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# EFFECTS OF INSECTICIDE TREATED NETS (ITNs) TO BEHAVIOUR CHANGES IN ADULT MOSQUITOES

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## ABSTRACT

Insecticide Treated Nets are being used as a strategy in controlling the adult mosquitoes in the control of malaria by mainly the populations living in malaria prone areas. Surveillance to evaluate their impact on control malaria is important. The aim of this study was to assess the effect of insecticide treated nets to behaviour changes in adult mosquitoes.

A longitudinal study was conducted in ten houses involving the collection of indoor resting vector densities monthly for eight months from September 2009 to April 2010. Species were identified both morphologically and by the (PCR) technique to determine the dominant malaria vector in the area

A total of 142 adult malaria mosquito vectors were collected with *An. gambiae s. s.* as the principal malaria vector. These results showed that the malaria transmission intensities are still reducing after the increased bed net use though the pace could be affected with the effects of the ITNs on the behaviour the people and adult mosquitoes. Thus monitoring should continue on the behaviour of the adult mosquitoes to establish their biting trend.

## *INTRODUCTION*

Surveillance to evaluate the impact of the interventions in the use of Insecticide Treated Nets (ITNs) to control malaria in Kenya has been going on since the early part of 1980. ITNs are being used as a strategy in controlling the adult mosquitoes in the control of malaria by mainly the populations living in malaria prone areas.

Malaria remains one of the major tropical disease challenges in the world today. According to the World Health Organization report (WHO 2003, 2005), half of the world's population (approximately 3.3 billion people) are at risk of contracting malaria. About 90% of all malaria deaths in the world today occur in Africa south of Sahara (Africa Malaria Report 2003). More than 1 million Africans, who die from malaria each year, are children under 5 years of age (WHO/UNICEF 2005). Studies focusing on under five children in African populations conclude that 600,000 children contract cerebral malaria yearly with a case fatality rate of 20%. Severe malaria due to anaemia occurs in 1.5-6.0 million African children with a case fatality rate of 15% (Hay *et al.,* 2001). Malaria is also a major threat to pregnant women and adversely affects foetal growth and newborn survival through low birth weight (Chandramohan *et al.,* 2005).

In Africa stable malaria predominates throughout the continent, however epidemics occur at the fringes of the endemic areas, particularly among communities at the northernmost latitudes, across the arid regions of North Africa, and mostly among the highlands of East, Central and Horn of Africa (Craig *et al.*, 1999). Malaria epidemics are common only in areas of unstable transmission where any slight modification in any of the transmission factors may upset the equilibrium (Ijumba *et al*., 2002). In East Africa highland malaria is classified as being unstable or epidemic occurring at altitudes above 1500m (Hay *et al.,* 2002). In East Africa Highlands, the threat is mounting as malaria outbreaks become more frequent in areas where malaria was previously rare (Beier *et al.,* 1999).

Malaria remains the leading cause of illness and deaths, accounting for about five percent of deaths and more than 30 percent or about six million out patient cases in hospitals (Kenya National Human Development Report of 2006). The report notes that ‘‘Although, the highland malaria is not as common as the lowland, malaria’s effects on the highland areas of Kenya is sporadic and yet devastating, with fatality rate of up to 50% if untreated”. In Western Kenya highlands, malaria did not exist until the second decade of the 20$^{th}$ century (Hay *et al.,* 2002). The epidemics normally occur at the valley bottoms, which are poorly drained thus providing permanent mosquitoes breeding sites (Githeko and Ndegwa, 2001). Man made environmental changes such as dams, water reservoirs; deforestation among others may greatly increase the breeding sites of mosquito, thereby triggering epidemics.

Assessing the effects of insecticide treated nets on the behaviour change of adult mosquitoes is paramount is necessary because these can be compared with the gains of mosquitoes control in 2006/2007 due to biological transmission of 1.68 infectious bites per person per year. It is along this background that this study was conducted to determine the effects of ITNs to behaviour change of the adult mosquitoes.

## MATERIALS AND METHODS

These studies were conducted in Emutete village, which is situated along the Kisumu-Majengo road, 5km past Maseno town (34°64 E, 00°22 N,) Western Kenya. Complementary experiments were conducted in CDC laboratories at the Centre of Global Health Research (CGHR), Kenya Medical Research Institute (KEMRI), Kisian which lies 13 km on the north western part of Kisumu city, Kenya.

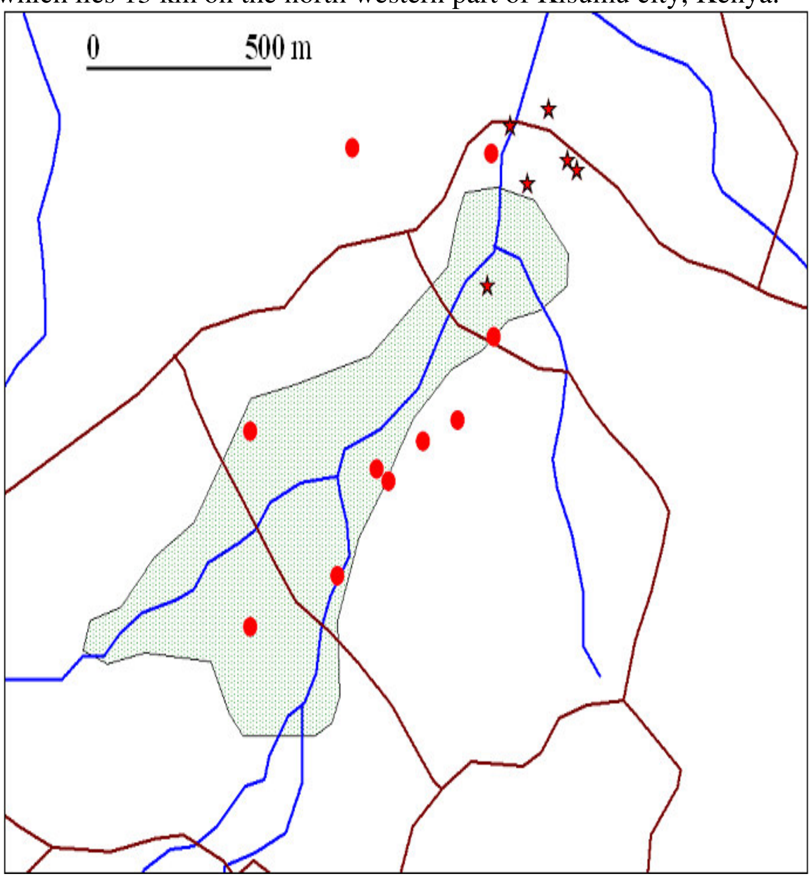


Figure 1: Map of Emutete village in Wekhomo location, in Emuhaya County (district) showing houses where adult mosquitoes were sampled using CDC light traps (□) and Pyrethrum Spray Catch collections (□).The houses are situated along roads (▯ ), river (▯ ) and swamp (░)

***Determination of indoor resting densities of malaria vector mosquitoes***

This involved selecting the houses, spraying in the houses, determining the human biting rates that night of collection and identifying the mosquitoes morphologically and on species level. The rainfall amounts and hospital data of the pre-control and post-scale up period (January 2005-April 2010) was obtained from Kenya meteorological department (Kakamega field station) and Linyang'inya health centre respectively.

***House Selection***

Houses were selected for Pyrethrum Spray Collections (PSC) and Centre for Disease Control (CDC) light traps basing on the population size and the area. The approximate household number in Emutete is eight people per household. The characteristics of the selected houses were one-two bed roomed medium sized, semi-permanent or grass thatched with open eaves and the numbers of occupants were children below ten years and adults. Ten houses were settled at randomly for PSC and six for CDC light trap collections (Fillinger *et al.,* 2009).

***Pyrethrum Spray Collection (PSC)***

A longitudinal study design was used in collecting the total number of mosquitoes resting indoors. This was done once a month for eight months from 0600hrs to 0900hrs. The method employed in the collection was Pyrethrum Spray Collection (PSC). The inhabitants of the house emptied the house, the floor surfaces and white sheets 2x2m were spread from wall to wall so that no space was left for a mosquito to be lost. The Pyrethrum extract [0.2% in kerosene] was sprayed starting from out on all the exist points like eaves, doors, windows before the space in the house was filled. The house was filled for 15 minutes, after which the mosquitoes were collected starting from the door and moving to the interior of the room. The sheets were held at the four corners and the mosquitoes moved gently in the middle. They were transferred out in the light and by use of the forceps the *Anopheles* mosquitoes were picked and placed on moist petri dishes (WHO, 2003), transported to KEMRI—Kisian laboratories transferred to silica gel in eppendorfs awaiting species identification. During picking of *Anopheles* mosquitoes, they were identified morphologically as having distinct black and pale scales on the wings while the *Culex* the scales were not distinct and were all brown. The female anopheleses were to have a non-plumose antenna as opposed to plumose for the males. (Beier *et al*, 1999).

***Mosquito Human Blood Biting Rates***

During the collection of mosquitoes from the houses, their gonotrophic status, fed and half gravid and the numbers of those people who slept in the house the night to the spraying were recorded. The total numbers of blood-fed and half gravid mosquitoes were divided by the number of persons who slept in the house that night to the spraying (Githeko *et al.;* 1993).

***Species identification***

The preserved *An. gambiae s. l.* was identified by use of a Polymerase Chain Reaction (PCR). A leg or a small part of the abdomen was used for identification. The main steps were DNA extraction with potassium acetate and DNA precipitation with 95% ethanol. DNA pellets from each sample were resuspended in 400 microlitre sterile $dH_2O$. Aliquots (1 micro-liter) from each DNA sample were used in the PCR reaction. All reagents used were obtained as a kit from Promega and the reactions were carried out using the PELTIER- THERMAL CYCLE 100 machines. All specimens were individually assigned to a species using rDNA probes in an rDNA-PCR diagnostic assay and ran over a programme consisting of 30 cycles for denaturation at 94 degrees centigrade; for 60 seconds, annealing at 50 degrees centigrade for 30 seconds and an extension at 72 degrees centigrade for 30 seconds. For each PCR reaction performed three controls were used; one negative control with the PCR mix and without any DNA template, one positive control for *An. arabiensis and An. gambiae s. s.* The final amplified product was then electrophoresed in 1.5% agarose- Tris-borate- ETDA gel containing ethidium bromide. All segments were visualized over U.V. Tran illuminator. Bromophenol blue was used as a dye front indicator and the fragment size were estimated by comparison with the size markers (Hay *et al.,* 1993).

***Data Analysis***

Data relative to species composition and abundance was analysed using the one way ANOVA. The monthly adult vector data were log transformed to stabilise variance. All these analyses were done using SPSS (Statistical Package for Social Sciences) Ver.18200 for Windows. Summary statistics were used to determine the mean monthly human biting rate.

One way ANOVA was used to compare the mean biting rates among the three species of the mosquitoes and a t-test used to ascertain the difference among the biting periods. Chi square test was used for equality of proportions in the data from bed net coverage, ownership and utilization. A 3-sample test for equality of proportions without continuity correction was used at X-squared = 34.7541, df = 2, p-value = 0.00000002839.

Poisson regression model was used controlling for calendar year (2005–2010), amount of rainfall, and months of the year to compute for the morbidity risk for malaria in the pre-control and post-scale periods.

**RESULTS**

**Determination of indoor resting densities of malaria vector mosquitoes**

The total number of female mosquitoes collected in eight months was 142, with *Anopheles gambiae s. l.* being 40 (28%), *An. funestus* 7 (5%), and *Culex* species 95 (67%).

***Vector densities***

The mean vector densities of *An. gambiae s. l.* were 0.13, *An. funestus* 0.03 and *Culex species* 0.18 densities per house for eight months sampling with *An. gambiae s. l.* having a wider difference between highest and lowest collections as shown in Figure 1. These results eliminates the *An. funestus* from the calculations of the EIR by still confirming that *An. gambiae s. l.* is the dominant malaria vector in the area.

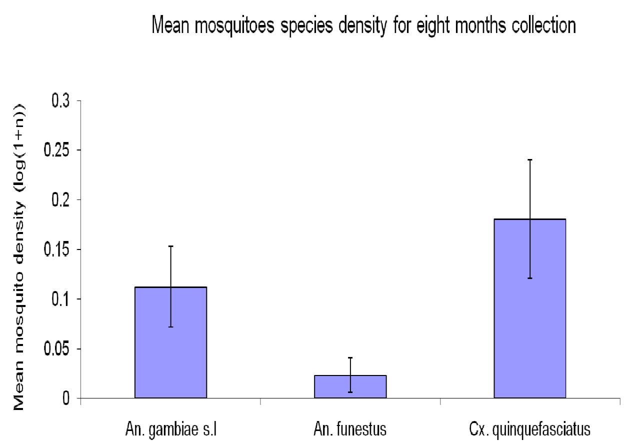


Figure 1: Mean mosquito density of vectors collected at Emutete in 2010.

*An. gambiae s. l.* indoor resting densities were highest in September and October 2009 and lowest in December 2009. These were the months with the highest amounts of rainfall and lowest amounts respectively as shown in figure 2. *An. gambiae s. l.* is the main dominant malaria vector in the study area.

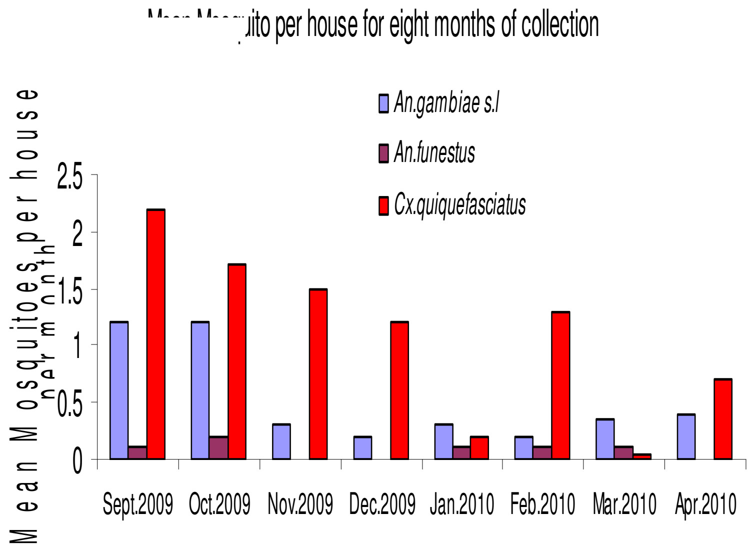


Figure 2: Mean monthly mosquitoe density at Emutete in the year Sept. 2009-April 2010.

**Hospital and rainfall data between the Pre-control and the post-scale period**

Comparing the pre- and post control period after controlling for calendar year, rainfall, and month, there was a lower morbidity of malaria in the post-scale period (risk ratio (RR)= 0.89) as shown in figure 3. This translates into 11% reduction in malaria cases in the post-scale period. This reduction was, however, not statistically significant (*p*=0.549). The analysis also indicated that a millimeter increase in the amount of rainfall led to a 0.08% increase in the chance of occurrence of malaria, but not significantly so (*p*=0.261159). Although there was a decrease in malaria cases in the other years as compared to 2005, a significant drop was only observed in 2006 (*p*=0.0002).

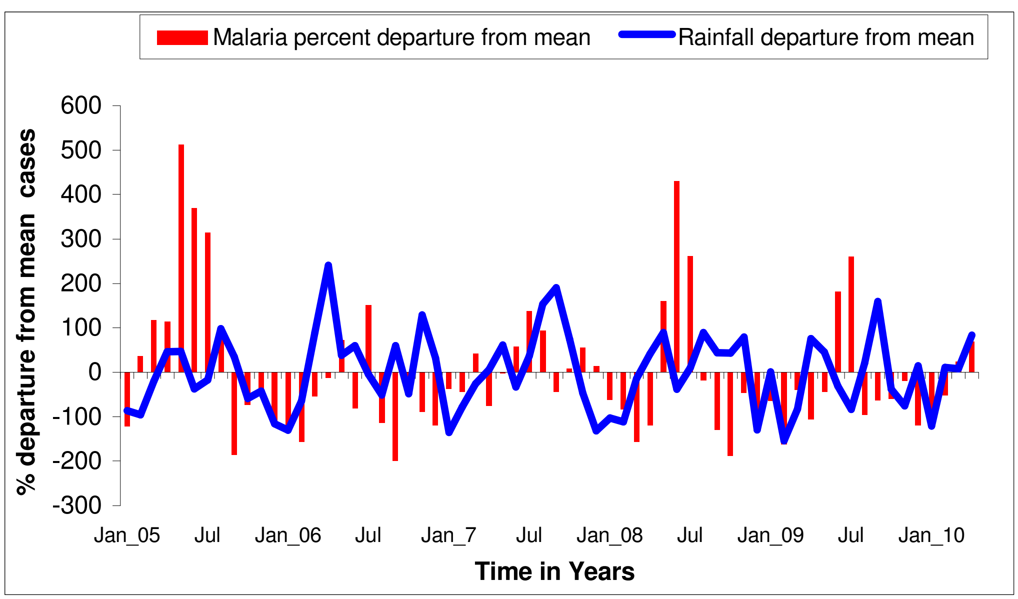


Figure 3: Comparison between the pre-control (2005/06) and post-scale period (2007/10).

**Mosquitoes human biting rates**

The total numbers of *An. gambiae s. l.* biting humans in the houses were summed up and an average for each month was determined, giving the mean monthly human biting rate for the eight months which was 0.12 bites per individual for eight months as shown in table 1.

**Table 1: Monthly human biting rates by *An. gambiae s. l.* as a factor for determining the EIR at Emutete in 2010**

| Month | Human biting rate |
|---|---|
| Sep-09 | 0.13 |
| Oct-09 | 0.07 |
| Nov-09 | 0.18 |
| Dec-09 | 0.07 |
| Jan-10 | 0.19 |
| Feb-10 | 0.28 |
| Mar-10 | 0.02 |
| Apr-10 | 0.06 |
| Overall Mean | 0.12 |

## DISCUSSIONS

In this study, *An.gambiae* complex is the main dominant malaria vector in Emutete area as compared to *An. funestus*, though *Culex quinquefasciatus* stands out high in numbers as a non- vector species of mosquitoes. Malaria morbidity has gone down in the post –scaled period as compared to the pre-scaled period, but an observed behavioural change in people and adult mosquito could have affected the degree at which transmission intensity has come down.

The study confirms *An. gambiae s. l.* as the principal malaria vector in Emutete village of Western province. This complex has been described previously in the same area as appearing in highest proportions among the other malaria vectors (Fillinger *et al.,* 2009). Pertinent to this study, a high proportion of *An. gambiae s. l.* has been in large proportions as compared to *An. funestus*. The indoor resting densities of this principal malaria vector were highest in September and October months that corresponded to high rainfall amount which confirms with previous findings in the same area (Fillinger *et al.,* 2009).

The dominant malaria vector *An. gambiae s. l.* had the highest early and late biting rates as compared to *An. funestus* and *Culex quinquefascitus*. Earlier studies at Miwani town in Kisumu district revealed a 2% in proportion of the *An. gambiae s. l.* (Githeko *et al.,* 1996) as compared to the present one of 20.8%. This is a 10 times increase in the proportion of early biting proportion. Also *An.gambiae s. l.* had a higher early biting proportion which was significantly different to that of *An. funestus,* this implies that *An. gambiae s. l.* is biting more in the early evening than late. This concurs with other trial studies in Papua New Guinea, Tanzania and in Kenya (Githeko *et al.,* 1996) on possible effects of ITNs on the time of biting of mosquitoes because the mosquito hosts had not yet gone to bed and were easily accessible. Although the mean early biting rate was not significantly higher than that of the late biting, still is a concern in the malaria control because the effectiveness of the bed nets was based on the observations that in the rural villages, majority of people are asleep by 9pm (Githeko *et al* 1993). Increased early biting by the *An. gambiae s. s.* can cause malaria infection and life threatening disease (Beier *et al*., 1999).

## Conclusions

This study has shown that *An. gambiae s. s.* is the main principal malaria vector in Emutete. The upscaling of ITNs in the area has resulted in the continual reduction of the malaria intensity though the reduction pace is much lower as compared to the earlier drop that was experienced in 2006 when the intial upscale was rolled out in the area. The effects of ITNs in the area have had a possible behavioural change in both people and the adult mosquitoes which has influenced the effectiveness of bed net trend.

**Recommendations**

1). The data should be collected over a long period of time both during the long and the short rainy seasons to allow time for a better comparison of the human biting rates during the two different seasons and be able to come up with a more comprehensive EIR.

2). An alternative method of estimating the human biting rates should be considered in future instead of the pyrethrum spray catch since houses with ITNs, mosquitoes are already deterred the night before the collection and this underestimates the biting rates.

3). In the comparison of the pre-scaled and post scaled period the denominator should be included in the calculation of the morbidity risk to determine the malaria cases in relation to other disease cases.

6). There is urgent need to inform the malaria national control program of the observed shifts in man-biting patterns in the major malaria vector *An. gambiae s. l.* in Western Kenya highlands so that more investigations are done to determine the trend.

10). Monitoring the behaviour of the vectors and the impacts of interventions on malaria control in the area should continue since this is an important part of integrated vector management.

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