Survey of container breeding mosquito larvae (Dengue vector) in Salvapan and Las Flores.

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ABSTRACT

Dengue is one of the most important vector borne diseases, with millions of cases occurring yearly and with billions found in risk areas. Eliminating water-holding containers where mosquitoes oviposit and develop can help manage urban disease-vector mosquitos. Thus, a water holding container eradication campaign was implemented by the Ministry of Health of Belize to eliminate the outburst of a dengue epidemic in known risk areas. A study was conducted in two selected communities, Salvapan and Las Flores, to determine the preferred container breeding habitat of the dengue vector and which of the site is more susceptible to a dengue epidemic. A number of households per site were randomly selected and a survey was carried out to identify water-holding containers breeding mosquito larvae and to identify the dengue vectors *Aedes aegypti* and *Aedes albopictus.* Mosquito larvae from different types of artificial and natural containers were collected and were identified using taxonomical keys. *Aedes aegypti* was the most abundant species in 74% of positive containers (water tanks, buckets, waste tire, etc). *Culex* spp. with 13%, *Aedes albopictus* with 12%, and *Anopheles* spp. with 0.48% in positive containers. The most abundant positive container was the bucket with 31% out of all positive containers. Twenty-six percent of the houses in Salvapan were positive compared to the thirty-seven percent in Las Flores. The study concluded that the dengue vector preferred breeding habitat was the bucket and that Las Flores is more prone to a dengue epidemic than Salvapan.

ACKNOLWDGEMENTS

The author Jonathan Funes thanks the residents of Salvapan and Las Flores for allowing me collect mosquito larvae in their yards. Also I thank my advisor Jair Valladarez for his unconditional support, guidance, and advice in the making of my thesis. In addition, I would like to thank Monica Hua her assistance in the field and Silvia Moralez for providing us with data on the socioeconomic status of the marginal areas of Belmopan from the Statistical Institute of Belize. Finally, but not least I would like to thank the members from the vector control program of the Ministry of Health namely Ricardo Jimenez, Hans Gutierrez, Eugene Lisbey, Edgar G. Escalante, and Kim Bautista for their assistance: in the field, in the identification of larva, and in making this project feasible.

INTRODUCTION

Dengue is one of the most important vector borne diseases, with millions of cases occurring yearly and with billions found in risk areas (World Health Organization, 2015). Eliminating water-holding containers where mosquitoes oviposit and develop can help manage disease-vector mosquitos (Dowling, et al., 2013). Therefore, a water holding container eradication campaign was implemented by the Ministry of Health of Belize to eliminate the outburst of a dengue epidemic in known risk areas. Known risk areas in Belize include many communities in the district of Cayo which is endemic for the vector disease. Although studies of vector mosquito fauna have been carried out in many major rural areas of Belize the studies have failed to include all the rural communities. Hence the reason for the present study where a mosquito larval survey was performed in water holding containers to investigate the distribution dynamics of the dengue vector in two know risk areas namely Salvapan and Las Flores, Belmopan City, Cayo District, Belize. Mosquito larvae survey was chosen for the study rather than mosquito adult survey since larva are true indicators of where the mosquito originates from.

Furthermore, the present study sought to achieve one of the objectives of the Ministry of Health for the district of Cayo. The objective was to conduct a mosquito larval survey in known risk areas in order to determine which risk area was more susceptible to a dengue epidemic. This may enable them to take preventative and control measures against it. This is in order, to decrease the chances for these communities specifically the one more prone to acquire the vector borne deadly disease. In addition, it enables the Ministry of Health the ability to canalize their resources effectively and efficiently in those needed areas. Finally, the study determined the dengue vector preferred breeding habitat which may serve as the basis for vector control management, educational campaign strategies, and decision making by the Ministry of Health in regards as a mean to effectively control dengue outbursts in such areas.

LITERATURE REVIEW

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of the world in recent years. Dengue virus is transmitted by female mosquitoes mainly of the species *Ae. aegypti* and, to a lesser extent, *Ae. albopictus*. The disease is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization. Dengue is one of the most important vector borne diseases, with millions of cases occurring yearly and with billions found in risk areas. Today, severe dengue affects most Asian and Latin American countries and has become a leading cause of hospitalization and death among children in these regions (World Health Organization, 2009).

Ae. aegypti exists in at least two forms (considered either subspecies or separate species according to different authors), namely *Ae. aegypti formosus* (the original wild type found in Africa) and *Ae. aegypti aegypti* (the worldwide urban form). The yellow fever mosquito, *Ae. aegypti aegypti*, has a worldwide distribution in the tropics and subtropics where it is the main vector of both dengue and yellow fever viruses. It can also transmit chikungunya and Zika viruses (Vector Base Organization, 2014). *Ae. aegypti* female can be described as a smallish, dark mosquito with conspicuous white markings and banded legs; the proboscis is all black although the palps are white tipped; the scutum has a dorsal pattern of white scales in the form of a 'lyre' with curved lateral and 2 central stripes contrasting with the general covering of narrow dark scales; wings are dark scaled; hind legs with femur pale scaled for basal three-quarters with dark scales dorsally on apical two-thirds and ventrally on apical third, tibia dark but tarsi with pale basal bands on 1-4 and 5 all pale; abdominal tergites with median and lateral white scale patches or bands (possibly some white scales on apical margins), sternites predominantly pale scaled with subapical bands on distal segments (Russell, 1996)

Adult *Ae. albopictus* are easily recognized by the bold black shiny scales and distinct silver white scales on the palpus and tarsi (Hawley 1988). The scutum (back) is black with a distinguishing white stripe down the center beginning at the dorsal surface of the head and continuing along the thorax. It is a medium-sized mosquito (approximately 2.0 to 10.0 mm, males are on average 20% smaller than females). Differences in morphology between male and female include the antennae of the male are plumous and mouthparts are modified for nectar feeding. The abdominal tergites are covered in dark scales. Legs are black with white basal scales on each tarsal segment. The abdomen narrows into a point characteristic of the genus Aedes. Field identification is very easy because of these distinct features (Rios & Maruniak, 2004). The worldwide distribution includes most of Asia and covers tropical and subtropical regions worldwide with introductions into the Caribbean (Morbidity and Mortality Weekly Report, 1989).

Ae. Aegypti and other mosquitoes (including *Ae. Albopictus*) have a complex life-cycle with dramatic changes in shape, function, and habitat. Female mosquitoes lay their eggs on the inner, wet walls of containers with water. Larvae hatch when water inundates the eggs as a result of rains or the addition of water by people. In the following days, the larvae will feed on microorganisms and particulate organic matter, shedding their skins three times to be able to grow from first to fourth instars. When the larva has acquired enough energy and size and is in the fourth instar, metamorphosis is triggered, changing the larva into a pupa. Pupae do not feed; they just change in form until the body of the adult, flying mosquito is formed. Then, the newly formed adult emerges from the water after breaking the pupal skin. The entire life cycle lasts 8-10 days at room temperature, depending on the level of feeding (Center for Disease Control and Prevention, 2014).

The *Ae. aegypti* mosquito is the primary vector of the dengue virus fallowed by *Ae. Albopictus* (Huang, 1972). The virus is transmitted to humans through the bites of infected female mosquitoes. *Ae. aegypti*; adult female mosquito taking a blood meal on human skin. After virus incubation for 4–10 days, an infected mosquito is capable of transmitting the virus for the rest of its life. Infected humans are the main carriers and multipliers of the virus, serving as a source of the virus for uninfected mosquitoes. Patients who are already infected with the dengue virus can transmit the infection (for 4–5 days; maximum 12) via Aedes mosquitoes after their first symptoms appear. The *Ae. aegypti* mosquito lives in urban habitats and breeds mostly in man-made containers. Unlike other mosquitoes *Ae. aegypti* is a day-time feeder; its peak biting periods are early in the morning and in the evening before dusk. Female *Ae. aegypti* bites multiple people during each feeding period (World Health Organization, 2009).

There are 4 distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3 and DEN-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes increase the risk of developing severe dengue (World Health Organization, 2015). Until now there is no vaccine available to give a permanent immunities or a specific medicine to cure the illness caused by dengue. But the vector can be controlled or suppressed by combating the mosquito population or minimizing the contact rate between mosquito and human with repellent (Aldila, Soewono, & Nuraini, 2012). Disposing of unused containers, placing useful containers under a roof or protected with tight covers, and frequently changing the water of animal drinking pans and flower pots greatly reduces the risk of dengue infections (Center for Disease Control and Prevention, 2014).

Therefore, eliminating water-holding containers (source reduction) where mosquitoes oviposit and develop can help manage urban disease-vector mosquitos (Dowling, et al., 2013).

According to Pan American Health Organization, dengue in the Americas, at the end of the year 2014, a total of 1,176,529 cases of dengue, 16,238 severe dengue cases and 761 deaths, were reported, for a case fatality rate (CFR) of 0.06%. The average incidence for dengue was 194 cases / 100,000 populations. Despite the historical increase in the number of cases reported by this disease, 2014 reported a reduction of approximately 50% in the number of cases, severe dengue cases, and deaths compared to the year 2013. However, the CFR maintained its same value (0.06%). In 2015, at the closure of the epidemiological week number (EW) 21, a total of 1,206,172 cases have been recorded in the entire continent, for an average incidence of 198 cases/ 100,000 populations. The data recorded to date have already exceeded the total number of cases reported in the end of 2014(Pan American Health Organization, 2015).

However, it is important to emphasize and highlight, that although there is an increase in the number of cases, the total number of severe cases (2,824) and deaths (459) have remained well below the values observed during 2014. The average CFR in the Americas is 0.04%. Brazil, Colombia and Mexico have reported the simultaneous co-circulation of the four serotypes of the dengue virus. Specifically, North and Central America: The total number of cases reported was 72,683 cases, with an average incidence of 43.4 cases/100,000 populations, with 1,415 reported cases of severe dengue and 3 deaths reported. Mexico (30,497) and Honduras (20,471) are the countries with the highest amount of cases in this sub-region, with the latter country also reporting the highest incidence. All the deaths reported in this sub-region occurred in Guatemala (Pan American Health Organization, 2015).

In Belize, the country wide dengue confirmed cases from the years 2006 to 2010 amounted to a total of 688 (Epidemiology unit Ministry of Health of Belize, 2011). Now in 2013 the amount of dengue cases was 372(Epidemiology Unit, 2016). However, recently in 2015 the dengue cases grew exponentially to 679 (Epidemiology Unit, 2016). This data illustrates that dengue is endemic to Belize.

Furthermore, a few studies have been done in the country of Belize in relation to the dengue vector. One of the mosquito studies concluded that the total number of mosquito species known to occur in the country was 111 species (including dengue vectors *Ae. aegypti* and *Ae.albopictus*) (PECOR, et al., 2002). Another study involved an entomological cross-sectional survey of the country where the findings of the study were the following: 17% of surveyed communities had a Breteau index below 2%. The mosquito density as measured by the Breteau, House and Container indices, gave an accurate indication of the existing level of risk of dengue transmission (Vanzie, 2008). Thirty-nine (39) percent had Breteau indexes between 2 and 5 per cent; this level of larvae infestation supports the maintenance of endemic dengue and low level outbreaks. Eight communities (44%) had larvae infestation as determined by the Breteau index, between 6 and 24 per cent; the probability of dengue outbreak is very high in these communities (Vanzie, 2008). But so far no study involving survey of container breeding mosquito larvae (Dengue vector) have been made in known risk areas including surrounding areas of Belmopan (Salvapan and Las Flores). Therefore, an information gap exist which can only be closed by carrying out the survey (study) mentioned before.

MATERIALS AND METHODS

Study Area

The study was conducted in the marginal areas of Belmopan in the Cayo district. The Cayo district is the biggest district in Belize and lies almost at the center of Belize. The district has an area of 2,061 square miles. It is bounded in the north by Orange Walk District, in the northeast by Belize district, in the west by Guatemala, in the southeast by Stann Creek, and in the south by Toledo district (Statistical Institute of Belize, 2016).

These marginal communities along the capital of Belize have been the epicenter for dengue and other mosquito disease outbreaks. These areas include: Las Flores, Salvapan, San Martin, and Maya Mopan (Epidemiology Unit, 2016). Now due to resource and time limitations only two areas were chosen and stratified based on the socio-economic status classified by the Statistical Institute of Belize (Statistical Institute of Belize, 2016). As shown in figure 1 below, the different areas, 1 to 7, are classified into 5 quintiles differentiated by colors with the top color being the lowest socio-economic classification and the bottom, the highest. Areas 5 (Salvapan) and 7 (Las Flores) were chosen as the two strata and the households within each strata were randomly selected. The two study areas were separated by 1.7km.

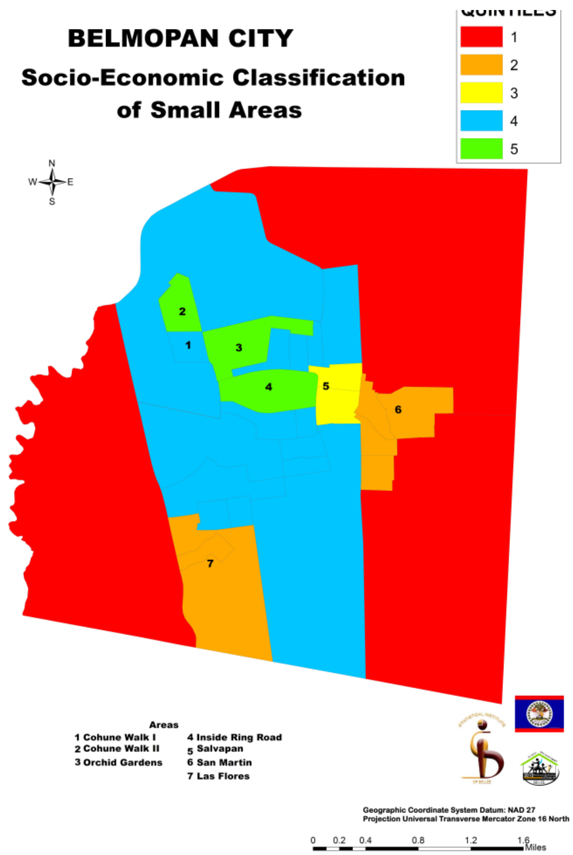


Figure 1: Showing small areas of Belmopan divided based on socioeconomic classification

Estimation of sample size for the entire selected area was determined using Epi Info and the sample size calculator function with a confidence limit of 10%, expected frequency of 50% and a confidence level of 95%. An additional 10% of household was then added to the sample size to allow for refusals, unusable data or other limitations. The population size of both areas totaled 929 households, the sample size calculated was 87 with an additional 9 household added to give a total of 96 households, the final sample size was decided to be 100 households. The number of sampling units was then distributed across the two strata by dividing the total number of sample household by the number of strata. Therefore, for each stratum, 50 households were aimed to be selected for the study (Hua, 2016).

Study Design*:*

Household Selection and Larval Collection

Mosquito larvae Surveys. At each household, we requested consent to survey (refer to Appendix A & B) the yard for all water-holding containers, larval mosquitoes, and acquire GPS location. Each house hold was given a code based on the initials of the name of their areas which consisted of the first three letters of the name of the community and the number of the household being surveyed. The survey was conducted from around 9:00am to 3:00pm on March 22nd, March 23rd, March 30th, and April 4th 2016. Approval was obtained from the Vector Control Unit, Ministry of Health before the start of the study. A modified point sampling was used to randomly select the houses in the two quartiles for the mosquito larval collection (survey).

The points were selected randomly using ArcGIS and Belmopan Street layers. Houses closest to the allocated points on the generated map were selected for survey. Two teams, each consisting of a Ministry of Health personnel and a student from the University of Belize, stopped at each of the houses and, after acquiring consent, surveyed the yard (Hua, 2016). During each survey, all water-holding containers were systematically searched for and enumerated on an existing table (refer to Appendix C). If the container classification was not found on the list of the table provided it was written down on the blank spaces provided in the same table. The number, type and water condition of containers which serve as a potential breeding site was examined and recorded using container index (CI). Container index = Number of container positive/ Number of container inspected X 100 (Rajesh, Dhanasekaran, & Tyagi, 2013).

The larvae collection was done by dipping technique using a dipper along with a pipette depending on the type of container and the amount of water present on it. For each positive container, all the larvae and pupae were collected with a dipper or a pipette and temporarily placed on a Nasco Whirl-PAK sample bag. The collected larvae and pupae were then transferred to a 1-liter polystyrene cup using a pipette which was then covered with a plastic cover. The cups were placed on a cooler for it to be taken to the San Ignacio Vector Control Unit Lab. Where the larva was transferred to mosquito breeding cups for adult emergence. The emerged adult mosquitoes were then pinned and identified.

Identification of collected Mosquito Larvae and Emerged Adult

The collected specimens were preserved in plastic vials for further identification. The mosquito larvae were collected by dipper method. Immature forms of the emerged adults were collected and stored in vials. In the Ministry of Health Vector Control Lab located at San Ignacio Hospital all the collected mosquitoes, both larvae (refer to Picture 2) and adult (refer to Picture 1), were identified to species using established pictorial keys (Rueda, 2004, Cutwa & O'Meara, Sai Gek, 2014).

Picture 1 Illustrating Mosquito Adult Species of *Ae. aegypti* (left) and *Ae. albopictus* (Alpuche, 2016)

Picture 2 Illustrating Mosquito Larva Species of Culex Spp.

Data Management and Data Analysis

Entomological data from the mosquito larvae survey was entered in Microsoft excel 2016 ® (Microsoft Corporation, 2016). The data collected was analyzed by the usage of bivariate analysis of CHI Square Goodness of fit test; it was calculated manually and then with Megastat add in ® (McGraw-Hill Higher Education, 2016). This consider two samples of categorical data. This type of analysis utilized tables, called contingency tables. Contingency tables focus on the dependency or association between column and row variables. It is the categorical equivalent of the scatterplot used to analyze the relationship between two continuous variables. The term contingency table was first used by the statistician Karl Pearson in 1904 (Dallal, 2000).

This CHI Square Goodness of Fit Test was used to calculate the Pearson P value of house index (total number of positive houses divided by total house surveyed X 100), Container Index, Positive Containers Per House, and Infestation rates (*Ae. Aegypti*, *Ae. Albopictus*, *Anopheles* Spp and *Cuelex* Spp). Furthermore, the Goodness of fit test was used to test the associations of the dynamic distribution of the dengue vector species between the two areas (Salvapan and Las Flores). This showcased which one of the two areas had a higher probability of an epidemic event of dengue to occur. Thus, by determining the P values the relationships between presence of larvae and their breeding habitat can be analyzed. Factors having a screening significance of $P < 0.05$ or more were not significant but if the P values were <0.05 it was classified as significant. Thus, the odds ratio was calculated to interpret the level of significance.

RESULTS

Mosquito Habitat Survey

Table 1. Entomological Indices of Two Areas in the Marginal Areas of Belmopan, Cayo District

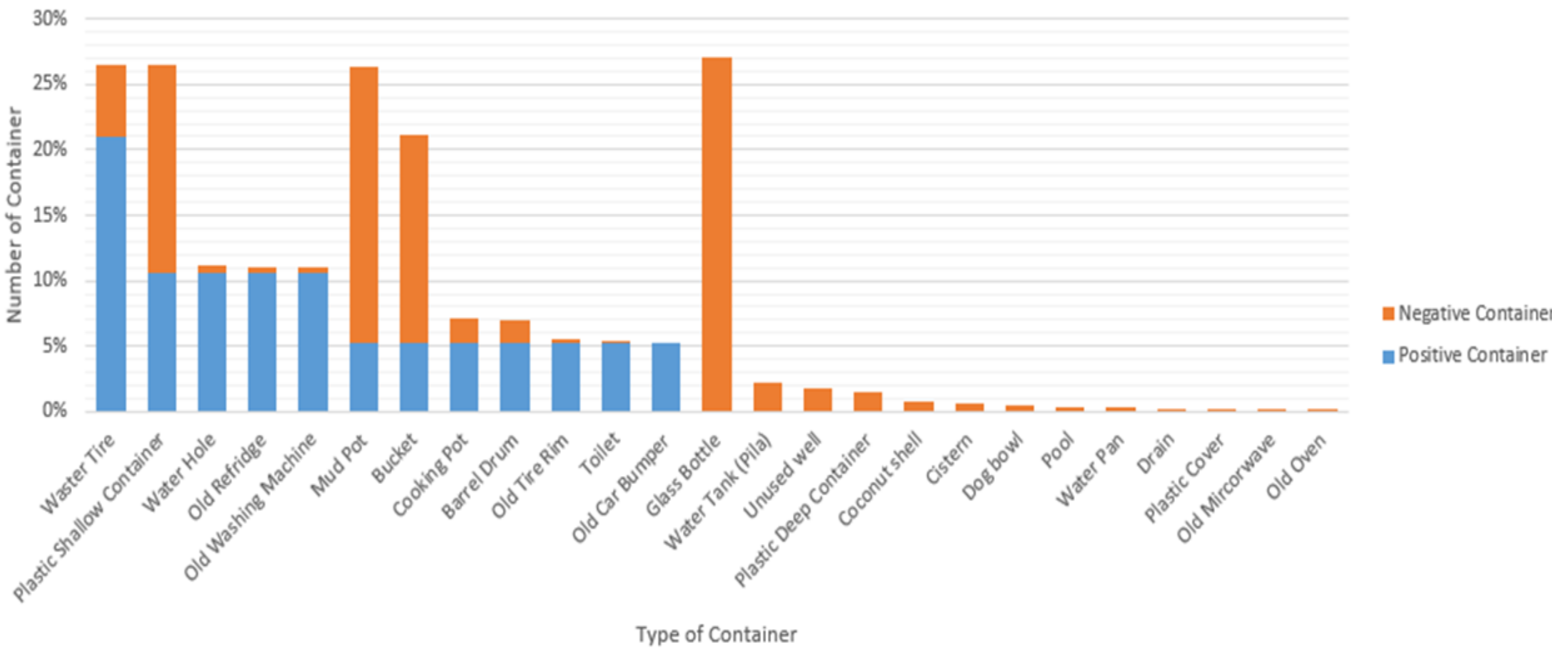
	Las Flores	Salvapan	Total	Odds Ratio	95% Confidence Limits	P values
Total Houses	41	43	84			
Total Containers	1615	788	2403			
House Index	37%	26%	62%	1.68	0.66-4.27	0.28
Container Index	1%	2%	4%	0.58	0.32-1.10	0.08
Positive Containers Per House	0.00035	0.00056	0.00046	2.89	0-1.59	0.98
Infestation rates						
Aedes Aegypti	82%	67%	74%	2.17	1.50-3.20	<0.001
Aedes Albopictus	8%	16%	12%	2.21	1.32-3.71	0.002
Anopheles	1%	0%	0.49%	0.00	0-0	0.08
Cuelex	9%	17%	13%	0.56	0.32-0.84	0.007

[a]Percentage houses with positive containers.
[b]Percentage of positive containers.
[c]Percentage of positive containers infested with species.
[d]P values are based on bivariate x^2 analyses

100 randomly selected households (50 from each community) were the stipulated number for the survey but due to the absence of selected house owners only 84 houses, 41 from Las Flores and 43 from Salvapan, were surveyed for mosquito larvae. Furthermore, in total there were 2403 water-holding containers found in respondent's yards, of which 4% were positive for mosquito larvae (Table 1). The majority of these positive containers were infested with *Ae. aegypti* (74%), followed by *Cuelex* spp (13%), *Ae. albopictus* (12%), and *Anopheles* spp (0.49%). *Ae. aegypti* larvae were found significantly more in Las Flores area than in Salvapan area (P=<0.001) and also the odds of encountering *Ae. aegypti* in Las Flores was 3 times greater when compared to Salvapan (95% confidence limits= 1.50- 3.20).

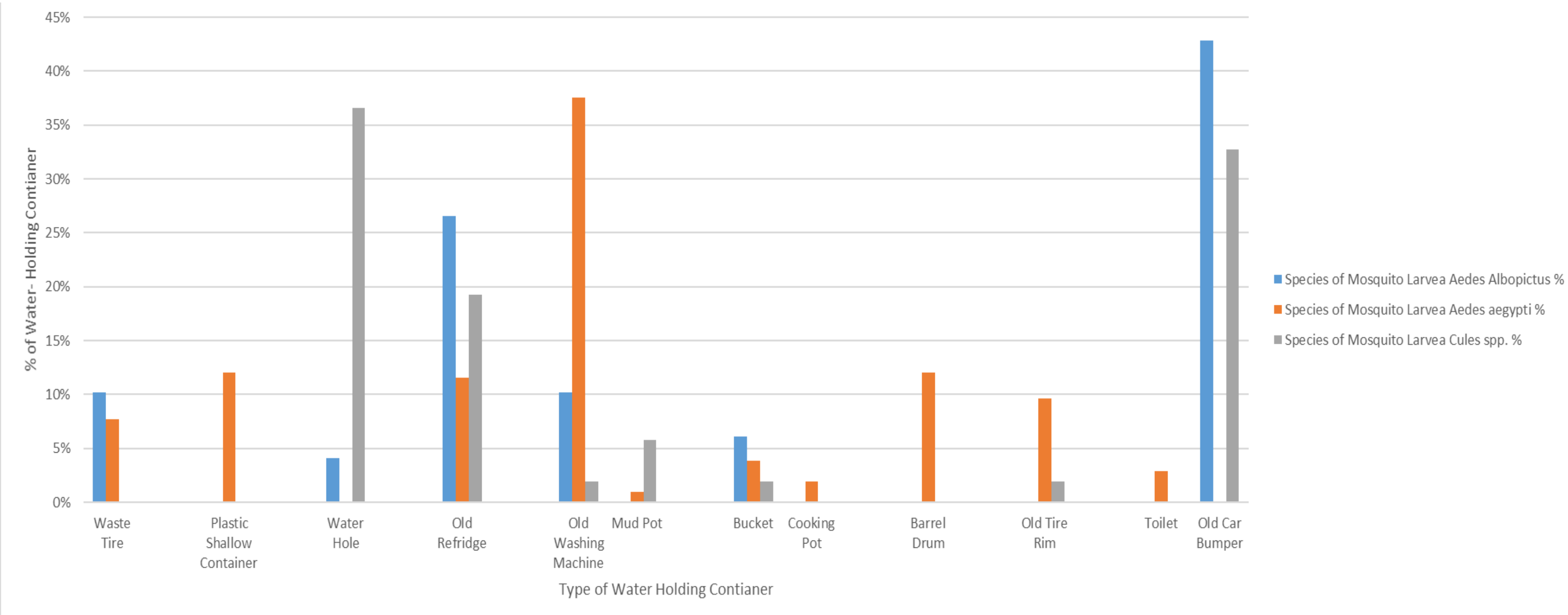
In contrast, *Ae. Albopictus* larvae were found to be significantly more in Salvapan area than in Las Flores area (P=0.002) and also the probability (odds) of encountering *Ae. albopictus* in Salvapan was 3 times greater when compared to Las Flores. Water-holding containers in Salvapan area were more likely to be infested with larvae than those in Las Flores area (P=0.08). The number of positive container per 100 houses were a little higher for Salvapan (0.00056) when compared to Las Flores (0.00035) (P=0.98). But it was more likely to find positive houses in Las Flores with positive containers than in Salvapan (P=0.28).

Figure 2. Number of larvae positive and negative containers per container type found in residential yards, Salvapan, Cayo.



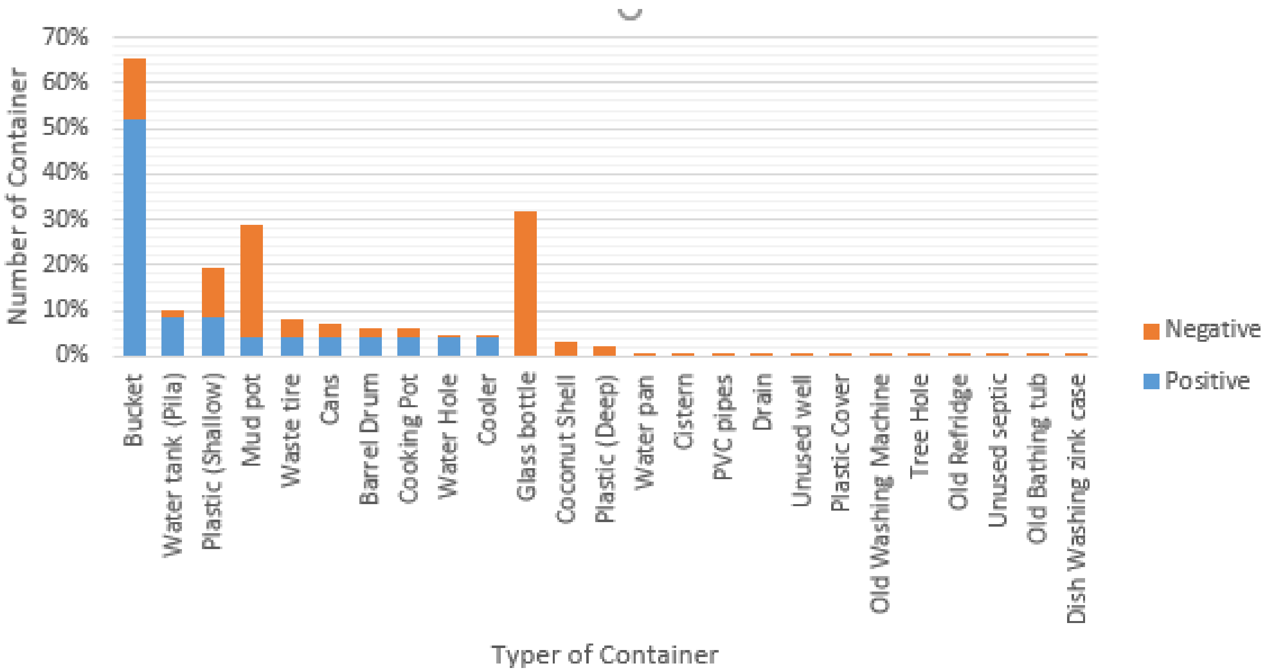
The twelve most common container types found to be positive for larvae in Salvapan included: Waste Tire (21% of positive containers), Plastic Shallow Container (11%), Water Hole (11%), Old Refridge (11%), Old Washing Machine (11%), Mud Pot (5%). Bucket (5%), Cooking Pot (5%), Barrel Drum (5%), Old Tire Rim (5%), Toilet (5%), and Old Car Bumper (5%) (Figure 2).

Figure 3. Percentage of Key Containers Types with *Ae. albopictus*, *Ae. aegypti*, and *Culex* spp larvae in Salvapan.



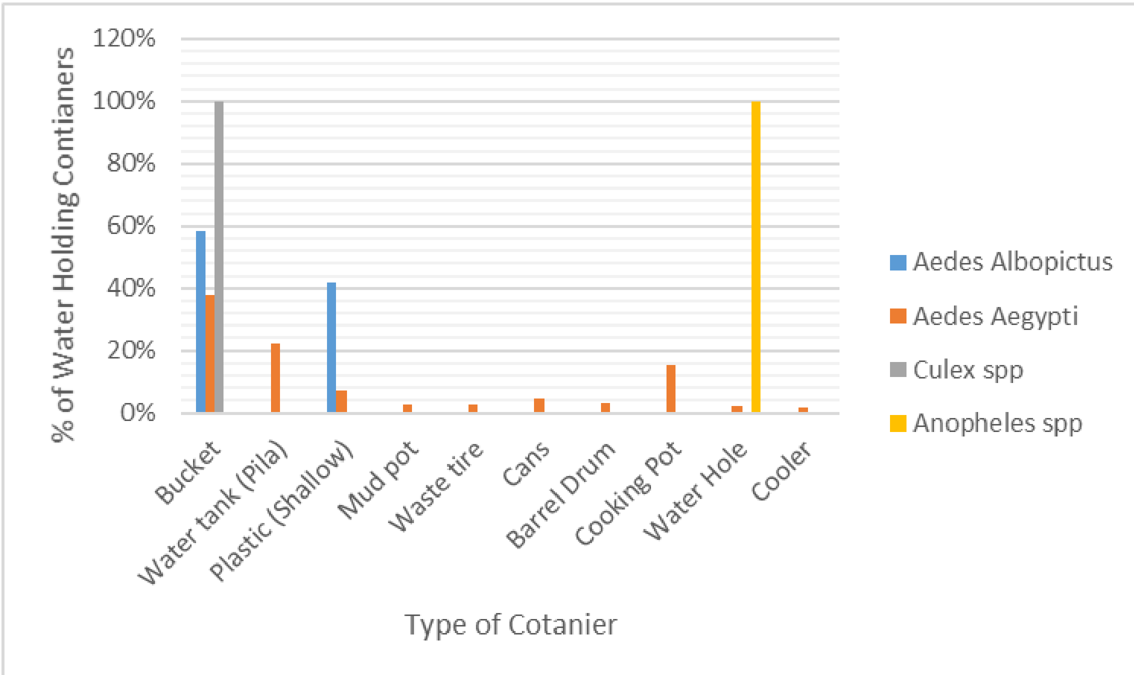
Larvae of *Ae. aegypti* inhabited mostly Old washing machine, barrel drum, old refridge, plastic shallow container, old tire rim, waste tire, bucket, toilet, cooking pot, and mud pot (Figure 2). Whereas *Ae. Albopictus* were more abundant in old car bumper, old refridge, waster tire, old washing machine, bucket, and water hole (Figure 2). Meanwhile, *Culex* spp were abundantly found water hole, old car bumper, old refridge, mud pot, bucket, old tire rim, and old washing machine (Figure 3).

Figure 4. Number of larvae positive and negative containers per container type found in residential yards, Las Flores, Cayo.



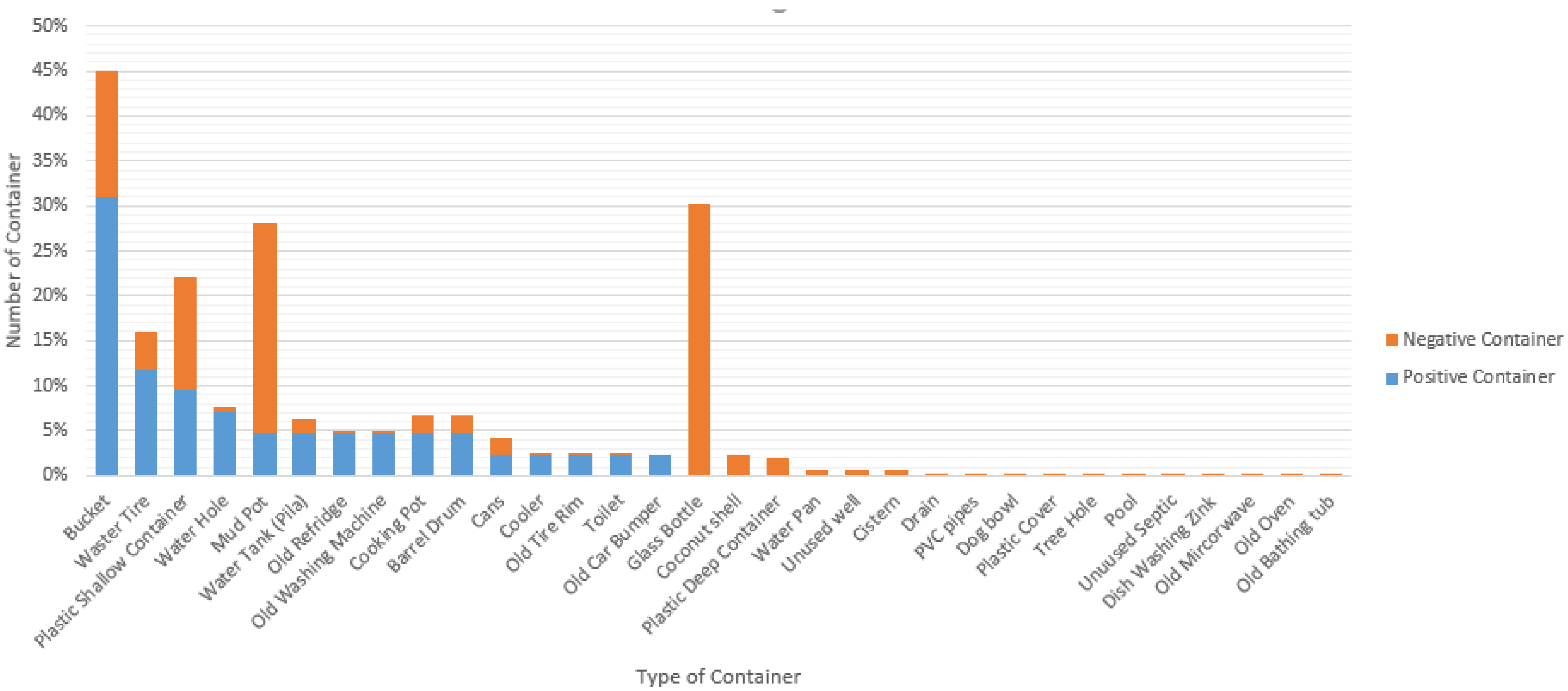
The ten most common container types found to be positive for larvae in Las Flores included: Bucket (52% of positive containers), Water Tank (Pila) (9%), Plastic Shallow (9%), Waste Tire (4%), Mud pot (4%), Cans (4%), Barrel drum (4%), Cooking Pot (4%). Water hole (4%), and Cooler (4%) (Figure 4).

Figure 5. Percentage of Key Containers Types with *Ae. albopictus*, *Ae. aegypti*, *Culex* spp, and *Anopheles* spp larvae in Las Flores.



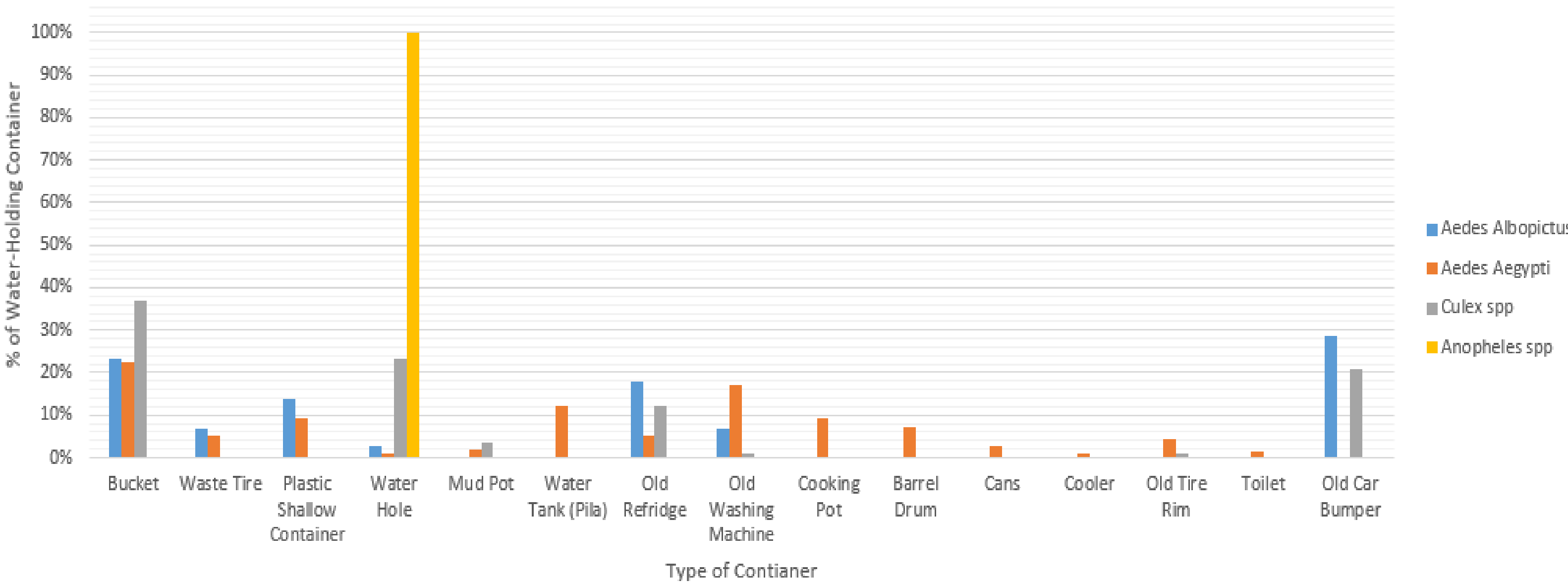
Larvae of *Ae. aegypti* inhabited mostly bucket, water tank (pila), cooking pot, plastic shallow container, cans, waste tire, mud pot, barrel drum, water hole, and cooler (Figure 5). Whereas *Ae. Albopictus* were more abundant in bucket, and Plastice Shallow container (Figure 5). *Culex* spp only inhabited bucket (Figure 5). *Anpheles* spp only inhabited a water hole.

Figure 6. Number of larvae positive and negative containers per container type found in residential yards, Salvapan and Las Flores, Cayo.



The fifteen most common container types found to be positive for larvae in both areas included: Bucket (31% of positive containers), Waste tire (12%), Plastic Shallow (10%), Water hole (7%), Mud pot (5%), Water tank (5%), Old refridge (5%), Old washing machine (5%), Cooking pot (5%), Barrel drum (5%), Cans (2%), Cooler (2%), Old tire rim (2%), Toilet (2%), and Old car bumper (2%) (Figure 6).

Figure 7. Percentage of Key Containers Types with *Ae. albopictus*, *Ae. aegypti*, *Culex* spp, and *Anopheles* spp larvae in Las Flores.



Larvae of *Ae. aegypti* inhabited mostly bucket, old washing machine, water tank (pila), cooking pot, plastic shallow container, barrel drum, waste tire, old tire rim, old refridge, cans, mud pot, toilet, cooler, and water hole (Figure 6). Whereas *Ae. Albopictus* were more abundant in old car bumper, bucket, old refridge, plastic shallow container, old washing machine, waste tire, and water hole (Figure 6). *Culex* spp inhabited mostly bucket, water hole, old car bumper, old refridge, mud pot, old tire rime, and old washing machine (Figure 6). *Anopheles* spp only inhabited water hole.

DISCUSSION

A previous study done in 30 major rural areas of the country of Belize concluded that about thirty-nine (39%) percent had Breteau indexes between 2 and 5 per cent; this level of larvae infestation supports the maintenance of endemic dengue and low level outbreaks. Eight communities (44%) had larvae infestation as determined by the Breteau index, between 6 and 24 per cent; the probability of dengue outbreak is very high in these communities. Therefore, stating that approximately 15 out of the 30 communities were at risk of a dengue epidemic (Vanzie, 2008). Thus, suggesting that about 50% of rural communities throughout the country of Belize are at risk of acquiring an outburst of dengue and that dengue is endemic throughout the country.

Compared to my study, it coincides with this study in that indeed dengue was endemic to rural communities (Las Flores and Salvapan) but it differs in that 62% (House index, refer to table 1) of the communities surveyed were at risk of a dengue epidemic due to the presence of dengue vectors (*Ae. aegypti* & *Ae. Albopictus*) larva accounting to 84% of the total larva collected from positive containers. Furthermore, it illustrates that Las Flores with 37 % (House Index, refer to table 1) and 90% of the total larval collected being the two major dengue vectors (*Ae. aegypti* & Ae. *Albopictus*) was more prone to a dengue outburst than Salvapan with 26% House Index with 83% of the total larva being the dengue vector. Moreover, my findings are supported by differences in the amount of confirmed dengue cases between the two study areas where there were 9 confirmed and 38 probable or suspected dengue cases in Las Flores compared to 6 confirmed and 27 probable or suspected dengue cases in Salvapan (Epidemiology Unit Ministry of Health of Belize, 2013).

In addition, my study illustrated that the most common mosquito larvae found in the two risk area was *Ae. aegypti* accounting to 74% (refer to table 1) of the total mosquito larva collected in the two sites. *Ae. aegypti* is the principle dengue vector (Rudnick, Tan, Lucas, & Omar, 1965). It also demonstrated that *Ae. albopictus* was the third most common mosquito larvae found with 12% (refer to table 1) in the two areas. *Ae. albopictus* is the secondary dengue vector (Rudnick, Tan, Lucas, & Omar, 1965). Moreover, *Ae. aegypti* larvae were found significantly more in Las Flores area than in Salvapan area (P=<0.001) and also the likelihood (odds) of encountering *Ae. aegypti* in Las Flores was 3 times greater when compared to Salvapan (95% confidence limits= 1.50- 3.20). In contrast, *Ae. Albopictus* larvae were found to be significantly more in Salvapan area than in Las Flores area (P=0.002) and also the probability (odds) of encountering *Ae. albopictus* in Salvapan was 3 times greater when compared to Las Flores (95% confidence limits= 1.32- 3.71). Thus, supporting that Las Flores is more prone to a dengue epidemic than Salvapan.

The level of the socioeconomic status can determine the likelihood of a community to be the epicenter of a dengue outburst. Communities with low socioeconomic status tend to have a higher probability of a dengue outburst due to high number of water holding containers found in it (Dowling, et al. 2013). Indeed, this trend was seen in my study where the community with high socioeconomic status (Salvapan) was seen to have a relative lower dengue vector larval infestations due to the low numbers of water holding containers (788, refer to table 1) than Las Flores with higher larval infestations due to high numbers of water holding containers (1615, refer to table 1). Thus, eliminating water holding containers which serve as a potential breeding site for the dengue vectors is extremely important in order to reduce the possibility of a dengue outburst in such communities.

Studies have shown that eliminating water-holding containers where mosquitoes lay eggs and develop can help manage disease-vector mosquitos (Dowling, et al., 2013). Thus, it is essential to eliminate water holding containers but to be more effective in doing so it is essential that the preferred breeding site of the dengue vector be determined. This is due to the fact that by eliminating such preferred breeding site guarantees that the dengue vector won’t be able to oviposit and thus preventing a dengue epidemic. Thus, my study showed that the dengue vectors were more abundantly found in the bucket (31% of positive containers) (which can be linked as their preferred breeding habitat), Waste tire (12%), Plastic Shallow (10%), Water hole (7%), Mud pot (5%), Water tank (5%), Old refridge (5%), Old washing machine (5%), Cooking pot (5%), Barrel drum (5%), Cans (2%), Cooler (2%), Old tire rim (2%), Toilet (2%), and Old car bumper (2%) (Figure 6). Where the dengue vector larvae of *Ae. aegypti* inhabited mostly bucket, old washing machine, water tank (pila), cooking pot, plastic shallow container, barrel drum, waste tire, old tire rim, old refridge, cans, mud pot, toilet, cooler, and water hole (Figure 6). Whereas *Ae. Albopictus* were more abundant in old car bumper, bucket, old refridge, plastic shallow container, old washing machine, waste tire, and water hole (Figure 6).

CONCLUSION & RECOMMENDATIONS

This is the first mosquito habitat survey study performed in Salvapan and Las Flores community. My study concluded that Las Flores was more susceptible to a dengue epidemic than Salvapan. This may enable the ministry of health to take preventative and control measures against it. Control measures such as spraying anti mosquito chemicals in the most needed area. This is in order, to decrease the chances for these communities to acquire this deadly disease. Thus, enabling the Ministry of Health ability to canalize their resources effectively and efficiently in the needed area. Finally, the study determined the dengue vectors were more abundantly found in the bucket which can be linked as their preferred breeding habitat. This may serve as the basis for vector control management, educational campaign strategies, and decision making by the Ministry of Health in regards as a mean to effectively control dengue outbursts in such areas. Where the ministry may distribute flyers and brochures to those areas on the importance of eliminating water holding containers form their yards and instructions on how to prevent dengue vectors from laying eggs in their potential preferred breeding site (more studies need to be done to determine this) the bucket. This is due to the fact that the bucket is one container that is used daily by the residents of such areas. Therefore, eliminating such containers is almost impossible but giving instructions on how to prevent mosquitos from laying eggs in the bucket can prevent or reduce a dengue outburst in such area.

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APPENDIX A

Consent Form (Formulario de consentimiento)

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Objective: To analyze the effects of dengue knowledge and attitudes on source reduction practice
(Objetivo: *Es analizar los efectos de los conocimientos y actitudes del dengue en la práctica de la reducción de fuentes.)*

I __________________________ am an adult (18 years and older) and I consent to take part in this study. I have had time to consider the information provided and asked questions when I wanted. I understood that the survey is entirely voluntary and I may leave the survey at any time without the need to give a reason. I also understand that I do not need to answer all the questions and that my name or any personal identifiable information will not be used. I fully understand that the survey will not be linked to the consent form. I also know that they will be testing the water body in the yard or around the yard and not in my house but I still have the option of declining that part of the survey. I am also entitled to receiving information about the publication if I want to.

(Soy __________________, soy mayor de edad (18 años y mayores) y doy mi consentimiento para participar en este estudio. He tenido tiempo para considerar la información proporcionada y preguntas cuando quería. Entendí que la encuesta es totalmente voluntaria y que puedo salir de la encuesta en cualquier momento sin la necesidad de dar una razón. También entiendo que no necesito responder a todas las preguntas y que no utilizarán mi nombre o cualquier

información de identificación personal. Entiendo perfectamente que la encuesta no estará vinculado al formulario de consentimiento. También sé que van a estar probando la masa de agua en el patio o alrededor del patio y no en adentro de mi casa, pero todavía tengo la opción de rechazar esa parte de la encuesta. Si quiero tengo derecho a recibir información acerca de la publicación.)

______________________	______________________	______________________
Name of Participant (Print)	Signature of Participant	Date
(Nombre del participante)	*(Firma del Participante)*	*(Fecha)*

Statement by Person Taking Consent ***(Declaración de la Persona Tomando el Consentimiento)***
I confirm that information sheet has been provided to the participants and the participant's questions have been answered correctly and to the best of my abilities. I confirm that the participants have not been bribed into giving consent and it is entirely voluntary. A copy of this consent form has been given to the participant.

(Confirmo que la hoja de información se ha proporcionado a los participantes y las preguntas de los participantes han sido contestadas correctamente y en la medida de mis capacidades. Confirmo que los participantes no han sido sobornados para dar su consentimiento y que es totalmente voluntario. Una copia del formulario de consentimiento se le ha dado al participante.)

______________________	______________________	______________________
Name of person (Print)	Signature of person	Date
(Nombre del participante)	*(Firma del Participante)*	*(Fecha)*

Observations:

(Observaciones)

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APPENDIX B

<u>Information Sheet (Hoja de información)</u>

We are a research team comprising of students from the University of Belize working in collaboration with the Belize Ministry of Health. We invite you to take part in our research study. This is entirely voluntary and you are not required to take part if you do not want to. Before deciding whether or not you want to participate it is important that you know what we are doing and what the study entails.

(Somos un equipo de investigación compuesto por estudiantes de la Universidad de Belice que trabajan en colaboración con el Ministerio de Salud de Belice. Te invitamos a participar en nuestro estudio de investigación. Esto es totalmente voluntario y no está obligado a participar. Pero antes de decidir si desea o no participar es muy importante que usted sepa lo que estamos haciendo y lo que implica el estudio.)

<u>**Please read the fallowing information carefully.** ***(Por favor lea la siguiente información cuidadosamente)***</u>

What is the study about? ***(¿Sobre qué es el estudio?)***
We are asking household members from Las Flores and Salvapan to answer a survey that will help us understand whether or not the educational campaigns of the Ministry of Health are working and to identify important knowledge gaps in order to improve the services provided by the ministry.

(Estamos pidiendo a los miembros del hogar de Las Flores y Salvapan para responder a una encuesta que nos ayudará a entender si las campañas de educación del Ministerio de Salud están trabajando. Y también para identificar importantes brechas de conocimiento con el fin de mejorar los servicios prestados por el ministerio.)

Why have I been chosen? ***(¿Por qué he sido elegido?)***
You have been chosen because you are a resident of Las Flores or Salvapan and is a consenting adult.
(Usted ha sido escogido porque usted es un residente de Las Flores o Salvapan y es un adulto.)

Is it mandatory to take part? ***(¿Es obligatorio para participar?)***
No, this is entirely voluntary and you will not be judged by refusing to participate. You may also leave at any time even if the survey is incomplete.
(No, esto es totalmente voluntario y no será juzgado por negarse a participar. También puede salir en cualquier momento, incluso si la encuesta no ha sido completada.)

What if I want to take part? ***(¿Qué pasa si quiero participar?)***
With your permission the surveyor will talk to you personally and take about 10 minutes of your time to ask you close and open ended questions. You may choose to write your answer or have the surveyor write for you. You may or may not answer all the questions asked. After the survey they may also take samples of water found around the yard with your permission. All surveys will be anonymous and your name will not be linked to the information you provided.

(Con su permiso, el inspector hablará con usted personalmente y tomará unos 10 minutos de su tiempo para preguntarle preguntas abiertas y cerradas. Usted puede optar por escribir su respuesta o que el topógrafo la haga por usted. Si usted quiere puede o no puede responder a todas las preguntas formuladas. Con su permiso, después de la encuesta también pueden tomar muestras de agua que se encuentra en el patio. Todas las encuestas serán anónimas y su nombre no estará vinculada a la información que ya ha proporcionado.)

Will you compensate me for the time this takes? ***(¿Me compensaran por el tiempo que tarde?)***
I'm sorry but no, we will not.

(Lo siento, pero no, no lo haremos.)

What are the disadvantage(s) and advantage(s) of taking part? ***(¿Cuáles son las desventajas y ventajas de tomar parte de esta encuesta?)***
The survey will take some of your time but it is advantageous because your answers will be important to the improvement of the health services supplied in Belize. It is also important to understand the epidemics of dengue.

(La encuesta le tomará un poco de su tiempo, pero es ventajoso debido a que sus respuestas serán importantes para la mejoría de los servicios de salud suministrados en Belice. También es importante para comprender las epidemias del dengue.)

What will happen to the results of this study? ***(¿Qué pasará con los resultados de este estudio?)***
Your name will not be used in the results of this study as it is just used to ensure the number of questionnaires are legitimate. The results will be shared through publication and through presenting at a symposium. If you would like to know the results of the study, please contact the team (details below) and we will share the information with you.

(Su nombre no será utilizado en los resultados de este estudio, ya que sólo se utiliza para asegurar que los cuestionarios sean legítimos. Los resultados serán compartidos a través de la publicación y mediante la presentación en un simposio. Si desea conocer los resultados del estudio, por favor, póngase en contacto con el equipo (detalles a continuación) y vamos a compartir la información con usted.)

APPENDIX C

Mosquito Larva Survey Table

Container Type	Number of Container Surveyed		Species of Mosquito Larvea							
	Pos.	Neg.	*Aedes albopictus*		*Aedes aegypti*		Other species		Other Species	
			N	%	N	%	N	%	N	%
Plastic (Deep)Container										
Waste Tire										
Barrel										
Mud pot										
Water Tank (Pila)										
Coconut Shell										
Plastic (Shallow Container)										
Tree Hole										
Unused well										
Glass Bottle										