High Aedes spp. larval indices in Kinshasa, Democratic Republic of Congo

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Research

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Abstract

BACKGROUND: Dengue, yellow fever, chikungunya and Zika are among the most important emerging infectious vector-borne diseases worldwide. In the Democratic Republic of Congo (DRC), increases in cases of dengue and outbreaks of yellow fever and chikungunya have been reported since 2010. The main vectors of these arboviruses, *Aedes aegypti* and *Aedes albopictus*, have been reported in DRC, but there is a lack of detailed information on their presence and spread to guide disease control efforts.

METHODS: In 2018, two cross-sectional surveys were conducted 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). Within the peri-domestic area of each household, searches were conducted for larval habitats which were then surveyed for the presence of *Aedes* larvae and pupae. A subset of the immature specimens were reared to adults for morphological identification followed by DNA-barcoding of the specimens to validate identifications.

RESULTS: The most rural commune (Mont Ngafula) had the highest pupal index of 246 (20) pupae/100 houses and a Breteau Index of 82.2 (19.5)/100 houses, while this latter was 21.5 (4.7), 36.7 (9.8) and 41.7 (7.5) in Kalamu, Lingwala and N’Djili in the rainy (and dry) season, respectively. The House Index was on average across all communes 27.5% (7.6%); and the Container Index 15.0% (10.0%) in rainy (and dry) season, respectively. The vast majority of *Aedes* positive containers were found outside the houses (adjusted OR 27.4 (95%CI 14.9-50.1)). During the dry season, the most productive containers were the ones used for water storage, whereas in the rainy season rubbish and tires constituted key habitats. Both *Ae. aegypti* and *Ae. albopictus* were found. *Anopheles* larvae were found in different types of *Aedes* larval habitats, especially during the rainy season.

CONCLUSIONS: In both surveys and in all communes, the larval indices (BI) were higher than the arbovirus transmission threshold values established by the World Health Organization. Management strategies for controlling *Aedes* in Kinshasa need to target the key containers, mainly located in outdoor spaces, for larval habitats destruction or reduction.

Background

Arboviruses are causing a variety of diseases, such as dengue, yellow fever, chikungunya and Zika, which are among the most important emerging infectious diseases worldwide [1–3]. The distribution of these diseases and their transmitting vector are well characterized in Latin-America and South-East Asia [4–6], but understanding of arbovirus ecology in Sub-Saharan Africa remains limited [7–9]. From dengue seroprevalence studies we know that there is or has been virus circulation, demonstrated by a 12.5% IgG positivity in Cameroon, 36% in Burkina Faso, 45% in Nigeria [7] and 50.6% in Tanzania [10], however few reports show the importance of dengue as a cause of acute fever in these settings. This is also the case for DRC, where the dengue virus is found in stored samples: the antigen dengue test was positive among three suspected chikungunya cases in Kinshasa in 2012, [11]; 0.6% of dried blood spots taken during a
Demographic Health Survey were positive in 2013-14 [12]; 3.5% of yellow fever suspect cases in the Bas Congo region between 2002 and 2013 were dengue positive [13]. More recently, in 2015-16, in Mont Ngafula (suburban area of Kinshasa) 8.1% of acute fever cases were dengue positive [14] and 30.2% of the 342 study participants had dengue IgG antibodies. Although no outbreak was reported, thus far, in DRC, the neighboring country, Angola, had a dengue outbreak with an estimated attack rate of 10% in 2013 [15]. Chikungunya, by contrast, is causing apparent outbreaks, such as the one in Kenya in 2004 [16], in Tanzania in 2013 [17], in Mozambique in 2018 [18], in Brazzaville (Republic of the Congo) in 2011 [19] and 2019 [20], and in Kinshasa, capital of the Democratic Republic of Congo (DRC) in 2000 [21], 2012 [22] and 2019 [23]. Such outbreaks can affect large populations, i.e. 67% of the population in Kenya [24]. Besides dengue and chikungunya, also other alpha-, flavi- and bunyaviruses were found in mosquito samples (Aedes and Culex) in Kinshasa in 2014 [25] Zika has been rarely detected in this African region [26], but several yellow fever outbreaks, the last major one in 2016, were described [27].

Within the sub-Saharan African region, the information on the presence and distribution of the Aedes mosquito is even more difficult to find than on the above pathogens. This lack of entomological data forces recourse to suitability maps, based on mathematical models, to estimate arbovirus transmission risk [28]. However, measures of real Aedes spp. infestation levels would give a more reliable insight for both risk and mitigation strategies [29]. Both Aedes aegypti and Aedes albopictus are found in the region, where Ae. aegypti is native and where Ae. albopictus was introduced from South-East Asia [30] in early 2000 [31] and in Kinshasa (DRC) in 2018 [32]. Both species were detected in human-domesticated environments [30], such as Kinshasa, a mega-city with a high population density and movement, but precisions on Aedes larval indices remain unknown. Without knowing the main location of Aedes larval habitats or the types of containers responsible for producing adult Aedes, it is impossible to define effective larval source management strategies for Kinshasa.

In this study, we evaluate the Aedes spp. larval indices, together with the characteristics of the preferred larval habitats, in order to have an evidence basis for guidance of Aedes control efforts and provide insight into the potential of the mosquito population to support local transmission of arboviruses in Kinshasa, the capital of DRC.

Methods

Settings

The surveys took place in Kinshasa, capital city of Democratic Republic of Congo (DRC), located in the Central-African region. Kinshasa lies at 279 m above sea level and is characterized by a tropical climate with a rainy season between October and May, and a dry season from June to September. The average temperature varies between 18°C and 32°C and the average monthly rainfall varies between 2 and 222 mm, in dry and rainy season respectively. Kinshasa encompasses 9965 km² and has an estimated population of almost 12 million people. The city is administratively subdivided into 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 purposively selected to capture diverse ecological, urbanization, water supply systems and epidemiological conditions (i.e. history of arbovirus outbreaks) (figure 1).

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

Kalamu II is a commune in the center of town, belonging to the Funa district, and is highly residential. The main economic activity is 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 erosions) and small valleys. The main economic activity is agriculture and the selling of agriculture products to Kinshasa city. Mont Ngafula I is emblematic of unplanned urbanization with deficient water supply system – in terms of both supply (i.e. as low as two times/week) and waste water disposal.

Lingwala is a commune in the center of the town, pertaining to the Lukunga district, with a lot of informal markets. It is a more urbanized area with fairly good water supply. Population density is estimated at 33 000 persons/km².

**Study design and data collection**

Two cross-sectional surveys were done, one in the rainy season (18 January – 16 February, 2018) and one in the dry season (2 – 27 July, 2018). To detect 10% of the houses being positive for *Aedes* spp. mosquitoes with 80% power, 3% precision and allowing for a 5% alfa-error, 400 houses needed to be surveyed in each survey site. 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. Each day, 80 houses 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 houses, starting on the right side of the avenue, each of the 4 teams inspected the selected houses up to reaching a maximum of 20 houses/day. When the avenue came to an end and the quota of 20 was not yet reached, the team turned back, approaching the houses on the other side of the street until the sample quota was reached. 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 next avenue (going left from the one of the previous day) was sampled. By this procedure, representative sampling was achieved. 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, pre-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 of mosquitoes (i.e. larvae or pupae) were observed, they were collected in plastic bottles (one bottle per larval habitat) and transported to the laboratory at INRB for genus identification (Anopheles, Aedes, Culex). The place, category and positivity/negativity of each container were recorded. For larvae, only positivity and negativity was recorded; for pupae, the number of pupae was counted per positive habitat. Surveys were implemented identically, but as samples were randomly selected, houses had equal probability of inclusion in one, both, or neither survey. Both surveys were largely realized by the same field team members.

Species identification: morphology and DNA-based

Each day, a random sample of 50 Aedes genus larvae/pupae were reared to adults in the insectarium to allow species identification using morphological keys [33,34]. F0 adults were stored at -20°C for DNA barcoding to validate the morphological identification of Ae. aegypti and Ae. albopictus and confirm the presence of the identified species in Kinshasa. Therefore five specimens of each species were randomly selected per survey site. DNA barcoding is a technique based on the amplification of a standard barcode - the partial mitochondrial cytochrome c oxidase subunit I gene for animals. Sanger sequencing of the 658 bp COI standard barcode was performed using the LCO1490 and HC02198 universal primers [35,36]. Amplifications were carried out in a 20 µl reaction mixture containing 2 µl of DNA template, 2 µl of 10X buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 0.4 µM of each primer, and 0.03 units/µl of PlatinumTM Taq DNA Polymerase (InvitrogenTM). PCR products and negative controls were checked on a 1.5% agarose gel, using a UV transilluminator and the MidoriGreenTM Direct (NIPPON Genetics Europe) method. Positive amplicons were purified using the ExoSAP-ITTM protocol and sequenced in both directions on an ABI 3230xl capillary DNA sequencer using BigDye Terminator v3.1 chemistry (ThermoFisher Scientific).

Subsequently, the generated sequences were compared to a library of reference sequences. A specimen was 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 of the protocol can be found in Additional File 2).

Data analysis

Data were entered in an Access database and 5% of the data were manually validated to detect errors. Data were cleaned and types of recipients regrouped into categories, adapted from guidelines used in dengue-endemic regions [37]: water storage tanks or cistern (> 15 L); small water deposits used for daily kitchen and cleaning activities (< 15 L); rubbish and discards; natural tree and bamboo holes; artificials
that are used in the households and cannot be destroyed (for example animal drinking pots); used tires; natural ground pools. 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 larval habitat 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 conducted and associated variables were identified based on a backwards conditional model, taking into account the clustering at household-level by inserting the household identification variable as a random factor in the model.

The number of larval habitats with at least one immature stage of *Anopheles* spp. was enumerated and its proportional importance calculated for each season and respective commune.

**Results**

The surveys sampled a total of 1,678 and 1,598 houses, in the rainy and dry season respectively. In the rainy season, 5,079 water-holding containers (potential larval habitats) were inspected compared with 1,657 in the dry season. The average number of containers per household varied across communes (p < 0.001): 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 rainy and dry season, 65.9% and 78.3% of the containers, respectively, were observed outside the sampled houses, i.e. in the open space around the house within the compound, (p < 0.001). The distribution of type of containers per location, commune and season is detailed in the Additional File 1 (table 1).

*Aedes* larval indices were higher in the rainy than in the dry season (p < 0.001, see table 2), 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, which was about four times higher than Kalamu, a commune that lies within the heart of the center of town. In the rainy season, the Pupal Index (number of *Aedes* pupae per 100 houses) reached 246 pupae/100 houses in Mont Ngafula, 126 in N'Djili, 90 in Lingwala, and 50 in Kalamu (Table 1). In the rainy season 99.3% of the positive larval habitats were outdoors versus 96.4% in the dry season. A wide variety of containers are occupied by the *Aedes* mosquito as aquatic habitat: water storage tanks, small water deposits, rubbish/dicards, bamboo/tree holes, non-destroyable artificial containers, used tires, natural ground pools (Figure 2). Tires were treated 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 pupal productivity of larval habitats, we observed a statistically significant difference between rainy and dry season (aOR 3.73, 95% CI (2.21-6.31); p < 0.001). In the dry season, we observed that water storage tanks were producing 20.3% of the pupae against 5.5% in the rainy season, indicating seasonal variability in aquatic habitat preference of the vector (Figure 3). In the rainy season 64.3% of all inspected containers were small water deposits, but they were only responsible for 46.4% of the pupae production, whereas used tires, representing only 11.1% of the inspected containers, were responsible for 35.0% of the pupae production. The containers used for water storage (big and small) contributed relatively more to the pupal productivity in the dry season than in the rainy season. Furthermore, we observed that productivity of artificial containers (mainly rubbish) was different across communities (p < 0.001) and season (p < 0.001) (Figure 4).

Positivity for *Aedes* was higher in the rainy than in the dry season with an adjusted OR (aOR) of 1.98 (95% CI 1.6 -2.4), and about 27 times (aOR 27.4 (95% CI 14.9 – 50.1)) more outdoors compared to indoors (p < 0.001). Mont Ngafula and Lingwala were statistically significantly more infested than N’Djili (p < 0.001, see table 2). The water container types most associated with *Aedes* infestation were used tires (aOR 4.6 (95% CI 3.5-6.1)) and rubbish/discards (aOR 1.9 (95% CI 1.4-2.5)) in comparison to water storage tanks (Table 2).

Based on the morphological identification of F0 adults, *Ae. aegypti* and *Ae. albopictus* were found in both seasons and all study sites. 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.

Among containers positive for *Aedes* spp. immature stages, 9.46% and 9.06% also contained immature stages of other genera, such as *Culex* and *Anopheles*, in the rainy and dry season respectively. In 99.3% of the outdoor recipients, and specifically in water storage tanks in the rainy season and in trash in the dry season, habitats were shared. In the rainy season, a total of 32 *Aedes* larval habitats were positive for *Anopheles* versus only two in the dry season. *Anopheles* were found in big and small water deposits, rubbish and used tires (Figure 6). In the rainy season *Anopheles* were observed in all communes whereas in the dry season *Anopheles* larvae were only found in small water deposits in Mont Ngafula, the most rural commune of the four survey 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 established by the World Health Organization (BI of 5) [38,39]. 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 larval habitats. In case an arbovirus is introduced in Kinshasa, the high larval and pupal Aedes densities suggest that transmission can rapidly occur and cause a major outbreak, such as the one caused by chikungunya in 2019 [23].

Having done surveys, following standardized procedures, in four different communes of Kinshasa during the rainy and dry season is the major strength of this study. The entomological team was trained beforehand and was largely the same for both surveys. A weakness is that the surveys took place over only one year time and only once per season. As inspection of larval habitats 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 survey-team. Due to operational issues we were not in a position to identify all larvae to species level, this was only done in a small subsample of the larvae, hence we could not calculate the specific relative importance of Ae. aegypti and Ae. albopictus, but only observe a tendency for an equal presence, neither could we calculate which species has predilection for which container type. These two mosquito species display different vector competences for the different arboviruses; and more detailed information on the occurrence of each vector would allow to develop more precise control measures in case of a specific arbovirus outbreak. AeDES aegypti - originated from Africa - is the main vector of arboviruses globally, but its vector competence is highly variable depending on the vector population, the virus isolate and the ecological context [28]. The presence of Ae. albopictus, which is an exotic species for Africa, might change the epidemiology for a number of arboviruses in Africa [40]. In several epidemics, Ae. albopictus has been shown to be the main driver of transmission for the chikungunya virus, especially the one of the ECSA lineage with the A226A, as shown in a recent outbreak in Kinshasa [41].

In a place like Kinshasa, where dengue is rarely reported [11–14] and chikungunya and yellow fever cause sporadic outbreaks [21,23,42], it is not expected to find such high Aedes larval and pupal indices. The observed indices are comparable to the ones of other African settings: south-eastern Tanzania has a HI of 4.9 – 6.6, CI of 14.6-18.9 [43]; Burkina Faso a HI of 70, CI of 35 and BI of 10 [44]; north-west Ethiopia a HI of 25.5, CI of 32.9 and BI of 48.4 [45]; Mozambique a CI of 22 [46]; and Angola a HI of 4.3 – 27.9, CI of 2.1-9.3 and BI of 5.8-42.2 [47]. However, the indices were much lower than the one observed in Kenya during a dengue outbreak in 2013-14, where BI reached a value of 270/100 houses [48].

In contrast to findings from Latin-America [49], in Kinshasa Aedes immature stages were found in 19.7% (916/4646 containers) of the outdoor containers against 0.5% (11/2090) of the indoor containers. This is a characteristic also seen in other African countries [50]. The prevalence of Aedes larval habitats outdoors, together with the behavior in this context (to stay during daytime in the backyard or in the open place in front of the house), suggests a close human-mosquito contact, favoring the development of the Aedes spp. cycle, by blood feeding during daytime [51]. The low presence of Aedes immature stages inside the homes can also be due to rapid use and cleaning of the containers found there. These results indicate that for controlling Aedes in Kinshasa, management strategies need to target outdoor spaces for larval habitats destruction or reduction.
Used car tires, water storage tanks and artificial larval habitats (type rubbish/dicards) were the main containers chosen by *Aedes* mosquitoes for the oviposition coinciding with other studies conducted in the African continent [44–48,50,52]. The water storage tanks 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 [53]. These containers used to store water are always filled (partially or fully) with water due to the deficient water supply system, not depending on rain, which makes them a preferred larval habitat, especially in the dry season, even despite being constantly subject to anthropogenic action. In the rainy season, in all survey sites, larval habitats are favored by rain and containers typically filled with rain water are the most productive ones for *Aedes* pupae. Old tires are illustrative: while they only represent 11% of the potential larval habitats, in the rainy season, about 35% of all pupae are found in them. Temperature, humidity and reduced light inside tires create a suitable environment for *Aedes* mosquito breeding and when tires are stored or discarded for long duration – without being scrubbed -, it makes them a prolific larval habitat [54–56]. Under these conditions, eggs can be attached to the tires for a long time, playing their role in the preservation of the *Aedes* mosquito population throughout the dry season [57]. This information on containers disproportionately responsible for containing pupae - and hence for producing adult *Aedes* -, can be used to identify the key larval habitats to be targeted in larval source management strategies applied to decrease arbovirus transmission risk.

In this study, *Aedes* species, a vector of chikungunya, dengue, Zika and yellow fever, were dominant in the inspected potential larval habitats, in and around the houses. Also other mosquito genera were found, such as *Culex* and *Anopheles*. While *Culex* may share larval habitats with urban *Aedes* species, it is unusual to find *Anopheles* species together with *Aedes* [58]. *Anopheles* usually prefers other types of larval habitats, such as ponds with static fresh water and are not particularly attracted to small containers [59]. The presence of *Anopheles* in urban settings was previously thought to be associated with urban agriculture, as in Mont Ngafula [60]. However, we found *Anopheles* in all four communes in the rainy season in the absence of agriculture. Further identification of *Anopheles* to species level is needed for the different communes, especially in the context of the invasion of *Anopheles stephensi* in Eastern Africa [61]. The observation of *Anopheles* larvae in man-made containers suggest that also *Anopheles* species can adapt to diverse containers, which suggests heightened transmission risk of urban malaria in Kinshasa.

**Conclusions**

*Aedes* spp. seem to be well established in all four survey communes of Kinshasa and are especially abundant in the sub-urban area of Mont Ngafula. This study – the first in its kind in Kinshasa – shows that an *Aedes* control strategy needs to target outdoor containers, specifically containers for water storage in the dry season and tires in the rainy season. Additional insights in the ecology of the adult *Aedes* mosquito and its insecticide susceptibility will support the design of a comprehensive *Aedes* control strategy to be implemented to prevent a next outbreak of arboviral infections in Kinshasa.
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

- Funding

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- Authors’ contributions

  - WTF, MMC and VV designed the study; MEZ, and VBW 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 VBW did the data
analysis; BJA, VBW, 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

**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

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### Table 1. *Aedes* spp. entomological indices of the four survey sites, in the rainy and dry season, Kinshasa, 2018.

| Nr of containers inspected | Rainy/dry season | TOTAL   | LINGWALA | NDJILI | MONT | NGAFULA | KALAMU |
|----------------------------|------------------|---------|----------|--------|------|---------|--------|
| Nr of containers inspected |                  | 5079/1657 | 821/180 | 2550/665 | 1164/634 | 544/178 |
| Container Index (%)        | Rainy season     | 14.98   | 17.90   | 7.84   | 28.18 | 15.81   |
|                           | Dry season       | 10.02   | 21.67   | 4.51   | 12.30 | 10.67   |

| Nr of houses inspected     | Rainy/dry season | 1678/1598 | 400/399 | 479/399 | 399/400 | 400/400 |
|----------------------------|------------------|----------|--------|--------|--------|---------|
| Breteau Index (/100H)      | Rainy season     | 45.35    | 36.75  | 41.75  | 82.21  | 21.50   |
|                           | Dry season       | 10.39    | 9.77   | 7.52   | 19.50  | 4.75    |
| House Index (%)            | Rainy season     | 27.53    | 22.25  | 27.97  | 44.86  | 15.00   |
|                           | Dry season       | 7.63     | 7.02   | 6.52   | 13.25  | 3.75    |
| Pupae Index (/100H)        | Rainy season     | 128.00   | 90.00  | 126.00 | 246.00 | 50.00   |
|                           | Dry season       | 15.00    | 13.00  | 9.00   | 20.00  | 19.00   |

### Table 2. Determinants of *Aedes* spp. positive larval habitats in Kinshasa, 2018.
| Parameter        | Category   | Total  | Positive N (%) | Multivariate OR (95% CI) | p-value |
|------------------|------------|--------|----------------|--------------------------|---------|
| **Season**       | Rainy      | 5079   | 761 (15.0)     | 1.98 (1.63-2.40)         | <0.001  |
|                  | Dry        | 1657   | 166 (10.0)     | 1                        |         |
| **Commune**      | Kalamu     | 722    | 105 (14.5)     | 0.97 (0.74-1.28)         | 0.857   |
|                  | Lingwala   | 1001   | 186 (18.6)     | 1.53 (1.20-1.96)         | 0.001   |
|                  | Mont Ngafula | 1798  | 406 (22.6)     | 2.67 (2.19-3.25)         | <0.001  |
|                  | N’Djili    | 3215   | 230 (7.2)      | 1                        |         |
| **Position**     | Exterior   | 4646   | 916 (19.7)     | 27.36 (14.9-50.1)        | <0.001  |
|                  | Interior   | 2090   | 11 (0.5)       | 1                        |         |
| **Container type** | Water storage tanks | 1080 | 94 (8.7)       | 1                        |         |
|                  | Small water deposits | 4373 | 395 (9.0)     | 0.99 (0.77-1.27)         | 0.918   |
|                  | Rubbish    | 533    | 134 (25.1)     | 1.89 (1.39-2.55)         | <0.001  |
|                  | Bamboo hole | 5     | 0 (0)          | 0                        | 1       |
|                  | Artificials not destroyable | 18 | 4 (22.2)       | 0.998 (0.32-3.13)        | 0.997   |
|                  | Used tires | 710    | 296 (41.7)     | 4.60 (3.50-6.06)         | <0.001  |
|                  | Ground pools | 17 | 4 (23.5)       | 2.06 (0.62-6.79)         | 0.236   |

Figures
Figure 1

The four survey communes in Kinshasa (in light grey) with localization of the sampling area (red dots), Kinshasa, 2018.
Figure 2

a. water storage tanks or cistern (> 15 L); b. small water deposits used for daily kitchen and cleaning activities (< 15 L); c. rubbish and discards; d. natural tree and bamboo holes; e. artificials that are used in the households and cannot be destroyed (for example animal drinking pots); f. used tires; g. natural ground pools.
Figure 3

The productivity of the larval habitats for Aedes spp. immature stages, Kinshasa, 2018
Figure 4

The geographical and seasonal difference of the most productive Aedes spp. larval habitats, Kinshasa, 2018.
Figure 5

The generated haplotypes of Aedes albopictus and Aedes aegypti of DRC specimens are highlighted in grey.
**Figure 6**

Types of larval habitats where Anopheles spp. were encountered in Kinshasa survey sites, 2018 (n=32 mixed larval habitats positive for Anopheles).

**Supplementary Files**

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