figure 1.

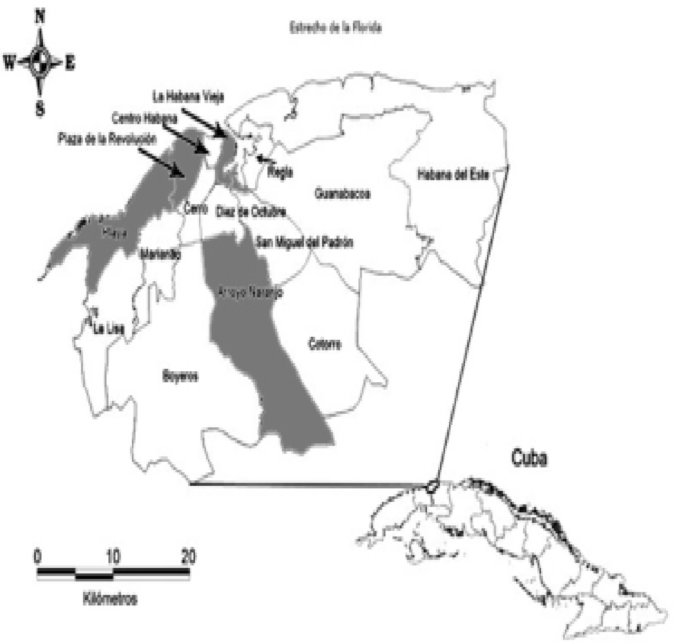

Figure 1: Sampled municipalities (grey color) localization in Havana province, Cuba.

Study design and data collection

A cross-sectional survey was undertaken (march-April 2019). During this period, 400 houses needed to be surveyed in each survey site. In each of the four-selected municipality, one neighborhood was randomly chosen (all the neighborhoods per municipality were listed, followed by a random number selection procedure) as the study site. Each day, 80 houses were inspected following a systematic sampling approach: Forty nine blocks were inspected, distributedas follows: 19 in Arroyo Naranjo municipality; 11 in Playa Municipality; 9 in Plaza de la Revolución municipality and 10 in Habana Vieja municipality. These blocks are characterized by having had a persistence of *Ae. Aegypti* infestation for several years. In each municipality, a total of 400 houses were inspected, which was about 33% of the total number of houses at the block sampled), totaling 1600 houses in the study. House-level sampling methodology used was as recommended by the program [18] and consisted in starting at the opening point of the house-block and continuing so that each house on your right is visited; upon arriving at the house, once the permit for sampling has been granted, starting by the yard to continue later with the rooms, always right-side and from the entry forward, in case one cannot inspect a room, as well as some closed housing, this is duly noted and inspection is done on another day. The data is collected in the established form for the program. The sampling was carried out by the Control Program staff belong to Havana province level When the inspection of one municipality had been completed; the staff went to another municipality and followed the same methodology. The inspection of all municipalities was achieved within a period of 4 weeks.

From each container positive for immature mosquito stages, all larval and pupae were collected in vials. The vials were labeled with the required information, as indicated by the national control program such as date, place of collection and container type [18]. For the species and genus identification of the samples, a morphological key [19] was used. Specimens identified were preserved in the Provincial Entomology Laboratory in Havana.

Container classification

The control program of dengue vectors in Cuba classifies the containers in 4 main groups [18]: 1) Group A: Water storage container ≥ 500 liters: Subdivided in: A1: Ground level water storage containers; A2: Elevated water storage containers; A3: Cisterns and wells; A4: water container < 500liters (basin, bucket, pots, small water storage container, ceramic container to store water, bathtub, etc.). 2) Group B: Other small miscellaneous artificial containers: subdivided in: B1: Non-destructible containers: spirituals vases, animal drinking bowls, flower vases, kitchen utensils in use, sanitary and domestic toilets (wash basin, sanitary tank), pots for plants etc. B2: Destructible containers: used car tires, bottles, cans, jars, metal scrap (abandoned auto parts and household electrical appliances). 3) Group C: Naturals containers like coconut shell, tree hole, hole in a stone, banana or another plant leaf, pools, etc. 4) Group D: Evacuation water like pits, sewers, drains, registers, etc.

Data analysis

Data were entered into the Microsoft Access database and 5% of the data were manually validated to detect errors. Data cleaning was undertaken and the types of recipients regrouped into categories, adapted from national control program guidelines [18]. We calculated, per round of visits and per municipality, the house index (HI; number of houses positive for at least one container with immature stages of Aedes spp. per 100 inspected houses), Breteau index (BI; number of containers positive for immature stages of Aedes spp. per 100 inspected houses), container index (CI; number of containers positive for immature stages of Aedes spp. per 100 inspected containers), and pupal index (number of Aedes spp. pupae per 100 inspected houses) [20,21]. The relative contribution to pupal productivity, defined as the total number of pupae of Aedes spp. per category of larval habitat divided by the total number of pupae of Aedes spp. collected per commune and per survey round, was calculated. A descriptive analysis was done. In order to evaluate the factors determining Aedes spp. immature stage positivity, a logistic regression model was used and associated variables were identified based on a backwards conditional model, taking into account clustering at the household level by inserting the household identification variable as a random factor in the model.

Results

Entomological study survey

A total of 6 647 containers were inspected from 1599 (Houses total sampled in Plaza de la Revolución municipality was 399) houses in the four study areas of which only 500 (7,5 %) were positive for *Ae. aegypti* immature stages. Aedes infestations levels was high with a general Bretau Index (BI) of 31,3 a Container Index (CI) of 7,5 and a House Index (HI) of 18,3 respectively. Arroyo Naranjo and Habana Vieja municipalities showed the higher index values, while Playa and Plaza de la Revolución municipalities showed the low index values. The number of *Ae. Aegypti* pupae present per 100 houses in total was 25, 6 with one variation between 7, 5 and 50, 3 in the municipalities (Table 1).

The number of deposits by type inspected, deposits with the presence of Ae. aegypti, deposits with pupae and the number of pupae by type of deposit in the different municipalities sampled, shown in (Figure 2); note that groups destructible containers (B2), water containers < 500 liters (A4) and non-destructible containers (B1) are those with the highest number of deposits inspected; those with the greatest presence of *Ae. Aegypti* correspond to B1 followed by A4 in Arroyo Naranjo and Habana Vieja respectively, being the reverse in the two remaining municipalities, regarding the number of deposits with pupae, group B1 is found in the four municipalities, however A4 is the one with the highest number of pupae by type of deposit in three municipalities and the second in the remaining municipality. According to the total of pupae by container type and container with *Ae. Aegypti* presence in general the inspection showed that A4 and B1 groups to be the primary producers of *Ae. Aegypti* followed by A1 and B2 (Figure 3).

Table 1: *Aedes aegypti* indicators of the four municipalities sampled, in Havana, Cuba, March -April 2019.

Indicators/Study, municipalities	Total	Arroyo Naranjo	Habana Vieja	Playa	Plaza de la Revolución
No. of houses inspected	1599	400	400	400	400
Houses with at least one container with mosquito immature stages	293	103	97	45	48
No. of containers inspected	6647	1691	1707	1685	1564
No. of container with mosquito immature stages	500	161	181	78	80
No. of pupae	410	201	105	74	30
Container Index (CI)	7.5	9.5	10.6	4.6	5.1
Breteau Index (BI)	31.3	40.3	45.3	19.5	20.1
House Index (HI)	18.3	25.8	24.3	11.3	12.0
Pupae Index (/100Houses)	25.6	50.3	26.3	18.5	7.5

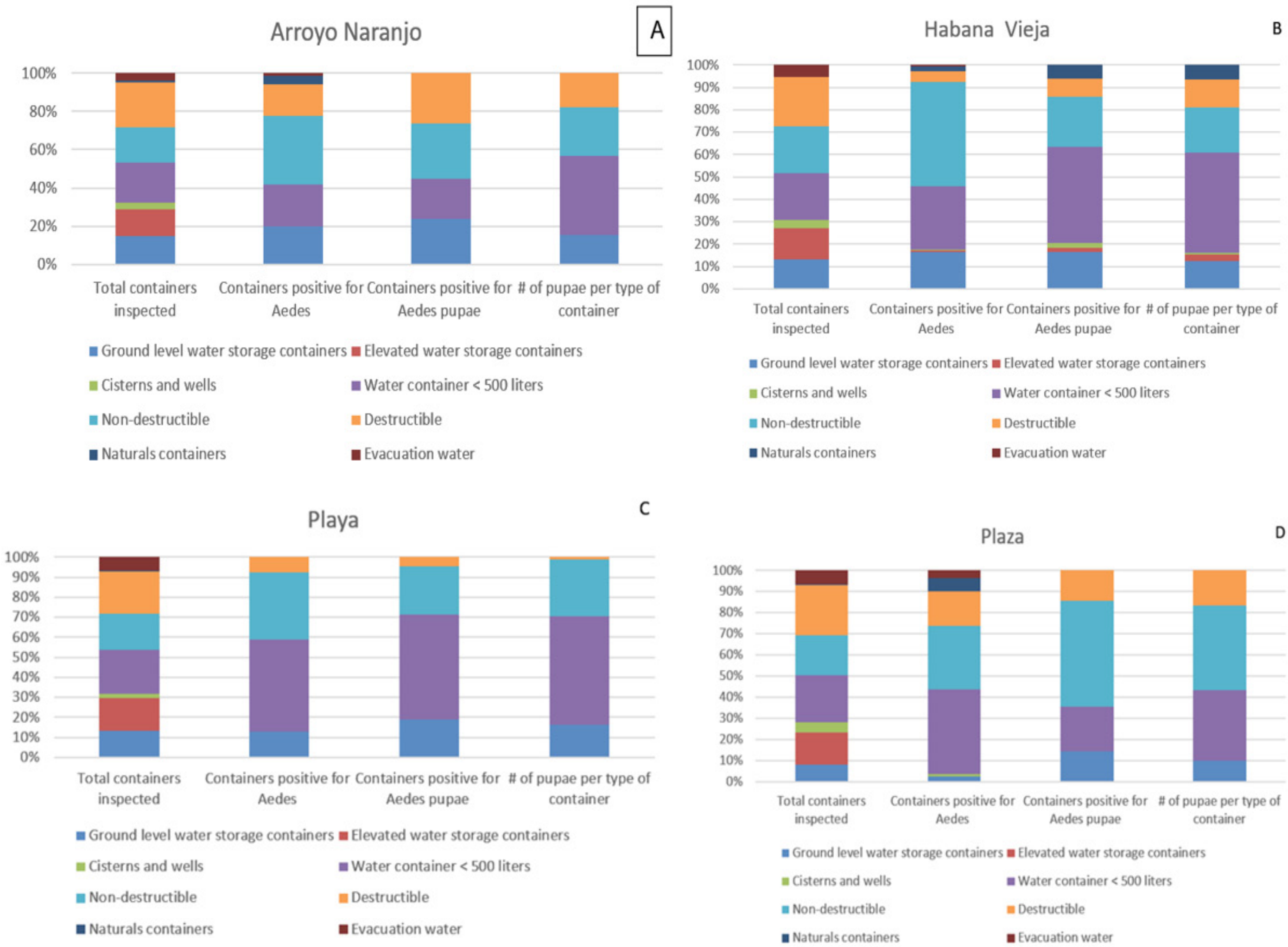

Figure 2: Number of deposits by type inspected, deposits with the presence of Ae.aegypti, deposits with pupae and the number of pupae by type of deposit in Arroyo Naranjo (A); Habana Vieja (B); Playa (C) and Plaza (D) municipalities march-april, 2019.

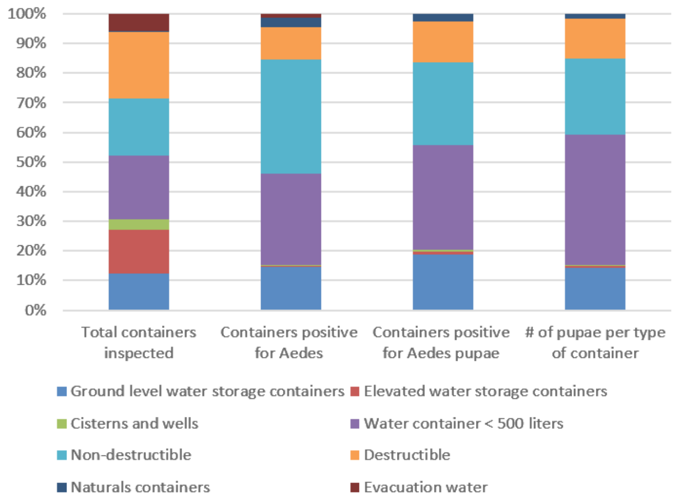

Figure 3: Number of deposits by type inspected, deposits with the presence of *Ae. Aegypti*, deposits with pupae and the number of pupae by type of deposit in all the studied municipalities, Havana province, March-April, 2019.

Statistical analysis showed that some factors influence the presence of *Ae. Aegypti* immature stages (Table 2). Indeed the municipality location (Habana Vieja, Playa, Plaza de la Pevolución) (OR: 1,2, 95% CI: 0.93-1.48, p= 0,179); (OR: 0,46, 95% CI 0.43-0.63, p< 0.001); (OR: 0.54. 95% CI: 0.41-0.72, p< 0.001) respectively showing similarity between the last two and differences of these with the rest of the municipalities, the position of the container in the housesit is two times more inside the houses(outside) (OR:0.86, 95% CI 0.67-1.12, p=0.266); and the container type (elevated water tank) (OR:0.01, 95% CI 0.002-0.08, p< 0.001); cistern and wells (OR:0.09, 95% CI 0.02-0.38, p=0.001); Water container< 500 liters (OR:1.31, 95% CI 0.98-1.76, p=0.072); non-destructible containers (OR:1.75, 95% CI 1.29-2.38, p<0.001);destructible containers(OR:0.43, 95% CI 0.29-0.63, p < 0.001); naturals (OR:9.49, 95% CI 4,60-19.57, p<0.001); evacuation water (OR:0.19, 95% CI 0.08-0.45, p<0.001) showing a presence of *Ae. Aegypti* nine times higher in natural sites and a similar presence in groups A4 and B1.

Of the 500 containers found with immature *Ae. Aegypti* stages during the study in the four municipalities 311 (62.2%) was inside the house and 189 (37.8) outside. All municipalities showed mainly presence inside the houses, being more marked in Habana Vieja y Plaza de laRevolución municipalities (75.2% and 24.8%) (76.5% and 23.5%) respectively while in Arroyo Naranjo and Playa the difference was less (54.7%and 45.3%) (52.2% and 47.9%) respectively The living room and kitchen presented the major number 114/311(36.6%); 91/311(29.2%) for a total of 205/311 (65.9%) inside while outside was the yard 159/189 (84.1%).

Only was found 12 of 500 (2.4%) of the positive containers associated with another mosquito species. Species associated with *Ae. Aegypti* in the container during the study was nine with Culex quinquefasciatus; two with *Ae. Albopictus* and one with Culex coronator.

Table 2: Factors influencing the Aedesaegypti immature stages presence in the municipalities studied in Havana, Cuba, March-April, 2019.

Factors	Modality	Total	Positive N(%)	OR (95% CI)	p-value
Municipalities	Arroyo Naranjo	1693	161 (9.5)	1	
	Habana Vieja	1707	181 (10.6)	1.2 (0.93-1.48)	0.179
	Playa	1685	78 (4.6)	0.4 6(0.43-0.63)	<0.001
	Plaza de la Revolución	1564	80 (5.1)	0.54 (0.41-0.72)	<0.001
Container position	Inside	2450	311 (12.7)	1	
	Outside	4191	189 (4.5)	0.86 (0.67-1.12)	0.266
Container Type	Ground tank water storage	824	74 (9)	1	
	Elevated tank water storage	980	1 (0.1)	0.01 (0.002-0.08)	<0.001
	Cisterns and wells	243	2 (0.8)	0.09 (0.02-0.38)	0.001
	Water containers < 500 liters	1430	154 (10.8)	1.31 (0.98-1.76)	0.072
	Non-destructible container	1270	192 (15.1)	1.75 (1.29-2.38)	<0.001
	Destructible container	1487	55 (3.7)	0.43 (0.29-0.63)	<0.001
	Naturals	35	16 (45.7)	9.49 (4.60-19.57)	<0.001
	Evacuation water	375	6 (1.6)	0.19 (0.08-0.45)	<0.001

Discussion

There are multiple factors that affect the arbovirus's transmission and operate simultaneously at different spatial and temporal scales, creating complex patterns of transmission, persistence, and dispersion [22-25]. So prioritizing prevention is an urgent necessity imposed by the epidemiological situation in America countries including the Caribbean area where Cuba is located.

On the other hand, although co-infections with arboviruses such as dengue and COVID-19 have not yet been well studied, [26] it is very likely that these cases occur in regions where there are outbreaks of dengue and COVID-19 at the same time. An example was what happened in Thailand where thirty-five cases of Covid-19 were confirmed; among which a case was reported of a patient who presented co-infection between these two viruses, with a fatal outcome [27] given this possibility, endemic areas could interfere in the diagnosis of both diseases, making it necessary to search for and implement methodologies for surveillance and monitoring of the dengue vector to gain speed in its control.

The results achieved using a reduction in the size of the area to be sampled without modifying the sampling methodology of the program, demonstrate that its evaluation could be feasible during the COVID-19.It was shown that valuable information can be obtained on the most important deposits of pupae and adult mosquitoes, as well as their location inside and outside the home of great importance when targeting the available control methods.

It was shown that when working with blocks historically with high infestation the index's gain in reliability by re-affirming the use of this indicator for to obtain information on *Ae. Aegypti* monitoring in an area in a fast and effective way, quite the opposite when they are generalized on a larger scale since it is known that mosquito populations fluctuate in time and space as well as the environmental conditions, which influence them [28].

As an additional result to be used by the program, it was found that the majority of the most productive pupae containers are mostly tanks with a capacity of less than 500 liters (A4), non-destructible artificial deposits(B1), followed by low tanks(A1); the first two are a group of deposits that generally lack physical protection (lids), which means that the program, need to review the educational messages to the population and emphasize the participation of the community in the control of the mosquito essential for this type of deposit.

In the case of the so-called ground tanks (A1), it should be noted that previously in a study carried out in Cuba, they were the most productive in Havana, which could be interpreted to have been gained in the control of this type of container in the community. In addition to informing the program staff to reinforce their inspection during the visit, taking advantage of an exchange with the residents, as it is shown that in any vector control strategy, it is essential to always include the component of community participation or social mobilization to guarantee a greater impact [29-31].Furthermore, at the current time of fighting COVID-19, where one of the measures is to stay at home, households should be encouraged to work together inside and around their homes to eliminate standing water, reduce solid waste and guarantee adequate coverage of all water storage containers, being carried out as a weekly family activity.

Regarding the high presence of *Ae. Aegypti* in natural sites it is well known that this species is a regular component of the tree hole fauna in some Africa countries where the species might have originated [32]. Observations of *Ae. Aegypti* developing in tree holes and other natural water containers are also reported from other regions of the world [33,34]. In the Pacific Islands, larvae and pupae were found in tree holes and coconut shells, but none were collected in leaf axils [35]. In the Americas, prior to the eradication campaign of the 1950s and 60s, *Ae. Aegypti* larvae were frequently found in human containers and sometimes in tree holes along city streets and parks, bamboo stems, coconut shells, and damaged papaya trees [36]. Since then, there have been only a few reports from natural containers, such as tree holes [37-39].Nevertheless the specie is highly adapted to the human environment, and artificial containers are the larval habitat most commonly used by *Ae. Aegypti* during the domestication favor by the human activity [40,41]. Although it is assumed that *Ae. Aegypti* breeds almost exclusively in artificial containers, potential larval habitats, such as tree holes, coconut shells, pools etc., may be overlooked by vector control personnel. Even though in urban environments, these natural habitats may appear to be comparatively scarce in relation to artificial containers, and their productivity may be low, they nevertheless may contribute to maintenance of populations of *Ae. Aegypti* and become relevant as re infestation sources if monitoring and control are not sustained.

On the other hand, the majority presence of breeding sites for Aedes mosquitoes inside the houses suggests a mosquito-man relationship favored by the permanence of the human population, mainly inside the houses where there are also the water tanks and other containers that are used in the development of domestic activities and other religious purposes. Besides results from various studies suggest that *Ae. Aegypti* female remain indoors or at resting sites for long period (36-50 hours) post blood feeding but the majority required a rest of at least 12 hours before taking a blood [42-44]. This human and mosquito behavior favors the development of the mosquito cycle since the female find breeding sites and at the same time sources of ingestion.

The low presence of breeding sites with the mosquito outside the houses is attributed to the fact that the work was carried out during the dry season in Cuba, which favors the shortage of useless artificial deposits in the courtyards of the houses, together with the possible rapid use of the water contained in the tanks that are filled by the community that implies a change that does not favor the development of the mosquito cycle.

It should also be noted that the effective control of *Ae. aegypti* in this neighborhoods would decrease the export of mosquitoes and viruses to other areas of the city, where the conditions for transmission are not so favorable, causing a decrease in the disease in untreated areas, constituting a tool for the program in prevention of epidemics and develop more efficient and cost-effective strategies [25,45].

Conclusions

The use of this type of sampling in the neighborhoods with high risk for *Ae. Aegypti* in Cuba helps the sustainability of the program at a time of COVID-19 when human, material and financial resources are limited since it would focus interventions more towards prevention and control in the event of transmission. On the other hand, prevention would be a key factor since it is known that in the case of dengue occurrence, there is a high asymptomatic transmission [46] that prevents the initial detection of outbreaks, which means that control actions historically always run after cases appear.

Therefore, carefully planned routine vector larval surveillance should still be an essential component of any dengue control program. Vector control authorities should be ready to see the high index values at times with low dengue incidence as vector breeding always precedes dengue incidence and takes time to precede the transmission, first silently and invisibly to reach outbreak proportions lately.

Due to the COVID-19 pandemic, the samplings were only carried out during the dry season in the country, however, we must clarify that the situation with the container for storage water in homes behaves in the same way in both seasons since the problems with the frequency of water in the sampled municipalities are maintained throughout the year. This behavior favors the number of deposits that could be used by *Ae. Aegypti* for laying remains more or less the same. Despite this limitation, the authors consider it interesting to show these results.

Declaration

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Availability of data and materials: The datasets supporting the conclusions of this article are included within the article. Raw data are available from the corresponding author on request.

Authors' contributions: MCMF, JABL and VV conceived and designed the study. MCMF, MPC, IPC, MAR, MRM, RMT, and MLS, identified the mosquitoes and collected data. MCMF, VV, JAB and MLS analyzed the data. VV. carried out the statistical analysis. MCMF and VV wrote the manuscript. All authors read and approved the final manuscript.

Consent for publication: This study was approved by ethical review committee of the Institute of Tropical Medicine, La Habana, Cuba and the ethical standards of the National Control Program of *Aedes aegypti* and *Aedes albopictus* established in Cuba. All the authors agree with the article publications.

Competing interests: The authors declare that they have not competing of interests.

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