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DETECTION OF YELLOW FEVER AND DENGUE VIRUSES IN MOSQUITOES BETWEEN 2014 AND 2015 IN BAYELSA AND BENUE STATES OF NIGERIA

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Abstract

Dengue (DEN) and yellow fever (YF) outbreaks have been previously reported in Nigeria. In order to avoid another epidemic, the identification of the vectors of these viruses at different locations is a necessity. Female adult mosquitoes caught in and around human dwellings in the Benue and Bayelsa States, Nigeria, using human-baiting and spray-sheet methods between January 2014 and December 2015, were examined for YF and DEN viruses using reverse-transcription polymerase chain reaction (RT-PCR).In total 172,010 adult female mosquitoes were identified, put into 9,110 pools of mosquitoes and tested for YF and DEN viruses. The hourly biting activities, true infection rate (TIR) and the density of infected mosquitoes (DIM) were estimated for the species positive to YF and DEN viruses. Among twelve identified species – *Aedes luteocephalus* (Newstead, 1907), *Ae. aegypti* (Linnaeus, 1762), *Ae. cumminsii* Theobald, 1903, *Ae. africanus* (Theobald, 1901), *Ae. albopictus* (Skuse, 1894), *Ae. vittatus* (Bigot, 1861), *Anopheles gambiae* (Giles, 1902), *A. nili* Theobald, 1904, *Mansonia africana* (Theobald, 1901), *M. uniformis* (Theobald, 1901), *Culex annulioris* (Theobald,1901) and *C. quinquefasciatus* (Say,1823), three mosquito species, *Ae. luteocephalus*, *Ae. aegypti* and *A. gambiae*, were positive for YF in Oju and Ega, while DENV-3 was detected in samples of *M. africana* from Ikarama. The biting patterns of these positive mosquito species showed both day and night activities, except the population of *A. gambiae* from Ega, which typically demonstrated nocturnal activity extended until dawn/early morning hours. *Ae. aegypti, A. gambiae* and *Ae. luteocephalus* could represent the primary vectors of YF in the Benue and Bayelsa States. The role of *M. africana* in transmitting DENV-3 also requires immediate investigation.

KEY WORDS: Dengue; yellow fever; *Aedes aegypti*; *Aedes luteocephalus*; *Anopheles gambiae*; *Mansonia africana*

Introduction

With a history of outbreaks of epidemic proportions in rural and urban communities, the yellow fever (YF) virus and dengue fever virus (DENV) are regarded as two of the most important arbovirus-causing diseases in Nigeria (Monath *et al.,* 1973; De Cock *et al*., 1988). Over 100 million Nigerians are reportedly at risk of YF, while DENV has been identified as an emerging cause of fever alongside the commonly known malaria (Oyero & Ayukekbong 2014). *Aedes* species' densities and biting rates have been sufficiently implicated in the outbreaks of YF and DENV (Diallo *et al*., 2008; Guzman *et al.,* 2010). Surveillance indicators commonly considered in the assessment of risk(s) of viral transmission include the level of human infection, the infection rate of field-collected mosquitoes, density of infected mosquitoes (DIM, estimates the frequency of contact between humans and infected mosquitoes within a given time (Gu *et al*., 2008)), biting preferences (Bustamante & Lord, 2010) and biting patterns (Korgaonkar *et al.,* 2012). In Nigeria, there is a lack of research employing screened adult female mosquitoes to determine human diseases incidence.

In this study, samples were screened using the reverse-transcription polymerase chain reaction (RT-PCR) for YF and DENV. The true infection rate (TIR) was calculated according to techniques reported by Diallo *et al*. (2000), while density of infected mosquitoes (DIM) was calculated based on methods by Ezenwa *et al.* (2006) and Gu *et al*. (2008). The use of both TIR and DIM in mosquito surveillance is necessary as the former does not give information on the transmission risks, but the latter does. Transmission risks are important in deciding the prioritization of a locality for vector control. Data on DIM are important to adequate interpretation of implications of infection rates.DENV is an RNA virus with five serotypes based on their antigenicity (DENV-1, DENV-2, DENV-3,DENV-4 and DENV-5) (WHO, 2009; Normile, 2013), but only DENV-1 and DENV-2 have been reported in Nigeria (Fagbami *et al*., 1977). The introduction of other serotypes is a possibility as infected travelers pose great risks (Messer *et al*., 2003).

In order to assess the potentials of a possible outbreak from an entomological perspective, two states were selected in Nigeria where a 2-year mosquito survey was carried out. One of the states had previously experienced an epidemic of YF, while the other is without a history of either YF or DENV outbreaks. The mosquito species caught in these study areas are presented, their infection rates as well as their indoor and outdoor hourly biting patterns. This study reports for the first time another DENV serotype in Nigeria; and identified potential vectors of YF and DENV in addition to the widely and commonly reported vectors.

Materials and Methods

Study area

Mosquitoes were collected from January 2014 to December 2015 from eight communities in four Local Government Areas (LGAs) from two states (Fig.1a): Benue State, which has a history of YF epidemic (Monath *et al*., 1973) and Bayelsa State, which is without any history of YF or DENV outbreak. Two LGAs, Oju [Oju (urban) and Ega (rural)] and Otukpo [Otukpo (urban) and Otukpoicho (rural)] in Benue State (7° 41'-10° 11' E and 6°25'-8° 8' N)) were selected for this study (Figs. 1 b, 1c). Benue State experiences two distinct seasons, the rainy season is from April to October, while the dry season begins in November and ends in March. Oju town is a commercial area with a large sprawling market, while Ega is a rural community with houses built with bricks and thatched roofs.

In Bayelsa State (5° 10'- 6° 55' E and 4° 45' - 5° 50' N), two LGAs [Yenagoa: Ekeki (urban), Ikarama (rural) and Ogbia: Immiringi (urban), Otuegila (rural)] were selected for this study (Figs. 1d, 1e). Bayelsa State, one

of the major oil-producing states in Nigeria, has a heavy-rainfall season of not less than 340 days in the year and a short dry season.

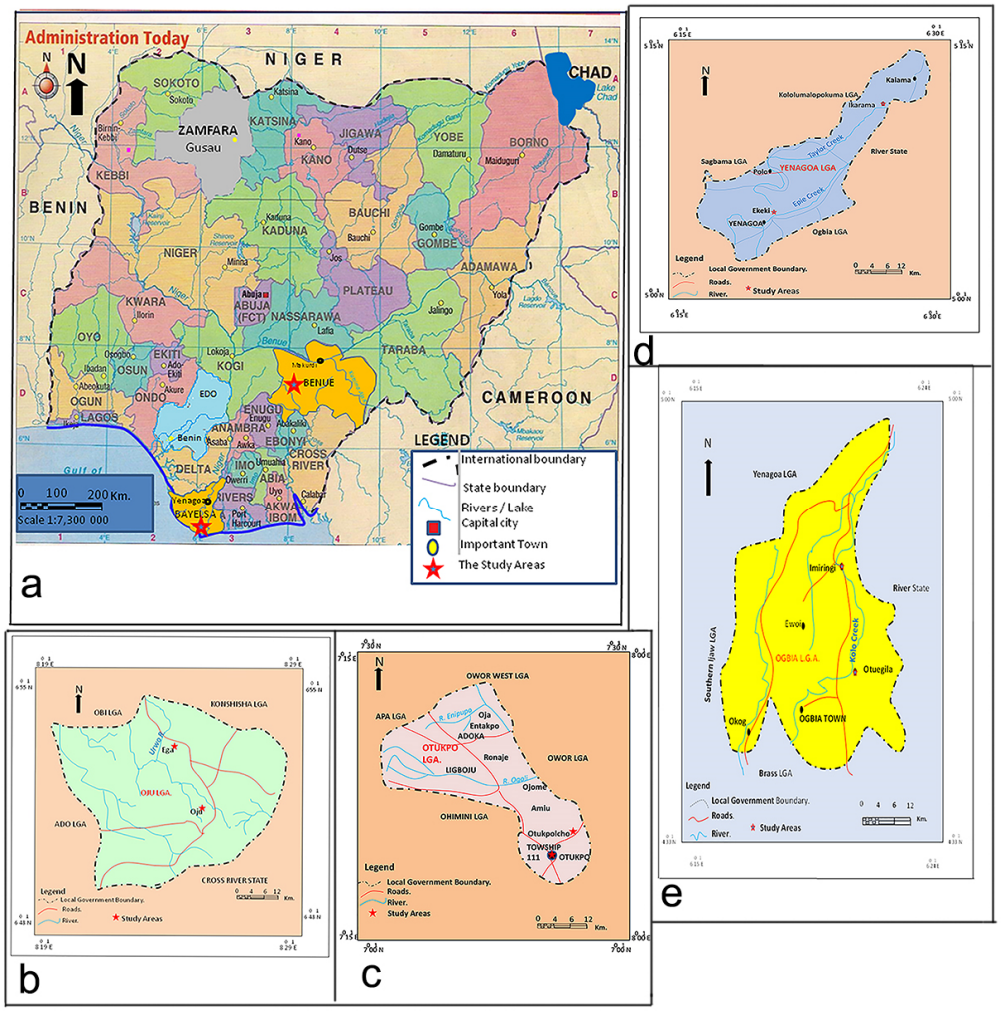

Figure 1. Maps. **a.** Map of Nigeria showing the two sampled states, Benue (with history of YF epidemic) and Bayelsa (without any history of YF or DENV outbreaks). **b.** Map of Oju LGA in Benue state showing the study areas, Oju (urban) and Ega (rural) communities. **c.** Map of Otukpo LGA in Benue state showing study areas, Otukpo (urban) and Otukpoicho (rural) communities. **d.** Map of Yenagoa LGA in Bayelsa state showing the study areas, Yenagoa (urban) and Ikarama (rural) communities. **e.** Map of Ogbia LGA in Bayelsa state showing study areas, Imiringi (urban) and Otuegila (rural) communities.

Ethical consideration

Before the mosquito survey, several visits to the study areas were made and meetings with the community heads and relevant groups took place. These meetings were aimed at obtaining permission as well as seeking cooperation during the mosquito collection exercise. Ethical permission was obtained from University of Nigeria Research Ethics Committee.

Mosquito collection

Two sampling methods were employed in the collection of adult female mosquitoes. These were the spray-sheet technique and the stationary direct human-bait catches. Thus, an entomological survey team was created consisting of two sampling groups, with four persons in each group. One group applied the human-bait sampling technique, while the other applied the spray-sheet adult sampling method. A total of 192 houses were chosen by random sampling (every other house) for the spray-sheet technique, while the human exposition was carried out in 24 houses selected randomly from the 192 houses. A room was regarded as one sample or sampling unit and only one room was sampled from each house. Each room with the corresponding identification number was sampled bi-weekly all year round (2014 and 2015).

Spray-sheet technique

Spray-sheet collection was carried out in line with Service (1976) approach. All the residents, furniture, food items and water containers were removed from the room. An average of 20 minutes was spent in each house for spraying and collection. The calico/china white sheets were laid over the entire floor, the bed surfaces and on different objects that were not removed. All doors and windows were closed and houses sprayed with pyrethrum-based insecticides by field workers wearing protective nose masks. The spray was directed at all potential escape routes such as doors, windows, roofs and ceilings. The mosquitoes fell on the white sheet and the sprayer returned to the hut 10 minutes later and collected the mosquitoes with a pair of forceps. The mosquitoes were identified, sexed, and females pooled into pools of 1-20 mosquitoes/pool to increase the accuracy of the mosquito infection rate as reported by Gu *et al*. (2008). Since mosquito infection rate calculations assume that one mosquito in each pool is positive, having pools of various smaller sizes increases the chances of accommodating more positive samples and increasing the accuracy of the calculations (Gu *et al*., 2008). The pools were stored in ice-packed cooler bags for onward transportation to the laboratory. In the laboratory, the method, location and date of collection were clearly written on each pool and these were stored at -70 °C until assayed for viruses.

Stationary direct human-bait catches

Adult female mosquitoes were collected using the stationary direct human-bait catches described by Service (1976). In this method, humans acted as both bait and collector. Four human baits were used for the study, and only mosquito scouts with valid immunization against YF were recruited. Two collectors seated on benches (36 cm high) were stationed side-by-side in a room (without mosquito netting) with its doors and windows open (indoors). Similarly, this was the arrangement in the open compound of the same house (outdoors).

The collectors folded their trousers, thereby exposing their legs, which are favored biting sites for numerous mosquito species. When settled, the mosquitoes were caught by carefully placing a test tube over them before they bit and plugging it with cotton wool. Torchlight was used for night collections. The human-baiting method was conducted once fortnightly for a 24-h period (daylight, crepuscular and night). The time each mosquito was caught was documented. Mosquito catches were then placed in hourly batches at the end of

each 24-h baiting. Collected mosquitoes were also identified, properly documented, noting the time of catches, total number caught per species and total man-hours spent during baiting. The man-hour biting rate was calculated using data generated and the adult female mosquitoes were put in pools as reported above and used for the detection of viruses.

Detection of viruses in mosquito pools

Detection of DEN was undertaken using primers described by Lanciotti *et al*. (1992) (Table I). The selection of the consensus primer D1 and the type-specific primers (TS1, TS2, TS3 and TS4) from the published sequence was done with the aid of sequence analysis computer program and synthesized by Inqaba Fermenters (USA) in South Africa. Amplifications were carried out in 25 µl of reaction mixture containing the following components: 5.0 µl water (Thermo Scientific, USA), 2.0 µl of RT enhancer (Thermo Scientific, USA) 10 µl of dNTPs PCR Reddy Mix (Biolab Innovative Research Technologies, Poland) and 1 µl of verso enzyme mix (Thermo Scientific, USA). The reactions were allowed to continue in a thermocycler (MJ Research, Marshall Scientific, USA) involving an initial conversion of RNA to DNA copy (cDNA) at 50 °C for 15 min and inactivation of enzyme (95 °C, 2 min). Thereafter, the reactions proceeded with 45 cycles of denaturation (95 °C, 2 s), primer annealing (60 °C, 30 s) and primer extension (72 °C, 1 min). Finally, the DNA strands that were separated under high temperature, attached to each of the created four strands under lower temperature conditions with subsequent primer extension (72 °C, 5 min). Meanwhile, for YF detection, primers described by Fulep *et al.* (1993) and Brozoni *et al.* (2005) were used (Table I). The same cycling conditions were followed as described for DEN except for the step of primer annealing (54 °C, 30 s). The PCR products were mixed with standard loading dye (1kb loading dye) and electrophoresed in a 2 % agarose gel stained with ethidium bromide (2 µg/ml); and then visualized and photographed under ultraviolet illumination. A negative control (distilled water) was included in each group of reactions.

Table I. DNA sequence of the primers used to amplify and type yellow and dengue viruses. D1= forward; TS (1, 2, 3 and 4) = reverse; FG1= forward; YF= reverse.

Organism	Primer	Sequence (5´ to 3´)	Genome position	Amplicon (bp)	References
Dengue virus (DENV)	D1	TCAATATGCTGAAACGCGCGAGAAACCG	134-161	511	Lanciotti *et al.,* 1992
DENV-1	TS1	CGTCTCAGTGATCCGGGGG	568-586	482	
DENV-2	TS2	CGCCACAAGGGCCATGAACAG	232-252	119	
DENV-3	TS3	TAACATCATCATGAGACAGAGC	400-421	290	
DENV-4	TS4	CTCTGTTGTCTTAAACAAGAGA	506-527	392	
Yellow fever (YF) virus	FG1	TCAAGGAACTCCACACATGAGATGTACT	8270-8297	958	Brozoni *et al*., 2005
	YF	TCAGAAGACCAAGAGGTCATGT	8502-8523	253	Fulep *et al*., 1993

Statistical analysis

The true infection rate (TIR, number of positive mosquitoes per 100 mosquitoes tested) was calculated with pools of between 1-20 mosquitoes per pool using the methods of Gu *et al.* (2008), Ezenwa *et al*. (2008) and Diallo *et al*. (2000). Here it was assumed that one mosquito was positive in each positive pool. Thus, the TIR = the number of mosquitoes tested/positive pools x100.The density of infected mosquitoes (DIM, product

of mosquito abundance and proportion of infected mosquitoes) of each species was estimated using the formula: Total number of adult mosquitoes of same species collected using the spray-sheet method, and human-bait catches (HBC)/Total days spent during human-baiting and spray-sheet catches x TIR, were calculated per species using the methods of Ezenwa *et al.* (2006) and Gu *et al.* (2008). The number of mosquitoes biting a bait/hour, otherwise known as the man-hour biting index, was calculated by dividing the total each for a specific mosquito species by the total man-hours during baiting. The Mann-Whitney rank sum test was used to estimate the differences between the outdoor and indoor biting patterns, while an unpaired T-test was applied on TIR data of seasonal and yearly variations. The statistical packages used for graph plot and data analysis were Graph Pad Prism version 5 and Sigma Stat.

Results

Infection rates of screened mosquitoes

A total of 172,010 adult female mosquitoes were caught from the Benue and Bayelsa States within a two-year period from January 2014 to December 2015. These were put into 9,110 pools and screened for YF and DENV infection. Thetotal number of mosquitoes caught by LGAs was: 31,644 in Oju and 49,251 in Otukpo (Benue); 46,687 in Yenagoa and 44,428 in Ogbia (Bayelsa). In Oju LGA (Oju town and Ega rural), the following mosquito species caught were identified: *Aedes luteocephalus* (3,838), *Ae. cumminsii* (151), *Ae. africanus* (1,819), *Ae. aegypti* (8,757), *Ae. albopictus* (2,663), *Anopheles gambiae* (7,531), *A. nili,* (134) and *Culex quinquefasciatus* (6,751). Among these, only three species (*Ae. luteocephalus, Ae aegypti* and *A. gambiae*) were positive for YF (Fig. 2a). In Otukpo LGA (Otukpotown and Otukpoicho rural), the following mosquito species were captured: *Ae. luteocephalus* (1,529), *Ae. africanus* (472), *Ae. vittatus* (1,556), *Ae. aegypti* (10,744), *Ae albopictus* (6,505), *C. quinquefasciatus* (8,384) and *A. gambiae* (20,061). None of the pools of these mosquitoes was positive for YF or DENV. However, in Yenagoa LGA (Ekeki and Ikarama communities), among the species of mosquitoes collected [*Ae. luteocephalus* (108), *Ae. africanus* (1,000), *Ae. aegypti* (1,962), *Ae. albopictus* (1,235), *Mansonia uniformis* (4,122), *M. africana* (9,347) *A. gambiae* (6,606) and *C. quinquefasciatus* (22,038), *C. annulioris* (269)], only some pools of *M. africana* from the 2015 collections in Ikarama were positive for DENV-3 (Fig. 2b). For Ogbia LGA (Imiringi town and Otuegila rural), none of the mosquitoes caught [*Ae. luteocephalus* (108), *Ae. africanus* (54), *Ae. aegypti* (2,694), *Ae. albopictus* (782), *M. uniformis* (6830), *M. africana* (15,522), *A. gambiae* (11,092) and *C. quinquefasciatus* (7,303)] was positive for YF or DENV.

The TIR of mosquitoes and DIM for 2014 and 2015 were estimated for the communities found positive for DENV and YF (Ega, Oju and Ikarama) (Table II). In the Ega community, data for the two years showed that *A. gambiae* had the highest TIR and DIM, while in Oju town, *Ae. aegypti* had the highest DIM, with a relatively lower TIR than *A. gambiae* in 2014.

In 2014, no YF-positive *Ae. luteocephalus* was detected in Oju. However, in 2015, YF-positive *Ae. luteocephalus* mosquitoes were recorded in Oju with significantly lower TIR and DIM in comparison to *Ae. aegypti* and *A. gambiae*. In addition, the TIR and DIM for *Ae. luteocephalus* in Oju was far lower than in Ega, while *Ae. aegypti* had significantly higher TIR and DIM in Oju town than Ega. In the Ikarama community, mosquitoes of *M. africana* positive to DENV-3 infection were recorded only in 2015, with a TIR of 0.78 %, while the DIM was 7.86 %.

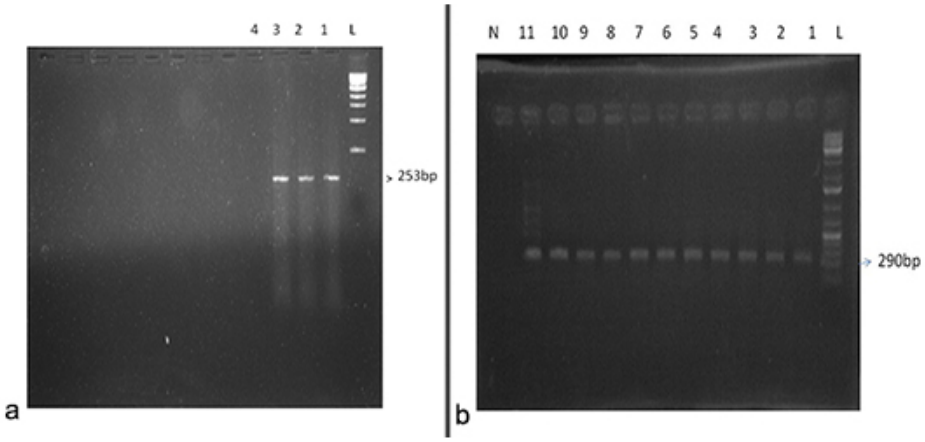

Figure 2. Representative photo gel of YF and DENV-3 positives by Reverse transcriptase PCR of mosquito samples. The PCR products were observed in ethidium bromide – stained agarose gel. Arrow point to obtained amplicon of expected size. **a.** Gel photo of YF positives *A. gambiae* (1), *Ae. aegypty* (2) and *Ae. luteocephalus* (3); 4- negative control; L- Ladder. **b.** Gel photo of DENV-3 positives (1-11) in *M. africana*; N- negative control; L- Ladder.

Table II: Mosquito infection rates and density of infected mosquitoes collected in Ega, Oju and Ikarama communities, using spray-sheet and stationary direct human bait techniques in 2014 and 2015.
Note: Data for Ega and Oju are related to infection with YF; data for Ikarama are related to infection with DENV-3; *TIR = True infection rate (estimated number of positive mosquitoes per 100 mosquitoes tested) according to Diallo *et al*. (2000); **DIM = Density of infected mosquitoes (Total no. of mosquitoes collected / no. of collection nights x TIR(384 collection nights was used for the calculations) according to Ezenwa *et al*.(2008); SE = standard error.

		2014				2015			
Community	**Mosquito**	**Total mosquitoes caught (No. of pools)**	**No. of positive pools**	**TIR*(SE) %**	**DIM**(SE) %**	**Total mosquitoes caught (No. of pools)**	**No. of positive pools**	**TIR*(SE) (%)**	**DIM**(%)**
Ega (rural)	*Ae. luteocephalus*	1661 (93)	32	1.92(0.22)	8.81	1836(102)	41	2.23(0.09	10.67
	A. gambiae	1710(95)	38	2.2(0.15)	9.93	2070(115)	58	2.80(0.37)	15.10
	Ae. aegypti	616(35)	11	1.78(0.17)	2.72	865(48)	16	1.85(0.14)	4.17
Oju (urban)	*Ae. luteocephalus*	89(5)	-	-	-	252 (14)	2	0.77(0.16)	0.51
	A. gambiae	1741(97)	46	2.64(0.28)	11.97	2010(112)	58	2.88(0.09)	15.18
	Ae. aegypti	2827(158)	62	2.19(0.02)	16.12	4449(248)	134	3.01(0.12)	34.87
Ikarama (rural)	*M.africana*	2291(127)	-	-	-	3870(215)	30	0.78(0.23)	7.86)

Daily biting activity of mosquito species positive to yellow fever and dengue fever viruses

In this study, *A. gambiae* in Ega and Oju exhibited multiple peaks of biting activity during the day, as demonstrated in both of the studied years (Fig.3a-3d). The highest peak of the biting activity of this species indoors and outdoors occurred during the same night period (between 02.00 and 03.00), except in Ega in 2015, where both peaks of indoor and outdoor activity were recorded an hour later (03.00-04.00).

Biting duration was higher indoors than outdoors in both localities, and in Oju it increased indoors. However, the biting duration period was longer in Oju in both years than Ega. It was within 13 h in Oju (18.00-07.00 h) and 11 h in Ega (21.00-08.00) (3a-d). *Ae. luteocephalus* exhibited single peaks in both locations with biting peaks mainly at the crepuscular period (Fig. 4a-4d). In both years, the indoor and outdoor activity peaks in Oju occurred within the same period (18.00-19.00). In Ega, in both years, the peak of the outdoor activity was recorded 2 h later (20.00-21.00) than in Oju.

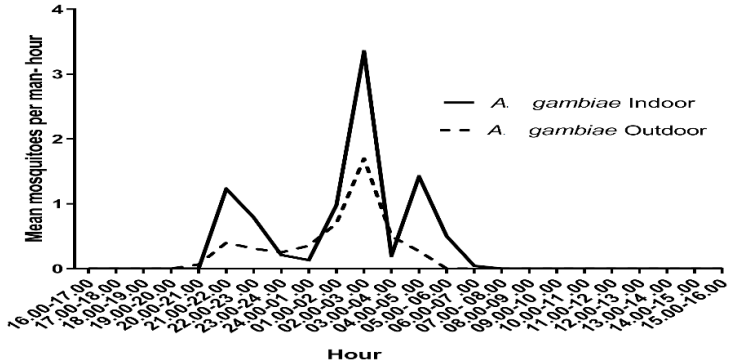

Figure 3a. Hourly biting pattern of *A. gambiae* in Ega for 2014.

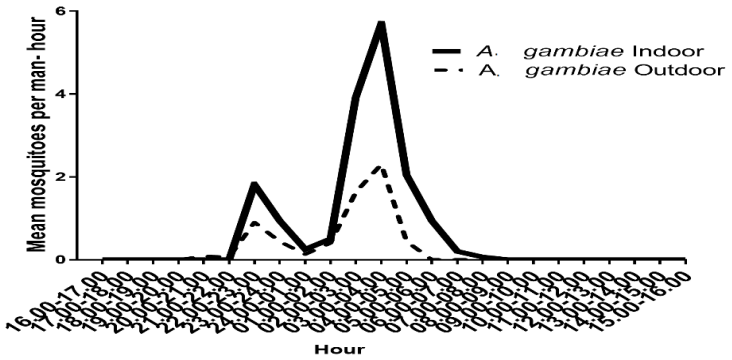

Figure 3b. Hourly biting pattern of *A. gambiae* in Ega for 2015.

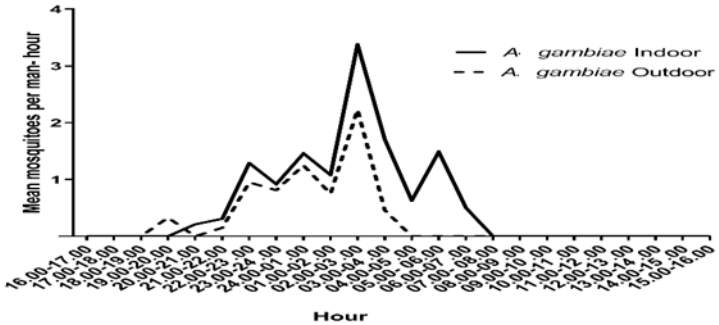

Figure 3c. Hourly biting pattern of *A. gambiae* in Oju for 2014.

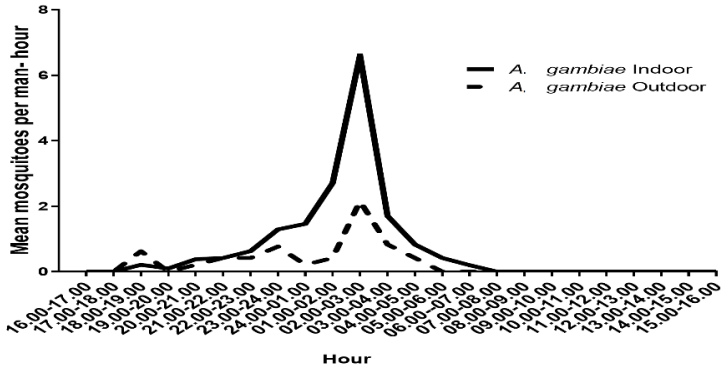

Figure 3d. Hourly biting pattern of *A. gambiae* in Oju for 2015.

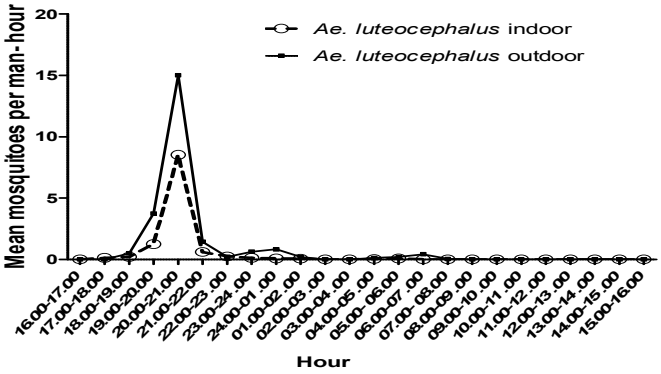

Figure 4a. Hourly biting activities of *Ae. luteocephalus* in Ega for 2014.

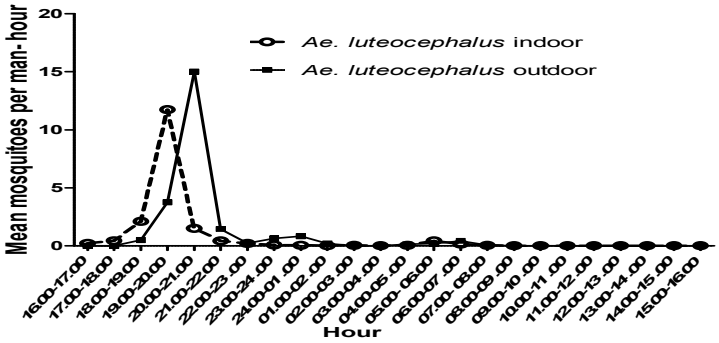

Figure 4b. Hourly biting activities of *Ae. luteocephalus* in Ega for 2015.

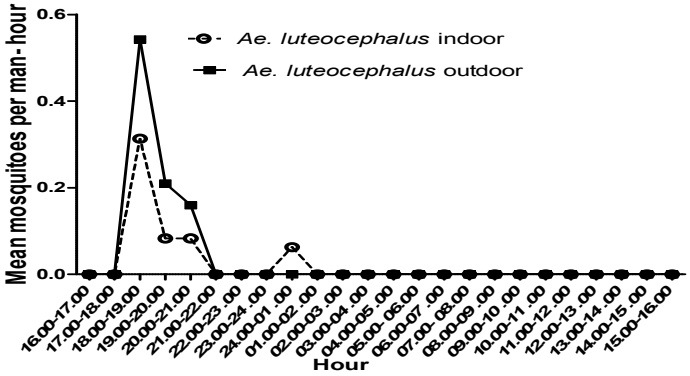

Figure 4c. Hourly biting pattern of *Ae luteocephalus* in Oju for 2014.

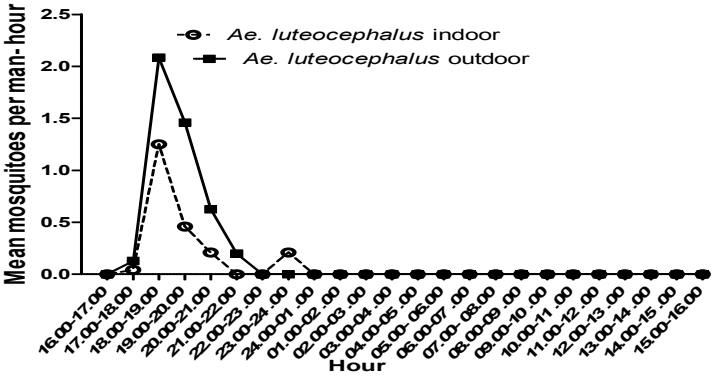

Figure 4d. Hourly biting pattern of *Ae luteocephalus* in Oju for 2015.

The indoor biting activity peak of *Ae. luteocephalus* in Ega coincided with the outdoor peak in 2014, while it occurred one hour earlier than the outdoor peak in 2015 (19.00-20.00). Biting duration in Ega was generally longer in 2015, andin this season the indoor activity was extended (15 h) than the outdoor activity (13 h). The indoor activity in 2015 was also significantly longer than the indoor activity recorded in 2014 (6 h). Biting duration outdoors was shorter in Oju (3 h in 2014 and 5 h in 2015) than in Ega (outdoor: 13 h in both years). Duration of the indoor biting activity in Oju was 7 h in both years; in Ega it was 6 h in 2014 but in 2015 it was extended to a longer period of 15 h. The biting activities of *Ae. aegypti* are shown in Figs. 5a-5d. The peak of the biting activity was an hour after crepuscular (19.00-20.00) indoors and outdoors in Ega in 2014, while in 2015, it was in the crepuscular period, an hour earlier. However, in Oju, the biting activity was recorded earlier, with a peak at 17.00-18.00 in both years. *Aedes aegypti* was more endophagic than exophagic, clearly feeding longer indoors than outdoors in both locations and both years. Indoor biting was longer in 2015 than in 2014 in both locations, and it was performed within the crepuscular, night and early morning periods. Biting occurred within 15 h in Ega, and 14 h in Oju. *M. africana* exhibited multiple biting peaks in both years (Figs. 6a-6b). *M. africana* was mainly exophagic biting longer (14-15 h) outdoors than indoors (11 h). Outdoor activity of this species demonstrated two major peaks: at 17.00-18.00 and in early morning hours (05.00-06.00). Major peaks of the indoor biting activity occurred in the same nocturnal period in both years (21.00-22.00 and 01.00-02.00).

The mosquito man-hour biting index of indoor and outdoor mosquitoes in Ega, Oju and Ikarama communities are shown in Table III. In Ega, the mean number of mosquitoes/man-hour outdoors and indoors was highest in *Ae. luteocephalus* as compared to *A. gambiae* and *Ae. aegypti*. The outdoor man-hour biting index of *Ae. luteocephalus* was significantly higher than indoors (T=36.00; P<0.001). Meanwhile, differences in the indoor and outdoor man-hour biting indices of *A. gambiae* and *Ae. aegypti* were not significant. In Oju town, *Ae. aegypti* had relatively higher indoor and outdoor man-hour biting indices than *Ae. luteocephalus* and *A. gambiae.* However, of the three mosquito species encountered, only *A. gambiae* indoor biting rates were significantly higher than outdoor (T=100; P<0.001). *M. africana* outdoor biting rates were significantly (T = 91; P<0.001) higher than indoors.

Table III. Man-hour biting index of mosquitoes collected using stationary human bait technique in Ega, Oju and Ikarama communities in 2014 and 2015.

Locations	Mosquito species	Mosquito man-hour biting index			
		Indoor		Outdoor	
		2014	2015	2014	2015
Ega	*Ae. luteocephalus*	0.47	0.73	0.97	1.05
	A. gambiae	0.37	0.68	0.19	0.26
	Ae. aegypti	0.06	0.36	0.09	0.18
Oju	*Ae. luteocephalus*	0.02	0.09	0.04	0.19
	A. gambiae	0.54	0.71	0.28	0.27
	Ae. aegypti	0.98	1.63	0.63	0.70
Ikarama	*M. africana*	0.623	0.87	0.91	1.48

Seasonal variation of infection in mosquitoes

The seasonal variation of infection expressed by the TIR of the three YF-positive mosquito species (*Ae. luteocephalus*, *Ae. aegypti* and *A. gambiae)* in Benue State is presented in Figs. 7 and 8. In 2014 in

Ega, none of the *Ae. luteocephalus* mosquitoes was infected during most of the dry season months; infection was recorded from Marchto October (Fig.7) and peaked in June (3.6 %) and August (2.8 %). In 2015, infection was recorded a month earlier, in February (1.1 %), and it peaked in April (3.0 %) and September (2.9 %). Infections of *Ae. aegypti* peaked in April (3.78 %) and October (2.0 %) in 2014; April (2.5 %) and September (1.53 %) in 2015 (Fig. 7). Infections of *A. gambiae* in the locality of Ega were continuously reported from February 2014 to December 2015 (Fig. 7) and the TIR reached the highest peaks in August in both investigated years (3.1 % and 3.6 % in 2014 and 2015, respectively). Differences in the TIR between dry and wet seasons were only significant for *Ae. luteocephalus* ($P<0.0001$).

In Oju, the infection of *Ae. luteocephalus* was recorded throughout 2014 and 2015 (Fig.8) and peaks were in August (2.21 %) in 2014 and October (3.0 %) in 2015.For *Ae. aegypti,* no infection was recorded in January and December 2014. The highest peak was recorded in March (3.0 %) in 2014, while in 2015 it coincided with *Ae. luteocephalus* since it was recorded in October (3.1 %) (Fig. 8). Meanwhile, for *A. gambiae*, infection was also all year round with peak infections in August, reaching TIR of 2.3 % and 3.2 % in 2014 and 2015, respectively (Fig. 8). The differences between the dry and wet seasons for the three mosquito species, and also between years (2014 and 2015) were not statistically significant. In addition, DENV infection of *M. africana* was recorded in Ikarama in 2015 (Fig. 9), in both dry and wet seasons, with the highest peaks of infection in June (2.5 %) and October (2.4 %). The mean difference in TIR between the dry and wet seasons was significant ($P=0.0052$).

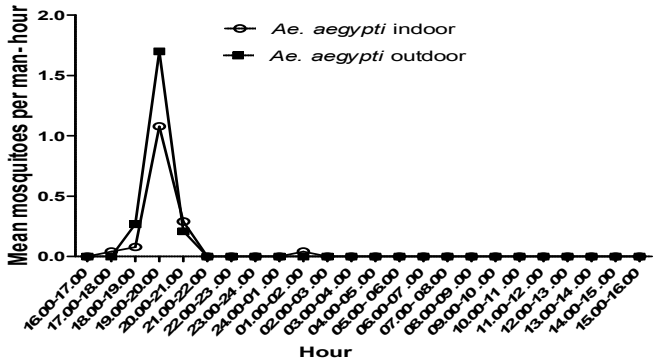

Figure 5a. Hourly biting pattern of *Ae. aegypti* in Ega for 2014.

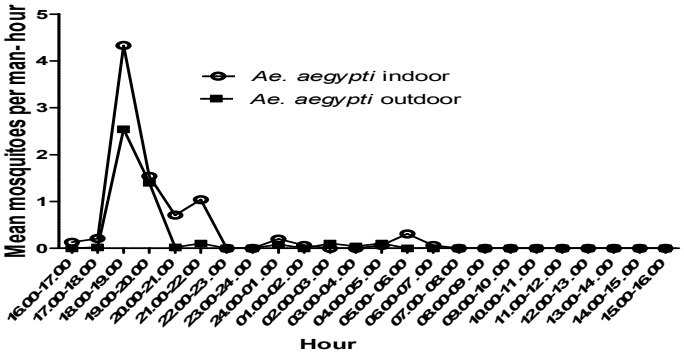

Figure 5b. Hourly biting pattern of *Ae. aegypti* in Ega for 2015.

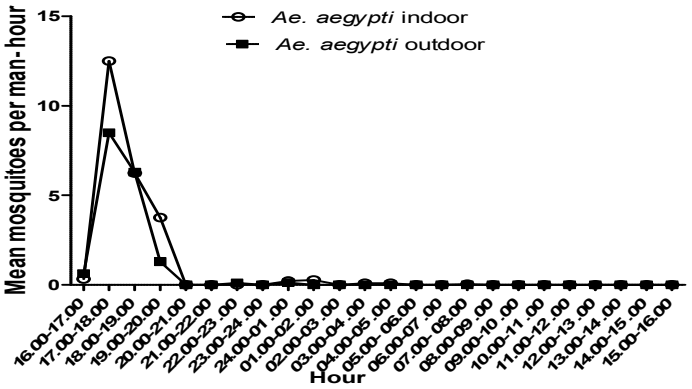

Figure 5c. Hourly biting pattern of *Ae. aegypti* in Oju for 2014.

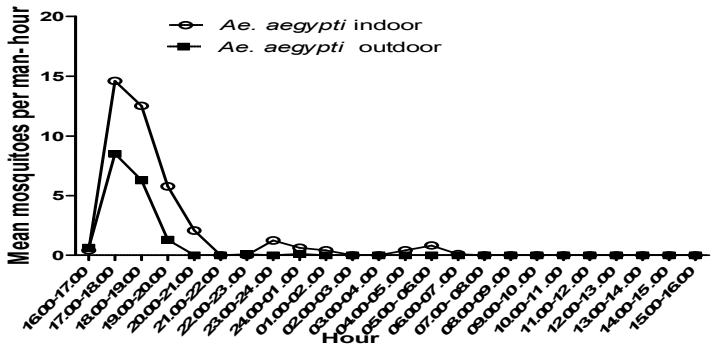

Figure 5d. Hourly biting pattern of *Ae. aegypti* in Oju for 2015.

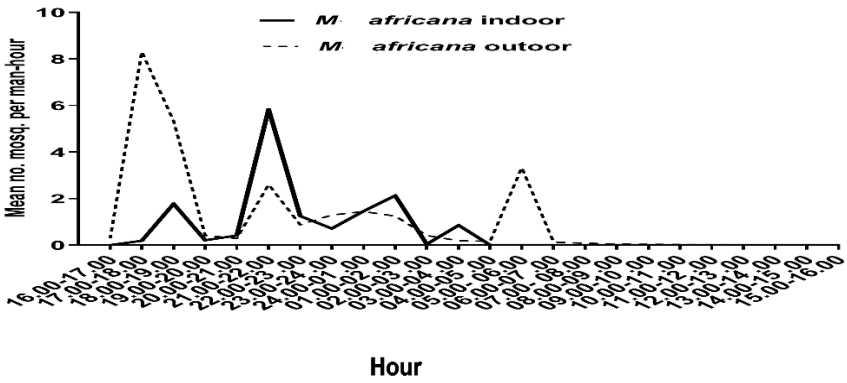

Figure 6a. Hourly biting pattern of *M. africana* in Ikarama for 2014.

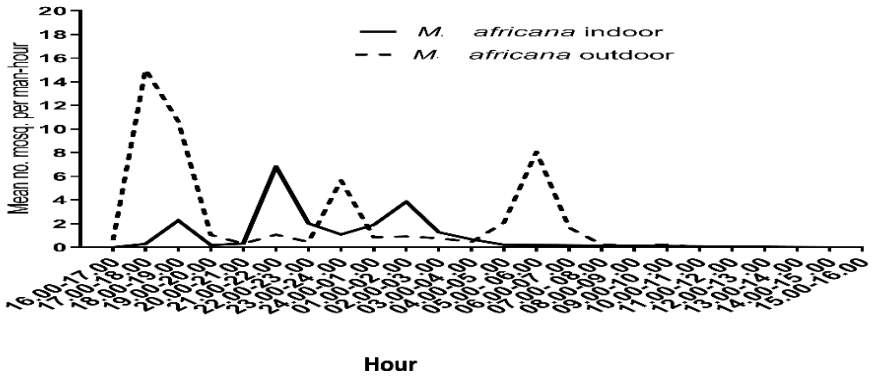

Figure 6b. Hourly biting pattern of *M. africana* in Ikarama for 2015.

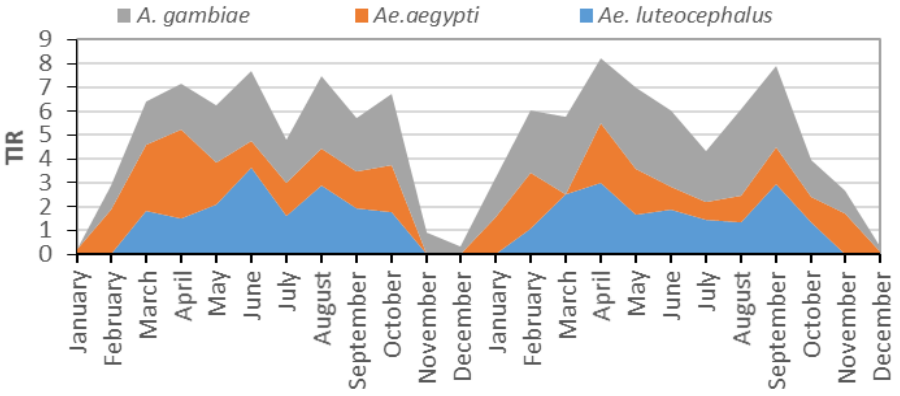

Figure 7. 24-month TIR pattern of three mosquito species positive for YF in Ega locality (2014-2015).

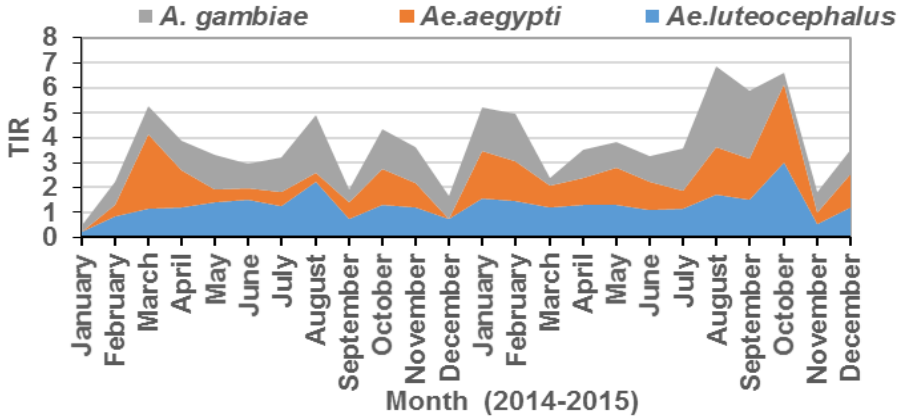

Figure 8. 24-month TIR pattern of three mosquito species positive for YF in Oju locality (2014-2015).

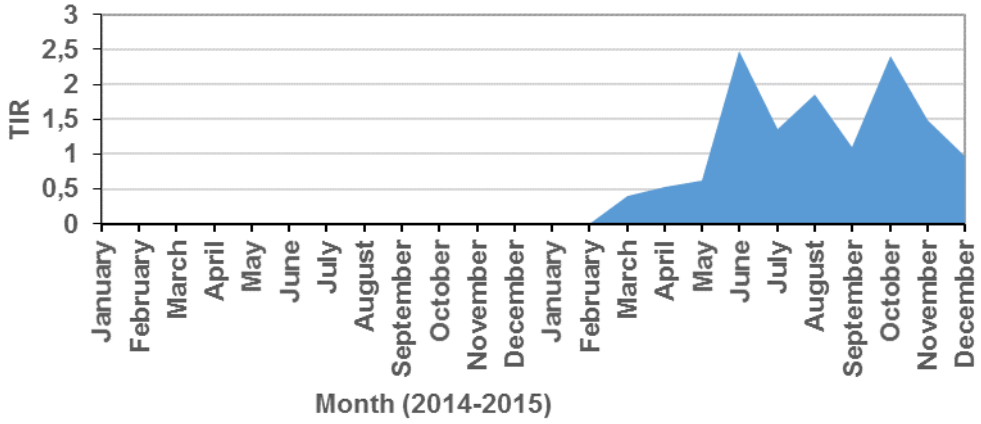

Figure 9. 24-month TIR pattern of *M. africana*, (DENV-3 positive mosquito species) in Ikarama (2014-2015).

Discussion

The survey of mosquitoes in the Benue and Bayelsa States yielded a greater number of members of the *Aedes* group than species of other genera. Five *Aedes* species were found: *Ae. luteocephalus*, *Ae. cumminsii, Ae. africanus*, *Ae. albopictus* and *Ae. vittatus*; while for the *Anopheles* group, only two species (*A. gambiae* and *A. nili,*) were caught. Two mosquito species from *Mansonia* genus (*M. africana* and *M. uniformis*) and the *Culex* group (*C. quinquefasciatus* and *C. annulioris*) were also identified. The arboviruses associated with these collected mosquitoes in the study sites have been widely reported (Nasidi *et al*., 1989; Appawu *et al.,* 2006; Weber, 2009). For instance, *Ae. aegypti* is generally known as the primary vector of DENV and urban YF, while *Ae. africanus*, *Ae vittatus* and *Ae. luteocephalus* are sylvatic YF vectors. *Ae. albopictus* is the primary vector of DENV in humans, while *Ae. cumminsii* has been associated with Pongola virus (Berger, 2015). *C. quinquefasciatus* is the vector of St Louis encephalitis in birds (Weber, 2009) and West Nile virus (WNV) in Kenya (Lutomiah *et al*., 2011), while *A. gambiae* has been linked with the transmission of o'nyong'nyong virus (Vanlandingham *et al.,* 2006) as well as harboring the Nyando-group virus (Lee & Moore, 1972). *A. nili, M. uniformis* and *M. africana* are not known vectors of arboviruses in Africa. (Boorman & Draper, 1968; Lutomiah *et al*., 2011).

The mosquito probe from Benue State identified three YF-positive species (*Ae. luteocephalus*, *Ae. aegypti* and *A. gambiae*) in both the rural and urban communities. Clearly this shows that transmission of YF may be ongoing but not at a rate it can be assumed of epidemic status as outbreaks have not been reported recently in Nigeria.Given the reported history of *Ae. luteocephalus* and *Ae. aegypti* in YF transmission (Berger, 2015), it is strongly suggested that these mosquitoes are the vectors of YF in Benue State (Vasilakis *et al.,* 2007; Vasilakis *et al*., 2008). However, quite unusually, *A. gambiae* mosquitoes that are known vectors of malaria were also positive for YF. This is in contrast to reports that *A. gambiae* are refractory to virus transmission except for the o'nyong'nyong virus (Keene *et al.,* 2004; Vanlandingham *et al*., 2006; Carrissimo *et al.,* 2015). One of the requirements for the establishment of viral infection in *A. gambiae* is that there must be an association of the virus with some bacterial flora; and this association has been quite remote because there is a protective antiviral siRNA pathway either in the midgut (Keene *et al.,* 2004) or in the systemic compartment (Carrissimo *et al*., 2015), being a characteristic component of the *A. gambiae* immune system.

Yellow fever, dengue and other arboviruses coinfections have been described in Nigeria (Baba & Talle, 2011) with the likelihood of promoting the exchange of genetic materials leading to mutant strains with relatively greater fitness and enhanced disease severity (review in Ayukekbong, 2014). We thus hypothesize the possibility of the ability of theYF strain to overcome the mechanism conferring antiviral protection in *A. gambiae.*

Similarly, this study reports for the first time the detection of DENV-3 in *M. africana* from Bayelsa State (Ikarama). The main arthropod vectors for the transmission of DENVs are *Ae. aegypti* and *Ae. albopictus* (McCall & Lehard, 2008) but none of these vectors, although present in the two States, tested positive for DENV infection. The detection of DENV-3 in *M. africana* should attract a lot of concern because if it is proven that this mosquito can transmit this virus in the field, then we have an emergency situation of control on our hands. Before now, only DENV-1 and DENV-2 have been reported in Nigeria with DENV-2 implicated in most epidemics (Carey *et al*., 1971; Lee & Moore, 1972). Phylogenetic studies suggested that DENVs were imported from India subcontinent (Messer *et al.,* 2003). Ikarama is home to many oil fields and consequently many multinationals operate here and a lot of these expatriates from Asia and other parts of the world moved to this town to begin operations (personal communication). Since we recorded no infection in mosquitoes in 2014, we may infer that it is likely that DENV-3 was imported within this period into Ikarama and thereafter established in *M. africana* from 2015. However, to confirm this suspicion, data from the human population areneeded.

The hourly indoor and outdoor catches of biting mosquitoes positive for YF or DENV sampled for a 24-hour period revealed that *Ae. luteocephalus* and *Ae. aegypti* were caught both indoors and outdoors in the village (Ega) and in the town (Oju), and they showed similar patterns, with the highest activity from late afternoon to midnight. The biting behavior of *Ae. luteocephalus* in the study areas does not alignwith the report from southeastern Senegal where no indoor mosquitoes in villages were caught (Diallo *et al.,* 2014). The biting period of *Ae. aegypti* is mostly in the daytime before sunset (Yasuno & Tonn, 1970) but we observed a deviation, and therefore report both day and night biting periods. For the potential vectors such as *A. gambiae* and *M. africana*, we recorded both indoor and outdoor catches and a stretch of a relatively longer biting period than that of *Aedes* species. Vector-control efforts should therefore be targeted at times when the mosquitoes' biting rates are at their peak for significant outcomes.

In Oju town, a higher biting rate and DIM were recorded for *Ae. aegypti* than for *Ae. luteocephalus* and *A. gambiae*. Moreover, a higher indoor biting rate than outdoor with a TIR > 2 % was seen for *Ae. aegypti* and *A. gambiae* in Oju. It is yet to be determined if this level of infection can constitute a risk for YF transmission because reports from other authors have pegged transmission risk at TIR > 4 to *Culex* species with WNV (Frank, 2004; CDPHC, 2009).

Conclusion

The study showed a rise in the *Ae. aegypti* infection rate from 2.19 % in 2014 to 3.01 % in 2015 in Oju LGA. Such a rise in TIR attests to the continuous transmission of YF; and if appropriate control measures are not put in place, this situation could pose a high risk of possibly unleashing another epidemic. Furthermore, the isolation of YF in *A. gambiae* in Oju and DENV-3 in *M. africana* in Ikarama present an additional threat, and therefore requires immediate intervention through research in ascertaining the abilities of these mosquito species in disseminating these viruses to humans.

Conflict of interest

The authors do not have any conflict of interest.

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ДЕТЕКЦИЈА ВИРУСА ЖУТЕ И ДЕНГА ГРОЗНИЦЕ КОД КОМАРАЦА У ДРЖАВАМА БАЈЕЛСА И БЕНУЕ У НИГЕРИЈИ У ПЕРИОДУ ОД 2014 ДО 2015 ГОДИНЕ

ЕКЕНМА ЈУЛИА АГВУ, КЛЕМЕНТ ИСАК и ИГО БЕНЏАМИН ИГБИНОЗА

Извод

Епидемије денга (DEN) и жуте грознице (YF) су раније пријављене у Нигерији. Да би се избегла нова епидемија, неопходна је идентификација вектора ових вируса на различитим локалитетима. Сакупљане су женке комараца у насељима и околини у државама Бајелса и Бенуе, Нигерија, користећи методе „људских мамца“ и запрашивања у периоду од јануара 2014 и децембра 2015. Јединке су затим тестиране на присуство вируса денга и жуте грознице коришћењем реверзне транскриптазе ланчане реакције полимеразе (RT-PCR). Идентификовано је 172 010 одраслих женки комараца а затим тестирано на присуство вируса. Број угриза, стопа инфекције (TIR) и густина инфицираних комараца (DIM) је процењена за врсте позитивне на вирусе жуте и денга грознице. Међу идентификованим врстама *Aedes luteocephalus* (Newstead, 1907), *Ae. aegypti* (Linnaeus, 1762), *Ae. cumminsii* Theobald, 1903, *Ae. africanus* (Theobald, 1901), *Ae. albopictus* (Skuse, 1894), *Ae. vittatus* (Bigot, 1861), *Anopheles gambiae* (Giles, 1902), *A. nili* Theobald, 1904, *Mansonia africana* (Theobald, 1901), *M. uniformis* (Theobald, 1901), *Culex annulioris* (Theobald,1901) и *C. quinquefasciatus* (Say,1823), три врсте *Ae. luteocephalus*, *Ae. aegypti* и *A. gambiae* су биле позитивне на вирус жуте грознице у Ојуи и Ега, док је денга вирус детектован у узорцима *M. africana* из Икараме. Динамика уједа врста које су биле позитивне на вирусе је била таква да су били активни и током дана и ноћи сем популације *A. gambiae* из Ега које су биле активне током ноћи све до раних јутарњих сати. *Ae. aegypti, A. gambiae* and *Ae. luteocephalus* могу представљати примарне векторе вируса жуте грознице у државама Бенуе и Бајелса. Такође, врста *M. africana* као преносилац вируса денга грознице захтева даље истраживање.

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