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# Susceptibility of *Culex quinquefasciatus* populations to deltamethrin in the Sefwi area of the western region of Ghana

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## ABSTRACT

Malaria vector control in Ghana includes the use of long-lasting pyrethroid treated nets. The high level of pyrethroid resistance reported in some West African Culex quinquefasciatus (Cx. Quinquefasciatus) populations may impede malaria control operations. The study sought to determine the susceptibility of Culex mosquito populations at Bekwai, Surano, Anwiam and Dwinase in the Sefwi Area of the Western region of Ghana to detamethrin - a pyrethroid insecticide. Bioassays were performed using WHO diagnostic test kits and procedures on 1-3 days – old unfed female mosquitoes reared from larval collections. The mosquitoes were identified using morphological keys. The results indicate that there was a significant variation in the KDRs between sites with the highest occurring at Surano (100%) and the lowest at Anhwian (69%) [p = 0.001]. Mortalities after 24 hour recovery period were 91%, 93%, 97% and 100% for the Anhwiam, Dwinase, Bekwai and Surano mosquitoes respectively. The results suggest that the Cx. Quinquefasciatus from Surano are highly susceptible to deltamethrin but there may be reduced susceptibility in the mosquitoes from Anhwiam, Dwinase and Bekwai to this pyrethroid, although biochemical assays may be necessary to confirm this observation.

Key words: Culex quinquefasciatus, deltametrin, pyrethroid, malaria, Ghana

## INTRODUCTION

Human malaria results from an infection with the protozoan parasites of the genus *Plasmodium* namely, *Plasmodium malariae*, *P. falciparum*, *P. knowlesi*, *P. ovale* and *P. Vivax* (1). Malaria due to *P. falciparum* is the most deadly and dominates in Africa. It is transmitted via the bite of infected female mosquitoes of over 30 anopheline species [2]. In Ghana, *P. falciparum* causes 90-98% of malaria cases and is transmitted mostly by *An. gambiae* s.s., *An. arabiensis* and *An. funestus* [3, 4, 5, 6, 7, 8].

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Malaria is among the tops 10 killer diseases reported in Ghana and the most vulnerable groups are pregnant women, children under age one year and non-immune visitors. The National Malaria Control Programme (NMCP) indicated in its strategic plan that 70% of the people rely on traditional medicines [9]. Lack of data on traditional healthcare provision implies that a major fraction of illness and deaths due to malaria are not reported. Yet, 3,849,536 clinical cases and 3,859 deaths were attributed to malaria in 2010 [2]. Also, the disease costs Ghana about US\$760 million in 2007, which included the cost of medication, treatment, loss of productive hours and movement of drugs which added up to 10% of Gross Domestic Product[10].

The strategy of the NMCP includes effective case management and the use of long-lasting insecticide treated nets (LLINs). Pyrethroid is the only insecticide class approved for treating nets due to its low toxicity to mammals, availability and affordability [11]. PermaNet<sup>®</sup> 2.0, which uses deltamethrin (a pyrethroid), is the key bed net in Ghana. However, the pyrethroid resistance reported in malaria vectors in several settings in the country and nearby countries calls for concern as this could impact negatively on the value of the LLINs for malaria control [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. There is also a high pyrethroid resistance in *Culex quinquefasciatus* in the Ivory Coast and Burkina Faso [13]. The *Culex* mosquito has been reported to be prevalent in several settings in Ghana [7, 24, 25]. Pyrethroid resistance in this mosquito may affect malaria control operations as the public may not perceive the personal protective effect of LLINs if this nuisance human-biting mosquito does not die. This is what necessitated this study on the susceptibility of the *Culex* mosquito to pyrethroids to guide the planning, implementation and evaluation of malaria vector control operations in Ghana. The current study sought to determine deltamethrin susceptibility status in *Culex* mosquito population in some communities at the Sefwi area namely, Anhwiam Bekwai, Surano and Dwinase in the Western Region of Ghana.

## MATERIALS AND METHODS

#### **Study Site**

The study was conducted at Sefwi Bekwai and Surano in the Bibiani/Anwiaso/Bekwai District and Dwinase and Anhwiam in the Sefwi Wiawso District. Surano occurs at latitude 15.681°N, longitude 21.567°W, a height of 699m above sea level and has human population of about 2,300. Bekwai is located at 11.674°N, 19.350°W and an elevation of 600m with a population of 12,942. Anhwiam is at 12.346°N, 30.559°W and an altitude of 543m, and there are 2,099 people. Dwinase is located at 13.211°N, 28.912°W and a height of 477m with a population of 4,166. Most of the people are Sefwi speaking Akans with Twi has the second local dialect. Farming is the key occupation of the natives with cocoa being the major cash crop. Other crops include rice, oil palm, plantain, banana, pineapple and maize. Malaria is prevalent in these areas due to the nature of the vegetation, presence of some rivers and streams as well as warm temperatures together with moderate to heavy rainfall and relatively high humidity. Surano is one of the communities under the Chirano Malaria Control Programme (an integrated malaria vector management instituted by the Chirano Gold Mines Limited, *A Kinross Company*). However, there are no organised large-scale malaria vector management operations at the rest of the study sites.

## **Mosquito Sample Collection**

Mosquito larvae and pupae were collected using the standard dipping and/or scooping technique. Breeding sites were identified by sampling gutters and small pools of stagnant water, shaded or exposed to sunlight. The *culicine* larvae were identified by their typical resting position, with the body inclined to the water surface [26]. The larvae and pupae from different collection points were collected into plastic containers and transported to the insectary for rearing.

#### **Mosquito Rearing**

The larvae were examined using a hand lens and non-mosquito species, that were predators, were discarded using hand-sucking pipette. The pupae were transferred into plastic cups and put in labelled cages for adult emergence. The larvae were maintained in water from the collection site and fed once a day on 'Cerelac', a cereal food product produced by Nestle Ghana Limited. Emerged pupae were picked and put in labelled plastic cages. The emerged adult female mosquitoes were used for the insecticide susceptibility tests. The larvae were reared to adults at a temperature of  $29^{\circ}$ C and a 12 hour photoperiod from the 18:00 hour to 18:00 hour supplied by fluorescent tube.

#### **Morphological Identification of Mosquito Species**

The adult female mosquitoes were identified as *Culex quinquefasciatus* species based on the morphological keys of Smart et al. [27].

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#### **Insecticide Susceptibility Tests**

Laboratory-bred live mosquitoes were held for up to four hours before testing and supplied with cotton balls soaked with 10% sucrose solution. The tests were performed using WHO test kits and method for measuring insecticide susceptibility [28]. Non-blood fed, one to three days old females were exposed to insecticide-treated filter papers in groups of 20 to 25. Each experiment consisted of at least three replicates. The mosquitoes were exposed for an hour with the assay cylinders in a vertical position. The number of mosquitoes knocked down after 5, 10, 15, 20, 30, 40, 50 and 60 minutes was recorded. After the exposure, mosquitoes were transferred into tubes with untreated papers and allowed a 24 hour recovery period after which mortality was recorded. All the bioassays were accompanied by negative control tests where mosquitoes were exposed to papers treated only with silicone oil for an hour. The bioassays were carried out at  $25\pm2^{\circ}$ C. The mosquitoes were supplied with a 10% sugar meal during the recovery period.

#### **Data Analysis**

Knockdown times (KDTs) were estimated using a log-time probit model [29] with the software Package for Social Sciences version 16.0 for Windows (SPSS Corporation, USA). Pearson goodness of fit test was used to test the hypothesis that the linear log-time probit model was a good fit to the data. Parallelism test was used to examine the hypothesis that the KDs of the mosquito populations assayed had a common slope. Chi-square test of homogeneity of proportions was used to determine the association between KDs and mortalities [30]. Susceptibility was based on the criteria that 98–100% mortality indicates susceptibility, 80–97% mortality implies potential resistance that needs to be confirmed via biochemical assays and <80% mortality implies resistance [28]. Since all controls showed no mortality, thus there was no need for the use of the Abbott's formula.

## RESULTS

#### Knockdown rates (KDRs)

A total of 337 mosquitoes were assayed and they consisted of 98 from Surano, 75 from Anhwiam, 104 from Dwinase and 60 from Bekwai. Knockdown rates (KDRs) due to exposure to the deltamethrin 0.05% for 60 min are shown in Figure 1. For the Surano, Dwinase and Bekwai mosquitoes, knockdown (KDs) began at the 15<sup>th</sup> min but KDs for the Anhwiam mosquitoes started at the 20<sup>th</sup> min. There were significant variations in KDRs between sites with the highest occurring at Surano (100%) and the lowest at Anhwiam (69%) [ $\chi^2 = 10.705$ , d.f. =3, p = 0.001].

The KDR at Surano was significantly higher than that of Anhwiam ( $x^2 = 6.217$ , d.f. = 1, p = 0.01) and Bekwai ( $x^2 = 5.908$ , d.f. = 1, p = 0.02) but not Dwinase ( $x^2 = 1.026$ , d.f. = 1, p = 0.31). Also, KDR at Dwinase was higher than that of Anhwiam ( $x^2 = 4.12$ , d.f. = 1, p = 0.04) and Bekwai ( $x^2 = 3.762$ , d.f. =1, p = 0.05). The KDRs at Anhwiam and Bekwai did not vary significantly ( $x^2 = 0.142$ , d.f. = 1, p = 0.71).



Fig 1: Knockdown rates of Cx. quinquefasciatus exposed to deltamethrin 0.05% for 60 minutes



Fig 2: Estimated 95% confidence intervals for the KDRs of Cx. quinquefasciatus population assayed

## Knockdown time (KDT<sub>50</sub> and KDT<sub>95</sub>)

Parallelism test showed that the KDs of the mosquito populations assayed had a common slope ( $\chi^2 = 2.189$ , d.f. = 3, p = 0.534). Thus, the KDTs were estimated together using study site as the factor in the log-time probit model. The Pearson goodness of fit test also showed that the log-time prohibit model used to estimate KDT<sub>50</sub> and KDT<sub>95</sub> fitted the distribution of percentage KD with time for all the mosquitoes ( $\chi^2 = 30.420$ , d.f. = 27, p = 0.296). The KDT<sub>50</sub> and KDT<sub>95</sub> estimated (with 95% confidence intervals) are shown in Tables 1. The highest KDTs, which were estimated for the Anhwiam population, were 2.9-fold the lowest KDTs estimated for the Surano population.





Fig 3: Percentage knockdown (KD) and mortality of *Cx. quinquefasciatus* after the 60 min exposure to deltamethrin 0.05% and the 24 hrs recovery period respectively

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Figure 4 shows the KDRs as well as the mortality rates 24 hours post-exposure to deltamethrin 0.05%. A hundred percent mortality was observed for the Surano mosquitoes tested. However, mortalities of 91%, 93% to 97% were observed for the mosquitoes from Anhwiam, Dwinase and Bekwai respectively. None of the mosquitoes used in the negative control experiment died during the recovery period. There was a strong positive linear correlation between KDRs and mortality rates (r = 0.755, p = 0.000).



Fig 4: Estimated 95% confidence intervals for the mortality rates of Cx. quinquefasciatus recorded

#### DISCUSSION

The National Malaria Control Programme (NMCP) is currently embarking on its nationwide distribution and hangup of long-lasting deltamethrin insecticide treated nets (LLINs) as part of the strategy to control malaria in the country. The widespread use of the pyrethroids such as deltamethrin in the public health sector may be due to its success in LLINs and indoor residual spraying among others [3, 31, 32, 33]. However, there is problem with the wide use of pyrethroids due to cross-resistance that results from pyrethroid insecticides formulated for use in the agricultural sector [14, 15, 18]. This calls for pyrethroid susceptibility/resistance related data to help in the planning, implementation and evaluation of pyrethroids and their role in mosquito borne disease control and/or prevention of nuisance associated with mosquito biting in general.

This study determined the status of deltamethrin susceptibility in *Culex quinquefasciatus* populations in the Sefwi area of the Western Region, Ghana. The *Cx. quinquefasciatus* is one of the most widespread nuisance causing and human-biting mosquitoes and the vector of lymphatic filariasis. The specific areas surveyed were Surano and Bekwai in the Bibiani-Anhwiaso-Bekwai District and Dwinase and Anhwiam in the Sefwi Wiawso District. The study made use of the World Health Organisation (WHO) Bioassays kits and insecticide impregnated paper on the mosquito.

*Culex quinquefasciatus* larvae were collected in April 2010 from Surano, which is within the Chirano Malaria Control Programme (CMCP). Larvae were also collected from Bekwai, Dwinasie and Anhwiam, which are outside the CMCP. The larvae were reared to adult and used for the deltamethrin susceptibly tests. The results of the study suggested that the Surano *Cx. quinquefasciatus* population is a 100% susceptible to deltamethrin 0.05% but the Anhwiam, Dwinase and Bekwai populations have reduced susceptibility to this pyrethroid as implied by mortalities  $\leq 97\%$  [28] that is, 91%, 93% and 97% respectively.

The deltamethrin susceptibility status found in the Anhwiam, Bekwai and Dwinase *Cx. quinquefasciatus* populations is consistent with the study carried out in the Kasena Nankana District (KND) which showed that *An. gambiae* and *An. funestus*, the main malaria vector mosquitoes are susceptible to the insecticides being used in the treatment of bed nets in the malaria control programme [34]. At both the KND and these three Sefwi Communities, however, there is the need for continuous monitoring of the pyrethroids in these areas as the efficacy is reduced. There is evidence that reduced susceptibility/resistance to one member of the pyrethroid class is enough evidence that reduced susceptibility/resistance to other chemicals in the same class will occur [35]. Therefore, the surveillance



study has been expanded to cover many more parts of the Sefwi area to determine susceptibility/resistance profiles in both the *Anopheles* and non-*Anopheles* populations to all the current insecticides approved for vector control.

The high susceptibility found at the Surano is not consistent with the baseline studies carried out in 2008 and 2009 before the commencement of the CMCP operations demonstrated high pyrethroid insecticide resistance in both malaria and non-malaria mosquitoes but high susceptibility to carbamates and organophosphates. Therefore, indoor residual spraying and larviciding under the CMCP were started with a Ficam<sup>®</sup> VC (a carbamate) and Starycide<sup>®</sup> SC (an insect growth regulator). It is therefore, possible that the high deltamethrin susceptibility found in the current study could be attributed to the ongoing insecticide resistance management in the area.

The reduced susceptibility in the Bekwai, Anhwiam and Dwinase *Culex* mosquitoes is also inconsistent with the high frequency of pyrethroid resistance reported in both malaria vectors and non-malaria mosquitoes in Ghana and the neighbouring countries such as Côte d'Ivoire and Burkina Faso [21, 22, 23]. There is no published data on the mechanism(s) underlying pyrethroid resistance in *Culex* mosquitoes in Ghana. However, studies in the Ivory Coast and Burkina Faso attributed resistance to permethrin partly to  $P^{450}$ -dependent oxidases [13]. Chandre et al. [13] also reported that knockdown resistance (*kdr*) associated with DDT cross resistance was responsible for a loss of permethrin knockdown effect on adults *Culex* mosquitoes. Thus, there is the need for further studies to confirm biochemically the reduced deltamethrin susceptibility or potential resistance found at Bekwai, Anhwiam and Dwinase.

The 95% confidence intervals (95% CIs) estimated for the KDT<sub>50</sub> and the KDT<sub>95</sub>% values indicated spatial variation in KDTs even between the three communities outside the CMCP (see Table 1). This spatial variation, indicated by the clear non-overlap of the 95% CIs, is noteworthy. This is because effectiveness of insecticides at controlling mosquitoes has sometimes been found to be focal in nature with susceptibility/resistance levels sometimes varying between communities short distances apart [36] and in different seasons of the year [16, 18]. Such focal nature of insecticide susceptibility/resistance has been attributed to selection pressure due to insecticide usage for agricultural and public health insect/pest control purposes [11, 16, 17, 19, 20, 21, 34, 37] or immigration of insecticide resistance genes [38].

The full impact of possible resistance development in the non-malaria mosquito population on nuisance biting prevention and malaria vector control efforts is not clear [39]. For example, studies in Côte d'Ivoire indicated that even where there is a high frequency of knockdown resistance mutation in the mosquito population, deltamethrintreated nets gave good protection against mosquito bites and hence disease transmission [40, 41]. This suggests that the *kdr* on its own may not be enough to confer resistance to an individual mosquito [36]. Thus, full resistance mechanism profiles of a mosquito population may be required to establish the absolute impact of resistance in that population on disease control. In the interim, while the effect of resistance development on nuisance biting, morbidity and mortality following continuing use of LLINs in resistant areas is yet to be studied [39], the reduced susceptibility found at Bekwai, Anhwiam and Dwinase should not be an obstacle to the current promotion and use of LLINs for protection against malaria transmission in these areas. The findings can rather guide the choice of appropriate doses of pyrethroids for intervention strategies such as curtain and net treatments.

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