MONTHLY ACTIVITY REPORT

March 2018

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ABSTRACT

The Belize Vector and Ecology Center has embarked on vector surveillance initiatives that will allow us to determine changes in distribution and density of vectors and obtain information about population changes. In turn this will allows us to monitor and evaluate adequate control methods. In this report, we have outlined the various activities that we have been conducting for the month of March.

The **Household Container Mapping** is being conducted by teams consisting of BVEC employees and Vector Control representatives. So far we have mapped approximately 20.61% of the total structures in Orange Walk Town. Mapping data was updated for the month of March based on the amount of structures we did.

Ovitrap Surveillance is being done in which 120 oviposition cups are set out to 60 pre-consented homes in Orange Walk Town. The hatch rate has been determined to be about 40 to 60% and there is interesting variation in the number of eggs collected per zone which may also be related to the position of ovicups and climatic conditions.

The **BG Sentinel Surveillance** is being conducted in order to collect live adult *Aedes* mosquitoes in the field. There are 12 BG Sentinels in the town at 12 pre-consented homes. The traps so far have collected a number of *Aedes* mosquitoes as well as some from the *Culex* species and other types of flies.

The adult *Aedes* mosquitoes collected in the traps are then sorted, identified and stored for testing using the **Dengue Antigen Kit**. Tests conducted so far have turned up negative and testing will continue while working on a pooling strategy depending on the number of adult females collected in the field**.**

Larval Resistance Testing is another key component to the surveillance which will be done in conjunction with the ovitrap surveillance initiative. Currently eggs are being pooled and hatched so that there is enough larvae to meet the standard testing methods.

BVEC also does **Presentations** and **Training** sessions in which we collaborate with schools and other groups to foster community engagement.

MAPPING (HOUSEHOLD CONTAINER MONITORING)

The MOH OW Vector Control Department has been undergoing an initiative in which we map the structures within Orange Walk Town by acquiring GPS data points and gathering structure information (i.e. level of structure, type, no. of doors, windows etc). This data is being linked to the daily *Aedes aegypti* yard inspection that the MOH is collecting. All the data is being collected with the use of an online form that is displayed on a tablet and utilized by BVEC research assistants. Data collection is done in teams with the presence of at least one vector control representative. Each structure is coded with a unique structure ID that is generated based on the zone, street name and number of the structure. The zonation is obtained from a previous database that the town council used in order to facilitate proper sanitation of the town. Orange Walk town is divided into 6 Zones labelled from A to F. The Ministry of Health uses these same zones so that each Vector Control representative has their designated zones that they are assigned to. New container data as well as the rate of positivity per container type was established. Below are data collected so far that has been analysed up to the month of March 2018.

Aedes Postive Containers	Zone A	ZoneB	Zone C	Zone D	Zone E	Zone F	Total
Vat/tank	3	0	0	0	0	1	4
Drum	14	2	1	0	5	5	27
Vases	2	0	0	0	0	0	2
Bucket	6	2	3	0	5	1	17
Tires	7	3	1	0	15	2	28
Bottle/cans	12	0	0	0	1	0	13
Coconut Shells	0	0	0	0	0	0	0
Treeholes/wells	1	0	0	0	0	0	1
Watering Pans	0	0	0	0	0	0	0
Other	10	6	5	0	16	3	40
Grand Total	55	13	10	0	42	12	132

Table 1. Aedes positive containers per zone

Common Containers Types	Zone A	Zone B	Zone C	Zone D	Zone E	Zone F	Total
Vat/tank	25	21	8	0	23	9	86
Drum	57	8	6	0	17	16	104
Vase	15	0	0	0	3	0	18
Bucket	121	57	25	0	103	47	353
Tires	69	10	4	0	68	13	164
Bottle/cans	235	172	142	0	307	91	947
Coconut Shells	26	0	0	0	2	0	28
Treeholes/wells	12	0	0	0	2	8	22
Watering Pans	50	0	0	0	14	0	64
Other	233	171	70	0	177	188	839
Grand Total	843	439	255	0	716	372	2625

Table 2. Number of different types of containers found per zone

Structures mapped	Total
House	988
House/store	40
Store	13
Apartment/Hotel	14
Lab	0
Church	11
Bar	3
House/bar	2
Restaurant	1
School	4
Cemetery	1
Office	3
Clinic	1
Business	15
Other	5
TOTAL STRUCTURES	5449
TOTAL MAPPED	1123
PERCENTAGE COMPLETED	20.61%

Table 3. Types of structures mapped along with percentage completion

Container Types	Rate of Positivity
Vat/tank	4.7%
Drum	26.0%
Vases	11.1%
Bucket	4.8%
Tires	17.1%
Bottle/cans	1.4%
Coconut Shells	0.0%
Treeholes/wells	4.5%
Watering Pans	0.0%
Other	4.8%

Table 4. Rate of positivity per container based on total positive over total encountered at the yard.

Note: Map displaying GPS points of structures mapped as of March 2018 is attached in the appendices on Page 32

OVITRAP SURVEILLANCE

The Belize Vector and Ecology Center is currently undergoing an ovitrap/ovicup surveillance program for six (6) months in Orange Walk Town to capture the eggs of *Ae. aegypti* and *Ae. albopictus*, key vectors in the transmission of Dengue, Zika, and Chikungunya. Homes were selected via a random selection process generated by QGIS software. A total of sixty (60) homes were selected throughout the six (6) zones in Orange Walk Town. Two (2) ovicups/ovitraps were placed in each home, giving a total of 120 ovicups/ovitraps placed throughout the entire town. Note that informed consent was obtained from all home/property owners to participate in the study under the supervision of a Ministry of Health (MoH) Vector Control Officer.

Ovitraps/ovicups are left in the field for a total of seven (7) days, after which the egg papers inside every ovitrap/ovicup is retrieved and taken to the BVEC insectary. Egg papers are carefully folded and placed in a plastic container, egg papers from each home remain separate from one another. The ovitraps/ovicups are cleaned with a scourging sponge and a new labelled egg paper and fresh water are added. Once in the BVEC insectary, the freshly collected egg papers are hung for a 24 hour period to remove any excess water. Once dry, the eggs are counted and the eggs are stored. Note that egg hatching for each zone is being done by pooling eggs from previous weeks, mainly to ensure that there are enough eggs to yield the number of larvae needed to perform a larvicide resistance test. Below is an actual picture of an Ovicup with egg paper in the field.

Figure 1. Oviposition cup with egg paper inside

Note: A copy of the consent form that was used is attached in the appendices on Page 36

When hatching eggs, egg papers for a particular zone are carefully inspected for eggs, and then segments of the egg paper containing the eggs are cut and placed in a container with water. The container with eggs is then placed in the vacuum hatcher for hatching. Use of the vacuum hatcher ensures a more synchronized hatch and same size larvae. After a few days, third and early fourth stage larvae are sorted for a larvicide resistance test.

Table 5 below shows the number of eggs collected for each zone for every week. Note that each zone is different in terms of geographic location within the town and whether it is more of a commercial or housing area. The different weather patterns experienced each week are also an important factor influencing oviposition each week. For this reason, the egg density will vary across zones and time.

Table 5. Number of eggs collected in each zone per week of surveillance for a total of four (4) weeks surveillance period. Zone E has the highest number of eggs for the entire surveillance period of four (4) weeks with n=2137. Zone F has the lowest number of eggs for the entire surveillance period with n=623.

ZONE	WEEK 21	WEEK 22	WEEK 23	WEEK 24	TOTAL
A	279	207	268	281	1035
B	201	180	180	346	907
C	337	78	180	196	791
D	432	205	754	241	1632
E	316	326	483	1012	2137
F	237	112	72	202	623
TOTAL	1802	1108	1937	2278	7125

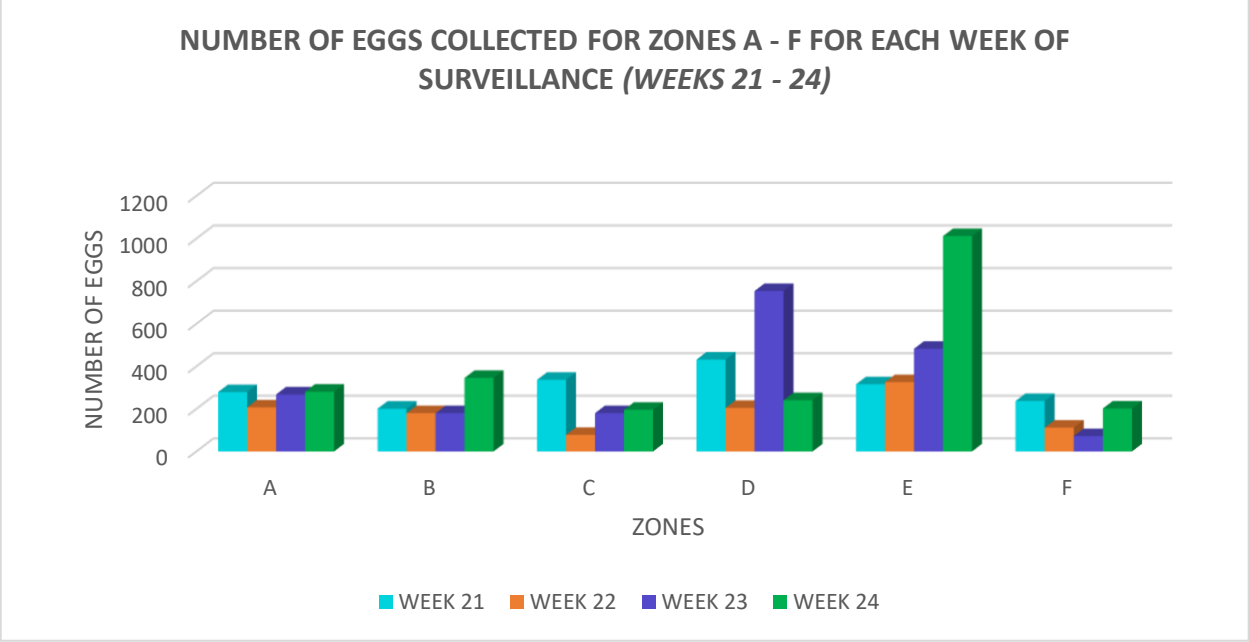


Figure 2. Number of eggs collected per week (4 weeks) for each zone.

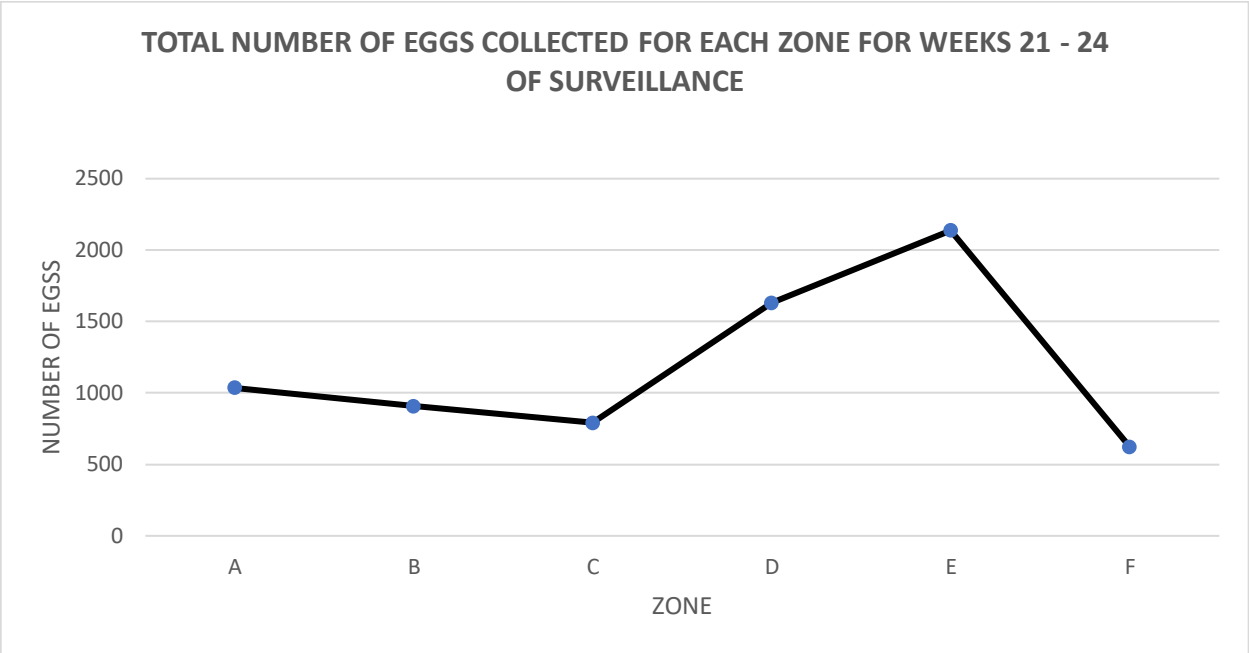
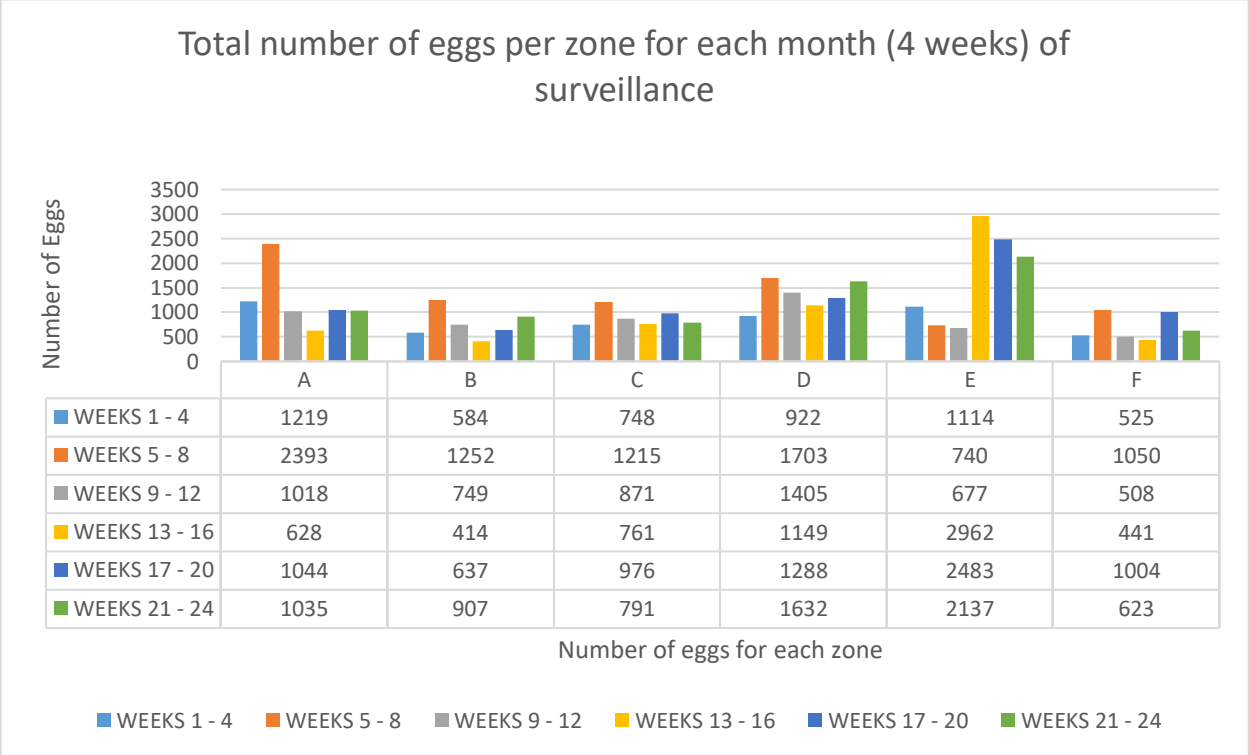


Figure 3. Number of eggs collected in each zone for the entire surveillance period of four (4) weeks/1 month.



	A	B	C	D	E	F
WEEKS 1 - 4	1219	584	748	922	1114	525
WEEKS 5 - 8	2393	1252	1215	1703	740	1050
WEEKS 9 - 12	1018	749	871	1405	677	508
WEEKS 13 - 16	628	414	761	1149	2962	441
WEEKS 17 - 20	1044	637	976	1288	2483	1004
WEEKS 21 - 24	1035	907	791	1632	2137	623

Figure 4. Cumulative data for each ovicup/ovitrap surveillance month, including latest month (weeks 21 – 24).

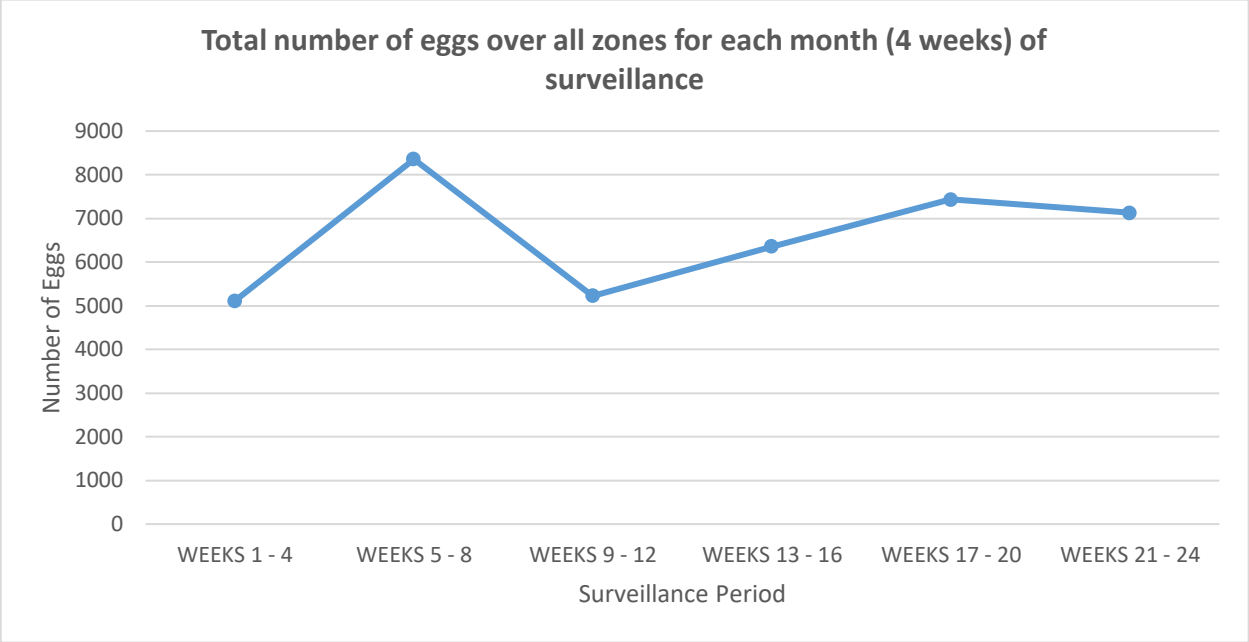


Figure 5. Number of eggs collected per month of surveillance for six (6) months surveillance period.

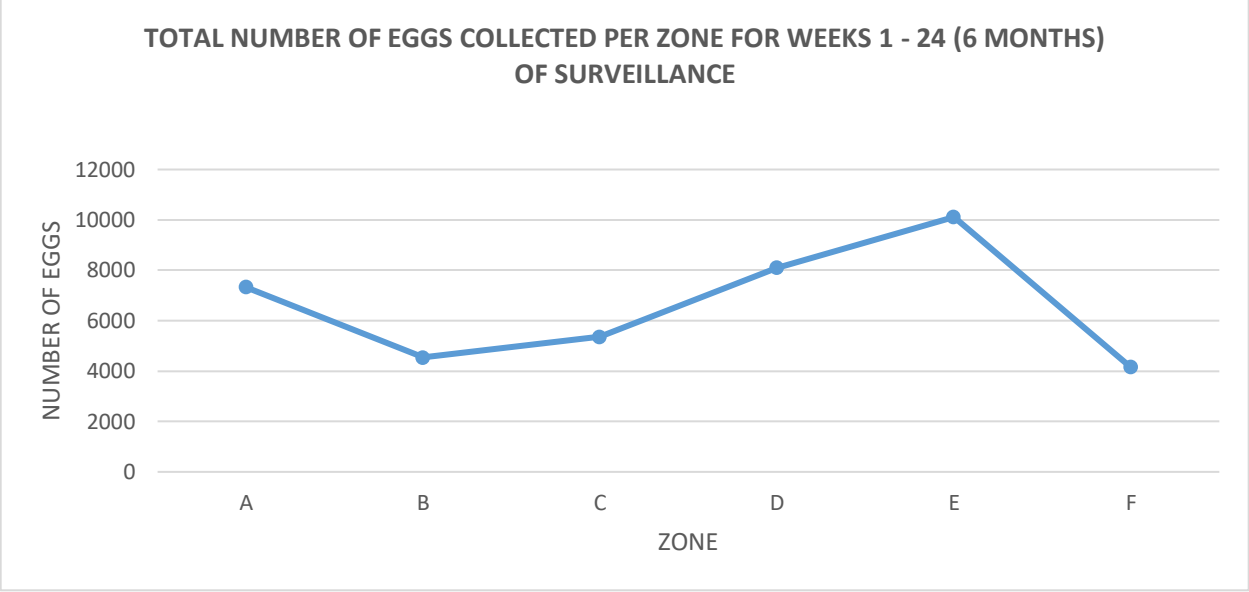


Figure 6. Number of eggs collected per zone for a period of six (6) months surveillance.

ZONE A OVITRAPS - Weeks 21 – 24

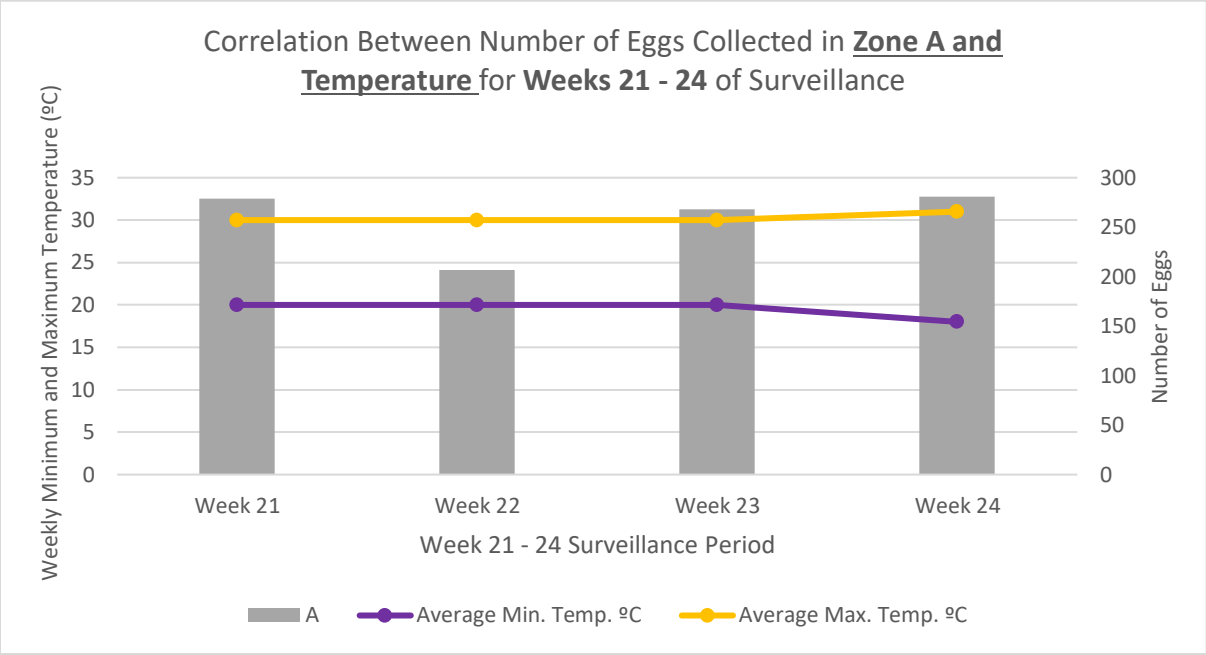


Figure 7. Number of eggs collected in Zone A and temperature data for weeks 21 – 24 of surveillance. Week 24 has the highest number of eggs collected (n=281) and Week 22 has the lowest number of eggs collected (n=207).

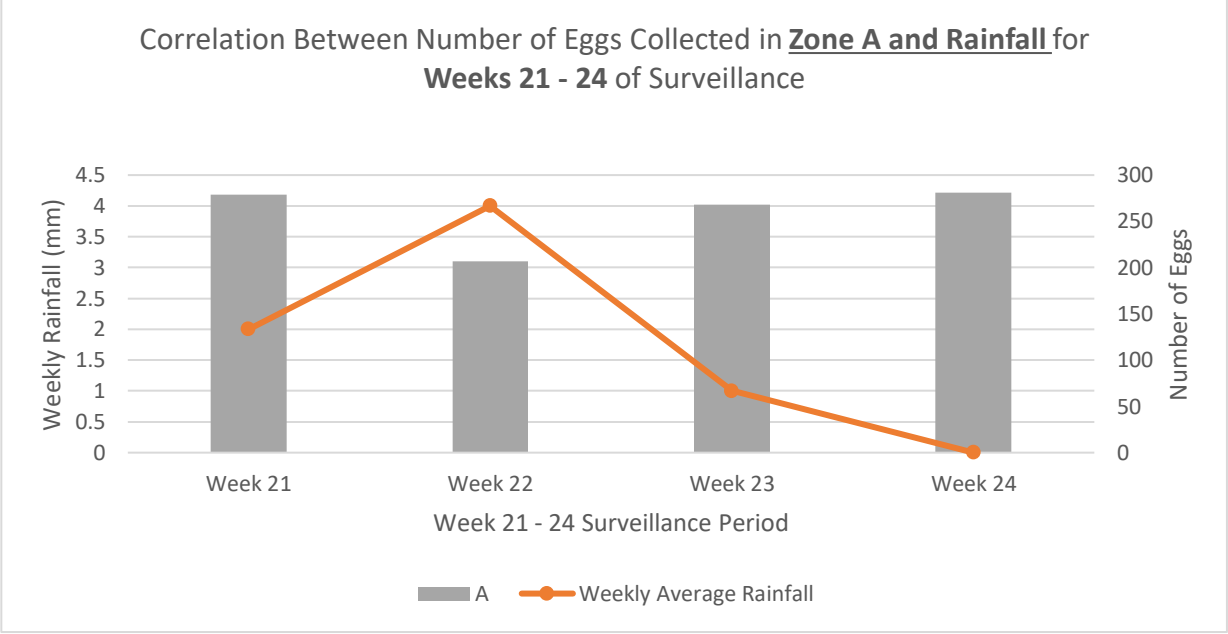


Figure 8. Number of eggs collected in Zone A and rainfall data for weeks 21 – 24 of surveillance.

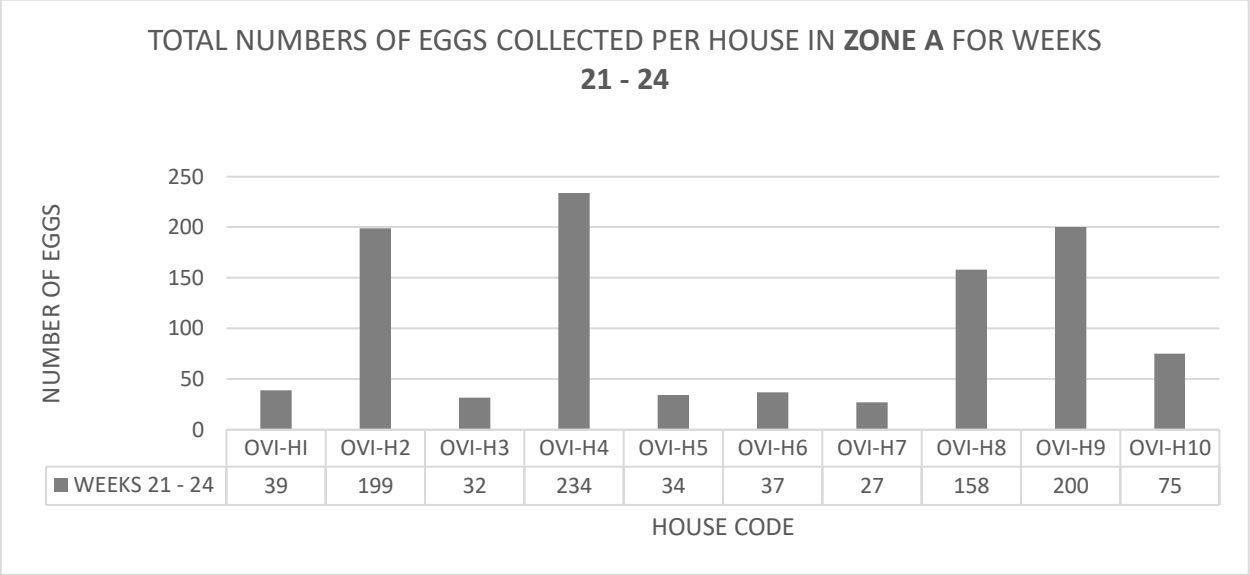


Figure 9. Number of eggs collected per house in Zone A for weeks 21 – 24 of surveillance. House 7 (OVI-H7) has the lowest number of eggs (n=27) and House 4 (OVI-H4) has the highest number of eggs (n=234).

ZONE B OVITRAPS

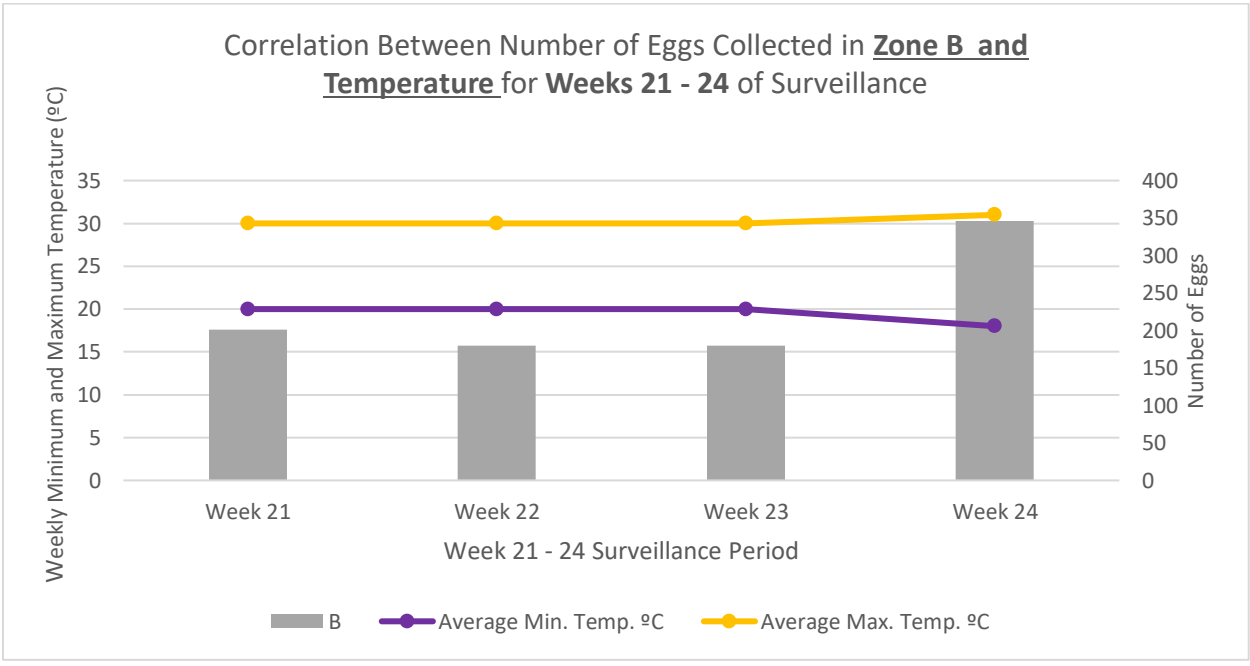


Figure 10. Number of eggs collected in Zone B and temperature data for weeks 21 – 24 of surveillance. Week 24 has the highest number of eggs collected (n=346) and Weeks 22 and 23 have the lowest number of eggs collected (n=180).

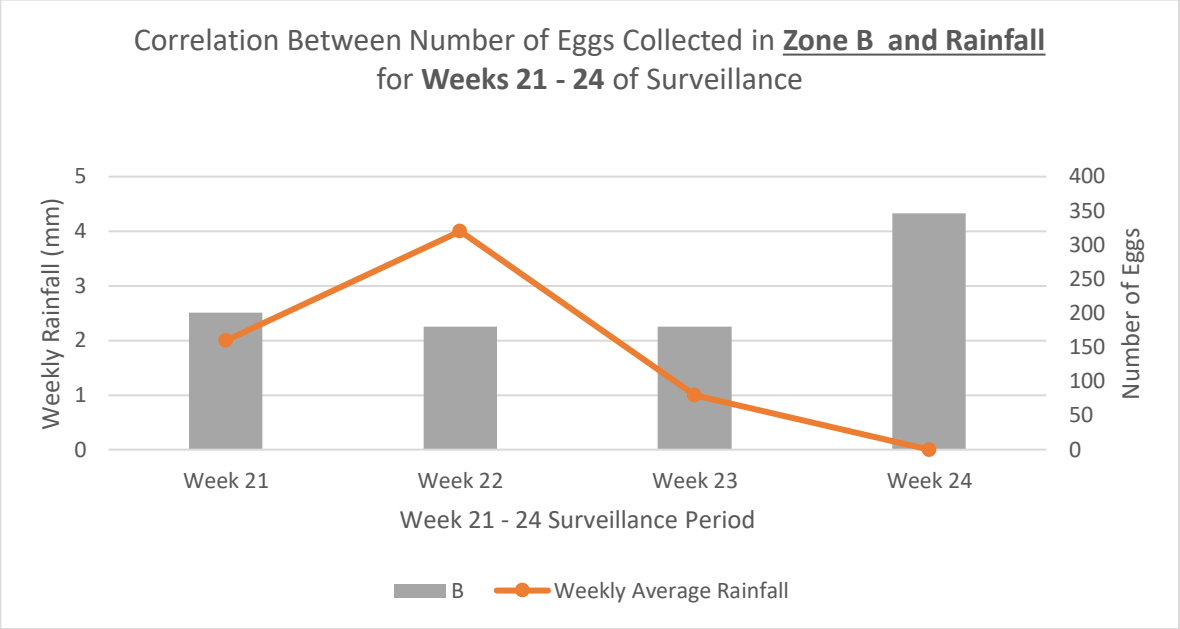


Figure 11. Number of eggs collected in Zone B and rainfall data for weeks 21 – 24 of surveillance.

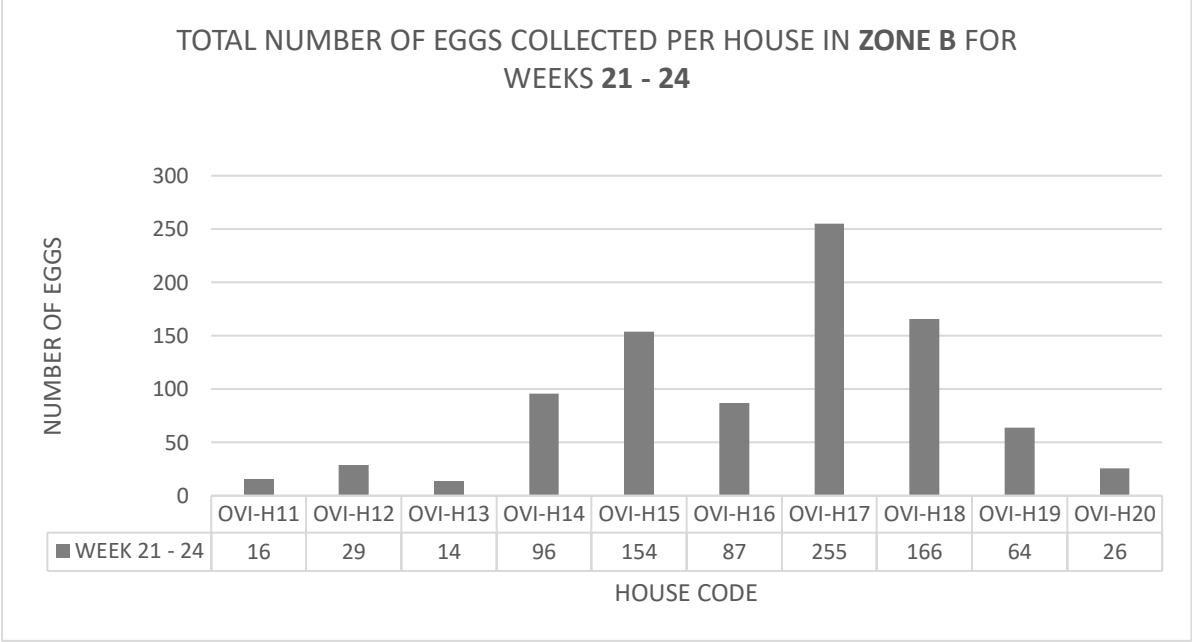


Figure 12. Number of eggs collected per house in Zone B for weeks 21 – 24 of surveillance. House 13 (OVI-H13) has the lowest number of eggs (n=14) and House 17 (OVI-H17) has the highest number of eggs (n=255).

ZONE C OVITRAPS

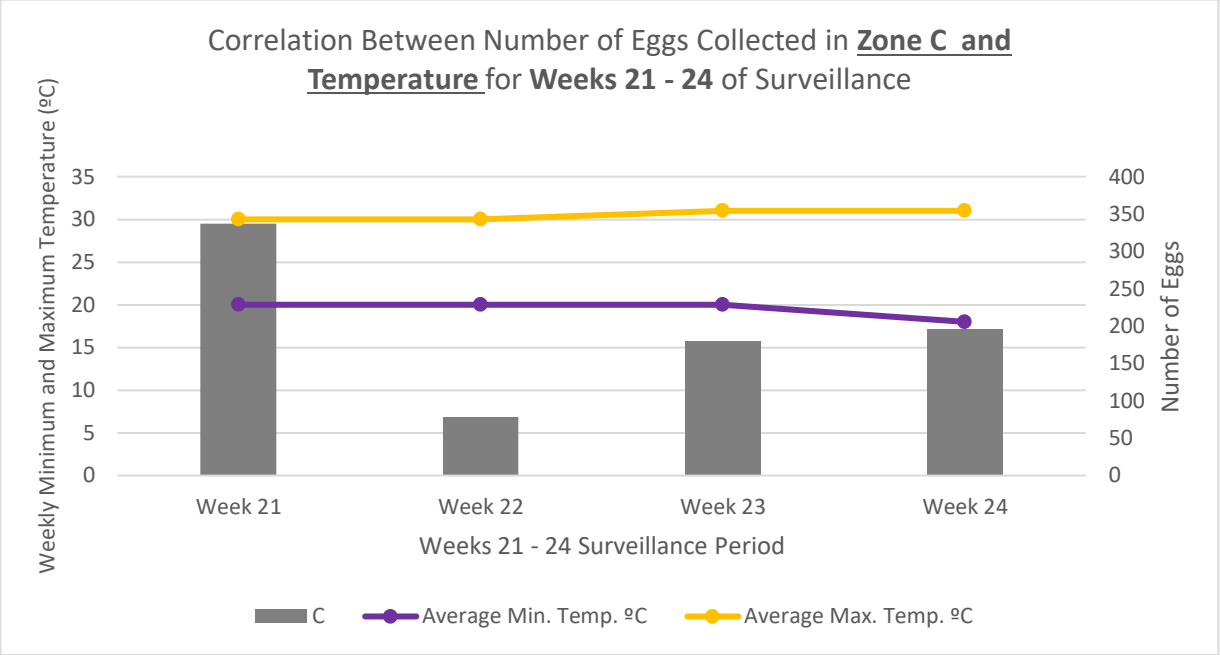


Figure 12. Number of eggs collected in Zone C and temperature data for weeks 21 – 24 of surveillance. Week 21 has the highest number of eggs collected (n=337) and Week 22 has the lowest number of eggs collected (n=78).

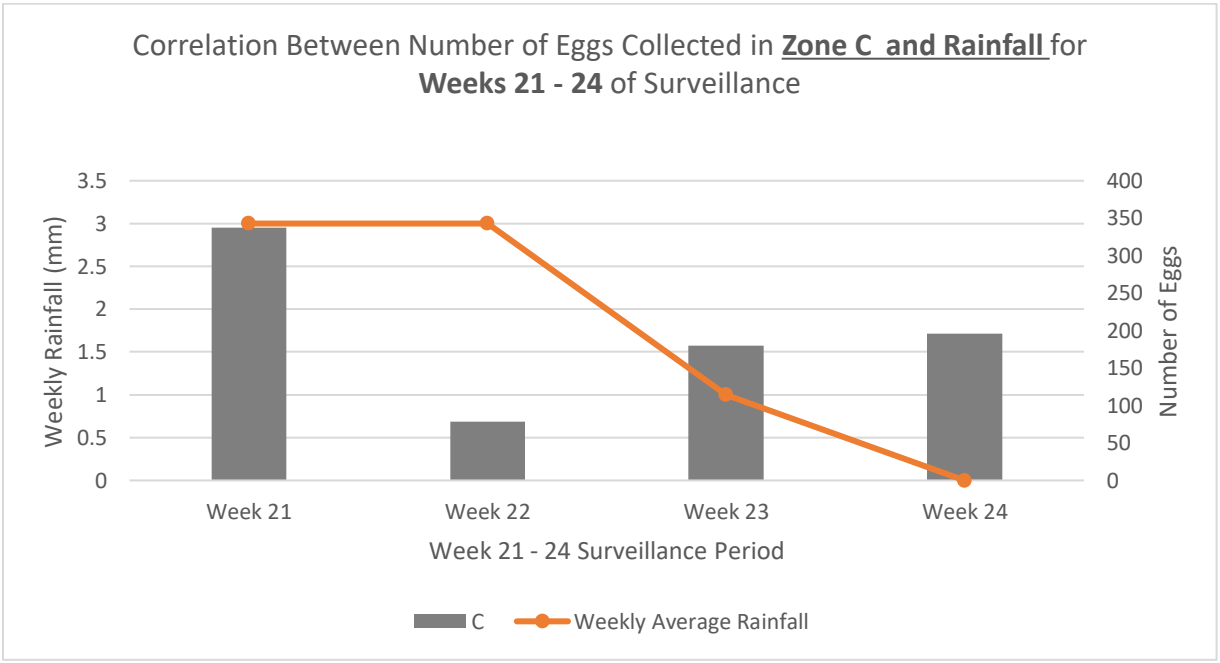


Figure 13. Number of eggs collected in Zone C and rainfall data for weeks 21 – 24 of surveillance.

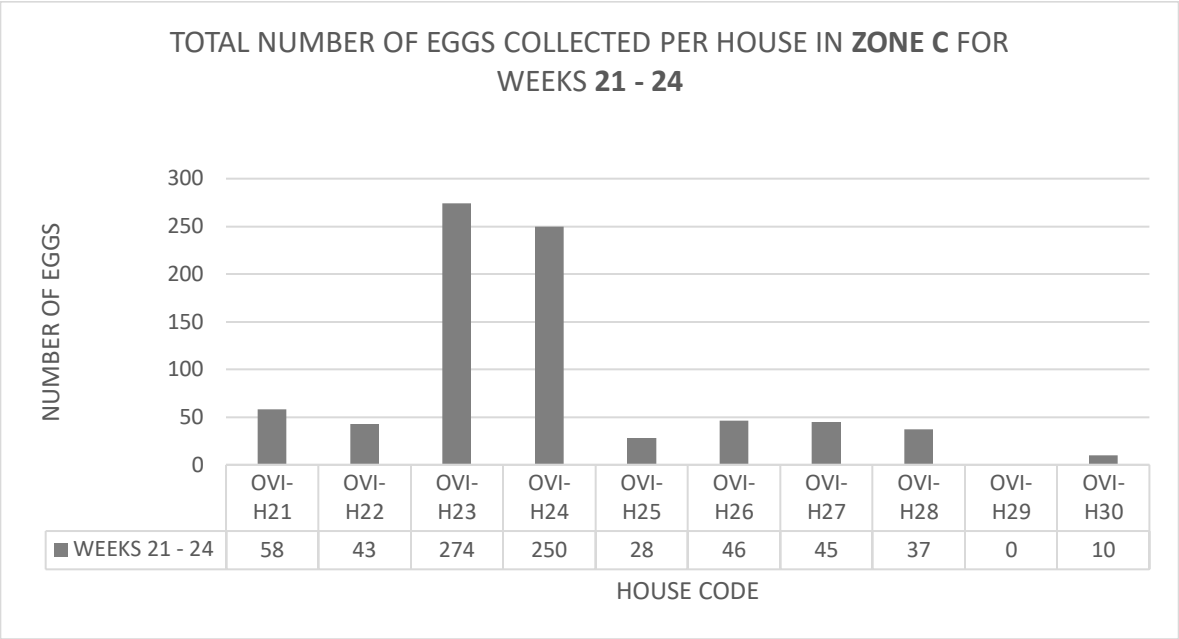


Figure 14. Number of eggs collected per house in Zone C for weeks 21 – 24 of surveillance. House 29 (OVI-H29) has the lowest number of eggs (n=0) and House 23 (OVI-H23) has the highest number of eggs (n=274).

ZONE D OVITRAPS

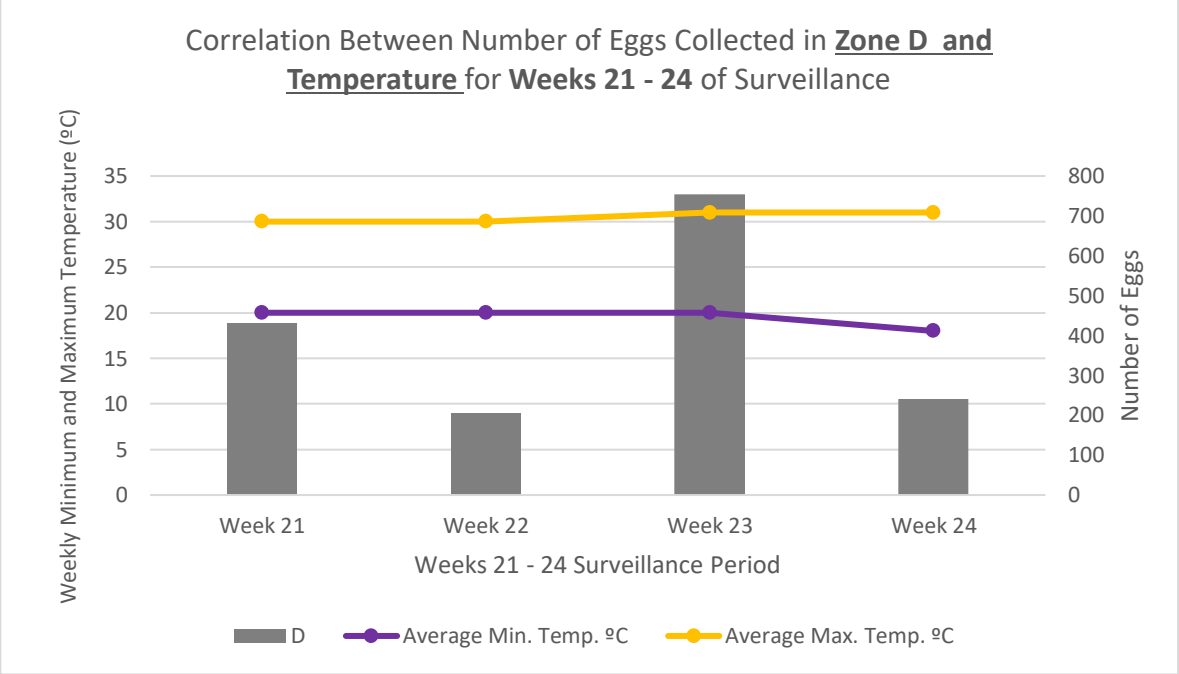


Figure 15. Number of eggs collected in Zone D and temperature data for weeks 21 – 24 of surveillance. Week 23 has the highest number of eggs collected (n=754) and Week 22 has the lowest number of eggs collected (n=205).

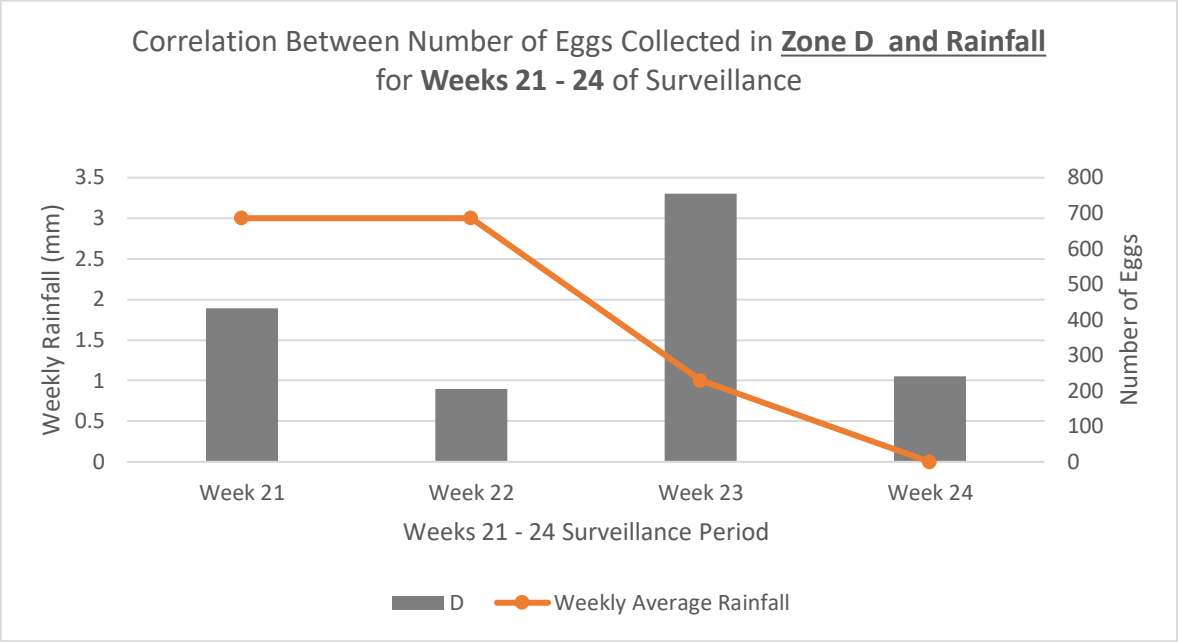


Figure 16. Number of eggs collected in Zone D and rainfall data for weeks 21 – 24 of surveillance.

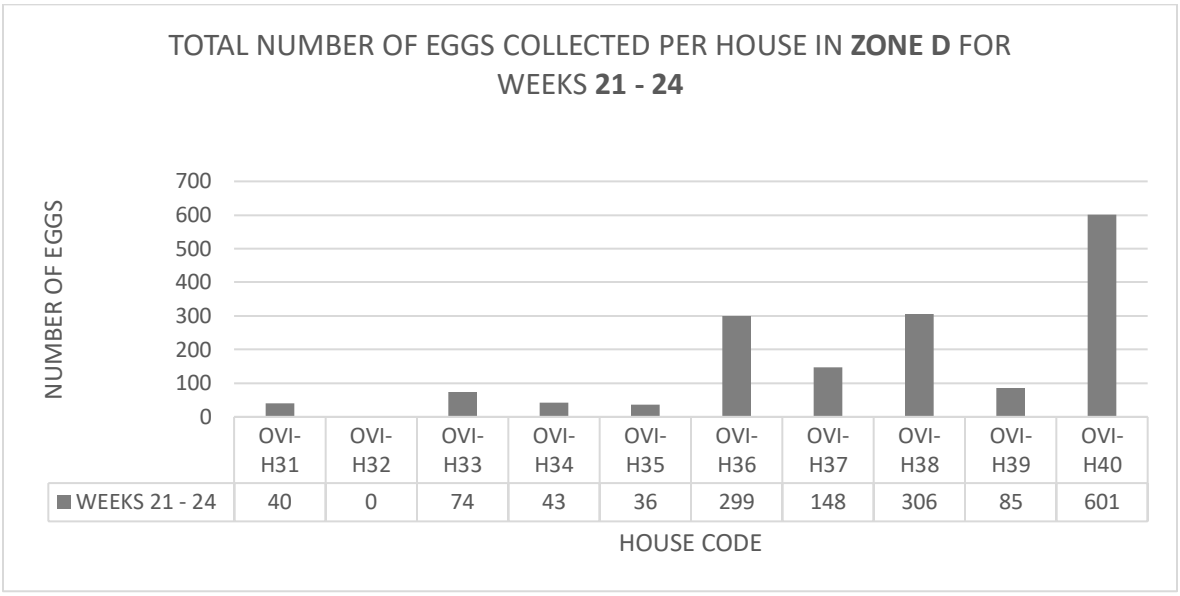


Figure 17. Number of eggs collected per house in Zone D for weeks 21 – 24 of surveillance. House 32 (OVI-H32) has the lowest number of eggs (n=0) and House 40 (OVI-H40) has the highest number of eggs (n=601).

ZONE E OVITRAPS

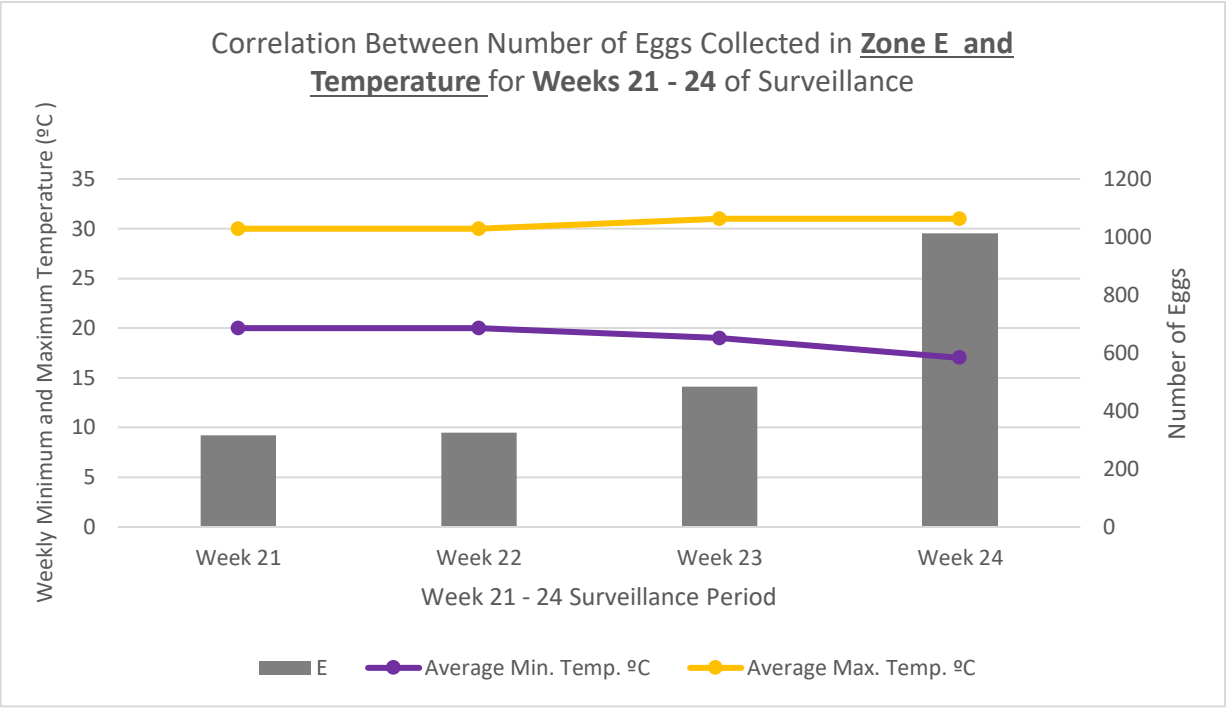


Figure 18. Number of eggs collected in Zone E and temperature data for weeks 21 – 24 of surveillance. Week 24 has the highest number of eggs collected (n=1012) and Week 21 has the lowest number of eggs collected (n=316).

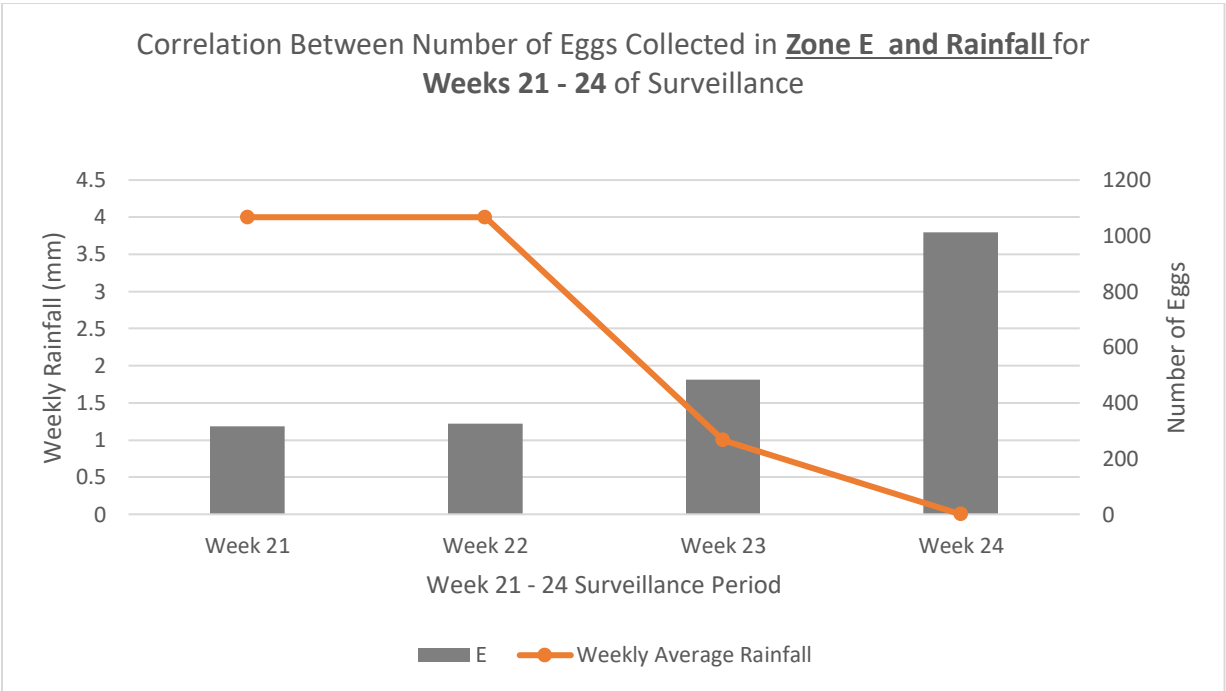


Figure 19. Number of eggs collected in Zone E and rainfall data for weeks 21 – 24 of surveillance.

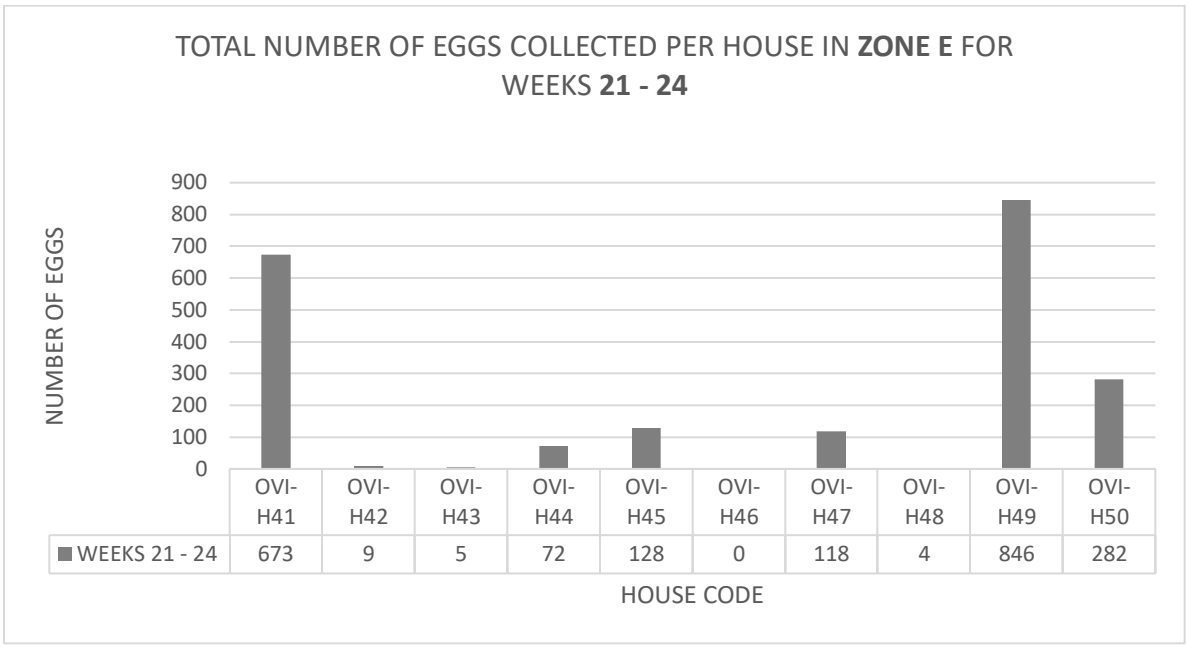


Figure 20. Number of eggs collected per house in Zone E for weeks 21 – 24 of surveillance. House 46 (OVI-H46) has the lowest number of eggs (n=0) and House 49 (OVI-H49) has the highest number of eggs (n=846).

ZONE F OVITRAPS

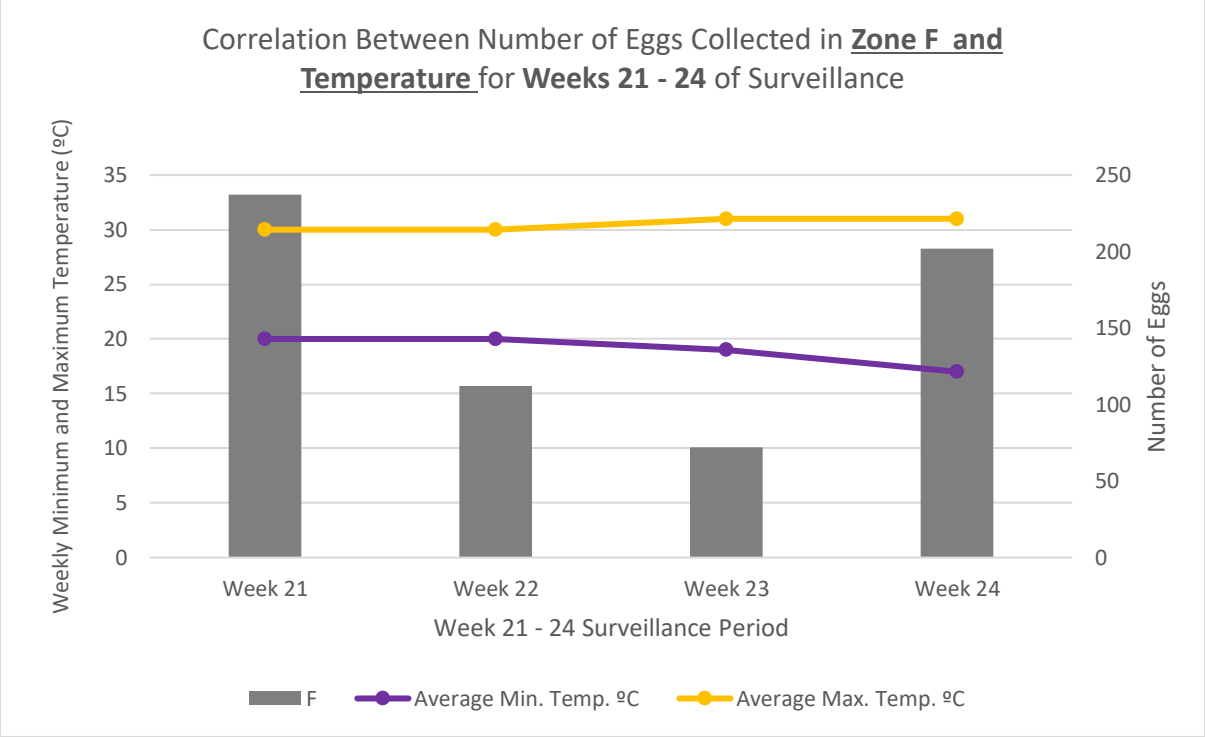


Figure 21. Number of eggs collected in Zone F and temperature data for weeks 21 – 24 of surveillance. Week 21 has the highest number of eggs collected (n=237) and Week 23 has the lowest number of eggs collected (n=72).

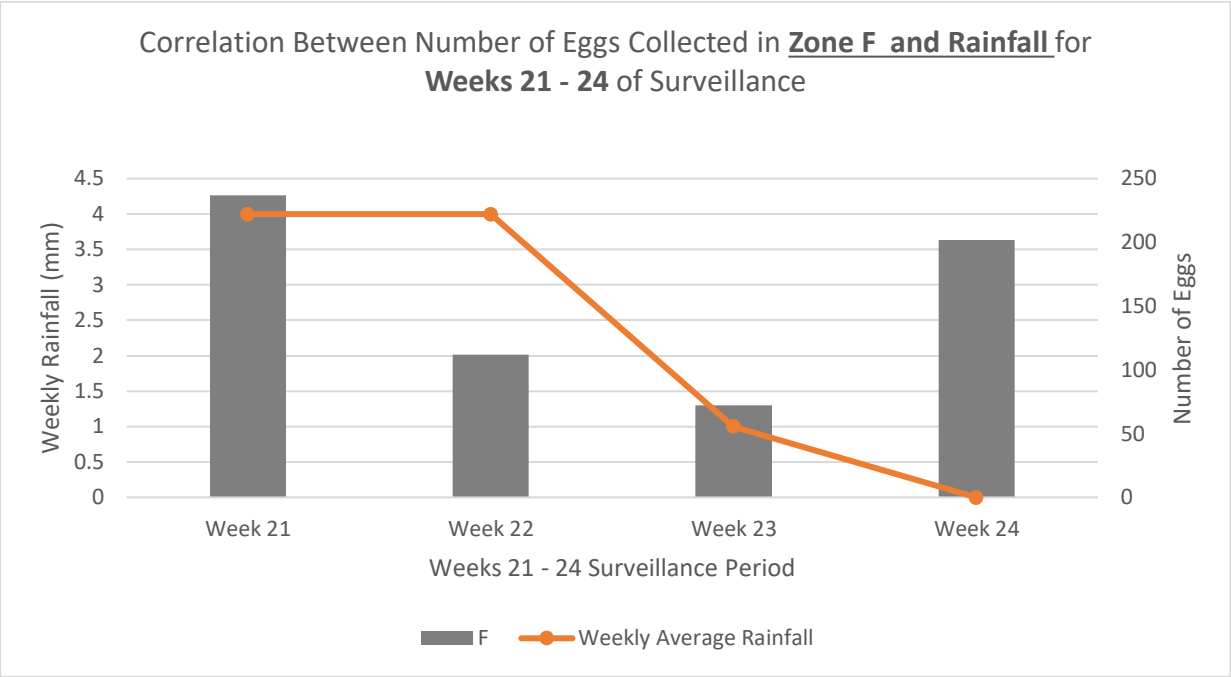


Figure 22. Number of eggs collected in Zone F and rainfall data for weeks 21 – 24 of surveillance.

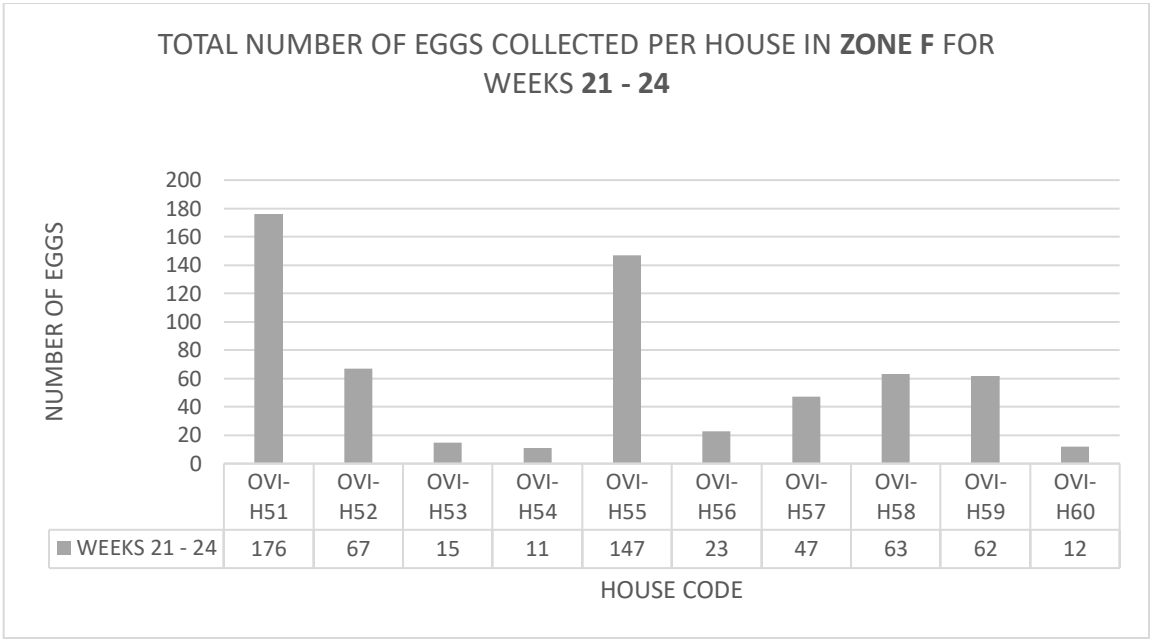


Figure 23. Number of eggs collected per house in Zone F for weeks 21 – 24 of surveillance. House 54 (OVI-H54) has the lowest number of eggs (n=11) and House 51 (OVI-H51) has the highest number of eggs (n=176).

BG SURVEILLANCE

For this particular surveillance activity, Biogent (BG) Sentinel traps are used to collect adult *Ae. aegypti* and *Ae. albopictus* mosquitoes in the field. A total of 12 homes (2 homes per zone) were selected via a random selection process generated by QGIS software. Informed consent was obtained from all home/property owners with the supervision of a MOH Vector Control representative.

BGS traps are set out in the field once a week. Note that traps are placed in the mornings and left to operate for 7-8 hours, and then retrieved in the afternoon of the same day. Below is an image of a BG Sentinel taken from one of the houses that is participating in the surveillance initiative.

Figure 24. BG Sentinel trap setup in the field.

The mosquitoes along with other insect specimens collected are taken to the BVEC laboratory and stored for later identification. Identification is done once a week. *Aedes Spp*. and *Culex Spp*. are stored, while any other insect specimens are discarded. Once mosquitoes are identified, they are stored back in the freezer until they can be processed for testing.

Table 6. Number of adult Aedes mosquitoes, both male and female, captured per house in each zone for each week of surveillance. Zone C has the highest number (n=29) of adult Aedes mosquitoes captured while Zone F has the lowest number of adult Aedes mosquitoes captured (n=3). In terms of weekly captures, week 24 had the highest number (n=39) of mosquitoes captured while week 22 had the lowest number of mosquitoes captured (n=16).

ZONE	HOUSE CODE	Total Adult Aedes (♂,♀) Collected Per Week				TOTAL
		Week 21	Week 22	Week 23	Week 24	
		02-Mar-18	09-Mar-18	16-Mar-18	23-Mar-18	
A	BG-H1	0	1	9	0	10
A	BG-H2	1	0	0	2	3
TOTAL		1	1	9	2	13
B	BG-H3	8	4	0	6	18
B	BG-H4	7	2	0	0	9
TOTAL		15	6	0	6	27
C	BG-H5	2	0	2	6	10
C	BG-H6	3	5	3	8	19
TOTAL		5	5	5	14	29
D	BG-H7	1	3	1	1	6
D	BG-H8	0	0	0	0	0
TOTAL		1	3	1	1	6
E	BG-H9	6	0	1	12	19
E	BG-H10	0	0	0	3	3
TOTAL		6	0	1	15	22
F	BG-H11	0	0	0	0	0
F	BG-H12	0	1	1	1	3
TOTAL		0	1	1	1	3
GRAND TOTAL		28	16	17	39	100

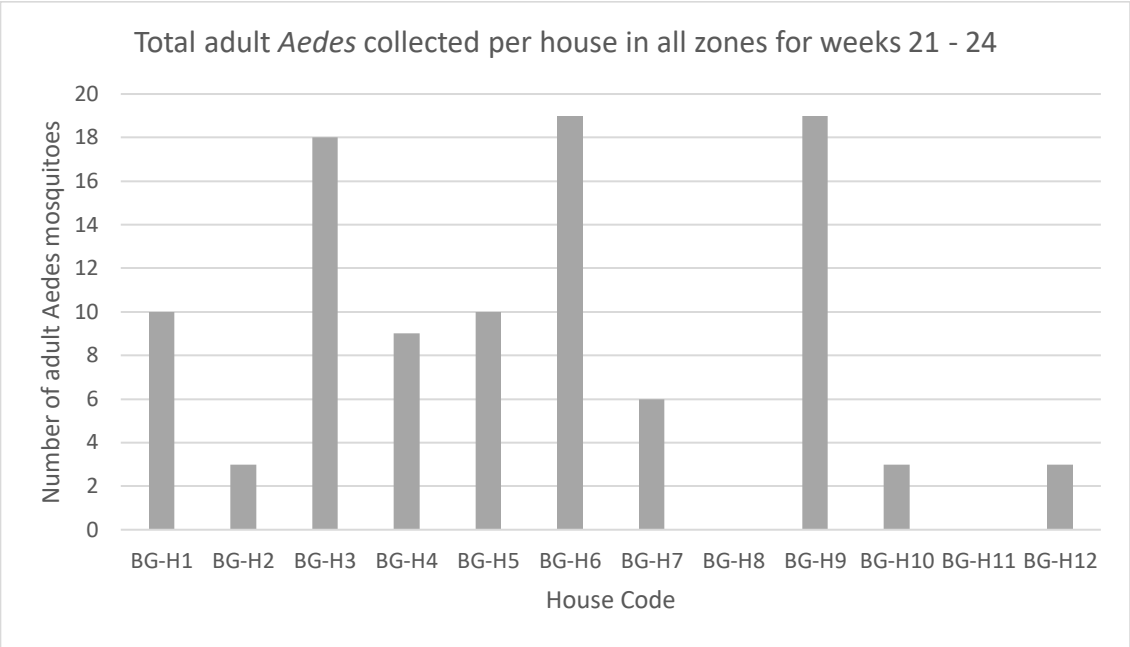
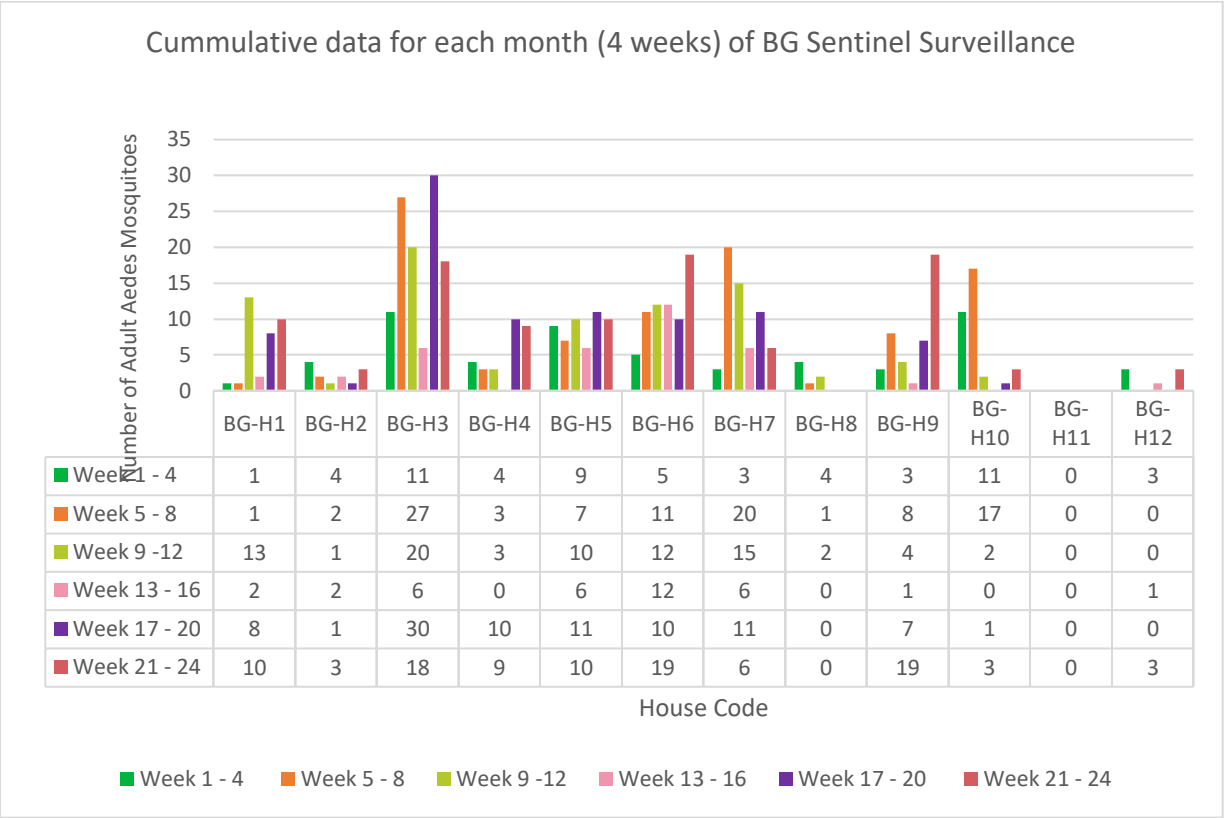


Figure 25. Number of adult Aedes mosquitoes captured per house for four (4) weeks pf surveillance. House 6 and 9 (BG-H6, BG-H9) have the highest number of mosquitoes captured (n=19) and Houses 8 and 11 (BG-H8, BG-H11) have the lowest number with zero mosquitoes captured during the four (4) weeks of BGS trap surveillance.

Table 7. Number of Aedes mosquitoes captured per house for every month (4 weeks) of BGS trap surveillance in all zones (A-F) in Orange Walk Town. The month with the highest number of mosquitoes captured is the sixth month (weeks 21-24) with one-hundred (n=100) mosquitoes. The month with the lowest number of mosquitoes captured is month 4 (weeks 13-16) with thirty-six (n=36) mosquitoes.

Cumulative Data - Total *Aedes* (♀+♂) mosquitoes captured per house for all zones for each month (4 weeks) of BG Sentinel Surveillance							
ZONE	**House Code**	**Week 1 - 4**	**Week 5 - 8**	**Week 9 -12**	**Week 13 - 16**	**Week 17 - 20**	**Week 21 - 24**
A	**BG-H1**	1	1	13	2	8	10
A	**BG-H2**	4	2	1	2	1	3
B	**BG-H3**	11	27	20	6	30	18
B	**BG-H4**	4	3	3	0	10	9
C	**BG-H5**	9	7	10	6	11	10
C	**BG-H6**	5	11	12	12	10	19
D	**BG-H7**	3	20	15	6	11	6
D	**BG-H8**	4	1	2	0	0	0
E	**BG-H9**	3	8	4	1	7	19
E	**BG-H10**	11	17	2	0	1	3
F	**BG-H11**	0	0	0	0	0	0
F	**BG-H12**	3	0	0	1	0	3
TOTAL		**58**	**97**	**82**	**36**	**89**	**100**



	BG-H1	BG-H2	BG-H3	BG-H4	BG-H5	BG-H6	BG-H7	BG-H8	BG-H9	BG-H10	BG-H11	BG-H12
Week 1 - 4	1	4	11	4	9	5	3	4	3	11	0	3
Week 5 - 8	1	2	27	3	7	11	20	1	8	17	0	0
Week 9 -12	13	1	20	3	10	12	15	2	4	2	0	0
Week 13 - 16	2	2	6	0	6	12	6	0	1	0	0	1
Week 17 - 20	8	1	30	10	11	10	11	0	7	1	0	0
Week 21 - 24	10	3	18	9	10	19	6	0	19	3	0	3

Figure 26. Number of adult Aedes mosquitoes captured per house in all zones for each month (4 weeks) of BG Sentinel surveillance thus far.

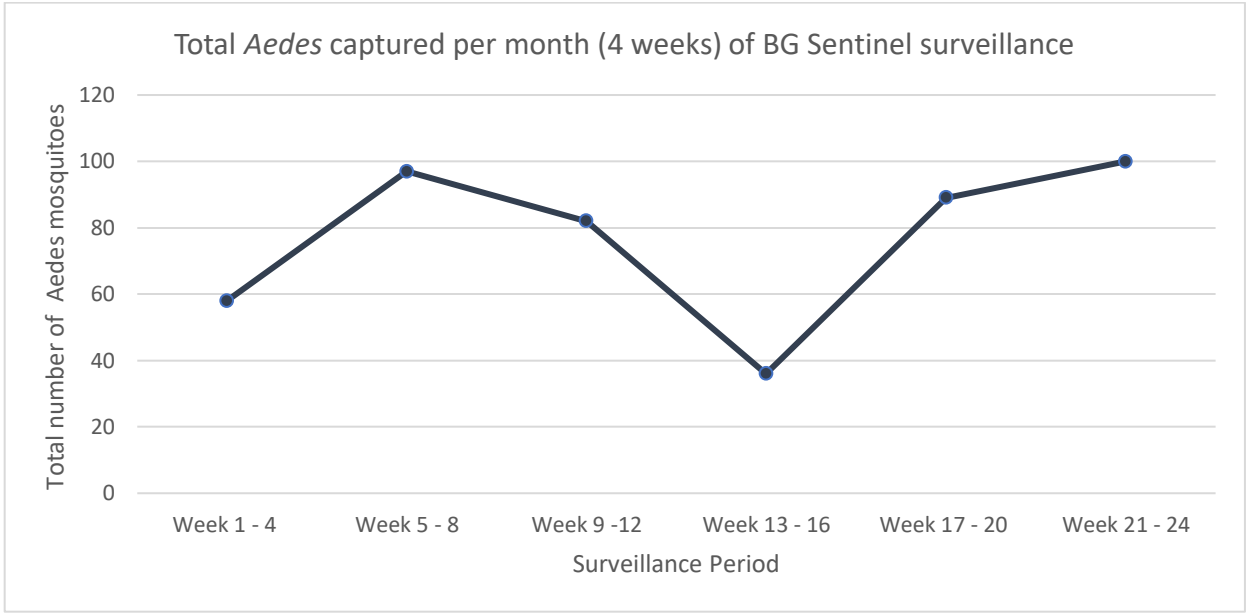


Figure 27. Number of adult Aedes mosquitoes captured per month (4 weeks) of BGS trap surveillance. Includes both male and female Aedes mosquitoes captured over a period of six (6) months.

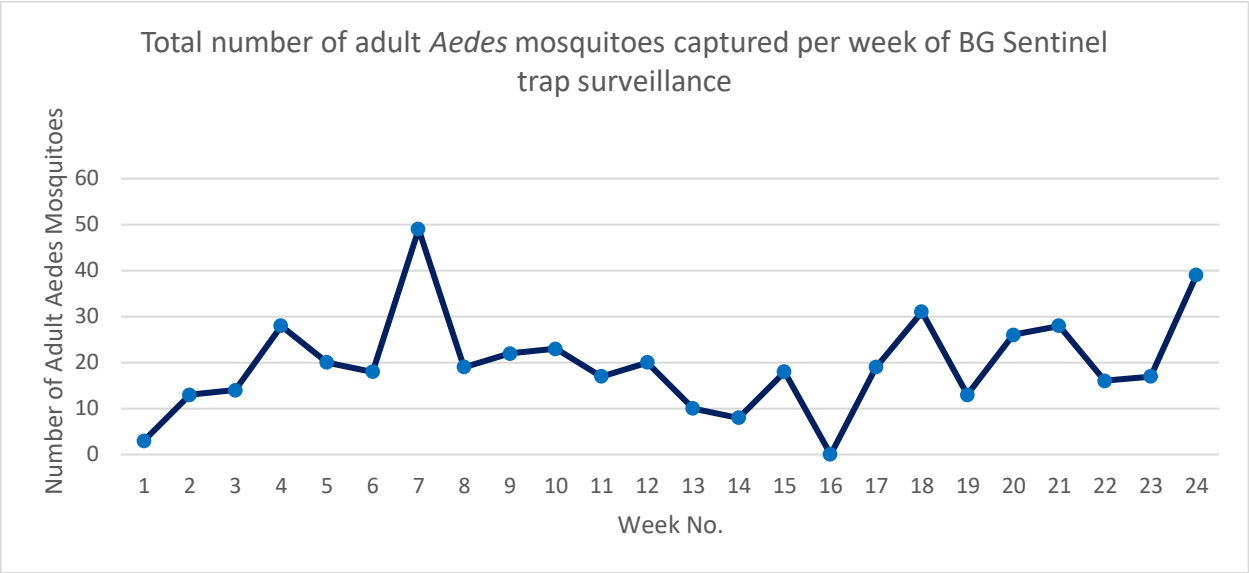


Figure 28. Number of adult Aedes mosquitoes captured per week of BGS trap surveillance. Includes both male and female Aedes mosquitoes captured over a period of 24 weeks (6 months).

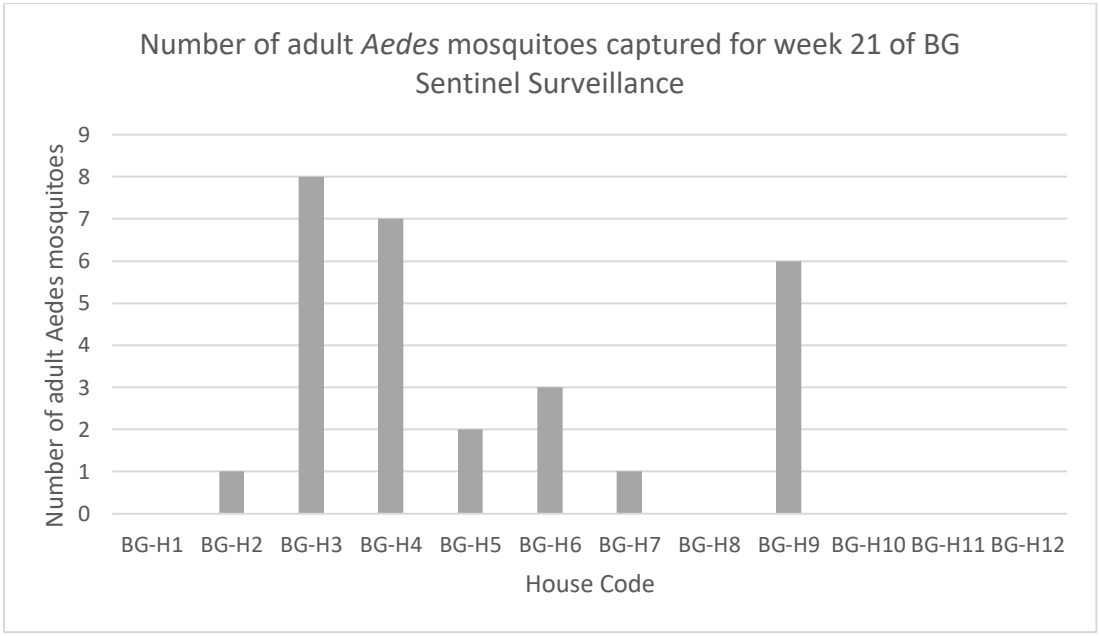


Figure 19. Number of adult Aedes mosquitoes captured per house in all zones for week 21 of BG Sentinel surveillance. House 3 (BG-H3) has the highest number (n=8) of adult Aedes mosquitoes captured for that day.

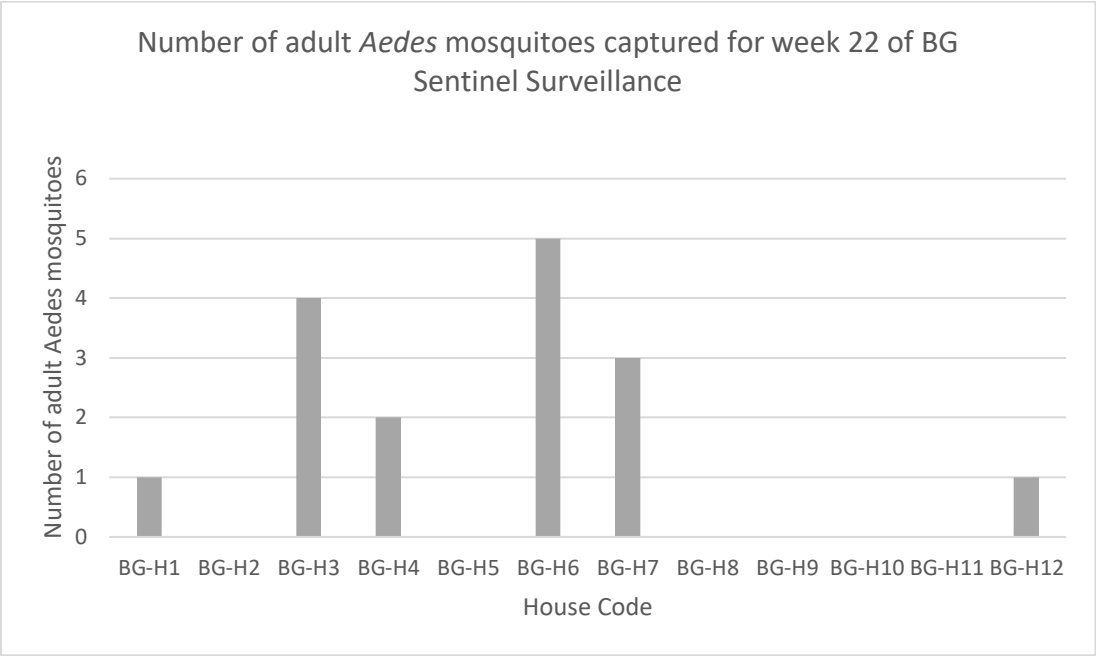


Figure 20. Number of adult Aedes mosquitoes captured per house in all zones for week 22 of BG Sentinel surveillance. House 6 (BG-H6) has the highest number (n=5) of adult Aedes mosquitoes captured for that day.

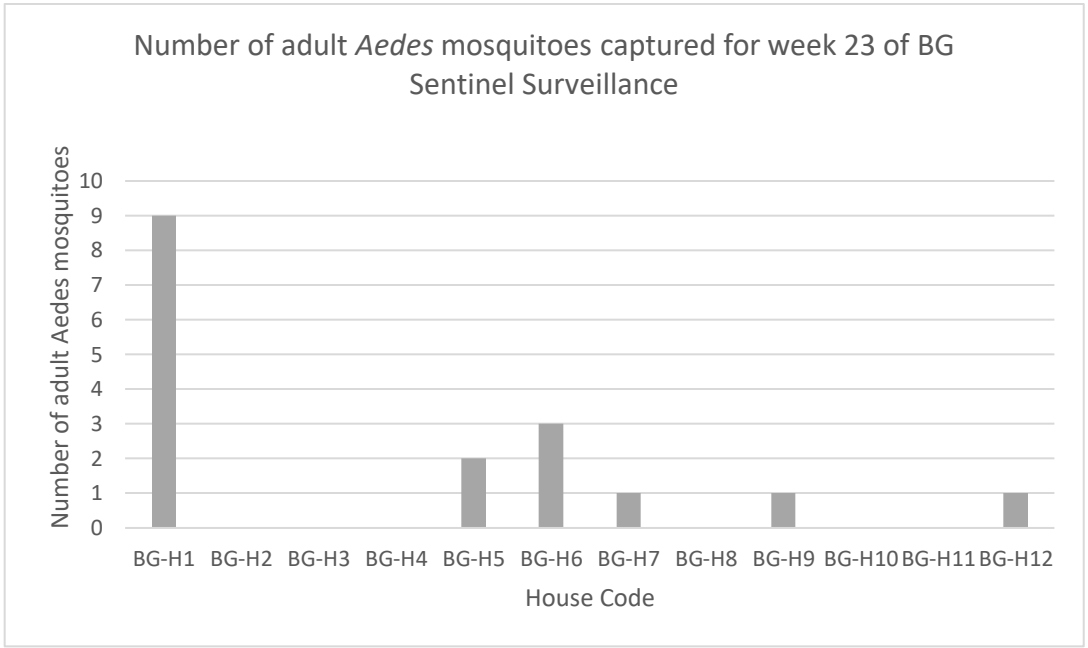


Figure 31. Number of adult Aedes mosquitoes captured per house in all zones for week 23 of BG Sentinel surveillance. House 1 (BG-H1) has the highest number (n=9) of adult Aedes mosquitoes captured for that day.

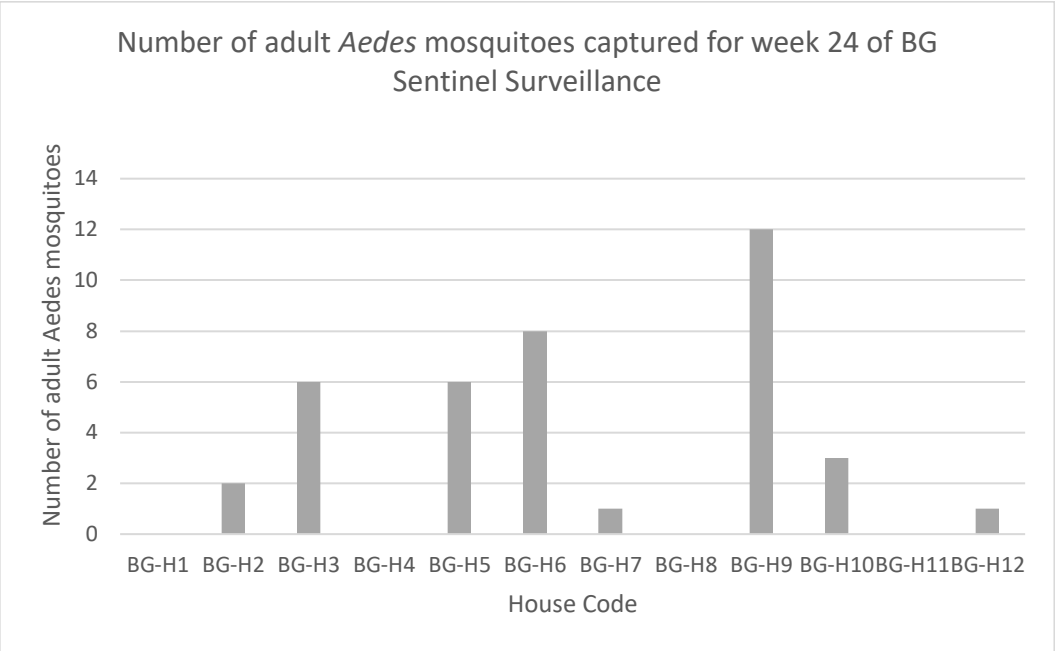


Figure 32. Number of adult Aedes mosquitoes captured per house in all zones for week 24 of BG Sentinel surveillance. House 9 (BG-H9) has the highest number (n=12) of adult Aedes mosquitoes captured for that day.

RESISTANCE TESTING

Insecticide resistance testing is being done currently on mosquito larvae that has been reared up from *Aedes* eggs collected via oviposition cups. The following procedure is being conducted based on the World Health Organization standard protocol for determining the susceptibility of mosquito larvae to insecticides. Third and fourth instar larvae are being tested against liquid Temefos (in a solution of 70% ethanol) at varying concentrations. Temefos is the active ingredient found in the granular Abate being used by the Ministry of Health to treat positive larval habitats.

Larvae are reared up in the BVEC insectary based on the zone that they were collected from. After reaching the appropriate size, the larvae are then prepped for testing by separating them into groups of 20 per container. For each trial, 5 containers are used (2 'controls' and 3 'treatments') per concentration per zone which equals to 100 larvae per trial. Containers being used for the test are small glass bowls. Ideally we want to obtain a total of 5 trials per concentration per zone. Depending on the amount of larvae obtained, that will determined how much trials can be done with varying concentrations at a certain time. The 5 glass bowl replicates, are labelled appropriately and are filled with 225ml distilled water. Using a micropipette, 1ml of 70% ethanol is added to both 'control' containers and 1ml of 'x-mg/l Temefos concentration solution' is are added to the three 'treatment' containers.

The contents of each container are stirred with a glass stirring rod for 30 seconds to ensure proper mixing. The larvae are then introduced into the containers and the investigator records time introduced, room temperature and relative humidity. These procedures are then repeated for every other trial. The investigator will then check for mortality after 24 hours and after 48 hours while recording any temperature and humidity changes. At the end of the experiment, larvae that have been exposed to the Temefos are then discarded in a large liquid waste disposal container while the control larvae reared up to adulthood and identified thereafter to verify species as either *Aedes aegypti* or *Aedes albopictus.*

The baseline data for all Zones were determined based on 24 hour mortality. There are some differences among zones but nothing too variable. On the next page are the graphs generated by QCal for each of the Zones in Orange Walk Town.

ZONE A (24 HOURS)

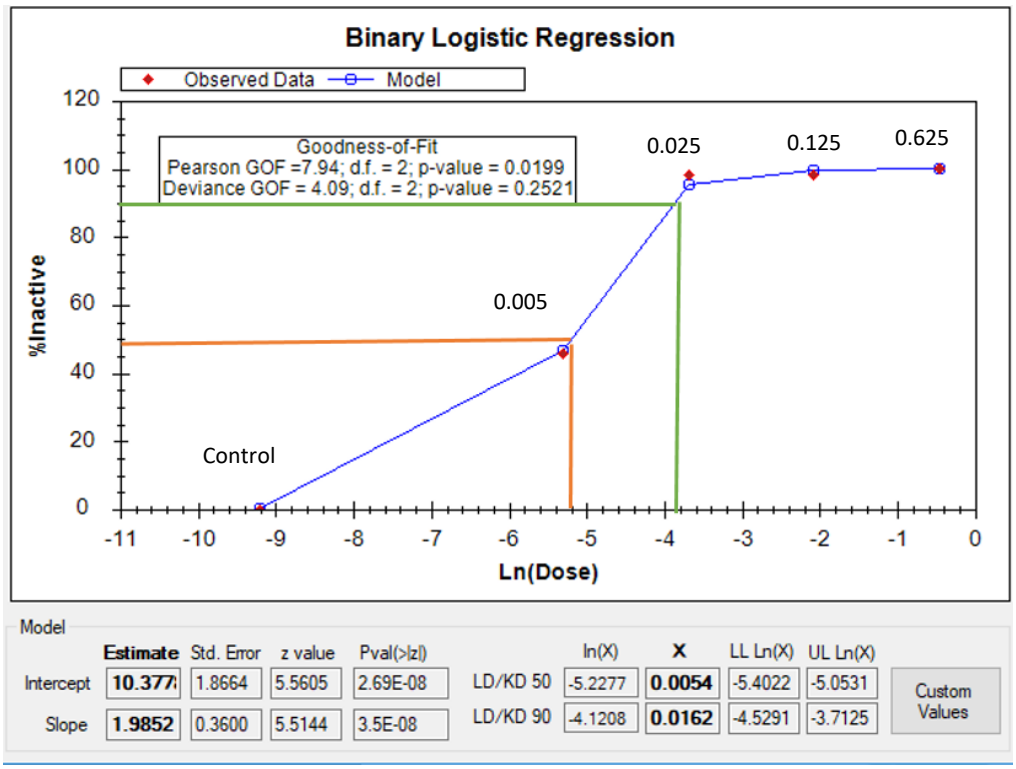


Figure 33. Larval Resistance Curve for Zone A after 24 hours mortality. LD 50 is 0.0054 mg/L while LD 90 is 0.0162 mg/L

ZONE B (24 HOURS)

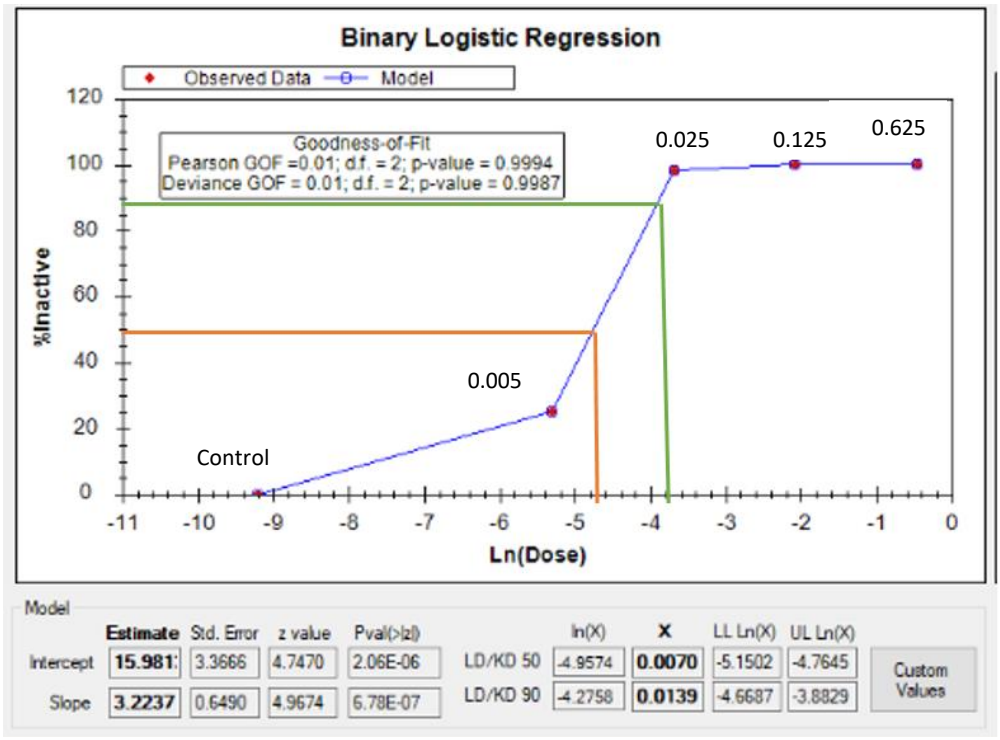


Figure 34. Larval Resistance Curve for Zone B after 24 hours mortality. LD 50 is 0.007 mg/L while LD 90 is 0.0139 mg/L

ZONE C (24 HOURS)

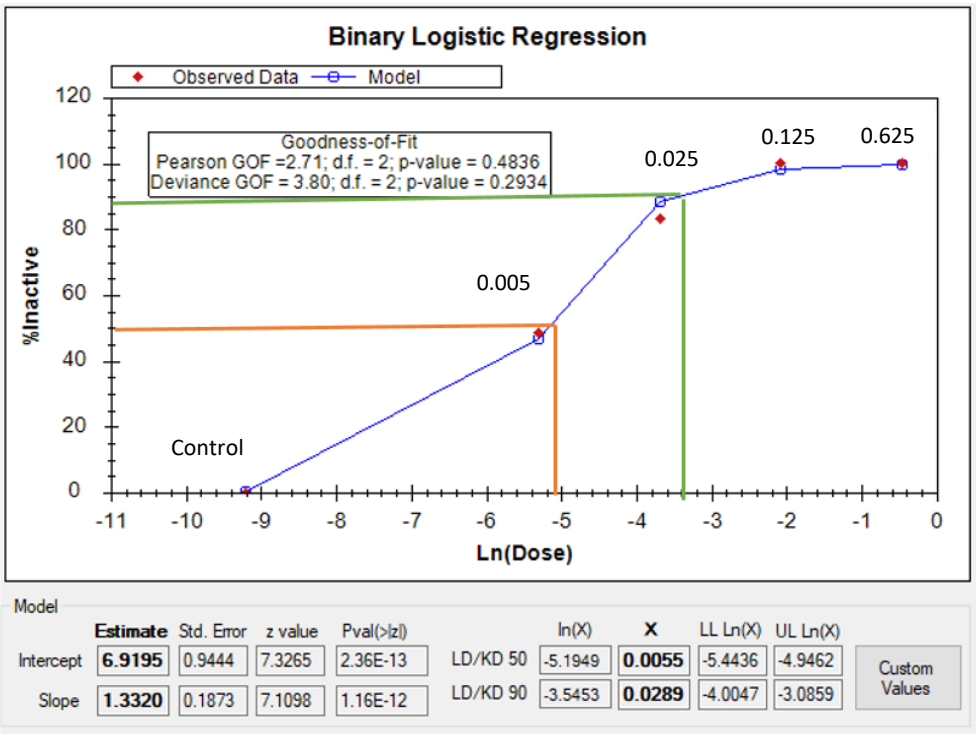


Figure 35. Larval Resistance Curve for Zone C after 24 hours mortality. LD 50 is 0.0055 mg/L while LD 90 is 0.029 mg/L

ZONE D (24 HOURS)

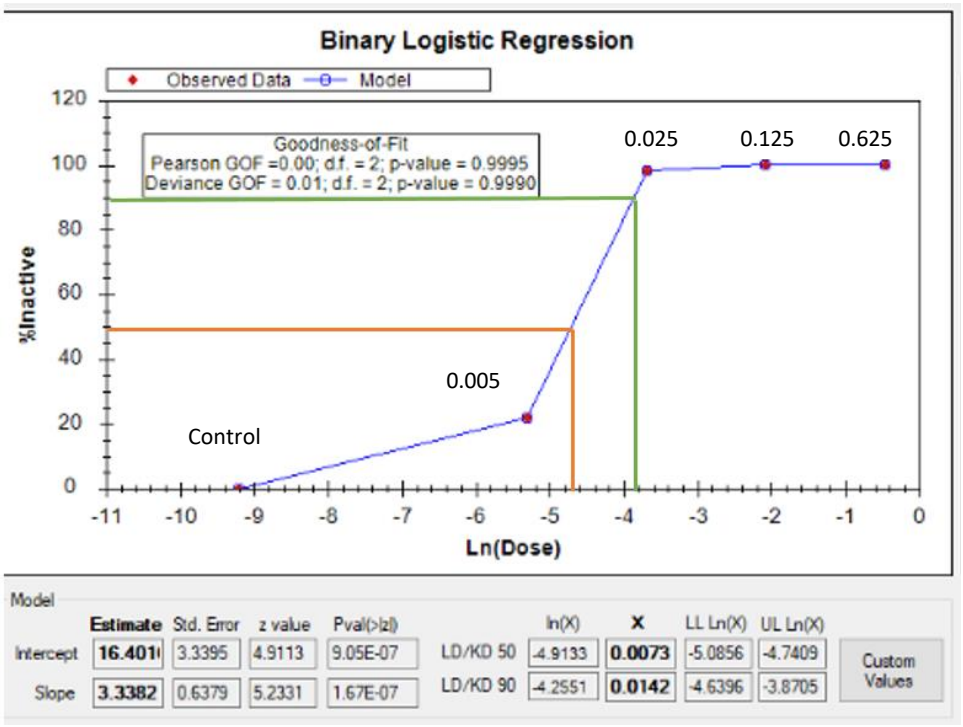


Figure 36. Larval Resistance Curve for Zone D after 24 hours mortality. LD 50 is 0.0073 mg/L while LD 90 is 0.0142 mg/L

ZONE E (24 HOURS)

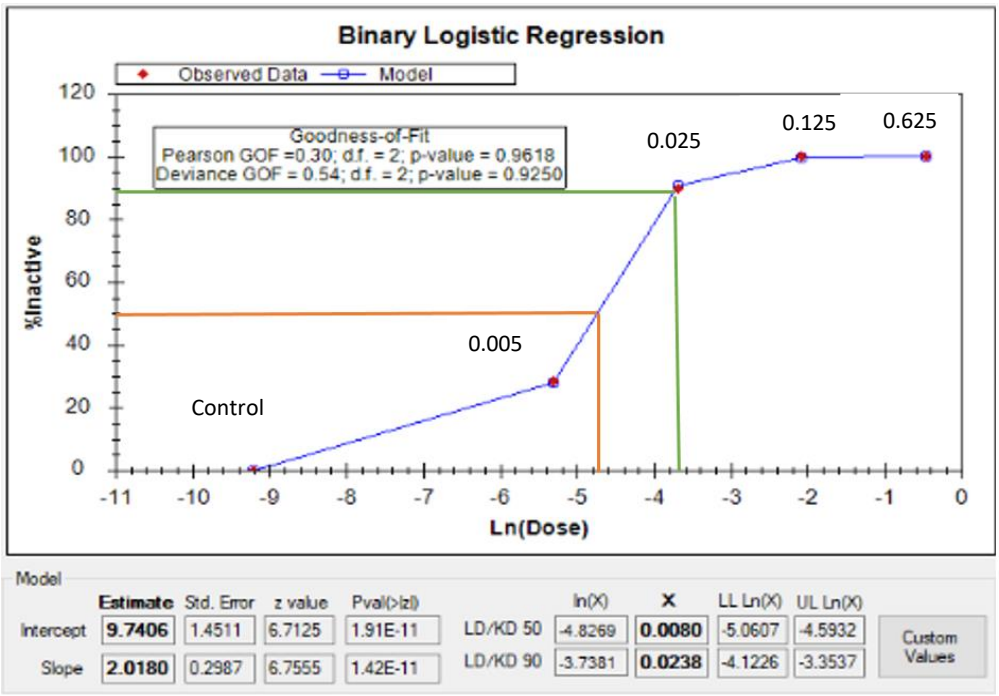


Figure 37. Larval Resistance Curve for Zone E after 24 hours mortality. LD 50 is 0.008 mg/L while LD 90 is 0.0238 mg/L

ZONE F (24 HOURS)

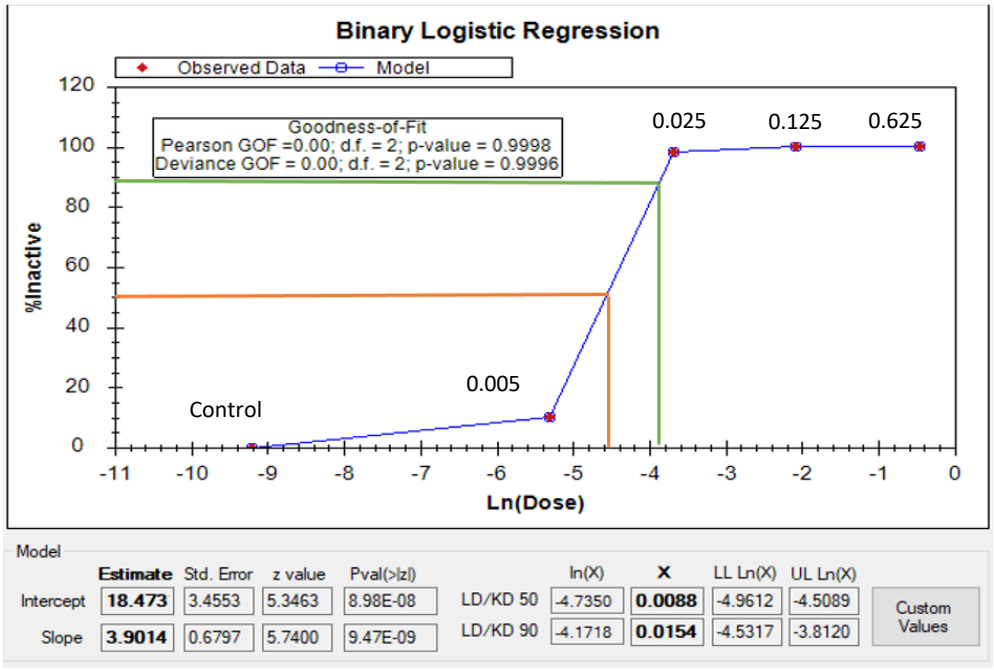


Figure 38. Larval Resistance Curve for Zone F after 24 hours mortality. LD 50 is 0.0088 mg/L while LD 90 is 0.0154 mg/L

Note: Sample datasheet used to record resistance data and model used to conduct testing are attached in the appendices on Pages 33-34

DENGUE ANTIGEN TEST

The adult female *Ae. aegypti* and *Ae. albopictus* mosquitoes that are collected from the BG Sentinel traps in the field are used in a Dengue Antigen Test to detect the presence of Dengue (DENV 1-4) in potentially infected Aedes mosquitoes. The VectorTest® Dengue Assay is a rapid test that identifies the presence or absence of antigens to any of the four Dengue serotypes in mosquitoes. The principle behind the VectorTest® Dengue Assay is the dual monoclonal antibody "sandwich" principle, similar to a pregnancy test, where there is a control zone that will always develop and a test zone that will only develop if there is antigen presence in the mosquito solution.

Prior to processing, all identified Aedes mosquitoes are stored at -20ºC. At no point in time are mosquito samples taken out of the fridge unless for processing. For samples of 25 mosquitoes or less, conical grinding tubes/Eppendorf tubes are used for grinding of mosquitoes. For samples of 26 – 50 mosquitoes, plastic culture tubes are used for the grinding of mosquitoes. Once mosquitoes are ground, test strips can be placed in the mosquito suspension and left to react for 20-30 minutes, after which the test results can be read and recorded. All dip sticks with test results are placed in a graphics template and a picture is taken for recording purposes.

Table 8. Number of female adult Aedes mosquitoes per zone, sorted by species, used in Dengue Antigen Detection Test.

ZONE	Female Adult Aedes for Weeks 21-24 used in VectorTest® Date of Test: April 6, 2018				# of Samples Run	# of Positive Samples
	Ae. aegypti	*Ae. albopictus*	Aedes Spp. (un-ID)	Total		
A	10	1	0	**11**	**3**	**0**
B	23	0	0	**23**	**2**	**0**
C	9	2	1	**12**	**4**	**0**
D	3	0	0	**3**	**1**	**0**
E	10	0	0	**10**	**2**	**0**
F	2	0	0	**2**	**1**	**0**
TOTAL	**57**	**3**	**1**	**61**	**13**	

Table 9. Number of female adult Aedes mosquitoes per house, sorted by species, used in Dengue Antigen Detection Test.

Surveillance Period: Weeks 21 - 24						
DATE OF TEST: April 6, 2018						
BG SENTINEL HOUSE INFORMATION			Number of Female Aedes Adult Mosquitoes Tested in VectorTest®			
ZONE	HOUSE CODE	BG SENTINEL NO.	*Ae. aegypti*	*Ae. albopictus*	*Aedes Spp (un-ID).*	TOTAL/HOUSE
A	BG-H1	1	7	1	0	8
A	BG-H2	2	3	0	0	3
B	BG-H3	3	15	0	0	15
B	BG-H4	4	8	0	0	8
C	BG-H5	5	3	2	1	6
C	BG-H6	6	6	0	0	6
D	BG-H7	7	3	0	0	3
D	BG-H8	8	0	0	0	0
E	BG-H9	9	8	0	0	8
E	BG-H10	10	2	0	0	2
F	BG-H11	11	0	0	0	0
F	BG-H12	12	2	0	0	2

NOTE: Diagram with test results on the dipsticks is attached in Appendices on Page 35

TRAINING ACTIVITIES

Training sessions are conducted by BVEC staff and International partners to collaborators that work and conduct research in Country. No Training activities we conducted in the month of March 2018

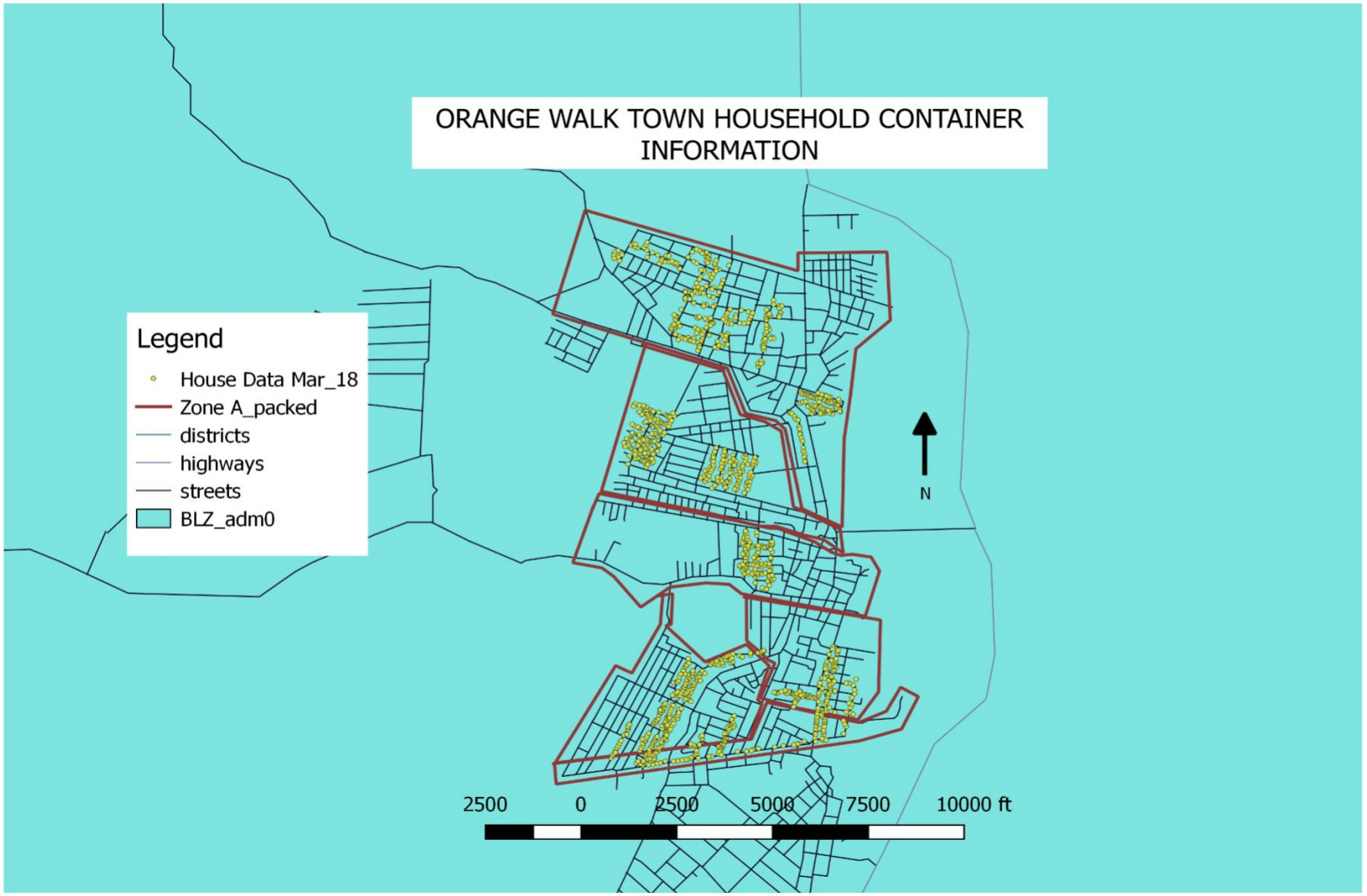
PRESENTATIONS

Presentations are given by BVEC staff and International partners to collaborators as well as local partners to promote community engagement. Below are a few presentations that have been given so far.

- **PAHO Regional Technical Meeting**
  - Date: March 2018
  - Venue: Radisson Hotel Conference Room
  - Purpose: Presentation on IMPACTS Initiative in Belize
  - Presenter/s: Donovan Leiva and Marla Magana

APPENDICES

APPENDIX I Surveillance Map



Appendix 1. Map of Orange Walk Town divided into zones and displaying points of structures mapped

APPENDIX II Resistance Datasheet

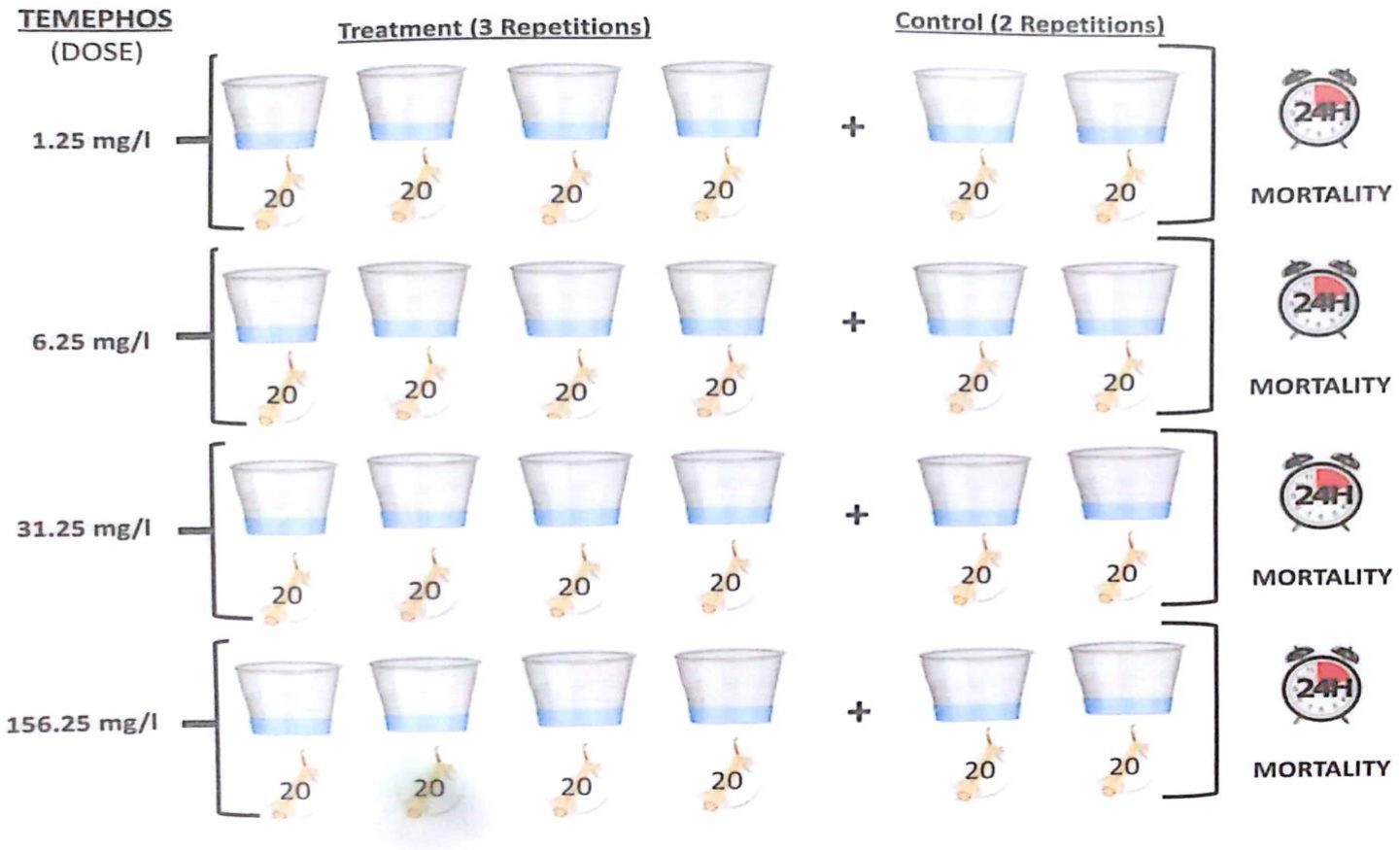
Small-scale field testing and evaluation of larvicides against mosquito larvae

Experiment No: 2 Starting date: 8/29/17 Location: Orange Walk Town
Investigator: _____ Assessment date: _____ Pre or days posttreatment:
_____ Types of Habitat: _____Species *Aedes aegypti*

Live larvae (L3-4) and pupae (P)/sample							
Treatment (Temephos)	Sample	Zone: C		Zone: D		Zone:----------	
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Control	1 10	L 10	L 9/ P 1	L 10	L 10		
	2 10	L 10	L 10	L 10	L 10		
	3 10	L 10	L 10	L 10	L 10		
	4 10	L 10	L 10	L 10	L 10		
	5						
	Total 40	40	19	40	40		
	Mean 10	10	9.5	10	10		
	%red						
T1 0.005mg/l	1 10	L 10	L 8/ P 1	L 10	L 9		
	2 10	L 10	L 9	L 10	L 10		
	3 10	L 10	L 9	L 10	L 7		
	4 10	L 10	L 10	L 10	L 8		
	5						
	Total 40	40	36	40	34		
	Mean 10	10	9	10	8.5		
	%red						
T2 0.025mg/l	1 10	L 5	L 2	L 6	L 5		
	2 10	L 6	L 6	L 5	L 5		
	3 10	L 3	L 2	L 2	L 8		
	4 10	L 6	L 1	L 7	L 2		
	5						
	Total 40	20	11	20	20		
	Mean 10	5	2.75	5	5		
	%red						

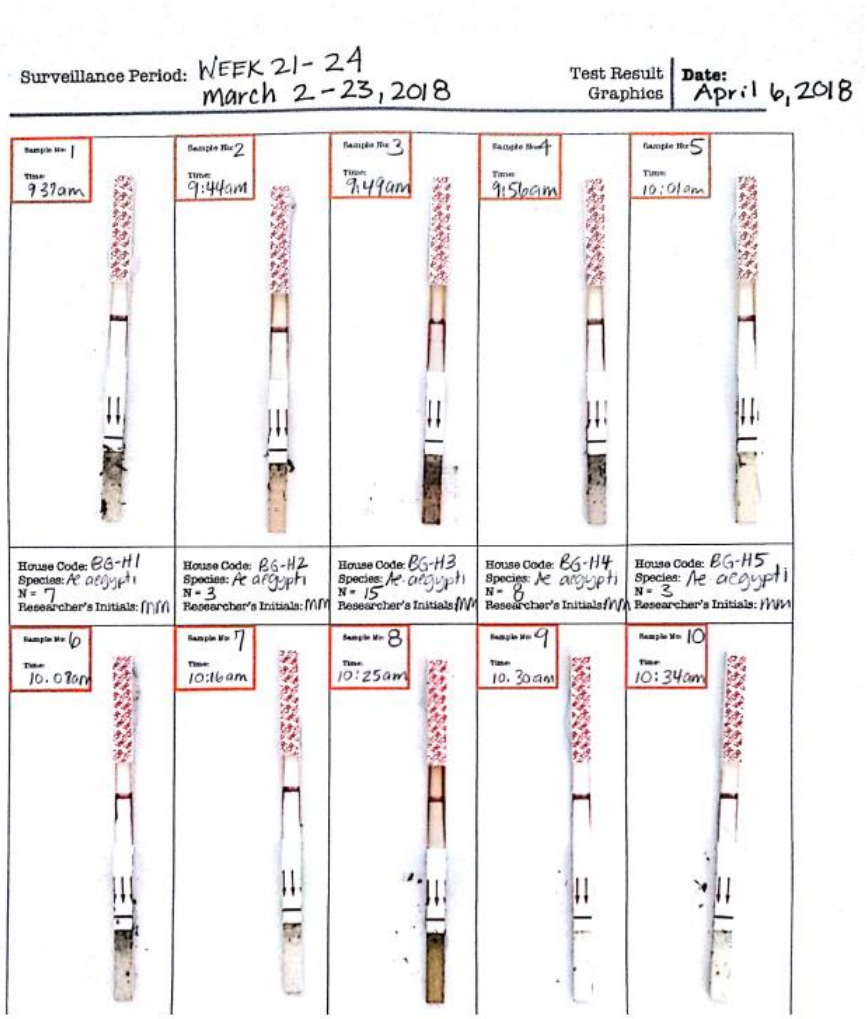
Appendix 2. Sample Datasheet showing results obtained from a small scale resistance test on Zone C and Zone D in Orange Walk Town. Two concentrations of Temephos were used (0.005mg/L and 0.025mg/L)

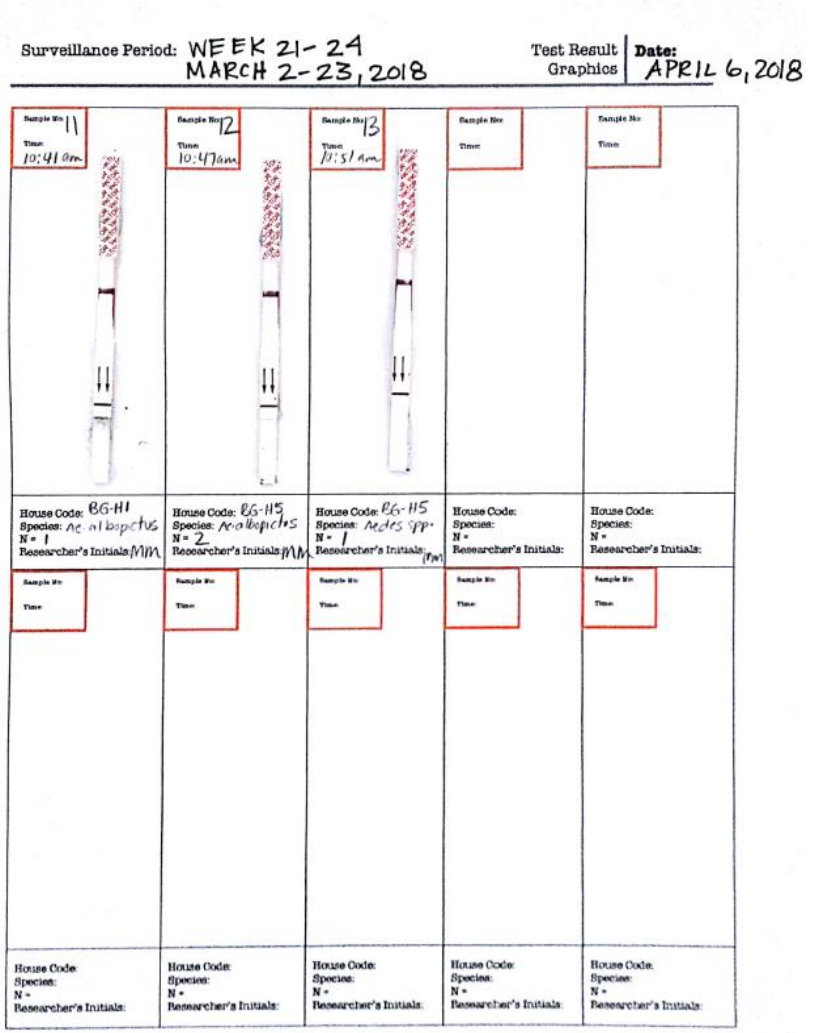
APPENDIX III Resistance Protocol



Appendix 3. Model outlining resistance testing protocol

APPENDIX IV Dengue Test Graphics





Appendix 4. Diagram of Vector Test dipsticks displaying results post testing.

APPENDIX V Consent Form

Consent for Participation in Research

Title: Ovicup/Ovitrap Surveillance for *Ae. aegypti* and *Ae. albopictus* eggs
Principal Investigator(s): The Belize Vector and Ecology Center (BVEC)

Introduction

The purpose of this form is to provide you with information that may affect your decision as to whether or not to participate in this research study. Read the information below and ask any questions that you might have before deciding whether or not to take part. The person(s) performing this study will answer any of your questions. If you decide to be involved in this study, this form will be used to record your consent.

Purpose of the Study

You have been asked to participate in this study by having ovicups/ovitraps set out in your yard for capturing *Ae. aegypti* and *Ae. albopictus* mosquito eggs. The eggs of *Ae. aegypti* and/or *Ae. albopictus* collected in this study will be collected from your yard and hatched in the BVEC laboratory to evaluate mosquito larvicide resistance that may impair the effectiveness of larvicide applications currently being used by the Belize Ministry of Health. This study will also help us to better understand the density of mosquito vectors in the area as well as the abundance of female *Ae. aegypti* and *Ae. albopictus*.

What will you be asked to do?

If you agree to participate in this study, you will be asked to provide us with access to your yard so that two ovicups/ovitraps can be placed outside and then retrieved after 7 days of installation. Therefore, ovicups/ovitraps will be out in your yard for an entire week to collect maximum amount of *Ae. aegypti* and/or *Ae. albopictus* eggs. You and individuals at your property will be asked to not move the ovicups/ovitraps from their location and/or disturb them so that the water in the cup or egg paper is not lost. The ovicups/ovitraps will remain in your yard for a total of 6 months. If the study is determined to need to be extended more than 6 months, we will ask if you would like to continue participating.

What are the risks involved in this study?

The risks involved with participation in this study are low and may include invasion of privacy. Risk level however, will be reduced by attempting to identify times of the day for ovicup/ovitrap placement and egg paper collection that fit homeowner habits (i.e., time of waking, time of preparing meals, returning from work).

How will your privacy and confidentiality be protected if you participate in this research study?

Your privacy and the confidentiality of the data from ovicups/ovitraps placed in your yard will be protected. Household locations will be coded so as not to use family names on data records. The signed consent forms will be stored in a locked, separate location from data records. Your name and any information that would allow you to be identified as an individual will not be used in data presentations, publications or reports resulting from this study.

If it becomes necessary to review study records, information that can be linked to you will be protected to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order.

What are the possible benefits of this study?

You will receive no direct benefit from participating in this study; however, there may be societal benefits as the data collected from this study may be used by the Belize Ministry of Health, Division of Vector Control to guide activities in improving or modifying mosquito larvicide control measures that target *Ae. aegypti* and *Ae. albopictus* mosquitoes that may have the potential to spread diseases such as Zika, dengue and Chikungunya.

Do you have to participate?

No, your participation is voluntary. You may decide not to participate at all or, if you start the study, you may withdraw at any time. Withdrawal or refusing to participate will not affect your relationship with the Belize Vector Ecology Center (BVEC) in anyway.

If you would like to participate in this study, sign and return the form to the principal investigator(s). You will receive a copy of this form.

Will there be any compensation?

You will not receive any type of payment, compensation or reimbursement for participating in this study.

Whom to contact with questions about the study?

Questions can be answered prior to, during or after your participation by contacting BVEC researchers Marla Magaña, Donovan Leiva or Jonathan Kay at 322 -1149 or send an email to bvec.bz@gmail.com.

Whom to contact with questions concerning your rights as a research participant?

For questions about your rights or any dissatisfaction with any part of this study, you can contact, anonymously if you wish, BVEC researchers Marla Magaña, Donovan Leiva or Jonathan Kay at 322 - 1149 or send an email to bvec.bz@gmail.com.

Participation

If you agree to participate please sign below and return the form to the primary researcher(s).

Signature

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are 18 years or older and you are not waiving any of your legal rights.

_____
Printed Name

_____ _____
Signature Date

As a representative of this study, I have explained the purpose, procedures, benefits, and the risks involved in this research study.

_____
Print Name of Person obtaining consent

_____ _____
Signature of Person obtaining consent Date

APPENDIX VI Glossary

Glossary of Terms

Types of Structures for Mapping Surveillance:

1. **House:** Any household dwelling unit.
2. **House/Store:** Is a household dwelling unit with a small grocery store attached to the structure.
3. **Store:** An independently established grocery store/supermarket/shop.
4. **Apartment/Hotel:** A structure that has rooms or living quarters for rent.
5. **Lab:** Any Medical diagnostic center or laboratory.
6. **Church:** A structure used for public worship.
7. **Bar:** An establishment that is authorized to sell solely alcoholic beverages and light food. May have entertainment such as slot machines and pool tables. Commonly known also as "Cool Spots" or "Lounge" or "Pubs")
8. **House/Bar:** A structure with the combination of a house and a bar.
9. **Restaurant:** An establishment that is authorized to sell food and drinks. May also sell alcoholic beverages. This also includes small vendor establishments.
10. **School:** An institution that is used for learning and higher education. Either Primary, Secondary or Tertiary level.
11. **Cemetery:** Grounds for which they deceased are laid.
12. **Office:** A government or non-governmental organization office/department. Example: (Immigration, Customs, etc.)
13. **Clinic:** Regional Hospital or any other privately owned establishment where outpatients are given medical treatment or advice, especially of a specialist nature.
14. **Business:** Refers to any other small business establishments such as beauty salons, barbershops, garages, car wash etc.
15. **Other:** Any structure that is distinct from the ones previously named

Types of Containers:

1. **Vat/Tank:** A large metallic or plastic container used for storing rain water at homes. Usually collects rain water from gutters that run at the end of roofs.
2. **Drum:** Refers to a metallic or plastic 50 gallon container used for rain water storage. May be used in place of a vat/tank.
3. **Bucket:** A small 1-5 gallon container used to store water (either rain or tap) on a temporary basis.
4. **Bottles/Cans:** Refers to any small empty plastic, glass, aluminum bottles or cans (usually soft-drink or beer) that is found at the premises.
5. **Tires:** Any discarded vehicle tires that do not contain a rim and are able to hold water.
6. **Tree-holes/wells:** Are holes that are natural or man-made in a tree (tree-holes) or in the ground (uncovered wells) that can collect water.
7. **Other:** Any container that is distinct from the ones previously named