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Characterization of anopheline (Diptera: Culicidae) larval habitats in Nouakchott, Mauritania

O. Ahmedou Salem Mohamed Salem[1, 2], M. Lekweiry Khadijetou[1], M. Hasni Moina[1], Konate Lassana[2], Briolant Sébastien[3], Faye Ousmane[2] & O. Mohamed Salem Boukhary Ali[1]

[1]*Laboratoire de Biotechnologies, Faculté des Sciences et Techniques, Université des Sciences, de Technologie et de Médecine, Nouakchott, Mauritanie;* [2]*Laboratoire d'Ecologie Vectorielle et Parasitaire, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar, Sénégal;* [3]*Institut de Recherche Biomédicale des Armées, Direction Interarmées du Service de Santé, Laboratoire de Parasitologie, CNR du Paludisme région Antilles-Guyane, Institut Pasteur de la Guyane, Cayenne cedex, France*

ABSTRACT

Background & objectives: Despite the increasing number of reported autochthonous malaria cases in Nouakchott and the identification of *Anopheles arabiensis* as the major malaria vector in this Saharan city, anopheline larval habitats have never been identified so far. The objective of this study was to identify and characterize anopheline larval habitats in Nouakchott.

Methods: During September and October 2012, samples from pools of rainwater, water discharged from standpipes and household drinking water tanks in the districts of Dar Naim, Teyarett and Arafat were analyzed for the presence/ absence of anopheline larvae and physicochemical characterization of breeding habitats.

Results: Of the 51 prospected water bodies, eight consisting of seven water discharged from standpipes and one household drinking water tank were productive for *Anopheles* sp. All emerged anopheline mosquitoes from the positive dipping were morphologically identified as members of the *An. gambiae* complex. Multivariate regression analyses showed that a salinity up to 0.1 g/l and a shaded situation were respectively protective factors against high larval density in breeding sites (adjusted odds ratio = 0.62, 95% CI [0.44–0.87], $p = 0.0052$ and adjusted odds ratio = 0.56, 95% CI [0.44–0.71, $p < 0.0001$] and a pH up to 7.61 was a risk factor for high larval density in breeding sites (adjusted odds ratio = 1.56, 95% CI [1.25–1.95], $p = 0.0001$).

Interpretation & conclusion: The study demonstrated in Nouakchott that despite an arid and dry climate, human practices have contributed to the establishment of favourable environmental conditions for the development of anopheline mosquitoes and, therefore, maintaining malaria transmission in this Saharan city. The core malaria vector control intervention as the use of long-lasting insecicidal nets (LLINs) could be complemented in Nouakchott by larval source control. In this area, appropriate larval control measures may be recommended in line with an integrated vector management (IVM) approach.

Key words *Anopheles gambiae* s.l.; habitats; larvae; Nouakchott; standpipes

INTRODUCTION

Malaria accounts for over 22% of the total outpatient morbidity in Mauritania, and about 51% of deaths in health facilities are due to malaria[1]. Almost 90% of the resident population of Mauritania is exposed to the risk of malaria. The endemic foci of the disease are located mainly in south and southeast parts of the country, especially in eight provinces of Hodh Elgharbi, Hodh Echarghi, Assaba, Brakna, Trarza (including Nouakchott), Gorgol, Tagant and Guidimagha[2]. Malaria cases in these areas are mainly caused by *Plasmodium falciparum*[2–3] except in Nouakchott where reported malaria infections are predominantly due to *P. vivax*[4–5]. Based on the WHO report of 2011, Mauritania is classified in the control phase of malarial control programme[2]. Despite its importance, little is known about the *Anopheles* mosquito species responsible for transmission of malaria in Mauritania. The only published entomological study dates back to 40 years[6–7]. More recently, Dia *et al*[8] studied the distribution, host preference and infection rates of three malaria vectors—*An. arabiensis*, *An. pharoensis* and *An. funestus* in five regions of Mauritania, namely Trarza, Brakna, Assaba, Hodh Elgharbi and Tagant. In Nouakchott, the capital city of Mauritania, despite the increasing number of reported autochthonous malaria cases[3–5] and the identification of *An. arabiensis* as the major malaria vector in this city[9], anopheline larval habitats have never been identified so far. Identification and characterization of anopheline mosquito breeding habitats have a critical role in malaria control programme. Usually urbanization has a great impact on the composition of the vector system and malaria transmission dynamics. In regard to breeding requirements, every *Anopheles* species has its preferred water bodies for oviposition. These preferences depend on the physical geography and human activities. Breeding sites can be natural or man-made, of various sizes, shaded or sunny and permanent or temporary. This study reports, for the first time, the existence of *An. gambiae* s.l. larval habitats in Nouakchott and describes some of their ecological and physicochemical characteristics.

MATERIAL & METHODS

Study area

Nouakchott (18°11' N–16°16' W) is situated in the Sahara zone, near the Atlantic coast, at an altitude of seven meters above sea level as measured at the centre of the city. It comprises of nine districts and houses 743,511 inhabitants corresponding to one fourth of the whole population of the country[4]. The wet season is short, extending from August to September, with little annual variation in the amount of rainfall (50 to 80 mm). The average annual temperature ranges from 20.7 to 33.3°C, and the average relative humidity from 33.4 to 79.1%. The vegetation consists of intra- and peri-urban orchards with multiple crops (date palm, Prosopis, legumes, mint and alfalfa). Due to the extension of informal urbanization and the lack of efficient drinking water network, most houses are supplied with drinking water either through public standpipes or building water tanks at home and only 21% of households are connected to the drinking water network[10]. The study sites were in Teyarett, Dar Naim and Arafat districts. Teyraett and Dar Naim districts were chosen due to the high incidence of malaria infections among their inhabitants and the presence of *An. arabiensis* as malaria vector compared to other Nouakchott districts[4,10] while report from Arafat district revealed little or no malaria infection and malaria vector has never been found.

Larval sampling and identification

Larval collections (once every two weeks) were conducted in September and October of 2012, corresponding to the end of the rainy season and the peak of malaria transmission. Three types of water bodies within the study sites were prospected in the following order: first, 40 pools of water collection from rainfalls were sampled and examined for the presence/absence of mosquito larvae. Then after two surveys, the absence of mosquito larvae in this natural water collections was observed, we became interested with the artificial water collections including three household drinking water tanks in Teyarett district, and eight tanks of discharged water from public standpipes spread over the three districts. To determine the occurrence of anopheline mosquito larvae at each site, a dip was made with a 350 ml of standard dipper and examined for the presence or absence of mosquito larvae. From each positive dipping, up to five anopheline larvae were collected and preserved in 95% ethanol for morphological identification. Furthermore, water samples of 350 ml were obtained from each site for adult anopheline mosquitoes' collection. Water samples were placed in crystallizers at room temperature and covered with a bednet. Emerged adult mosquitoes were then collected every two days. Larval (III and IV instars) and adult mosquitoes identification were performed with a stereo microscope (Optika, Italy) using the keys of Gilles and Coetzee[11].

Physicochemical characteristics of larval habitats

Simultaneously with larval sampling, the environmental characteristics of each larval habitat were measured. Habitat length, width and depth were measured using a metal ruler. Water pH and temperature were determined using hand-held pH meter HM-20P (DKK-TOA Corporation, Japan), and turbidity was measured using a TB-25A portable turbidity meter (DKK-TOA Corporation, Japan). Salinity and conductivity were determined using the EC-21P conductivity meter (DKK-TOA Corporation, Japan). Dissolved oxygen was determined using the handheld DO meter DO-24P (DKK-TOA Corporation, Japan). Light/shadow of the sampled water from natural and artificial collections and the presence or absence of vegetation was also noted. All samples and measurements were taken between 1200 and 1400 hours.

Statistical analysis

Data were recorded using Excel and were checked for consistency before statistical analysis by using R-software (version 2.10.1). All qualitative variables were constructed to have equilibrated effectives in the groups. The larval positivity of water collections was analyzed as a dependent variable according to individual water collections characteristics using a logistic regression model. First, a descriptive analysis of the independent variables was performed and bivariate analysis was conducted by entering each independent variable in a logistic regression model. The larval density of breeding site was analyzed as a dependent variable according to individual breeding site characteristics using a Poisson regression. First, a descriptive analysis of the independent variables was performed, and bivariate analysis was conducted by entering each independent variable in a Poisson regression model. In both logistic and Poisson regression models, variables were retained for the multivariate analysis when their effect had a p-value <0.25. A backward stepwise selection procedure with a minimization of Akaike Information Criterion was applied to retain the significant (p <0.05) independent variables and their interactions in the final model.

RESULTS

During the study period, in September and October 2012, 51 water collections (40 rainfall water and 11 man-made) were prospected in Teyarett, Dar Naim and Arafat districts. None of the 40 rainfall water collections were found positive for mosquito larvae, while eight out of the 11 man-made water collections consisting of seven water discharged from standpipes and one household drinking water tank were productive for *Anopheles* sp. Table 1 presents the names, habitat type, geographical position, larvae abundance and instar stages of the artificial habitats in Nouakchott. The altitude of these sites ranged between one and six meters above the sea level. Only one surveyed water collection discharged from standpipes in Arafat district was found positive for anopheline larvae. Larval abundance ranged from 20 to 80 collected larvae (mean of five samples per breeding site). III and IV instar larvae were found in all the positive habitats except the EI mechrou breeding site where only I and II instar larvae were isolated. All the sampled anopheline larvae and 132 adult mosquitoes emerged from positive water collections were morphologically identified as members of *An. gambiae* s.l. Table 2 summarizes the characteristics of positive anopheline larval habitats in Nouakchott. Habitat depth, width and length ranged from 25–150, 60–170 and 30–250 cm respectively. Temperature of breeding sites during the larval collection ranged from 25–29°C. Chemical analysis showed that anopheline larvae tolerate wide range of conditions. The pH varied little between habitats (7.41–8.22). Water salinity ranged from 0.07 g/l for El mechrou samples to 0.2 g/l for samples from Lycée Teyarett 1 and 2. The conductivity of tested water samples is generally low with Ould Badou breeding site from Dar Naim district showed the highest conductivity level (1949 μs/cm) compared to other positive anopheline larval habitats. Water sample from EI mechrou (household drinking water tank) was clear (turbidity = 0.98 NTU), Hay Saken and Mgueizira samples (water discharged from public standpipes) were slightly turbid (24 and 25 NTU respectively) and the remaining samples, all water discharged from public stand-pipes, were trouble with a turbidity ranging from 70 to 157 NTU. The lowest dissolved oxy-

Table 1. Geographical location and larval abundance and instar stages of anopheline breeding sites in three districts of Nouakchott

District	Sites	Habitat type	Longitude	Latitude	Altitude (m)	Larval abundance	Larvae instar stages
Dar Naim	Ould Badou	Water discharged from standpipes	18°06′60″N	15°55′20″W	2	50	III, IV
	Hay Saken	Water discharged from standpipes	18°07′27″N	15°55′60″W	6	80	III, IV
Teyarett	Mgueizira	Water discharged from standpipes	18°06′59″N	15°56′21″W	4	30	III, IV
	Lycée Teyarett (1)	Water discharged from standpipes	18°07′37″N	15°56′13″W	2	20	III, IV
	Lycée Teyarett (2)	Water discharged from standpipes	18°07′36″N	15°56′12″W	1	25	III, IV
	Dar el Barka	Water discharged from standpipes	18°08′22″N	15°55′29″W	3	40	III, IV
	EI Mechrou	Household drinking water tanks	18°08′05″N	15°55′58″W	3	50	I, II
Arafat	Mallah	Water discharged from standpipes	18°04′08″N	15°56′50″W	5	25	III, IV

Table 2. Physicochemical characteristics of positive anopheline larval habitats in Nouakchott

Sites	Depth (cm)	Width (cm)	Length (cm)	Conductivity (μsec/cm)	Turbidity (NTU)	Temp. (°C)	Salinity (g/l)	pH	Dissolved oxygen (mg/l)	Sunlight situation
Ould Badou	45	80	170	1949	88	26	0.10	7.41	7.87	Full sunlight
Hay Saken	110	170	30	252	24	25	0.10	7.61	7.10	Full sunlight
Mgueizira	30	60	60	275	25	27	0.10	7.50	8.01	Partial sunlight
Lycée Teyarett (1)	130	150	150	437	70	29	0.20	7.60	7.20	Partial sunlight
Lycée Teyarett (2)	65	150	250	378	157	28	0.20	7.69	7.50	Partial sunlight
Dar el Barka	80	150	150	182	80	25	0.10	8.04	7.05	Partial sunlight
El Mechrou	150	150	200	135	0.95	26	0.07	8.22	0.90	Shaded
Mallah	25	100	200	131	92	25	0.10	7.52	7.04	Partial sunlight

Table 3. Multivariate logistic regression analysis of larval positivity in water collections in Nouakchott city

Characteristics	N	P	OR	95% CI	*p*-value
Depth					
≤ 25 cm	32	1	1	1.39–354.42	0.0282
> 25 cm	19	7	22.22		
pH					
< 7.7	24	6	1	0.0–1.11	0.0587
≥ 7.7	27	2	0.06		
Sunlight					
Full sunlight	42	2	1	1.71–346.47	0.0185
Shaded	9	6	24.34		

N = Total; P = Larvae positive water collections; OR = Adjusted odds ratio; 95% CI= Confidence interval 95% of OR.

gen concentration (0.9 mg/l) was observed in El mechrou water sample originated from a house-hold drinking water tank and the highest oxygen content (8.01 mg/l) was scored in Mgueizira sample. All sampled water collections, except that of El mechrou, were with partial or full sunlight and free from vegetation. In order to determine key factors governing larvae positivity of prospected water bodies from one hand and larvae density of breeding sites from another hand, obtained data were subjected to regression analyses. The multivariate logistic regression analysis (Table 3) has shown that the depth and the sunlight situation of the water collections in Nouakchott city were independently associated with the larvae positivity. A water collection pH ≥ 7.61 showed a tendency to be a protective factor (adjusted odds ratio—OR = 0.06, 95% CI [0.0–1.11], $p = 0.0587$) and the shaded situation of the water collection and a depth >25 cm were risk factors (OR = 24.34, 95% CI [1.71–346.47], $p = 0.0185$ and OR = 22.22, 95% CI [1.39–354.42], $p = 0.0282$). The multivariate Poisson regression analysis (Table 4) has shown that the salinity, pH and the sunlight situation of the breeding sites were independently associated with the larval density in Nouakchott city. A salinity up to 0.1 g/l and a shaded situation were respectively protective factors against high larval density in breeding sites (OR = 0.62, 95% CI [0.44–0.87], $p = 0.0052$ and OR = 0.56, 95% CI [0.44–0.71, $p < 0.0001$] and a pH up to 7.61 was a risk factor for high larval density in breeding sites (OR= 1.56, 95% CI [1.25–1.95], $p = 0.0001$).

Table 4. Multivariate Poisson regression analysis of larval density in breeding sites in Nouakchott city

Characteristics	N	OR	95% CI	*p*-value
Salinity				
≤ 0.1 g/l	6	1	0.44–0.87	0.0052
> 0.1 g/l	2	0.62		
Sunlight				
Full sunlight	2	1	0.44–0.71	< 0.0001
Shaded	6	0.56		
pH	2			
< 7.61	4	1	1.25–1.95	0.0001
≥ 7.61	4	1.56		

N = Total; OR = Adjusted odds ratio; 95% CI = Confidence interval 95% of OR.

DISCUSSION

This study was undertaken to identify and characterize larval habitats of anopheline mosquitoes in Nouakchott. As a Saharan city with arid and dry climate, Nouakchott has been regarded malaria-free for many years. Results revealed the presence of anopheline larvae in all prospected water collections except those created from rains. The absence of anopheline larvae in such water pools could be explained by the sandy texture of the soil of prospected sites which are not able to preserve the precipitation as surface water. In contrast, all sampled human made water pools were productive for anopheline larvae and emerged adult mosquitoes were morphologically identified as *An. gambiae* s.l. Their specific identification will be done using the polymerase chain reaction method. Malarial vectors in the *An. gambiae* complex are known to use diverse water bodies as larval habitats. These habitats differ in physical as well as chemical and biological characteristics, which directly influence the distribution and abundance of mosquito larval populations in nature. Physicochemical characteristics of the evidenced anopheline breeding sites generally agree with established habitat description for *Anopheles* larvae. Larvae of *An. gambiae* complex, particularly *An. arabiensis* and *An. gambiae* s.s. are usually associated with small and shallow freshwater pools[11]. They are known to prefer open sunlit habitats without vegetation for oviposition and often with turbid and low conductive water[12–13]. Their presence in water collections resulting from human activities has also been reported[14–15].

It is, therefore, clear that in Nouakchott, the development of public standpipes and the households' use of water storage tanks have generated favourable conditions for the development of aquatic stages of anopheline mosquitoes in this Saharan city. Further investigations are necessary to better understand how females select habitats for oviposition and what environmental factors influence the production of adults from those habitats. In summary, this study has established for the first time, habitat characteristics of *An. gambiae* s.l. in the city of Nouakchott. It demonstrates that in Nouakchott, despite an arid and dry climate unfavourable for the development of mosquitoes, human practices consisting of the construction of tanks to retain water discharged from public standpipes or to store drinking water in households have contributed to the establishment of environmental conditions favourable for anopheline mosquitoes breeding and, therefore, maintaining malaria transmission in this Saharan city. Results provide useful information that can be used as a guide for selection and implementation of adequate larval control interventions with regard to these unexpected artificial anopheline habitats. The core malaria vector control intervention like use of LLINs could be complemented in Nouakchott by larval source control. In this area, appropriate larval control measures may be recommended in line with an IVM approach. Finally, future research in this field should focus on mapping the malaria risk in order to spatially focus malaria control in the areas that are at greater risk levels.

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Correspondence to: O. Mohamed Salem Boukhary Ali, Department of Biology, Faculty of Sciences and Techniques, University of Sciences, Technology and Medicine, Nouakchott, Mauritania.
E-mail: alimedsalem@gmail.com

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