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QWeCI

Quantifying Weather and Climate Impacts on Health in Developing Countries

D5.2a – Report on entomological survey of malaria data for each field season, with maps of larval and adult mosquito habitats, and map of malaria risk areas

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Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	PP
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

The report is made up of the following:

1. Entomological Survey for the period February - July, 2011
2. Entomological Survey for the period July – November, 2011
3. Entomological Survey for the period March – May, 2012

1. Entomological survey for the period February to July, 2011 at three Micro-ecological Zones

Entomological survey began in February at communities within the catchment areas of the various study hospitals (peri-urban, urban and rural). Mosquitoes are being collected from March to date and are being processed.

Pyrethrum Spray Catch method is being used to carry out in the mornings between 5.30am and 10.am in bedrooms of residents in the study catchment areas. In each month, a total of ten bedrooms per study site were sprayed and mosquitoes collected for examination and analysis.

In the rural catchment areas, between 2 to 37 mosquitoes per room were collected from the bedrooms of residents, in the peri-urban, between 1 to 30 mosquitoes per room and 3 to 29 were collected per room from urban catchment areas.

All mosquitoes collected were morphologically identified under stereomicroscope and examined for the presence of blood and degrees of blood meal digestion. Three genera were identified, *Anopheles*, *Culex* and *Mansonia*. The *Anopheles* was further identified into species, *gambiae* and *funestus*. Over 75% of the mosquitoes have taken blood meal and over 80% was human blood. The *Anopheles* genus was the pre-dominant in all the study sites with little variations at the different study sites. For the anopheline mosquitoes collected, only some were dissected and examined for the presence of sporozoites. The monthly sporozoite rate is 3.2% with a human biting rate of 3.8%.

Month, 2011	Species of Mosquitoes				Total
	A.gambiae	A.funestus	Mansona	Culex	
February	64	14	31	11	120
March	73	9	24	2	108
April	57	11	12	12	92
May	82	7	17	5	114
June	67	23	23	13	119
July	91	17	14	4	126
Total	434	81	121	47	679

Eco-zone	A.gambiae	A. funestus	Mansona	Culex	Total
Urban	91	18	31	9	149
Peri-urban	147	23	35	17	222
Rural	196	35	55	21	307

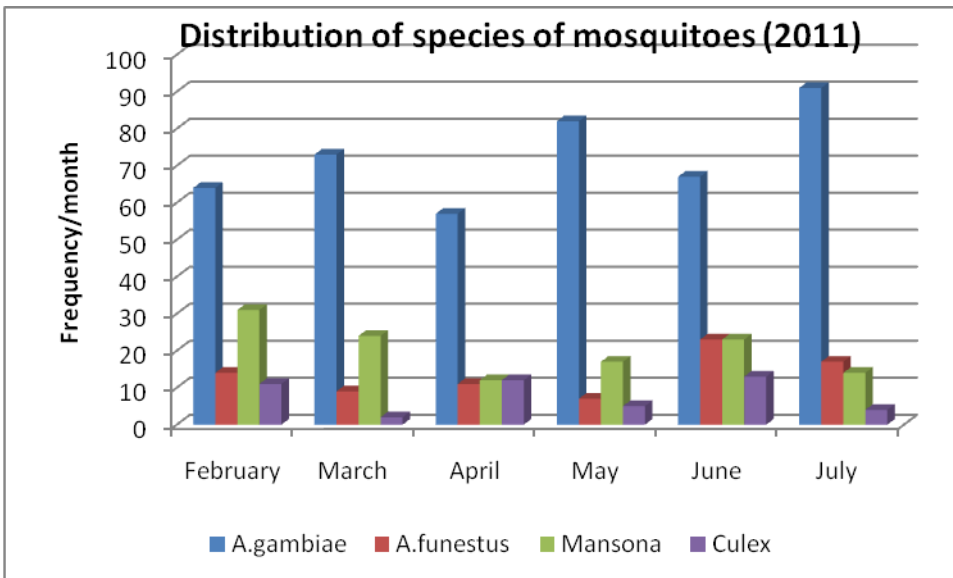


Fig. 1: Distribution of species/genera of mosquitoes per month

The Fig. 1 shows the distribution of the different species of mosquitoes collected for the period of February to July, 2011. This shows that, *A. gambiae* is the predominant species while *Culex* was least distributed genus through the study months. There have been marked variations in the species abundance at different months.

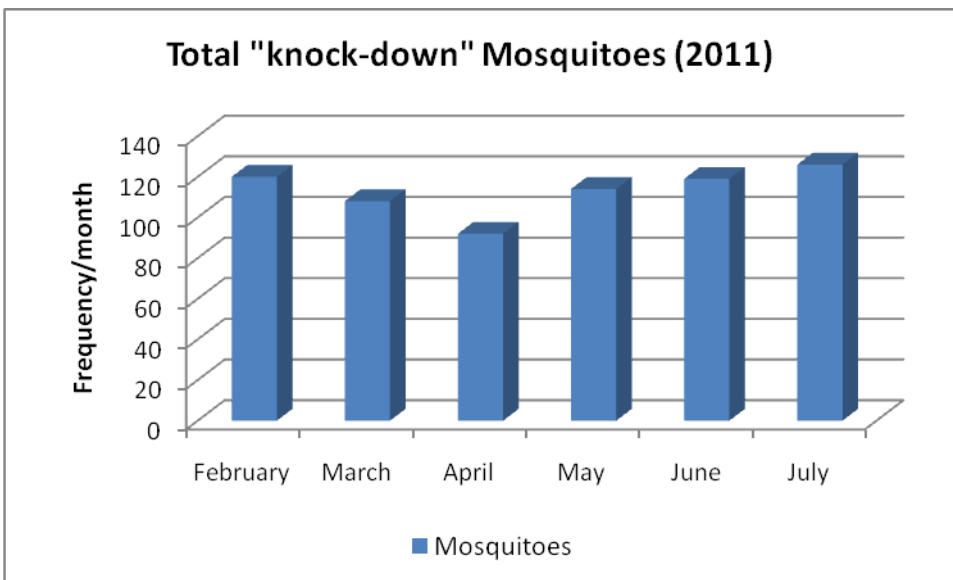


Fig. 2: Total mosquitoes "knock-down" per month

Fig. 2 above shows the monthly knock-down mosquitoes collected from rooms of residents in all the study sites. There was a little variation in the mosquito's abundance during the study months.

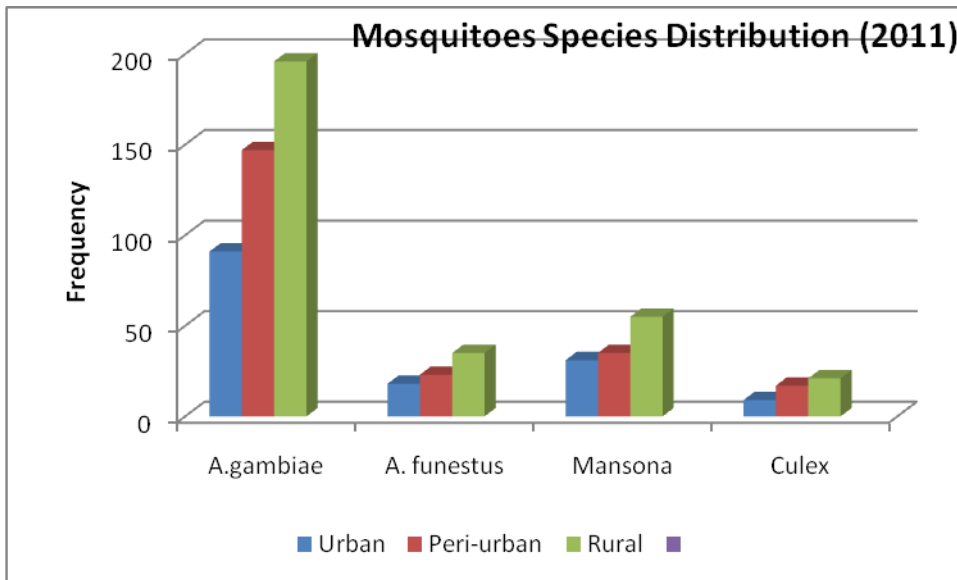


Fig. 3: Mosquitoes species /genera distribution per Eco-zones

The mosquito species /genera were generally more abundant in the rural eco-zone than the other sites and fairly distributed in the three different eco-zones.

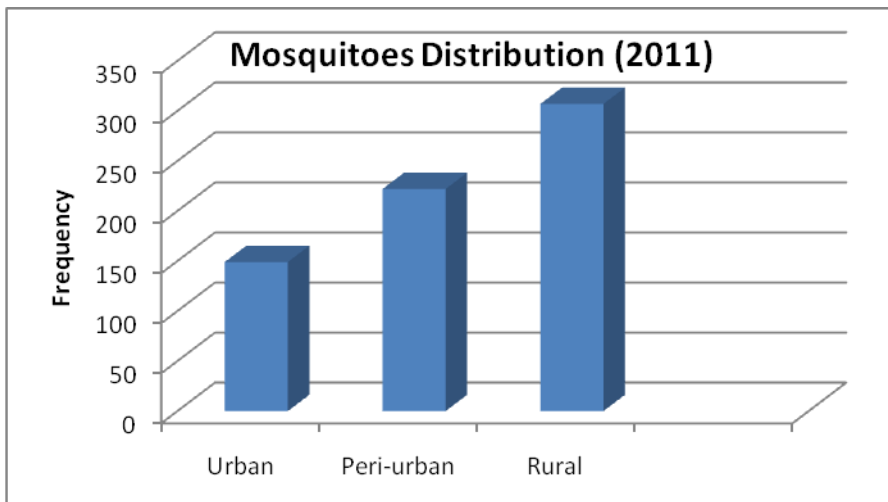


Fig. 4: Mosquito Distribution in the three Eco-zones

There was generally high mosquito abundance in the rural eco-zone followed by the peri-urban and urban respectively as shown in **Fig. 4** above.

2. Entomological Survey for the period July – November, 2011

a. Mosquito Spray and Collection

Mosquito collection was carried out at the entire study site (Figure 1) using the World Health Organization recommendation method popularly called the spray catch method. For effective collection a spraying machine hired from the university sanitary section was used for mosquito spraying.

The knocked-down mosquitoes were collected with forceps and stored in pyrethrum solution in a

well labeled test tube. The following procedure was followed during the mosquito spray and collection task.

- Environmental conditions of the study area were first observed e.g. presence of water body.
- Houses to be sprayed (PSC houses) were identified and systematically selected after permission from household heads was sought.
- Questionnaires prepared was administered to household heads
- Thereafter spraying, collection and storage of adult mosquitoes was carried out.

b. Search for open water bodies

In each community, an inventory was made of the potential anopheline breeding sites (see Figure 5). These sites were described according to characteristics such as degree of exposure to sunlight, conductivity, salinity of water in these areas and their pH. Global positioning systems (GPS) of the villages were also recorded using a 12 channel GPS (Garmin) machine (Figure 1).

c. Identification of communities and systematic selection of houses to be sprayed

Malaria morbidity data from the study hospitals were used to determine specific communities within the study site where selected houses will be sprayed. Ten houses per each study site were systematically selected for spraying

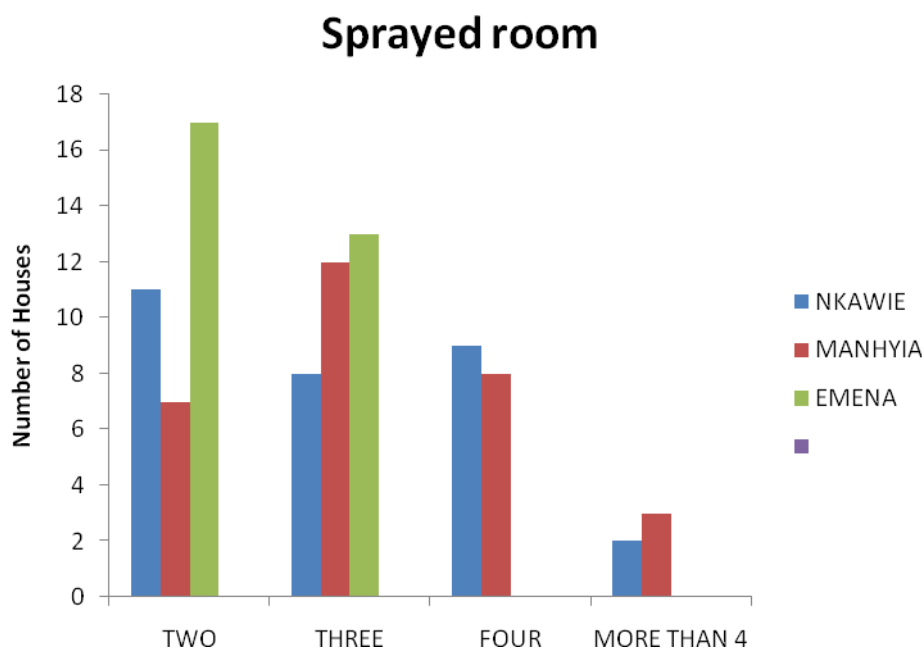


Figure 2: Number of people living in the sprayed room

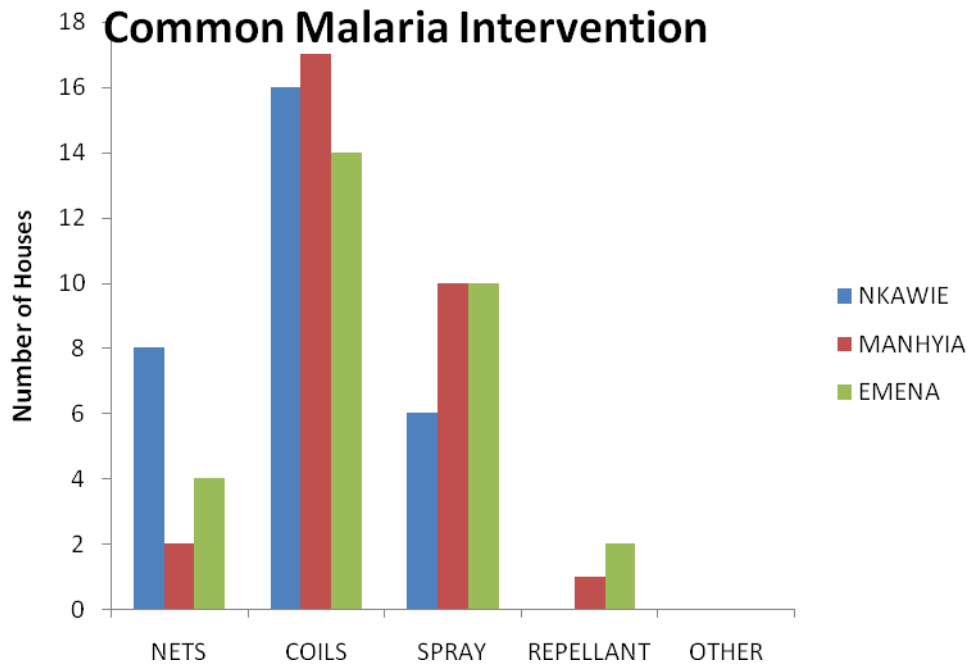


Figure 3: Malaria intervention used in the selected study site

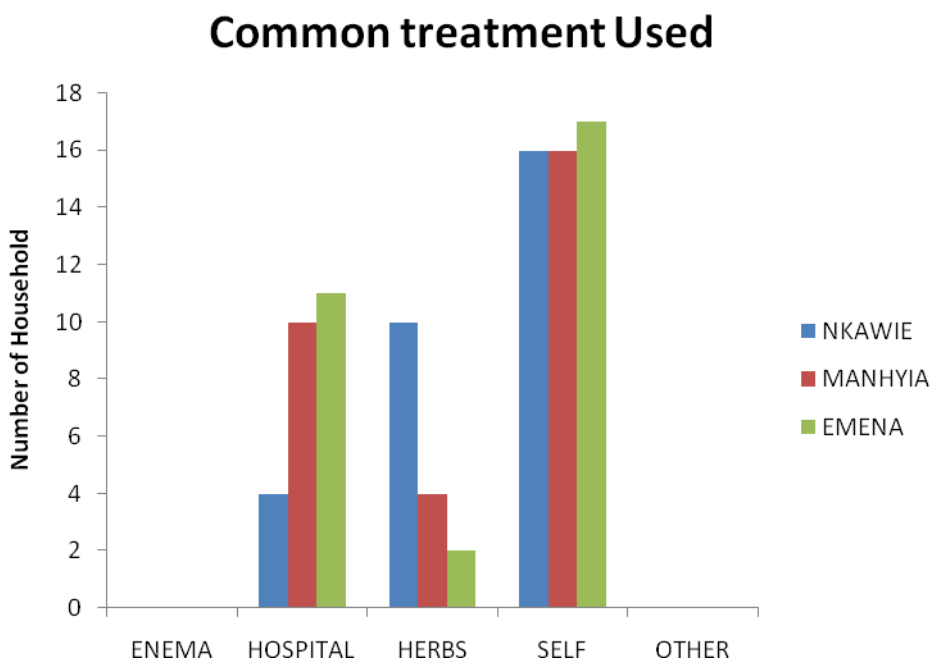


Figure 4: Malaria treatment methods used according to respond from the questionnaire

d. Seeking informed consent from head of the household

Informed verbal consent was sought from household heads of selected houses. The purpose of the research was explained to the household head and when consent was given, one bed room in which at least two or more persons sleep in was sprayed the following day. Whenever the household head

refuses, the next house was chosen.

e. Administering questionnaires to household heads

A structured questionnaire was used to solicit information on number of inhabitants per household sprayed, socio-demographic characteristics of the family head (age, educational level, house type etc.) and information relating to malaria control methods used in the household. Household response to questionnaire are shown in Figures 3 and 4.

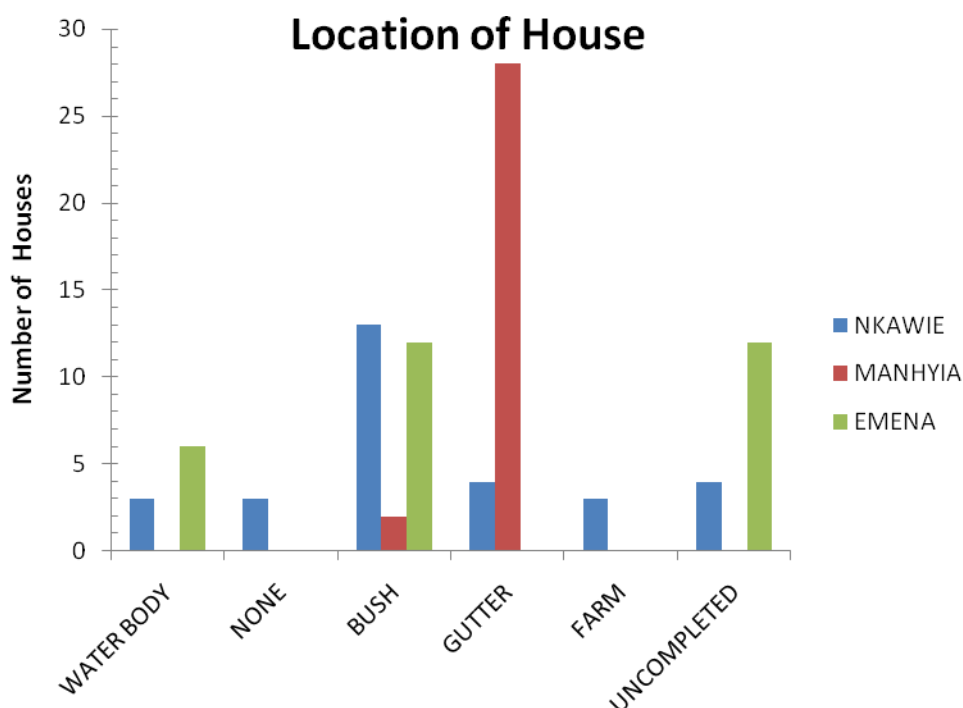


Figure 5: Location of the houses sprayed in the three communities

2.6. Spray catches procedures, collection and storage of adult mosquitoes

A room was prepared for spraying as follows:

- All food and small items of furniture were removed.
- All openings and eaves were covered with cloth or mosquito netting
- The white sheets were then spread so that they completely covered the floor and all flat surfaces of remaining furniture. Sheets were also spread under tables, beds and other places where mosquitoes may hide.
- All windows and doors were then closed.

The pyrethrin solution was prepared at a concentration of 0.2% in kerosene. Two parts of pyrethrin solution was added to 98 parts of kerosene.

A member of the team walked round the outside of the room and sprayed in open spaces or holes in the walls and eaves. Another member of the team then entered the room, closed the door and, moving in a clockwise direction, applied spray towards the ceiling until the room was filled with a fine mist. The operator then left the room quickly and made sure that the door remained closed for at least 10 minutes.

After 10 minutes, starting from the doorway, the sheets were picked up one at a time by their corners and carried outside. All knocked down mosquitoes were collected outside in daylight using forceps. Collected mosquitoes were placed in labeled test tubes with a layer of damp cotton wool and filter paper on top of the cotton wool. Separate test tubes were used for each house and will be labeled with all the essential information. These were then placed in the ice box for transportation to the laboratory for further processing

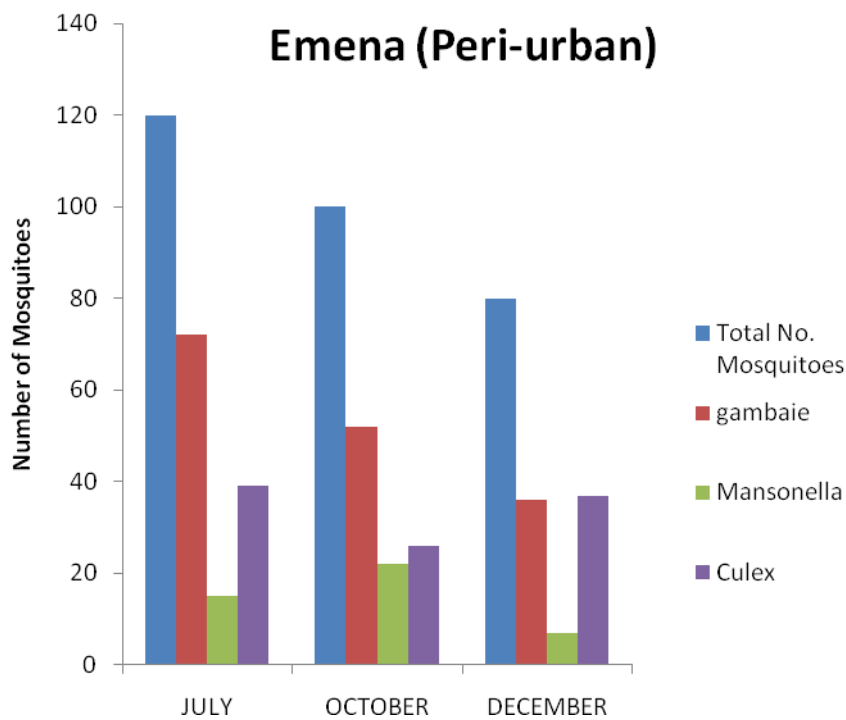


Figure 6: Mosquito types found in Emena (peri-urban)

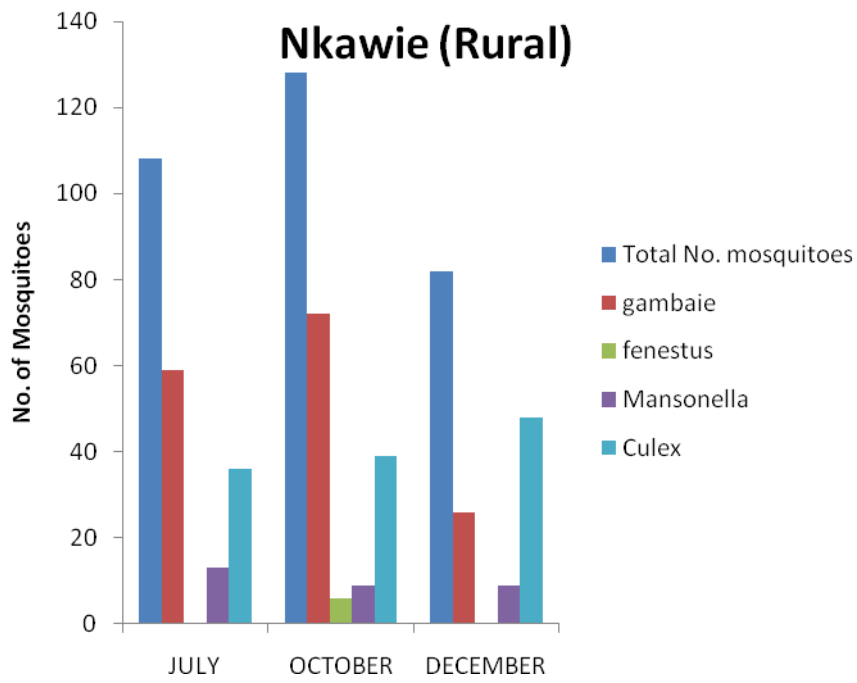


Figure 7: Mosquito types found in Nkawie (rural)

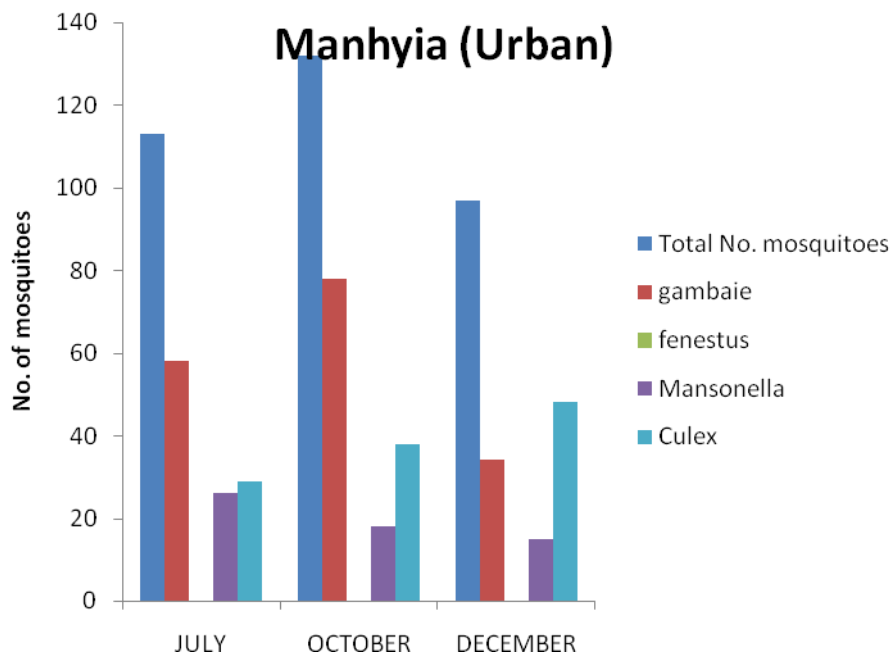


Figure 8: Mosquito types found in Manhya (urban)

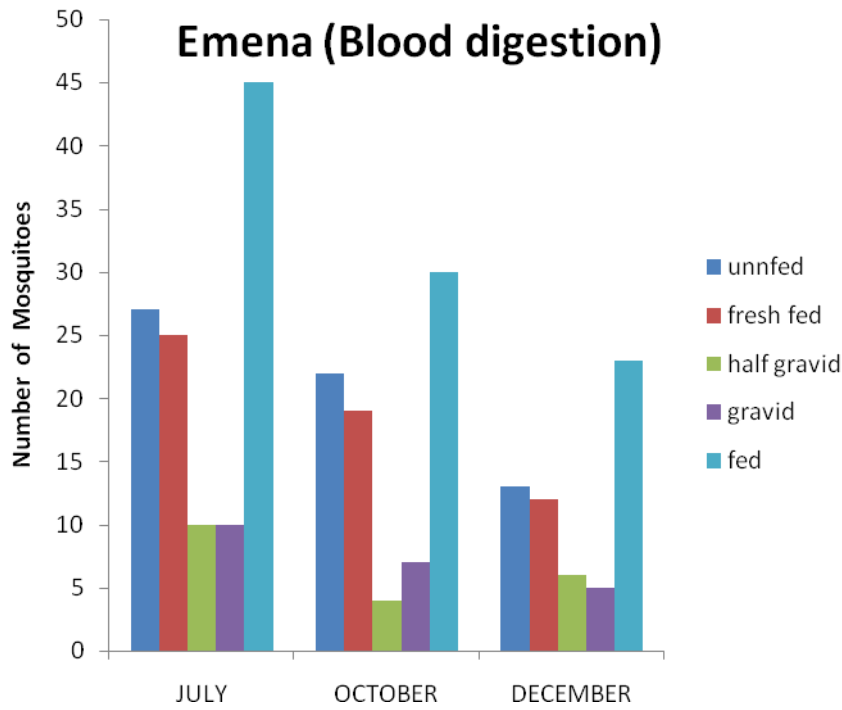


Figure 9: Mosquito blood digestion information in Emena

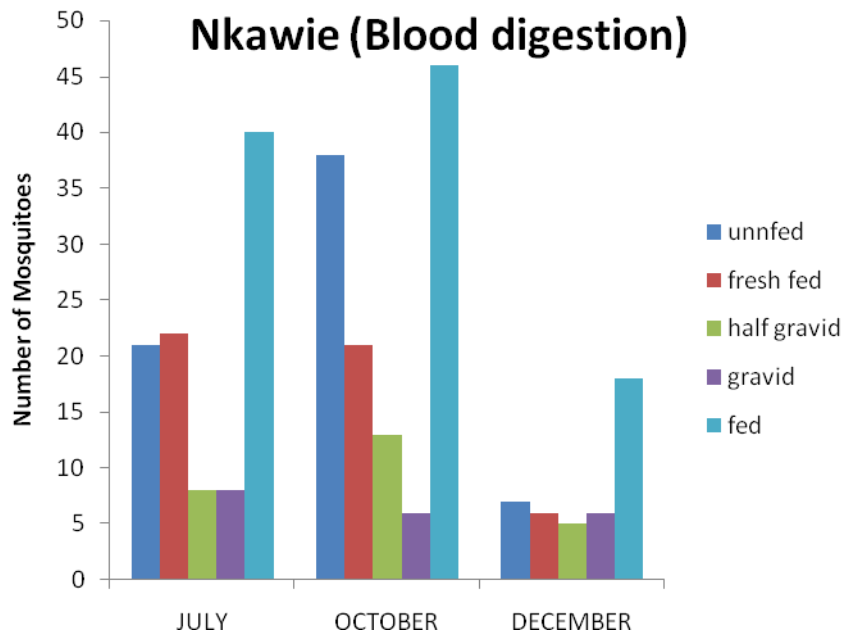


Figure 10: Mosquito blood digestion information in Nkawie

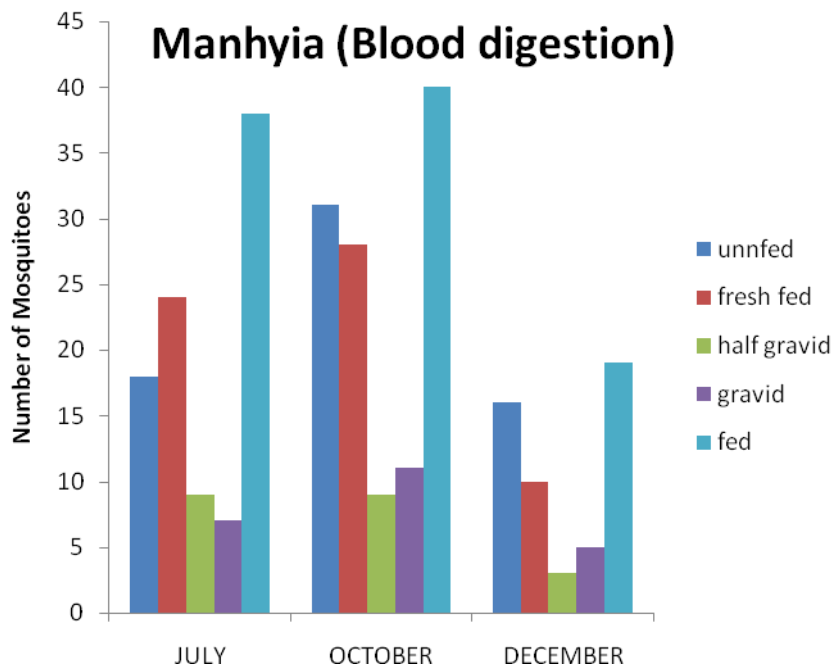


Figure 11: Mosquito blood digestion information in Manhya

All mosquitoes' collectors were required to take all precautionary measures to ensure their safety and others. The necessary safety precautions when handling pyrethrin were observed such as wearing nose masks, an overall dress and were kept away from the reach of children. Enough time was allowed for air circulation into the sprayed room before occupants of the rooms were allowed into it.

All mosquitoes were morphologically identified under stereomicroscope using keys (Gillet 1972, Gillies and DeMeillion 1968, Gillies and Coetzee 1987). Four (4) breeds of mosquitoes were identified, these are anopheles gambaie, fenestus, mansonella and culex. It was observed that anopheles gambaie were the most common type of mosquitoes (see Figures 6 – 8).

Using a hand lens, the blood digestion stage of each mosquito was determined and grouped as unfed, freshly fed, half-gravid, and gravid (see Figures 9 – 11) using the following criteria:

- Unfed - The abdomen is flattened.
- Freshly fed - The abdomen appears bright or dark red from the blood in the midgut. The ovaries occupy only a small area at the tip of the abdomen and this part is not red; it includes only two segments on the ventral surface and at most five segments on the dorsal surface.
- Half-gravid - The blood is dark in colour—almost black—and occupies three to four segments on the ventral surface and six to seven on the dorsal surface of the abdomen. Ovaries occupy most of the abdomen.

- Gravid - The blood is reduced to a small black patch on the ventral surface or may be completely digested. The ovaries occupy all the rest of the abdomen.

2.7. Determining human bite rate

Mosquito blood samples were collected on a 25x75 inch microscope slide by squashing fed mosquitoes. A drop of human immunoglobulin was added to the blood and emulsified. After 60 seconds, the slide is examined under the microscope under low power. Agglutination is positive for human blood. The human blood index was calculated as follows

$HBI = \frac{\text{Number of Mosquitoes with Human Blood}}{\text{Total Number of Mosquitoes with blood}}$. The corresponding human biting rate was

determined as $HBR = \frac{\text{Number of Mosquitoes with Human Blood}}{\text{Number of Occupants in the sprayed room}}$.

2.8. Enzyme linked immunosorbent assay (ELISA)

Each Anopheles mosquito was placed on a clean slide and using a stereo microscope at X32 magnification and in a drop of 1XPBS, the salivary glands were carefully removed and preserved in microtitre plates containing 250 ul of 1XPBS on ice to prevent the degradation of their circumsporozoite (CS) proteins. The salivary glands were stored at -20°C for the detection of CS plasmodium falciparum antigens.

Each assay was performed in a 96- well microtitre plate employing p. falciparum circumsporozoite antibodies (CS capture Abs) (Kirkegaard and Perry laboratories) and read using the OPTEX ELISA reader. The entomological inoculation rate (EIR) was calculated as
 $EIR = HBR \times \text{Sporozoite Rate}$ infective bites/per person/night.

2.9. Results from the three study sites

2.9.1 Nkawie-Rural

A total of 108 mosquitoes were collected from April to July 2011 out of which 59 (54.6%) were Anopheles gambiae s.l. out of these 21 were unfed while 38 were fed. There was an average circumsporozoite protein rate of 6% while 35 out of 38 mosquitoes had fed on human blood. There were 28 inhabitants in the 10 rooms sprayed and had a human bite rate of 1.25 bites per night and an average entomological inoculation rate of 273.8 infectious bites per person per year.

Between August and November, 2011, a total of 128 mosquitoes were collected out of which 72 were *Anopheles gambiae* s.l and 6 were *Anopheles fenestus*. Out of these 38 were unfed while 40 were fed. There was an average circumsporozoite protein rate of 5.6% while 37 out of 40 mosquitoes had fed on human blood.

There were 32 inhabitants in the 10 rooms sprayed and had a human bite rate of 1.2 bites per night and an average entomological inoculation rate of 245.3 infectious bites per person per year.

For the rest of the months, (December 2011 to February 2012), representing the dry season in Ghana, a total of 82 mosquitoes were collected out of which 26 were *Anopheles gambiae* s.l. Out of these 7 were unfed while 17 were fed. There was an average circumsporozoite protein rate of 2.6% while all the *Anopheles* mosquitoes caught had fed on human blood.

March to May 2012, there were 28 inhabitants in the 10 rooms sprayed and had a human bite rate of 0.6 bites per night and an average entomological inoculation rate of 56.9 infectious bites per person per year.

Most of the household heads were women with basic education, divorced or separated, living in block houses with cement floors and aluminum sheets. They depended heavily on mosquito coils for malaria prevention and resorted to self medication as a treatment option for malaria. The commonest mosquito breeding site close to the houses were bushes.

2.9.2 Manhyia-Urban

A total of 113 mosquitoes were collected from April to July 2011 out of which 58 were *Anopheles gambiae* s.l. Out of these 18 were unfed while 40 were fed. There was an average circumsporozoite protein rate of 6.6% while all the fed mosquitoes had fed on human blood.

There were 33 inhabitants in the 10 rooms sprayed and had a human bite rate of 1.2 bites per night and an average entomological inoculation rate of 269.1 infectious bites per person per year.

Between August and November 2011, A total of 132 mosquitoes were collected out of which 78 were *Anopheles gambiae* s.l . Out of these 31 were unfed while 46 were fed. There was an average circumsporozoite protein rate of 4.8% while 42 out of 46 mosquitoes had fed on human blood.

There were 33 inhabitants in the 10 rooms sprayed and had a human bite rate of 1.3 bites per night and an average entomological inoculation rate of 227.8 infectious bites per person per year.

For the rest of the months, representing the dry season in Ghana, (December 2011 to February 2012), a total of 97 mosquitoes were collected out of which 34 were *Anopheles gambiae* s.l. Out of these 16 were unfed while 18 were fed. There was an average circumsporozoite protein rate of 5.0% while all the *Anopheles* mosquitoes caught had fed on human blood.

March to May 2012, there were 31 inhabitants in the 10 rooms sprayed and had a human bite rate of 0.6 bites per night and an average entomological inoculation rate of 109.5 infectious bites per person per year.

Most of the household heads were Muslim men with secondary education, married but polygamous, living in block houses with cement floors and aluminum sheets. They depended heavily on mosquito coils for malaria prevention and resorted to self medication as a treatment option for malaria. The commonest mosquito breeding site close to the houses were gutters.

2.9.3 Emena-Peri Urban

A total of 120 mosquitoes were collected from April to July 2011 out of which 72 were *Anopheles gambiae* s.l. Out of these 27 were unfed while 45 were fed. There was an average circumsporozoite protein rate of 3% while 44 out of 45 mosquitoes had fed on human blood.

There were 23 inhabitants in the 10 rooms sprayed and had a human bite rate of 1.9 bites per night and an average entomological inoculation rate of 208.1 infectious bites per person per year.

Between August and November 2011, A total of 100 mosquitoes were collected out of which 52 were *Anopheles gambiae* s.l. Out of these 22 were unfed while 30 were fed. There was an average circumsporozoite protein rate of 5% while all 30 fed mosquitoes had fed on human blood.

There were 25 inhabitants in the 10 rooms sprayed and had a human bite rate of 1.2 bites per night and an average entomological inoculation rate of 219 infectious bites per person per year.

For the rest of the months (December 2011 to February 2012), representing the dry season in Ghana, A total of 80 mosquitoes were collected out of which 36 were *Anopheles gambiae* s.l. Out of these 13 were unfed while 23 were fed. There was an average circumsporozoite protein rate of 4% while all the *Anopheles* mosquitoes caught had fed on human blood.

March to May 2012, there were 23 inhabitants in the 10 rooms sprayed and had a human bite rate of 0.9 bites per night and an average entomological inoculation rate of 134.3 infectious bites per person per year.

Most of the household heads were males with tertiary education, married, living in block houses with cement floors and aluminum sheets. They depended heavily on mosquito coils for malaria prevention and resorted to self medication as a treatment option for malaria. The commonest mosquito breeding sites close to the houses were bushes and uncompleted buildings.

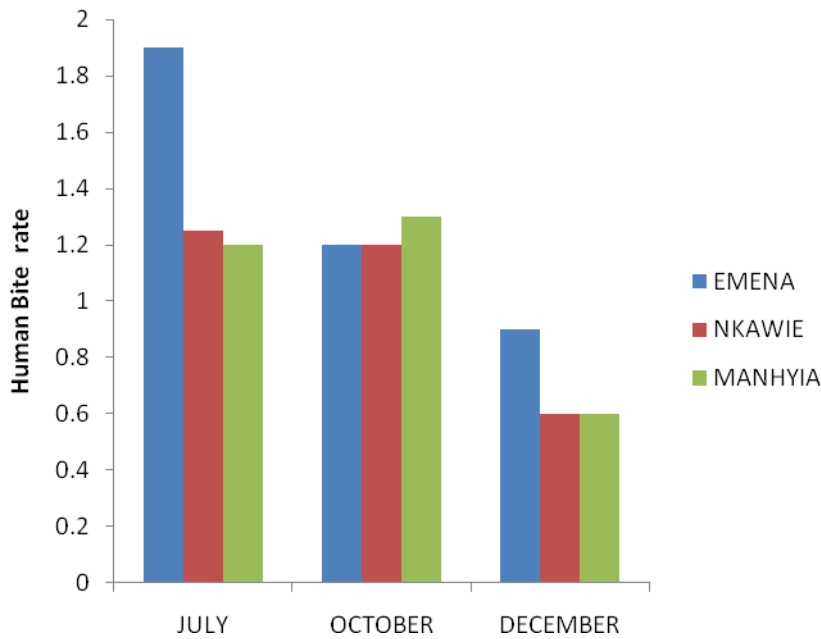


Figure 12: Human bite rate at the various study sites

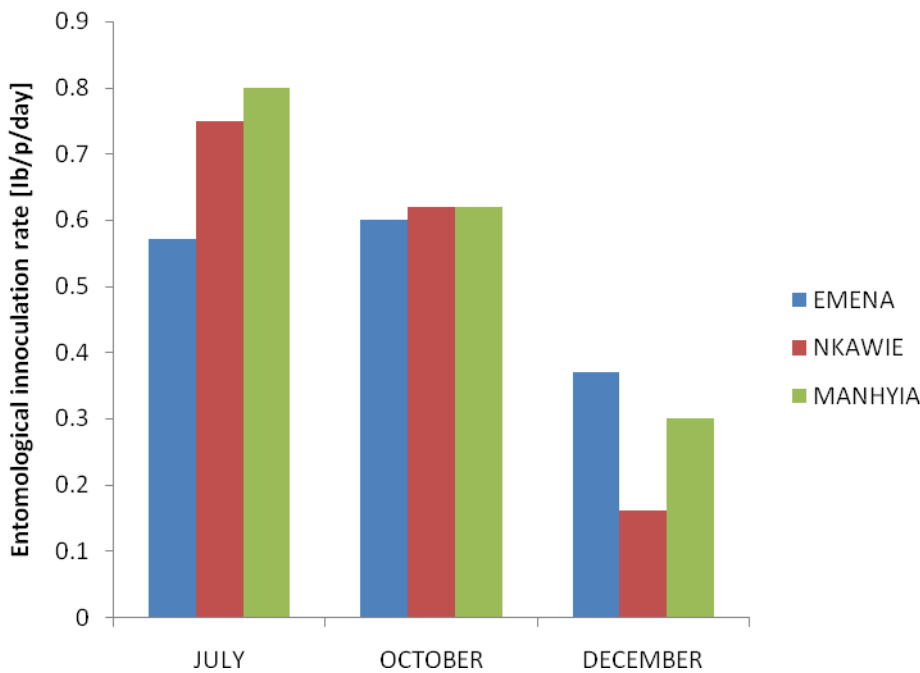


Figure 13: Daily Entomological inoculation rate at the various study sites



Figure 14: Annual Entomological inoculation rate at the various study sites

3. Entomological Survey for the period March – May, 2012

A further entomological survey was carried out in the communities of the health facilities or study sites for the period March to May, 2012. Pyrethrum Spray Catch method is being used to carry out in the mornings between 5.30 a.m and 10 a.m in bedrooms of residents in the study catchment areas. In each month, a total of ten bedrooms per study site were sprayed and mosquitoes collected for examination and analysis.

The results are shown below:

Mosquitoes Species Identified

Agogo Hospital (Rural)	Mosquitoes Species	Number
	Gambaie	73
	Funestus	48
	Mansonella	2
	Culex	33
	Total:	156

Nkawie (Rural)	Gambaie	63
	Funestus	4
	Mansonella	10
	Culex	47
	Total:	124

Manhyia (Urban)	Gambaie	60
	Funestus	0
	Mansonella	24
	Culex	32
	Total:	116

Circumsporozoite Rate

Agogo (Rural)	6.7
Nkwawie (Rural)	6.2
Manhyia (Urban)	6

Human Bite Rate

Agogo (Rural)	B/N	1.9
Nkwawie (Rural)	B/N	1.5
Manhyia (Urban)	B/N	1.3

Entomological Inoculation Rate

Agogo (Rural)	lb/p/day	1.3
	ib/p/yr	464.6
Nkawie (Rural)	lb/p/day	0.93
	ib/p/yr	339.5
Manhyia (Urban)	lb/p/day	0.78
	ib/p/yr	284.7

