Risk of transmission of viral haemorrhagic fevers and the insecticide susceptibility status of *aedes aegypti* (linnaeus) in some sites in Accra, Ghana

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SUMMARY
Background: Dengue is one of the emerging diseases that can mostly only be controlled by vector control since there is no vaccine for the disease. Although, Dengue has not been reported in Ghana, movement of people from neighbouring countries where the disease has been reported can facilitate transmission of the disease.
Objective: This study was carried on the University of Ghana campus to determine the risk of transmission of viral haemorrhagic fevers and the insecticide susceptibility status of *Ae. aegypti* in some sites in Accra, Ghana.
Design: Larval surveys were carried to inspect containers within households and estimate larval indices and adult *Aedes* mosquitoes were collected using human landing collection technique. WHO tube assays was used to assess the insecticide susceptibility status of *Aedes* mosquitoes.
Results: *Ae. aegypti* were the most prevalent species, 75.5% and followed by *Ae. vittatus,* 23.9 %. *Ae. albopictus* and *Ae. granti* were in smaller numbers. Household index (HI), Breteau index (BI), and container index were calculated as 8.2%, 11.2% and 10.3% respectively with man-vector contact rate of 0.67 bites/man-hour estimated for the area. The mortalities recorded for *Ae. aegypti* from WHO tube assays was 88%, 94%, 80% and 99% for DDT (4%), deltamethrin (0.05%), lambdacyhalothrin (0.05%) and permethrin (0.75%) respectively.
Conclusion: The survey results indicated that the density of *Aedes* mosquitoes was considered to be sufficient to promote an outbreak of viral haemorrhagic fevers on Legon Campus. *Aedes* mosquitoes were found to be resistant to DDT, deltamethrin and lamdacyhalothrin, but susceptible to permethrin.

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Keywords: Mosquitoes, *Aedes aegypti*, insecticide, risk, VHF transmission, Ghana

INTRODUCTION
Dengue and Yellow fevers are viral haemorrhagic fevers (VHF) of grave public health concern. About 50 to 100 million cases of Dengue Fever (DF) and a few hundred thousand cases of Dengue Haemorrhagic Fever (DHF) occur globally every year.[1] Currently, Yellow Fever (YF) infects about 200,000 people killing about 30,000 of them annually. Dengue has been reported in 18 of the 46 countries in the WHO African Region. These include seven countries in West Africa. *Aedes aegypti* is considered to be the most important vector of YF and DF and occurs throughout West Africa from sea-level up to at least 1220 m in Nigeria and from the coastal swamp zone to Northern Guinea savannah.[2] This species has been recorded from various potential breeding sites including crab holes, tree holes, fallen leaves, rock pools, domestic containers, snail shells, leaf axils, rain pools, and latex cups in rubber plantations. Transportation and urbanisation of new areas are the major causes of the spread of *Ae. Aegypti*.[2] Whilst a vaccine exist for the control of YF fever disease, the conventional methods normally used during outbreak to control VHF especially Dengue is larval reduction. However, in most cases insecticide based vector control remains the only option. However, the pyrethroid resistance in *Ae. aegypti* is widespread in many areas.[3,4]

In Ghana, DF has not been reported but has been detected in neighbouring Côte d'Ivore and Burkina Faso. YF was earlier reported in the Northern, Upper East and Upper West regions from 1969 to 1970.[5]

The recent YF case that claimed at least 2 lives occurred in Ghana between October 2011 and February 2012.[6] The trends in YF outbreak seems to have a 10-year cycle in endemic African and South American countries.[7] It is thus possible that VHF outbreak may occur in Ghana. Indeed the northern regions of Ghana were reported to be a high risk area of VHF transmission.[8]

The University of Ghana Campus is a community located in southern Ghana comprising students, families and dependants of university staffs, staffs, food vendors and petty traders. The university has a student population of about 42,000. These students are predominantly Ghanaians (96%). However, it is possible that some students from Dengue endemic countries may help establish a dengue transmission cycle in the university community if the vectors for the transmission of the disease could be found on campus. Many *Aedes* breeding sites have earlier been identified on the University of Ghana campus and many student complain of mosquito bites late in the afternoon when mostly outdoors (Dadzie, personal communication, April 8, 2012). There was therefore an urgent need to assess the risk of DF transmission on the University of Ghana campus.

Pyrethroid resistance of *An. gambiae*, the malaria vector is common in most areas in Ghana [9-12] and in view of the fact that VHF control is mainly by the use of insecticides, there was the need to conduct susceptibility tests of the potential vectors occurring in the area

METHODS

Mosquito collection sites

Aedes larvae and adults were collected in five areas located within the University of Ghana campus at Legon. The survey areas were South Legon, East Legon, Ayido Flat, Little Legon and Lower Hill. The larval survey and insecticide susceptibility test was done during the dry season from December 2012 to February 2013. Dry season was chosen for the survey because previous work showed that larval indices were high during the dry season when people tended to store water[8]. Legon is located in the coastal savannah zone of the Greater Accra Region at coordinates 5°39′3″N and 0°11′13″W. The study sites experience two rainy seasons. The major rainy season occurs from April to July and the minor from September to November. Average rainfall ranges between 350 and 1200 mm annually.

Household surveys

All households were examined for water storage containers for the presence of pre-adult stages of *Aedes* mosquitoes to estimate larval indices. Flower pots in households were also examined and abandoned lorry tyres were also inspected.

Three indices were used to assess *Aedes* mosquito density. These were House index (HI), Container index (CI) and Breteau index (BI). HI was expressed as the percentage of houses infested with *Aedes* larvae; CI as the percentage of containers infested; and BI as the number of positive containers per 100 inspected houses.

Oviposition traps

Oviposition traps were set in order to detect the presence of *Aedes* mosquitoes and to estimate the *Aedes* population density. Ten black plastic paper cups ($9.5 \times 6.2 \times 12.4 cm^3$) half-filled with water were positioned at vantage points in each study area for a week, and examined every morning for the presence of *Aedes* eggs. Ovitrap index (OI) expressed as the percentage of traps infested was calculated.

Adult mosquito collection

Human Landing Catches (HLC) was carried out twice in each area. Adult *Aedes* mosquitoes were collected off three people who exposed their legs for a period of three hours between 15:30-18:30. All mosquitoes which landed on the collectors, and thus presumed to be biting, were collected, transferred into paper cups and killed in a refrigerator for species identification. The man-vector contact rate was estimated as the number of *Aedes* mosquitoes biting a person per unit time.

Species identification

Pre-adult *Aedes* mosquitoes obtained from ovitraps and larval surveys were raised to adults and identified. Adult mosquitoes were identified using morphological characters as described by Huang[13,14] and (bioinfo-prod.mpl.ird.fr/identiciels/culmed/html/images/adulte/txaevi1a.jpg, accessed 13/12/2014).[15]

WHO insecticide susceptibility tube assays

WHO insecticide susceptibility tests were conducted using 2-5-day-old non-blood-fed female *Aedes* mosquitoes that emerged from pupae obtained during the survey[16]. Dichloro diphenyl trichloroethane, DDT (5%) and three pyrethroids: deltamethrin (0.05%), permethrin (0.75%), and lambdacyhalothrin (0.05%) were selected for the tests. Three (3) replicates and 2 controls were used for each insecticide. The mosquitoes were exposed to each insecticide for 1 hour.

While they were exposed, the number of mosquitoes knocked down was recorded. If the number of knockdown mosquitoes was less than 80% after an hour of exposure to the insecticide, the mosquitoes in each tube were transferred to their corresponding holding tubes and observed for another 20 minutes before recording the last knockdown values.

The mortality 24 hours after the exposure was subsequently recorded. *Ae. aegypti* population resistant to each insecticide was estimated from the calculated % mortality using WHO Insecticide Susceptibility criteria.

Data analysis

The risk of VHF at each site was estimated using the WHO criteria as follows: An area where BI, HI, and CI exceeded 50, 35 and 20 respectively, the risk of *Ae aegypti*-transmitted VHF was considered to be high; an area where BI was between 5 and 50, the density of *Ae. aegypti* was considered to be sufficient to promote an outbreak of VHF disease; an area where BI, HI and CI were less than 5, 4 and 3 respectively, it was considered to be unlikely for VHF transmission to occur[17]. Ovitrap index (OI) expressed as the percentage of traps infested was also calculated. WHO insecticide susceptibility levels were estimated using the WHO criteria[16]. With 98% mortality, a test population was considered susceptible; mortalities between 90% and 97% suggested a possible resistance in the mosquito population; and below 90% the test population was resistant.

RESULTS

Species identification

A total of 985 adult *Aedes* mosquitoes were obtained from human landing catches (HLC) and Ovitraps. Out of the total, HLC yielded (36) with the remaining (949) collected by Ovitraps. The most common species was *Ae. aegypti* (75.5%), followed by *Ae. vittatus* (23.9%). The *Ae. granti* and *Ae. albopictus* formed 0.5% (5/985) and 0.1% (1/985) respectively of the total population of *Aedes* mosquitoes obtained during the study.

Larval and ovitrap indices

One hundred and seventy (170) households were surveyed during the study period. The overall HI, BI and CI estimated were 8.2%, 11.2% and 10.3%, respectively (ure 1A-C). A higher HI was observed in East Legon (23%), followed by South Legon (18%). The other three sites (Ayido flats, Little Legon and Lower Hill) had HI lower than 5% (Figure 1A) .This trend was also observed for BI and CI (Figure 1B-C). The higher larval indices observed in those two sites as compared to Ayido Flats, Little Legon and Lower Hill sites may be attributable to larger numbers, 85%of used and abandoned car tires in those sites which could serve as breeding sites. Figure 2 shows the ovitrap index estimated for the four sites. The Ovitrap Index was highest in Little Legon.

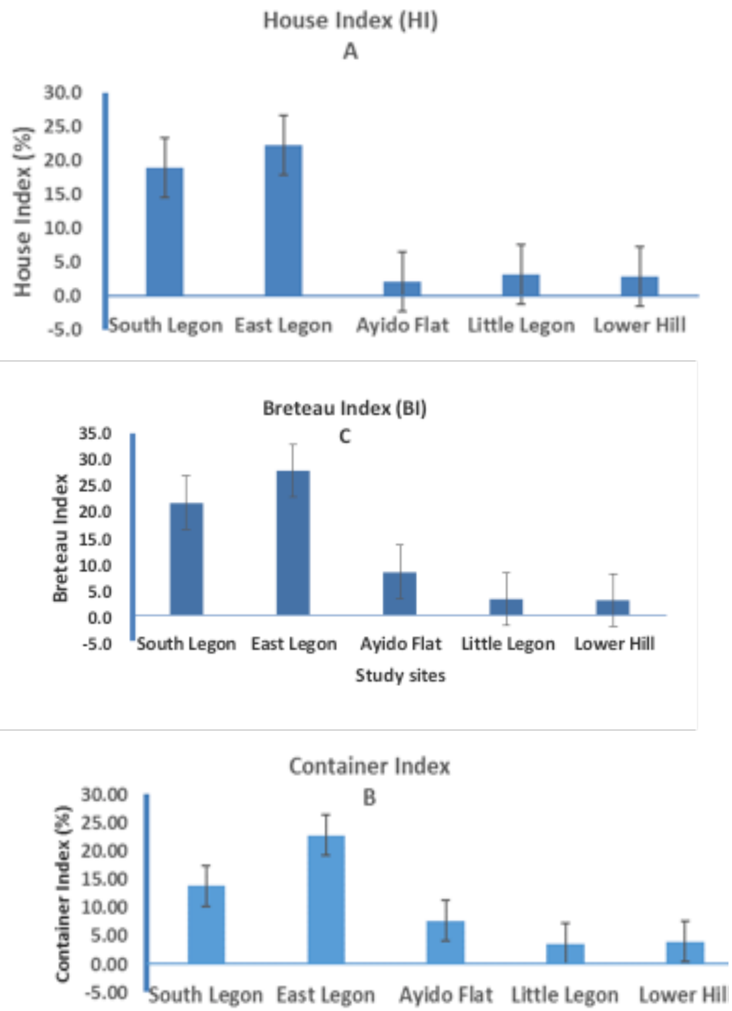

Figure 1 Larval indices A) House Index (HI), B) Container Index (CI) and C) Breteau Index (BI) estimated for *Aedes* mosquitoes at the University of Ghana Campus

Man-vector contact

A total of 36 adult *Ae. aegypti* was collected during 54 man-hours. A man-vector contact rate of 0.67 bites per man-hour was estimated in the area and this was lower than WHO-guided rate of high dengue transmission. Man vector counts exceeding two female mosquito bites per man-hour were considered to be a significant risk of VHF transmission.

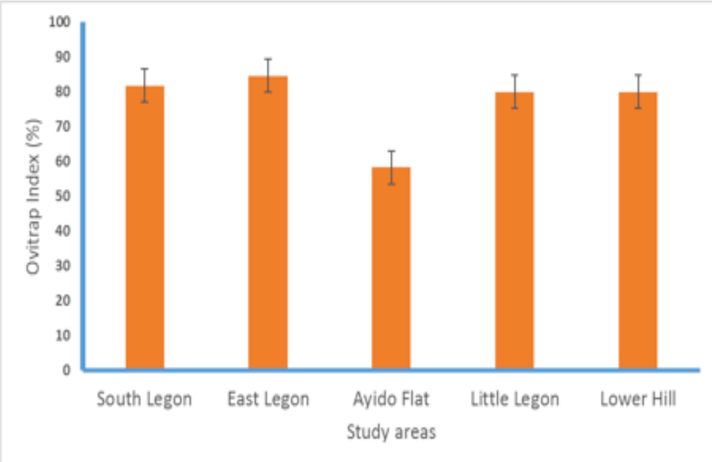

Figure 2 Ovitrap Index estimated for *Aedes* mosquitoes at the University of Ghana Campus

WHO Susceptibility Test

The WHO susceptibility test revealed that the *Aedes* mosquito populations were suspected to be resistant to deltamethrin, resistant to lambdacyhalothrin but susceptible to permethrin. The percentage mortality after 24 hours exposure to DDT, deltamethrin, lambdacyhalothrin and permethrin were 85.4%, 93.6%, 81.6% and 98.6% respectively (Table 1). The knock down rate (KD_{60}) of DDT was lower (43%) than those of pyrethroids (72-76%).

Table 1 DDT and pyrethroid susceptibility of *Aedes aegypti* mosquitoes from the University of Ghana campus

Insecticide class	Type of insecticide	Number Tested	Number KD* after 15 min (%)	Number KD after 30 min (%)	Number KD after 60 min (%)	Number KD after 80 min (%)	Mortality after 24 hours (%)	Corrected % mortality after 24 hours	Mortality of Controls after 24 hours (%)	Conc
Organochlorine	DDT - 4.0%	77	2 (0.03)	10 (0.13)	43 (55.84)	46 (59.74)	68 (88.31)	85.39	4 (20.0)	Re
Pyrethroids	Deltamethrin - 0.05%	78	22 (28.3)	64 (82.05)	76 (97.44)	-	73 (93.59)	-	0 (0.0)	Sus Re
	Permethrin - 0.75%	72	7 (9.7)	62 (86.11)	72 (100)	-	71 (98.61)	-	0 (0.0)	Susc
	Lambdacyhalothrin -0.05%	76	1 (0.01)	44 (57.89)	74 (97.37)	-	62 (81.58)	79.53	2 (10.0)	Re

DISCUSSION

The results from the larval indices indicated that *Aedes* population on the University of Ghana Legon campus was sufficient to promote the outbreak of VHF especially dengue and yellow fever. An earlier study in Yellow fever epidemic prone areas in Northern Ghana had estimated much higher HI and BI and indicated that the risk of VHF transmission was higher in the dry season than in the rainy season.[8] The high risk was attributed to water storage practices where many inhabitants tended to store water in containers during the dry season. The study also found that most of the *Aedes* mosquitoes were breeding in containers outside houses especially in lorry tires, and in choked gutters around the campus. Thus removing abandoned containers and old lorry tyres as well as desilting choked gutters will help reduce the population of *Ae. aegypti* mosquitoes in the area.

Ae. albopictus, originally from Asian was identified among the *Aedes* mosquitoes collected and this is the second reported case of the species in Ghana. Unpublished data[18] established one *Ae. albopictus* species also found close to the South Legon settlement on the University of Ghana Campus.

Although, only one *Ae. Albopictus* species was identified, it is necessary to increase surveillance on the species in the country. This is because *Ae. albopictus* is a very competent vector of many viruses including dengue fever[19] and Eastern equine encephalitis virus.[20] *Ae. albopictus* was originated in Asian countries but can now be found globally in many places including the United States, almost the entire South American continent, and some of European countries.[21] *Ae. albopictus* has recently invaded several Central African countries[16]. Importantly, *Ae. Albopictus* was reported in Anambra, Benue, Delta, Enugu and Imo states of Nigeria.[22] The global distribution and continuous spread of *Ae. albopictus* has been principally due to the transport of eggs in used tires by air and sea transport.[22-24]

This study also revealed that the *Aedes* mosquito population were susceptible to permethrin and resistant to lambdacyhalothrin and DDT. In contrast, *An. gambiae*, the major malaria vector has been reported in many areas in the country to be resistant to both permethrin and DDT.[25,26]

It had been reported that in West and Central Africa, most resistance data on *Ae. aegypti* date back more than 30-40 years.[26] Resistance of *Ae. aegypti* populations to DDT has been reported in Gabon.[25] In this study, the source of DDT resistance is not very clear. It may be as results of permethrin use in LLINs or indoor residual spraying and for agricultural purposes.

The reason why *Ae. aegypti* was susceptible to permethrin in this part of the country need to be further investigated. DDT and pyrethroids share similar mechanism of action and mutations at the sodium channel gene were reported to confer resistance to DDT and pyrethroids.[27]

Cross resistance to DDT and permethrin is also known to occur.[28] It is also known that up-regulation of cytochrome P450 gene expression also causes DDT resistance in insects.[29] Based on the results, two hypotheses may be postulated. The first hypothesis may be that detoxification mechanisms of DDT and permethrin insecticides are different in *Ae. aegypti.* In this case, up-regulation of cytochrome P450 gene expression may have played a major role in conferring resistance to the mosquitoes. Secondly, unlike *Anopheles* mosquitoes that are nocturnal and to a great extent endophagic and thus frequently exposed to pyrethroids, *Aedes* mosquitoes are diurnal and therefore take blood meals usually outdoors where they may have little or no exposure to pyrethroids. Our result suggest that permethrin among pyrethroids could be used for *Aedes* control on the University of Ghana Campus.

CONCLUSION

This is the first report that provides information on risk of transmission and insecticide susceptibility of *Ae. aegypti* population in a community in Southern Ghana. The presence of *Ae. albopictus* in the area requires active surveillance on the species in the country. The man-vector contact rate did not support a possible outbreak of VHF, however, the larval indices were sufficient to promote outbreak of VHF. The study showed that permethrin could be a candidate insecticide to be used in the control of *Aedes* mosquitoes in the area. More detailed information on the surveillance of risk of transmission of VHF and the insecticide susceptibility of *Ae. aegypti* is needed across the country to inform future control efforts.

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