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Molecular detection of arboviruses in culicidae in some sites of Côte d'Ivoire

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Abstract

An upsurge in the cases of some arboviruses (dengue, yellow fever, chikungunya and zika) has recently been reported in Côte d'Ivoire. These arthropod-borne diseases are mostly transmitted by several species of Culicidae of the genus *Aedes* (*Ae*). The objective of this study is to evaluate the prevalence of arboviruses in Culicidae in Côte d'Ivoire. This study was conducted in Côte d'Ivoire from 2018 to 2019 in ten sites grouped under primary (human settlement areas) and secondary (forest zones) sites. The collection of Culicidae was conducted using oviposition traps (ovitraps), larval mosquito collections, trapping under a double mosquito net and aspiration. Subsequently, monospecific mosquito pools were made and sent to the Pasteur Institute in Côte d'Ivoire to identify the viral genomes of arboviruses using the real time quantitative polymerase chain reaction (rt-qPCR). The following Culicidae were identified: *Ae. aegypti*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. opok*, *Ae. simpsoni*, *Ae. metallicus*, *Ae. vittatus*, *Eretmapodites* (Er) *chrisogaster* and *Er. quinquevittatus*. In total, 4,813 Culicidae divided into 686 monospecific pools were obtained from the study sites. Two pools of females of the species *Ae. aegypti* from surveys of breeding sites tested positive for dengue 2 and amaril viruses. These mosquitoes that tested positive were collected from Vapleu and Tron Touba sites. The presence of arboviruses and their vectors constitute a significant health risk for the human populations living in these sites. The findings of this study are useful for the development of an entomo-epidemiological surveillance program and for the planning of effective and sustainable vector control strategies.

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Introduction

Arboviruses (dengue, yellow fever, Zika and Chikungunya) are vector-borne diseases with high epidemic potential (WHO, 2017). These arboviruses belong to the families Flaviviridae and Togaviridae. They are RNA viruses transmitted from vertebrates to vertebrates by mostly several species of mosquitoes belonging to the genus *Aedes*. Infact, arboviruses are mostly found in tropical and subtropical regions, with more or less similar clinical manifestations, ranging from a simple fever to sometimes very severe forms (Aubrey and Gaüzer, 2018). In many countries, they represent a considerable health burden (Biardeau, 2012; Bhatt *et al.*, 2013; Wikan and Smith, 2016; Fortuna *et al.*, 2017). The epidemiology of dengue virus (DENV), yellow fever virus (YFV), zika virus (ZIKV and chikungunya virus (CHIKV) is undergoing significant changes, particularly in relation to the globalization of trade, movement of people, climate change, intercontinental distribution of vectors and the urbanization of many cities (Kulkarni, 2016; Rodhain, 2017; Failloux, 2019). In Côte d'Ivoire, these factors have greatly contributed to the resurgence of diseases, such as dengue and yellow fever (Attoh-Touré *et al.*, 2010; Akoua-Koffi *et al.*, 2011).

In addition, several studies have indicated the presence of the zika and chikungunya viruses (Mondet and Montagne, 1993; Akoua-Koffi *et al.*, 2001; Faye *et al.*, 2013) in Côte d'Ivoire. In urban areas, particularly in Abidjan, *Ae. aegypti* and *Ae. Albopictus* have been reported as the major vectors involved in the transmission of arboviruses (Coulibaly, 2015; Fofana *et al.*, 2019). In rural and forest areas, several other potentially dangerous Culicinae including: *Ae. africanus*, *Ae. fucifer*, *Ae. luteocephalus*, *Ae. opok*, *Ae. usambara* and *Ae. vittatus* have been identified (Mondet and Montagne, 1993; Akoua-Koffi *et al.*, 2001; WHO, 2014).

The presence of arboviruses and its vectors constitute a permanent threat to the health of human populations. Thus, in order to prevent any risk of epidemics, the World Health Organization (WHO) advocates vector control as the safest means. In the absence of specific treatments against arboviruses, the control of vector populations could reduce the disease burden (WHO, 2017). However, for vector control to be effective, it must be guided by entomo-epidemiological indicators. These indicators are provided by increased and regular epidemiological and entomological surveillance data (Barré-Cardi *et al.*, 2016; Rousseau *et al.*, 2016).

However, entomological surveillance is an essential tool that serves as an early warning for most vector-borne diseases outbreaks, but has not been operational for more than a decade in Côte d'Ivoire. However, its implementation could allow early detection of the threat and a rapid, efficient and coordinated organization of vector control, especially in rural areas that are difficult to access. Several papers have published on the ecological aspects of the vectors of arboviruses and the estimation of the transmission risk for its spillover (Beugré *et al.*, 2020; Zagui *et al.*, 2020), but little has been published on its molecular detection in mosquitoes within Côte d'Ivoire (Agboli *et al.*, 2021). The objective of the present study is to contribute to the epidemiology of arboviruses by evaluating its prevalence in Culicidae in some sites in Côte d'Ivoire.

Materials and methods

Study area

This study was carried out in Côte d'Ivoire from 2018 to 2019 in ten sites (Table 1). These sites were grouped into primary sites (anthropized environments) and secondary sites (forest) (Fig. 1). The study sites had different eco-geographical characteristics in terms of relief, vegetation, rainfall, temperature and culture. Also, these sites are known for the regular epidemics of arboviruses and are frequently visited by people.

Primary sites

Banco National Park

The Banco National Park is located in the heart of Abidjan, between the municipalities of Adjamé, Attécoubé, Abobo and Yopougon. It covers an area of 30 km² (OIPR, 2011a; Sangné *et al.*, 2018).

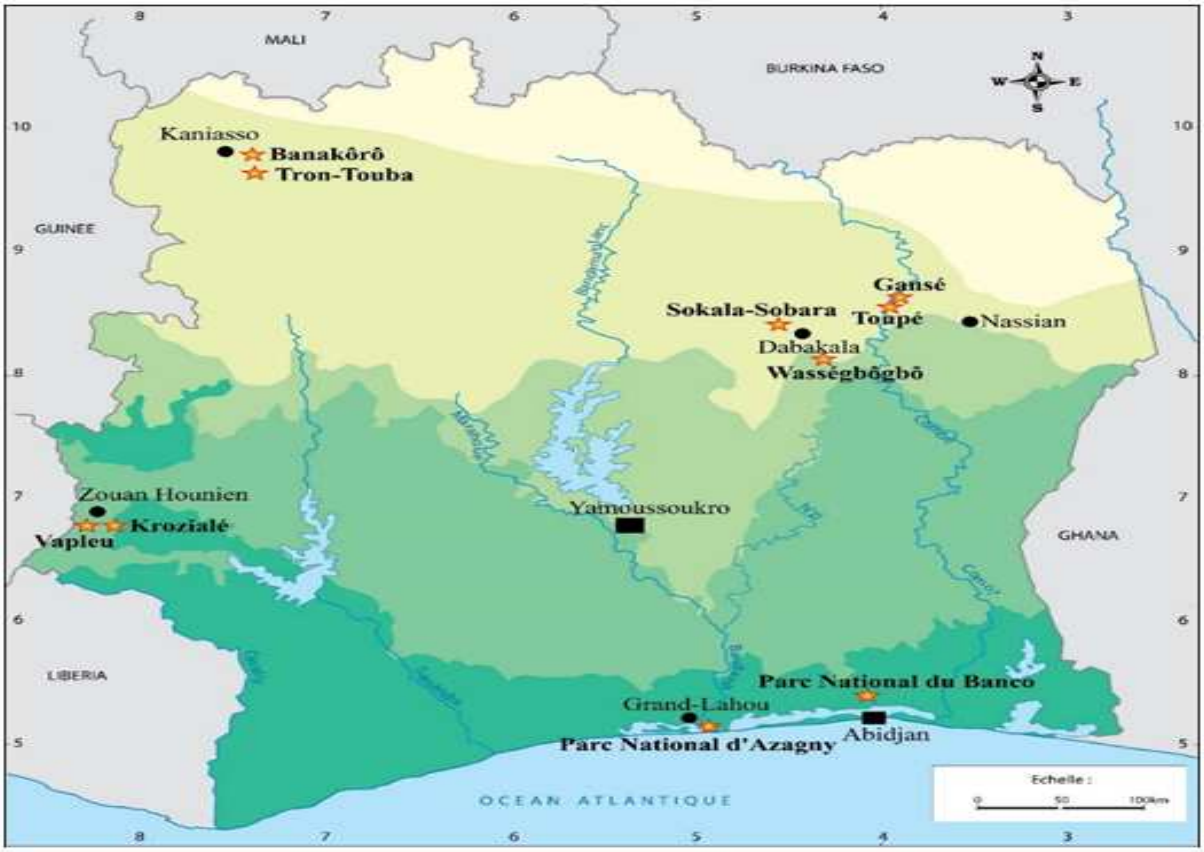

Fig.1. Map of Côte d'Ivoire showing the ten study sites (orange stars).

In this site, entomological studies have indicated the presence of certain arbovirus vectors including: *Ae. aegypti*, *Ae. africanus* and *Ae. opok* (Guindo-Coulibaly *et al*., 2019).

Vapleu Village

The Vapleu village is located in the department of Zouan Hounien, precisely in Bin Houyé municipality. Here, a girl was tested positive for the Dengue 2 virus after returning from Krozialé with her parents in 2002. Entomological investigations at the time had not implicated any mosquito vector.

Tron Touba Village

This site is located in the Folon region, Tron Touba belongs to the administrative district of Denguélé in the municipality of Kaniasso. In this village, a yellow fever epidemic caused the death of several people in 2009. This epidemic raised several questions, particularly on the epidemiological cycle of the disease but also on the species involved in the transmission of the Amaril virus (Konan *et al*., 2011).

Toupé Village

The Toupé village is located in the municipality of Dabakala, in the heart of the Comoé National Park (Gauze *et al*., 2014). In the Comoé National Park, a German died following an infection with the amaril virus in 1999. The results of serological and entomological investigations revealed the presence of the amaril and zika viruses in the human population and in several Culicinae of the genus *Aedes* (Akoua - Koffi *et al*., 2001).

Sokala Sobara village

Sokala-Sobara belongs to the department of Dabakala. It is home for a heterogeneous population consisting of non-nationals and nationals (INS, 2014).

The Sokala Sobara site was an entomo-epidemiological surveillance station of ORSTOM (Office for Scientific and Technical Research Overseas) today IRD (Research Institute for Development). The results of published

entomological research indicated the circulation of amaril and dengue viruses in mosquitoes of the genus *Aedes* (Cordellier and Boucheti, 1983; Cordellier *et al.*, 1988).

Secondary sites

Azagny National Park

The Azagny National Park is located at the mouth of Bandama, 100 km from the city of Abidjan. It covers an area of 19,400 hectares (OIPR, 2011b). Before the post-electorial crisis, the Azagny National Park was an important touristic site (Vergnes and N'Gbesso, 2012; Gboméné, 2015). This site has already served as a sentinel site for the 2012 entomological surveillance program.

Krozialé forest

The Krozialé forest is located three kilometers from the Vapleu village, in the department of Zouan Hounien. It is accessible by foot, this forest harbors vast cocoa, coffee and rubber plantations. Logging is in full swing in this forest. The first case of dengue fever diagnosed in the Vapleu village came from this forest.

Banakôrô forest

The Banakôrô forest is located in the Banakôrô village, located ten kilometers from Tron Touba, in the Kaniasso community. During the yellow fever epidemic in the Tron Toubavillage, people who tested positive for the amaril virus originated from Banakôrô. Most of them regularly visited the forest.

Gansé forest

The Gansé forest is located in the Comoé National Park. It is a place for worship where only initiated persons belonging to this village have access to. Every year, rituals are undertaken there to prepare for the yam harvest. In this forest, entomological investigations were carried out after the death of a German researcher following an infection with the amaril virus. Gansé is adjacent to the Comoé National Park and is separated from Toupé by the Comoé River. It belongs to the Nassian municipality (Gauze *et al.*, 2014).

Wasségbôgbô forest

The Wasségbôgbô forest is eight kilometers from Sokala Sobara, which is difficult to access in the rainy season. In this locality, several cases of yellow fever have been reported by the public health services. These cases had visited the forest at least once. It is an important, low-density place of worship, crossed by a shallow stream.

Culicidae collection methods

Collection of mosquito eggs

The *Aedes* spp. eggs were sampled using the standard WHO ovitrap method (Bonizzoni *et al.*, 2013). Ninety ovitraps were used and were divided into primary sites (n=70) and secondary sites (n=20). At each primary site, 30 ovitraps were used in the villages around human settlement areas and 40 traps in the forests around villages (low human settlement areas). After three nights depending on the sampling sites, the water in these traps was collected and observed for possible egg hatching (Konan *et al.*, 2011; Koné *et al.*, 2013). The collected mosquito samples were allowed to dry for 10 days. Two consecutive waterings were carried out at 5 days intervals. Larvae from hatched-eggs were reared to adult mosquitoes. Imagos were identified at the genus and species level using published keys (Edwards, 1941; Yiau-Mi, 2004; Harbach, 2007).

Collection of mosquito larvae and pupae

Larvae and pupae of mosquitoes were sampled using the World Health Organization (WHO) standard equipment that has already been described by Zahouli *et al.*, (2017). The sampling of mosquito breeding sites was only carried out in primary sites. This consisted of the inspection of all the places that could serve as breeding grounds for mosquitoes.

These inspections were made by direct observation with the naked eye and the collection of water from the breeding sites to check for possible mosquito larvae and/or nymphs (Fofana *et al.*, 2019). The protential breeding sites checked for larvae of mosquitoes were domestic storage utensils (kettles, barrels, barrels, cans, basins, bowls, buckets),

abandonned storage containers/objects (disposable cups, abandoned plates, boxes, abandoned calabashes, toys, cut cans, broken basins), and natural deposits (holes on tree trunks, tree leaves, holes on rocks). Spoons of approximately 300 ml were used to collect immature stages of mosquitoes from larger breeding sites. Using transfer pipettes, the larvae and /or nymphs were put in plastic containers and transported to the laboratory. In the laboratory, the larvae were reared to the imago stage. Morphological identification was carried out upto the genus and species levels using published keys (Edwards, 1941; Yiau-Mi, 2004; Harbach, 2007).

Adult mosquito sampling

The sampling of adult mosquitoes was carried out using human-baited double-net traps (HBDNT) and the indoor morning aspiration techniques. The human-baited double-net trapping was conducted from 3 p.m. to 7 p.m. (period of high *Aedes* activity) (Hervé, 2003; Zahouli *et al.*, 2017). In each primary site, three (3) capture points were chosen for trapping. The collection device was designed such that blood meal seeking mosquitoes crosses the first mosquito net whose height above the ground is approximately 0.25 m. The second mosquito net completely covering an individual (human bait) prevents human-mosquito contact. Trapped mosquitoes were collected using a sucking tube. Regarding the morning aspiration method, it was conducted inside the households located in the primary sites, between 6 a.m. and 7:30 a.m. (Hervé, 2003). This approach consisted of collecting all the adult mosquitoes using a sucking tube.

The suction was carried out for two successive days in 12 households chosen at random. The collected mosquitoes were put in tubes and then sent to the laboratory for identification using standard keys (Edwards, 1941; Yiau-Mi, 2004; Harbach, 2007).

Vector potential indices

The mosquito species identified in this study were distributed according to four indices (ranging from 0 to 3) (Pradel *et al.*, 2007) as such:

0= Species not involved in the transmission or lack of information concerning the species

1= Species naturally infected

2=Species naturally infected and competent in the laboratory

3=Species naturally infected and competent in a natural environment.

Construction of monospecific mosquito pools

In addition, all the adult mosquitoes identified were grouped into monospecific pools ranging from 1 to 20 mosquitoes per species. The mosquito pools were constructed by considering the sampling techniques, the collection sites and the sex of the species. The mosquito pools were stored in liquid nitrogen at -80 ° C and then sent to the Pasteur Institute in Côte d'Ivoire for the detection of arboviruses using rt-qPCR.

Extraction and amplification of viral RNA

Nucleic acid (viral RNA) extraction was performed from 140 µL of mosquito pool ground supernatant using the QIAamp Viral RNA kit (Qiagen Catalog # 52904) according to the manufacturer's recommendations. The supernatant was obtained after centrifugation at 8000 rpm for one minute of a mixture of phosphate buffered saline (PBS) and ground mosquito pool. The different processes (amplification and detection) were carried out in the same reaction tube using the real-time rt-PCR trioplex developed by the Center for Disease Control and prevention (CDC) for the dengue, zika and chikungunya viruses (CDC, 2017) and the OneStep rt-qPCR Kit (QIAGEN) for amaril virus (Faye *et al.*, 2017). The rt-PCR trioplex for the detection of dengue, zika and chikungunya viruses required a final volume of 25 µL made of 0.5 µL of sterile water (Nuclease-free water), 12.5 µL of 2X buffer, 1.5 µL of primers and probes specific to the different genes to be amplified, 0.5 µL of RNase (enzyme) and 10 µL of RNA extract (CDC, 2017). The One Step rt-qPCR for the detection of the Amaril virus was carried out in a final volume of 25 µL made of 12.5 µL of 2X buffer, 6.5 µL of sterile water (Nuclease-free water), 2.5 µL of primers, 0.5 µL of probe, 1 µL of 25X enzyme and 2

µL of the extracted RNA (Faye *et al*., 2017). PCR was followed by a transcription reaction. The actual amplification of the viral genomes was performed in a thermal cycler (Applied Biosystems 7500 Fast Dx Real time PCR) using SDS software, in 45 PCR cycles following the manufacturer's instructions (CDC, 2017). The list of primers and probes used in the PCR reaction are provided in Table 2. Regarding the Dengue virus, the typing of the different serotypes (DEN 1, 2, 3 and 4) was carried out using specific primers and probes (Table 3) (Johnson *et al*., 2005). The positive pools for the different viral types were determined as a function of the fluorescences emitted by the fluorophores, on an amplification curve of a real-time rt-PCR reaction. The validity of the test is based on the negative and positive controls of the reaction. If at least one of the controls is not valid, the test must be repeated. On the other hand, if all the controls (positive and negative) are valid, the results can then be interpreted.

Data analysis

Data was analysed using SPSS version 21.The exact Fisher test was used to compare the proportions of the different monospecific pools created to test for the presence of the viral disease. The level of significance was set at 5%.

Results

Identification of potential arbovirus vectors

Fig.2 shows the vector potential of each mosquito species identified in this study. It shows that majority of the species could be infected with at least one of the four arboviruses (dengue, amaril, zika and chikungunya). Mosquitoes belonging to the genus *Aedes*: *Ae. aegypti*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. simpsoni*, *Ae. vittatus* and genus *Eretmapodites*: *Er. chrysogaster* and *Er. quinquevittatus* were identified. Among the 28 mosquito species identified during entomological studies, *Ae. aegypti*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. simpsoni*, *Ae. vittatus* and *Er. chrysogaster* had the highest vector potential with index of three (3). Beside these species, *Ae. opok*and *Er. quinquevittatus* showed indices of one (1) and two (2) respectively. The monospecific pools from the different sampling sites were recorded in Table 4. In total, 686 monospecific mosquito pools were made from 4813 Culicidae collected. These pools belonged to five (5) genera of mosquitoes: *Aedes*, *Anopheles*, *Coquillettidia*, *Culex* and *Eretmapodites*.

Table 1. Study sites and their geographical coordinates.

Geo-location	Site	Geographical coordinates
South	Banco National Park	5°23'N; 4°03'W
	Azagny National Park	5°12'N; 4°53'W
West	Vapleu Village	6°49'N; 8°16'W
	Krozialé Forest	6°48'N; 8°14'W
North-West	Tron Touba Village	9°43'N; 7°23W
	Banakôrô Forest	9°49'N; 7°23'W
North-East	Toupé Village	9°06'N; 3°43'W
	Gansé Forest	8°37'N; 3°54'W
Center	Sokala Sobara Village	8°27'N; 4°31'W
	Wasségbôgbô Forest	8°30'N; 4°30'W

The genus *Aedes* had 4,203 individuals including 17 species (*Ae. Aegypti*, *Ae. Africanus*, *Ae. Apicoargenteus*, *Ae. Denderensis*, *Ae. Dendrophilus*, *Ae. Fraseri*, *Ae. Haworthi*, *Ae. Lilii*, *Ae. Longipalpalis*, *Ae. Luteocephalus*, *Ae. Metallicus*, *Ae. Opok*, *Ae. Palpalis*, *Ae. Simpsoni*, *Ae. Schwetzi*, *Ae. Unilineatus*, *Ae. Vittatus*) distributed in 592 (86.3%) monospecific pools. Among these pools, 2 tested positive for arboviruses. The pools of the genera *Anopheles* (3.94%; n = 27), *Coquillettidia* (0.15%; n = 1), *Culex* (5.53%; n = 38) and *Eretmapodites* (4.08%; n = 28) recorded no positive result for arboviruses. The Fisher's test on the proportions of the monospecific pools revealed a significant difference ($p < 0.001$) (Table 5).

Table 2. The list of primers and probes used for the rt-PCR.

Primers and probes	Sequences of primers and probes (5' à 3')	Target regions	Reference
DENV-F	TAGTCTRCGTGGACCGACAAG	5'UTR	CDC (2017)
DENV-R	CAGTTGACACRCGGTTTCTC		
DENV-R	GGGTTGATACGCGGTTTCTC		
DENV-P	FAM-CGYCTWTCAATATGCTGAAACGCG-BHQ1		
CHIKV-F	ACCATCGGTGTTCCATCTAAAG	nsP1	CDC (2017)
CHIKV-R	GCCTGGGCTCATCGTTATT		
CHIKV-P	HEX-ACAGTGGTTTCGTGTGAGGGCTAC-BHQ1		
ZIKV-F	CCGCTGCCCAACACAAG	E	CDC (2017)
ZIKV-R	CCACTAACGTTCTTTTGCAGACAT		
ZIKV-P	CALFluorRed610-AGCCTACCTTGACAAGCAGTCAGACACTCAA-BHQ2		
YFV-F	ATTGAGGTGYATTGGTCTGC	5'UTR	Weidmann *et al.,* (2010)
YFV-R	GTCRRTTCTCTGCTAATCGCTCA		
YFV-P	6FAM-TCCTTCTCCCAGTCAGCCCCAC-BBQ		

Dengue virus (DENV); yellow fever virus (YFV); Zika virus (ZIKV and Chikungunya virus (CHIKV); -F : forward primer; -R : reverse primers; -P : probe.

The monospecific pools for Culicidae of the genus *Aedes* (592) were divided into 339 pools of females and 253 pools of males (Table 5). Two (2) pools tested positive for dengue and amaril viruses. These pools concerned females of the species *Ae. aegypti*. These Culicinae were from the larvae obtained from mosquito larval studies (Table 6). The dengue and amaril viruses were identified in mosquitoes from the primary sites of Vapleu and Tron Touba (Table 7).

Table 3. The primers and probes of the serotypes of dengue virus used for the rt-PCR.

Primers and probes	Sequences of primers and probes (5' à 3')	Reference
DENV 1-F	CAAAAGGAAGTCGTGCAATA	Johnson *et al.,* (2005)
DENV 1-R	CTGAGTGAATTCTCTCTACTGAACC	
DENV 1-R	FAM-CATGTGGTTGGGAGCACGC-BHQ1	
DENV 2-F	CAGGTTATGGCACTGTCACGAT	Johnson *et al.,* (2005)
DENV 2- R	CCATCTGCAGCAACACCATCTC	
DENV 2-P	HEX-CTCTCCGAGAACAGGCCTCGACTTCAA-BHQ1	
DENV 3-F	GGACTGGACACACGCACTCA	Johnson *et al.,* (2005)
DENV 3-R	CATGTCTCTACCTTCTCGACTTGTCT	
DENV 3-P	TR-ACCTGGATGTCGGCTGAAGGAGCTTG-BHQ-2	
DENV 4-F	TTGTCCTAATGATGCTGGTCG	Johnson *et al.,* (2005)
DENV 4-R	TCCACCTGAGACTCCTTCCA	
DENV 4-P	Cy5-TTCCTACTCCTACGCATCGCATTCCG-BHQ-3	

DENV: dengue virus; R: reverse primer; F: forward primer.

Discussion

The species of mosquitoes capable of transmitting pathogens, in this case arboviruses- dengue, amaril, zika and chikungunya are widespread in endemic settings of Côte d'Ivoire. It was therefore necessary to establish the species composition of mosquitoes that could be more or less involved in the transmission of arboviruses. The vector potential of Culicidae was estimated from published indices ranging from 0 to 3. This approach aims to provide a list of species of public health interest for more in-depth studies. These are among others: *Ae. aegypti*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. opok*, *Ae. simpsoni*, *Ae. metallicus*, *Ae. vittatus*, *Er. chrisogaster* and *Er. quinquevittatus*. Of course, a vector species involved or not in the transmission of pathogens in a given

environment does not mean that it will be so in another. However, it is important to emphasize that the development of a vectorial system is favored by the existence of a multitude of factors related to the modification of habitats and interfaces between vectors and hosts (Rodhain, 2008, De La Rocque *et al.*, 2011).

Its success therefore results from the encounter and compatibility between the different compartments of the transmission cycle (Desenclos *et al.*, 2009). Moreover, even within vector species, the levels of competence and vectorial capacity are very variable and depend, among other factors, on the adaptation of the vector-virus couple.

Table 4. Positivity of monospecific pools of mosquitoes to arboviruses.

Genus	Number of individuals	Number of species	Mosquito pools	
			Number of pools (%)	Positive pools (%)
Aedes	4 203	17	592 (86.3)	2 (100)
Anopheles	216	3	27 (3.94)	0 (0)
Culex	253	5	38 (5.53)	0 (0)
Coquillettidia	1	1	1 (0.15)	0 (0)
Eretmapodites	140	2	28 (4.08)	0 (0)
Total	4 813	28	686 (100)	2 (100)

This adaptation is undoubtedly the outcome of the coevolution between populations of vertebrate hosts, vectors and viruses (Bagny, 2009; Desenclos *et al.*, 2009). In a study conducted in France (Rhone-Alpes region) (Pradel *et al.*, 2007), a list of species likely to be involved in the onset of arbovirus epidemics were classified based on their vectorial potental using a semi-quantitave scale from 0 to 3. According to these authors, the attribution of an index of vector potential by considering the abundance of each species, makes it possible to establish a methodological framework leading to a hierarchy based on the relative importance of these species. To this, other criteria (seasonality, biology, species ecology) could be added to further refine the list or adapt it according to the objectives envisaged (Pradel *et al.*, 2007).

Table 5. Positivity of monospecific pools of Culicidae of the genus *Aedes* to arboviruses**.**

Aedes spp. and other Culicinae	Sex	Number of species	Number of pools	Number of monospecific pools positive for arboviruses				
				Den2	Amaril	Zika	Chikungunya	Total
Ae. Aegypti	Female	2 284	230	1	1	0	0	2
	Male	1 108	164	0	0	0	0	0
Ae. africanus	Female	1	1	0	0	0	0	0
	Male	0	0	0	0	0	0	0
Ae. luteocephalus	Female	31	9	0	0	0	0	0
	Male	15	6	0	0	0	0	0
Ae. metallicus	Female	10	3	0	0	0	0	0
	Male	8	2	0	0	0	0	0
Ae. opok	Female	1	1	0	0	0	0	0
	Male	1	1	0	0	0	0	0
Ae. simpsoni	Female	1	1	0	0	0	0	0
	Male	0	0	0	0	0	0	0
Ae. vittatus	Female	24	4	0	0	0	0	0
	Male	20	3	0	0	0	0	0
Other Culicinae of the genus *Aedes*	Female	449	90	0	0	0	0	0
	Male	250	77	0	0	0	0	0
Total	Female	2 801	339	0	0	0	0	0
	Male	1 402	253	0	0	0	0	0
Overall total		4 203	592	1	1	0	0	2

In Côte d'Ivoire, dengue, amaril and zika viruses have been isolated at least once from several species of mosquitoes of the genus *Aedes* including *Ae. aegypti*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. opok*, and *Ae. vittatus* (Cordellier *et al.*, 1988; Faye *et al.*, 2008; Faye *et al.*, 2013). The results of rt-PCR viral analysis in this present study revealed the presence of dengue and amaril viruses in *Ae. aegypti* only. The implication of this species in the emergence of arbovirus epidemics around the world no longer needs to be demonstrated because there is a great deal of research that attests to it (Eisen *et al.*, 2014; Guillaumot, 2014; Perez-Castro *et al.*, 2016; Severson and Behura, 2016; Ngugi *et al.*, 2017; Serrato *et al.*, 2017; Ferreira and Camara, 2018). In Côte d'Ivoire, dengue and yellow fever epidemics are recurrent. From the outcome of the present study, the positive pool of *Ae. aegypti* to the amaril virus on the Tron Touba site indicate a worrying health situation. This species is believed to be responsible for the yellow fever epidemic that occurred in 2009 in this locality. During this epidemic, entomological investigations revealed the presence of a number of potentially dangerous vectors in addition to *Ae. aegypti*.

Table 6. Positivity of monospecific pools of mosquitoes with different collection approaches. Human-baited double-net trap (HBDNT).

Mosquito collection method	Number of species	Number of pools	Number of pools positive for arboviruses				
			Dengue 2	Amaril	Zika	Chikungunya	Total
Ovitraps	889	199	0	0	0	0	0
Larval prospection	2 841	315	1	1	0	0	2
HBDNT	473	78	0	0	0	0	0
Morning aspiration	0	0	0	0	0	0	0
Total	4 203	592	1	1	0	0	2

It was *Ae. luteocephalus* and *Ae. opok* (Konan *et al.*, 2011). However, no arboviral research has been conducted on these species to confirm or deny their involvement in the epidemic. However, the decline in immunization coverage has been identified as the main cause (Konan *et al.*, 2011). According to Attoh-Touré *et al.*, (2010), the resurgence of the yellow fever in Côte d'Ivoire is partly due to the abandonment of mass vaccination associated with uncontrolled urbanization, migration and logging or cultivable land. At the Vapleu site, no arbovirus epidemic has been reported to date. Nevertheless, a case of dengue was reported by the health service of Zouan Hounien in 2002.

It concerned a girl who returned from Krozialé with her parents. Previous entomological surveys conducted by the National Institute of Public Hygiene (INHP), under the Ministry of Health and Public Hygiene, did not reveal any circulation of the virus in mosquitoes. Arboviral analysis in this study indicates the presence of dengue 2 virus in *Ae. aegypti*. This result could reflect a high epidemic risk, especially if all conditions are met (presence of larval breeding sites and vertebrate hosts) to ensure the maintenance and sustainability of the vector. Dengue virus serotype 2 has already been demonstrated in *Ae. aegypti* but in urban areas, in Abidjan particularly during various epidemics (WHO, 2009; L'Azou *et al.*, 2015; INHP, 2017).

All the monospecific Culicidae pools from the Sokala Sobara Wasségbôgbô, Toupé, Gansé and Banco and Azagny National Parks sites tested negative for arboviruses. However, previous studies conducted in some of these sites have indicated the opposite. In the Comoé National Park, the death of a German national from yellow fever raised fears of the risk of an epidemic.

In 2001, the results of multidisciplinary investigations (serology, epidemiology and entomology) revealed the presence of zika and amaril viruses in humans but also in species of mosquitoes of the genus *Aedes* including *Ae. aegypti* and *Ae. opok* (Akoua-Koffi *et al.*, 2001). Also, at the Sokala Sobara

site, the dengue 2 virus was detected from pools of four species of potential vectors of yellow fever, selvatic and primatophilic in West Africa notably: *Ae. furcifer*/taylori, *Ae. luteocephalus*, *Ae. opok* and *Ae. africanus* (Cordellier *et al.*, 1988). The negative test results for species such as *Ae. africanus*, *Ae. luteocephalus*, *Ae. opok*, *Ae. simpsoni*, *Ae. metallicus* and *Ae. vittatus* could be justified by their relatively low sample numbers. The pools of *Ae. aegypti* positive for dengue and amaril viruses all came from surveys in breeding sites. This confirms vertical or trans-ovarian transmission in this kind of mosquito. Contamination of the developmental stages of this mosquito shows its role as a biological vector (Bocquet and Morelo, 1996). This mode of transmission allows viruses to persist in areas where the duration of the dry season is greater than the lifespan of the vectors (Cordellier, 1991). However, the real incidence of this transmission is, on the epidemiological level, strongly attenuated by the low rate of infection of the offspring (Cordellier, 1991). According to Fontenille *et al.* (1998), the natural vertical transmission of arboviruses has two important epidemiological consequences. For the first consequence, *Aedes* females contaminated by the vertical route are probably infectious much earlier, from their first blood meals a few days after emergence, without waiting for the completion of a classic extrinsic viral cycle of 8 to 12 days after a blood meal on a viraemic host.

Table 7. Positivity of *Aedes* with sampled sites. BNP: Banco National Park; ANP: Azagny National Park.

Sites		Number of individuals	Number of pools	Number of pools positive for arboviruses				
				Dengue 2	Amaril	Zika	Chikungunya	Total
Primary sites	PNB	342	71	0	0	0	0	0
	Vapleu	778	130	1	0	0	0	1
	Tron Touba	1 053	105	0	1	0	0	1
	Toupé	1 162	136	0	0	0	0	0
	Sokala Sobara	527	77	0	0	0	0	0
	Total site 1	3 862	519	1	1	0	0	2
Secondary sites	PNA	11	6	0	0	0	0	0
	Krozialé	24	12	0	0	0	0	0
	Banakôrô	130	23	0	0	0	0	0
	Gansé	145	26	0	0	0	0	0
	Wasségbôgbô	31	6	0	0	0	0	0
	Total site 2	341	73	0	0	0	0	0
Total		4 203	592	1	1	0	0	2

The number of infesting meals and the proportion of females capable of transmitting are thus increased. This mode of transmission favors the amplification of the epidemic by adding to the classic horizontal transmission during which only “old females” transmit the virus at the end of the completion of the extrinsic cycle (Fontenille *et al.*, 1998). Secondly, the virus can persist from one rainy season to the next, in dormant eggs. Thus, in the absence of vaccination in a region largely beyond an epidemic zone, the virus can reappear in the following year in a neighboring region and cause a new epidemic if there has been no control campaign (Fontenille *et al.*, 1998). This mode of transmission should be taken into account in the design and implementation of vector control programs.

Conclusion

Mosquitoes (*Ae. Aegypti*, *Ae. Africanus*, *Ae. Luteocephalus*, *Ae. Opok*, *Ae. Simpsoni*, *Ae. Metallicus*, *Ae. Vittatus*, *Er. Chrisogaster*, *Er. quinquevittatus*), potential vectors of arboviruses are present in the study sites. Among them, only *Ae. aegypti* from the breeding sites was positive for amaril virus and dengue virus serotype 2. The presence of vectors and arboviruses constitute a significant health risk for the human populations living in these sites. The results of this study are therefore useful for the development of an entomo-epidemiological surveillance program and for the

planning of effective and sustainable vector control strategies. Such a system will provide reliable information to the public health services for the strategic implementation of measures for the prevention and control of arboviruses.

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