

International Journal of Mosquito Research

ISSN: 2343-5906 CODEN: IJMRK2 IJMR 2024; 11(4): 01-09 © 2024 IJMR www.dipterajournal.com

Received: 02-04-2024 Accepted: 05-05-2024

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Use of ovitraps for seasonal entomological surveillance of the arbovirus vector *Aedes aegypti* and some abiotic parameters influencing the distribution of this vector in the health district of Grand Lomé, Togo, West Africa

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DOI: https://doi.org/10.22271/23487941.2024.v11.i4a.780

Abstract

This study aims at exploring the possibility of using ovitraps as a tool for effective *Aedes* mosquito surveillance in Togo.

Ovitraps were deployed from May 2022 to March 2023 in houses in the 13 communes of the health district of Grand Lomé for *Aedes aegypti* mosquito eggs collection. An inter-seasonal comparison of the quantity of eggs collected was carried out using Student's t-test, and the influence of abiotic parameters was assessed using Spearman's correlation test.

A total of 20,273 eggs exclusively of the *Aedes aegypti* species were collected. Entomological calculations indicate that there is a high risk of arbovirus transmission in the Grand Lomé health district. Spearman's correlation revealed that only rainfall had a positive and slightly significant influence on egg distribution (r = 0.3351; P = 0.0382).

Ovitraps could therefore contribute to Aedes vector control in the health district grand lomé.

Keywords: Ovitraps, Aedes aegypti, arbovirus, entomological surveillance, Lomé, Togo

Introduction

Aedes aegypti, a mosquito of medical and economic importance, is well known for its role in the transmission of various pathogens responsible for dengue fever, chikungunya, Rift Valley fever, yellow fever and Zika fever ^[1]. These insects also represent major sources of nuisance for humans and animals^[2]. By 2022, the World Health Organization (WHO) has highlighted that almost half of the world's population is at risk of dengue virus transmission ^[3]. This risk spreads over almost a hundred countries worldwide, with an estimated 390 million cases per year, including more than 25,000 deaths per year, and remains a public health concern, especially in West Africa ^[4, 5, 6]. Between January and December 2023, a total of 171,991 suspected cases of dengue fever, including 70,223 confirmed cases resulting in 753 deaths, were recorded in 15 member states of the African Union. Eleven West African countries, including Togo, were affected ^[7]. These countries account for about 88% of all dengue cases in West Africa, which shows the extent of the disease's spread. This significant increase shows the urgent need for immediate action to manage and prevent this vector-borne disease. In the sub-region, the fight against the spread of mosquito-borne infections relies primarily on the use of insecticides (aerosols, coils, etc.) in public health, and Long-Lasting Impregnated Mosquito Nets (LLINs), in line with the WHO guidelines ^[3]. Unfortunately, efforts in vector control strategies have been hampered by the growing insecticide resistance of Aedes mosquitoes in several West African countries, including Burkina Faso^[8, 9], Senegal^[10], Côte d'Ivoire^[11], Ghana^[12], and Togo^[13]. Moreover, urban areas, characterized by larval habitats conducive to Aedes mosquitoes, high population density, and complicit migratory patterns, are

favorable foci for the rapid spread of arboviruses [14-16].

Togo, benefiting from a tropical and humid climate, offers a favorable environment to the breeding of *Aedes* mosquitoes. Rapid and uncontrolled urbanization in certain communes of Grand Lomé has also altered natural landscapes, creating conditions favorable to the development of *Aedes* mosquitoes. The massive influx of materials related to industrialization, ecological changes, species adaptation, international trade development especially the trade in used tires, and demographic growth have favored the proliferation of the vector and the spread of the dengue virus across different countries, especially in West Africa ^[17, 5]. Moreover, the proximity of Togo to dengue-endemic countries such as Benin, Burkina Faso, and Ghana, exposes the country to increased risks of arbovirus epidemics.

Considering the increase in insecticide resistance and the favorable conditions for the proliferation of *Aedes* mosquitoes, it is necessary to adopt adapted, less costly, and easy-to-use methods for entomological surveillance in urban and peri-urban areas. These low-cost strategies will enable us to assess vector dynamism to prevent possible epidemics. The

use of ovitraps, long considered appropriate, offers significant advantages for assessing the relative population density/abundance of *Aedes spp* in various environments ^[18]. However, Togo is yet to harness the potential of ovitraps as a monitoring tool. It is in this context that this study was initiated to showcase the potential of ovitraps in monitoring seasonal infestations of *Aedes aegypti* mosquitoes, as well as the abiotic parameters influencing their distribution in the health district of Grand Lomé.

Materials and Methods

Study Area

The study took place in the health district of Grand Lomé (6° 7' 54.998" N 1° 13' 22. 001" E), covering an area of 425.6 km² and subdivided into 13 communes: Bè-Est (Golfe 1), Bè Centre (Golfe 2), Bè Ouest (Golfe 3), Amoutiévé (Golfe 4), Aflao-Gakli (Golfe 5), Baguida (Golfe 6), Aflao-Sagbado (Golfe 7), Agoè-Nyivé (Agoènyivé 1), Légbassito (Agoènyivé 2), Vakpossito (Agoènyivé 3), Togblekopé (Agoènyivé 4), Sanguera (Agoènyivé 5), and Adétikopé (Agoènyivé 6). Table 1 shows the geographic coordinates of ovitrap locations.

Table 1:	Geographical	coordinates	of ovitran	locations
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Ovitrap locations	Latitude	Longitude	Altitude (m)
Anfame	6.161750	1.271089	18
Adakpame	6.169470	1.286414	16
Dabalakondji	6.177067	1.275710	31
Lycee de be-kpota	6.162799	1.262033	26
Attiegou kloklovicope	6.196750	1.268450	06
Bè-lagune	6.151667	1.254300	09
Ablogame	6.136496	1.253961	10
Akodessewa avelime	6.148481	1.263371	12
Zorro-bar	6.159901	1.285677	16
Carrefour dva	6.201336	1.264600	16
Togo 2000	6.178009	1.251250	21
Tokoin n'tifafa	6.151517	1.233683	23
Hedzranawoe	6.180619	1.236488	23
Nukafu	6.159839	1.238788	20
Forever	6.159949	1.231081	40
Ceg attiegou	6.190555	1.266505	27
Tokoin-elavagnon	6.146740	1.228876	12
Doumassesse	6.158032	1.217636	14
Kegue	6.206365	1.237514	43
Massouhoin	6.196116	1.211943	46
Campus ul	6.176385	1.214340	08
Amoutieve	6.141950	1.232406	16
Gbadago	6.145850	1.216383	21
Above	6.156500	1.232583	15
Bè-klikame	6.175533	1.204800	27
Kodjoviakope	6.120575	1.205249	11
Hanoukope	6.139027	1.217164	12,48
Nyekonakpoe	6.134734	1.202410	8
Avenou	6.176259	1.190245	38
Avedji	6.202200	1.174427	39
Cassablanca	6.153350	1.200233	32
Djidjole	6.180333	1.196167	46
Amadahome	6.207937	1.155091	46
Gblinkome	6.189868	1.182547	34
Agbalepedo	6.193410	1.200541	28

Lycee baguida	6.178417	1.330422	19
Devego	6.196015	1.345357	40
Baguida-kpota	6.186040	1.353619	39
Cite des anges-bgbk	6.166192	1.339224	19
Kagome	6.175501	1.303281	-07
Adamavo	6.175767	1.310483	23
Camp baguida	6.172768	1.360301	24
Cite boad	6.169324	1.317079	14
Logote	6.193915	1.127160	30
Ceg-lankouvi	6.179147	1.131250	28
Akato avoeme	6.187050	1.093351	38
Apedokoe	6.210519	1.143400	46
Segbe-zavie	6.195010	1.114544	44
Camp fir	6.246802	1.179342	57
Adjougba	6.232149	1.217477	35
Cacaveli	6.221549	1.200807	63
Lycee de sogbossito	6.259980	1.178059	31
Legbassito-akanhoukope	6.269155	1.148940	59
Somayaf-sogbossito ewlissime	6.262423	1.160539	56
Camp gp	6.239035	1.166358	47
Vakpossito	6.214392	1.165242	59
Assigonme	6.230040	1.164248	62
Agoe-telessou	6.216987	1.167871	36
Togblekope-mairie	6.274727	1.209544	26
Togblekope-gakpoto	6.281177	1.207687	29
Tsikplonou-pia annexe	6.297205	1.214780	27
Fidokpui	6.268020	1.226058	25
Sanguera-klikame	6.245163	1.117058	47
Zanguera-kleme	6.225917	1.126747	39.37
Kohe-zossime	6.228084	1.144795	58.98
Sanguera	6.231539	1.102443	41
Adetikope-kpotave	6.304940	1.234536	50
Adetikope-bar temps aux temps	6.318969	1.230557	50
Sita-kpamonou pia	6.335725	1.215091	40
Adetikope-novissi	6.326500	1.204152	49

The health district of Grand Lomé is Togo's most populated region, with an estimated population of 2188376 in 2022 that is 27% of the country's total population ^[19]. Grand Lomé is limited to the south by the Atlantic Ocean, to the west by the Aflao-Ghana border and the Avé prefecture, to the north by the prefecture of Zio and to the east by the prefecture of Lacs (Figure 1). To ensure adequate representation of environmental diversity, each commune was considered as a potential unit for ovitrap location. This approach made it possible to cover a variety of urban, and peri-urban contexts (settings, landscape) in the area. The scarcity of agricultural land, due to land pressure, is a notable trend. Apart from peri-urban communes (Golfe 6, 7, Agoè-Nyivé 2, 5, and 6), where a few crops are still grown, agriculture in Grand Lomé mainly consists of market gardening.

The study area is characterized by a humid tropical climate with two dry seasons (December-March and August-

September) and two rainy seasons (April-July and October-November). The average temperature is 28 °C, with annual rainfall varying between 1,000 mm and 1,600 mm. The average relative humidity is above 75% due to the impact of the sea on the climate ^[20]. The study was carried out during the two long rainy and dry seasons.

Selection criteria of ovitrap locations

The selection of houses for ovitrap locations was determined by some key factors. Firstly, the population density: the distance between two (2) ovitraps which must be at least five hundred meters (500 m) to minimize the collection of the same species. Secondly, topographical variations, such as proximity to the sea, ponds, or wetlands were also considered, as they can influence mosquito larval habitats. Finally, the presence of market gardening and green spaces around houses were considered as influential elements.

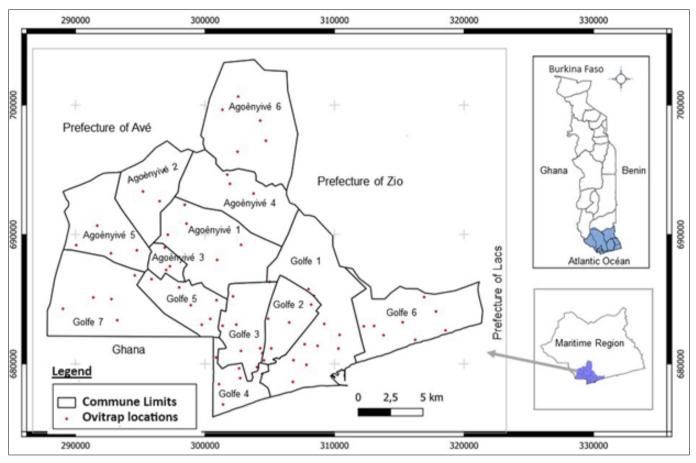


Fig 1: Map of the study area showing ovitrap locations.

Ovitrap installation on sites Ovitrap components

Egg sampling was done using ovitraps, which are suitable for collecting the eggs of *Aedes* mosquitoes ^[21]. The ovitraps consisted of black plastic cups of 750 ml (Figure 2A), filled with 500 ml of tap water. Inside each cup, special egg-laying papers (Anchor Paper Co-Minneapolis, Minnesota, USA; Figure 2B) or Wattman papers (Figure 2C) were placed half-submerged inside against the cup wall.

Mosquito egg collection

A specific number of ovitraps were randomly placed in houses in each of the 13 communes of the health district of Grand Lomé, such as: Golfe 1 (n = 10), Golfe 2 (n = 7), Golfe 3 (n = 4), Golfe 4 (n = 7), Golfe 5 (n = 7), Golfe 6 (n = 8), Golfe 7 (n = 4), Agoènyivé 1 (n = 6), Agoènyivé 2 (n = 2), Agoènyivé 3 (n = 2), Agoènyivé 4 (n = 3), Agoènyivé 5 (n = 5), and Agoènyivé 6 (n = 5). Consent was obtained from house owners before the ovitraps were set up. A total of 70 ovitraps were placed. Egg collections were carried out during both the main rainy season (late May to September 2022) and the main dry season (December 2022 to early March 2023). The geographical coordinates of the location of each ovitrap were recorded using a Garmin 64S GPS (Global Positioning System) and georeferenced on a base map using QGIS 3.28.9 software.

The ovitraps were placed on the ground in a shady area and protected from the rain. Houses were visited every five (5) days to collect mosquito eggs. Water in the ovitraps was top upped or renewed if required, at each visit. Each paper was labeled according to the house and date when the ovitraps was set, then returned to the Laboratory of Ecology and Ecotoxicology at the University of Lomé (LaEE/UL). The eggs were dried at room temperature and then counted using a stereomicroscope.

At the insectarium of LaEE/UL, eggs were reared to adults in tanks (30 x 15 x 10 cm). Each day, the nymphs were sorted and placed in a cage (30 x 30 x 30 cm) until the adults emerged. Adults were morphologically identified using the keys of Edwards ^[22] and Hopkins ^[23].

Adults were then recovered and preserved, according to their geographical origin, in Eppendorf tubes containing silica gel, then stored at -20 °C for subsequent analysis. Rearing conditions were Temperature = 26 ± 2 °C, Relative Humidity (RH) = $85 \pm 5\%$ and Photoperiodicity = 12 L: 12 D. Translated with DeepL.com (free version)

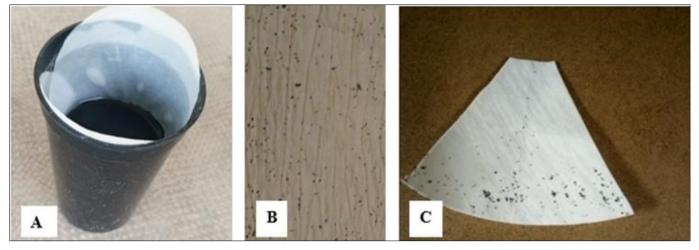


Fig 2: Black plastic trap door (ovitrap) containing the papers (A); *Aedes* spp eggs on the egg-laying paper (Anchor Paper Co-Minneapolis, Minnesota, USA) (B) and on the blotting paper (Wattman) (C)

Abiotic parameters

Data on rainfall, temperature, and relative humidity were collected from the National Meteorological Agency of Togo (ANAMET) in 2022 to see if there was a significant positive correlation between these abiotic parameters and the number of eggs collected (Figure 5).

Statistical analysis

The Ovitrap Positivity Index (OPI) and Egg Density per Ovitrap (EDO) were calculated according to formulae 1 and 2 ^[24]:

$$OPI = \frac{\text{Number of positive ovitraps}}{\text{Total number of ovitraps}} X100 \text{ (Formula 1)}$$
$$EDO = \frac{\text{Number of eggs}}{\text{Total number of positive ovitraps}} \text{ (Formula 2)}$$

Data was entered into an Excel office 2019 spreadsheet. To compare the number of eggs obtained over the two seasons, we performed a Student's t-test using DATAtab. Spearman's correlation test was performed using R software version 4.3.1 to assess the influence of abiotic parameters (rainfall, temperature, and humidity) on the number of eggs collected during the two seasons in *Aedes aegypti* mosquitoes, and to compare the number of eggs per season. All tests were considered statistically significant when p < 0.05.

Results

Entomological indices (OPI and EDO)

From May 2022 to March 2023, a total of 20,273 eggs were collected in Grand Lomé. These eggs came exclusively from the *Aedes aegypi* species and were confirmed by specific morphological identification of the emerged adults. The presence of eggs from other mosquito species such as *Culex* sp and *Anopheles* sp was not detected in the ovitraps.

The ovitrap positivity index (OPI) ranged from 37.5 to 100 in the Golfe communes and from 0 to 100 in the Agoènyivé communes, while the EDO ranged from 8.04 to 48.96 in the Golfe communes and from 0 to 7.75 in the Agoènyivé communes (Table 1). The average egg density per ovitrap and per collection period in the health district of Grand Lomé was 14.84. However, the egg density showed substantial variations from one commune to another, ranging from 0 to 48.96 (Table 1). Entomological indices indicate that there is a potential high risk of arbovirus transmission in Grand Lomé.

Table 2: Entomological index values obtained for communes in
Grand Lomé in Togo.

Communes	OPI (%)	EDO
Golfe 1	70	13.13
Golfe 2	57.14	12.79
Golfe 3	100	48.96
Golfe 4	64.28	33.58
Golfe 5	49.99	29.5
Golfe 6	87.5	29.63
Golfe 7	37.5	8.04
Agoè-nyive 1	35	3.46
Agoè-nyive 2	100	7.75
Agoè-nyive 3	0	0
Agoè-nyive 4	0	0
Agoè-nyive 5	10	3.5
Agoè-nyive 6	10	2.63

Seasonal variation in the number of Aedes aegypti eggs

The number of Aedes aegypti eggs varies significantly according to season and commune ($X^2 = 2994.1$; p < 0.001) and was high in the Golfe communes and low in most of the Agoènvivé communes (Figure 3). During the rainy season (May to September 2022), a total of 14,032 eggs were obtained, with significantly different values between communes ($X^2 = 9586.4$; p = 0.0001), representing around 69.22% of the total for all seasons combined, with an average of $1,079 \pm 932.88$ for all communes. The highest values were recorded in the communes of Golfe 6 (Baguida, n = 3,575; 17.63% of the total), Golfe 3 (Bè-Ouest, n = 3,422; 16.87% of the total), and Golfe 4 (Amoutiévé, n = 2,204; 10.87% of the total) which are the most populated communes in the Grand Lomé, with 33 (47.14%) positive ovitraps. During the same period, no eggs were collected at Agoènyivé 1, 3, 4, 5, and 6 (Figure 3).

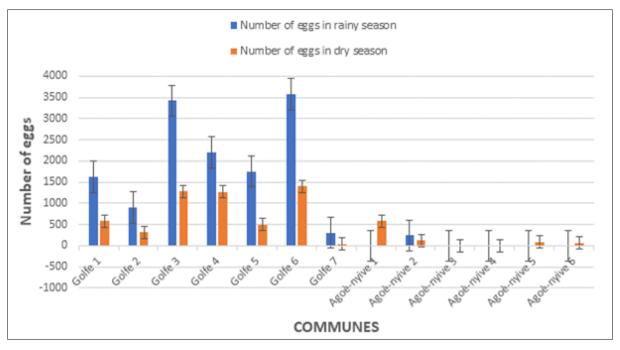


Fig 3: Seasonal variation in the number of Aedes aegypti eggs by commune

During the dry season period (December 2022 to March 2023), 43 ovitraps (61.42%) were positive. The highest numbers of eggs were collected in the communes of Golfe 6 (Baguida, n = 1,398), Golfe 3 (Bè-Ouest, n = 1,272), and Golfe 4 (Amoutiévé, n = 1,275) (Figure 3). The total number

of eggs (n= 6,241; 30.78% of the total) was low at all communes ($X^2 = 3,355.7$; p = 0.0001), with an average of 480 \pm 368.78; a significant decrease compared with the rainy season (p = 0.028) (Figure 4).

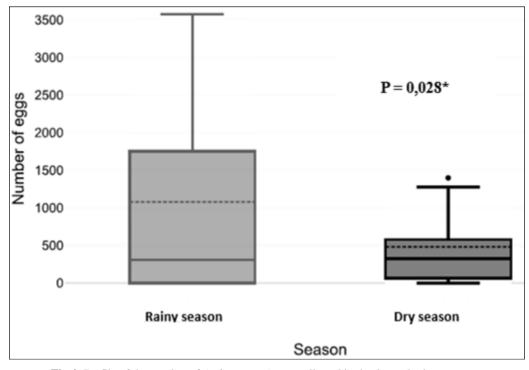


Fig 4: Profile of the number of Aedes aegypti eggs collected in the dry and rainy seasons.

Seasons had a strong influence on the number of *Aedes* mosquito eggs distribution, with a significant drop in the number of eggs obtained in the dry season at all collection sites (Figure 3).

Analyses of climatic factors' data (precipitation, temperature, and relative humidity) showed that only precipitation exerted

a slightly significant influence (r = 0.3351; p = 0.0382) on the number of eggs collected. The correlation with temperature and egg number was negative (r = 0.527; p = 0.005). No significant correlation was observed between humidity and the number of mosquito eggs over the entire collection period (r = 0.0012; p = 0.912) (Figure 5).

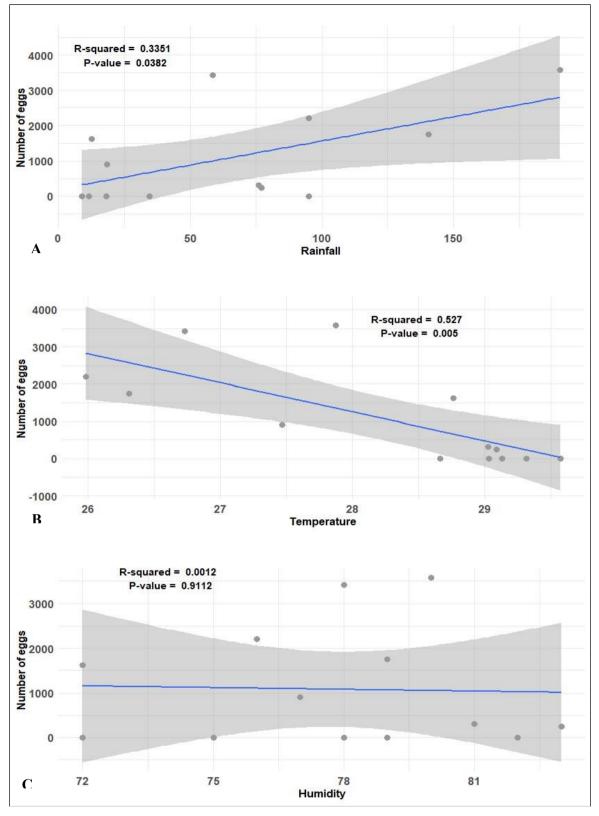


Fig 5: Influence of climatic factors (Rainfall = A, Temperature = B, and Humidity = C) on the number of eggs collected

Discussion

This study focused on the use of ovitraps as a tool for analyzing the seasonal distribution of *Aedes aegypti* eggs. Data collected provided essential information to guide dengue vector surveillance and the risk of dengue transmission management, particularly in an urban context.

Results showed the exclusive presence of eggs of the Aedes aegypti species in ovitraps positioned in the health district of

Grand Lomé, corroborating the findings of previous studies ^[25, 26]. These findings are also in line with the known preference of *Aedes* mosquitoes for water containers as oviposition sites, reinforcing the usefulness of this method in monitoring and controlling these vectors. Given that only the eggs of *Aedes aegypti* were observed in the ovitraps, and not the eggs of *Aedes albopictus*, *Anopheles* spp or *Culex* spp, this suggests that there is a species-specific predominance of

Aedes aegypti. The absence of Aedes albopictus could indicate a limited geographical distribution of this species in the commune, or simply its temporary absence during the study period. Further investigations may be required to deepen our understanding of Aedes mosquito population dynamics in Grand Lomé and other regions of Togo.

A high number of eggs was observed in some of the most populated communes notably the communes of Golfe 6, 3, 4, 5, and 1. This increase suggests a possible correlation between human population density and Aedes aegypti egg-laying activity. This increase in the number of eggs in these communes may be linked to the poor management of garbage and tin cans, the presence of ponds and flood-prone areas. Studies by ^[27] and ^[28] reported that human populations can influence the availability of dengue vectors. In addition, other authors have reported that, Ae. aegypti exhibits a more anthropophilic behavior, being adapted to the daily life of humans and thus preferring to inhabit city centers [29]. According to [30], the process of urbanization favors an increase in the prevalence and abundance of species of the Aedes genus, as it increases the number of artificial breeding sites, such as tires, cans, or water storage containers. Barely ten vears ago, had increased urbanization begun in Grand Lomé, affecting particularly many Golfe communes, with building constructions and road rehabilitations, starting from the center of the city and extending out to the outskirts. Unfortunately, this urbanization is poorly controlled, with inadequate sewage facilities. These conditions have created and maintained breeding sites for Aedes mosquitoes, particularly in Golfe communes.

On the other hand, the total absence of egg collection during both dry and rainy seasons in the communes of Agoènyivé 3 and 4 raises questions about the possible factors contributing to this observation. Further investigations are needed to better understand this phenomenon.

The positive correlation between rainfall and the number of eggs showed that rainfall exerted a slightly significant positive influence on the number of eggs, favoring high values for OPI and EDO. These results could explain the significant difference between the number of eggs obtained during wet and dry seasons (p = 0.028). Rainfall can induce the formation of new oviposition sites (small water containers, tires, etc.), thereby increasing oviposition opportunities for Aedes aegypti. The significant reduction in oviposition sites observed during the dry period could lead to a considerable drop in the number of eggs, hence underlining the importance of environmental conditions in the dynamics and proliferation of Aedes aegypti. Study by ^[31] in north-eastern Brazil showed the same trends. Egg densities per ovitrap ranged from 0 to 48.96, whereas a single female of Aedes spp. can lay between 300 and 400 eggs during its lifetime ^[32]. This result seems to be linked to the fact that not all females lay their eggs at the same site [33], which would be conducive to the species' longevity. Similar results were obtained in the Air-France district of the city of Bouaké, where egg densities per trap were obtained inside and outside houses ^[34]. According to ^[35], if the EDO is less than 10, there is no risk of dengue transmission in each area. In fact, the average density of eggs/ovitraps is 14.84 in Grand Lomé, which raises a concern about the dissemination of arboviruses, particularly the dengue virus.

Conclusion

Ovitrap is an effective tool of monitoring Aedes aegypti in

Grand Lomé, providing information to guide arbovirus prevention initiatives, particularly dengue transmission. Rainfall was identified as the main factor influencing mosquito breeding, so the risk could be higher in the rainy season. The number of eggs was high in the populated communes. However, specific communes showed a complete absence of eggs, requiring further investigations. This study provides essential data to guide *Aedes aegypti* surveillance in Grand Lomé.

Acknowledgments

The authors would like to thank the heads of the households visited in the neighborhoods. Special thanks to Dr. Kossi B. AMEGNAGLO for assisting with the mapping. Sincere thanks to Dr. Mamachi MELILA, Associate Professor in biochemistry and nutrition, for the blotting papers. Finally, thanks to Delagnon ASSOU and Komlan TOUDJI for helping deploy the ovitraps and collect the eggs.

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