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Blaise Atioumounan KONE
National Institute of Public Hygiene (NIPH), Human Disease Vector Control Service. PO. BOX V14 Abidjan, Côte d'Ivoire.

Lambert Kouassi KONAN
Pasteur Institute of Côte d'Ivoire (PICI). Abidjan 01, Côte d'Ivoire.

Diakaridia FOFANA
National Institute of Public Hygiene (NIPH), Human Disease Vector Control Service. PO. BOX V14 Abidjan, Côte d'Ivoire.

Alexandre Franklin KOFFI
National Institute of Public Hygiene (NIPH), Human Disease Vector Control Service. PO. BOX V14 Abidjan, Côte d'Ivoire.

Daouda COULIBALY
National Institute of Public Hygiene (NIPH), Human Disease Vector Control Service. PO. BOX V14 Abidjan, Côte d'Ivoire.

Joseph Bi Vroh BENIE
National Institute of Public Hygiene (NIPH), Human Disease Vector Control Service. PO. BOX V14 Abidjan, Côte d'Ivoire.

Correspondence
Alexandre Franklin KOFFI
National Institute of Public Hygiene (NIPH), Human Disease Vector Control Service. PO. BOX V14 Abidjan, Côte d'Ivoire.

Entomological evaluation of the risk of spread of the dengue 3 epidemic in the health district of Cocody (Abidjan, Côte d'Ivoire)

Blaise Atioumounan Kone, Lambert Kouassi Konan, Diakaridia Fofana, Alexandre Franklin Koffi, Daouda Coulibaly and Joseph Bi Vroh Benie

Abstract
As directed by the Ministry of Health and Public Hygiene, this study aims to assess the extent of the viral circulation of dengue 3 in the health district of Cocody-Bingerville and the risk that this represents for the populations.
To do this, adult anthropophilic mosquitoes were captured by the double mosquito net technique and a larval prospecting around the dengue case was performed. Mosquito samples were made for viral research.
A total of 64 mosquitoes were captured and *Aedes aegypti* constituted approximately 97% (62/64) of the culicidian fauna. Thus, 7 samples were formed, including 6 with *Aedes aegypti*. In addition, *Aedes aegypti* constituted 93% (200/216) of emergences in the laboratory. At the emergence level, 21 samples were formed, including 20 with *Aedes aegypti*. Among these different samples, 2 from the catches and 1 from the emergences, were positive for the dengue virus. In Cocody, the Breteau index was estimated at 297, the container index at 41%, and the house index at 82%. While in the municipality of Bingerville, these indices were respectively 213, 35% and 69%.
Stegomyian index showed that larval densities were very high in both communes, reflecting a high risk of spreading dengue viruses.

Keywords: Entomology, *Aedes aegypti*, risk of spread, dengue, Cocody, Côte d'Ivoire

1. Introduction
Dengue is a tropical arbovirosis caused by a virus belonging to the family Flaviviridae and the genus *Flavivirus*. The dengue virus (DENV) or arbovirus (arthropod borne virus), is an enveloped RNA virus, of which we distinguish 4 distinct serotypes (DEN-1, DEN-2, DEN-3, DEN-4). The vector is a mosquito of the genus *Aedes*, mainly *Aedes aegypti*, but also *Aedes albopictus* (WHO, 2009) [1].
In Côte d'Ivoire, in 2008, an epidemic of dengue, accompanied at the same time by another yellow fever, was detected in Abidjan and its surroundings [2]. In addition, 3 imported cases of dengue fever by people returning from Côte d'Ivoire, were diagnosed in France and Japan between May and August 2008 [3, 4]. In July 2010, Ivorian health authorities reported 12 dengue cases, including 1 death, in the cities of Abidjan, Grand Bassam and Bouaké. Serotype DEN-3 was diagnosed by PCR in several patients [5]. In 2017, dengue epidemics were reported in Côte d'Ivoire and Sri Lanka [6]. In Côte d'Ivoire, this epidemic of dengue 3 has been declared since April 2017 in Abidjan, in the health district of Cocody-Bingerville. The laboratory of the Pasteur Institute of Côte d'Ivoire, confirmed on April 28, 2017, that the index case presented a dengue type 3.
Faced with this situation, the Ivorian health authorities immediately took action to fight and preventit. These consisted of sensitizing the population and controlling the density of vector populations on the one hand, and strengthening epidemiological and biological oversight on the other. This is why this study aimed to assess the risk factors for the spread of dengue fever 3 in the Abidjan district. Ultimately, it should enable the development of prevention and control strategies for the epidemic.

2. Materials and methods

2.1 Study areas

The study was conducted in the health district of Cocody-Bingerville, that is to say, in the communes of Cocody (5°20'56" North Latitude and 4°00'42" West Longitude) and Bingerville (5°21' North Latitude and 3°54' West Longitude). Figure 1 shows the areas and sites of the study, located in the autonomous district of Abidjan (Côte d'Ivoire, West Africa).

In the Bingerville study area, 12 neighborhoods were prospected. In the area of Cocody, 9 neighborhoods were surveyed.

2.2 Harvest adult mosquitoes

Harvesting of adult mosquitoes was only done in the neighborhood where the case was declared. Three capture points were chosen. This capture was carried out for three consecutive evenings between 3 pm and 8 pm, in the course of the dwellings. Indeed, a trained volunteer, was placed on a removable bed and covered with a device of double mosquito net. All the mosquitos looking for a blood meal, were trapped between the two mosquito nets. Using a vacuum cleaner mouth, a flashlight and hemolysis tubes clogged with cotton, the volunteer collected all mosquitoes at intervals of 60 minutes (50 minutes of trapping and 10 minutes of collection).

2.3 Identification of adult mosquitoes

Mosquitoes from different captures were identified using a binocular loupe and identification keys up to the species level [7, 8]. Mono-specific batches of identified adult mosquitoes were made and sent to the laboratory in a liquid nitrogen container.

2.4 Research of the virus by the molecular biology technique

Monospecific batches of mosquito samples were analyzed at the Pasteur Institute of Côte d'Ivoire (PICI), for virological research, by viral isolation using the method of molecular biology PCR.

2.5 Data processing

The Stegomyian indices recommended by the WHO have been calculated (House index, Container index and Breteau index)

XLSTAT software version 2018.2.504991 and Stat EL were used to compare abundances and proportions. The Chi² independence test was used for linkage analysis between qualitative variables.

2.6 Ethical considerations

All participants present in the field, as part of the capture of adult mosquitoes, were trained on the experimental protocol of the study. They all gave their informed consent orally.

3. Results

3.1 Viral isolation

3.1.1 Capture of adult mosquitoes under double mosquito nets in the area of the declared case

The capture of anthropophilic mosquitoes, between 3:00 pm and 8:00 pm, has resulted in a total of 64 mosquitoes, divided into 2 species. They are *Aedes aegypti* (N = 62) and *Culex quinquefasciatus* (N = 2). The difference was highly significant (*p-value* < 0.0001). *Aedes aegypti*, the major vector of the dengue virus, was the most abundant of the culicidian fauna caught under the double mosquito net in this neighborhood. It was equally caught in all time bands, while *Culex quinquefasciatus* was caught between 6:00 pm and 7:00 pm. Seven (7) mono-specific lots were established, including 6 of *Aedes aegypti* and 1 of *Culex quinquefasciatus*

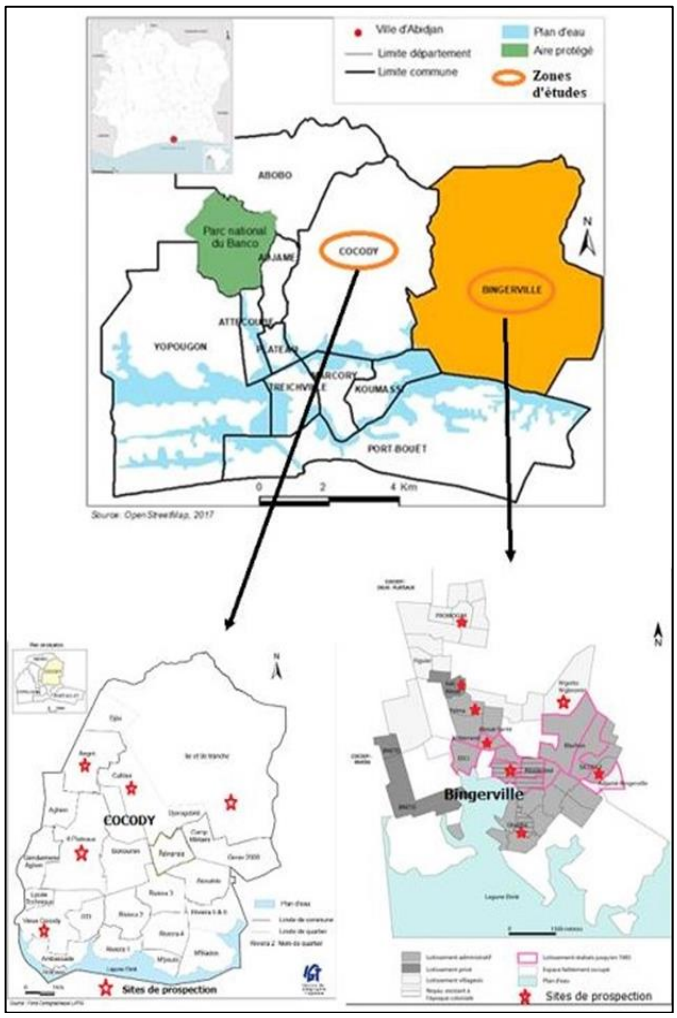

Fig 1: Location of study areas and sites in the health district of Cocody-Bingerville

3.1.2 Emergence of adult mosquitoes obtained after rearing the larvae collected in the district of the declared case

The larvae cultured at the National Institute of Public Hygiene insectarium, yielded 216 adult mosquitoes, including 200 of *Aedes aegypti* and 16 of *Culex quinquefasciatus* (*p-value* < 0.0001). A total of 21 lots have been established, including 20 for *Aedes aegypti* and 1 for *Culex quinquefasciatus*.

All these mosquito batches were sent to the Institute Pasteur in Côte d'Ivoire for viral research. Dengue virus 3 was isolated from 3 batches of *Aedes aegypti* mosquitoes by the real-time PCR technique. Among the 3 batches, one is derived from the mosquitoes obtained after the emergence of the larvae harvested during the larval survey and the other two from the catch under double mosquito net.

3.2 Evaluation of the risk of dengue virus spread from larval breeding sites prospecting

3.2.1 Distribution of larval breeding sites according to their geographical location

In Cocody, 1282 breeding sites at *Aedes aegypti* have been inventoried, including 913 (71%) inside the concessions and 369 (29%) outside. The difference is significant (*p-value* < 0.001). Domestic coverts dominate in Cocody. Bingerville also, of the 1984 larval breeding sites surveyed, 1659 (84%) were domestic and 325 (16%) were peri-domestic. In the two localities, the majority of breeding sites found were found inside the concessions visited (*p-value* < 0.0001). Domestic breeding was the most abundant.

3.2.2 Diversity of identified larval breeding sites

The breeding sites listed in and near some dwellings in the communes of Cocody and Bingerville are not all of the same nature. They have been as diverse as many. They have been grouped into five main types: drinking water storage containers (RS), abandoned containers (RA), old used tires (Pn), natural lodgings (GN) and other lodgings (AG). Figure 2 shows the abandoned containers as being the most varied. The difference is significant (*p-value* < 0.0001). In this group, 52 and 57 different types of deposits were respectively inventoried in Cocody and Bingerville.

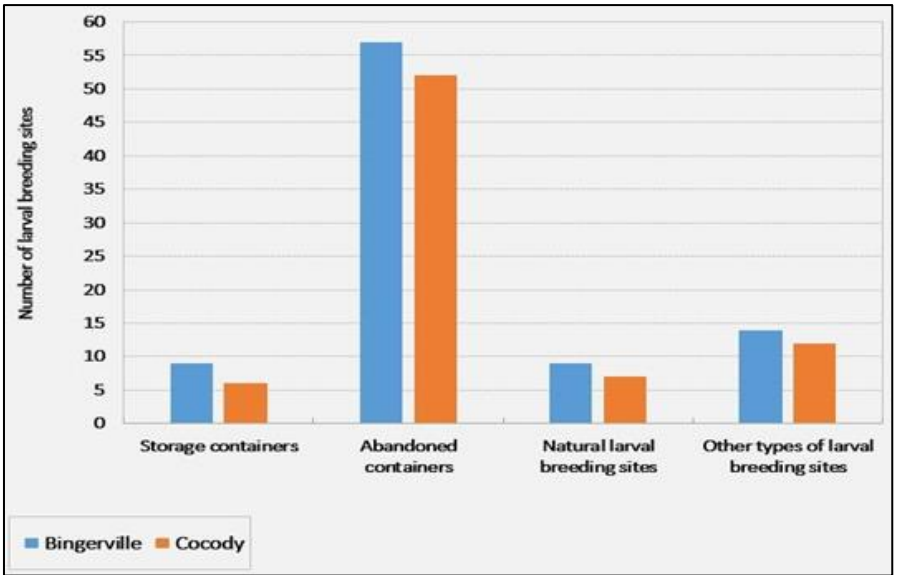

Fig 2: Abundance of larval breeding sites at *Aedes aegypti* in Cocody and Bingerville

3.2.3 Abundance and infestations of identified larval breeding sites

Of a total of 1282 coverts surveyed in the commune of Cocody, 521 were positive at *Aedes aegypti*. In the commune of Bingerville, 691 out of a total of 1984 larval breeding sites, were found positive in *Aedes aegypti*. (Table 2). In the two communes, the domestic breeding sites were the most colonized by *Aedes aegypti larvae*. The difference is very highly significant (*p-value* < 0.0001). The abandoned containers appeared the most colonized, with respectively 245 and 335 positive deposits, respectively in Cocody and Bingerville (*p-value* < 0.001). However, the percentage of infestation of breeding sites showed that used tires were the most colonized by *Aedes aegypti* larvae. In these, 87% and 92% of breeding sites found were positive, respectively, in Cocody and Bingerville.

In addition, in the town of Bingerville, 88% of the water storage containers were found to be positive. In the commune of Cocody, on the other hand, it is the group of houses called "other deposits", which was found mostly positive (Table 1).

3.2.4 Larval breeding sites productivity, with *Aedes aegypti* larvae

Of all the types of larval breeding sites surveyed, the most productive were the abandoned containers, the drinking water storage containers (domestic lodgings) and the used tires that strew the streets (roost homes). In fact, the greatest number of *Aedes aegypti* larvae were harvested the most in abandoned containers, with 8091 and 10170 larvae respectively, in the communes of Cocody and Bingerville (*p-value* < 0.0001). The drinking-water storage containers were also colonized by *Aedes aegypti* larvae, with 3969 and 5441 larvae, respectively, for Cocody and Bingerville communes. As for used tires, they produced 3938 and 2514 larvae respectively in the communes of Cocody and Bingerville (*p-value* < 0.001).

In addition, nymphs (N = 996) were harvested in larger numbers in the abandoned containers of the commune of Cocody (*p-value* < 0.0001). In Bingerville, on the other hand, drinking water storage containers recorded the highest number of nymphs (Table 2).

3.2.5 Stegomyian indices of epidemic risk

The population density of *Aedes aegypti* was assessed using the "House" index, the "Container" index and the Breteau index. Overall, the various indices calculated were very high (Table 3). In fact, the calculated "House" indexes were 69 and 82%, respectively in the communes of Bingerville and Cocody. For calculated "Container" indices, the values obtained in Bingerville and Cocody were respectively 35 and 41%. The "Breteau" index, estimated at Cocody and Bingerville, was respectively 297 and 213.

The calculated Stegomyian indices, are relatively high and are at levels 9 and 8-9, respectively in Cocody and Bingerville. The two indices (Containers and Breteau) that give a fairly good view of the danger of an epidemic in a given region are higher than the threshold set by the WHO in Cocody and Bingerville.

4. Discussion

Concerning the viral isolation of mono-specific batches, catches of adult mosquitoes under double mosquito nets, showed the presence of 2 species that were *Aedes aegypti* and *Culex quinquefasciatus*. *Aedes aegypti*, was mainly present in the catches, with a proportion

of 97% (62/64) of the total numbers. Similarly, of the 216 larvae harvested during larval survey, 200 were from *Aedes aegypti* and 16 were from *Culex quinquefasciatus*. These larvae were harvested in and around the houses. This corroborates the high number of adult mosquitoes of *Aedes aegypti*, during catches under double mosquito nets, as well as the strong implantation of this species in the communes of Cocody and Bingerville. Indeed, *Aedes aegypti* is a mosquito very dependent on humans and its larvae develop in water storage containers, abandoned containers and used tires, which constitute consumer waste. It also has a great preference for small and medium water collections [9].

Table 1: Distribution of breeding sites containing *Aedes aegypti* larvae in the communes of Cocody and Bingerville.

Nature of larval breeding sites	Cocody			Bingerville		
	Number of larval breeding sites	Positive larval breeding sites	Infestation rate (%)	Number of larval breeding sites	Positive larval breeding sites	Infestation rate (%)
Storage container	150	87	**58**	184	161	**88**
Abandoned container	693	245	**35**	441	335	**76**
Tires	129	112	**87**	72	66	**92**
Natural larval breeding sites	224	19	**8**	1047	16	**2**
Other larval breeding sites	86	58	**67**	240	113	**47**
Total	**1282**	**521**	**41**	**1984**	**691**	**35**
Domestic	913	405	**44**	1659	616	**37**
Peri domestic	369	116	**31**	325	75	**23**

Table 2: Productivity of deposits containing *Aedes aegypti* larvae in the communes of Cocody and Bingerville

Nature of larval breeding sites	Cocody			Bingerville		
	Number of positive larval breeding sites	Number of larvae	number of nymphs	Number of positive larval breeding sites	Number of larvae	number of nymphs
Storage container	87	3969	303	161	5441	1046
Abandoned container	245	8091	996	335	10170	2046
Tires	112	3938	324	66	2514	508
Natural larval breeding sites	19	1037	91	16	512	114
Other larval breeding sites	58	1702	144	113	2899	496
Total	**521**	**18737**	**1858**	**691**	**21536**	**4210**

Table 3: Estimated levels of risk of dengue transmission in the Cocody health district.

Communes	Stegomyian indices							
	Number of visited houses	Number of houses with positive larval breeding sites	Number of larval breeding sites	Number of positive larval breeding sites	House index (%)	Container index (%)	Breteau index	Density scale
Bingerville	175	144	1282	520	**69**	**35**	**213**	**8 - 9**
Cocody	325	223	1984	691	**82**	**41**	**297**	**9**

The strong implantation of this vector (*Aedes aegypti*) in the neighborhoods of the two communes could also be explained by the lack of knowledge of the vector and its places of reproduction, by the populations. To this, could be added a deficiency of surveillance of the vector.

The dengue 3 virus was isolated from 3 mosquito samples, 2 of which were from double net catches and the other, from larvae harvested during larval prospecting. These results could, on the one hand, lead us to think of interpersonal transmission [10] and, on the other hand, confirm the transovarian spread of the dengue virus [11]. They thus come to help confirm the existence of vertical transmission of the dengue virus by the mosquito vectors *Aedes aegypti* [12], as has been demonstrated for the yellow fever virus [13]. The fact that the juvenile stages of *Aedes aegypti* are carriers of the dengue virus suggests that these vectors play not only a role of transmitter, but also, a role of reservoir of this virus [14]. This situation could contribute to the maintenance of the endemic, or its expansion during epidemic and inter-epidemic periods. The dengue virus would then be able to spread wherever the major vector *Aedes aegypti* is present in the commune, and therefore, in all the autonomous district of

Abidjan and its surroundings. This could then increase the risk of spreading the virus.

For the assessment of the risk of dengue virus spread, the calculated "House" indexes were 69 and 82%, respectively in Bingerville and Cocody communes. These values were significantly higher than the normal WHO guideline of 35%. For calculated "Container" indexes, the values obtained in Bingerville and Cocody were respectively 35 and 41%. These values were also above 20%, the threshold value set by WHO. The "Breteau" index, estimated at Cocody and Bingerville, was respectively 297 and 213. These values were higher than 50. These 3 calculated indices, showed that the risk of transmission or propagation of dengue, by *Aedes aegypti*, is very high in these two communes and in Abidjan.

The calculated Stegomyian indices were relatively high and were found at levels 9 and 8-9, on the WHO density scale [15], respectively in Cocody and Bingerville. According to WHO estimates, the density index reflects an epidemic risk from value 2 ("Container" index > 3%, "Breteau" index ≤ 5) and high risk from value 6 ("Container" index ≤ 20%, "Breteau" index ≤ 35) [15]. The two indices ("Container" and "Breteau") which give a good idea of the danger of an epidemic occurring in a given region were, in Cocody and Bingerville, above the thresholds set by WHO.

In the majority of neighborhoods in the communes of Cocody and Bingerville, inventoried larval breeding sites were found largely in the yards of dwellings. They represented respectively 71 and 84% of the deposits found in Cocody and Bingerville. Indeed, in the course of their work, Lanoix and Roy, and Romeo-Vivas and Falconar [9, 16], observed that *Aedes aegypti* was a vector that almost always coexisted with humans. The multiplication of breeding places in the houses could be related to the ignorance of these, by the populations.

The larval breeding sites found were numerous and diverse. The majority (54% in Cocody and 22% in Bingerville) consisted of containers abandoned by the population, inside and outside the homes. However, in the commune of Bingerville, the presence of numerous strains of banana found throughout the city has significantly influenced the total number of listed larval breeding sites. With regard to positive larval breeding sites, older used tires were the most numerous, with 40 and 34%, respectively in Cocody and Bingerville. *Aedes aegypti* was the most popular species at the level of the different deposits surveyed in the 2 communes. On the other hand, although some types of larval breeding sites, such as abandoned containers, were probably the most numerous and the most positive, they were not the most productive. In fact, used tires were the most colonized by the larvae of this species. Beside these used tires, water storage containers for domestic use, have been in number, the most positive deposits. This observation was also made by Lutomiah [17], during the entomological investigation that was carried out following the dengue epidemic in Mombasa, Kenya. Water storage had become a common practice in most homes visited during this study.

Concerning the productivity of the larval breeding sites of the communes of Cocody and Bingerville, it is the abandoned containers and the storage containers of water, which were the most productive. Indeed, the high number of *Aedes aegypti* larvae harvested, reflects their preference for these man-made artificial deposits.

In addition, larval densities of *Aedes aegypti*, obtained in the communes of Cocody and Bingerville, were very high. "House", "Container" and "Breteau" indices, calculated, have been very high and range between 8 and 9, on the WHO density scale [14]. In 2004, in Cixi, China, a "Breteau" index equal to 326, was recorded during an epidemic of dengue transmitted by *Aedes albopictus*, before stabilizing at less than 5, after 10 days of larval control [18, 19]. This descriptive study, carried out in the communes of Cocody and Bingerville, confirms the existence and the implantation of dengue fever in highly urbanized areas. Thus, galloping urbanization and the absence or weak control measures of vector populations, would be important factors in the growth of this arbovirosis [20]. These 2 communes would therefore be exposed to the danger of an outbreak of dengue fever [21]. Indeed, the risk of transmission and / or spread of this arbovirosis, is reflected by the high larval density observed, and the detection of the virus in the juvenile and adult stages of *Aedes aegypti* [22].

5. Conclusion

The entomological investigation initiated around the reported case of dengue 3 at Cocody, made it possible to isolate the dengue virus 3, from 3 mosquito samples, 2 of which came from the capture of adult mosquitoes under double mosquito nets, and the other, emergence of larvae put in breeding with the insectarium of the National Institute of Public Hygiene (NIPH). Larval surveys have revealed the presence of breeding sites inside houses. The majority of these consisted of abandoned containers and water storage containers. These are diverse and numerous. Around the dwellings, it was used tires that were the most numerous. On the whole, they were the most positive. On the other hand, in terms of productivity, it was in the abandoned containers and the water storage containers that the greatest number of larvae was counted. The densities of larval populations of *Aedes aegypti* in the communes of Cocody and Bingerville were very high. In fact, the estimated "House", "Container" and "Breteau" indices were very high and ranged between 8-9 and 9 on the WHO density scale, respectively in Bingerville and Cocody. As a result, the risk of dengue spread was very high in Abidjan, where the virus was already circulating, at the level of the mosquito vectors collected around the residence of the patient declared positive. Finally, it can be noted that the behavior, the habits of the populations and their lack of knowledge of mosquito breeding sites, have created favorable conditions for the emergence and maintenance of *Aedes aegypti*.

6. Declaration of Intrest

The authors declare that they have no conflicts of interest in relation to this article.

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