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Typology of domestic larval habitats of *Anopheles* in the rural savanna of northern Côte d'Ivoire

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Abstract
Vector-borne diseases impose a heavy burden on at-risk populations. Better management of vectors via larval control is essential; thus, understanding the effects of man-made domestic containers on mosquito breeding behaviour is necessary. From March 2016 to May 2017, four villages, divided into water sources presence (WSP) and water sources absence (WSA), were selected in the rural savanna of northern Côte d'Ivoire. In-house man-made water containers were classified into four types according to the base material: iron, clay, rubber and sand–soil. During rainy season, the lowest mean larval density was reported in sand–soil containers while the lowest density during dry season was observed in clay containers, irrespective of WSA or WSP groups. During the rainy season, the larval density of *Anopheles* was higher in iron, clay and rubber larval habitats compared with sand–soil larval habitats. However, during the dry season, the larval density of *Anopheles* was lower in iron and clay compared with the sand–soil larval habitat. *Anopheles* larval densities were found to be similar in iron, clay and rubber larval habitat during the dry season. Whatever the proximity to water bodies, the current study indicates seasonal difference of *Anopheles* density in man-made domestic containers. Specially, some preference of *Anopheles* to dirty water spread onto village streets was observed. Sensitization and prevention strategies must be developed to tackle the different types of domestic rural larval habitat.

Keywords: *Anopheles*, larvae, containers, habitat, Côte d'Ivoire

1. Introduction
Climatic factors such as temperature and rainfall determine the presence and relative frequency of *Anopheles* vector species in rural tropical Africa [1, 2]. The main vectors inoculating the malaria parasite are widespread in most in sub-Saharan African countries, and have the capacity to adapt to different environments [3]. Moreover, in large dry savannas and semi-desert areas, malaria vector species can maintain malaria transmission during periods of drought [4, 5]. The ability of the main malaria vector to adaptation to extreme weather conditions, and to even survive extended and intense phases of drought in some parts of Africa, is an extremely important consideration when reinforcing vector control strategies [6]. In these areas prone to water shortages and droughts, the water necessary for *Anopheles* larval development is rare during 6 to 8 months between October and May [7, 8]. However, despite the lack of breeding sites, malaria continues to affect a large amount of people that are seeking care in rural health facilities, as previously observed during the dry season in northern Côte d'Ivoire [9]. The malaria prevalence reported in 2017 from two districts in this region– Niakaramandougou and Kiémou – is 7.13 % and 3.08 % during dry season and 31.22 % and 15.05 % during the rainy season [10]. Larvae from domestic breeding sites are likely to play a key role in maintaining malaria transmission during dry season [5]. According to the preference–performance hypothesis, insects without parental care perform oviposition in locations that are most suitable for their offspring, to optimize their performance [11]. Consequently, during periods of severe drought *Anopheles* tend to oviposition in domestic water collection containers around or inside human houses owing to their suitability [5, 12]. While species oviposition sites choice in relation to water physicochemical cues has been widely studied [13-16], little is known about the container typology of domestic water where larvae have been found. The main objective of the current study was to understand the effects of the material of man-made domestic containers on the mosquito oviposition site preferences and offspring.

2. Materials and Methods

2.1 Study area

Larval collection was carried out in the Niakaramandougou (8°40 North and 5°17 West) and Kiémou (9° North and 5° West) district, in a rural savanna environment, located in Korhogo, northern Côte d'Ivoire. Four villages namely Longo in Niakaramandougou and Gossonkaha, Bémavogo, Nalourgokaha in Kiémou (Fig. 1) were chosen to be assessed for this study based on easy accessibility, and the availability of complete information on malaria cases during the dry and rainy season in the health administration. Villages were aggregated into two sites according to the proximity to permanent water sources Group WSP (presence of water bodies) included Longo and Gossonkaha located 1.5 km from Bandama River, while group WSA (absence of water bodies) was represented by the villages Bémavogo and Nalourgokaha, near which there is no river within a radius of 5 km. The distance between the two groups was approximately 8.5 km. The landscape is dominated by uplands – the so-called 'plateaux rigueux'. The rainy season stretches from June to September (4 months), and the dry season from October to May (8 months), dominated by the 'Harmattan' (dry and hot wind blowing from north to south), building the Sudanese climate in this area. The average annual rainfall ranges from 1200 to 1400 mm, the average annual temperature is 26°C and the average monthly relative humidity lies between 35 % and 79 %. Savanna is the most dominant vegetation.

2.2 Larval sampling

From March 2016 to May 2017, all domestic water containers were screened monthly for mosquito breeding site identification. Larval density was assign to each larval habitat belonging to a certain container material using the classical dipping technique of 60 ml capacity [17]. Breeding sites that contain only anopheline larvae were designated exclusive breeding sites, while those in which *Anopheles* larvae coexisted with other mosquito larvae (i.e. *Aedes* and *Culex*) were designated mixed breeding site. The containers of larval breeding sites were categorized into iron (e.g. open barrels and cans), rubber (e.g. buckets and landry tub), clay (e.g. clay pots and jars) and sand–soil (e.g. laundry water flow, shower water flow and dishwater) (Fig. 2). The collected larvae were reared in the University Nangui Abrogoua entomology laboratory at 24°C and relative humidity around 80 %. Larvae were fed with powdered cat food, and morphological identification of emerging adults was based on the Mattingly key [18] for *Anophelinae*, and the Gillies and Meillon key [19] for Culicinae.

2.3 Statistical analysis

Data was entered in Excel version 2013. Statistical analyses were performed using STATA version 14 (Stata Corporation; College Station, TX, USA). Larval density was expressed as the average number of larvae per dipper. Negative binomial regression was applied to explain variation in larval density among breeding sites container material [20]. Incidence rate ratio (IRR) with 95% confidence interval was reported rather than coefficient for ease of comparison between categories. The threshold of significance was set to 5 %.

3. Results

3.1 Seasonal distribution of *Anopheles* larval habitat

A total of 137 *Anopheles* breeding sites were identified with 70.8 % (n = 103) during the rainy season and 24.8 % (n = 34) during dry season for both study groups (Table 1). During rainy season, natural water collection accounted for 67.9 % (n = 38) of larval breeding sites compared with 32.1 % (n = 18) of artificial breeding sites in the WSP group. In the WSA group, 75.6 % (n = 31) of breeding sites surveyed during the rainy season were found in natural water bodies compared with 24.4 % (n = 10) in artificial ones. However, in dry season, all *Anopheles* positive larval habitats were found in artificial domestic larval breeding sites across both study groups (Table 1).

3.2 Proportion of *Anopheles* positive larval habitat by container material

During the rainy season, the proportion of larval breeding sites in rubber containers was highest (61.1 %; n=11/18 and 40.0 %; n=4/10 for WSP and WSA group, respectively), followed by iron containers (Table 2). In dry season, sand–soil containers represented half of all larval habitats (50 %; n=12/24), followed by rubber (20.8 %; n=5/24 in WSP group and 25.0 %; n=4/16 in WSA group) (Table 2).

All domestic larval habitats that were found positive for *Anopheles* larvae in the dry season were mixed breeding site whereas in rainy season natural and artificial domestic larval habitats were exclusive breeding sites.

3.3 Relationship between larval density of *Anopheles* and the material of domestic larval habitat

The variation in mean larval density per dipper by season is presented in Fig. 3. For the WSP group, the lowest mean larval density during rainy season was reported as 12 larvae per dipper in sand–soil larval breeding sites (95 % CI 9–22), while during dry season, the lowest value of 5 larvae per dipper was found in clay containers (95 % CI 2.5-8.0). In the WSA group, the lowest mean larval density during the rainy season estimated at 11 larvae per dipper, was observed in sand–soil larval breeding sites (95 % CI 7–15), while in the dry season the lowest value of 4 larvae per dipper was reported in clay containers (95 % CI 2–7).

In the rainy season and compared with sand–soil larval habitat, the larval density of *Anopheles* was 1.35 times higher in iron larval habitat (IRR = 1.35, 95 % CI 0.99–1.84, $P = 0.05$), 1.75 times higher in clay larval habitat (IRR = 1.75, 95 % CI 1.24–2.35, $P = 0.001$) and 1.64 times higher in rubber larval habitat (IRR = 1.64, 95 % CI 1.20–2.23, $P = 0.002$). In contrast, in the dry season, again compared with sand-soil larval habitat, the larval density of *Anopheles* was 0.51 times lower in iron larval habitat (IRR = 0.51, 95 % CI 0.33–0.81, $P = 0.004$), 0.32 times lower in clay larval habitat (IRR = 0.32, 95 % CI 0.20–0.50, $P< 0.001$) and 0.81 times lower in rubber larval habitat (IRR = 0.81, 95 % CI 0. 55–1.18, $P = 0.27$). *Anopheles* larval densities were found to be similar in iron, clay and rubber larval habitats in each group during dry season.

4. Discussion

The aim of this study was to evaluate the effects of larval habitat container materials on larval density during rainy and dry season. Knowledge of local malaria vector preferences for artificial breeding sites around human housing, and the productivity of those breeding sites in relation to the seasons, are essential to implement effective vector control strategies. Different container materials in human houses, where mosquitoes have ovipositioned, have been identified. Indeed, it is well known that insects adapt to changes in habitat due to

climatic variations such as drought and fluctuating temperatures [21]. During rainy season, rainfall causes small water enclaves that are colonized by mosquitoes once the rain stops. A high occurrence of *Anopheles* breeding sites has previously been described to coincide with the peak of rainfall [22]. However, in the dry season, when the habitats are drying, mosquitoes perform physical and behavioural adaptations to survive. It has been shown previously that the characteristics of the habitat and the season have an effect on the mosquito abundance in containers [21].

Moreover, clinical cases and deaths related to malaria infections were found to be more frequent in dry season [23].

The current observations during the dry season demonstrated a substantial amount of domestic larval habitats in man-made containers with mixed mosquito genera. This is probably due to the rarity of the preferential oviposition site generating a competition of mosquitoes in the colonization of the remaining water source. Indeed, several studies have shown previously that domestic breeding sites are not only host to *Aedes*, but can also contain *Anopheles* larvae [12, 24]. However, breeding sites that *Anopheles* do not usually use but that are often colonize by *Culex*, were observed and designated as *Anopheles* atypical breeding sites [25, 26]. During dry season, regardless of the nature of man-made containers used as mosquitoes breeding sites, all of them were in the immediate vicinity of rural populations and it has been verified to be a strong predictor of *Anopheles* abundance [26]. Moreover, anopheline larval habitat selection depends essentially on the physicochemical components of water in the container [13, 14, 15]. Anopheles, especially the *gambiae* species is endowed with an incredible genetic plasticity that allows adaptation to variable environmental conditions. This adaptive potential is largely related to the presence of polymorphic chromosomal inversions [28]. During the dry season, facing the scarcity of running water, rural inhabitants get water from the village hydraulic pump and store it a various container often for more than a week for their daily needs [12]. This water storage period is sufficient to allow the *Anopheles gambiae* life cycle to finish [29]. Despite water scarcity, people must pursue their most essential water-based daily activities such as washing and drinking. However, the absence of sewage disposal system leads to the spread of dirty water collections (sand–soil) from these different activities in the village streets. Being the most abundant and most easily accessible source of water during dry season, *Anopheles* has no alternative breeding site, which explains the high abundance of these kind of breeding sites harbouring larva in the current study. High larval density has been observed during the rainy season at all sites, which might be potentially involved in high levels of malaria transmission [30].

Furthermore, sand–soil material has demonstrated a higher anopheline larvae production compared with iron, clay and rubber materials during the dry season. Seasonal difference of *Anopheles* density in man-made domestic containers was observed conversely to findings from a study carried out in urban north and central Côte d'Ivoire, reporting similarity in domestic larval breeding sites [12]. This difference might be explained by the study area where the lack of water is greater in rural than urban areas.

Table 1: Seasonal diversity of *Anopheles* larval breeding sites

		Rainy Season	Dry Season	Total
Study sites groups	**Type of larval habitat**	**N (%)**	**N (%)**	**N (%)**
WSP	Natural	38 (67.9)	0(0.0)	38 (47.5)
	Artificial	18 (32.1)	24 (100)	42 (52.5)
	Total	56 (100)	24 (100)	80 (100)
WSA	Natural	31 (75.6)	0 (0.0)	31 (54,4)
	Artificial	10 (24.4)	16 (100)	26 (45.6)
	Total	41 (100)	16 (100)	57 (100)
	Overall	97 (70.8)	40 (24.8)	137 (100)

Table 2: Seasonal abundance of domestic *Anopheles* larval habitats

		Rainy season	Dry season	Total
Study site groups	**Larval breeding site Maters**	**N (%)**	**N (%)**	**N (%)**
WSP	Iron	4 (22.2)	3 (12.5)	7 (16.7)
	Clay	2 (11.1)	4 (16.7)	6 (14.3)
	Rubber	11 (61.1)	5 (20.8)	16 (38.1)
	Sand-soil	1 (5.5)	12 (50.0)	13 (30.9)
	Total	18 (100)	24 (100)	42 (100)
WSA	Iron	3 (30.0)	1 (6.2)	4 (15.4)
	Clay	2 (20.0)	3 (18.7)	5 (19.2)
	Rubber	4 (40.0)	4 (25.0)	8 (34.6)
	Sand-soil	1 (10.0)	8 (50.0)	9 (34.6)
	Total	10 (100)	16 (100)	26 (100)

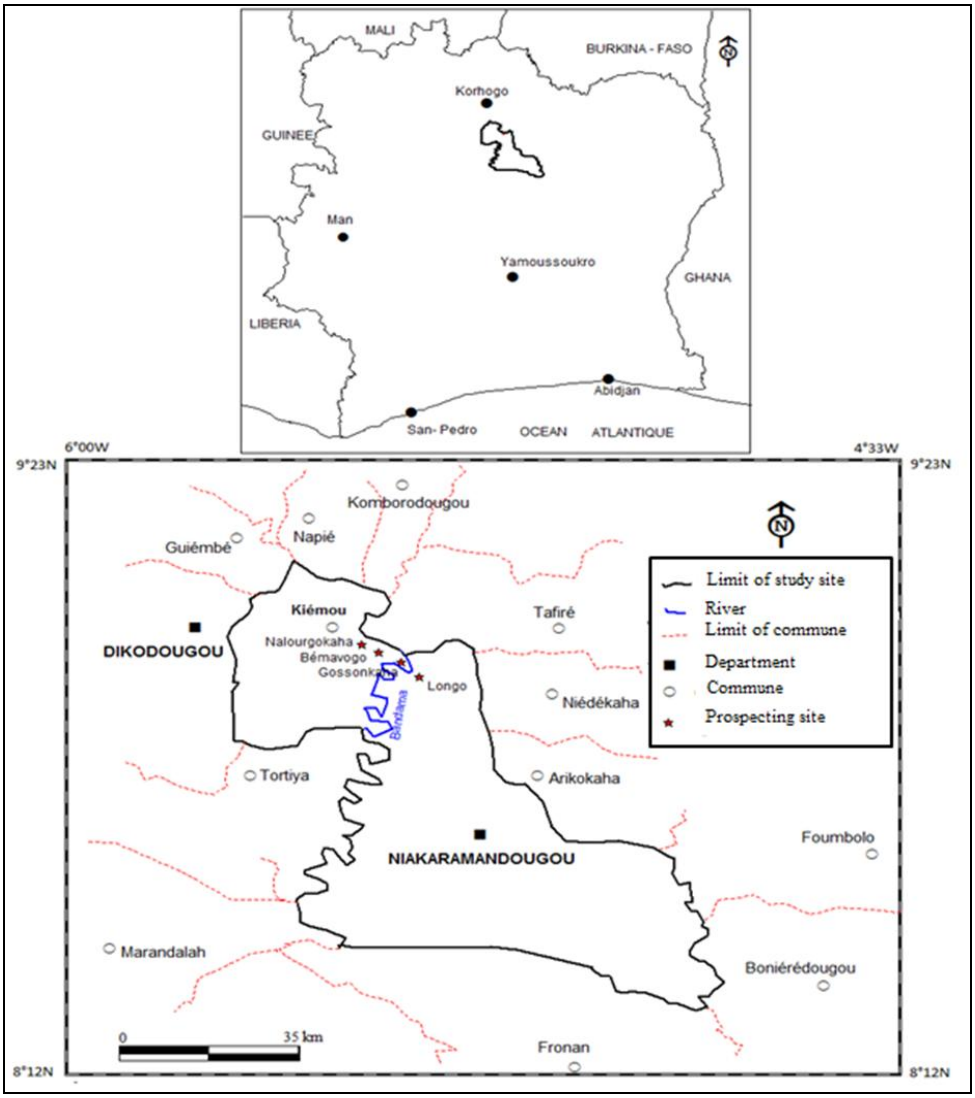

Fig 1: Study area

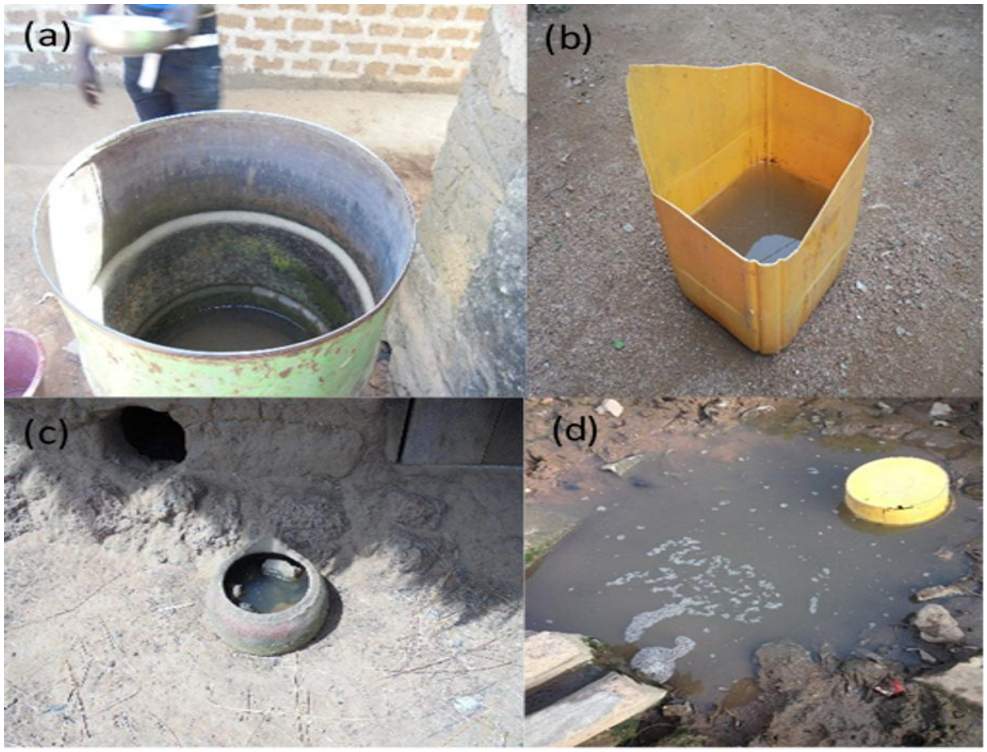

Fig 2: Water in different containers identified as larval habitat. (a) Water barrel made of iron. (b) Water container made of rubber commonly used for goats and sheep. (c) Clay pot used for providing chickens water. (d) Water accumulation in sand-soil in the street.

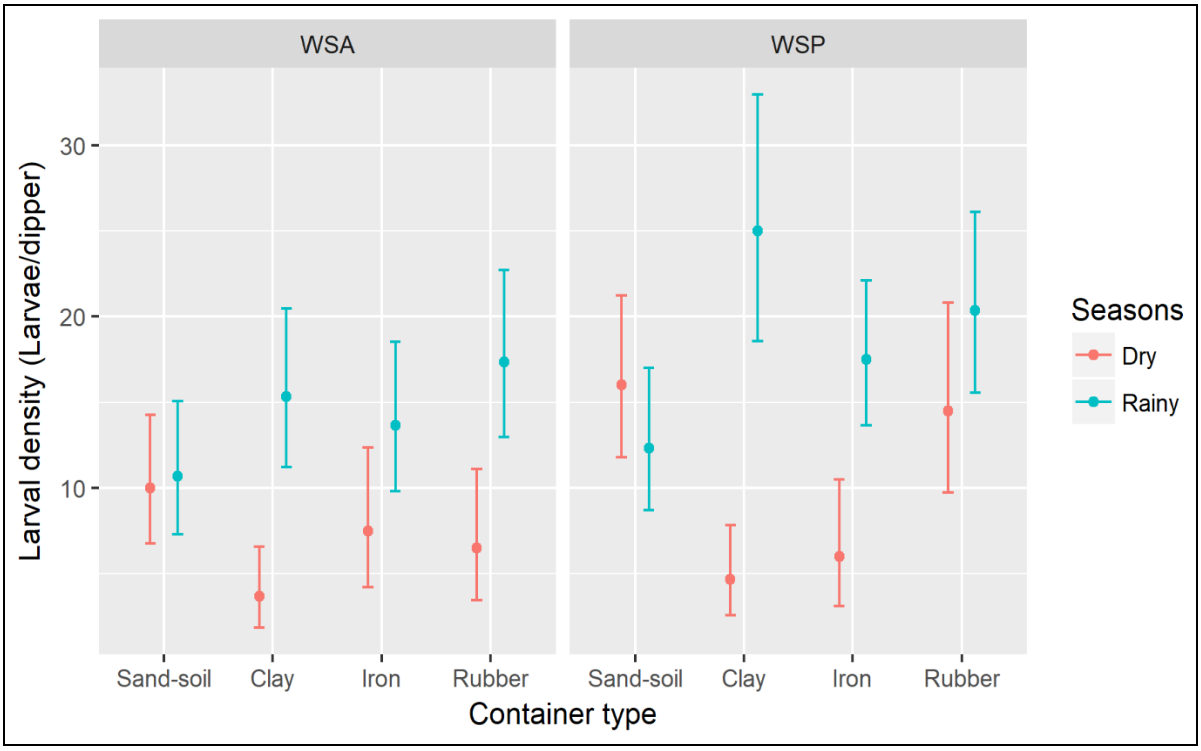

Fig 3: Larval density in different water container types per study site groups. (The dots represent the mean larval density, while the whiskers represent the confidence interval)

5. Conclusion

The adaptation of mosquitoes to different materials for water containers as breeding sites remains a risk in vector-borne disease transmission regardless of the season, and especially for malaria. Although there was no difference in larval density whatever the proximity to water bodies, a substantial number of larvae colonized man-made containers calling for special attention. Any sensitization efforts must include a focus on the maintenance of domestic water storage containers, such as jars and barrels. Weekly emptying of the water container in order to eliminate mosquito eggs and the distribution of containers with a rigid and well-fitting cover could counteract the establishment of such man-made breeding sites and significantly lower transmission during the dry season.

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