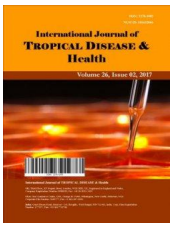

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An Assessment of the Effects of Insecticide-Treated Livestock Protective Fences (LPF) for Protecting Humans from Anthropophilic Mosquitoes and Malaria Transmission in a Suburb of Kumasi in the Forest Zone of Ghana

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study investigated whether a 100 cm high livestock protective fence (LPF), effectively protects humans against anthropophilic mosquitoes and hence malaria.
Study Design: Four experimental segregated, half-roofed shelters with concrete floors, each measuring 6m x 7m, separated from each other by 500m, fenced by 100cm high chicken wire, one of them enclosed by an LPF, were used.
Place and Duration of Study: Work was done on Boadi Cattle Farm by Kumasi Centre for Collaborative Research in Tropical Medicine, Kwame Nkrumah University of Science and Technology, Ghana, for four weeks.
Methodology: Human landing catches of mosquitoes were conducted twice a week. Two groups of two mosquito collectors worked at each of the four shelters during the same night; one group collected from 1800h to midnight, the second group from midnight to 0600h. One collector collected inside as the other collected outside at a distance of about 20m.
Results: Altogether 6118 mosquitoes were collected, of which 773 *Anopheles gambiae*, 11 *A. funestus*, 874 *A. ziemanni* and 4460 Culicinae. There were insignificant (P = 0.30) and significant (P = 0.0003) decreases in numbers of *A. ziemanni* and culicines entering the shelters with LPF respectively. However, significantly more *A. gambiae* entered the LPF fenced shelters than in unfenced shelters (P = 0.0008). A variation of hourly biting activities of *A. gambiae* with a peak between 0100 and 0400 at Boadi and between 1100 and 0300 at two sites at Anwomaso, was observed. *Plasmodium falciparum* infections were detected in only 1% of *A. gambiae* but not in *A. ziemanni*. All 47 *A. gambiae* s.l. randomly selected and tested using Polymerase Chain Reaction were identified as *A. gambiae* s.s.
Conclusion: LPF protects humans against some mosquitoes but not the malaria vector, *A. gambiae.*

Keywords: Mosquitoes; Anopheles gambiae; Plasmodium falciparum; malaria; shelters.

ABBREVIATION

LPF: Livestock Protective Fence.

1. INTRODUCTION

Insecticide-treated livestock protective fences have been successfully employed to protect zero-grazed dairy cattle against tsetse flies transmitting animal trypanosomiasis. Farmers noticed that nuisance by mosquitoes was alleviated [1] when 1.50m high fence material treated with beta-cyfluthrin was used. In a similar study carried out in 2005 on the cattle farm of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, a deltamethrin-treated fence 100 cm high fixed around experimental pens was tested for its potential to protect cattle and humans against mosquitoes, biting and nuisance flies of veterinary and medical relevance. It was shown that the insecticide-treated fence efficiently reduced the numbers of Muscinae and Stomoxyinae biting flies [2]. The effect of the deltamethrin-treated livestock protective fence (LPF) on mosquitoes attracted to humans was apparently obscured by the presence of cattle. Given that three times as many *A. gambiae* were caught by human landing catches (HLC) in pens without cattle than in pens with cattle irrespective of whether they were protected by a treated LPF or not [3], a follow-up study using the same shelters without cattle but with vector collectors only was carried out in 2006. Exclusion of the cattle avoided the possibility of the latter serving as zooprophylaxis; attracting mosquitoes to themselves and away from the human collectors to reduce the HLC [4]. The study aimed to investigate if the LPF would prevent anthropophagic mosquitoes from entering the shelters, thereby possibly reducing malaria transmission.

2. METHODOLOGY

The study was conducted at the Cattle Farm of the Kwame Nkrumah University of Science and Technology (KNUST) at Boadi, a peri-urban area south-east of Kumasi. The four experimental segregated shelters A to D (Fig. 1), separated from each other by a distance of 500m, constructed for the study in 2005 [3] were again used in 2006. The shelters measuring 6m x 7m had a concrete floor, were half-roofed with corrugated iron sheets and were fenced by 100cm high chicken wire (Fig. 2). In order to assess the effect of the deltamethrin-treated LPF on numbers of mosquitoes entering the shelters,

one of the four was protected by the LPF (black, 150 denier polyester fibre with a 2x2 mm mesh impregnated with 100 mg deltamethrin/m^2). The treated LPF was fixed on the chicken wire surrounding the shelter using hand gloves. Human landing catches of mosquitoes were conducted twice a week for four weeks during the minor rainy season from 9th October to 3rd November 2006. This was the same time period as the previous study [2]. Two groups, each made of two mosquito collectors worked at each of the four shelters during the same night; one group collected from 1800h to midnight, the second group from midnight to 0600h.

One of the collectors always collected inside the shelter and the second one outside at a distance of about 20m. To compensate for possible differences of mosquito densities at the four locations, the LPF was moved after each catching night from one shelter to the next one. Also, the collectors rotated from shelter to shelter, from the inside to the outside positions as well as before and after midnight on each sampling day to compensate for differences in their attractiveness to mosquitoes. Care was taken to ensure that the same collectors did not follow the treated fence as it was moved from shelter to shelter. Altogether, six full-night catches were carried out inside and outside each shelter without LPF (a total of 24 catches) and two full-night catches at shelters with LPF (8 catches). For comparison, six supplementary catches were conducted in locations E and F (0.7 and 1.3 km northeast of shelter C) in the nearby village of Anwomaso (Fig. 1).

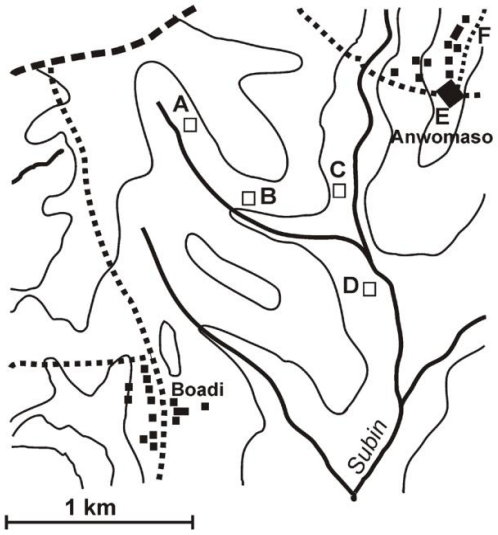

Fig. 1. Map of boadi cattle Farm (N 6° 41'; W 1° 32') showing locations of experimental shelters A to D constructed along tributaries of the Subin river and supplementary catching sites E and F in nearby anwomaso. Broken lines = roads, strong broken line = main Kumasi to Accra road

Fig. 2. Fitting the black deltamethrin treated net to the one-meter high chicken wire fence around the semi-roofed shelter. In the background, the gallery forest of a tributary of the Subin River is seen

The hourly collections were brought to the laboratory the next morning. Mosquitoes were counted and separated into Anophelinae and Culicinae. The former were further identified using the keys of Gillies and Coetzee [5], the culicines were counted and discarded. *Anopheles* females were dissected under a stereomicroscope, mid-guts and ovaries were removed and the latter examined under a compound microscope to determine parity by inspection of the ovarian tracheoles [6]. The head and thorax of all females were examined for the presence of circumsporozoite (CS) of *Plasmodium falciparum* antigen using the enzyme-linked immunosorbent assay (ELISA) developed by Wirtz et al. [7]. A polymerase chain reaction (PCR) was conducted to identify members of the *A. gambiae* complex [8].

Stata 12.0 for Windows, 2011 (StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA) was used for the statistical analysis of the results at 95% confidence intervals. Differences between percentages and likelihood Chi^2 values were analysed. The Chi^2 test of homogeneity was employed to compare hourly biting patterns.

3. RESULTS

3.1 Mosquito Density in the Various Pens and Effects of the LPF

Altogether 6118 mosquitoes were collected at the Boadi cattle farm, of which 773 were *Anopheles gambiae* s.l. (67% parous), 11 were *A. funestus*, 874 were *A. ziemanni* and 4460 were Culicinae. Total numbers of *A. gambiae*, *A. ziemanni*, Culicinae caught inside and outside shelters with LPF and mean numbers per catch obtained inside and outside shelters without LPF are shown in Table 1. While there was some, although not significant, decrease in numbers of *A. ziemanni* entering the shelters with LPF (P = 0.3015) (SD =1.380; SE = 0.131), there was a significant decrease in numbers of culicines entering the shelters with LPF (P = 0.0003) (SD = 2.873; SE =0.218). On the other hand, significantly more *A. gambiae* were caught in the LPF fenced shelters than in unfenced shelters (P = 0.0008) (SD = 2.185; SE = 0.174). The LPF had no effect on the numbers of *A. ziemanni* (P =0.20) (SD =2.557; SE =0.209) and culicines (P =0.53) (SD =5.126; SE =0.382) collected outside the shelters. However, significantly more *A. gambiae* were again caught outside the LPF fenced shelters than outside unfenced shelters (P =0.03) (SD =1.303; SE =0.111).

3.2 *Plasmodium* Infections and *A. gambiae* Species Identification

On the Boadi Cattle Farm, 8 (1.0%) of the 773 *A. gambiae* caught were found to be positive for *P. falciparum* sporozoites by the ELISA technique. Using the means of all catches inside and outside the shelters, daily and monthly biting rates of 12.1 bites per person per night (b/p/n) and 375 b/p/m respectively, as well as an entomological infective biting rate (EIR) of 3.9 ib/p/n, were estimated for the one-month study period in October/November 2006. None of the 11 *A. funestus* and 874 *A. ziemanni* was sporozoite positive. All 47 *A. gambiae* s. l. processed in the polymerase chain reaction (PCR) were identified as *A. gambiae* s.s. At Anwomaso, 5.6% of 71 *A. gambiae* were sporozoite positive.

3.3 Parous Rates and Hourly Biting Pattern

The parous rate of *A. gambiae* was 67% (773) at Boadi Cattle Farm and 89% (71) at Anwomaso. The parous rate of *A. ziemanni* at Boadi was 66% (874).

In Anwomaso, the biting activities of *A. gambiae* at site F peaked between 23 and 02 hours. However, at site E, the biting pattern prescribed a minor peak between 23 and 24 hours and a major peak between 02 and 03 hours (Fig. 3). This pattern is similar to that on the Boadi Cattle Farm which peaked in the early morning between 01 and 04 hours. The biting activity of *A. ziemanni* on the cattle farm was highest between 24 and 02 hours (Fig. 4).

The biting patterns of the different species of mosquitoes were significantly different from each other (p = 0.00). The LPF also impacted the biting activities of the different mosquito species differently (P = 0.0019).

4. DISCUSSION

The study showed that a 1m high LPF surrounding an experimental shelter does not prevent *A. gambiae* s.s. mosquitoes from entering. It is difficult to explain why significantly higher numbers of *A. gambiae* were collected in shelters with LPF. Possibly it is not the insecticide, but the net alone which changed the environmental conditions for the host-seeking mosquitoes. The partially roofed pens enclosed by the dark netting may mimic conditions

attractive for endophilic species. Obviously, in contrast to the *Stomoxyinae* and *Muscinae*, which fly close to the ground and are killed by the netting [2], the *A. gambiae* were not intercepted. This is further corroborated by the fact that in a much similar experiment using 100 cm high, 150 denier polyester fences with 100 mg/m^2 deltamethrin, tsetse population was reduced by over 90% [9]. A recent study [10], also reported a decrease in numbers of flies including tsetse, biting and other nuisance flies in test pens protected by LPF compared to unprotected ones. In this context, it is of interest that *A. gambiae* was classified in an intermediate group between low and high flying mosquitoes by Gillies and Wilkes [11]. Snow [12] observed that *A. gambiae* is only slightly affected by increasing wall height and enters houses at eaves level. Therefore, they may not get in contact with the LPF when entering the pens. The effectiveness of impregnated LPF around cattle enclosures on *Anopheles* populations and malaria transmission [1,3,13] may be explained by contact of the vector with the LPF after feeding on cattle when searching for a resting site. The biting pattern of *A. gambiae* can differ with peaks from midnight until early morning. In northern Ghana, it peaked early at 22h to 24h and continued until daybreak [14]. This correlates with the finding by Abonuusum et al. [15] at Afamanaso and Kona. However, though Boadi (Fig. 3) is about 40 km from Afamanaso and Kona, the biting pattern of *A. gambiae* differed markedly from these reports. The biting patterns at the two sites of Anwomaso reflect the variability even in close proximity (Fig. 3).

The low malaria transmission in the Boadi area despite the high anopheline density may be due to low vector-human host contact. While *Anopheles* mosquitoes bite mainly during the night [16,14,15], workers are on the farm during the day. At night only one or two security personnel remain. Again, given that the farm is a university cattle research centre, there is high malaria awareness and the people can afford both preventive and treatment measures to reduce transmission.

The abundance of *A. ziemanni*, which is highly zoophilic [17], can be attributed to the presence of cattle. Considering the low infection rate of the most efficient malaria vector, *A. gambiae*, in the area [17], the absence of infected *A. ziemanni* was not surprising as the latter is considered to be a secondary, inefficient malaria vector. This result conforms to the observations by Ribeiro et al. [18] and Ribeiro and Ramos [19], who did not detect sporozoite infections in 846 *A. ziemanni* in Angola. However, it is interesting to note that recent studies have implicated *A. ziemanni* as an important malaria vector. A report from Goulmon, Chad, [20], where *A. ziemanni* was found to be one of the most efficient malaria vectors with a human biting rate of1.3 b/p/n and circumsporozoite protein rate of 0.5%. Another more recent example is the findings by Tabue et al. [21] in the northwestern region of Cameroon that with a daily human biting rate ranging from 6.75 to 8.29 b/p/n and an infective inoculation rate from 0.028 to 0.063 ib/p/n, *A. ziemanni* is a better malaria vector than *A, gambiae.*

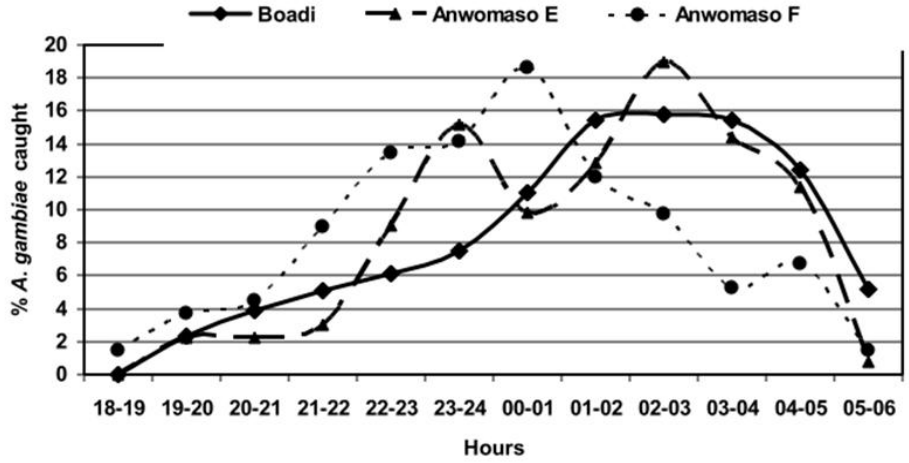

Fig. 3. Hourly biting activities of *A. gambiae* at Boadi cattle farm and sites E, F at Anwomaso

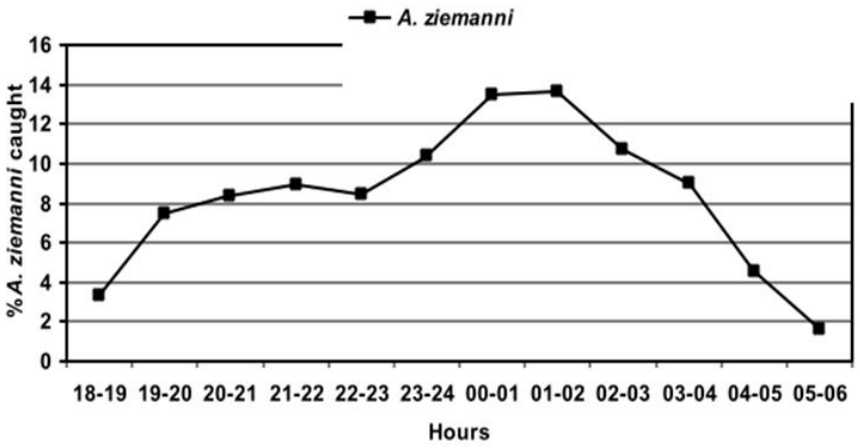

Fig. 4. Hourly biting activities of *Anopheles ziemanni* at Boadi cattle farm

Table 1. Total numbers and means of *Anopheles gambiae* (Ag), *A. ziemanni* (Az) and culicinae (Cc) caught per night in- and outside shelters with LPF (8 catches) and without LPF (24 catches), change (%) of mean numbers caught when shelters were surrounded by an LPF and P values. *A. funestus* (11 specimens) caught during the study is not included

Location	Inside			Outside		
Species	**Ag**	**Az**	**Cc**	**Ag**	**Az**	**Cc**
LPF on the pen (total)	175.0	18.0	280.0	77.0	165.0	692.0
Mean	21.9	2.3	35.0	3.2	9.6	86.5
LPF not on the pen (total)	104.0	36.0	450.0	70.0	195.0	713.0
Mean	13	4.5	56.3	8.8	24.4	89.1
Totals	279.0	54.0	730.0	147.0	360.0	1405.0
Change (%)	+68.0	-50.0	-38.0	10.0	-15.0	-2.9
Standard error	0174	0.131	0.218	0.111	0.209	0.382
Standard deviation	2.185	1.380	2.873	1.303	2.557	5.126
P-value	0.0008	0.3015	0.0003	0.0325	0.1997	0.5312

5. CONCLUSION

The LPF was found to be effective in protecting humans against *A. ziemanni* and the Culicinae but not the malaria vector, *A. gambiae.* The biting pattern of the latter was also found to vary at sites not far from each other, suggesting changing malaria transmission trends at closed distances.

CONSENT

Mosquito collectors were enrolled on verbal informed consent as approved by the KCCR-IRB. This was chosen because most volunteers did not speak English and could not read and write. Following the IRB approved protocol, a meeting was held with all participants in their local language explaining the project outline and participatory risk. The meeting was documented with the signature of all participants and of two witnesses. Diagnosis and treatment were offered during the entire period of the project and one month after conclusion, though none of the volunteers became sick during or at least four weeks after the catching period.

ETHICAL APPROVAL

Ethical approval was obtained from Kumasi Centre for Collaborative Research Institutional Review Board (KCCR–IRB), certificate number: KCCR/IRB/063/11.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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