Important Aedes Spp. Infestation Levels In Kinshasa, Democratic Republic Of Congo

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Research

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Abstract

BACKGROUND

Arboviruses such as dengue, yellow fever, chikungunya and Zika are among the most important emerging infectious diseases worldwide. Yellow fever and chikungunya outbreaks, and few dengue cases have been reported in Democratic Republic of Congo (DRC) in recent years. Although the main vectors of these arboviruses, Aedes aegypti and Aedes albopictus, were reported in DRC, the lack of detailed information on their presence and spread hampers transmission risk assessments in this region.

METHODS

In 2018, two cross-sectional surveys were realized in Kinshasa province (DRC), one in the rainy (January/February) and one in the dry season (July). Four hundred houses were visited in each of the four selected communes (N’Djili, Mont Ngafula, Lingwala and Kalamu). Breedings sites were recorded, larvae and pupae collected and reared to obtain adults for genus and species identification. A subset of specimen was DNA-barcoded for species validation.

RESULTS

The most rural commune (Mont Ngafula) had the highest infestation levels, with a Breteau Index of 82.2 and 19.5/100 houses in rainy and dry season, respectively. The Breteau Index in the other communes Kalamu, Lingwala and N’Djili elevated to 21.5 (4.7), 36.7 (9.8) and 41.7 (7.5) in the rainy (and dry) season respectively. The House index was on average 27.5% and 7.6%; and the Container Index 15.0% and 10.0% in rainy and dry season, respectively. The vast majority of Aedes positive containers were found outside the houses (aOR 27.3 (95%CI 14.9-50.0)). The main breeding sites were used tires, water storage containers and trash. Anopheles larvae were also found in Aedes breeding sites in all four communes in the rainy season.

CONCLUSIONS

These results show that Kinshasa is highly infested with Aedes spp. which indicates a high potential for arbovirus transmission in the area. The present study evidences that Aedes breeding sites are mainly located outdoors. The most productive containers (for Aedes pupae production) during the dry season are the water storage containers, while over the rainy season these are the artificial containers, especially tires. This will have an impact on the design of control strategies for these vectors in Kinshasa.

Background

Arboviruses transmitted by Aedes mosquitoes, such as dengue, yellow fever, chikungunya and Zika, are among the most important emerging infectious diseases worldwide. The distribution of these diseases is fairly well known in Latin-America and South-East Asia, but information on their occurrence in Sub-Saharan Africa remains scarce and scattered. Most information is coming from seroprevalence studies
for dengue, showing that there is or has been a non-negligible circulation, demonstrated by a 12.5% IgG positivity in Cameroon, 36% in Burkina Faso, 45% in Nigeria (1) and 50.6% in Tanzania (2). Chikungunya, instead, is mainly reported during outbreaks, such as the one in 2004 in Kenya (3), in 2013 in Tanzania (4), in 2018 in Mozambique (5), and in 2011 (6) and 2019 (7) in Brazzaville (Republic of the Congo). In Kenya it has been shown that a chikungunya outbreak can infect up to 67% of the population (8). Chikungunya outbreaks have also been repeatedly reported in Kinshasa, Capital of the Democratic Republic of Congo (DRC), namely in 2000 (9), 2012 (10) and 2019 (11). There are four reports on dengue transmission in DRC: one is from 2012, where the antigen dengue test was positive in three suspected chikungunya cases in Kinshasa (12), a second is an evaluation of arboviruses among dried blood spots taken during a Demographic health Survey in 2013–14, where 0.6% was positive for dengue (13), a third one detected some dengue cases (3.5%) among yellow fever suspect cases in the Bas Congo region (14), a last report comes from a cross-sectional study in Mont Ngafula (suburban area of Kinshasa) showing that among acute undifferentiated fever, dengue was responsible for 8.1% of the cases and chikungunya for 0.9% (15). On the contrary, in the neighboring country, Angola, an important dengue outbreak with an estimated 10% of the population having had contact with the virus was described in 2013 (16). This country was also already exposed to Zika (17) and together with DRC had a yellow fever outbreak in 2016 (18). Finally, several alpha-, flavi- and bunyaviruses were found in mosquito samples (Aedes and Culex) from Kinshasa in 2014 (19).

Within the sub-Saharan African region, the information on the presence and distribution of the Aedes mosquito is even more difficult to find, and according a recent comprehensive review, one better uses suitability maps for the African continent to estimate risk for disease transmission (20). Both Aedes aegypti and Aedes albopictus are found in the region, where Ae. aegypti is native, whereas Ae. albopictus is native of South-East Asia. The latter was reported for the first time in Central Africa in early 2000 (21) and in Kinshasa (DRC) in 2018 (22), but is nowadays considered omni-present in Central Africa (23). Both species can be found in human-domesticated environments (24). However, real Aedes spp. infestation levels are more reliable than suitability maps, which are based on mathematical models, to estimate the risk for transmission of arboviruses (25). These infestation levels are unknown for Kinshasa, a mega-city with a high population density and movement.

In this study, we evaluate the Aedes spp. infestation levels, together with the characteristics of the preferred breeding sites, in order to have an evidence basis for guidance of Aedes control efforts and for transmission estimation in Kinshasa, the capital of DRC.

Methods

Settings

The study took place in Kinshasa, capital city of Democratic Republic of Congo, located in the Central-African region. Kinshasa lies on 279 m above sea level and the climate is tropical with a rainy season between October and May, and a dry season from June to September. The average temperature is 25.5 °C
and the average annual rainfall is 1368 mm. Kinshasa is expanded over 9965 km² and has an estimated population of almost 12 million people. The city is administratively subdivided in 24 communes, which are grouped in four districts: Tshangu in the East, Lukunga in the North, Mont Amba in the South-East and Funa in the Center-West. In this study, four communes were selected in different geographical zones of the city having a different ecology, urbanization, water supply systems and history of arbovirus outbreaks (figure 1).

N’Djili is a peri-urban commune in the East of the city, pertaining to the Tshangu district, and where many informal economic activities, specifically vehicle repair shops, are located. Urban infrastructure is deficient, such as waste water infrastructure and garbage collection. 97% of the houses have a water supply system in their compound, but an important proportion of them has problems with quality and amount of water availability. The population density of this area is estimated at 39 000 persons/km².

Kalamu II is a commune in the centre of town, belonging to the Funa district, and is a more residential place with as main economic activity technical service provision. It has an estimated population density of 47 000 persons/km².

Mont Ngafula I is situated in the South of the city, bordering Mont-Amba district, and is an example of a semi-urban area with an estimated population density of 730 persons/km². It is geographically characterized by the presence of hills (and accompanying erosions) and small valleys. The main economic activity is agriculture and the selling of agriculture products to Kinshasa city. The place is characterized by unplanned urbanization with a typical deficient water supply system—with in some areas supply frequency as low as two times/week—and a deficient waste water disposal.

Lingwala is a commune in the centre of the town, pertaining to the Lukunga district, with a lot of informal markets. Population density is estimated at 33 000 persons/km².

**Study design and data collection**

Two cross-sectional surveys were done in the four selected communes, one in the rainy season (18 January–16 February, 2018) and one in the dry season (2–27 July, 2018). In each of the four selected communes, one neighborhood has been randomly chosen (all neighborhoods per commune listed, followed by random number selection procedure) as study site. In each study site 400 houses were randomly selected to be surveyed. The sample size was calculated based on an estimation of 10% of the houses being positive for *Aedes spp.* mosquitoes with a precision of 3% and allowing for a 5% alpha-error. Each day 80 houses (four teams inspecting each 20 houses/day) were inspected, using a systematic sampling approach: on a landmark (roundabout or main road) random points were identified for each team as their starting point to enter the (smaller) avenues. With a sampling interval of three, starting on the right side of the avenue, each team inspect the selected houses up to reaching 20 houses/day. When the avenue came to an end and the sampling size of 20 was not yet reached, the team turned back entering the houses on the other side of the street up to reaching the daily sample size. In each selected
house, the entire house was inspected inside and outside. If there was more than one house per compound, a random house was chosen to inspect, but the entire outside part of the compound was inspected. The next day, the avenue on the left side of the previous day was sampled. By this procedure, an extensive part of the neighborhood was covered with the survey. When one commune was finalized, the four entomological teams went to another commune and followed the same methodology. All communes were covered in four weeks time. Each entomological survey team consisted of three persons, previously trained by the entomology department of the 'Institut National de Recherche Biomédicale' (INRB), one entomologist of the INRB (supervisor) and one community health worker.

In each compound, all water holding containers were inspected and if immature stages (larvae or pupae) of mosquitoes were observed, they were collected in plastic bottles (one bottle per breeding site) and transported to the laboratory at INRB for genus identification (Anopheles, Aedes, Culex). The place, category and positivity/negativity of each container was reported. For larvae, only positivity and negativity was recorded; for pupae, the number of pupae was counted per positive breeding site. Both surveys were done in a similar way, but starting points of avenue and house selection differed and it could be that a same house was visited in both surveys, but this was based on randomness and was not aimed at. Both surveys were largely realized by the same field team members.

Species identification: morphology and DNA-based

Each day, a subsample of Aedes genus larvae/pupae were reared to adults in the insectarium to allow species identification using morphological keys (26,27). F0 adults were stored at ~20°C for genetic analyses. Five mosquitoes of both Aedes species were randomly selected per study site for further DNA-based identification technique to validate species identification and confirm the presence of the identified species in Kinshasa.

Indeed, a critical step in any monitoring project is the correct species identification of the sampled specimen. Therefore, a subset of specimen was DNA barcoded (28). DNA-barcoding is a technique based on the amplification of a standard barcode - the partial mitochondrial cytochrome c oxidase subunit I gene for animals. Subsequently, the generated sequences were compared to a library of reference sequences. A specimen is identified by analyzing its percentage sequence similarity with these reference sequences under the assumption that genetic diversity is lower within than between species. A rooted Neighbour-Joining tree was constructed including a sub-selection of the Ae. albopictus and Ae. aegypti barcodes available from online repositories, together with the newly generated haplotypes (full details in Additional File 2).

Data analysis

Data were entered in an Access database and 5% of the data were manually checked to evaluate inconsistencies. Data were cleaned and types of recipients regrouped into categories as following: Big
water deposits (> 15 L), Small water deposits (< 15 L), Artificial -trash, Natural sites, Artificial not-destroyable, Tires, Water evacuation/ ponds. Data were analyzed using IBM SPSS Statistics, version 25. We calculated per round and per commune house index (number of houses positive for at least one container with immature stages of Aedes spp. per 100 inspected houses), Breteau index (number of containers positive for immature stages of Aedes spp. per 100 inspected houses), Container index (number of containers positive for immature stages of Aedes spp. per 100 inspected containers), and pupal index (number of Aedes spp. pupae per 100 inspected houses). The relative contribution to pupal productivity was calculated and defined as the total number of pupae of Aedes spp. per category of breeding site divided by the total number of pupae of Aedes spp. collected per commune and per survey round. A descriptive analysis was done. In order to evaluate the factors determining Aedes spp. immature stage positivity, a logistic regression model was made and associated variables were identified based on a backwards conditional model.

The identification of Ae. aegypti or Ae. albopictus was done on daily subset of samples, and analysis was restricted to calculation of proportions. The number of breeding sites with at least one immature stage of Anopheles spp. was enumerated and its proportional importance calculated for each season and respective commune.

**Results**

The survey in the rainy and dry season allowed to investigate/sample a total of 1678 and 1598 houses, respectively. In the rainy season, 5079 water-holding containers, which were potential breeding sites, were inspected against 1657 in the dry season. The average number of containers per household was different across communes: for example in the rainy season, an average of 1.4 (Standard deviation SD 1.3) in Kalamu, 2.0 (SD 1.7) in Lingwala, 2.9 (SD 2.3) in Mont Ngafula and 5.3 (SD 2.6) in N'Djili. In both seasons and in all communes, containers were mostly observed outside (in the space around the house, but within the compound) than inside the inspected houses. In the rainy and dry season, all communes taken together, 65.9% and 78.3% of inspected containers were located outside, respectively. In the Additional File 1 (table 1), the distribution of type of containers per location, commune and season is detailed.

*Table 1. Aedes spp. entomological indices of the four study sites, in the rainy and dry season, Kinshasa, 2018.*
Aedes infestation levels were higher in the rainy than in the dry season, with a Breteau Index (BI) of 45.35 versus 10.39/100 houses, a Container Index (CI) of 14.9% versus 10.02 % and a House Index (HI) of 27.53% versus 7.63%, respectively (Table 1). Mont Ngafula, a rural sub-urban area in the Southern edge of Kinshasa had the highest infestation levels amongst all visited communes with a BI of 82.21 and 19.50/100 houses in the rainy and dry season respectively, and was about four times higher than Kalamu, a commune that lies within the heart of the center of town. The number of Aedes pupae present per 100 houses reached in the rainy season 246 pupae/100 houses in Mont Ngafula, 126 in N'Djili, 90 in Lingwala, and 50 in Kalamu, which were two to 14 times higher than in the dry season. In the rainy season 99.3% of the positive breeding sites were outdoors against 96.4% in the dry season. A wide variety of containers is used by the Aedes mosquito to breed: big water deposits, small water deposits, artificial-trash, natural sites, artificial not destroyable, tires, water evacuation/ponds (Figure 2). Tires were taken as a separate group, as they are frequently present and it is difficult to know if they are just put aside for re-use/temporary storage or to be destroyed.

When analyzing the productivity of recipients, we observe that in the rainy season 64.3% of all inspected recipients were small water deposits, but they were only responsible for 46.4% of the pupae production, whereas tires represented only 11.1% of the inspected recipients, but were responsible for 35.0% of the
pupae production. In the dry season, we observed the same trend, but less pronounced: the big water deposits were producing 20.3% of the pupae against 5.5% in the rainy season, indicating seasonal variability in breeding preference of the vector (Figure 3). The breeding type category “water storage” contributed relatively more to the pupal productivity in the dry season compared to the rainy season. Further, we observed in some communes that artificial containers (mainly trash) were the most productive types in the rainy season (Figure 4).

Positivity for Aedes breeding was higher in the rainy than in the dry season with an adjusted OR of 1.99 (95% CI 1.6 –2.4), and about 27 times (aOR 27.3 (95% CI 14.9—49.9)) more outdoors compared to indoors. Mont Ngafula and Lingwala were statistically significantly more infested than N’Djili. The water container types most associated with Aedes infestation were tires (aOR 4.6 (95% CI 3.5–6.1)) and trash (aOR 1.9 (95% CI 1.4–2.5)) in comparison to big water deposits (Table 2).

**Table 2. Determinants of Aedes spp. positive breeding sites in Kinshasa, 2018.**
| Parameter       | Category      | Total | Positive N (%) | Bivariate OR (95% CI) | p-value | Multivariate OR (95% CI) | p-value |
|-----------------|---------------|-------|----------------|-----------------------|---------|--------------------------|---------|
| Season          | Rainy         | 5079  | 761 (15.0)     | 1.58 (1.32-1.89)      | <0.001  | 1.99 (1.64-2.41)         | <0.001  |
|                 | Dry           | 1657  | 166 (10.0)     | 1                     |         | 1                        |         |
| Communauté      | Kalamu        | 722   | 105 (14.5)     | 2.21 (1.73-2.83)      | <0.001  | 0.96 (0.74-1.25)         | 0.788   |
|                 | Lingwala      | 1001  | 186 (18.6)     | 2.96 (2.40-3.65)      | <0.001  | 1.51 (1.21-1.89)         | <0.001  |
|                 | Mont Ngafula  | 1798  | 406 (22.6)     | 3.78 (3.18-4.50)      | <0.001  | 2.65 (2.19-3.21)         | <0.001  |
|                 | N'Djili       | 3215  | 230 (7.2)      | 1                     |         | 1                        |         |
| Position        | Exterior      | 4646  | 916 (19.7)     | 46.41 (25.51-84.31)   | <0.001  | 27.31 (14.9-50.0)        | <0.001  |
|                 | Interior      | 2090  | 11 (0.5)       | 1                     |         | 1                        |         |
| Container type  | Big water deposits | 1080 | 94 (8.7)     | 1                     |         | 1                        |         |
|                 | Small water deposits | 4373 | 395 (9.0)     | 1.04 (0.82-1.32)      | 0.735   | 0.99 (0.77-1.26)         | 0.916   |
|                 | Artificials (trash) | 533  | 134 (25.1)    | 3.52 (2.64-4.70)      | <0.001  | 1.89 (1.39-2.55)         | <0.001  |
|                 | Natural       | 5     | 0 (0)          | 0                     | 0.999   | 0                        | 0.999   |
|                 | Artificials not | 18   | 4 (22.2)     | 3.00 (0.97-9.29)      | 0.057   | 1.00 (0.32-3.13)         | 0.997   |
In both seasons, both *Ae. aegypti* and *Ae. albopictus* were found. The morphological identifications were validated by comparing the generated sequences of a subset of specimens against the Identification System of BOLD, with Species Level Barcode Records. The obtained similarity percentages ranged from 99.69 to 100%. The five and 14 haplotypes of *Ae. albopictus* and *Ae. aegypti*, respectively, are clustering only with conspecific sequences from specimens collected worldwide, supported with maximum bootstrap support (Figure 1 of Additional File 2). The generated sequences were deposited in GenBank with following accession numbers: MT345349-MT345426.

9.46% and 9.06% of the total number of containers positive for *Aedes* spp. immature stages, contained also immature stages of other species, such as *Culex* and *Anopheles*, in the rainy and dry season respectively. This was in 99.3% of the cases observed in outdoor recipients and specifically in big water deposits in the rainy season and in trash in the dry season. Of them, in the rainy season, a total of 32 *Aedes* breeding sites were positive for *Anopheles* against only two in the dry season. *Anopheles* was found in big and small water deposits, trash and tires (Figure 6). In the rainy season *Anopheles* was observed in all communes whereas in the dry season larvae were only found in small water deposits in Mont Ngafula, the most rural commune of the four study sites (Figure 7).

**Discussion**

In both surveys and in all communes, the larval indices (HI, CI, and BI) were higher than the arbovirus transmission threshold values (BI of 5 set out by the World Health Organization) (29)(30). The Breteau index was on average 45 per 100 houses in the rainy season, and in comparison to a house index of on average 27%, it is clear that one house can have different *Aedes* spp. positive breeding sites. These high larval and pupal *Aedes* infestations suggests that if an arbovirus starts circulating, transmission can rapidly occur in Kinshasa. This has been confirmed during the recent chikungunya outbreak in 2019 (11).

The strength of this study lies in the use of standardized procedures in four different communes of Kinshasa during the rainy and dry seasons. The entomological team was trained beforehand and was largely the same for both surveys. A weakness is that the study took place over only one year time and only once per season. As inspection of breeding sites depends on the rigor and professionality of the team doing the fieldwork, quality control was established, namely a fixed supervisor was available in the
field site during the survey and there was regular extra control from the international integrant of the study-team. Due to operational issues we were not in a position to identify all larvae to species level, which was another weakness, hence we could not calculate the relative importance of *Ae. aegypti* and *Ae. albopictus*, neither which species has predilection for which container type.

The larval indices are remarkable high for a place without important endemicity of arboviruses and only sporadical reporting of cases of dengue (14)(12)(15)(13), outbreaks of chikungunya (11)(9) and yellow fever (31). The level of infestation is on the higher end of what has been published in other African settings: in south-eastern Tanzania with HI of 4.9–6.6, CI of 14.6–18.9 (32); in Burkina Faso with HI of 70, CI of 35 and BI of 10 (33); in north-west Ethiopia with HI of 25.5, CI of 32.9 and BI of 48.4 (34); in Mozambique with CI of 22 (35); and in Angola with HI of 4.3–27.9, CI of 2.1–9.3 and BI of 5.8–42.2 (36). But the Breteau Index in Kinshasa was much lower than the one observed in Kenya during a dengue outbreak in 2013–14, where BI reached a value of 270/100 houses (37).

In contrast to findings in Latin-America (38), in Kinshasa *Aedes* breeding sites were mainly found outdoors, a characteristic also seen in other African countries (39). The prevalence of *Aedes* breeding sites outdoors suggests a close human-mosquito contact. The inhabitants stay most of their day-time outside the house, in the backyard or in the open place in front of their house, where also the water storage is for domestic activities. This human behavior favors the development of the mosquito cycle since the mosquito finds there breeding sites and at the same time sources of blood, as *Aedes spp.* bites during day-time. The low presence of *Aedes* immature stages inside the homes can also be due to rapid use and cleaning of the few containers found there. Outdoors, emptying and cleaning the many potential breeding sites is a daunting job that needs to be realized all weeks of the year if one wants to stop the mosquito development cycle. These results indicate that for controlling *Aedes* in Kinshasa, management strategies need to target outdoor spaces for breeding sites destruction or reduction.

Used car tires, water storage containers and artificial breeding sites (type trash) were the main containers chosen by *Aedes* mosquitoes for the oviposition coinciding with other studies conducted in several countries of the African continent (33–37,39)(40). The water storage containers were also found to be the most productive for *Aedes* pupae, which is a stage in the mosquito cycle which does not need nutrients and which is just before the adult stage of the mosquito. These containers are all always filled (partially or fully) with water, also when there is no rain to fill the small trash deposits outdoors. This makes them a preferred breeding site, even despite being constantly subject to anthropogenic action. In the rainy season, in Lingwala and N’Djili, artificials, favored by rain, are most productive for *Aedes* pupae, while in Mont Ngafula and Kalamu, small water containers, where rain water is captured, are most productive. In the dry season, it is clear that water storage containers (whether big or small) are the most productive, pointing out that water storage is important for the households due to a deficient water supply system, especially in the communes of N’Djili and Mont Ngafula. In the rainy season, about 35% of all pupae are found in tires, while tires are only representing 11% of the potential breeding sites. In the dry season this is less pronounced as expected since they contain water dependent on the occurrence of rainfall. Discarded tires might also be stored for longer durations and harbor mosquito larvae undisturbed,
making them prolific breeding containers (41). Moreover, the weather conditions inside tires, such as temperature, humidity, and reduced light, create a suitable environment for *Aedes* mosquito breeding (42,43). Eggs attached to the tires may also play a role in the preservation of the *Aedes* mosquito population throughout the dry season (44). These containers preserve water, organic and mineral substances for a long time and allow vectors to reach adulthood. The importance of used tires in the maintenance of *Aedes* mosquitoes populations is well documented by several previous works in different parts of the world, and it is well described how to handle them, such as recycling (39) or storing them under a roof to avoid filling with rainwater.

Small water deposits were found in large number in all the study sites and were also the main pupae producing containers. A slightly lower productivity by this group of container type was seen in the rainy season, in comparison to the dry season, and can be due to their short-term use for water storage and being subject to frequent emptying and cleaning, which effectively interrupt the breeding cycles of *Aedes* mosquitoes.

In this study, *Aedes* species were dominant in the inspected potential breeding sites, in and around the houses. Also other mosquito genera were found, such as *Culex* and *Anopheles*. *Culex* species are common species in urban settings using similar breeding sites as the urban *Aedes* species. It is of note that *Anopheles* species were found together with *Aedes* in the same breeding sites. *Anopheles* usually prefers other types of breeding sites, such as ponds with clear water and are not particularly attracted to small containers. The presence of *Anopheles* in urban settings is primarily associated with urban agriculture, as in Mont Ngafula (45), though we found *Anopheles* in all four communes in the rainy season, also in the center of Kinshasa.

**Conclusion**

*Aedes* spp. seem to be well established in all four study communes of Kinshasa and are especially abundant in the sub-urban area of Mont Ngafula. This study—the first in its kind in Kinshasa - provides inside on how to organize a control strategy, targeting outdoors and in the dry season the water storage containers, while in the rainy season artificial should be targeted, especially tires. This study needs to be complemented by (i) an adult mosquito survey to be able to identify their habitat/environmental preferences, which is important for control activities to stop transmission, (ii) and by a study evaluating the susceptibility of *Aedes* to chemical and biological control methods. Based on these local evidences, effective and contextual control strategies for these vectors can be designed and implemented in Kinshasa to prevent a next outbreak of arboviral infections.

**List Of Abbreviations**

DRC Democratic Republic of Congo

INRB Institut National de Recherche Biomédicale
BI Breteau Index
CI Container Index
HI House Index
SD Standard Deviation
OR Odds Ratio
aOR adjusted Odds Ratio
CI Confidence Interval
ITM Institute of Tropical Medicine, Antwerp

Declarations

- Ethics approval and consent to participate

The study protocol was approved by the ‘Comité d’éthique de l’Université de Kinshasa’ (authorization number: ESP/CE/032/2018). Before starting the survey in each commune, the study was presented to the ‘Médecin Chef de Zone’ and the local mayor, in order to have their approval for realizing the study in their area of responsibility. An informed consent was asked to the head of the households of the sampled houses and an oral approval was obtained. Different quality control measures were put in place: in each commune an entomological expert supervised the work of the field teams, the project-leader verified at the end of each day a subset of the data collection forms on completeness and an external entomological expert (Cuban expert) did ad hoc supervisions of the field work and of the laboratory activities.

- Consent for publication

NA

- Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

- Competing interests

The authors declare that they have no competing interests

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- Authors’ contributions
  - WTF, MMC and VV designed the study; MEZ supervised survey activities and the laboratory work; FS organized practically the fieldwork; BMZ, IG and MTR realized the fieldwork; MMC, BMZ, IG and VW did laboratory work; SN did the DNA barcoding; VV and VW did the data analysis; BJA, VW, MMC interpreted the results. All authors did a part of the manuscript writing. All authors read and approved the final manuscript.

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- Authors’ information (optional)

NA

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Figure 1

Distribution of Anopheles spp. positive breeding sites in the four study communes in the rainy and dry season, Kinshasa, 2018 (n=32 mixed breeding sites positive for Anopheles).
**Figure 2**

Types of breeding sites where Anopheles spp. was encountered in Kinshasa study sites, 2018 (n=32 mixed breeding sites positive for Anopheles).
Figure 3

Neighbour-Joining tree including the six medically important Aedes species of the subgenus Stegomyia occurring in the Afrotropical region. The generated haplotypes of Aedes albopictus and Aedes aegypti of DRC specimens are highlighted in grey.
| RAINY SEASON          | DRY SEASON          |
|-----------------------|---------------------|
| LINGWALA              |                     |
| big water deposits    | big water deposits  |
| small water deposits  | small water deposits|
| artificals            | artificals          |

| NDJILI                |                     |

| MONT NGAFULA          |                     |

| KALAMU                |                     |

**Figure 4**
The geographical and seasonal difference of the most productive Aedes spp. breeding sites: The pie charts show the relative contribution of the breeding site categories to the pupal productivity, Kinshasa, 2018.
Figure 5

The productivity of the breeding sites for Aedes spp. immature stages, Kinshasa, 2018
Figure 6

Illustration of various breeding sites identified/investigated in the study area: a. big water deposits; b. small water deposits; c. artificials-trash; d. natural sites; e. artificials not destroyable; f. tires; g. water evacuation/ponds
Figure 7

The four study communes in Kinshasa (in light grey) with localisation of the sampling area (dark grey dots), Kinshasa, 2018.

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