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Short Communication

Preliminary Evaluation of Insecticide-Impregnated Ceiling Nets with Coarse Mesh Size as a Barrier against the Invasion of Malaria Vectors

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SUMMARY: We evaluated the effectiveness of installing the Olyset® Net on the ceiling in preventing the invasion of malaria vectors. This study was conducted in houses in western Kenya. The number of resting mosquitoes inside the houses reduced when the ceiling and eaves of the houses were covered with the net. The mosquito densities remained low for 9 months, until the nets were removed.

Olyset® Net, which is made of polyethylene netting material (mesh size, 20 holes/cm^2), with permethrin (2%) incorporated into the polymer before monofilament yarn extrusion, is one of the most successful long-lasting insecticide-treated net (LLITN) products recommended by the World Health Organization. In the present study, we evaluated the effectiveness of Olyset® Net as a barrier against the invasion of malaria vectors. We propose that the use of Olyset® Net with a coarse mesh size to cover the ceiling and the eaves is a novel, economical, and environment-friendly protective measure against the entry of mosquitoes.

Two net materials identical to Olyset® Net were provided by Sumitomo Chemical Co. Ltd. (Tokyo, Japan). While one net material was impregnated with 2% permethrin, the other material was untreated. Each net material was cut and sewn into sheets measuring 7 × 5 m. To facilitate fixation under the ceiling, ring bands were attached to the diagonal positions of the nets.

The study was performed in 3 houses in Nyandago village, Gembe East, Mbita division, Suba district of Nyanza province, western Kenya. The Gembe East area has a population of ca. 13,000 with 2,700 households over an area of 46.2 km^2. The primary malaria vectors found in the area are *Anopheles gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. Both *An. gambiae* s.s. and *An. arabiensis* belong to the *An. gambiae* complex (*An. gambiae* s.l.). These 3 species were recently reported to have developed multimodal pyrethroid resistance (1). *An. rivulorum*, which is a sibling species in the *An. funestus* complex, is a minor vector in the area. We selected 3 residential houses (NYAND 6, 8, and 11) with a high mosquito density, as determined by preliminary mosquito collection. The houses were of standard sizes and had standard structures (Table 1), with 1 entrance door, 1 or 2 small windows, and eaves, which are the gaps between the top of the wall and the roof and are very common house structures in Africa. The distance between each house was less than 80 m. The houses were surrounded by farms of maize, carrot, tomato, etc., and were located near a swampy coastal area of Lake Victoria, which was the main breeding area of the Anopheline mosquitoes. Two houses (NYAND 8 and 11) were used as the intervention houses, while the third (NYAND 6) constituted a control house. NYAND 8 and 11 have 1 bedroom and 1 living room divided by a partition, and NYAND 6 has 2 bedrooms and 1 living room divided by partitions. The residents were informed about the study and their written consent was obtained before the intervention. Data on the residents' sleeping conditions and their daily use of bed nets were recorded and maintained during the intervention. NYAND 8 and 11 received intervention with permethrin-impregnated ceiling nets on May 26, 2010. One-and-a-half ceiling nets (7 × 5 m plus 3.5 × 5 m) were installed to cover the ceiling of each house. The bottom edges of the ceiling nets were stapled onto the edge of the eaves or mud walls to close the openings of the eaves (Fig. 1). The ceiling nets were kept hanging for ca. 9 months and were then removed on February 11, 2011. To compare the effect of the ceiling nets with or without permethrin-treatment, untreated nets were placed on the ceilings 1 week after removal of the permethrin-impregnated nets (February 18, 2011), in the same manner as described above. Finally, new permethrin-impregnated ceiling nets were reinstalled in the 2 intervention houses on February 25, 2011.

Mosquito collection was performed in each of the 3 houses in the morning (07:00–09:00). Anopheline mosquitoes resting on the walls, under the furniture, etc., were collected by 3 different persons for ca. 20 min per house using a battery-powered aspirator (C-cell

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Table 1. Houses used for the study

House ID	Household size (m) Width × Length × Height[1)]	No. of residents[2)]	Bed nets used during intervention[3)]	Intervention
NYAND 6	5.5×4.5×1.8 (3.5)	1 adult and 4 children	Olyset® Net+Untreated Net	Control
NYAND 8	7.0×5.5×1.8 (3.8)	2 adults and 1 child	Olyset® Net+Untreated Net	Ceiling Net (1.5 Sheet)
NYAND 11	6.5×5.5×1.9 (4.0)	2 adults and 2 children	Untreated Net	Ceiling Net (1.5 Sheet)

1): Height from floor to eaves. Figures in parenthesis indicate height from floor to the top of roof.
2): Adult, >18 years old; Child, <10 years old.
3): Bed nets had been used by the residents before the intervention of ceiling nets and were kept using during the intervention.

Fig. 1. Intervention with the permethrin-impregnated ceiling net.

aspirator; BioQuip Products, Rancho Dominguez, Calif., USA). The collected mosquitoes were microscopically examined on the basis of the identification keys developed by Gillies and Coetzee (2). Individual species within *An. gambiae* s.l. and *An. funestus* s.l. were identified using the multiplex PCR method described by Scott et al. (3) and Koekemoer et al. (4). Mosquitoes were collected 3 times prior to intervention with permethrin-impregnated ceiling nets; 6 times after intervention with the permethrin-impregnated ceiling nets; 5 times after removal of the permethrin-impregnated ceiling nets, 5 times after intervention with permethrin-untreated ceiling nets; and 4 times after reintervention with new permethrin-impregnated ceiling nets.

The mosquito density was calculated as the mean number of female mosquitoes collected daily per house. Square root of the ratio of the mosquito density in the intervention houses (NYAND 8 and 11) versus that in the control house (NYAND 6) was converted into arcsin values. A generalized linear mixed model (GLMM) designed using the R package lme4 (http://www.R-project.org) was used to examine whether the interventions of nets explained the number of mosquitoes collected. Multiple comparison of the ratio was performed using the Tukey's HSD test using the package multcomp in R.

The dominant species collected were *An. arabiensis* and *An. funestus* s.s. A total of 40 female *An. gambiae* s.l. were collected in NYAND 8, 11, and 6 in the preliminary collection before intervention with nets; of these 40, *An. arabiensis* comprised 97.5%, with *An. gambiae* s.s. comprising the remaining 2.5%. In contrast, 1,088 female *An. funestus* s.l. were collected, of which *An. funestus* s.s. comprised 88.8%, while *An. rivulorum* comprised 11.2%. The number of mosquitoes decreased dramatically 1 day after intervention with ceiling nets in NYAND 8 and 11. The mosquito densities remained low for 9 months, until removal of the nets (February 11,

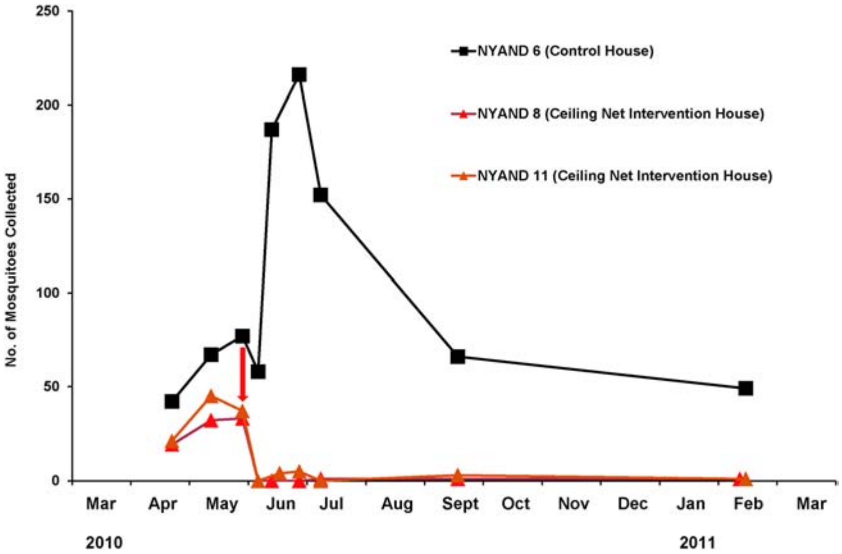

Fig. 2. Changes in the number of mosquitoes collected in the intervention houses (NYAND 8 and NYAND 11) and the control house (NYAND 6). The red arrow indicates the day of intervention (May 26, 2010).

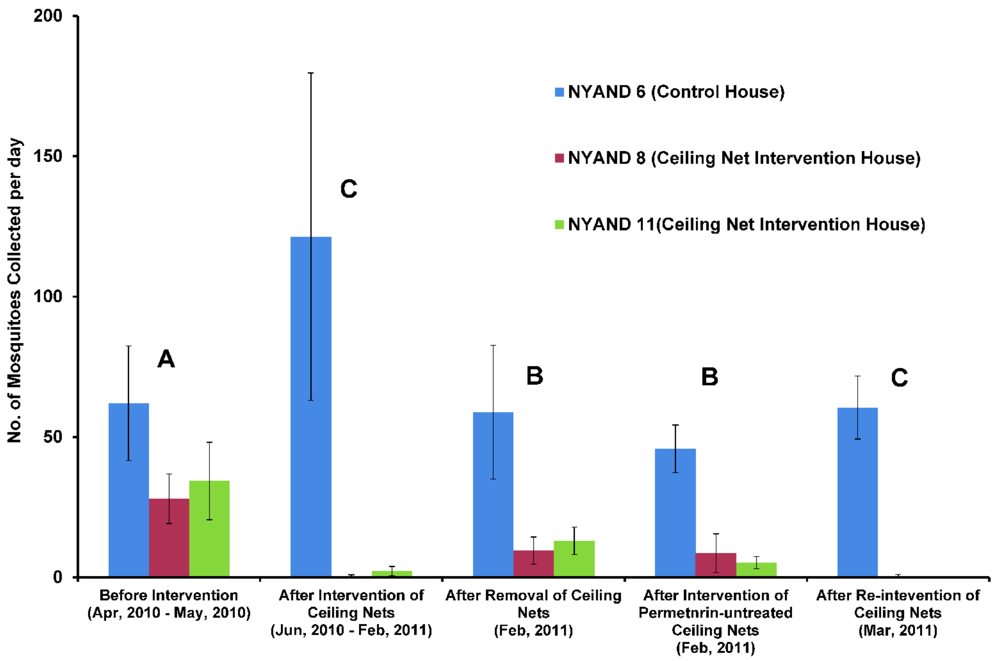

Fig. 3. Average number of mosquitoes collected prior to intervention with permethrin-impregnated ceiling nets, after intervention with the permethrin-impregnated ceiling nets, after removal of the permethrin-impregnated ceiling nets, after intervention with permethrin-untreated ceiling nets, and after re-intervention with new permethrin-impregnated ceiling nets. Bars indicate 95% confidence limits. The same letters indicate no significant difference when the square root of the ratio of the number of mosquitoes collected in the intervention houses versus that collected in the control house was converted into arcsin. The multiple comparison of the ratio was performed by Tukey's HSD test (P = 0.05).

2011). Throughout the study period, the mosquito density in the control house (NYAND 6) remained at a high level (Fig. 2). The total numbers of female *An. gambiae* s.l. collected in the intervention houses (NYAND 8 and 11) and the control house (NYAND 6) during this period were 18, 18, and 96, respectively, while the total numbers of female *An. funestus* s.l. were 151, 188, and 1,590, respectively. Differences in the ratio of the number of female mosquitoes collected in NYAND 8 and 11 and those collected in NYAND 6 was significant with relation to the intervention (ANOVA, df = 4, $\chi^2 = 65.8$, $P < 0.0001$). On multiple comparison testing, significant differences were observed between the following values: the ratio of mosquito density after intervention with permethrin-impregnated ceiling nets and that after reintervention with permethrin-impregnated ceiling nets; the ratio of mosquito density after intervention with the permethrin-untreated ceiling nets and that after removal of the nets; and the ratio of the data group prior to intervention with the permethrin-impregnated ceiling nets (Fig. 3).

Bed nets are effective against malaria vectors when the vector mosquitoes are endophagous and their feeding time corresponds to the time when people are asleep inside the bed net. The most important limitation for the effective use of bed nets is that the nets are effective only when people are sleeping inside it. Bed net usage is strongly affected by sleeping arrangements and by the availability of suitable locations for hanging the bed nets (5). The ease of hanging a bed net is particularly important in the case of children, who often sleep in the living room, where net hanging is difficult. Daily usage of bed nets may therefore be troublesome for residents, and is sometimes limited to persons sleeping in a bedroom (parents and babies). The remaining family members (including children >5 years old) often sleep in the living room with no bed net, resulting in a high incidence of *Plasmodium falciparum*-positive cases.

Eaves, the gaps between the top of the wall and the roof, are a very common structure in houses in Africa and are the most important entrance for malaria vectors (6). Changes in house design, such as screening or closing of eaves, can be effective in reducing human exposure to malaria vectors (7). However, restructuring of houses or physical closing of eaves is expensive. Moreover, these steps may cause deterioration in the living environment by blocking ventilation. Screening of the ceiling and closing of eaves with nets is likely to be well accepted and would offer the greatest benefit in moderating disease transmission (7,8). The use of nets with a coarse mesh size, such as Olyset® Net, will have minimal effect on the living environment by allowing maximum ventilation. Lindsay et al. (7) reported little difference in the protective effects of insecticide-treated and untreated screen nets. In contrast, the present study emphasizes the importance of using insecticide-impregnated nets as a chemical barrier (Fig. 3). Permethrin might act as a chemical barrier against vector mosquito entrance. Mosquitoes could enter through the windows or the entrance door. However, the number of such mosquito entries was low, because the windows and doors were normally closed during the night for security reasons and for keeping warm.

Olyset® Net was shown to be a promising candidate for new self-protection techniques against the entry of mosquitoes. The excito-repellency of the slow-release permethrin might reduce the human-vector contact and thus the blood-feeding success, resulting in biorational vector control with maximum reduction in mosquito biting and minimum risk of resistance.

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Conflict of interest H. Kawada and K. Ohashi have applied for a patent concerning the invention of the ceiling nets described in the present paper. The applicant is Sumitomo Chemical Co., Ltd. and our patent right has already been transferred to Sumitomo Chemical Co., Ltd. Therefore, H. Kawada and K. Ohashi have none to declare the conflict of interest.

REFERENCES

1. Kawada, H., Dida, G.O., Ohashi, K., et al. (2011): Multimodal pyrethroid resistance in malaria vectors, *Anopheles gambiae* s.s., *Anopheles arabiensis*, and *Anopheles funestus* s.s. in western Kenya. PLoS One, e22574.
2. Gillies, M.T. and Coetzee, M. (1987): A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region). South Afr. Inst. Medical Res., No. 55.
3. Scott, J.A., Brogdon, W.G. and Collins, F.H. (1993): Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg., 49, 520–529.
4. Koekemoer, L.L., Kamau, L., Hunt, R.H., et al. (2002): A cocktail polymerase chain reaction (PCR) assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. Am. J. Trop. Med. Hyg., 66, 804–811.
5. Iwashita, H., Dida, O.G. and Futami, K. et al. (2010): Sleeping arrangement and house structure affect bed net use in villages along Lake Victoria. Malar. J. 9, 176.
6. Njie, M., Dilger, E., Lindsay, S.W., et al. (2009): Importance of eaves to house entry by anopheline, but not culicine, mosquitoes. J. Med. Entomol., 46, 505–510.
7. Lindsay, S.W., Jawara, M., Paine, K., et al. (2003): Changes in house design reduce exposure to malaria mosquitoes. Trop. Med. Int. Health, 8, 512–517.
8. Kirby, M.J., Ameh, D., Bottomley, C., et al. (2009): Effect of two different house screening interventions on exposure to malaria vectors and on anaemia in children in The Gambia: a randomized controlled trial. Lancet, 374, 998–1009.