Journal of Medical Entomology, 57(4), 2020, 1239–1245
doi: 10.1093/jme/tjaa024
Advance Access Publication Date: 29 February 2020
Research

Biting Behavior and Molecular Identification of *Aedes aegypti* (Diptera: Culicidae) Subspecies in Some Selected Recent Yellow Fever Outbreak Communities in Northern Ghana

Millicent Captain-Esoah,[1,2] Philip Kweku Baidoo,[2] Kwadwo K. Frempong,[3] Delphina Adabie-Gomez,[4] Joseph Chabi,[5] Dorothy Obuobi,[5] Godwin Kwame Amlalo,[5] Francis Balungnaa Veriegh,[6] Martin Donkor,[7] Victor Asoala,[8] Eric Behene,[3] Daniel Adjei Boakye,[3] and Samuel Kweku Dadzie[3,9]

[1]Department of Applied Biology, University for Development Studies, Navrongo, Ghana, [2]Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Department of Parasitology, Noguchi Memorial Institute for Medical Research (NMIMR), College of Health Sciences, University of Ghana, Legon, Accra, Ghana, [4]Department of Basic Veterinary Medicine, College of Basic and Applied Science, University of Ghana, Legon, Accra, Ghana, [5]Vestergaard-NMIMR Vector Laboratory, Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [6]Bio-Medical and Public Health Research Unit, Center for Scientific and Industrial Research, Water Research Institute (CSIR-WRI), Accra, Ghana, [7]Department of Chemistry and Biochemistry, University for Development Studies, Navrongo, Ghana, [8]Department of Entomology, Navrongo Health Research Centre, Navrongo, Ghana and [9]Corresponding author, e-mail: sdadzie@noguchi.ug.edu.gh

Subject Editor: Douglas Norris

Received 21 June 2019; Editorial decision 27 January 2020

Abstract

Aedes aegypti (L.) (Diptera: Culicidae) is a diurnal feeder that lives in close association with human populations. It is the principal vector of yellow fever, dengue fever and the Zika Virus. Issues of arboviral diseases have been on the ascendency in most countries including Ghana where *Aedes* mosquito is the main vector of yellow fever. A comparative study of the biting behavior of *Ae. aegypti* and the identification of subspecies were undertaken using molecular technique. Standard human landing technique was used to collect both indoor and outdoor biting mosquitoes at three zones located in the Upper East (Bolgatanga), Upper West (Nadowli), and Northern (Damongo) Regions of Ghana during the dry and rainy seasons between 0600 and 1800 Greenwich Mean Time (GMT). All collected mosquitoes were identified morphologically using taxonomic keys. random amplified polymorphic DNA polymerase chain reaction was used to categorize *Ae. aegypti* into subspecies. Adult female *Aedes* mosquitoes identified formed 62% (n = 1,206) of all female mosquitoes collected. *Aedes aegypti* 98% and *Aedes vittatus* 2% were the only *Aedes* species identified. Bolgatanga recorded the largest number of *Ae. aegypti* 42%, whereas Nadowli 22% recorded the least. *Aedes vittatus* was observed in Nadowli. *Aedes aegypti* exhibited a bimodal biting behavior peaking at 0600–0800 GMT and 1500–1600 h GMT. Molecular findings revealed 69% *Ae. aegypti aegypti* and 31% *Ae. aegypti formosus* as the two subspecies (n = 110). This information is important for implementing effective vector control programs in the three regions of the northern Ghana.

Key words: Biting behavior, *Aedes aegypti*, Northern Ghana, Yellow Fever Outbreak, molecular identification

Arboviral diseases, most of which are transmitted by *Aedes* species have increased in recent times (Figueiredo 2007). Some of these arborvial diseases are dengue fever, chikungunya virus, Zika Virus and yellow fever. In Ghana *Aedes* mosquitoes are known to transmit yellow fever, with different species transmitting at different locations (Mouchet 1971). In forest areas for example *Aedes africanus* and *Aedes luteocephalus* are the main vectors. In the urban areas the predominant vectors are *Aedes aegypti* (L.) (Diptera: Culicidae). Other vectors of yellow fever are *Aedes vittatus*, *Aedes metallicus*, *Aedes simpsoni*, and *Aedes furcifer-taylori* (Mouchet 1971, Appawu et al. 2006, Captain-Esoah et al. 2014). In the Northern parts of Ghana, the potential *Aedes* species are *Ae. aegypti* and *Ae. africanus* (Appawu

et al. 2006). Recent work done by Captain- Esoah et al. (2014) revealed the presence of *Ae. simpsoni* in the Upper regions of northern Ghana. *Ae. albopictus* has also been identified recently by Suzuki et al. (2016) in Accra. *Aedes aegypti*, a highly domestic mosquito prefers biting humans but they also bite dogs and other domestic animals, mostly mammals (Edward 1984, WHO 1986, Clements 1992b). Female *Aedes aegypti* are highly anthropophilic and have a propensity for feeding on humans (Harrington et al. 2001). It is capable of long flights if host on which it feeds are unavailable locally (Liew and Curtis 2004), but rarely disperse >200 m from point of emergence or release (Harrington et al. 2005). However, under certain circumstances *Ae. aegypti* females disperse further probably to locate a bloodmeal (Maciel-de-Freitas et al. 2010). According to Harrington et al. (2001), the unique isoleucine concentration of human blood is associated with *Ae. aegypti*'s unusual affinity to feed preferentially and frequently on humans. This helps to intensify their fitness level, energy reserves synthesis capabilities, and contact with human hosts, hence making them actual sources of human pathogens. *Aedes* mosquito is a diurnal feeder that bites both indoors and outdoors. The bite usually occurs when their potential hosts are less protected (Karunamoorthi 2012). *Aedes* mosquitoes are normally active within 2 h of day break and at dawn. However, they can bite in the night when there is light. For instance, *Ae. simpsoni* complex species bites mainly in the mid-afternoon, *Ae. africanus* bite at dusk and *Ae. vittatus* bite throughout the day (Gillett 1972). A study by Captain-Esoah et al. (2014) in the Upper East Region of Ghana, revealed that the peak biting time of *Ae. aegypti* in the afternoon was 1700–1800 h. A recent study conducted in Upper West Region of Ghana by Adzika and Arhin (Unpublished thesis), revealed that biting by *Aedes aegypti* peaked within 0600—0800 and 1800–1900 h, whereas *Ae. vittatus* peaked at 0500–0600 h, no *Ae. vittatus* were found biting in the evening. *Aedes aegypti* have modified their peak biting activities in the early morning and early evening, which can lead to disease transmission in some areas in the northern parts of Ghana (Captain-Esoah et al. 2014). In Ghana, the Ministry of Health, reported a yellow fever outbreak in three districts; Builsa and Kassena-Nankana-West in the Upper East Region and Kintampo-South in the Brong Ahafo Region (MOH 2011). These outbreaks involved three laboratory-confirmed cases and two deaths (WHO 2012a). Within the same year (2011), three cases were reported in Nadowli, Jirapa, and Wa East in the Upper West Region and Dikatami in the Sawla-Tuna-Kalba District in the Northern Region which resulted in one death (GNA 2011). A recent outbreak in the West Gonja District, of Northern Region has resulted in 3 deaths with 12 people infected with the yellow fever disease (Ghana Web 2016). This study aimed at comparing the biting behavior of *Aedes* species in (Bolgatanga), Upper East Region (Damongo), Northern Region (Nadowli), and Upper West Region of Ghana and to identify the subspecies of *Ae. aegypti* molecularly in order to prepare for any future outbreaks and also to implement appropriate vector control programs.

Materials and Methods

Study Sites

The study sites were Damongo (located within West Gonja district and lies on the lat. 8°32′ and 10°2′ North and long. 1°5′ and 2°58′ West), Bolgatanga (located within Bolgatanga Municipal district and lies on the lat. 10°30′ and 15°5′ North and long. 0°33′ and 1°00′ West), and Nadowli (located within Nadowli-Kaleo district and lies on the lat. 10.8°28′ and 9.8°18′ North and long. 2.7°10′ and 1.9°10′ West of Sahelian Savannah; Fig. 1). The study sites were selected based on reports of recent outbreaks of yellow fever. and these are the regions also share border with Burkina Faso that had reported yellow fever and dengue fever outbreaks in the recent past prior to this study. The areas are mostly savannah with mean annual rainfall between 950 and 1,100 mm and average temperature of 36°C. The inhabitants are mainly farmers (MOFA 2010, GSS 2014).

Study Design

One hundred houses were selected from each of the three study areas: Damongo, Bolgatanga and Nadowli communities using stratified sampling method (Neyman 1934, Creswell 2009). *Aedes* mosquitoes were collected from indoors and outdoors between 06:00 and 18:00 h GMT using the Human Landing Catches Method for a period of 8 mo in 2015 and 2016. The 100 houses were surveyed for mosquito larvae twice every month for the entire duration of the study. The mosquitoes were collected from some rooms of two randomly selected houses by trained collectors using the human landing catches technique. Landing catches were performed by eight collectors (four indoors and four outdoors) for three consecutive days from 6 am to 6 pm, with 10-min breaks every hour. Mosquito collectors wore long sleeve shirts to ensure that blood-seeking mosquitoes had access to only their lower legs which were exposed. Using a test tube, mosquitoes were collected once they landed on the exposed lower legs of the collectors, but before biting commenced and transferred to prelabelled holding cups. Collections were done both in the dry (January to April 2015 and 2016) and the rainy (July to October 2015 and 2016) seasons. Morphological identification of the *Aedes* mosquitoes was done using the keys as described by Gillett (1972) and Huang and Ward (1981). Briefly, the Adult *Aedes aegypti* were differentiated by the patterns of white scales on the dorsal side of the thorax. In *Aedes aegypti*, the pattern consisted of two straight lines surrounded by curved lyre-shaped lines on the side, whereas in *Ae. Vittatus* three pairs of distinct, small white spots of narrow scales on anterior, middle and posterior part of the scutum were observed. Each mosquito was preserved dry in a perforated 1.5-ml microcentrifuge tube kept in zip-lock bags containing silica gel for molecular studies.

DNA Extraction and purification

Genomic DNA was extracted from 110 *Aedes aegypti* samples randomly selected from different areas in Bolgatanga, Damongo and Nadowli in the rainy and dry seasons of 2015 and 2016 using the Qiagen kit (Invitrogen, Carlsbad, CA) protocol.

Random Amplified Polymorphic DNA Polymerase Chain Reaction

The *Aedes aegypti* subspecies was identified using the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) developed by Ballinger-Crabtree et al. (1992) with slight modifications. Amplification of DNA fragments was done in a mix of 20 µl containing 3 µl of extracted DNA, 1× PCR reaction buffer, 1.5 mM $MgCl_2$, 0.25 mM of the deoxynucleotide triphosphates (DNTPs) mix, 0.25 µM of each of the oligonucleotide primers (see Table 1), and 1 U of Taq polymerase. The primer sequence and the expected fragment sizes of the RAPD-PCR products are shown in Table 1 (reagents for PCR from Invitrogen, Carlsbad, CA). Amplification was done using PCR MAX Alpha cycler range (Cole-Parmer Ltd) using the following cycling conditions: 94°C for 4 min, followed by 45 cycles of 94°C for 1 min, 35 °C for 1 min, 72°C for 2 min, and a final cycle at 72°C for 5 min.

The amplified products were analyzed on a 1% agarose gel stained with 0.17 mg/ml of ethidium bromide using 10 µl of each PCR product at 100 V for 40 min. Fragments were visualized using BioDoc–It Imaging Systems, (Ultra Violet Product Ltd, Cambridge, United Kingdom)

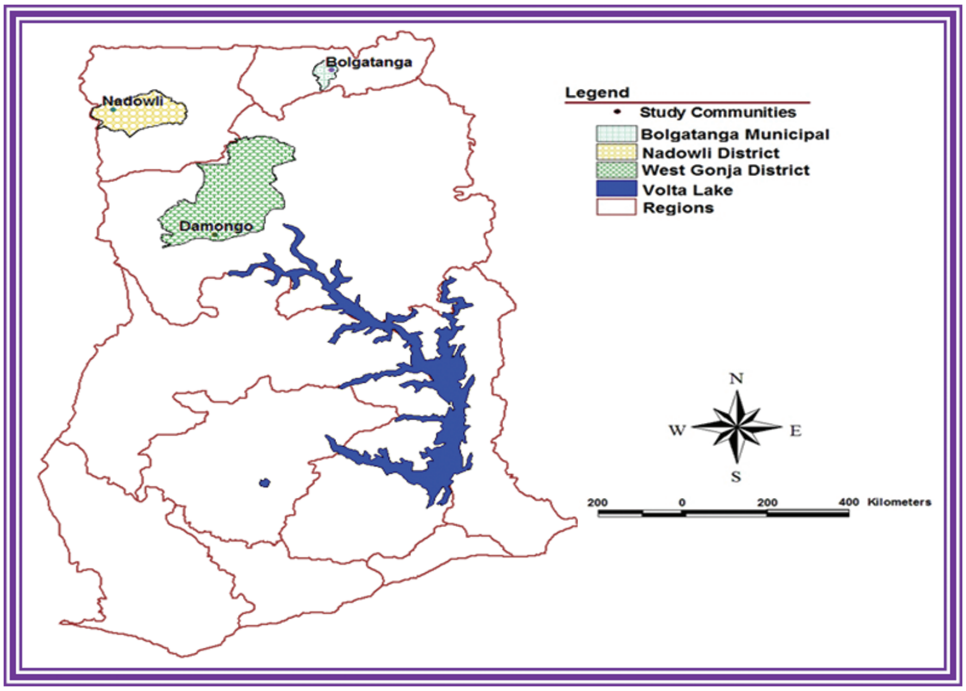

Fig. 1. Map of Ghana showing the sampling sites or locations (Source: Derf networks, 2015)

Table 1. Summary of oligonucleotide primer details used for molecular identification of *Aedes aegypti* subspecies

Primer	Sequence (5′–3 ′)	Melting Temperature (°C)	Band Sizes (bp) and sub species
Aedes B3	CATCCCCCTG	34	493–515 (*Ae. aegypti aegypti*) 1,085–1,121 and 1,707–1,773 (*Ae. aegypti formosus*)

Data Analysis

Data collected from all the study sites were analyzed using STATA version 13.0 statistical software package. Data for mosquito human landing catches were subjected to analysis using Kruskal–Wallis tests. The difference in the proportions of the *Aedes aegypti* subspecies were determined using χ^2 test. The man-vector contact (human biting rate) used to determine the potential risk of transmission was based on adult density estimated as the number of biting adult female *Aedes* mosquitoes per person-hour. Man–vector count exceeding two female bites per man hour was indicative of a significant risk of transmission of the disease (WHO 1971, WHO 1986). The overall potential risk of transmission for the areas were determined using the WHO criteria (WHO 1971, WHO 1986).

Results

Distribution and Abundance of Different Mosquito Species

In total, 1,206 adult female mosquitoes were collected in 2015 (n = 385) and 2016 (n = 821). Out of this total, 556 (46.1%) representing the largest collection was obtained from Bolgatanga, followed by 376 (31.2%) from Damongo and 274 (22.7%) from Nadowli. In terms of species distribution, *Aedes* mosquitoes collected were 749 (62.1% of total), *Anopheles* species were 122 (10.1%), and *Culex* species 335 (27.8%) (Table 2).

Distribution and Abundance of *Aedes* Mosquito Species

Out of the 749 *Aedes* mosquitoes collected, 314 (42%), 258 (34%), and 177 (24%) were collected from Bolgatanga, Damongo, and Nadowli, respectively. In total, 236 was collected from 2015 to 513 from 2016. *Aedes aegypti* was the predominant species biting man and representing 98%, whereas *Ae. vittatus* accounting for 2% (n = 749). The 2.0% *Ae.vittatus* was all collected outdoors from Nadowli during the dry and wet seasons in 2015 and 2016 (Table 3). In 2015, 96.2% of the *Aedes* collected was *Aedes aegypti* and 3.8% *Aedes vittatus* (n = 236). Relatively in 2016, the percentage of *Aedes aegypti* observed was higher with 98.8% and *Aedes vittatus* lower with 1.2% (n = 513). Small numbers of *Aedes* were caught both indoors and outdoors from all the study areas. The largest number, of *Aedes* species was collected from the Bolgatanga Municipality during the rainy season whereas the least number was collected from Nadowli. Data obtained further showed that in both 2015 and 2016, more *Aedes* mosquitoes were collected outdoors (568, 76%) than indoors (181, 24%) across all three study sites (Table 3).

The mean biting rate of *Aedes aegypti* was lower in 2015 (2.4 bites/person/h) than 2016 (5.3 bites/person/h), but that of *Aedes vittatus* was 0.1 bites/person/h in both years (Table 2). *Aedes* species collected in the rainy season were significantly higher than numbers collected in the dry season (P = 0.000; Table 2) in both years.

Biting Pattern of *Aedes* Mosquito Species

The predominant species biting man during the day was *Ae. aegypti* with peak biting period between 0600 and 0800 and 1500 and 1600 h GMT in 2015 and 2016 both in the rainy and dry seasons (Figs. 2 and 3). Small numbers of *Aedes aegypti* were collected in

Table 2. Mosquitoes species collected from the study areas in 2015 and 2016

Mosquito Species	Dry season				Rainy season				Total	Percentage	Mean biting rate bites/ person/hour
	Jan.	Feb.	Mar.	Apr.	Jul.	Aug.	Sep.	Oct.			
Aedes aegypti	7/19	4/15	11/28	13/28	52/121	49/86	56/132	35/78	227/507	59/61.8	2.4/5.3
Aedes vittatus	0/0	0/0	0/0	0/0	4/0	0/0	2/3	3/3	9/6	2.3/07	0.1/0.1
Anopheles **spp.**	0/0	0/0	0/3	3/9	6/15	6/16	15/26	11/12	41/81	10.6/9.9	0.4/0.8
Culex **spp.**	6/9	6/7	6/12	9/16	24/49	25/73	16/39	16/22	108/227	28.1/27.6	1.1/2.4
Total	13/28	10/22	17/43	25/53	86/185	80/175	89/200	65/115	385/821	100.0/100.0	4.0/8.6
Percentage	3.4/3.4	2.6/2.7	4.4/5.2	6.5/6.5	22.3/22.5	20.8/21.3	23.1/24.4	16.9/14.0		100.0/100.0	

Table contains number of mosquitoes collected in 2015 and 2016. Numbers collected in both years have been separated with a bar. Figures on the left side of the bar represent numbers collected in 2015 and those on the right side are numbers collected in 2016.

Reference criteria for WHO biting rate: <2 per an hour (unlikely occurrences of urban transmission) and >2 per an hour (high risk of urban transmission)

Table 3. *Aedes* species and their preferred biting locations in the three study areas

Study site	Species	HLC dry season		HLC wet season		Total
		Indoor (%)	Outdoor (%)	Indoor (%)	Outdoor (%)	
Bolgatanga	*Aedes aegypti*	9 (20.0)	36 (80.0)	80 (29.7)	189 (70.3)	314
	Aedes vittatus	0	0	0	0	0
	Aedes simpsoni	0	0	0	0	0
	Aedes africanus	0	0	0	0	0
Damango	*Aedes aegypti*	12 (22.2)	42 (77.8)	47 (23.1)	157 (76.9)	258
	Aedes vittatus	0	0	0	0	0
	Aedes simpsoni	0	0	0	0	0
	Aedes africanus	0	0	0	0	0
Nadowli	*Aedes aegypti*	4 (15.4)	22 (84.6)	29 (21.3)	107 (78.7)	162
	Aedes vittatus	0	9 (100)	0	6 (100)	15
	Aedes simpsoni	0	0	0	0	0
	Aedes africanus	0	0	0	0	0
Total		25	109	156	459	749

HLC, human landing catches.

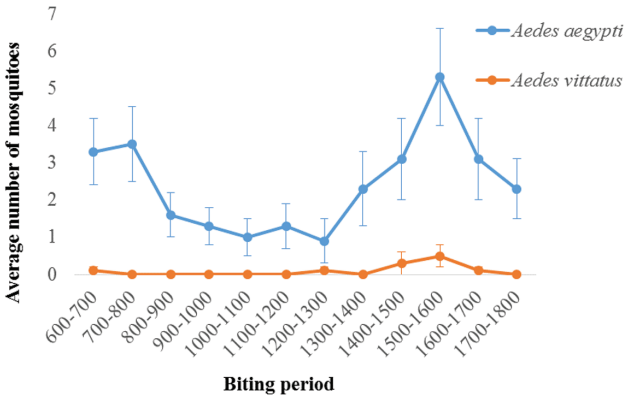

Fig. 2. Mean numbers of *Aedes* species and their biting patterns in 2015.

2015 compared with 2016. In 2015, it was observed that *Ae. aegypti* started biting from 0600 to 0700 h and peaked at 0700 and 0800 h GMT. Biting decreased between 0800 and 0900 h GMT. *Aedes vittatus* was found biting almost at the same time between 1500 and 1600 h GMT, but with a lower biting activity (Fig. 2). In 2016, biting peaked for *Ae. aegypti* between 0600 and 0700 h and decreased over a period of 7 h with another peak at 1500 and 1600 h GMT. *Aedes vittatus* had similar biting period, 1500 and 1600 h GMT but with very low biting activity (Fig. 3). No *Ae. simpsoni* and *Ae. africanus* were captured in 2015 and 2016.

Molecular Identification of *Aedes aegypti* Subspecies

Out of 110 subspecies identified, 69% were *Ae. aegypti aegypti* and 31 % were *Ae. aegypti formosus* (Table 4). *Aedes aegypti aegypti* obtained in the rainy season was 66% compared with 34 % *Ae. aegypti formosus* (Fig. 4 shows a gel image of results analyzed by RADP-PCR). Relatively small numbers of *Ae. aegypti aegypti* were collected during the dry season 22 (29 %) from all the study sites than in the rainy season 54 (71 %). There was no significant difference in the number of *Ae. aegypti aegypti* and *Ae. aegypti formosus* obtained both in the rainy and dry seasons (P = 0.209).

Discussion

The yellow fever outbreaks within the Upper West Region (GNA 2011) and Northern Region in 2011 and 2013, respectively, have necessitated the need for such vector studies in these areas. Although yellow fever is known to have been eliminated, these outbreaks may indicate a reemergence of the disease. It is important to have surveillance in such areas to monitor transmission especially within the northern regions which share borders with Burkina Faso where there were similar outbreaks in the recent past. Transmission of vector-borne diseases mainly depend on the abundance of vector species and man–vector contact rate (WHO 1971, Appawu et al. 2006).

In Ghana, several species of *Aedes* mosquitoes are known to be potential vectors of yellow fever with different geographical locations harboring different species. *Aedes africanus* and *Ae. luteocephalus* are mostly found in the forest areas whereas the urban areas have *Ae. aegypti* as the predominant vector (Vainio and Cutts 1998). Although this study focused on *Aedes* species, other mosquito species such as *Anopheles* spp. and *Culex* spp. were observed during the collection. The collections also showed that *Aedes* mosquitoes are abundant within the study areas which is a factor that can contribute to such outbreaks. Two main *Aedes* species were observed and these include *Ae. aegypti* and *Ae. vittatus*. These two species have also been reported by Mouchet (1971), Appawu et al. (2006) and Captain-Esoah et al. (2014) who worked on similar study in that part of Northern Ghana. The predominant species recorded in all three study sites was *Ae. aegypti* confirming earlier findings by Appawu et al. (2006) and Suzuki et al. (2016). Large numbers of *Ae. aegypti* observed in almost all the study sites could be due to the abundance of container breeding sites which are suitable sites for *Ae. aegypti* (Okogun 2003, Agha et al. 2017).

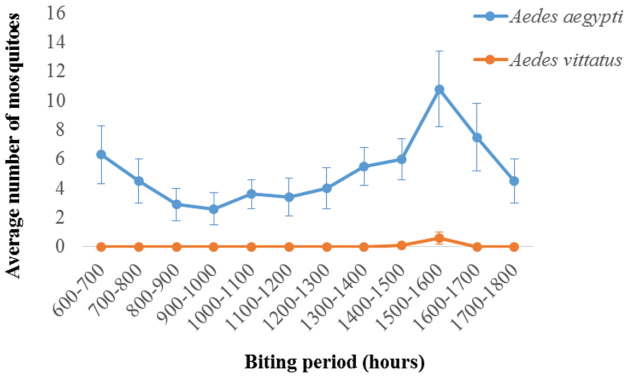

Fig. 3. Mean numbers of *Aedes* species and their biting patterns in 2016.

The molecular findings from this study revealed the presence of two *Ae. aegypti* subspecies (*Ae. aegypti aegypti* and *Ae. aegypti formosus*) in Northern Ghana as reported earlier by Ballinger-Crabtree et al. (1992). Larger numbers of *Ae. aegypti aegypti* were obtained during the rainy season compared with the dry season. Although, the differences in numbers were not statistically significant (P = 0.209), the presence of these subspecies identified in all the study sites should be monitored. The molecular method we use (RAPD-PCR) is an efficient tool for differentiation and genetically isolated of *Aedes* population (Ballinger-Crabtree et al. 1992). Nonetheless, there are some limitations which include a repetition of sample not giving similar band patterns due to random primer, although the interpretation of results will be the same. Additionally, mismatch between the primer could result in total absence of PCR products. It is possible that some of our unsuccessful reactions could have been analyzed using a more sensitive tool such as the one based on the mitochondrial gene cytohrome oxidase *c* subunit I or II (COI or COII) as described (Cook et al. 2005).

Ae. aegypti aegypti is reported to be mostly anthropophilic (WHO 1986) and relatively larger numbers were recorded biting, therefore the risk of urban yellow fever transmission if an infection is introduced into the communities is highly probable. Furthermore, the relatively higher biting rate values observed in all the study sites in 2015 and 2016 showed that the values are above the WHO threshold which consequently depicts high risk of urban transmission in all the areas (Table 2).

Adult *Aedes* spp. are diurnal feeders, with both endophilic and exophilic feeding behavior during the early or late hours of the day. They usually bite and rest outdoors before and after feeding (WHO 1986). Peak biting times are usually altered to times when their potential hosts are less protected (Karunamoorthi 2012).

Table 4. *Aedes aegypti* subspecies abundance in rainy and dry seasons.

Subspecies	Bolgatanga		Damongo		Nadowli		Total
	Rainy	Dry	Rainy	Dry	Rainy	Dry	
Aedes aegypti aegypti	14	6	22	0	18	16	76
Aedes aegypti formosus	16	1	6	0	6	5	34
Total	30	7	28	0	24	21	110

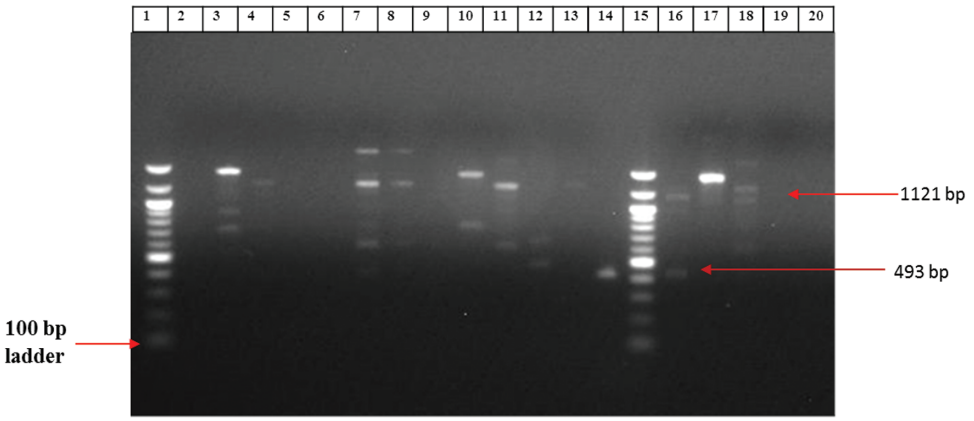

Fig. 4. Identification of *Aedes aegypti* subspecies by gel electrophoresis ethidium bromide stained 1% gel electrophoregram of amplified DNA of *Aedes aegypti s.l.* Lanes 1 and 15 are 100-bp DNA ladder. Lanes 7, 8, 12, and 14 are all *Aedes aegypti aegypti*. Lane 16 and 17 are *Aedes aegypti formosus*. Lane 19 is a negative control. Bands analysis (493–515 base pair fragment diagnostic for *Ae. aegypti aegypti* and 1,085–1,121 and 1,707–1,773 base pair fragments are diagnostic for *Ae. aegypti formosus*).

It was observed in this study that majority of mosquitoes were biting outdoors both in the dry and wet seasons. These findings support the observations made by WHO (1986) and Service (1996) who obtained larger numbers of *Aedes* species biting outdoors than indoors. Suzuki et al. (2016) reported that *Anopheles* mosquitoes are nocturnal and mostly endophagic while *Aedes* mosquitoes are diurnal and therefore take bloodmeal usually outdoors and rest outdoors (exophilic and exophagic).

Aedes aegypti is a highly domestic and diurnal feeder and has recently been shown to have modified period of their peak biting activities (Karunamoorthi 2012). This study revealed generally two main peak biting period (bimodal) from all the study sites (Figs. 2 and 3). It was observed from the study areas that, most of the community members who are mainly farmers usually performed many activities outdoors in the morning before leaving to their various farms. They often return home by 1600 and 1800 h GMT. The bimodal biting pattern observed may be an adaptation to coincide with the human activities in the various homes in order to ensure bloodmeal. The different minor peak biting periods, recorded in all the study sites, could be due to persistent biting and several partial blood meals taken within the day (Canyon et al. 1998, Scott et al. 2000, Ritchie 2014).

The intensity of disease transmission is therefore expected to be highest between the hours of 0600–0800 and 1500–1600 h GMT. This findings are significant to the formulation of future vector control programs within the study areas. This means that adulticiding such as fogging at the peak of biting could significantly reduce the vector populations especially where resources are limited. The use of protective clothing to reduce man-vector contact at these times of the day could also reduce transmission. Furthermore, vector control programs targeted at these times of the day could prove effective in reducing vector population and consequently disease transmission. It was observed that more *Ae. aegypti* were found biting in the rainy season, whereas few *Ae. vittatus* were recorded. This could probably lead to a high risk of urban cycle of yellow fever transmission as well as intermediate cycle of transmission in the rainy season.

The study areas have been recognized as potential risk areas for transmission of yellow fever in particular and other *Aedes aegypti*-borne diseases as a whole. This is because there have been reports of an outbreak of yellow fever across neighboring Burkina-Faso (WHO 2005) in the past. In later years, there were reports of yellow fever cases in the Upper West (GNA 2011) and some areas in the Northern region of Ghana (GHS 2012, WHO 2012a). These high-risk areas can also be confirmed from this study showing high man-vector contact rates recorded in the communities in the 2015 and 2016.

Conclusion

Aedes aegypti subspecies identified from all the three regions of the north are *Ae. aegypti aegypti* and *Ae. aegypti formosus*. *Aedes aegypti* has a bimodal peak biting period of 0600 and 0800 h and 1500 and 1600 h GMT in both the rainy and dry seasons. The man–vector contact rate supports a possible outbreak of viral hemorrhagic fevers if measures are not taken. The use of protective measures to reduce man-vector contact at these peak biting times of the day could be very appropriate measures in reducing disease transmission. Community-wide surveillance for *Aedes* infectivity with virus should be implemented in all the study areas to prevent further outbreaks. The information from all the study areas could be useful for future vector control programs within the three regions of the north and other parts of Ghana where *Aedes* species are abundant.

Acknowledgments

We wish to express our gratitude to the University for Development Studies for the financial support and providing laboratory space for the morphological identification of mosquitoes. We are thankful to the Noguchi Memorial Institute for Medical Research (NMIMR) especially staff of the Parasitology and Virology Departments for the laboratory support in the molecular identification of *Aedes* mosquitoes. From these Departments, we specifically want to acknowledge the support of the following individuals: Dr. Jewelna E. B. Akorli, Mba Tissomah-Mosoore, Dr. Sellase Pi-Bansa, and Joseph Harold Nyarko Osei as well as the staff of Virology Department, NMIMR, particularly, Dr. Kofi Humphrey Bonney and Dr. Evangeline Obodai, and staff and students of Vestergaards laboratory- NMIMR, Legon-Accra. We are grateful to Edmound Tampugre of the Navrongo Health Research Centre for his assistance in our field and laboratory work also on morphological identification of mosquitoes. We thank the staff of Queen of Peace Senior High School (QUEENSEC), Nadowli, District Health Directors of Damongo, Dr. Chrysantus Kubio and Hajia Fuseina Sulemana. Our gratitude also goes to the staff of the Mole National Park and Damango Senior High School (DASS) specially, Seidu Ibraimah for their tremendous support in the field work.

References Cited

Agha, S. B., P. D. Tchouassi, A. D. S. Bastos, and R. Sang. 2017. Assessment of risk of dengue and yellow fever virus transmission in three major Kenyan cities based on Stegomyia indices. PLoS Negl. Trop. Dis 1: 20.

Appawu, M., S. Dadzie, H. Abdul, H. Asmah, D. Boakye, M. Wilson, and D. Ofori-Adjei. 2006. Surveillance of viral haemorrhagic fevers in Ghana: entomological assessment of the risk of transmission in the Northern Region. Ghana Med. J. 40 (4): 137–141.

Ballinger-Crabtree, M. E., W. C. Black IV, and B. R. Miller. 1992. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Ae. aegypti* subspecies and populations. Am. J. Trop. Med. Hyg. 47 (6): 893–901.

Canyon, D. V., J. L. Hii, and R. Muller. 1998. Multiple host-feeding and biting persistence of *Aedes aegypti*. Ann. Trop. Med. Parasitol. 92: 311–316.

Captain-Esoah, M., S. Dadzie, C. A. Kyorku, D. A. Adabie-Gomez, M. Appawu, and D. A. Boakye. 2014. The ecology of potential yellow fever vectors in the paga community, kassena-nankana district, upper east region of ghana. J. Ghana Sci. Assoc. 2 (15): 72–83.

Clements, A. N. 1992a. The biology of mosquitoes: development, nutrition and reproduction. Chapman and Hall, London, United Kingdom, pp 1–8.

Clements, A. N. 1992b. Feeding behavior, natural food and nutritional relationship of mosquito larva. J. Vec. Ecol. 50 (2): 30–37.

Cook, S., M. Diallo, A. A. Sall, A. Cooper, and E. C. Holmes. 2005. Mitochondrial markers for molecular identification of *Aedes* mosquitoes (Diptera: Culicidae) involved in transmission of arboviral disease in West Africa. J. Med. Entomol. 42: 19–28.

Creswell, J. W. 2009. Research design. Qualitative, quantitative, and mixed methods approaches. SAGE Publication Inc. 3rd ed. pp 145–2230.

Derf networks. 2015. Graphics design centre. Navrongo, Ghana

Edward, E. D. 1984. Biting behavior by a haemolymph-borne factor in *Aedes*. J. Insect Physiol. 30 (2): 179–183.

Figueiredo, L. T. 2007. Emergent arboviruses in Brazil. Rev. Soc. Bras. Med. Trop. 40: 224–229.

Ghana Health Service. 2012. Yellow fever immunization. Available from http://www.ghanaweb.com/Ghana Homepage/News Archive/artikel.php?ID=224091. Accessed 4 December 2018.

Ghana Health Service. 2016. Yellow fever cases: health news. Available from https://www.Yellow-fever-cases-GHS-says-no-cause-for-alarm-445254. Accessed 19 October 2018.

Ghana News Agency. 2011. Yellow fever immunization. Available from ghananewsagency.org/health/uwr-records-three-yellow-fever-cases-35535. Accessed on 5 May 2018.

Ghana Statistical Service. 2014. 2010 Population and housing census: district analytical report: Bolgatanga Municipality, Upper East Region, pp. 1–75.

Ghana Web. 2016. Yellow fever kills 3 in West Gonja District. Available from http://www.ghanaweb.com/GhanaHomePage/health/Yellow-fever-kills-3-in-Gonja-406024. Health news, Friday 8 January 2016. Accessed 28 January 2017.

Gillett, J. D. 1972. Common African mosquitoes and their medical importance. William Heinemann Medical Books Limited, London, United Kingdom, pp. 1–103.

Harrington, C. L., D. J. Edman, and T. W. Scott. 2001. Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? J. Med. Entomol. 38: 411–422.

Harrington, L. C., T. W. Scott, K. Lerdthusnee, R. C. Coleman, and A. Costero. 2005. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. Am. J. Trop. Med. Hyg. 72: 209–220.

Huang, Y. and Ward, R. A. 1981. A pictorial key for the identification of the mosquitoes associated with yellow fever in Africa. Mosq. Syst. 13: 138–149.

Karunamoorthi, K. 2012. Yellow fever encephalitis: an emerging and resurging global public health threat in a changing environment. INTECH. 3: 207–230.

Klowden, M. J., and A. O. Lea. 1978. Blood meal size as a factor affecting continued host-seeking by *Aedes aegypti* (L.). Am. J. Trop. Med. Hyg. 27: 827–831.

Liew, C., and C. F. Curtis. 2004. Horizontal and vertical dispersal of dengue vector mosquitoes, *Aedes aegypti* and *Aedes albopictus*, in Singapore. Medical and Veterinary Entomology 18: 351–360.

Maciel-de-Freitas, R., R. Souza-Santos, C. T. Codeco, and R. Lourenco-de-Oliveira. 2010. Influence of the spatial distribution of human hosts and large size containers on the dispersal of the mosquito *Aedes aegypti* within the first gonotrophic cycle. Med. Vet. Entomol. 24: 74–82.

McClelland, G. A. H. 1960. A preliminary study of the genetics of abdominal colour variations in *Aedes aegypti* (L.) (Diptera: Culicidae). Ann. Trop. Med. Parasitol. 54: 305–320.

McClelland, G. A. H. 1974. A worldwide survey of variations in scale pattern of the abdominal tergum of *Aedes aegypti* (L.) (Diptera: Culicidae). Trans. R. Entomol. Soc. Lond.126: 239–259.

McKight, P. E., and J. Najab. 2010. Kruskal-wallis test. The Corsini encyclopedia of psychology. 1–1. doi:10.1002/9780470479216.corpsy0491.

Ministry of Food and Agriculture. 2010. Profile of Nadowli in the upper west region of ghana. Available from mofa.gov.gh/site/?page_id=1677. Accessed on 15 March 2018.

Ministry of Health. 2011. Yellow fever in Ghana. Available from http://www.who.int/csr/don/2012_02_03b/en/. Accessed on 7 November 2018

Ministry of Health. 2016. Ghana: 2016 Yellow fever – cases reported in the northern regions. Available from http://www:yellow-fever/743939-ghana-2016-yellow-fever-cases-reported-in-the-northern-regions Accessed on 9 January 2018.

Mouchet, J. 1971. Preliminary report on potential yellow fever vectors in Ghana. World Health Organization, Geneva, Switzerland, WHO/VBC/71.267, pp 1–11.

Neyman, J. 1934. On the two different aspects of the representative method: the method of stratified sampling and the method of purposive selection. J. Roy. Stat. Soc. 97: 558–625.

Okogun, G. R. A., B. E. B. Nwoke, A. N. Okere, J. C. Anosike, and A. C. Esekhegbe. 2003. Epidemiological implications of preferences of breeding sites of mosquito species in Midwestern Nigeria. Ann. Agric. Environ. Med. 10: 217–222.

Ritchie, S. A. 2014. Dengue vector bionomics: Why *Aedes aegypti* is such a good vector, pp. 455–480. *In* D. J. Gubler (ed.), Dengue and dengue hemorrhagic fever. 2nd ed. James Cook University, Cairns, Australia.

Scott, T. W., A. C. Morrison, L. H. Lorenz, G. G. Clark, D. Strickman, P. Kittayapong, H. Zhou, and J. D. Edman. 2000. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: population dynamics. J. Med. Entomol. 37: 77–88.

Service, M. W. 1996. Medical entomology for students. Chapman and Hall, London United Kingdom. pp. 1–80.

Smallegange, R. C., N. O. Verhulst, and W. Takken. 2011. Sweaty skin: an invitation to bite? Trends Parasitol. 27: 143–148.

Suzuki, T., J. H. Osei, A. Sasaki, M. Adimazoya, M. Appawu, D. Boakye, N. Ohta, and S. Dadzie. 2016. Risk of transmission of viral haemorrhagic fevers and the insecticide susceptibility status of *Aedes aegypti* (linnaeus) in some sites in Accra, Ghana. Ghana Med. J. 50: 136–141.

Vainio, J., and F. Cutts. 1998. Yellow fever. World Health Organization, Geneva, Switzerland, WHO/EPI/GEN/98. Vol. 11, pp. 1–87.

World Health Organization. 1971. Technical guide for a system of yellow fever surveillance. Wkly Epidemiol Rec. 46: 493–500.

World Health Organization. 1986. Prevention and control of yellow fever in Africa. Geneva, pp. 1–89.

World Health Organization. 2005. The yellow fever situation in Africa and South America in 2004. Wkly Epidemiol Rec. 80: 250–256.

World Health Organization. 2012a. Yellow fever in Ghana. Available from www.who.int/csr/don/2012_12_03b/en/index.html. Accessed on 19 November 2015.

World Health Organisation. 2012b. Global plan for insecticide resistance management in malaria vectors (GPIRM). Available from www.int/malaria/publications. Accessed on 12 November 2016.

World Health Organization. 2014b. Yellow fever: fact sheet. World Health Organization, Geneva, Switzerland 2002, Fact sheet No. 100. http://www.who.int/mediacentre/factsheets/fs100/en/. Accessed on 11 November 2016.