Impact of visual features on capture of Aedes aegypti with host decoy traps (HDT)

Joseph Y. Tang
University of Notre Dame
Jackline Kosgei
KEMRI/CDC Research and Public Health Collaboration
Eric Ochomo
KEMRI/CDC Research and Public Health Collaboration
Bryson A. Ndenga
Kenya Medical Research Institute
Roya Ghiaseddin
University of Notre Dame
Neil F. Lobo
University of Notre Dame
Frances M. Hawkes
University of Greenwich
Joseph E O'Tousa (jotousa@nd.edu)
University of Notre Dame

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Abstract

Background

Aedes aegypti is the major mosquito vector of many burdensome human diseases. While behavioral research has focused primarily on understanding the importance of olfactory stimuli in its host-seeking abilities, *Ae. aegypti*'s vision has also been shown to contribute significantly to locating a human host. In this semi-field study conducted in Kisian, Kisumu County, Kenya, we explored the role of visual properties in *Aedes* host-seeking by testing four different visual characteristics presented in host decoy traps (HDT). This surveillance trap presents a combination of visual, thermal, and odor stimuli to attract bloodmeal-seeking mosquitoes. This was also the first test of HDT for sampling *Aedes* mosquitoes, having previously been shown to be effective for capture of other vector genera such as *Anopheles*, *Mansonia*, and *Culex* in field settings.

Results

Our results show that the HDT is an effective means of capturing *Ae. aegypti* mosquitoes, with a per trial capture rate of up to 69% across four visually distinct HDTs deployed simultaneously in a semi-field arena. Of these four, a solid black HDT (HDT B) captured more mosquitoes than HDTs with: black-white stripes (HDT S), black-white checkerboard patches (HDT P), and a solid white color (HDT W). Across 16 replicates wherein 200 mosquitoes were released per trial, HDT B caught more mosquitoes than HDTs S, P, and W by a factor of 1.9, 1.7, and 1.5 respectively. In all cases, mosquito capture was not evenly distributed on the HDT surface, with captures on the HDT's outer half, away from the odor delivery, exceeding captures on the inner half facing towards the odor delivery by a factor of 4.8, 3.7, 3.7, and 5.1 on HDTs B, S, P, and W respectively.

Conclusions:

Our results establish that in semi-field conditions, the HDT is effective for the capture of *Ae. aegypti* mosquitoes and provides a flexible platform to test experimental parameters pertinent to the host-seeking behavior of mosquito vector species. We show that *Ae. aegypti* makes use of dark, but not light, high contrast visual information while responding to both the olfactory and thermal stimuli associated with hosts. The results further show that the solid black surface of the original HDT design is more effective than the other surfaces (white or black/white patterns) for the capture of *Ae. aegypti*.

Introduction

The oppressive human diseases of yellow fever, dengue fever, chikungunya, and Zika are all vectored by the *Aedes aegypti* mosquito, creating a heavy disease burden in *Ae. aegypti*-endemic regions [1, 2]. Control of *Ae. aegypti* and other vector species include insecticide-treated nets (ITN), indoor-residual spraying (IRS), and larvicide techniques. Interventions such as these knock down local mosquito populations, thereby curbing the transmission of a number of mosquito-borne diseases [3, 4, 5, 6].

Aedes aegypti's geographical distribution necessarily informs the application of vector control. *Aedes* species propagate exclusively in tropical and subtropical climates and tend to be found in and around densely populated cities [7]. Global climate change and exploding rates of transcontinental human travel and interconnectedness could benefit *Ae. aegypti* and its propagation worldwide, necessitating diligent vector surveillance and refinement of distribution maps [8, 9].

Effective surveillance traps apply principles of *Ae. aegypti*'s navigation, oviposition, and host-seeking mechanisms. With respect to host-seeking, laboratory studies have described the effects of a range of sensory stimuli on the host-seeking behavior of *Ae. aegypti*. The major contributor to long-range host detection is the olfactory system, with CO_2 plumes originating from hosts dictating the flight direction of *Ae. aegypti* females from a distance [10]. Once the mosquitoes have closed the distance to the source of stimuli, however, visual and thermal cues begin to play a significant role, with dark, warm, and visually contrasting objects being most attractive to the *Ae. aegypti* females [11, 12, 13]. Moreover, studies have shown that these cues act in concert. For instance, CO_2 detection is a prerequisite for mosquitoes' responses to host-derived odors such as lactic acid [14], whereas the detection of dark visual cues is a prerequisite for thermotaxis [15].

Surveillance traps make use of multiple sensory cues relevant to the behavior of target mosquitoes that is induced by particular physiological statuses. One such trap, the host decoy trap (HDT), primarily targets female mosquitoes seeking human targets for bloodmeals. The HDT, in its original design, is a black cylindrical structure presenting a warm surface and the odors of a live host to mosquitoes. The surface of the HDT is adhesive, allowing mosquitoes to be captured if they land on the HDT. The effectiveness of the HDT has been established for the capture of the malaria mosquito *Anopheles gambiae* s.l., as well as *Mansonia* and *Culex* species [16]. Iyaloo, et al. [17] demonstrated *Aedes albopictus* are captured by a different multisensory cylindrical trap, the BG-Sentinel. In the latter study, traps composed of black cylinders and black lids were shown to capture more mosquitoes than traps containing white cylinders/lids and traps presenting contrasting black and white colors on the cylinder and lids.

In this study, we evaluated both the effectiveness of the HDT as a means of capturing *Ae. aegypti* and the effect of different visual stimuli on the HDT surface in attracting *Ae. aegypti*. To determine this, we compared the capture rates of four HDTs with varying visual appearances. These alterations included a solid black surface, a black-white striped surface, a black-white checkerboard surface, and a solid white surface. In doing so, this study demonstrates the importance of visual cues in the host-seeking behavior of *Ae. aegypti*.

Methods

Study Site. This study was conducted at the Centre for Global Health Research (CGHR) of the Kenya Medical Research Institute (KEMRI) in Kisian, Kisumu County, Kenya through the support of the Eck Institute of Global Health at the University of Notre Dame. This study was carried out by conducting 16

daily trials over the course of four weeks during the rainy season of May-June 2019, in semi-field screen house arenas at the Kisian Campus.

Setup of Host Decoy Traps. All HDTs used in this study were manufactured by BioGents AG and used primarily as described in Abong'o et al. [16] with the following minor modifications. The original HDT design employed a solid black surface color. This study introduced three additional visual modifications in HDTs to evaluate the impact of visual stimuli on *Ae. aegypti* host-seeking behavior. These visual patterns were printed onto vinyl fabric that was wrapped around each of the drums (Fig. 1A). In addition to the original solid black surface ("HDT B"), the study used a solid white surface ("HDT W"), a striped pattern consisting of evenly alternating black and white stripes each of 6 cm width ("HDT S"), and a checkerboard pattern consisting of 6 cm x 6 cm alternating black and white square patches ("HDT P"). The sides of each of these drums was wrapped with a transparent adhesive plastic sheet that provided the same tactile surface for the capture landed mosquitoes (FICS Film, Barrettine Environmental Health, Bristol, UK).

HDTs B and W were presented as single targets for host-seeking mosquitoes that contrasted visually with the surrounding grass, soil, and rocks. These visual designs adapted the methods of numerous laboratory studies such as Muir et al. [11] and van Breugel et al. [12] that presented visually contrasting landing targets to *A. aegypti* females released in an enclosed chamber. On HDTs S and P, the black stripes and patches were visually juxtaposed with the white stripes and patches on HDTs S and P respectively. These stripes and patches were hypothesized to present to mosquitoes as discrete, individual targets, thus introducing an additional layer of potential visual contrast conducive to host-seeking navigation. Ultimately, all four designs presented unique visual elements whose impact on host-seeking could be evaluated via simultaneous deployment in a semi-field arena.

Semi-field arena. The arena used in this study was constructed using untreated Optinet netting material permeable to air and draped over a 20 m long x 8 m wide x 3 m high galvanized steel frame. Figure 1B depicts the arrangement of PVC pipes and connectors directing airflow to the four HDTs in the arena. To ensure that the sensory cues of each HDT were as spatially distinct as possible, the traps were symmetrically positioned such that the distance between each pair of HDTs was at least 4 m, and that the distance between the HDTs and the arena walls was at least 2 m. Mosquitoes were released from the center of the arena, equidistant from each of the HDTs. Due to the terrain over which the semi-field arena was constructed, the four corners of the arena possessed different vegetation cover and soil types. Moreover, the direction of the sunset cast varying levels of light intensity upon each trap position. To minimize the impact of these and other variables on observed capture rates, we employed a Latin square design in which all four HDTs were systematically rotated to different positions for each trial such that each HDT was placed at each position the same number of times.

Natural host odors were transferred to the HDTs from a ventilated canvas tent (Pop Up Tent, Sports God) 2 m outside the arena (Fig. 1C). The same human volunteer sat in the tent for the duration of each of the 16 trials conducted. A high-speed 12 V DC fan (Delta) powered by a 12 V, DC 7 ah rechargeable lead acid

batteries (ExpertPower) was attached via duct tape to a PVC pipe (10 cm in diameter). This end of the pipe was placed inside the tent. The PVC pipe was directed into the arena and sealed at its entry point with cement. Each of the four pipe exit points was sealed with untreated mosquito netting. An HDT unit was positioned 10 cm away from each opening to allow odors and CO_2 from the tent to ventilate over each HDT. Figure 1B depicts the arrangement of PVC pipes and connectors directing airflow to the four HDTs in the arena. The 12 V fan provided a wind speed of approximately 1.26 m/s at each exit point, delivering approximately 600 l/min of human odors to each HDT. These parameters were set to approximate those used by Abong'o et al. [16].

Mosquito Rearing. *Aedes aegypti* mosquitoes present at sites around Kisian, Kisumu County were captured using ovitraps and used to establish a breeding colony within the rearing facility. Larvae were fed on Super Brewers Yeast Tablets (Pharmadass Ltd., Healthaid House, Marlborough Hill, Harrow, Middlesex, HA1 1UD, United Kingdom) ground into powder and deposited in water as required. The resulting F1 male and females adults were fed a 10% solution of sugar. These adults were aged for five days to provide an opportunity for females to mate. The sugar solution was removed 6 hours prior to the collection of females.

Trial Operation and Data Collection. KEMRI insectary staff maintained production of adult mosquito rates sufficient to provide 200 female mosquitoes per trial, the same number used in previous semi-field studies [18, 19]. 100 females were aspirated into each of two paper cups (a total of 200) before each experimental trial and transported to the arena. At the start of each trial, the temperature of the HDT surface, the wind speeds at pipe exit points, the light intensity in the arena, and the arena's temperature and humidity were measured and recorded. The mosquitoes were then released from the center of the screen house (Fig. 1C). To align with the day-biting behavior of *Ae. aegypti* [20], each trial began between 12:00 h and 13:00 h, and ended at 18:00 h, lasting a total of 5–6 hours. The same set of environmental parameters were re-measured at the conclusion of each trial. Mosquitoes not captured on the HDTs during the trials were collected from the arena using a battery-powered aspirator (Prokopack Model 1419, The John W. Hock Company).

At the end of each trial, the adhesive sheet on the surface of each HDT was wrapped with plastic food wrap, sandwiching the captured mosquitoes between the adhesive sheet and the wrap. The adhesive sheets were labeled using permanent marker to denote the HDT type, the position of the black and white regions on the striped and patched HDTs, and the location of the pipe outlet.

Data Analysis. Mosquitoes captured on the HDT adhesive sheets and recaptured from the arena were killed in a -20^{o}C freezer overnight. The number of landed mosquitoes per HDT type were then counted. For all adhesive sheets, the count of mosquitoes on each HDT's inner half (facing the pipe outlet) and outer half (facing away from the pipe outlet) was determined. For HDTs S and P, the count of mosquitoes captured on their black and white regions was also determined. Finally, the mosquitoes recaptured in the plastic cup were sorted and counted.

The data were analyzed using SPSS Statistics software, version 26.0.0. Confounding variables were assessed via ANOVA and Kruskal-Wallis analyses. Linear regression assessed the effect of ambient environmental variables, odor wind speeds, and HDT surface temperatures on the catch of mosquitoes on HDTs. To address the primary research question (i.e., which HDT type had the highest capture rate), χ^2 goodness of fit analysis determined if differences in capture rates between the HDT types were statistically significant. Then, pairwise post hoc comparisons between the traps were conducted among pairs of HDT types to determine the source of the capture rate incongruence, and thus statistical differences between HDT types. Similar pairwise comparisons were made to determine whether there were landing preferences on the black and white regions of the striped and patched HDTs (S and P). An independent samples comparison was used to assess mosquito preference for landing on the outer half of the HDT surface.

Results

HDT B is more effective at capturing **Ae. aegypti** females than HDTs S, P, and W. Table 1 summarizes the capture data from the 16 replicates of this study. Of the total of 3200 released mosquitoes, 1096 (34%) were captured by the HDTs.

Table 1

Compilation and χ^2 GOF statistical analysis* of mosquito capture data in the 16 trials of the study. *11 (bolded) out of 16 demonstrate significant difference in capture counts between the four HDTs. All tests were performed with three degrees of freedom, with a null hypothesis of expected counts being equal for all four HDT types.

HDT type	Black (B)	Striped (S)	Patched (P)	White (W)	Total captured on HDTs	χ^2	p-value
Trial 1	14	2	2	4	22	18.00	**< 0.001**
Trial 2	40	17	16	17	90	18.18	**< 0.001**
Trial 3	31	10	12	14	67	16.64	**0.001**
Trial 4	16	10	15	10	51	2.41	0.491
Trial 5	9	6	7	16	38	6.42	0.093
Trial 6	11	18	17	22	68	2.11	0.302
Trial 7	37	10	22	16	87	11.67	**< 0.001**
Trial 8	36	30	12	17	95	6.32	**0.001**
Trial 9	18	14	9	17	57	3.38	0.337
Trial 10	20	11	15	19	65	3.12	0.373
Trial 11	24	4	15	10	52	16.21	**0.001**
Trial 12	32	18	16	14	80	10.00	**0.019**
Trial 13	11	10	5	23	49	14.27	**0.003**
Trial 14	49	19	36	34	138	13.13	**0.004**
Trial 15	23	9	25	15	72	9.11	**0.028**
Trial 16	26	17	13	9	65	9.77	**0.021**
Mean	24.81	12.81	14.81	16.06	**68.5**	78.64	**< 0.001**
Total	**397**	**205**	**237**	**257**	**1096**		

χ^2 goodness of fit analysis of the capture counts of the four HDT types revealed that there is a significant overall difference between the HDT types' mean capture rates ($\chi^2 = 77.82, p = 9.014 \times 10^{-17}$), and 11 out of 16 χ^2 GOF tests on individual trials revealed significant differences in the HDTs' capture rates (Table 1).

HDT B captured the most mosquitoes, with a mean of 24.81 per trial (95% CI: 18.54–31.09) compared to

16.49), the 14.81 by HDT P (95% CI: 10.49–19.13), and the

16.06 by HDT W (95% CI: 12.44–19.69) (Fig. 2A). Paired analyses of differences in captures amongst HDT types in each trial revealed that HDT B's capture counts significantly exceeded those of HDTs S, P, and W by a mean difference of 12 (95% CI: 6.6–17.4), 10 (95% CI: 5.3–14.7), and 8.75 (95% CI: 2.7–14.8) respectively (Fig. 2B). These analyses revealed no significant differences between the mean capture counts per trial between HDTs S, P, and W (Fig. 2B).

Aedes aegypti have no preference for landing on black stripes and patches on HDTs S and P respectively. Though the 6 cm black and white stripes and patches were intended to provide closer range visual contrast of HDTs S and P, respectively, the results showed no difference in capture effectiveness for these surface designs. In addition to not significantly outperforming HDT W in terms of mean catch (Fig. 2B), the black portions of these HDTs did not capture significantly more mosquitoes than the white portions, with a mean difference in captures between black and white portions of 0.214 (95% CI: -1.91–2.34) on HDT S and 1.467 (95% CI: -0.72–3.65) on HDT P. Moreover, χ^2 goodness of fit analysis of HDT P's capture counts on the black patches (84 captured) and white patches (85 captured) indicated no significant difference in captures ($\chi^2 = 0.0059, p = 0.9387$). The same analysis performed on the black stripes (133 captured) and white stripes (107 captured) of HDT S also indicated no significant difference in captures ($\chi^2 = 2.8167, p = 0.0933$).

Aedes aegypti prefer to land on the downwind half of the HDT. During post-trial analysis, the location of each captured mosquito on the HDT surface was categorized by whether they landed on the inner-upwind half, facing the odor delivery pipe, or outer-downwind half of the HDT that faced away from the odor delivery pipe (Fig. 3A). Trials 1 and 3 were excluded from this analysis due to heavy rain that washed away the labels on the sticky sheets. Amongst the remaining 14 trials, the distribution of *Ae. aegypti* landings on the HDTs' surfaces indicated significant skew towards the outer-downwind half compared to the inner-upwind half for each HDT type (Fig. 3B). The inner halves of HDTs B, S, P, and W caught an average of 4.36, 3.36, 2.94, and 2.79 mosquitoes respectively, whereas the outer halves of B, S, P, and W caught 20.79, 12.5, 10.86, and 14.29 respectively. Based on a comparison of means analysis pairing landing rates on the two halves of the HDTs for each trial, captures on the outer halves exceeded that of the inner halves by a mean of 11.25 (95% CI: 9.05–13.45, $p < 0.0001 < / > cript >$). Captures on outer halves also significantly exceeded captures on inner halves when a similar analysis was performed individually for each HDT type.

Ae. aegypti have no preference for landing on black stripes and patches on HDTs S and P respectively. Though the 6 cm black and white stripes and patches were intended to refine the visual contrast of HDTs S and P respectively, the results showed no difference in capture effectiveness for these surface designs. In addition to not significantly outperforming HDT W (Fig. 2B), the black portions of these HDTs did not capture significantly more mosquitoes than the white portions, with a mean difference in captures between black and white portions of 0.214 (95% CI: -1.91–2.34) on HDT S and 1.467 (95% CI: -0.72–3.65) on HDT P. Moreover, χ^2 goodness of fit analysis of HDT P's capture counts on the black patches (84 captured) and white patches (85 captured) indicated no significant difference in captures (

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Ae. aegypti prefers to land on the outer half of the HDT. During post-trial analysis, the location of each captured mosquito was categorized by whether they landed on the inner, facing the odor delivery, or outer half of the HDT that faced away from the odor delivery site (Fig. 3A). Trials 1 and 3 were excluded from this analysis due to heavy rain that washed away the labels on the sticky sheets. Amongst the remaining 14 trials, the distribution of *Ae. aegypti* landings on the HDTs' surfaces indicated significant skew towards the outer half compared to the inner half for each HDT type (Fig. 3B). The outer halves of HDTs B, S, P, and W caught an average of 4.36, 3.36, 2.94, and 2.79 mosquitoes respectively, whereas the inner halves of B, S, P, and W caught 20.79, 12.5, 10.86, and 14.29 respectively. Based on a comparison of means analysis pairing landing rates on the two halves of the HDTs for each trial, captures on the outer halves exceeded that of the inner halves by a mean of 11.25 (95% CI: 9.05–13.45, $p < 0.0001 < / > cript >$). Captures on outer halves also significantly exceeded captures on inner halves when a similar analysis was performed individually for each HDT type.

Capture of **Ae. aegypti** by HDTs was not significantly affected by environmental variables.

To determine if the position of the HDT within the screen house influenced capture rates, the data for all HDT types were stratified by location (Table 2). When no distinction was made with respect to the HDT type at each position, the differences in captures at each position was not significant ($F = 2.185, p = 0.099$). For three of the four HDT types, differences in capture rate based on position in the screen house showed no statistical significance. The fourth, HDT S, did show a significant difference in capture between positions 2 and 3, the positions closest to the entrance of the arena. However, given the lack of statistical significance for all other tests involving positions 2 and 3, this finding was not expected to alter conclusions regarding the mosquito's attraction to HDT trap type.

Table 2
Mosquito capture rates analyzed* with respect to position of the HDTs in the screen house.

Trap Position	Black (B)	Striped (S)	Patched (P)	White (W)	Total
1	52	65	56	55	228
2	120	33	29	50	232
3	115	75	84	60	334
4	110	32	74	92	308
Total	397	205	237	257	1096
P-value	0.112	0.031	0.149	0.107	

*Two different statistical tests were used to determine if position was responsible for differences in capture rates. For HDT S and HDT W, because variances were equal at the four positions, ANOVA analysis was used, while for HDT B and HDT P, where the variances were unequal, Kruskal-Wallis was employed. The statistical analysis showed that only the HDT S data showed significant differences in capture due to trap position. Post-hoc tests determined that this difference was due to the HDT S data collected from positions 2 and 3.

We found no evidence that ambient light intensity, temperature, and humidity conditions influenced the total mosquito capture during the 16 trials. Linear regression analysis indicates no significant correlation between a trial's total HDT capture and: (1) the mean light intensity ($p = 0.697$), (2) the trial's mean ambient temperature ($p = 0.669$), and (3) the mean relative humidity ($p = 0.521$) respectively. Similarly, a lack of significant correlation was observed with regression analyses of the values of each of these environmental variables at the beginning and end of each trial. Moreover, for each HDT type, no correlation was observed between capture counts and the mean surface temperatures ($p = 0.157$) and wind speed ($p = 0.853$) at the pipe exit points. Based on this analysis, the potential confounding variables explored did not influence the experimental results.

Discussion

The goal of this study was to characterize the impact of altering visual characteristics on the landing frequency of *Ae. aegypti* females. The HDT paradigm allows for a host mimic target to present variations in the parameters of host-associated stimuli. The original HDT design outlined in Hawkes et al. [21] used a solid black surface color. This choice is supported by laboratory studies that point to *Ae. aegypti*'s visual preferences. Muir et al. [11] demonstrated *Ae. aegypti*'s preference for landing on dark targets. More recent studies uncovered details on the interactions between different types of stimuli. Van Breugel et al. [12] demonstrated that CO_2 detection activates a strong attraction for dark targets on a light background. Liu & Vosshall [15] also showed *Ae. aegypti's* preference for landing on a single dark spot on light background in the presence of CO_2. These studies support the attractiveness of black-on-white surface designs integrating with the HDT's non-visual cues to contribute to the trap's overall attractiveness to *Ae. aegypti*. Little research has been conducted on whether light targets on dark backgrounds (which theoretically generate the same degree of contrast) result in the same behavior patterns as dark targets on light backgrounds. Between night and day, the level of light intensity differs by around 11 orders of magnitude. However, irrespective of this, visual contrast remains constant [22]. Our semi-field results suggest that while *Ae. aegypti* are attracted to and will land on light targets, dark targets result in a greater landing rate; this may be because the light reflected from the background mosaic of soil, vegetation, and other naturally occurring material is lighter overall, thus providing stronger contrast relative to a dark target than a light one.

Mixed surface designs (HDTs S and P) did not improve capture rates. Each HDT presented a target with an experimental visual property along with a set of constant nonvisual cues (detectable heat signature,

 egypti [12, 15]. The black stripes and patches on the surface

designs of HDTs S and P in this study could in principle represent individual, visually contrasting dark targets that may affect the visual attractiveness of HDTs S and P. However, this study indicates such patterns reduce the capture rate of *Ae. aegypti* compared to that of both plain trap coverings, HDTs B and W, suggesting that the solid white surface of HDT W provides greater attractive visual stimuli than the patterned traps. A possible explanation for this is that the black and white portions of HDTs S and P, which each occupy 50% of the HDT surface area, do not resolve in *Ae. aegypti* visual fields as distinct targets as well as a single black spot on a mostly white field does. Another possibility is that the spectrum of visual cues on the ground surrounding the traps, such as the different colors and textures of grass, soil, and rocks, are varied enough to mask the mixed visual cues of black and white stripes and patches.

The stripes and patches present on the HDTs were 6 cm in width. *Ae. aegypti* require a minimum optical angle of between 4º and 8º to perceive distinct objects [23]. At a distance of 1 m, the angle of perception of a 6 cm wide stripe would be 3.4º and thus may not be well resolved by the mosquito. Increasing the sizes of the stripes and patches would increase the distance at which *Ae. aegypti* would visualize the HDTs S and P patterns, while providing the most extreme visual contrast that does not rely on variable natural backgrounds. Comparisons between the capture rates of the black stripes with those of the white stripes on HDT S, notably, yielded a low p-value of 0.093. Repeating the study with wider black stripes could reveal a significant preference for landing on black stripes over white stripes. Ultimately, studies altering the dimensions of stripes and patches, or any similar mixed patterns, are needed to understand these properties and the effects they may have on close-range host orientation and landing by *Ae. aegypti*.

Mosquito capture was not evenly distributed on the HDT. This study found a greater concentration of mosquitoes captured on the HDTs outer half, facing away from the odor delivery pipe. This suggests that the three-dimensional surface of the HDT was not a uniform surface for capture. Clearly there was a nonuniform distribution of odors across the surface of the HDTs since the odors were vented from a single exit point directed toward the HDT. Because odors were deposited at 10 cm away from the base of the HDT, the surface of the HDT facing the exit point received the odor immediately as it was dispensed from the pipe. An important consideration is that the odor was vented from the pipe at a speed of approximately 1.26 ± 0.18 m^{-s}, which may have created sufficient directional air flow to induce upwind flight in *Ae. aegypti*. Geier et al. [24] showed that *A. aegypti* increase their upwind flight activity upon exposure to plumes of both CO_2 and host odors both independently and in combination. Because odor delivery through the pipe creates a wind current, mosquitoes would have first encountered the HDT surface facing away from the pipe due to this tendency to fly upwind while tracking odor plumes. Additionally, the wind velocity resulting from airflow out of the pipe may have limited the ability of the mosquito to reach the surface facing the pipe. Further investigation of the dynamics of airflow onto and around the HDT's cylindrical drum may illuminate these mechanisms as well as alternative methods of odor delivery to improve overall capture rates.

The HDT is a useful tool for both mosquito surveillance and behavioral studies. By presenting multiple host-associated cues, the HDT primarily samples female *Ae. aegypti* that engaged in host-seeking behavior. The compact materials of the HDT allows it to be transported to the vicinities of remote human communities with difficult terrains and climates where surveillance of mosquito prevalence is most lacking, and little supervision is required once set up is complete. Data provided by the HDT can effectively serve as important indicators of regional species prevalence and host preferences and will contribute to further refinements of probability models of *Ae. aegypti* and other species' geographical distributions [7]. The importance of such models was demonstrated in a study that aligned the known distributions of several *Aedes* species throughout Thailand and the distributions of human incidences of dengue, chikungunya, and Zika infections [25]. Continuously updating information on vector prevalence is crucial to combatting short-term disease outbreaks and to observing long-term trends in geographical shifts in vector species distribution.

This study further documents the value of the HDT as an experimental tool in field settings. In terms of capture effectiveness, cattle-baited HDTs were demonstrated to capture significantly more *Anopheles* mosquitoes in the wild than HLCs, though further studies must take place to replicate this finding for *A. aegypti* and human hosts. More importantly, in terms of design, HDTs have the intrinsic advantage over HLCs in that they present negligible risk of mosquito bites to researchers. The properties of the HDTs can also be modified to present different stimuli that may arise from hosts, manipulating experimental variables related to thermal, olfactory and visual properties of hosts so that the effect of these properties on host-seeking behavior of field populations can be studied. We have demonstrated the HDT's ability to safely and effectively capture *Ae. aegypti* females, extending the work of Abong'o et al. [16] showing effective capture of *Anopheles* and *Culex* species in field settings.

The presence of attractive visual cues on traps can contribute to mosquito control. Many current vector control methods such as insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS), though effective at hampering human contact with mosquitoes, do not eliminate mosquito populations [26]. The sustained use of insecticides consistently leads to mosquito resistance [27, 28, 29]. Mosquito surveillance traps could be adapted as alternative vector control tool. The BG-Sentinel, for example, is a multisensory surveillance trap that was shown to be effective at controlling populations of *Ae. mediovittatus*, a mosquito vector native to the Caribbean [27]. Moreover, with regards to its visual design, Iyaloo et al. [17] demonstrated *Ae. albopictus'* preference for landing on a solid black BGS over other black-white surface patterns. Similarly, optimizing the HDT's visual design could be an avenue of introducing it as a vector control tool. This study's findings constitute a step towards not only optimizing the visual design of the HDT, but also elucidating general patterns of vision-based behavior that can inform the design of novel vector control methods. Ultimately, any vector control methods that take advantage of sensory cues instead of or in addition to the traditional chemical cues would diversify the set of stimuli that place selective pressure on mosquito evolution and mitigate the development of resistance to vector control [30].

Conclusions

The study demonstrates that the HDT can sample *Ae. aegypti* mosquitoes in semi-field settings. Furthermore, amongst the four visual designs tested, the original black surface of the HDT was the most effective design for capturing *Ae. aegypti* females seeking bloodmeals. The disparities in capture rates amongst the four HDTs demonstrates that the HDT's visual design is an important determinant of its ability to capture mosquitoes. This study provides evidence that, all other variables held equal, field populations of host-seeking *Ae. aegypti* females do discriminate visually and are attracted to dark, visually contrasting targets in the wild, compared to light contrasting targets or those with light/dark stripes or patches. The nonuniform distribution of odors and air flow on the HDT surface is also a factor in mosquito capture. Overall, the flexibility of the HDT's design means that it is a powerful tool for experimental purposes and offers a system for surveilling and potentially controlling the transmission of disease by *Ae. aegypti* and other vectors, as well as field-testing behavior of wild vector populations. Further studies on the HDT involving multiple mosquito species, host odors, visual designs, and deployment methods should be explored.

Abbreviations

KEMRI
Kenya Medical Research Institute
CGHR
Centre for Global Health Research
PVC
polyvinyl chloride
HDT
host decoy trap
HDT B
solid black HDT
HDT S
black/white striped HDT
HDT P
black/white patched HDT
HDT W
solid white HDT
HLC
human landing catch
GOF
chi-square goodness of fit statistical test
RNA
ribonucleic acid

BGS
Biogents-Sentinel trap
ITN
insecticide-treated bed nets
IRS
indoor residual spraying

Declarations

Ethics Approval and Consent to Participate:

The study was approved by the Scientific and Ethics Review Unit at the Kenya Medical Research Institute (KEMRI/SERU), case number 3877. Individuals assisting with the trials gave written consent regarding the risks of mosquito exposure.

Consent for Publication

(as stated in acknowledgments): We thank the KEMRI Director General for granting permission for this manuscript's publication.

Availability of data and material:

All materials and data sets associated with this manuscript will be made available by contacting the corresponding author.

Competing interests:

The authors have no competing interests regarding this manuscript.

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Author Contributions:

JYT, NFL, and JEO conceived the study. JYT, JK, EO, BAN, NFL, FMH, and JEO designed experimental protocols. JYT performed the experiments. JYT, RG, FMH, and JEO analyzed the data. JYT, FMH and JEO wrote the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgments

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Figures

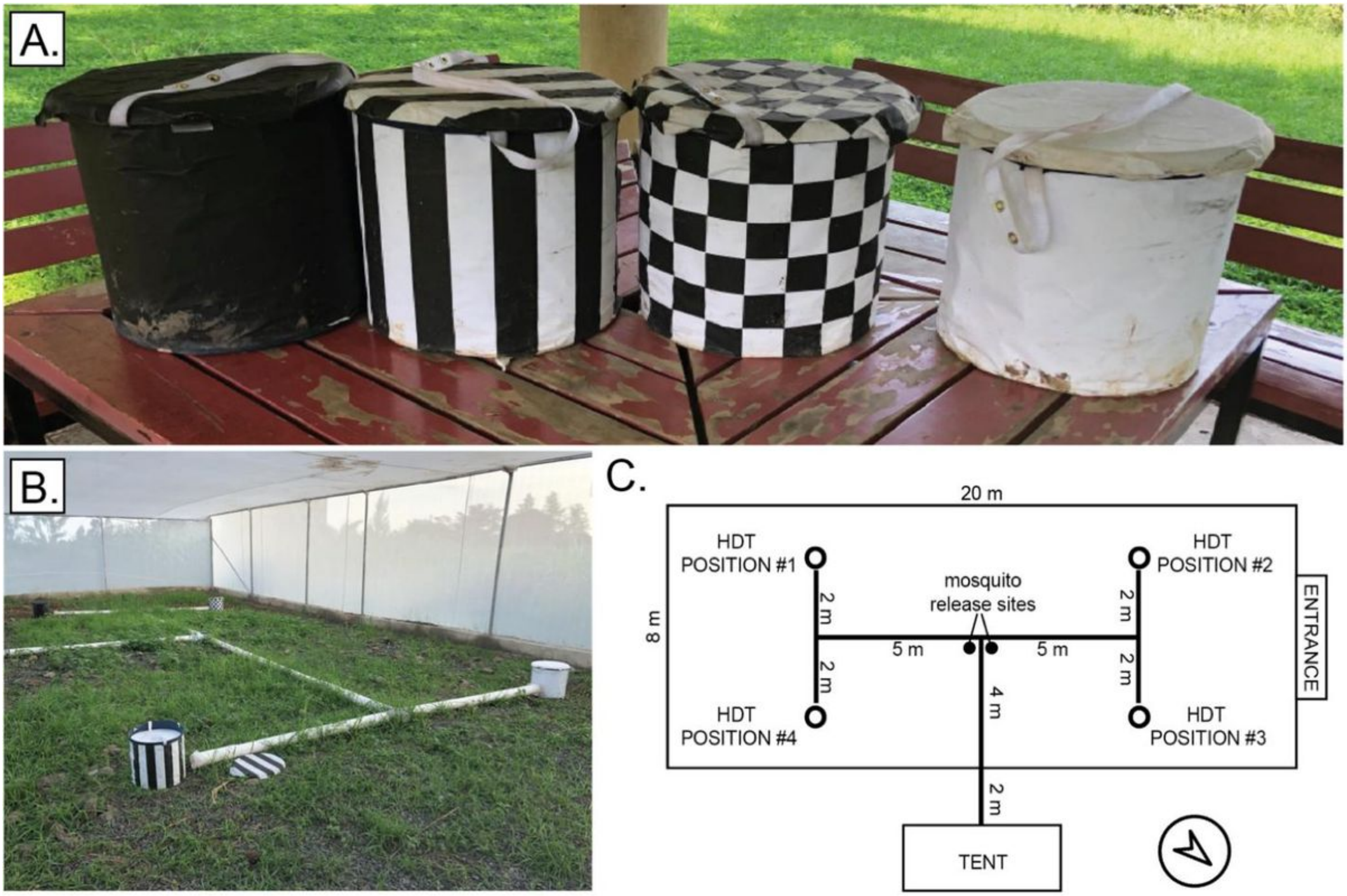

Figure 1

Visual features on the HDTs and layout of the experimental facility. (A) Test traps showing the visual feature designs for (left to right) the black, striped, patched and white HDTs. (B) Example arena set up of the four HDTs and the pipework delivering the human odors from the tent to each of the HDTs. (C) Diagram showing the dimensions of the screen house and other elements of the experimental set up. A North arrow indicates the approximate cardinal orientation of the arena. The pipework delivers odors from a human subject residing in the tent to symmetrically placed HDTs within the screen house.

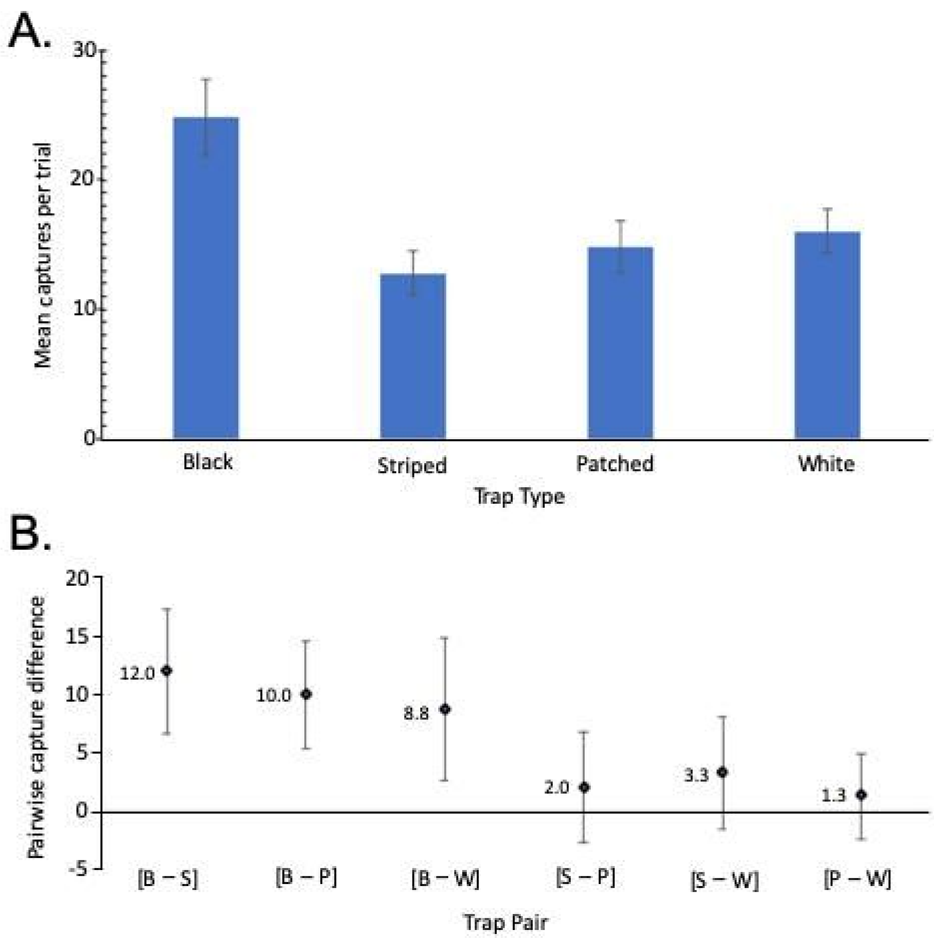

Figure 2

Figure 2

Comparisons between mean capture rates of HDTs B, S, P, and W. (A) Mean capture rates of each HDT type. Error bars indicate standard deviation. (B) Pairwise differences in mean capture by trial between each pair of HDTs depicting the 95% confidence bands. Bands that do not include 0 indicate with 95% confidence that there is a non-zero difference in captures between the pair of HDT types, i.e. a statistically significant difference. By this metric, HDT B captured significantly more mosquitoes than the other three DHT types, while the differences between HDTs S, P, and W are not significant.

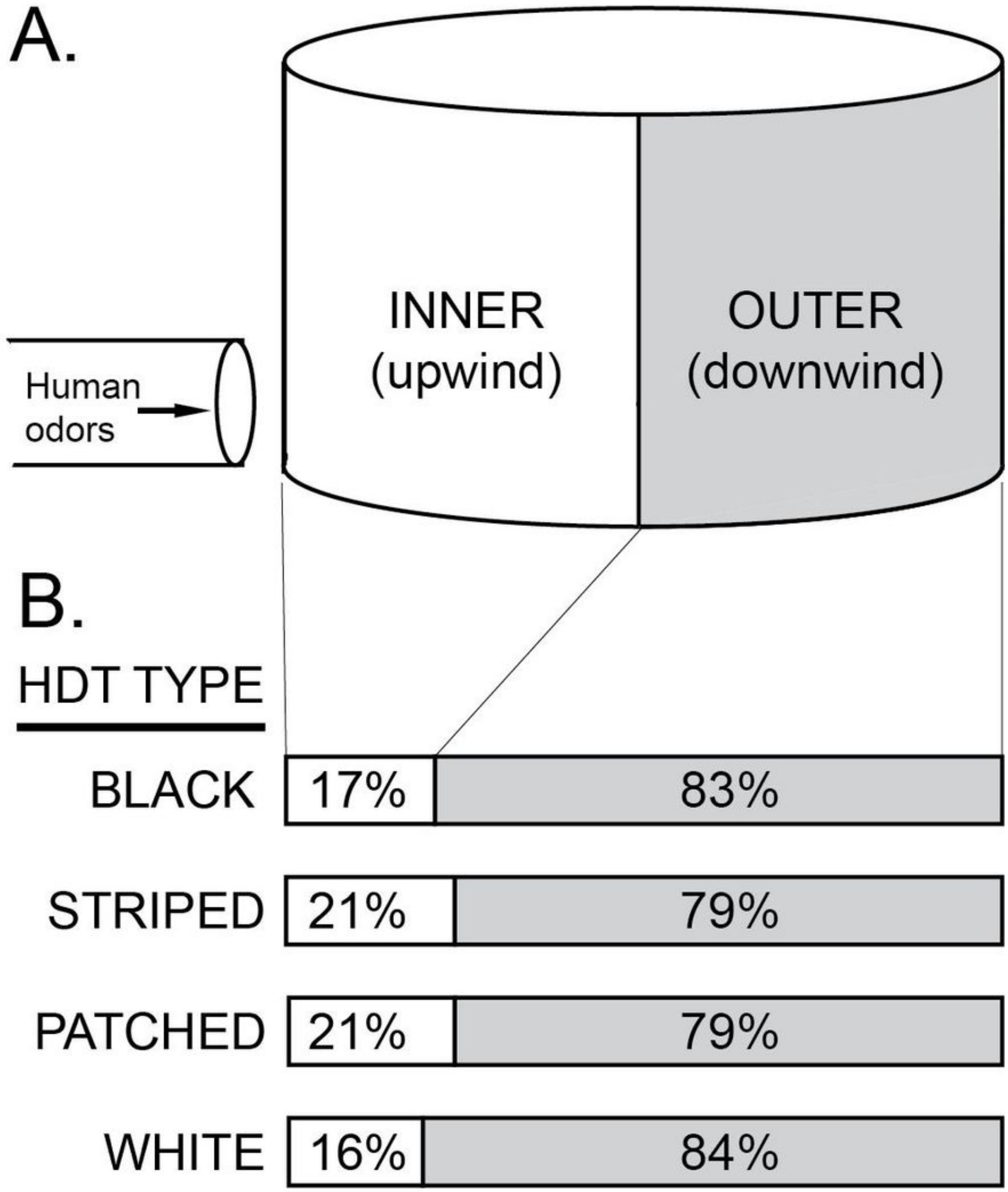

Figure 3.

Figure 3

Comparisons of mosquito capture on outer and inner halves of the HDTs. (A) An illustration designating the inner and outer halves of the HDTs. The terms upwind and downwind denote the direction of the wind currents generated by odor delivery through the pipe. (B) The percent capture determined for the inner and outer surfaces of each HDT type.

Supplementary Files

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