Entomological Monitoring for the US President's Malaria Initiative in Kenya
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Abstract

The Kenya National Malaria Control Program (NMCP) is scaling up vector control for the prevention of malaria throughout western Kenya. In addition to insecticide treated nets, the last IRS campaign was conducted in 2012 with mass insecticide treated net (ITN) distribution in 2014/2015. These interventions require susceptible populations of vector mosquitoes to be maximally effective. It is therefore essential to monitor mosquito populations for insecticide resistance and temporal changes related to the intervention. We performed baseline mosquito collections in 6 districts so that we can measure the impact of ITNs and IRS on vector densities in the houses longitudinally. We also assessed insecticide resistance in 4 districts to assess changes in the mosquito populations related to the implementation of ITNs and IRS. The data generated is important for the NMCP in making decisions on where to implement IRS and which insecticide to use given the rise in insecticide resistance following the deployment of ITNs and IRS.

1.0 Background and significance:

Since the year 2000, a concerted campaign against malaria has led to unprecedented levels of intervention coverage across sub-Saharan Africa with the impact being a marked reduction in morbidity and mortality due to malaria [1]. Current malaria vector control efforts in Africa have emphasized the use of insecticide treated nets (ITNs) and indoor residual spray (IRS) for the prevention of mosquito bites and transmission of malaria. In western Kenya, ITNs have been shown to reduce malaria transmission by 90% when measured by entomological measures namely vector density and sporozoite rates [2] and by 74% when measured by the incidence of new infections in cohorts of infants [3]. This, in turn, results in large reductions in child morbidity and mortality [4, 5]. Between 2008 and 2012, the Kenya National Malaria Control Program (NMCP) expanded the coverage of IRS from small hotspots in highland endemic districts to complete coverage in some of these highland districts and also some lowland endemic districts

The widespread use of insecticide based control tools has led to the emergence of insecticide resistance in *Anopheles* mosquitoes, which has been shown to reduce the effectiveness of these control measures. *Anopheles gambiae* from Burkina Faso and Kenya have been shown to survive lethal exposure to insecticide treated ITNs [6, 7]. Pyrethroid resistance has forced multiple countries to shift to more expensive insecticides for their IRS programs, often resulting in a loss of coverage or, in some cases, a complete abandonment of IRS [8]. In western Kenya where this work was conducted, pyrethroid resistance in mosquitoes that transmit malaria was first observed in the context of a small scale ITN trial [9] and has steadily increased[10]. In addition, there is evidence that the L1014S-*kdr* allele is becoming fixed in *An. gambiae* s.s. [11]. The West African genotype, L1014F-*kdr* allele has also been observed in a recent report in western Kenya [11]. Understanding how genes associated with insecticide resistance arise and spread is important for developing means to detect and respond to them.

Anopheles funestus and *An. gambiae* s.s. were the predominant vectors of malaria before the rollout of the mass ITN campaigns. After high coverage with ITNs was achieved in the early 2000s, *An. funestus* levels dropped to below 1% while *An. arabiensis* replaced *An. gambiae* as the predominant vector [12]. However, recent studies have reported the re-emergence of *An. funestus* [13]. Being a more anthropophlic vector and given the observations of high levels of insecticide resistance, there is a chance *An. funestus* could contribute to higher levels of transmission.

We monitored the impact of ITNs and IRS on the populations of these vectors, as well as changes in their susceptibility to insecticides. Entomological collections for vector surveillance were carried out in the IRS districts: Rachuonyo, Migori, Nandi South, Kericho, Kisii and other districts Nyando, Rarieda, Gem, Karemo, Bungoma, Busia, Bundalangi, Kakamega, Kisumu West. ITNs are widely used in all of these districts, though coverage varies between them.

1.1 Specific Aims:
i.   To determine malaria vector density in several districts in western Kenya including the districts targeted for IRS by the NMCP
ii. To monitor the levels and mechanisms of insecticide resistance of local malaria vector populations in western Kenya
iii. To determine the extent of modulation of the *Anopheles* vector population structure under various intensities of ITN use in endemic malarious areas of lowland western Kenya.

2.0 Research Design and Methods
To monitor the changes in *Anopheles gambiae* populations, we performed monthly pyrethrum spray collections (PSC) from about 40 houses in each district. These made up 2 clusters of 20 houses with each cluster located in a different part of the district. Prior to mosquito collection, a short questionnaire was administered to determine the number of people who slept in the house the previous night, whether the house was sprayed by IRS, whether nets are present in the house and some characteristics of the house including wall, roof and floor type and presence of open or closed eaves. PSCs were done by laying white sheets upon the floor and over the furniture within the house. Two collectors, one inside the house and one outside, sprayed around the eaves with 0.025% pyrethrum emulsifiable concentrate with 0.1% piperonyl butoxide in kerosene. The collector inside the house then sprayed the roof and walls. The house was closed for 10-15 minutes after which dead mosquitoes were collected from the sheets and transferred to the laboratory on moist filter paper inside petri dishes.

In addition to the PSC collections, light trap collections were also conducted simultaneously in separate houses within the cluster. The traps were hung inside houses next to sleeping areas, just above the bednets on the evening before the PSC collections are conducted. The trap ran the whole night and mosquitoes collected the next morning.

The species of mosquito were identified using conventional PCR, and then (if female) the remainder of the carcass was analyzed for malaria infection using sporozoite ELISA (head), parity status (age grading), and plans are underway to determine the source of host if blood was present, using a mitochondrial cytochrome B PCR method. Additional entomological samples were collected throughout western Kenya where there are differences in climate and ecology, vector control practices and species composition of mosquitoes. These data will be incorporated into models assessing the impact of vector control and ecological settings as well as the potential impact of climate change on vector distribution and composition. Larval and live adult malaria vectors were collected from all the study clusters once annually. Larvae were collected from larval habitats in each cluster and brought back to be reared in the Entomology insectary. Emerging adults were exposed to insecticides using WHO tube bioassay technique and part of the specimen used to measure insecticide resistance intensity using the CDC bottle bioassay technique. Given the difficulty in sampling *An. funestus*, live adult mosquitoes vectors were collected from the clusters in all the counties using aspirators. The mosquitoes were placed in holding cups and transported to the laboratory to determine resistance status using WHO susceptibility tests.

2.1 Data handling and analysis

All entomological data were collected on PDAs which are designed with buttons, drop down menus, and data quality checks to limit entry errors in the field. All collections included information about the head of household, the unique household ID, and the latitude and longitude of the collection site. Each mosquito collection was assigned a unique ID based upon the date of collection, the name of the PDA (each is assigned a different letter), and an automatic counter. A check variable based upon the district, village, and name of the head of household was also included. Individual mosquitoes are labeled with pre-printed barcodes and are linked to the field data by the collection ID which is automatically assigned in the database. Additional tests on individual mosquitoes, including sporozoite ELISAs, species identification by PCR and PCR identification of blood meal sources were linked by the unique barcode label. Data entry was done on pre-tested data entry screens designed to limit data errors through drop down menus and automatic data checks. For data sharing, all data was merged into a single file and checked to ensure a proper merge.

3.0 Progress Sep 2014- June 2015

3.1 Monitoring Vector Densities
In all districts, the mosquitoes were mostly a mixture of *An. arabiensis* and *An. funestus* (Table 1)

Table 1: Total numbers of Anopheles mosquitoes collected during the study period

District	*An. gambiae* s.l.	Proportion of *An. arabiensis*	*An. funestus*
Gem	295	0.48	401
Homa Bay	62	1	111
Kisumu East	96	0.89	124
Migori	51	1	111
Nyando	324	0.99	389
Rachuonyo	358	0.99	99
Rarieda	111	0.61	57
Siaya	333	0.53	629
Total	1630		1921

An. funestus were observed to have greater numbers than members of the *An. gambiae* s.l. complex in most of the districts except for seasonal peaks associated with rainfall. By PCR, *An. gambiae* s.s. remained rare in most sites (only about 10% of the *An gambiae* s.l. population overall) (Figure 1 and 2).
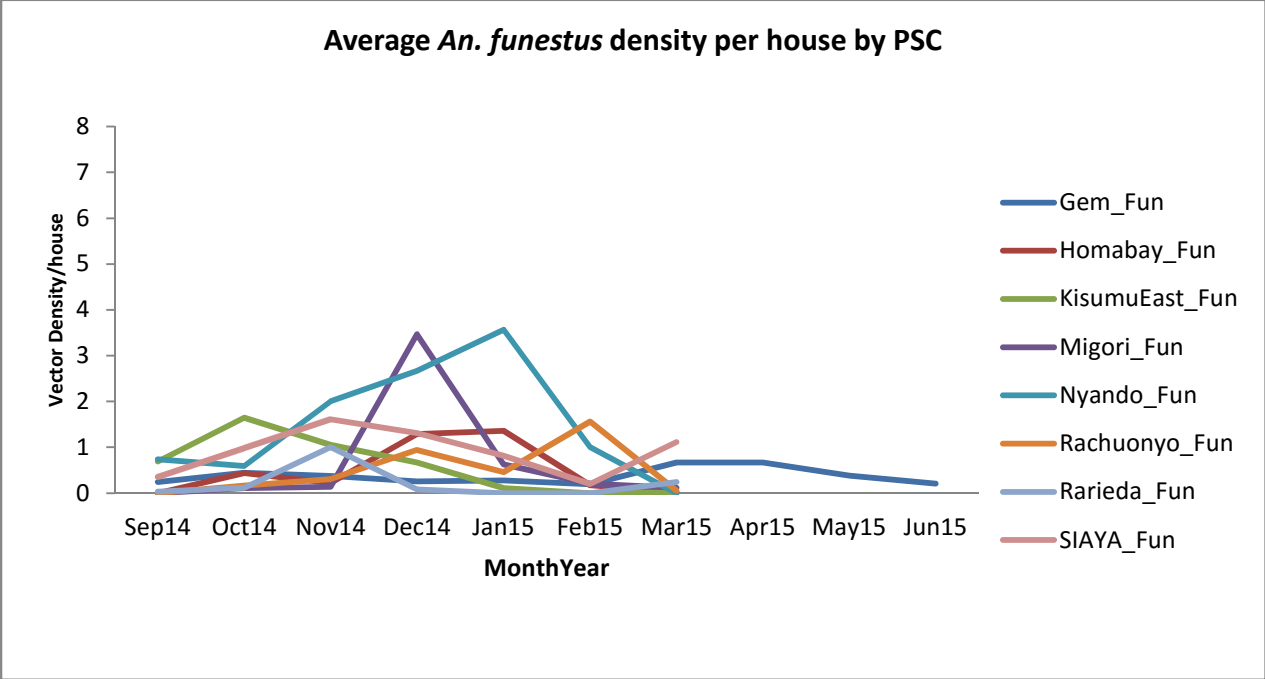
Figure 1. Average *An. funestus* density per house in each of the districts
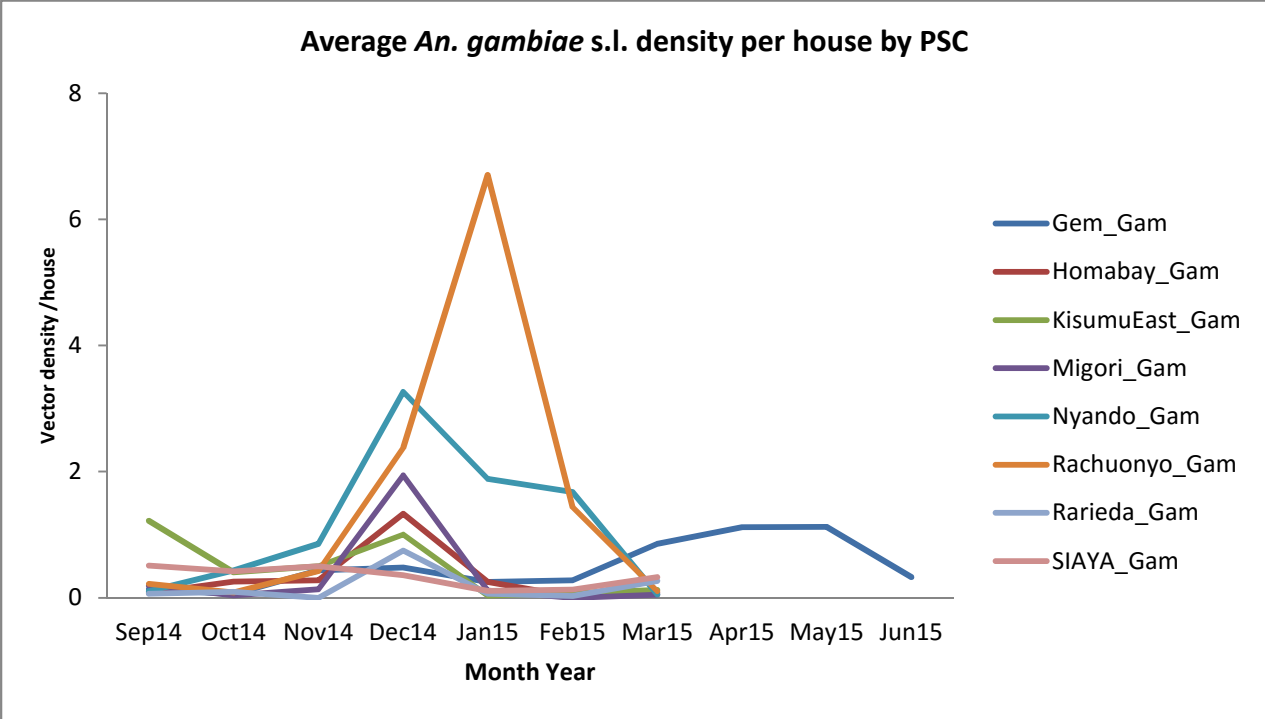

Figure 2. Average *An. gambiae* s.l. density per house in each of the districts

Light trap catches resulted in higher mosquito densities per house compared to PSCs and also in higher *An. funestus* catches in all sites except for Migori (Figure 2).
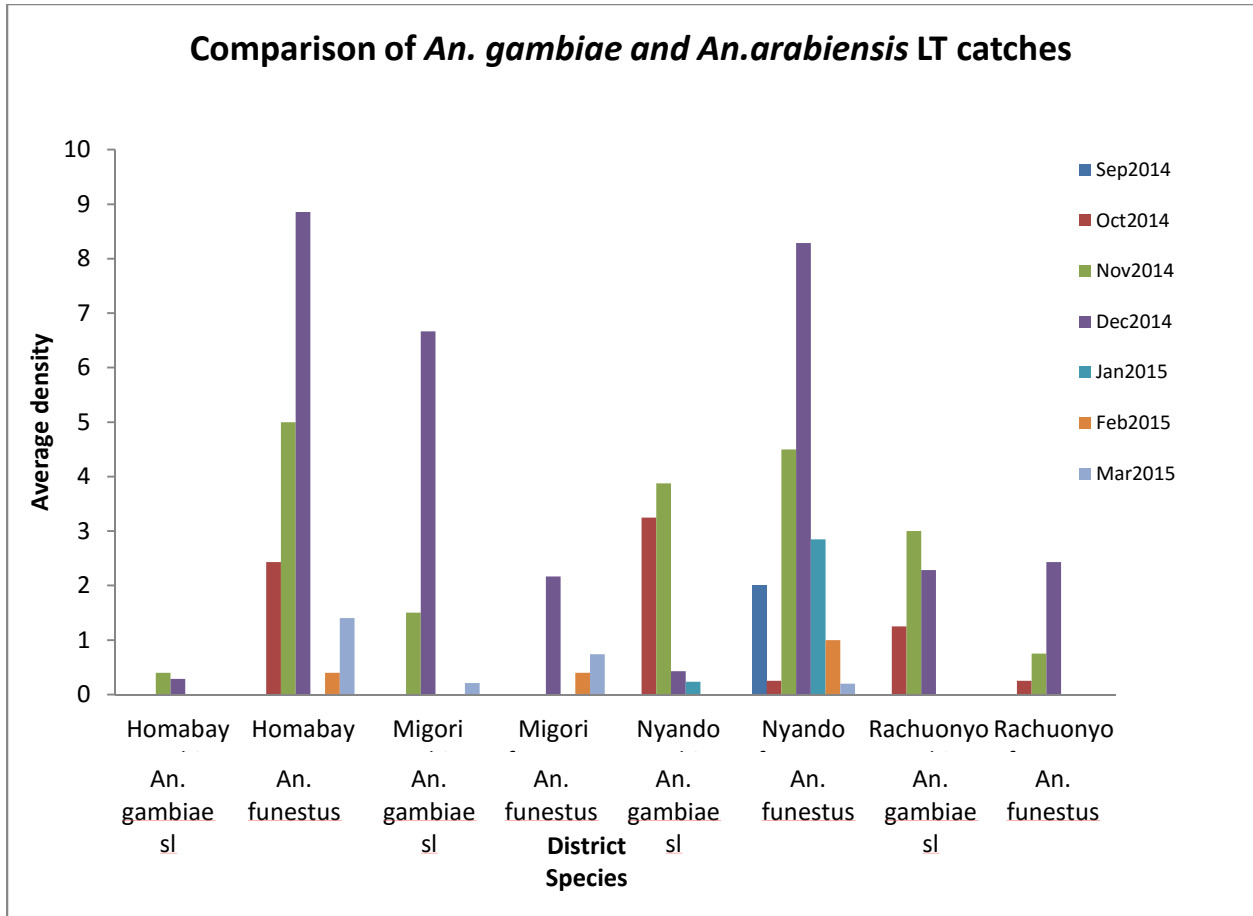

Figure 3: Comparison of light trap catches by district

3.2 Insecticide Resistance Monitoring

Between January and May 2015, insecticide resistance was measured in 4 districts—Siaya, Gem, Migori and Ugunja. In all the districts, mosquitoes tested were a mixture of *An. gambiae* s.s. and *An. arabiensis* although from a recent publication [10], these were likely to be above 95% *An. arabiensis*. High levels of resistance to pyrethroids was observed with probable resistance to bendiocarb detected in Siaya. There was full susceptibility to malathion in all sites tested (Figure 4).
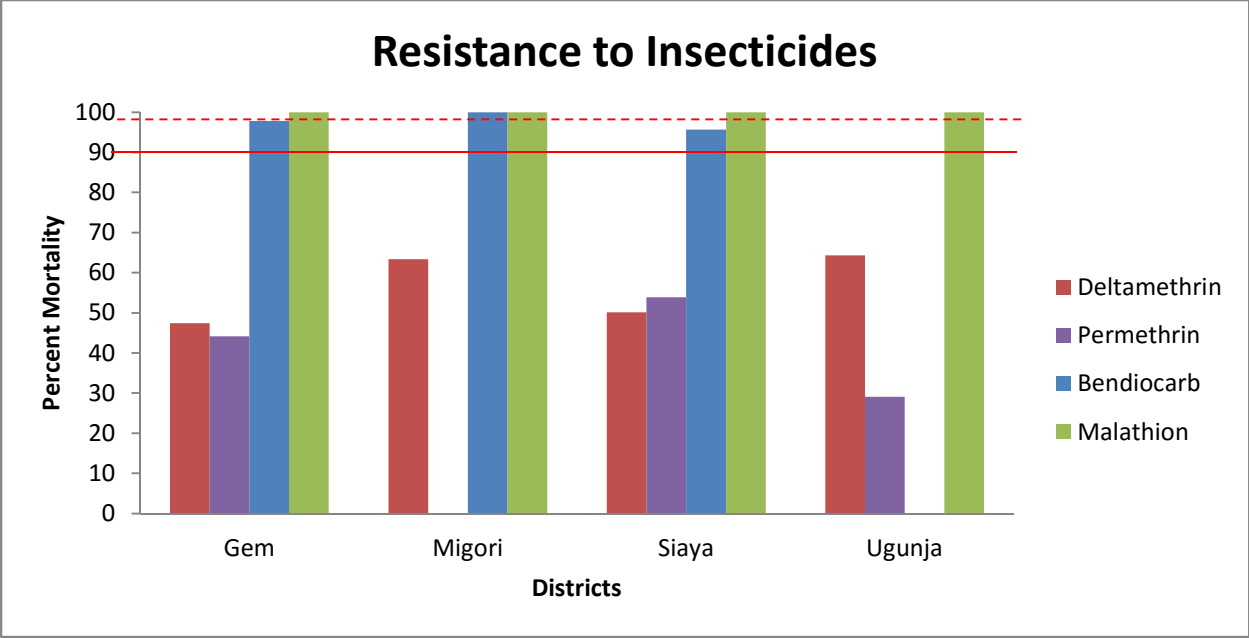
Figure 4: Resistance to insecticides in *An. gambiae* s.l. in the study districts in 2015. Bendiocarb was not tested in Ugunja, neither was permethrin in Migori. The red continuous line represents the WHO cutoff for insecticide resistance while the dotted line shows 98% mark for susceptibility

3.2.2 Comparison of historical resistance to deltamethrin data in the districts.

We compared resistance data collected from 2011 to 2014 in four districts to understand if there were any clear trends of insecticide resistance to deltamethrin (Figure 5). In Bondo, we observed increasing resistance over time.
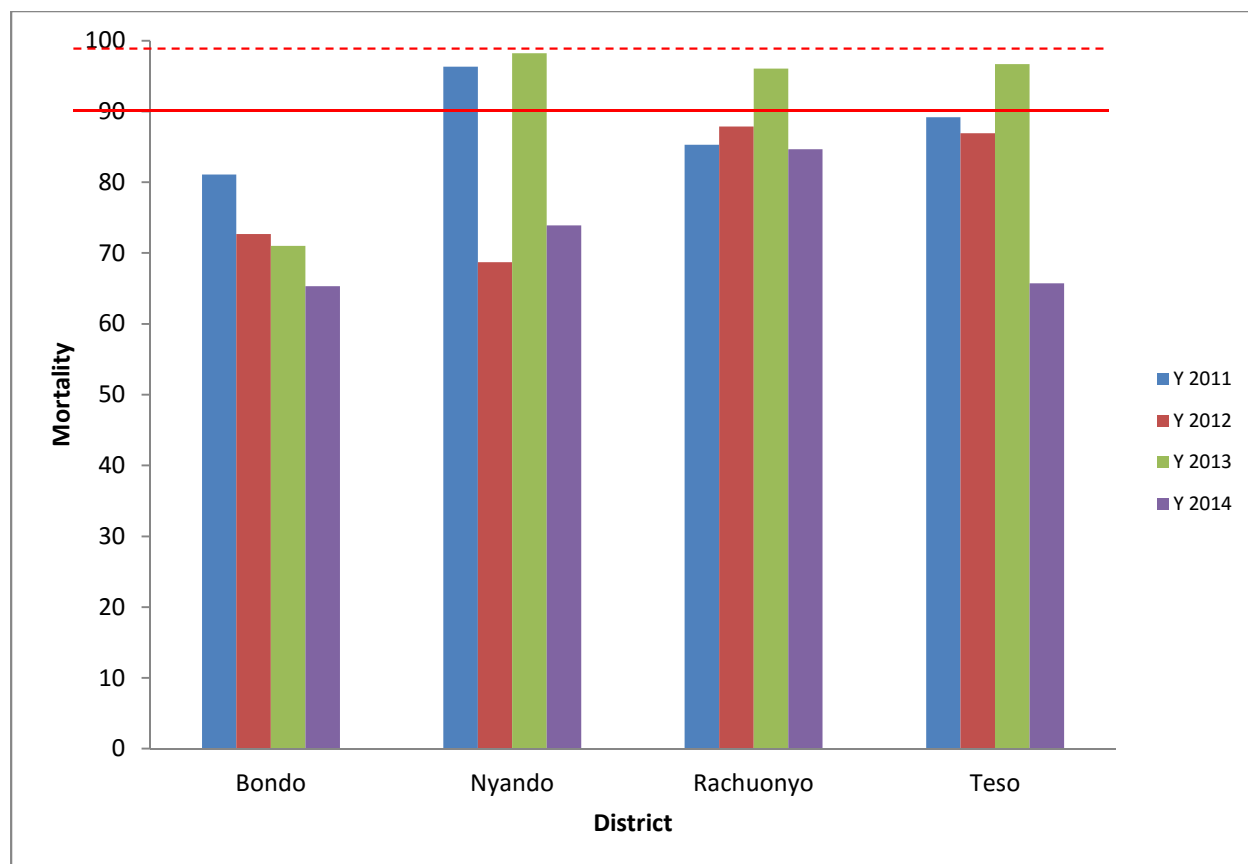
Figure 5: Trends of insecticide resistance from 2011-2014.The red continuous line represents the WHO cutoff for insecticide resistance while the dotted line shows 98% mark for susceptibility

3.2.3 Comparison of resistance to deltamethrin between wild *An. gambiae* s.l. and *An. funestus*

Given the difficulty in rearing *An. funestus* in the insectary, we exposed a sample of 100 wild unfed *An. funestus* and *An. gambiae* collected in the sites indicated in Nyando and Rachuonyo and evaluated them for deltamethrin resistance using the WHO tube bioassay technique. We observed lower levels of resistance in the *An. funestus* in most sites.
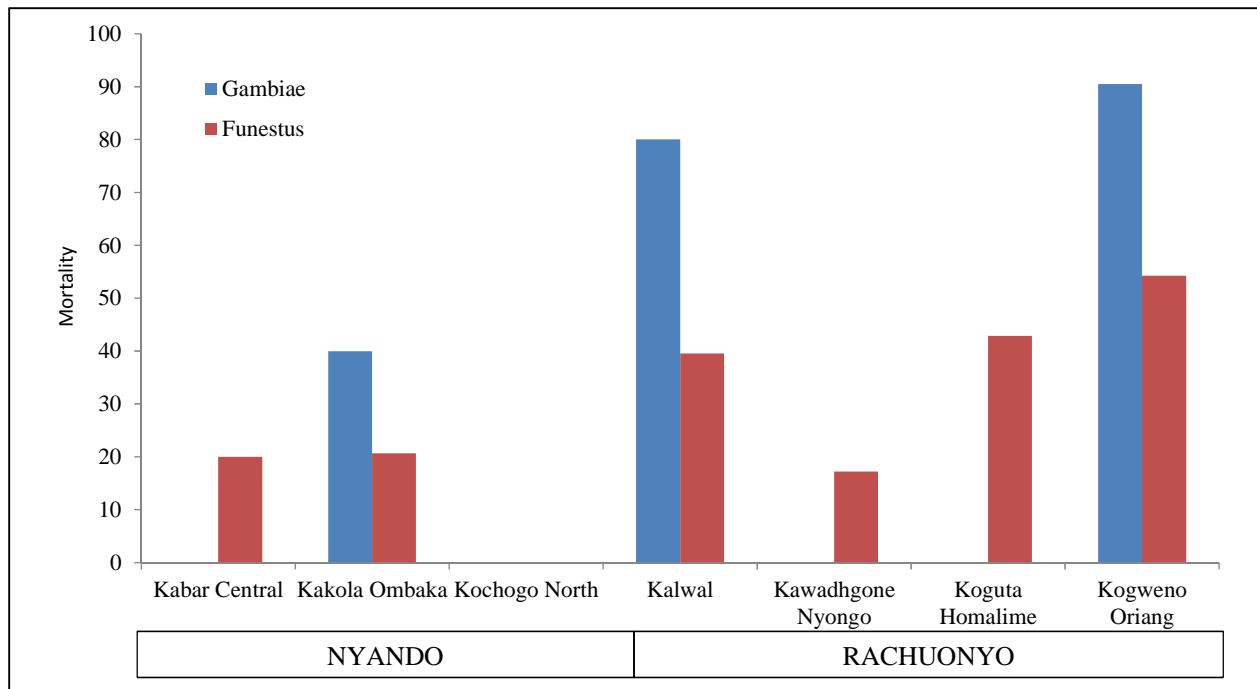
Figure 6 Mortality to deltamethrin in wild *Anopheles* mosquitoes. No *An. gambiae* mosquitoes were collected in Kabar Central, Kawadhgone Nyongo and Koguta Homalime. Mosquitoes collected in Kochogo North were all resistant to deltamethrin

4.0 Discussion of Results

Monthly monitoring of mosquito populations has been going on in the 6 study districts of Rachuonyo, Nyando, Migori, Kisumu East, Homa bay and Gem. Routine collections through pyrethrum spray catches and light traps have previously shown a dramatic impact of indoor residual spraying in Nyando and Rachuonyo, and Migori . It is now clear that *An. funestus* has re-emerged as a predominant vector of malaria in all districts except Rachuonyo as has been documented in a previous publication [13]. Other studies currently being conducted at the Entomology Section at CGHR, KEMRI indicate *An. funestus* in Siaya have up to 5 times the sporozoite rates of the *An. arabiesis* population meaning they could potentially be the predominant vector of malaria in Siaya and therefore a reason for the stagnated malaria transmission in parts of western Kenya [14, 15].

When compared to light traps, PSC performed poorly in collection of mosquitoes inside the house. This means that mosquitoes are coming into the house but do not rest on the walls as traditionally thought. There is therefore a need to capture exiting mosquitoes, assess their abdominal status at the time of exiting in order to understand whether they still feed indoors even though they may choose to rest elsewhere. In addition there is a need to collect mosquitoes outdoors as there is potential transmission could be taking place outside the house. Tools such as exit traps, suna traps, tent traps, pots and plastic drums need to be assessed and included in monitoring programs for collection of outdoor vectors.

It is important to evaluate the blood meal sources of these mosquitoes since as they are exiting; there is a chance they could feed on non-human hosts outdoors. Even though recent reports point to late night biting [16], it is important that the biting behaviour and location of human is constantly monitored as interventions are scaled up. Already there are reports of shifts in the biting patterns of *An. gambiae* s.s [17] and it is important that emphasis is placed in such behavioural studies. If a behavioural shift in biting were to occur so that mosquitoes no longer bite at times when humans are under LLINs, or in houses where they are protected by IRS, there is a real chance transmission could increase to pre-intervention levels [18, 19].

Insecticide resistance especially to the pyrethroid class of insecticdes continues to increase in almost all the district. In the recent past, there have been reports of emergence of insecticide resistance in western Kenya [10, 13] and the spread of new resistance genes into the local populations [11]. There has also been a report of resistance undermining vector control using ITNs [6]. Insecticide resistance monitoring including determining the levels of resistance to the different insecticides used for vector control and unraveling the resistance mechanisms adopted by the different vector species are important measures to be put in place especially as the NMCP considers the introduction of a new class of insecticides for IRS. It is important that surveillance for resistance markers is done presumptively to avoid fixation of resistance genes and to ensure insecticide resistance mitigation strategies are put in place promptly.

5.0 Recommendations

It is important that WHO's Global Plan for Insecticide Resistance Management (GPIRM) policies are put into action to stop the spread of insecticide resistance particularly to the pyrethroid group of insecticides. More vector control paradigms need to be evaluated and put in place to further reduce vector densities and malaria transmission in western Kenya. Vector surveillance tools need to be improved to include tools that can provide information on vector behaviour right from house entry, exiting and outdoor behaviour and feeding patterns. This would enable us to come up with better targeted interventions. There is a need for improved insecticide resistance surveillance, especially of the *An. funestus* which are a challenge to colonize in the insectary. The intensity of resistance needs to be evaluated to enable the understanding of what level of resistance is likely to result in control failure. In addition, there is need to identify PCR based markers that can be directly correlated with insecticide resistance to facilitate tracking of the flow of insecticide resistance. There is a need to evaluate the new tools currently available in the market such as next generation LLIS (Olyset plus®, Permanet 3.0®, Interceptor G2®, attractive toxic sugar baits (ATSBs), mass drug administration with endectocidal drugs such as ivermectin, treatment of cattle with insecticides, topical and spatial repellents among others

6.0 Challenges

Project activities were stopped abruptly in April 2015 following financial hurdles at the institution and is the reason we were only able to conduct resistance surveillance in only 4 of the 6 districts and stopped surveillance in April for most sites. However, in one site, Gem, we managed to collect data for another 2 months. Plans have now been put in place to ensure proper financial management.

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