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Effect of insecticide-treated bed nets on house-entry by malaria mosquitoes: The flight response recorded in a semi-field study in Kenya

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ABSTRACT

Insecticide-treated nets are currently a major tool to reduce malaria transmission. Their level of repellency affects contact of the mosquito with the net, but may also influence the mosquito's entry into the house. The response of host-seeking malaria mosquitoes approaching the eave of an experimental house was recorded within a large screen house. We compared entry- and exit rates in relation to the presence in the house of different insecticide-treated bed nets (ITNs) with an untreated net. Mosquitoes were lured towards the house by dispensing a synthetic host-odour blend from within the net in the house. Complementary WHO bioassays revealed that the treated nets caused high knock-down- and mortality responses to the *Anopheles gambiae* sensu stricto strain tested.

The proportion of mosquitoes that came into view of the cameras and subsequently entered the house did not differ between treated nets and the untreated net. Treated nets did not affect proportions of mosquitoes that exited the house and departed from view around the eave. However, the percentage of house-leaving and re-entering mosquitoes when an insecticide- treated net was present, was lower than in the presence of an untreated net.

Our results indicated that there was no spatial repellent effect from pyrethroid-treated nets that influences house-entry at eave level. It is argued that the toxic effect of treated bed nets resulted in a reduced number of mosquitoes re-entering the house, which could thereby affect malaria transmission in neighbouring, unprotected houses.

1. Introduction

Declines in malaria cases over the last decade are mainly attributed to the use of insecticide- treated nets (ITNs), indoor residual spraying (IRS) and better case management (Bhatt et al., 2015). Increased resistance against insecticides and drugs is considered a major threat to the ability to sustain or further decrease malaria and therefore the continuous assessment of the efficacy of existing tools and the development of new products have been put high on the agenda (WHO, 2016). For example, lure-and kill technologies, repellents and genetic tools promise to provide tools complementary to the existing insecticide-based strategies (Benelli and Mehlhorn, 2016; Hammond et al., 2016; Homan et al., 2016; von Seidlein et al., 2017).

ITNs have been proven effective by reducing contact between humans and mosquitoes infected with the *Plasmodium* parasite (Lengeler, 2004; Lindsay et al., 1989). The effectiveness of ITNs is dependent on the susceptibility of the local mosquito population to the chemicals used (e.g. Malima et al., 2009). In addition, house design and the quality of housing are critical (Tusting et al., 2015). The design of houses together with the use of IRS and the presence of ITNs inside houses are other important factors influencing the vector capacity of mosquitoes (Grieco et al., 2000; Koffi et al., 2015; Massue et al., 2016). Continuous monitoring of intervention tools is necessary to measure whether the methods used are still effective or can be optimized. Behavioural adaptions of malaria vectors to control measures have been observed, but there are few studies done that provide supporting data (Gatton et al., 2013; Mathenge et al., 2001; Takken, 2002). Such adaptations could reinforce residual malaria transmission through mosquitoes biting outdoors. This is of concern for further improving malaria control or eradication (Bradley et al., 2016; Killeen, 2014; Killeen et al., 2016; Russell et al., 2013).

Early studies on the effectiveness of ITNs reported the repellent and

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killing effect of pyrethroids on these nets (Miller et al., 1991; Lengeler, 2004). Malima et al. (2009) reported that carbosulfan, a carbamate, prevents mosquitoes from entering a house. Tungu et al. (2010) found a deterrent effect of unwashed PermaNet 3.0, but not for PermaNet 2.0 or washed nets. Behavioural data supporting these effects are scarce (Miller and Gibson, 1994), and conclusions on repellent effects sometimes differ from studies based on indirect behavioural observations that rely on collections from experimental huts (Siegert et al., 2009). More recent studies implemented behavioural recording techniques, and in these studies the repellent effect of pyrethroids is less evident (Cooperband and Allan, 2009; Parker et al., 2015; Spitzen et al., 2014; Sutcliffe and Yin, 2014; Sutcliffe and Colborn, 2015). The change in interpretations of the repellency of nets may not only be due to more advanced recording techniques. The production process of bed nets has also evolved and repellent effects described earlier could possibly be explained by the emulsifiers used to impregnate the nets (Lindsay et al., 1991). Current nets often lack these components and the active ingredients are incorporated into the net (Spitzen et al., 2014). There is an urgent need for evidence-based studies on the repellent effect of pyrethroids on bed nets, given the rapid development of insecticide resistance in a large number of malaria vectors (Hemingway et al., 2016; Ranson and Lissenden, 2016; WHO, 2016).

Recent advances in tracking techniques make it possible to provide detailed information on flight behaviour around hosts, bed nets and human dwellings without the need for interception traps (Parker et al., 2015; Spitzen et al., 2014; Sutcliffe and Colborn, 2015; Angarita-Jaimes et al., 2016; Spitzen et al., 2016). We investigated if the presence of three different types of pyrethroid-treated nets affected house-entry behaviour of mosquitoes by recording their flight paths near the eave of an experimental house. We hypothesize that, based on short range studies as mentioned above, mosquitoes approaching the eave do not distinguish between the presence inside an experimental house, of an untreated net or a net with insecticides incorporated in the net fibre. The technique used provides information on mosquito behaviour prior to house entry, without the barrier of an interception trap, and can be further developed to support testing and evaluating new generation ITNs, push-pull systems (Menger et al., 2015) and house designs (Tusting et al., 2016).

2. Materials and methods

2.1. Experimental set-up

The study was carried out in West Kenya, Mbita Point township, at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (*icipe*-TOC). We video-recorded the approach and house-entry of female mosquitoes on one side of an experimental house that was constructed inside a large screen house. The experimental house had, like many African houses, a wide gap between the top of the wall and the roof. These so-called 'eaves' measured approximately 15 cm in height and were found at both sides of the house. Two windows and a door remained closed during the experiment. For technical details about the set-up we refer to Spitzen et al., 2016 and Supplementary online material, Fig. S1). Exit and entry rates were compared in relation to the presence of different bed nets inside the house: 1) an untreated net, 2) PermaNet 2.0, a long lasting insecticidal net (LLIN) with deltamethrin (dose of 1.4–1.8 g/kg ± 25%) incorporated into a coating on the filaments, 3) PermaNet 3.0, a LLIN with deltamethrin in the side panels (2.1–2.8 g/kg ± 25%) and deltamethrin (4.0 g/kg ± 25%) and piperonyl-butoxide (PBO) (25 g/kg ± 25%) in the top panel and 4) Olyset, a LLIN with permethrin (20 g/kg ± 15%) incorporated into the filament material. Bed nets were kindly provided by Vestergaard Frandsen SA (Lausanne, Switzerland) or obtained from the local market in Kenya. The samples were tested in random order and kept strictly separate in aluminium bags (Lifesystems®, United Kingdom) between experiments. Host odour was standardized using an attractive synthetic blend based on procedures described by Menger et al. (2014) and Smallegange et al. (2010) and consisted; Ammonia 2.5% (v/v), L-(+)-lactic acid 85% (w/w), Tetradecanoic acid 0.0025 g/l, 3-methyl-1-butanol 0.000001% (v/v), Butan-1-amine 0.001% (v/v) including CO_2 produced via sugar fermenting yeast which was dispensed from inside the bed net using an MM-X trap (Supplementary Fig. S1).

2.2. Experimental procedures

2.2.1. Mosquitoes

Mosquitoes were obtained from the *Anopheles gambiae* Giles sensu stricto (Mbita strain) insectaries. Three times a week, the colony was blood fed on a human arm for ten minutes. Rearing procedures are described in Spitzen et al., 2016.

2.2.2. WHO bio-efficacy tests

The bio-efficacy of the tested nets (top panels) was examined using 3 min WHO tube assays (WHO, 2006) and these were done with non-blood-fed, 5–7 day old *An. gambiae* s.s. Ten replicates per treatment were done with an average group size of five mosquitoes per tube. After exposure, the groups of mosquitoes were placed in a single 1 L cup and provided with 6% glucose solution *ad libitum*. Their knock-down status was measured 60 min post exposure and mortality was recorded after 24 h.

2.2.3. Behavioural assays

Five experimental nights per treatment were scheduled. For the analysis, we included experimental nights that were video-recorded for four hours only. Two-hundred non-blood-fed mosquitoes, 3–8 days old, were selected 8–10 h before release in the screen house and placed in 1L plastic cups, provided with a paper towel soaked in water. The MM-X trap dispensed odour from 20:00 h onwards, just before the moment mosquitoes were released and video recorded. At 24:00 h the cameras were stopped and the bed net was removed from over the trap, allowing the mosquitoes to enter the trap. Mosquitoes caught in the trap were collected and counted the next morning (Spitzen et al., 2016). During control experiments, there was no bed net present and the trap started running at 20:00 h so that mosquitoes could be trapped from the moment of mosquito release. This treatment was added to provide basic information on the number of mosquitoes that enter the house in the absence of a bed net. The odour-baited trap thus served as a proxy for a human being asleep in the house. Due to differences in trapping hours, we did not use data from the house without a bed net to compare the entry/exit rates with the other four treatments.

2.3. Data analyses

For mosquitoes that came into view of the cameras, observations of (re-)entry and exit behaviour were compared between treatments using a Generalized Linear Model (GLM) with binomial distribution and logit link function. The effects of sampling day, mean temperature and humidity over 4 h of filming were estimated and fitted as parameters when significant ($P < 0.05$). The fitted model per response category is presented in Supplementary material, Table S1. A pairwise comparison test of least significant difference (LSD) was used in case a significant effect of net type on entry and exit behaviour was observed. SPSS Statistics version 22 (IBM corp., USA) was used for the statistical analyses.

3. Results

3.1. WHO bio-efficacy tests

The effect of the various bed nets on the knock-down and mortality of *Anopheles gambiae* was recorded after exposure to netting in a WHO