



High density in dry season and resistance to pyrethroids of *Aedes aegypti* populations in Bouaké, Côte d'Ivoire

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Abstract

Yellow fever and dengue are the most prevalent arboviral diseases in Côte d'Ivoire. The data on the resistance to insecticides of *Aedes aegypti*, main vector of these diseases, are insufficient in the country. The study aims to determine the dynamic and assess the level of insecticides resistance of *Ae. aegypti*, in Bouaké city. Sampling was performed from November 2016 to August 2017 using the WHO layer-traps method to collect *Aedes* eggs and WHO tubes tests for assessing *Ae. aegypti* susceptibility to insecticides. The study revealed the existence of three potential arboviruses vectors in the urban areas of Bouaké: *Ae. aegypti*, the urban vector, and *Ae. luteocephalus* and *Ae. unilineatus*, two sylvatic vectors. The highest densities of *Aedes* mosquito eggs were recorded during the rainy season and at the beginning of the dry season. The populations of *Ae. aegypti* showed resistance to deltamethrin and lambda-cyhalothrin (mortality rates ranging from 56 to 94%) in all the study sites. Meanwhile, in Kôkô and Air France 1, susceptibility was observed with permethrin. The *Ae. aegypti* populations exhibited susceptibility to bendiocarb and propoxur (carbamates) and chlorpyrifos-methyl (organophosphates) in all the study sites apart from Air France 1 neighbourhood where its showed resistance to propoxur. We conclude the existence of three potential arboviruses vectors in Bouaké city. *Ae. aegypti* the most abundant species recorded, was resistant to pyrethroids, insecticides commonly used to control arboviruses vectors. Monitoring of vectors resistance to insecticides would be essential to arboviruses vectors control and surveillance systems in Côte d'Ivoire.

Keywords *Aedes aegypti* · Seasonal dynamic · Insecticides resistance · Bouaké · Côte d'Ivoire

Introduction

Yellow fever and dengue, commonly reported in West Africa, caused heavy social and economic burdens (Braack et al. 2018). Despite an effective preventive vaccine against yellow fever, the disease continues to be a public health problem in tropical areas where 90% of cases occur (Tomori 2004). Dengue is the most widespread arboviral disease. In

recent years, dengue outbreaks have been increasingly prevalent in some West African countries (CEDEAO 2019).

Côte d'Ivoire, a country also located in West Africa, an endemic zone of these two arboviral diseases, has known since 2008 single or joint outbreaks of yellow fever and dengue. Indeed, Abidjan the economic capital has faced recurrent outbreaks of these diseases (WHO 2009, 2017, ECDC 2020). Likewise, the northern part of the country was also marked by a resurgence of yellow fever cases in 2009 (Konan et al. 2011) and 2010 (Konan et al. 2014).

Mosquitoes in the genus *Aedes* are the main vectors of these arboviral diseases. Several *Aedes* species were collected in urban and rural areas of the south eastern Côte d'Ivoire during entomological investigations, where *Aedes aegypti*, the major vector of dengue and yellow fever was predominant (Guindo-Coulibaly et al. 2010; Zahouli et al. 2016; Konan et al. 2017). The control of these diseases relies essentially on vectors control which is generally based on

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the suppression of breeding sites and the use of insecticides. Unfortunately, the vectors of these diseases have developed widespread resistance to insecticides (Maestre-Serrano et al. 2014; Kawada et al. 2016; Badolo et al. 2019) that can limit the success of chemical control. However, in Côte d'Ivoire little data are available on the resistance to insecticides of the main vector *Ae. aegypti* and they were achieved mainly in the city of Abidjan (Konan et al. 2012; Guindo-Coulibaly et al. 2014). Besides, although recent outbreaks of yellow fever and dengue occurred mainly in the city of Abidjan, the possibility of the spread of these diseases in other areas of the country remains a considerable risk. The risk of spread is mostly considerable for the city of Bouaké which is a crossroads city with important commercial exchanges and intense movement of population coming from Abidjan and neighbouring countries that have experienced arboviral diseases outbreaks (Ridde et al. 2016). Thus, face to the increasing risk of arboviruses circulation and transmission across the country, specifically in the city of Bouaké, this study was implemented with the general objective to determine the dynamic and assess the level of resistance to insecticides of *Ae. aegypti* in this city.

Materials and methods

Study sites

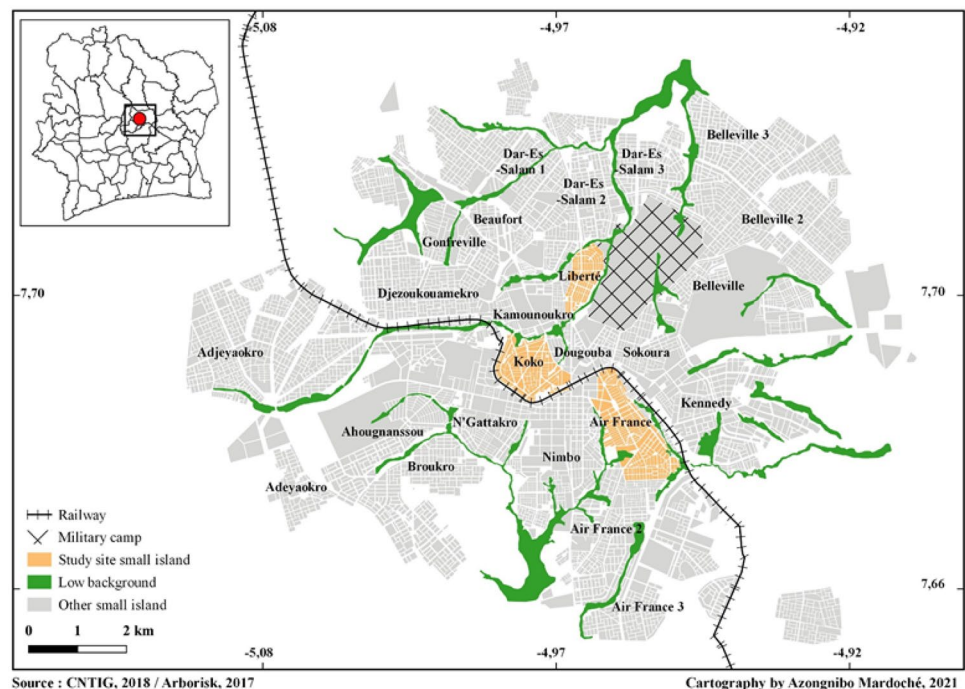
This study was carried out in the center part of Côte d'Ivoire specifically in the city of Bouaké. Bouaké is located about

350 km from Abidjan (07°41.139 N, 05°01.678 W) with over 1 million inhabitants (INS 2015). The climate of this city is characterized by one rainy season which extends from March to October and one dry season from November to February with monthly average precipitation of 916.5 mm and 26.8 °C of average temperature in 2016 (Data source: SODEXAM: Ivorian Society for Development and Exploitation of Meteorological Parameters). This study was conducted in Bouaké because Bouaké is crossing city where populations from great agglomerations (Abidjan, Bobo-Dioulasso, Ouagadougou, Bamako) transit regularly. Thus, the flux of people between Bouaké and these agglomerations can constitute a factor of virus spreading in the city of Bouaké. Furthermore, in 2010 yellow fever outbreak occurred in Bouaké where *Ae. aegypti*, the common vector with dengue is well established (Komono 2012).

The surveys were implemented in three neighbourhoods of Bouaké city named Kôkô, Liberté and Air France 1 (Fig. 1: Map showing the study sites in the city of Bouaké).

Kôkô (07°41.390 N and 05°02.119 W) is located in the center of Bouaké. It is a popular neighbourhood with common courtyard type dwellings. Kôkô displays the train station receiving goods from sub-region which constitutes a gateway for arboviruses. Likewise Kôkô, Liberté (07°42.390 N and 05°01.474 W) is a popular neighbourhood situated in the northern part of Bouaké. This neighbourhood accommodates the biggest market of Bouaké city where take place commercial and economic business. Some goods from countries of the sub-region are stored in this market, thus representing a spreading

Fig. 1 Map showing the study sites



risk of arboviruses. Contrary to Liberté and Kôkô, Air France 1 (07°40.775 N and 05°01.085 W) is a residential neighbourhood located in the southern part of Bouaké. The populations of this residential neighbourhood commonly use flower pots containing water which is favourable to the development of urban vectors of arboviruses.

Study design

The study was performed from November 2016 to August 2017 in three neighbourhoods of Bouaké. Four surveys were achieved in each neighbourhood with two during the dry season (November representing the beginning of the dry season and February the end of this season) and two during the rainy season (May and August). Twenty layer-traps were used to collect *Aedes* eggs in each study site. The position of each layer-trap was generated randomly by the software QGIS version 2.14. The geographic coordinates (longitude and latitude) of random points were entered into a Global Position System (GPS) version Garmin eTrex that was used to find these points during surveys. The layer-traps were set at their same places during sampling in each study site. A total of 80 layer-traps (20 layer-traps/surveys) were used through all surveys for each study site. The maps were generated with QGIS 2.14.

Mosquito eggs sampling and rearing

Aedes mosquito eggs were collected using the standard WHO layer-traps method (Fay and Eliason 1966). The layer-trap is made of an empty metallic box of 33 cl, with hole in the third upper part and painted in black to attract gravid female *Aedes* mosquitoes in search of egg laying grounds. At the time of setting the trap, a wooden paddle was placed inside as an oviposition substrate, then filled with rain or well water. The layer-traps were set at 1.5 m above the ground and removed seven days after they were installed. The wooden paddles were dried in the Transrisk laboratory of Institut Pierre Richet during ten days and the collected eggs were counted under a binocular magnifying glass. The wooden paddles were wetted and after hatching of eggs, larvae were reared to emergence as described previously (Guindo-Coulibaly et al. 2010). Larvae and pupae collected during removal of the traps were reared like those obtained after hatching of collected eggs. Emerged mosquitoes were identified using identification key of Huang (2004). In laboratory, all the stages of mosquitoes were reared under room temperature varying from 24 to 27 °C and relative humidity from 70 to 80%.

Insecticides susceptibility tests

The tests were performed with unfed females of *Ae. aegypti* (F1), aged 3–5 days, using the WHO tubes tests. *Ae. aegypti* (F1) females were obtained by rearing of collected field eggs in the three study sites. The *Ae. aegypti* SBE strain originating from Benin was used as the susceptible reference strain (Ngoagouni et al. 2016). The tests were performed on the F1 of susceptible SBE strain from eggs provided by the MIVEGEC laboratory of Montpellier University in France. The doses of insecticides used for *Aedes* susceptibility tests were those applied for accessing susceptibility of anopheline mosquitoes (OMS 2017). Thus, six insecticides were tested namely three pyrethroids (permethrin 0.75%, deltamethrin 0.05% and lambda-cyhalothrin 0.05%), two carbamates (bendiocarb 0.23% and propoxur 0.1%) and one organophosphate (chlorpyrifos-methyl 1.03%). The papers impregnated with insecticides used were supplied by the WHO collaborating centre, vectopole directed by MIVEGEC laboratory. For each insecticide, one hundred females of each strain were tested distributed in four batches of 25. Females of different batches were firstly observed for one hour for viability with untreated papers. Thereafter, they were exposed to insecticide during one hour. After one hour of exposure, mosquitoes were transferred into observation tubes and fed with wet cotton of 10% honey solution. The mortalities were recorded after 24 h and the susceptibility status of populations was graded according to the WHO protocol (OMS 2017). The mosquito population is susceptible if mortality rate is $\geq 98\%$, resistant if mortality rate is $< 90\%$ and exhibiting probable resistance if the mortality rate is between 90–97%.

Statistical analysis

Data were entered and cross-checked using Excel 2010. The statistical analysis of entomological data was carried out using the software GraphPad (Version 5.01). Mann–Whitney (U) test was used for the comparison of densities between two independent groups and the Kruskal–Wallis (KW) test was used for the comparison between more than two independent groups. Analysis of *Aedes* eggs spatial distribution was performed with the software ArcGIS 10.3. The spatial autocorrelation test of Morans was used to assess the distribution of collected *Aedes* eggs. Statistical test significance was set at the 5% level.

Ethics Statement

The study protocol was approved by the National Committee for Ethics and Research of Côte d'Ivoire (04 August 2015; No. 040/MSLS/CNER-dkn). This study also received

approval from the Director General of Health of Côte d'Ivoire and health district authorities of the study municipalities. In each study site, permission to work was granted by the chief of the municipality. Community members were briefed in detail on the objectives, procedures, and potential risks and benefits of the study. Guides were solicited in each study site to assist surveys teams during the study period. Before setting layer-traps to collect mosquitoes eggs, permission was requested from owners of houses and private lands.

Results

Inventory of mosquito fauna from eggs

A total of 3,275 mosquito eggs were collected distributed in 1,016 in Liberté, 849 in Air France 1 and 1,410 in Kôkô. Rearing of these eggs yielded 2,046 adult mosquitoes of which 565, 942 and 539 were recorded respectively in Liberté, Kôkô and Air France 1. All the mosquitoes belonged to only *Aedes* genus and are distributed in three species: *Ae. aegypti*, *Ae. luteocephalus* and *Ae. unilineatus*. *Ae. aegypti* and *Ae. luteocephalus* were recorded in all the study sites. While *Ae. unilineatus* was recorded only in Kôkô neighbourhood. The three species identified are potential arboviruses vectors. *Ae. aegypti* was the most abundant species recorded representing more than 99% of species identified in each study site (Table 1).

Seasonal variation of *Aedes* eggs density

Overall, 3,275 eggs were collected in Bouaké city during the study period with an annual density of 14.36 eggs/layer-trap. Specifically, annual density of 11.32 eggs/layer-trap was recorded in Air France 1 (N = 849); 13.03 eggs/layer-trap in Liberté (N = 1,016) and 18.81 eggs/layer-trap in Kôkô (N = 1,410). These annual densities recorded were comparable (KW = 2.128; P = 0.345). The seasonal dynamic of eggs density in Bouaké city showed high densities at the outset of the dry season (November) and during the rainy season (May and August) and these densities were comparable (U = 1422; P = 0.084). The same trend was observed in all the neighbourhoods (Table 2).

Spatial distribution of *Aedes* eggs in the study sites

Figures 2 and 3 show the spatial distribution of *Aedes* eggs in the three study sites (Fig. 2: Spatial distribution of *Aedes* eggs during the dry season in the three neighbourhoods of Bouaké; Fig. 3: Spatial distribution of *Aedes* eggs during the rainy season in the three neighbourhoods of Bouaké). The maps generated showed that at the beginning of the dry season (November), the positive layer-traps (layer-trap containing at least one *Aedes* egg) spread throughout the Liberté neighbourhood. However in Kôkô and Air France 1 neighbourhoods, these were located in peripheral areas. At the end of the dry season (February), the positive layer-traps were found in the periphery of all the study sites. During the rainy season (May and August), the majority of the layer-traps were positive in *Aedes* eggs. They were homogeneously distributed over all the sites. The spatial Morans autocorrelation test revealed that *Aedes* eggs were randomly distributed for each quarterly sampling performed in November (I = 0.0518; P = 0.7464), in February (I = 0.1015; P = 0.4891), in May (I = 0.1713; P = 0.2020) and in August (I = 0.0448; P = 0.4714) in all the study sites.

Susceptibility status of *Ae. aegypti* populations

The tests performed with the pyrethroids showed that all the populations of *Ae. aegypti* were resistant to deltamethrin and lambda-cyhalothrin (with mortality rates from 56 to 94%) whereas with permethrin susceptibility was observed apart from Liberté neighbourhood (77%). The *Ae. aegypti* populations were significantly more resistant to deltamethrin and lambda-cyhalothrin in Liberté than Kôkô and Air France 1 (deltamethrin: $\chi^2 = 39.58$, df = 2, P = 0.0001; lambda-cyhalothrin: $\chi^2 = 31.04$, df = 2, P = 0.0001), while those of Kôkô and Air France 1 showed similar resistance (deltamethrin: $\chi^2 = 1.607$, df = 1, P = 0.2049; lambda-cyhalothrin: $\chi^2 = 3.092$, df = 1, P = 0.0787). For carbamates, *Ae. aegypti* populations were susceptible to bendiocarb in all the study sites with mortality rates ranging from 99 to 100%. However, with propoxur resistance was observed in Air France 1 with mortality rate of 92.16%.

Table 1 Composition of *Aedes* species collected in the three neighbourhoods of Bouaké city

Species	Liberté		Kôkô		Air France 1		Total	
	n	%	n	%	n	%	n	%
<i>Ae. aegypti</i>	564	99.82	939	99.68	537	99.63	2,040	99.7
<i>Ae. luteocephalus</i>	1	0.18	1	0.11	2	0.37	4	0.2
<i>Ae. unilineatus</i>	0	0	2	0.21	0	0	2	0.1
Total	565	100	942	100	539	100	2,046	100

n number of mosquitoes, % percentage

Table 2 Density of *Aedes* eggs collected in the three neighbourhoods of Bouaké city from November 2016 to August 2017

Sampling month	Liberté			Air France 1			Kôkô			Total		
	n	ED	CI (95%)	n	ED	CI (95%)	n	ED	CI (95%)	n	ED	CI (95%)
November 2016 (Dry season)	359	17.95	6.63–29.26	206	11.44	4.74–18.14	445	22.25	6.09–38.40	1,010	17.41	10.63–24.20
February 2017 (Dry season)	36	2	0.45–4.45	36	2	0.90–4.91	44	2.2	0.16–4.23	116	2.07	0.74–3.40
May 2017 (Rainy season)	396	19.8	7.09–32.50	457	22.85	12.49–33.20	601	30.05	15.13–44.96	1,454	24.23	17.20–31.26
August 2017 (Rainy season)	225	11.25	5.75–16.74	150	7.89	3.32–12.46	320	21.33	1.62–44.28	695	12.87	6.38–19.35
Total	1,016	13.03	8.44–17.61	849	11.32	7.62–15.01	1,410	18.8	11.49–26.11	3,275	14.36	11.27–17.46

n number of eggs, ED eggs density, CI confidence interval

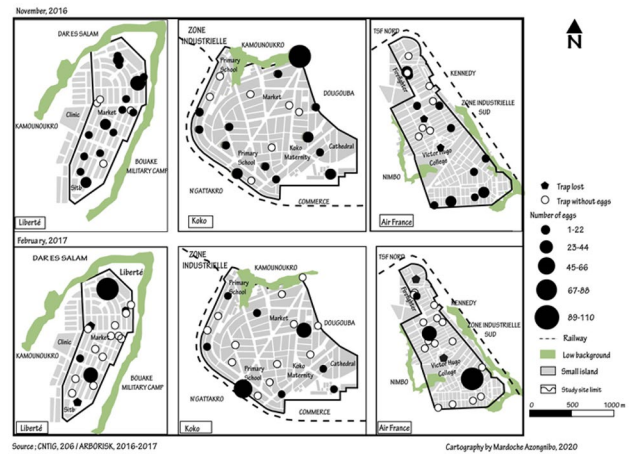


Fig. 2 Spatial distribution of *Aedes* eggs during the dry season in the three neighbourhoods of Bouaké

Regarding chlorpyrifos-methyl from organophosphate family, susceptibility was observed in all the study sites with mortality rates ranged between 98 and 100% (Table 3).

Discussion

Yellow fever and dengue are the most prevalent arboviral diseases in West Africa (Braack et al. 2018). *Ae. aegypti*, one of the main vector of these two arboviral diseases has been highlighted repeatedly in Côte d’Ivoire (Guindo-Coulibaly et al. 2010; Zahouli et al. 2016; Konan et al. 2017). This species exhibits a widespread resistance to insecticides (Maestre-Serrano et al. 2014; Kawada et al. 2016; Badolo et al. 2019) that limits the success of chemical control. However, studies referring to the resistance to insecticides of this vector in the country were insufficient

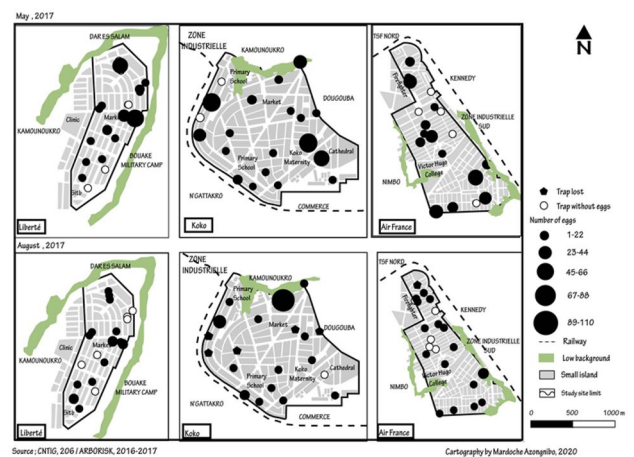


Fig. 3 Spatial distribution of *Aedes* eggs during the rainy season in the three neighbourhoods of Bouaké

Table 3 Mortality rate of *Aedes aegypti* populations 24 h after exposure to insecticides

Insecticides	SBE			Kôkô			Air France 1			Liberté		
	n	%M	St	n	%M	St	n	%M	St	n	%M	St
Permethrin 0.75%	100	100	S	101	100	S	100	98	S	100	77	R
Deltamethrin 0.05%	100	100	S	100	94	R	100	89	R	100	62	R
Lambdacyhalothrin 0.05%	100	100	S	100	89	R	100	80	R	100	56	R
Propoxur 0.1%	100	100	S	100	100	S	102	92.16	R	100	98	S
Bendiocarb 0.23%	100	100	S	100	100	S	100	100	S	100	99	S
Chlorpyrifos-methyl 1.03%	100	100	S	100	100	S	100	98	S	100	98	S

n number of mosquitoes tested, %*M* mortality rate, *St* resistance status, *S* susceptible, *R* resistant

and were achieved mainly in Abidjan. Thus, this study aims to provide a database necessary for efficient control and surveillance methods to mitigate the circulation and transmission risk of arboviruses in Bouaké, where there is a considerable transmission risk of yellow fever and dengue virus.

The layer-traps technique used to collect mosquitoes in the city of Bouaké showed the presence of *Ae. aegypti*, an urban species, and two sylvatic species (*Ae. luteocephalus* and *Ae. unilineatus*). Given mosquitoes can move above 400 m from their natural habitat (Honório et al. 2003), the presence of forest relics nearby the study sites could justify the existence of sylvatic vectors in the study sites. This result corroborates those of Guindo-Coulibaly et al. (2019), which found the sylvatic vector *Ae. africanus* in the 200 m radius from the Banco National Parc in Sagbé neighbourhood of Abidjan city. Moreover, the three *Aedes* species collected are well involved in the transmission of dengue, yellow fever, zika and chikungunya (Robert et al. 1993; Tariq et al. 2010; Diallo et al. 2014). These facts increase the risk of these arboviral diseases spreading in case of outbreaks in Bouaké city. The risk of diseases spreading is also high for other cities or neighbouring countries that realize many exchanges with Bouaké.

The high densities of *Aedes* eggs were observed during the rainy season and at the beginning of the dry season in all the study sites. Commonly, high densities of vectors are observed during the rainy season, as our study revealed. However, besides the rainy season, the beginning of the dry season proves to be favourable for the development of vectors. This fact could be explained by the presence of many available breeding sites such as trash, discarded containers and used tires that can retain water during the rainy season and will remain until the beginning of the dry season, the period following the rainy season. The studies of Pichón and Gayral (1970) and Appawu et al. (2006) also showed high densities of *Aedes* eggs at the beginning of the dry season. Based on our data, vector control would be necessary before and at the end of the rainy season; this

would reduce the high densities of *Aedes* eggs observed during this season and at the beginning of the dry season.

Analysis of *Aedes* eggs spatial distribution by the spatial Morans autocorrelation test revealed that *Aedes* eggs were distributed randomly in all the study sites. This random distribution observed across all the study sites could be due to common factors to these study sites like the problem of drinking water supply. Indeed, Bouaké knows constant water shortages cause of eventual dryness of the Loka barrage that supplies the city (Bétio et al. 2012). This situation leads populations to stock water in the containers often opened, which are preferential breeding sites of *Aedes* mosquitoes (Mukhtar et al. 2018). The random distribution observed in all the study sites is confirmed by the annual densities of eggs comparable between the study sites.

The study also revealed areas of high productivity of *Aedes* eggs. These areas, considered of high entomological risk, were more pronounced at the periphery of the different sites. This finding could be explained by specific environmental factors such as the probable presence of breeding sites resulting from a lack of waste management system. Our results were identical to those of Moura et al. (2020) performed in Brazil where they found also areas of high entomological risk at the periphery. The peripheral areas of high productivity of *Aedes* eggs must be taken into by vectors control programs for more effective control.

The insecticides susceptibility tests showed that *Ae. aegypti* populations were resistant to pyrethroids in all the study sites. The resistance of *Ae. aegypti* to pyrethroids is widespread and is commonly associated to the target cible modification notably sodium channel that confers knock-down (*kdr*) resistance and the metabolic resistance through detoxification enzymes (Dusfour et al. 2015; Estep et al. 2017). In this study the resistance to pyrethroids observed in all the study sites could probably result of larval breeding sites contamination around the houses through the high pressure of insecticides used in agricultural practices such as the market garden crops. The study of Ouattara et al. (2019) also highlighted the resistance to pyrethroids of *Ae. aegypti*

from Burkina Faso due to the effect of insecticidal pressure through the agricultural practices in Bobo-Dioulasso and Boromo. The use of long-lasting insecticidal nets for malaria vectors control by populations could likewise justify the resistance of *Ae. aegypti* in all the study sites (Jirakanjanakit et al. 2007; Ayorinde et al. 2015).

Our results also revealed that the *Ae. aegypti* populations of all the study sites are susceptible to bendiocarb (carbamates) and chlorpyrifos-methyl (organophosphates). The susceptibility of *Ae. aegypti* to these two insecticides could be explained by the poor use of these insecticides for vectors control. This susceptibility to these insecticides suggests that they are an alternative option for controlling arboviral diseases vectors. Indeed, a trial showed in Mexico that bendiocarb was more effective than some pyrethroids for indoor residual spraying (Vazquez-Prokopec et al. 2017). Thus, these results could help vectors control programs in the implementation of more efficient control strategies in the city of Bouaké.

Conclusion

This study showed the presence of *Ae. aegypti*, *Ae. unilineatus* and *Ae. luteocephalus*, three potential vectors of arboviruses in Bouaké city. The periods of eggs abundance were observed during the rainy season and at the beginning of the dry season in all the study sites. The more productive layer-traps in *Aedes* eggs were located in peripheral areas. The populations of *Ae. Aegypti* from all the study sites were resistant to pyrethroids which are commonly used for vectors control. Vectors control programs should then consider adopting new control strategies with for instance the use of new molecules or some carbamates and organophosphates such as bendiocarb and chlorpyrifos-methyl in the control of arboviruses vectors. The resistance to pyrethroids observed within *Ae. aegypti* populations requires the detection of resistance mechanisms involved in the resistance of *Ae. aegypti* to these insecticides, that could be useful for implementing alternatives vectors control strategies. Also, given the presence of three vectors recorded in Bouaké city, further investigation are needful in order to determine the infection rate of these vectors.

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Authors' contribution Conceptualization, AMA, NG-C, AMNK and AY; Methodology, AMA, NG-C and AMNK; Software, AMNK,

SMDK and KRMA; Validation, AMA, NG-C and AMNK; Formal analysis, AMNK, SMDK and NG-C; Investigation, AMNK, SMDK, FKA, DDZ and KRMA; Writing-Original Draft Preparation, AMNK; Writing-Review & Editing, AMA, NG-C, AMNK, AMCS-K, SMDK, DDZ, FKA and AY; Supervision, AMA and NG-C, Project Administration, AMA, Funding acquisition, AMA and NG-C. All authors have read and agreed to the published version of the manuscript.

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Declarations

Ethical approval The study protocol was approved by the National Committee for Ethics and Research of Côte d'Ivoire (04 August 2015; No. 040/MSLS/CNER-dkn).

Conflict of interests The authors declare that they have no conflict of interests.

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