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Effects of long-lasting insecticidal nets and zooprophylaxis on mosquito feeding behaviour and density in Mwea, central Kenya

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Abstract

Background & objectives: Zooprophylaxis is a strategy that can control malaria by attracting mosquitoes to domestic animals that act as dead-end hosts. The objective of this study was to establish the effects of zooprophylaxis and long-lasting insecticidal nets (LLINs) on malaria transmission in an agro-based ecosystem with seasonal transmission.

Methods: The mosquito samples were collected indoors using the space spray catch method before and after intervention between October 2005 and March 2006 to determine the mosquito densities and the feeding patterns of *Anopheles* spp in Mwea, Kenya.

Results: A total of 4148 mosquito samples were collected, out of which 11 (0.2%) were tested positive for sporozoites. Ten were *Anopheles gambiae* species and one was *An. funestus* species. Results on blood meal ELISA showed that in the household categories that used bednets and kept one cow there was a decrease in relative change ratio (post-/pre-intervention) of 87.5 and 19.6% ($p<0.05$) in human and cattle blood intake respectively. For households that kept 2–4 cattle and used bednets, there was a decrease in cattle blood index (CBI) by 61.9% and an increase in human blood index (HBI) by 2%, which was not significant ($p>0.05$). In households with >4 cattle and bednet, there was significant reduction ($p<0.05$) in CBI of 37.5% as compared to the reduction of 10.3% in HBI. The ratios of man biting rates (MBR) decreased significantly, as you move up from households with one cattle with or without LLINs to households with more than four cattle with or without LLINs with a regression coefficient of –0.96; SE = 0.834; $p = 0.017$. Similarly, the HBI decreased significantly with the regression coefficient of 0.239; SE = 0.039; $p = 0.015$ ($p<0.05$) especially in households with >4 cattle.

Interpretation & conclusion: This study demonstrated that there were additive effects of zooprophylaxis and LLINs in the control of mosquito density and reduction of human risk to the mosquito bites. However, in Integrated Vector Management (IVM), the number of animals per household should not be more than four.

Key words Cattle biting index – human biting index – long-lasting insecticidal nets – man biting rates – mosquitoes – zooprophylaxis

Introduction

Malaria is a public health problem with an estimated 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly a million deaths, mostly of children <5 years. A total of 109 countries were endemic for malaria in 2008, 45 within the African region[1]. In Africa, approximately 10% of hospital admissions and 20–30% of outpatient visits are due to malaria[2]. The disease is endemic in Kenya affecting >4 million people with highest incidences being recorded in the western and coastal regions, parts of Rift Valley, central and eastern provinces[3]. It continues to increase in severity and frequency since early 1980s, with 4 and 2-fold increase in risk of disease and death rate, respectively, due to increased parasite virulence and drug resistance.

The resurgence of malaria in some regions may be linked to the expansion of breeding grounds resulting from global changes in the environment and habitats[4,5]. The degree of transmission depends on the number of mosquitoes, the frequency with which a particular species bites man and the longevity of the adult mosquitoes. Longevity is particularly important because it can take 10 or more days for parasites to mature in the host mosquito before it is passed on to infect the next human host. However, since the resurgence of malaria is compounded by the spread of drug resistance, prevention of infections and selective vector control have become quite significant[6]. Among these, the insecticide treated nets (ITNs) and curtains have emerged as the most practical methods of control[7]. Pyrethroids are the only group of insecticides currently recommended for use on nets and ordinary bednets need to be treated and retreated after every six months. However, not every one can afford to buy a synthetic pyrethroid, hence there is need to implement the long-lasting insecticidal nets (LLINs), which are known to last for five years.

Zooprophylaxis is a strategy that intends to control vector-borne infections by diverting vectors to domestic animals that act as dead-end or decoy hosts[8]. For instance, in the case of malaria, domestic animals such as cattle can serve as a dead-end host because human malaria parasites cannot develop in cattle.

The choice of blood meal is influenced by several factors including host availability, nutritional requirements, intrinsic host preferences of the species and vector density[9–12]. The two species of *Anopheles* found in Mwea, Nigeria that are known to transmit malaria are *Anopheles arabiensis* (Diptera: Culicidae) and *An. funestus* with the former preferring to feed on cattle[13]. Livestock husbandry is beneficial in availing an alternative source of blood meal for the female mosquitoes other than man, and has long been recommended as a protective measure against malaria by the World Health Organization[14]. The fact that *An. arabiensis* has been shown to prefer feeding on cattle over humans makes cattle an effective mosquito attractant. However, there is a lot of concern about the practice since some studies have shown that the presence of cattle may instead increase malaria prevalence[8,15]. Control of vector-borne diseases such as malaria is often directed at controlling vectors with insecticides and larvicides. Insecticide treated bednets are known to kill and/or repel mosquitoes. Thus, the aim of this study was to establish whether when mosquitoes seeking human blood are repulsed by LLINs or they would seek an alternative blood meal source such as cattle within the compound. The objective was to examine whether combining the two methods described above will have additive effects on the control of malaria transmitted by *An. arabiensis*.

Methods

Study site: The studies were conducted in Mwea rice irrigation scheme in central Kenya, 100 km northeast of Nairobi, Kenya. Mwea rice scheme covers an area of approximately 13,600 ha with a population of 150,000 people in 2500 households[16]. Mwea Division is situated on the east of Mount Kenya at an altitude of 1100 to 1350 m above sea level. It is the centre of Mwea rice irrigation scheme, a settlement scheme that produces 75–90% of the rice that is consumed in Kenya (National Irrigation Board Report). Water from the two major rivers: Nyamindi and Thiba irrigate the rice-fields in a network of primary and secondary feeder canals. The villages are discrete units made up of homesteads/compounds with varying numbers of households. The majority of families keep domestic animals such as cattle, goats, sheep, donkeys and chicken among others[13].

Three villages, namely Nguka, Mukou and Kirogo were randomly selected within the irrigation scheme where 75% of each village land is under rice cultivation. In these villages, a cross-sectional survey was set to investigate the combined effects of LLINs and cattle in the compound (zooprophylaxis) on malaria transmission in Mwea. In each of the village selected, all households were included in the study. In each of the three villages, 40 households were issued LLINs. For zooprophylaxis, households were grouped according to the number of cattle they had within the compound. The grouping included 0 cows, 1 cow, 2–4 cows and >4 cows. Ten households in each of the three villages with no intervention (without LLINs and cattle) were treated as control. In each case, the selection was random as summarized in Table 1. The impact of the interventions (LLINs and zooprophylaxis) was determined by assessing man biting rate (MBR), human blood index (HBI) and cattle blood index (CBI) and comparing it with the controls. The study design was a 2 x 4 factorial design since it was comparing households with cattle grouped into (0, 1, 2–4, >4) and those with or without bednets.

Collection and processing of mosquitoes: Selected households with ordinary bednets were issued with the Olyset^{TM} LLINs for reinforcement. Vector collection was done using space-spray catch. Synthetic pyrethroid insecticide (0.5% Permethrin) was sprayed and the houses were checked after 5–10 min for the knocked-down adult mosquitoes. The knocked-down *Anopheles* mosquitoes (both males and females) falling on the white bed sheets spread on the floor were collected using fine forceps and transferred temporarily into plastic petri-dishes and then labelled with the household number and village name. The number of persons who slept in the house the previous night was also recorded. Household items and people returned to the houses after one hour. This activity was carried out before and after interventions and the results were recorded. In the laboratory, the mosquitoes were identified using morphological keys[17,18]. Abdominal stages were classified as fed, gravid, half-gravid or unfed. The mosquitoes were desiccated over anhydrous calcium sulphate and stored at room temperature till further processing. In the laboratory at Kenya Medical Research Institute (KEMRI), the mosquito samples were subjected to enzyme-linked immunosorbent assays (ELISA) for determination of both blood meal and sporozoite.

Vector host preference: Vector host preference is usually determined by analyzing the sources of mosquito blood meals. The MBR is the average number of bites per person per night by a vector species and its estimation involves both the feeding habits of the vector and the night time habit of the local people. MBRs can be calculated directly from the man landing catches and indirectly from spray sheet collection like in the case of this study. HBI is the proportion of mosquitoes with human blood. HBI in a vector species can be used as an indication of the degree of anthropophily of that particular species. HBI is calculated using the formula below:

$$HBI = \frac{\text{Number of mosquitoes with human blood}}{\text{Total number of mosquitoes with blood}}$$

Data analysis: Data were entered and verified using Epi-Info® software version 3.4.1 (CDC, Atlanta, Georgia, USA). For the analysis, Mantel-Haensel Chi-square was used to compare the exposure type, mosquito species, mosquito density, mosquito infectivity and HBI. The level of significance was estimated by category and overall.

Ethical considerations: Written consent for participation was sought from the household heads after meeting the criteria for participation in this study. Ethical clearance was sought from National Ethical Review Committee (NERC) and the Ministry of Education, Science and Technology in Kenya. Participation in the proposed study was voluntary and could be withdrawn if one so wished at any time, without penalty or loss of benefit to which they were otherwise entitled.

Results

Based on the questionnaire response, 80 households were selected from each study village. The selection criterion was based on the cattle numbers and bednet use. Based on the cattle numbers and bednet usage,

Table 1. Cattle groupings and bed net use per household

House-hold category	Level	Animals	LLINs	Average number of people per household
0,0	L0	No cattle	No bednet	3.61
0,1		No cattle	With bednet	4.13
1,0	L1	One cattle	No bednet	4.23
1,1		One cattle	With bednet	4.27
2,0	L2	2–4 cattle	No bednet	4.47
2,1		2–4 cattle	With bednet	4.36
3,0	L3	>4 cattle	No bednet	4.63
3,1		>4 cattle	With bednet	4.61

the households were coded and the average number of occupants per household determined (Table 1). A total of 4148 mosquitoes were collected, whereby 1533 mosquitoes were collected before (0) and 2615 after (1) intervention from the three study villages.

Vector host preference: The experiment demonstrated that there was increased protection from mosquito bites in the households with less than five (≤4 animals) animals and using bednets (L3). This was observed in the MBR and from the HBI (Figs.1 and 2) respectively.

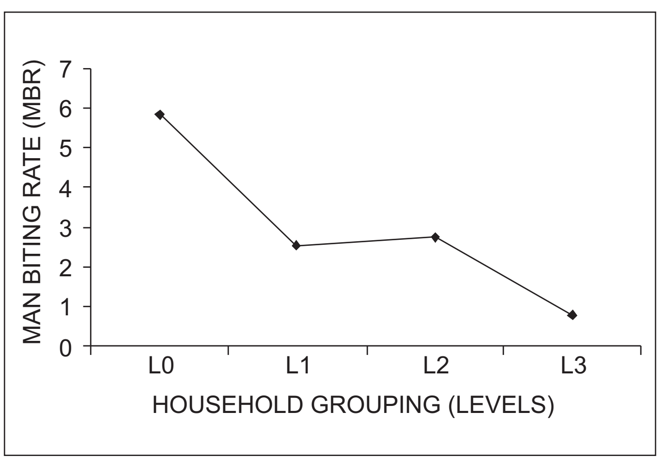

Fig.1: Change in the rate ratios of man biting rates (MBR) before and after intervention by animal groupings and LLINs use (Levels). L0: 0,0 and 0,1 households; L1:1,0 and 1,1 households; L2: 2,0 and 2,1 households; L3: 3,0 and 3,1 households

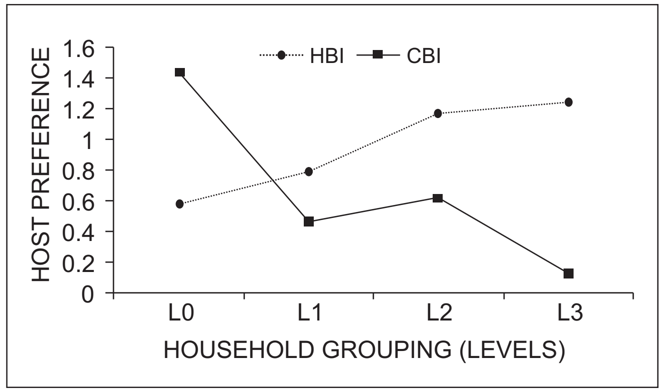

Fig. 2: Changes in the rate ratios of HBI and CBI before and after intervention by animal groupings and ITNs use. The categories L0, L1, L2 & L3 are as described in Fig. 1.

Man biting rates: Intervention levels had significant effects on host seeking behaviours of the mosquito vectors especially for the human hosts (Fig. 1). For levels 0 (control group) through to levels 1, 2 and 3 there was a significant decrease in MBR with regression coefficient of –0.96 (MBR = 6.38; –0.96* level) (df = 3; SE = 0.834; $p = 0.017$), which indicates that intervention provided significant protection from mosquito bites.

Human biting index: In this study, risk ratios of HBI before and after intervention were compared in different household groupings with various interventions. Significant protection was observed at all levels (Fig. 2), however, protection decreased gradually with the increase in the number of animals with regression coefficient of 0.239 (HBI = 0.589; +0.239* level) (df = 3; SE = 0.073; $p = 0.015$), which indicates that intervention provided significant protection from mosquito bites.

Cattle biting index: There was a decrease in CBI (1.44–0.46) for L0 to L1. When the animals were increased to 4 (L2), the CBI increased slightly from 0.46 to 0.62. However, when the cattle in the household were >5 (L3) the CBI decreased steadily from 0.62 to 0.13 (Fig. 2). The risk ratios of CBI before and after intervention were compared in different household groupings with various interventions. Protection to animals was not significant at all levels

Table 2. Comparison of the mean human risk ratio by household codes

Household codes	Risk ratio	Mean human risk ratio (MHRR)	*p*-value
0,0 0,1	0.5<0.58<0.67	$\chi^2 = 10$, df = 1	<0.05*
1,0 1,1	1.13<1.25<1.38	$\chi^2 = 21.7$, df = 1	<0.05*
2,0 2,1	0.88<0.96<1.05	$\chi^2 = 0.61$, df = 1	>0.05
3,0 3,1	1.71<1.94<2.21	$\chi^2 = 101$, df = 1	<0.05*

*p <0.05 shows there was significant reduction in the risk ratio comparing levels 0,0 and 0,1 through to 3,0 and 3,1. (Household codes have been defined in Table 1)

(p >0.05). However, protection decreased gradually with the increase in the number of animals with regression coefficient of 1.144 (MBR = 0.949; +1.144 level) (df = 3; SE = 0.445; p = 0.167).

Discussion

The study revealed that cattle kept within the compound protected the household occupants from mosquito bites. Results also demonstrated an additive protection where LLINs were used and four or less animals were kept in the compound. The results indicated that households that kept lesser animals and used LLINs had significant low HBI as compared to those with more than five animals. This supports the findings of the studies which were carried out in Pakistan[15].

It was clear that zooprophylaxis and LLINs had a direct impact on the mosquito feeding behaviour. The use of bednets in the presence of few animals decreased the indoor biting rates within the households. For MBR, there was a significant increase in protection from mosquito bites with maximum protection at L3. The same scenario was observed in HBI, where significant protection occurred at all levels. Yet, a gradual increase in risk was observed from L1 to L3. The use of LLINs in the presence of cattle within the homesteads showed significant reduction in risk ratio. This suggests that the insecticide treated bednets had repelling effects on mosquito vectors from the houses. This then discouraged the mosquitoes from feeding on humans and hence diverting them to the alternative blood meal sources. These findings support previous reports[7,19] that the presence of insecticide treated bednets in good condition provides protection to inhabitants within the compounds and hence reducing the parasitaemia. Increased HBI in the presence of many cattle may have been attributed by the fact that animals were too close to the human dwellings, hence increasing the risk of humans being bitten by mosquitoes within the proximity. It seems that mosquitoes were attracted to cattle due to their size and numbers, but they preferred to feed on humans rather than cattle.

Increased HBI in the presence of LLINs could also be attributed to individuals staying longer at night before going to sleep under bednets. In this case, the community should be advised to use zooprophylaxis together with other control measures such as repellents.

Results obtained from this study have further revealed that as the number of animals increases, the level of protection decreases. This effect could be attributed to the increased mosquito densities. As the number of animals within the homesteads increased, it acted as a ready source of blood meal for the mosquitoes, which in turn increased their breeding potential. This strategy, therefore, suggests that zoo- prophylaxis provides readily available blood meals for mosquitoes, and especially when present in large number in the presence of LLINs. As you move from L1 to L2 there was increased CBI, but at L3, it decreased while at the same level, the HBI increased. When animals are in large number, they lead to the increase in vector densities, hence increasing the chances of HBI, and reducing the CBI. This is possible since domestic animals may not only serve in the reduction of malaria but may also serve as amplifiers of the disease pathogens[20]. The results support the phenomenon of 'compound effect'[15] which showed that the families that kept cattle in their houses recorded higher prevalence of malaria than those did not. The results obtained from this study suggest that if zooprophylaxis has to be incorporated into IVM, the number of animals per household should not be more than four.

The study has revealed that in the households that used LLINs, the impact of mosquito survival was low as seen in households 0,0 and 0,1. The presence of animals and LLINs within the compound also decreased the risk ratios. This indicates that bednets enhanced zooprophylaxis. The additive effects of the two methods (zooprophylaxis and LLINs) operating simultaneously, led to an increase in vector density. Additive effects also have been demonstrated in the HBI and MBR. A strong relationship between the HBI and zooprophylaxis has been demonstrated in Pakistan[15].

Bednets also decreased the proportion of mosquitoes that successfully fed the previous night on humans in the houses that had bednets with no animals. This could be attributed to the mortality of the adult vector. The insecticide-treated bednets shorten vector longevity, therefore, decreasing the probability of the parasite completing its development[21]. Reduction in the daily survival rate has a profound effect on malaria transmission. In addition, LLINs provide a barrier, thus, reducing the man-vector contact. On the other hand, use of LLINs may further be enhanced by keeping animals far from the homesteads and more so in the rice paddies so that the *Plasmodium* cycle may be restricted to the irrigation schemes far from the human dwellings. Experimental hut studies have shown a reduction in feeding success of the mosquitoes in the households that were using bednets[21]. Entomological monitoring during intervention trials showed a reduction in the number of indoor resting mosquitoes that were blood fed in the villages and were using bednets[22].

Conclusion

In conclusion, this study has demonstrated the significance of studying mosquito behaviour in the households using LLINs and with cattle within the homesteads. The results have shown the importance of domestic animals in the fight against malaria. This implies that integrated control with the two tools when well-organized could reduce malaria in endemic areas and thus reducing the morbidity and mortality. It therefore seems that the potential impact of LLINs and zooprophylaxis can be integrated in the programmes for IVM.

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