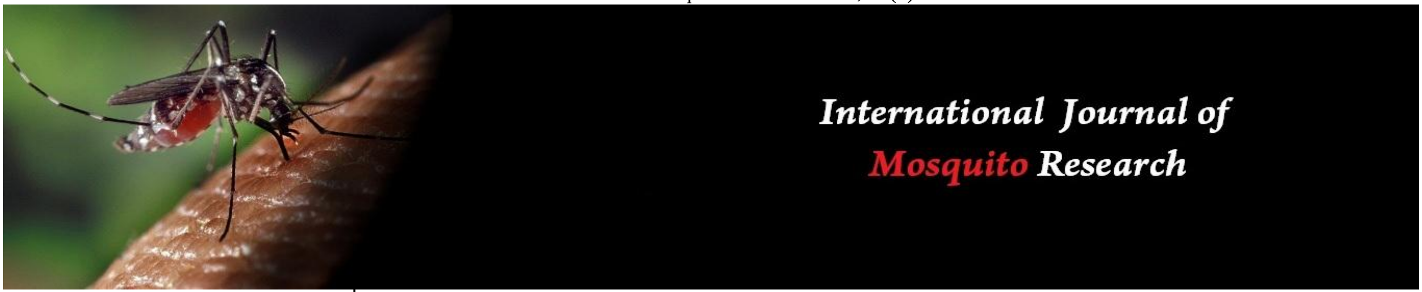

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Identification and characterisation of breeding sites of *Aedes aegypti* (Linnaeus, 1762), a vector of yellow fever and dengue fever in three districts of the town of Korhogo in Côte d'Ivoire

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Abstract
Entomological monitoring to identify the larval sites of *Aedes aegypti* to assess the risk of outbreaks of dengue and yellow fever was carried out in three neighbourhoods of the city of Korhogo, in northern Côte d'Ivoire, from April 2019 to June 2019 during the rainy season. Larval surveys and breeding of mosquito progeny indicate a total of 362 potential artificial larval roosts identified. Tyres were the most important type of lodging (N-205: 56.62%) highest productivity (44.25%). The highest rates of emergence ranged from 70% to 100%. In the kôKô neighbourhoods (55.97%) and Dem (75.65%) *Aedes aegypti* was the most abundant. Characterization based on the types of larval deposits found that watering cans, water troughs and boxes were representative of the genus *Culex*, while the specific habiats of *Aedes aegypti* were canaries, buckets, jars and tyres. The distribution of mosquitoes has varied with the site.
Larval habiats characteristic of *Aedes aegypti* are likely to pose a risk in the occurrence of dengue and yellow fever outbreaks in Korhogo.

Keywords: *Aedes aegypti*, characterization of larval habitats, risk of epidemics, Korhogo

Introduction

Aedes aegypti is a mosquito in the Arthropoda phylum, the Insect class, the Diptera order and the Culicidae family [1]. This mosquito is a tropical species and is also present in subtropical regions of the world. *Aedes aegypti* is the main vector of dengue fever, Chikungunya and yellow fever. These three diseases have a particularly worrying impact on global health [2]. It is also involved in the transmission of the Zika virus, which recently caused an epidemic in Brazil [3]. This medical interest has led the scientific community to take an interest in this vector. This has led to the accumulation of a wealth of ecological, behavioural, physiological and genomic data. *Aedes aegypti* is thought to have originated in Africa [4]. According to the WHO, 200,000 new cases of yellow fever occur every year, with almost 30,000 deaths, mainly in sub-Saharan Africa. Thirty-four countries are at risk [5].Several cases have been identified in Côte d'Ivoire. In 1999, a fatal case occurred in the Comoé Park in the north of the country [6]. In 2001, an epidemic in the west of the country reached Abidjan [7]. Three cases were reported in 2002, including two in Alépé in the south-east and one in Sassandra in the south-west. In 2006, cases were reported in Korhogo in the north and Ouragahio in the west [8]. Abidjan was hit again in 2008 [9]. Finally, in 2009, ten cases were observed in Minignan and Odienné in the Denguélé region in the north-west of the country [9].According to Gubler [10], dengue fever is the most widespread arbovirosis in the world. It is therefore a major public health problem. Every year, between 50 and 100 million people are infected. Severe forms of the disease have been observed (500,000 cases), and between 10,000 and 20,000 deaths have been identified [11].The particular ecology of the vector means that this disease could lead to epidemics of yellow fever and dengue fever. It is therefore important to conduct studies to assess the risks. With this in mind, the town of Korhogo was chosen as the study site.
The general objective of this study was to identify *Aedes aegypti* breeding sites in order to assess the risk of epidemics occurring in the town of Korhogo.

Materials and Methods
Study Site
The study was carried out in the districts of Sossoribougou, Kôkô and Dem in the city of Korhogo (Figure 1). The choice of study sites was motivated by the permanent presence of water points used for the production of market garden crops and shea butter, which could contribute to the creation of classic potential breeding grounds for Culicidae (market gardens, rice fields, puddles, dams). In addition, there are polluted water sources, such as waste water from septic tanks, residual shea butter effluent and waste from mechanical activities (tyres), which have contributed to the factors of choice, etc. In addition, the Sossoribougou neighbourhood is close to a dam around which intensive market gardening has developed. In these study sites, the potential breeding sites (puddles) result either from the actions of the population in terms of discharging waste water (washing clothes and dishes) or from rainwater.

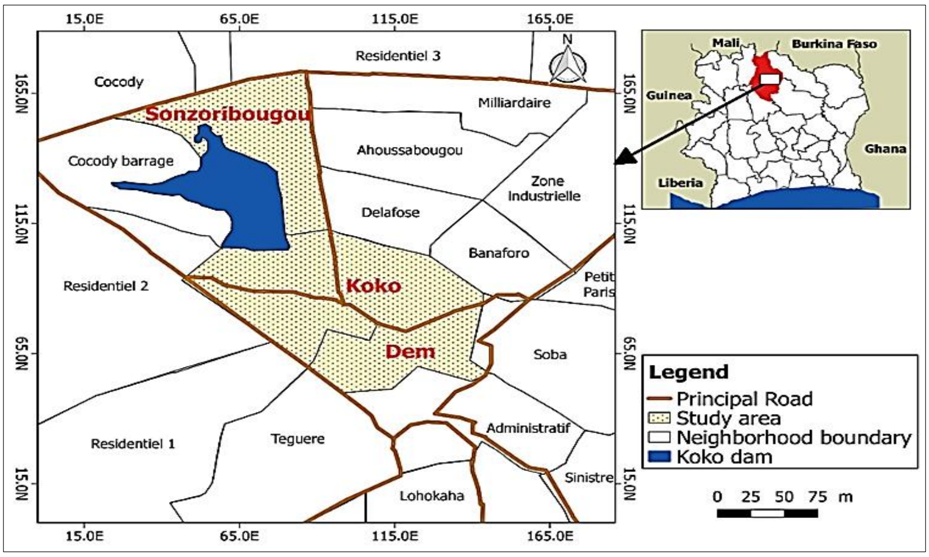

Fig 1: Location of study sites in the study area

Methods
Larval Surveys
The larval surveys were carried out at the start of the rainy season in June 2019, in the districts of Dem, Kôkô and Sossoribougou (Korhogo). These surveys consisted of searching for natural and artificial breeding sites. Once these sites had been identified, the 'dipping' method (Service, 1983) was used to collect larvae and pupae. To do this, larvae and pupae were taken from the sites using a dipper. The larvae and pupae collected were identified using a World Health Organisation identification key [12] and counted using a pipette according to the type of lodging and by neighbourhood.

After identification, the larvae and pupae were placed in jars. These jars were covered with mosquito netting for rearing in the laboratory.

Data Analysis
Prior to any analysis, the normal distribution of our data was verified using the Shapiro-Wilk normality test. Analysis of variance was used to compare larval abundances. Following this analysis, the Newman-Keuls test was used to compare and rank abundances. Principal component analysis was used to characterise larval breeding sites as a function of sites and larvae. The Generalised Linear Model was used to test the effects of site and type of site on larval distribution. All these statistical tests were carried out using Statistica (Version 7.1) and Past (Version 1.0) software.

Results
Identification of potential breeding sites
A total of 362 potential breeding sites in the study area. These potential breeding sites consisted of tyres, boxes, buckets, Water troughs, cans, flower pots, canaries, jars, thermos, shuttles, watering cans, bottles, mortars and calabashes (Figure 2 and 3). Overall, a comparison of the types of deposit shows that tyres were the most important type of deposit in numerical terms (N=205: 56.62%). Boxes, buckets and water troughs were moderately represented (N=47-28: 12.98%-7.73%), while the remaining types were poorly represented (N=8-1: 2.2%-0.27%). These are flower pots, cans, canaries, jars, thermos, shuttles, watering cans, bottles, motars and calabahes (Figure 4).

The spatial distribution of the types of potential larval breeding sites according to neighbourhood shows that tyres are the predominant type of breeding site whatever the neighbourhood (N=88-44: 65.67% - 47.09%). In the neighbourhoods of Sossoribougou and Dem, watering cans, buckets and boxes are the types of lodging that are moderately represented (N=19-3: 14.17% - 4.1%), while in Kôkô boxes come second (N=36: 23.22%). The other types of shelter are poorly represented in the study area (N=4-0: 5.47% - 0%) (Figure 5).

Spatial characterisation of potential breeding sites
A study of the spatial distribution of potential roost types according to neighbourhoods, based on principal component analysis (Figure 6), reveals that two major groups are observed when axis 1 is considered (58.23% contribution). To the left of this axis, the types of lodging are least represented (watering cans, jars, bottles, thermos, canaries, cans and shuttles), whereas to the right of this axis, the other types of lodging (boxes, water troughs, buckets and tyres) are the most represented.

When we look at the second axis (8.76% contribution), a clearer segregation appears in the distribution of the types of shelter according to neighbourhood. In the Kôkô and Sossoribougou neighbourhoods, boxes and troughs are the most characteristic types of shelter, while in Dem, tyres and buckets are the most representative. The Generalised Linear Model confirms this observation by revealing that the distribution of types of shelter varied with the site (GLM: ddl=2; W=8.62; $p<0.05$).

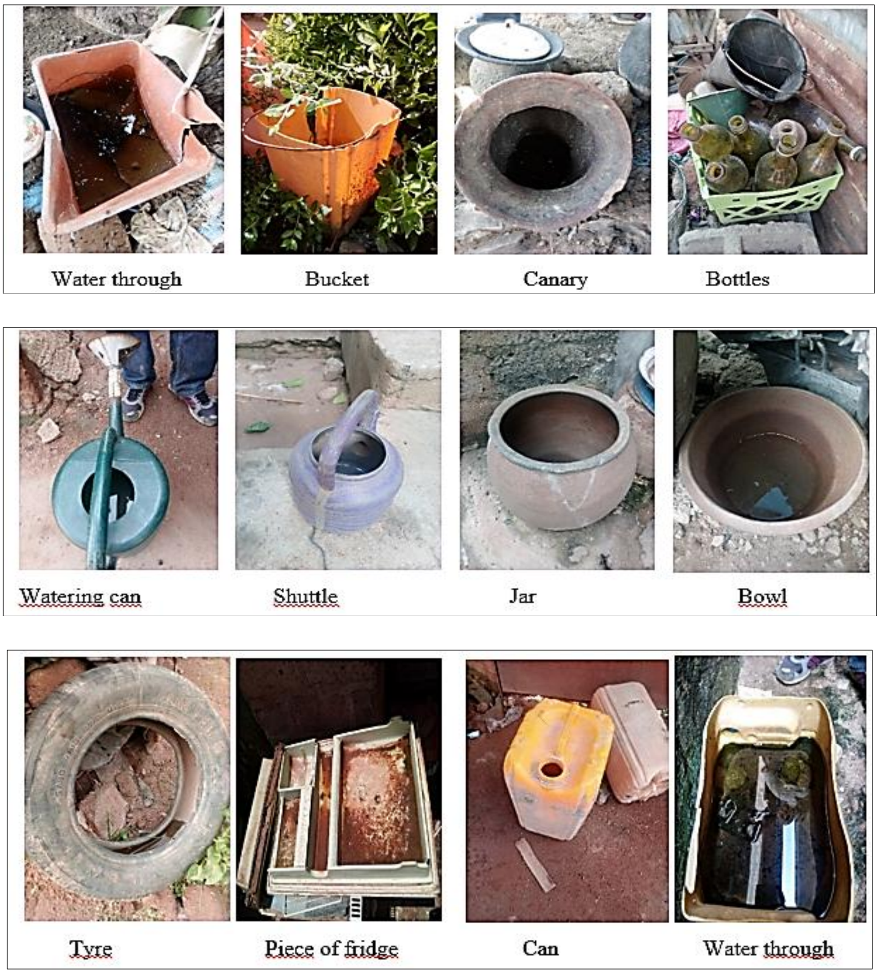

Fig 2 : Map of the different types of gîtes identified in the study area

Fig 3: Map of the different types of gîtes identified in the study area

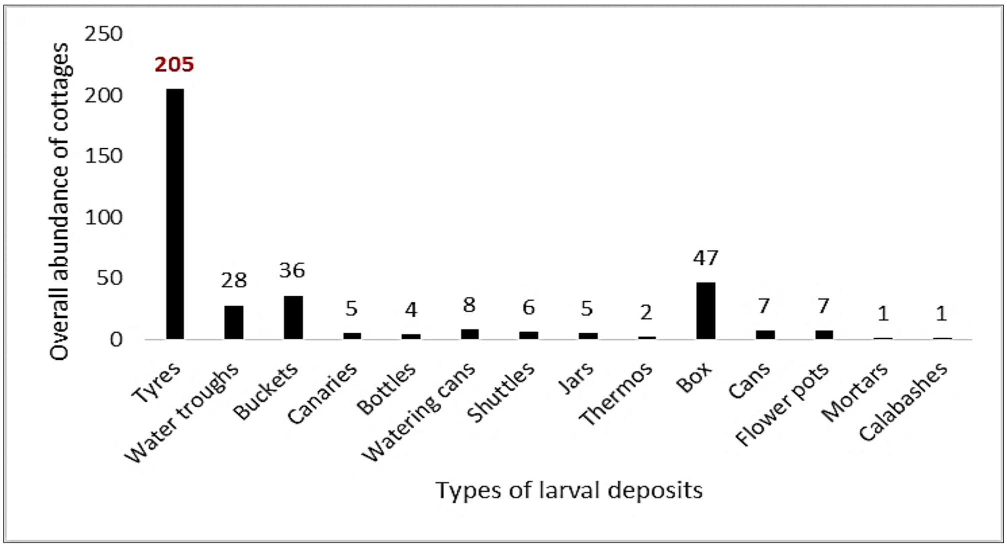

Fig 4: Overall abundance of types of nesting sites in the study area

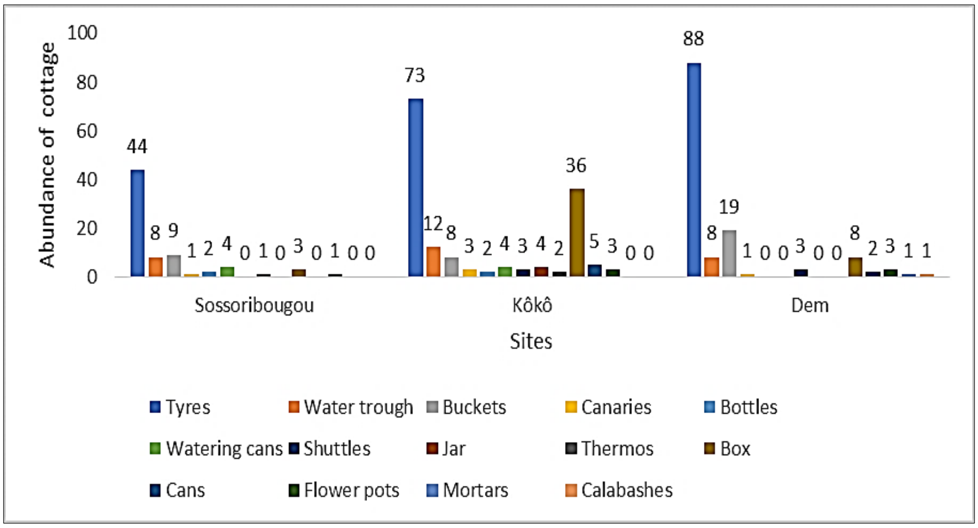

Fig 5: Spatial abundance of types of nesting sites in the study area

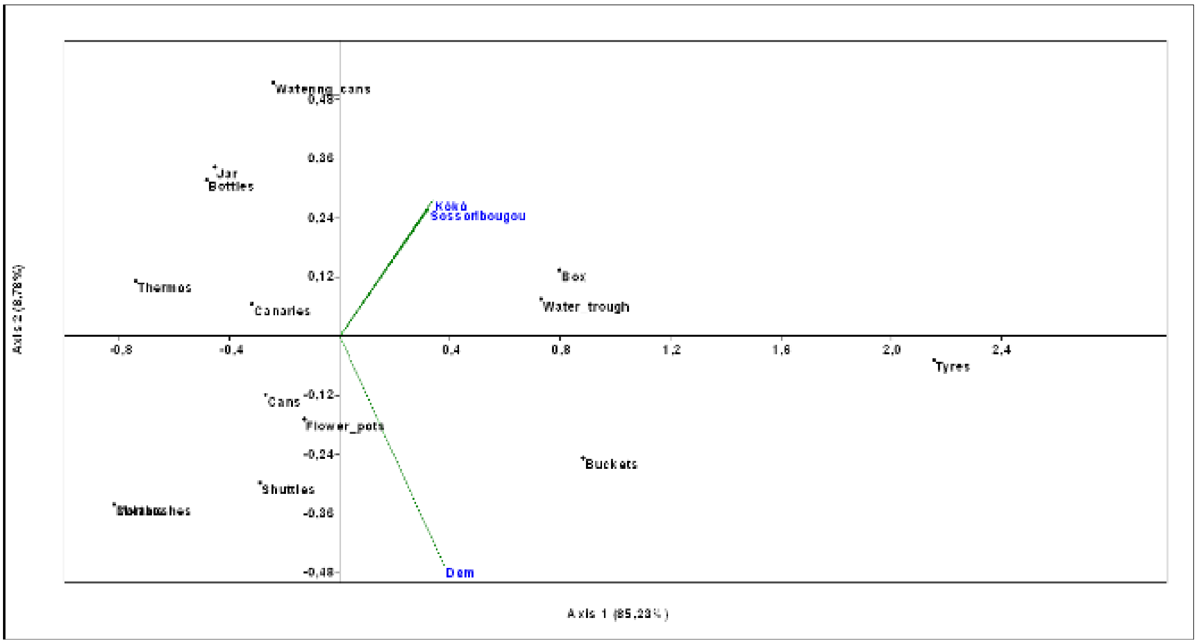

Fig 6: Spatial distribution of potential nesting sites

Positivity rate of potential breeding sites

The various potential breeding sites identified during the larval surveys (N=362) were divided into two main groups on the basis of the presence or absence of larvae. Overall, the observations revealed that 254 sites contained larvae. The proportion of positive sites was therefore 70.16%. On the other hand, larvae were absent from the remaining 108 potential sites, giving a negativity rate of 29.84% (Figure 7).

Variations in the rates of positivity and negativity of larval breeding sites by neighbourhood (Figure 8) show that, whatever the neighbourhood, larval breeding sites containing larvae are better represented (65.76%-77.62%) than sites without larvae, i.e. tested negative (22.38%-34.24%).

Productivity and characterisation of breeding sites

Productivity as a function of type of site

A study of the productivity of larval breeding sites as a function of the type of site produced the results shown in Figure 9. Overall, the highest productivity was observed in the sites formed by tyres (44.25%). Average productivity was recorded in boxes (22.03%), while low productivity levels ranging from 0.37% to 7.62% were obtained in the other types of shelter. These included drinking troughs, buckets, canaries, bottles, watering cans, bowls, jars, thermos flasks, cans, flowerpots, mortars and calabashes.

The productivity of the types of larval breeding sites according to district shows that the greatest number of larvae was observed in tyres in Sossoribougou (53.15%) and Dem (55.52%), while in Kôkô the greatest abundance was recorded in boxes (31.27%) and tyres (28.67%). Average productivity was observed in boxes at Sossoribougou (15.27%) and Dem (16.40%) and in jars (13.27%) at Kôkô. The rest of the caches had low productivity (0% - 9.29%) on all three sites (Figure 10).

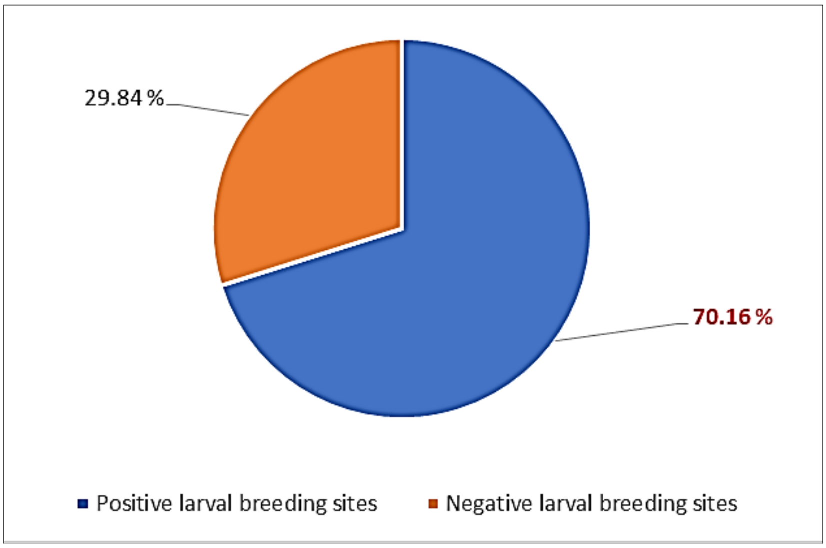

Fig 7: Overall positivity rate of breeding sites in the study area

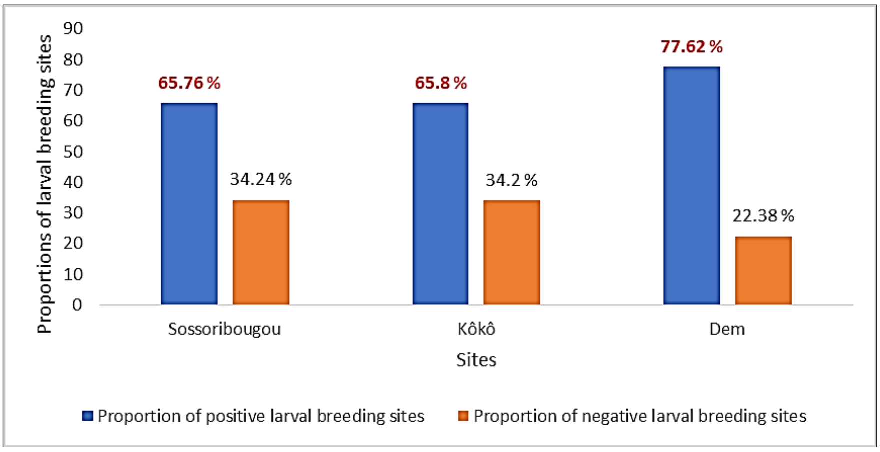

Fig 8: Spatial variations in larval deposit positivity rates

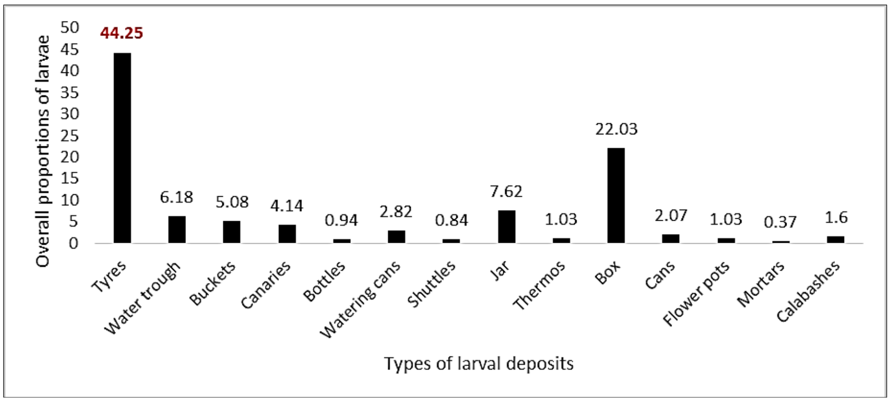

Fig 9: Overall variations in larval abundance by type of site

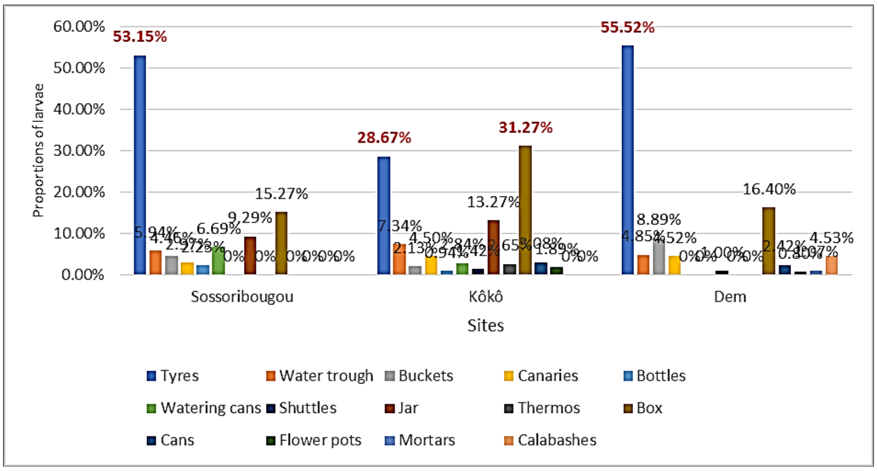

Fig 10: Spatial variation in larval abundance by type of site

Characterisation of breeding sites

A study of the spatial distribution of larval site types using principal component analysis (Figure 11) reveals that there are two main groups when axis 1 is considered (85.23% contribution). To the left of this axis, the types of larval breeding sites (calabash, mortar, watering cans, flower pot, thermos, bottle and watering can) are the least represented, whereas to the right of this axis, the types of breeding sites (jar, buckets, canaries, water troughs, box and tyres) are the most representative.

When we consider the second axis (8.76% contribution), a discrimination appears in the distribution of the types of cottages according to neighbourhood. In the Dem district, buckets, canaries, water troughs, boxes and tyres are the most representative sites, whereas in Kôkô and Sossoribougou, larvae are more abundant in jars. The Generalised Linear Model confirms this observation by revealing that the neighbourhood-type of site association influenced the distribution of larval abundance (GLM: ddl=26; W=150.2; p<0.0001).

Rate of emergence of adult mosquitoes

The emergence rates of adult mosquitoes by type of breeding site obtained after larval rearing are shown in Figure 12. The highest emergence rates were between 70% and 100%. The types of breeding sites concerned were tyres, water troughs, buckets, canaries, bottles, watering cans, shuttles, jars, cans, mortars and calabahes. An average rate of emergence was observed in flower pots (63.63%), while the lowest rate of emergence was observed in larvae housed in thermoses (11.11%).

Diversity, abundance and distribution of adult mosquitoes

Mosquito diversity and abundance

Identification of adult mosquitoes after emergence identified the species *Aedes aegypti* and the genus *Culex*.

The abundances of identified mosquitoes are shown in Figure 13. The abundances of *Aedes aegypti* (N=479) are slightly higher than those of the genus *Culex* (N=477). However, comparison of the abundances by analysis of variance revealed that there was no significant difference between these abundances (ddl=1; F= 0.06; p >0.05).

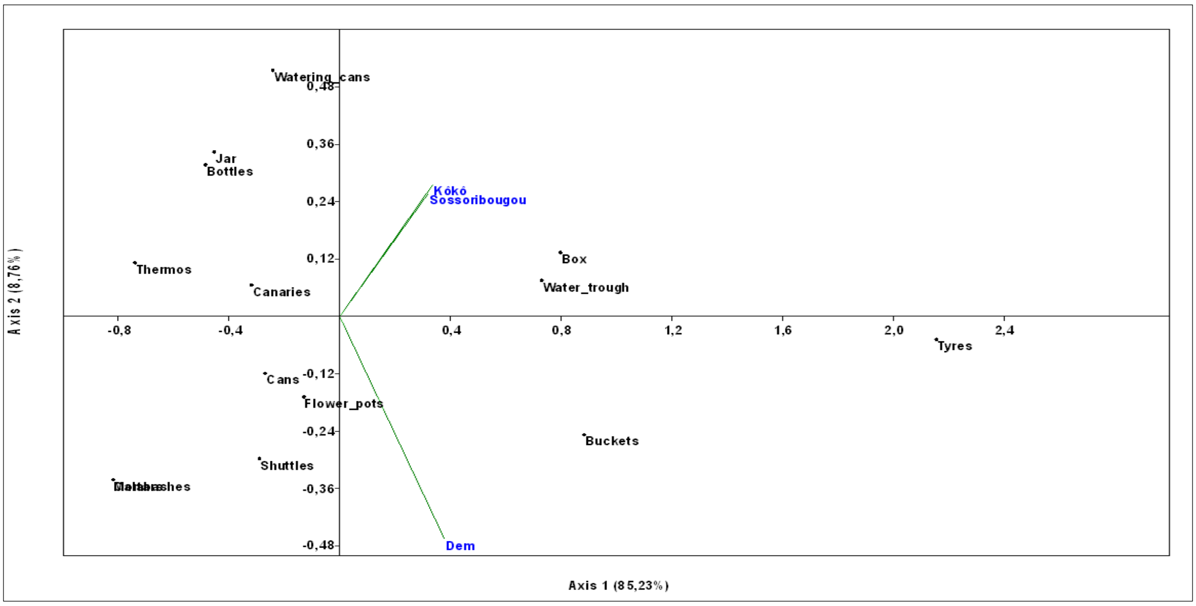

Fig 11: Distribution of larval breeding sites by neighbourhood

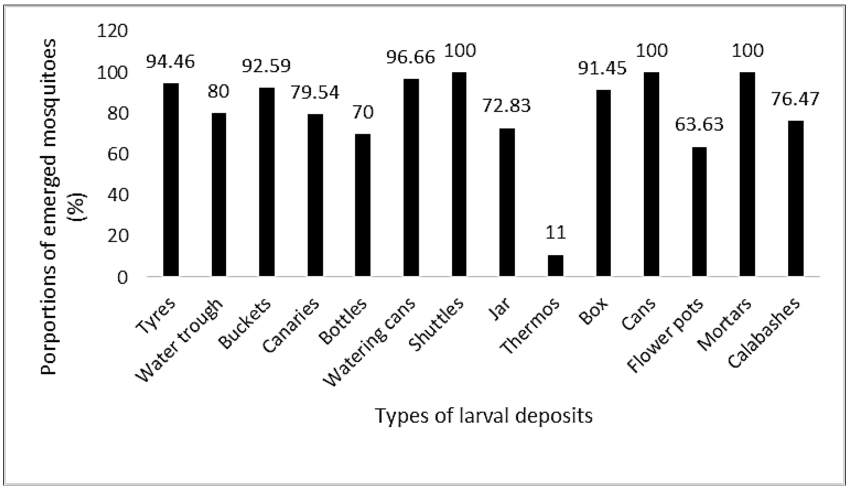

Fig 12: Rate of emergence of adult mosquitoes by type of breeding site

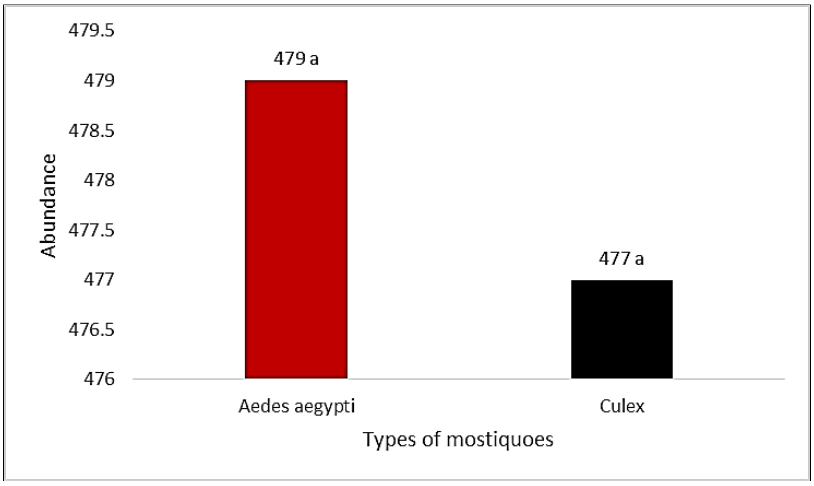

Fig 13: Overall abundance of *Aedes aegypti* and *Culex* genera

The spatial abundances of the species Aedes aegypti and the genus Culex are shown in Figure 14. The abundance of *Aedes aegypti* was highest in Kôkô (55.97%) and Dem (75.65%), while in Sossoribougou the genus *Culex* was best represented (93.92%). An analysis of variance comparing the abundance of *Aedes aegypti* and *Culex* in Kôkô (ddl=1; F=11.30; p<0.0001), Dem (ddl=1; F=29.04; p<0.0001) and Sossoribougou (ddl=1; F=45.93; p<0.0001) was highly significant (p<0.0001). The Newman-Keuls test revealed that the abundance of the Culex genus was highest only in the Sossoribougou district, while the *Aedes aegypti* species was best represented in the Kôkô and Dem districts. The Generalized Linear Model confirmed these observations by revealing that the distribution of mosquitoes varied with site (GLM: ddl=2; W=25.04; p<0.0001).

Spatial distribution of adult mosquitoes

The principal component analysis (Figure 15) used to characterise adult mosquito species according to the type of larval site of emergence revealed two main groups, taking into account the first axis (80.84% contribution). On the left-hand side of this axis, larval sites such as gourds, thermos, shuttles, mortars, flower pots and bottles were the least used. To the right of axis 1, the most characteristic larval habitats were boxes, water troughs, watering cans, jars, buckets, canaries and tyres.

When axis 2 is taken into account (19.16% contribution), a clearer segregation takes place by associating species with types of lodging. Thus, watering cans, water troughs and boxes were characteristic of the *Culex* genus, whereas jerry cans, canaries, buckets and jars were specific to the *Aedes aegypti* species (Figure 15). The Generalized Linear Model confirmed this observation, revealing that the distribution of mosquitoes varied with the type of bed (GLM: ddl=13; W=90.74; p<0.0001).

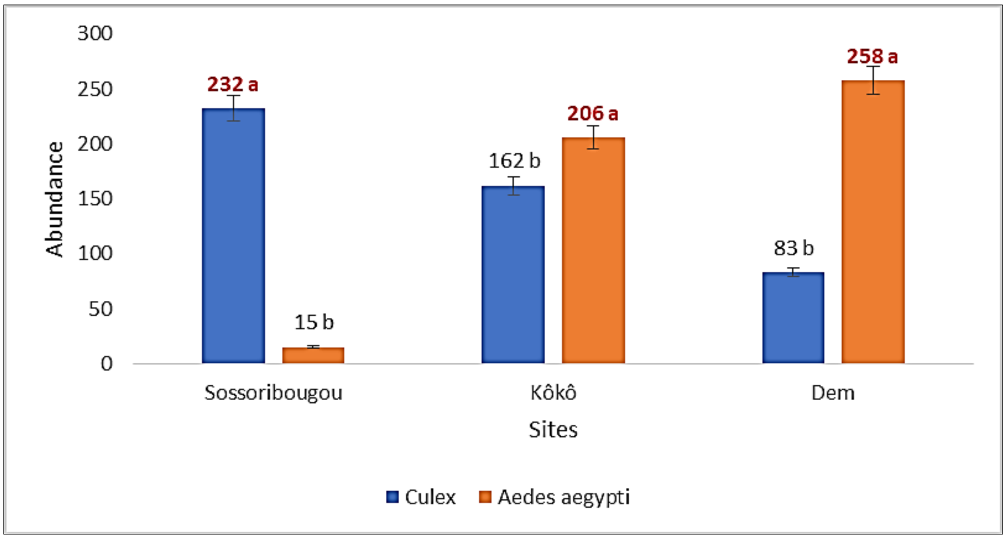

Fig 14: Spatial abundance of *Aedes aegypti* and *Culex* genera

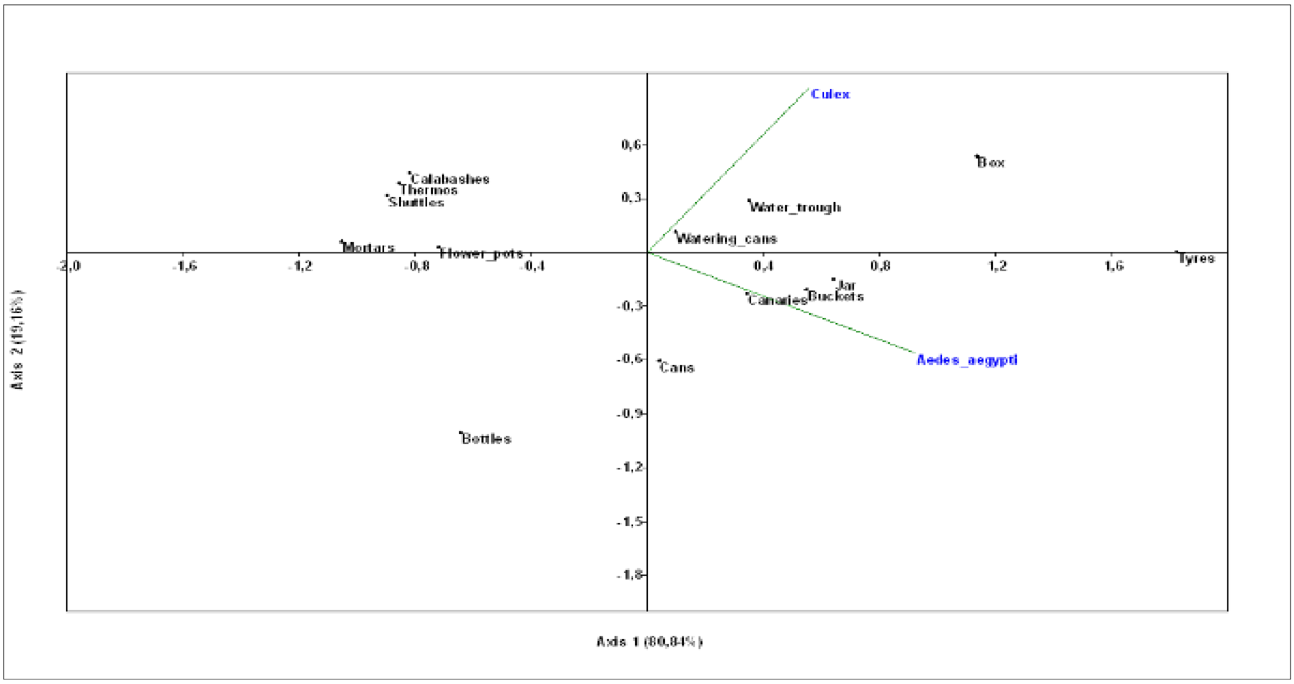

Fig 15: Distribution of *Aedes aegypti* and *Culex* genus breeding sites

Discussion

Potential gites identified in this study were tyres, boxes, buckets, troughs, cans, flower pots, canaries, jars, thermos flasks, bowls, watering cans, bottles, mortars and gourds. In Womi in Cameroon, potential breeding sites were gutters, streams, tyre tracks, wells and water storage containers [13]. These differences could be linked, on the one hand, to the seasons and, on the other, to the various human activities carried out in these localities, which would have contributed to the establishment of the said sites. Indeed, by modifying their environment and lifestyle, humans create favourable conditions for the establishment of larval breeding sites [14, 15, 16].

The different sites identified were artificial sites and peri-domestic sites, with a predominance of tyres. Our results are similar to studies conducted on the characterisation of *Aedes aegypti* habitats in northern Côte d'Ivoire and Togo [9, 17]. Tyres also made up the largest portion [18]. In contrast, the most important Anopheles gambiae breeding sites were puddles in Korhogo, while in Oussou-Yaokro, rivers were predominant [16].

Human activities and insalubrity linked to a lack of sanitation in the living environment favour the multiplication of breeding grounds [15, 16]. This would lead to the proliferation of major vectors, with the resulting health risks of diseases such as chikungunya, filariasis, dengue fever, yellow fever and Zika [19, 16]. Some diseases are linked to sanitation problems [20].

The highest productivity was observed in the tyres. This observation is contrary to the data obtained at Manoka in Cameroon on *Anopheles gambiae*. Indeed, the highest productivity was recorded in containers [13]. This finding may be linked to the fact that unsanitary conditions (box, bottles and tyres) create shallow breeding sites, which are the most productive [16].

The productivity of the breeding sites was high. This could be linked to the large number of sites associated with the rainy season, during which our study took place. This season is favourable for mosquito breeding [21]. *Aedes aegypti* and *Culex* mosquitoes were the most abundant. The abundance of *Culex* mosquitoes could be linked to their resilience and ability to adapt to different habitats due to their plasticity [22, 23]. In the case of *Aedes aegypti,* their numbers would be linked to the nature of the breeding sites. Indeed, within the framework of this study, a variety of types of breeding sites for this species were identified.

These included jars, buckets, canaries, drums and tyres. *Tia et al* [16] also observed the nature of the breeding sites.

Conclusion

Larval surveys carried out in the town of Korhogo identified a total of 362 potential artificial breeding sites. These included tyres, boxes, buckets, water troughs, cans, flowerpots, canaries, jars, thermos, shuttles, watering cans, bottles, mortars and calabashes. Tyres provided the most important breeding sites. Boxes and water troughs were characteristic of the Kôkô and Sossoribougou neighbourhoods, while tyres and buckets were representative of the Dem neighbourhood. A positivity rate of 70.16% was determined for larval breeding sites. The high productivity of the breeding sites was observed in the tyres. In the Sossoribougou district, the highest productivity was observed in tyres, while in Kôkô, the highest abundances were recorded in boxes and tyres. Characterisation of larval breeding sites showed that in the Dem district, the characteristic larval breeding sites were buckets, water troughs, canaries, cans and tyres, while in the Kôkô and Sossoribougou districts, they were jars.

The highest rates of emergence were observed in tyres, water troughs, buckets, canaries, bottles, watering cans, shuttles, jars, cans, mortars and calabashes. In the districts of Kôkô and Dem, the species *Aedes aegypti* was the most abundant, while in Sossoribougou the genus *Culex* was the most important. Characterisation by type of larval site showed that watering cans, water troughs and boxes were representative of the *Culex* genus, while the specific sites of *Aedes aegypti* were canaries, buckets, jars and tyres. These breeding sites, which are characteristic of *Aedes aegypti*, are therefore likely to constitute a risk in the occurrence of dengue and yellow fever epidemics in the town of Korhogo.

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