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Distribution and identification of *Aedes* mosquitoes, vectors of Dengue virus in Abéché, Chad

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Abstract

Aedes mosquitoes, vectors of dengue, chikungunya, Zika, and yellow fever, represent a significant threat to public health due to their role in transmitting these diseases. Their emergence in Chad, driven by increasing urbanization and demographic expansion, raises considerable concerns. The aim of this study is to better understand the diversity and the role of *Aedes* mosquitoes in dengue transmission in the city of Abéché, Chad.

This is a cross-sectional study conducted over four months, from July to October 2024. Mosquitoes were collected using human bait techniques in nine neighborhoods of Abéché. The samples were then subjected to morphological identification, followed by viral RNA extraction and RT-PCR analysis to detect dengue virus.

The results show that out of 370 mosquitoes captured, 85.9% (n = 318) belonged to *Aedes* genus, of which 95% (n = 305) were *Aedes albopictus* and 4.6% (n = 13) were *Aedes aegypti*. There is no evidence of dengue virus in all analyzed mosquitos' samples.

This study highlights the significant presence of *Aedes* mosquitoes, particularly *Aedes albopictus*, in the city of Abéché, Chad, where they constitute the dominant species. Despite their high abundance and known role as vectors of dengue, none of the analyzed mosquitoes tested positive for the dengue virus. These findings underscore the importance of continued entomological surveillance and further research to better understand the distribution, dynamics, and potential role of *Aedes* mosquitoes in disease transmission in Chad.

Such efforts are crucial for developing effective vector control strategies and mitigating the risk of dengue outbreaks in the region.

Keywords: Distribution; Identification; Mosquito; *Aedes*; dengue; Abéché; Chad

1. Introduction

Mosquitoes belong to the class Insecta, the order Diptera, and the suborder Nematocera. According to the Mosquito Taxonomic Inventory, there are 3,727 species distributed across 111 genera worldwide as of 2024 [1]. These insects represent one of the most significant groups of vectors for pathogens transmissible to humans, including zoonotic diseases [2].

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Female mosquitoes require blood for egg development during their reproductive cycle. Certain species, such as *Aedes aegypti*, exhibit strong anthropophilic tendencies, making them particularly effective vectors of arboviruses, including dengue, chikungunya, Zika, and yellow fever. These diseases collectively impact millions of people annually, causing a substantial number of deaths. Indeed, mosquitoes are responsible for over 700,000 deaths per year, making them the deadliest animals for humans [3]. While mosquito-borne diseases are predominantly concentrated in tropical and subtropical regions, urbanization, globalization, and international travel have facilitated their spread to previously unaffected areas [4].

Mosquitoes of the genus *Aedes*, particularly *Ae. albopictus* and *Ae. aegypti*, are the primary vectors of dengue and other arboviruses. While *Ae. aegypti* is largely confined to tropical and subtropical zones, *Ae. albopictus* has successfully adapted to temperate regions. These hematophagous mosquitoes typically prefer human blood and act as efficient transmitters of arboviruses between vertebrate hosts [4]. Dengue, though known for centuries, has emerged dramatically in recent years as a major global public health concern. According to WHO, approximately 40% of the global population lives in areas at risk of dengue [5]. *Aedes albopictus*, an invasive species originally from Asia, is now found on all continents, including Europe, North and South America, Africa, and in numerous islands in the Pacific and Indian Oceans [2].

In Chad, the presence of *Ae. aegypti* was first reported in 2013, associated with cases of yellow fever [6]. Dengue, however, was only reported for the first time in the third quarter of 2023. On August 15, 2023, the Ministry of Public Health officially declared a dengue epidemic. Since then, several suspected cases, including fatalities, have been recorded. Laboratory testing using RT-PCR confirmed dengue virus in 47 blood samples, of which 26 were positive, collected from the provinces of Ouaddaï, Wadi Fira, and N'Djamena [7].

However, limited studies have been conducted on the distribution and entomological parameters of mosquito vectors in Chad. Understanding their role in dengue virus transmission is critical to guiding vector control strategies. The main objective of this research is to study the distribution and identification of *Aedes* mosquitoes and to analyze the presence of dengue virus in mosquitoes captured in Abéché.

In a time of ongoing surveillance in Chad to reduce the risk of Dengue infections, the study will contribute to monitoring strategies by providing the genetic diversity of circulating dengue virus serotypes and their vector, on the way to achieve the goal of diseases prevention.

2. Methodology

2.1. Study Area

The study was conducted in Abéché (Figure 1), the capital of Ouaddaï Province, one of Chad's 23 provinces. Abéché covers an area of 3,600 hectares and is located on the Ouaddaï massif, approximately 960 kilometers east of N'Djamena, the capital of the country. Geographically, it lies between 13°45'0'' and 14°0'0'' N latitude and 20°40'0'' and 21°0'0'' E longitude, with an altitude ranging from 500 to 600 meters above sea level [8].

The province experiences a Sahelo-Sudanian climate, characterized by a long dry season (October to June) and a short rainy season (July to September), with rainfall ranging from 200 mm to 600 mm annually, peaking in August. The hydrographic network in Ouaddaï consists of surface runoff and groundwater. Wadis and seasonal ponds, primarily fed by rainwater from the mountains dominate the region. Key wadis, such as Chao-Rimé, Djileney-Bouboula, Enné, and Haddad, support temporary water storage for up to 45 days post-rainfall, primarily serving livestock and irrigation needs. Temperatures are moderately high, with the hottest months being March to May and cooler temperatures recorded between November and January.

Abéché's population was 138,684 in 2009 and has grown rapidly due to factors such as the Darfur crisis and the establishment of institutions like Adam Barka University. As of 2016, the population reached 178,893, and it is now estimated at 200,000. This yearly growth (3.6%) has driven significant spatial expansion of the city [8].

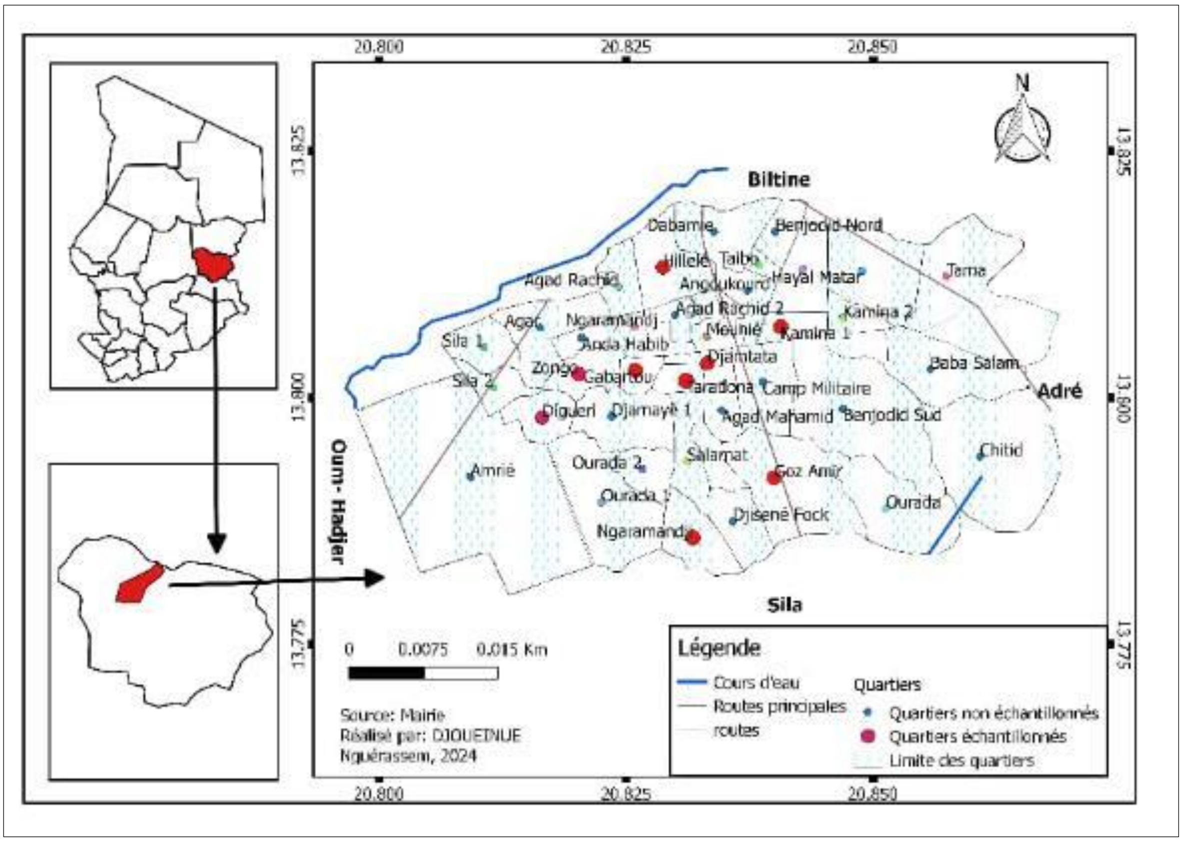

Figure 1 Map showing sampling sites in Abéché in Eastern Chad

2.2. Mosquito Collection and laboratory work

The study was conducted from July to October 2024. Sample collection was performed in several neighborhoods of Abéché, while morphological identification and PCR based method were carried out respectively at the Centre Hospitalier Universitaire La Reference Nationale" (CHU-RN) and the Laboratoire National de Biosûreté et des Epidemies de N'Djamena (LaBiEp).

2.3. Collection Methods

Aedes genus including *Aedes albopictus* and *Aedes aegypti* mosquitoes were targeted for this study. The Human landing catch sampling technique was employed. It consisted of volunteers exposing their arms or legs [9], [10]. Mosquitoes landing on the skin were caught before biting. Collections were conducted during peak activity times for *Aedes* mosquitoes, from 6:00–9:00 AM and 3:00–6:00 PM.

Captured mosquitoes were placed in tubes containing ethanol, labeled with date, time, and location, and stored at -20°C before transportation to CHU-RN at 2–8 °C to prevent RNA degradation. They were then stored at -80°C until further processing.

2.4. Mosquito Identification and RNA Extraction

Identification was performed at CHU-RN using morphological keys [11]. Key features such as body coloration, scutal ornamentation, and antennae structure were examined under a stereomicroscope. Specimens were sorted by species, sex, and collection site, then stored at -80 °C for further analysis.

RNA extraction was conducted using Qiagen kit according to the manufacturer's protocol. Eluates were then stored at -20°C for short-term use or -80 °C for long-term storage.

2.5. Detection of Dengue Virus RNA in Mosquitoes Using RT-qPCR

The presence of Dengue virus RNA in mosquitoes was detected using Reverse Transcriptase-Quantitative Polymerase Chain Reaction (RT-qPCR) [12]. The reaction mixture was prepared in a designated Master Mix room at LaBiEp using

1.5 mL microtubes. The composition of the Master Mix and the PCR program varied depending on the virus being tested. Ready-to-use mixes containing all necessary components for RT-qPCR were used, requiring only the addition of the dengue-specific probe PanDenp1_LNA (5'-Fom-TAGCGTCAATATG-C-TG-T-TT-BHQ1-3') and primers DEN2f 177 (5'-GTCCAAGGACGTATTAAGAAG-3'), DEN13forw (5'-TTGAGCAAACCGTGCTGC-3'), and DEN24rev (5'-GAGACAGCAGGATCTCTGGTCT-3'), alongside the RNA to be amplified (Table 1). 20 µL of the prepared Master Mix was dispensed into each PCR tube. To each PCR tube, 5 µL of extracted RNA was added [13].

RT-qPCR was performed using the MIC4qPCR Cycler, a magnetic induction real-time thermocycler known for its rapid PCR cycling. Results were obtained within 127 minutes. The linked software facilitated data analysis and report generation, detailing the applied parameters and results for interpretation.

Table 1 Reagents used for the RT-qPCR

Reagents	Volume per reaction (µL)
2x Reaction Mix	12.5
Water without RNase	3
DEN2f 177(10µM) Forward Primer	1
DEN13 (10µM) Forward Primer	1
DEN24 (10µM) Reverse Primer	1
PanDenp1_LNA (10µM) Probe	0.75
SuperScript III qRT-Mix	0.75
Total MasterMix Volume	20
Sample Volume (Eluate)	5
Final Reaction Volume	25

2.6. Data analysis

Our data were analyzed using Microsoft Excel 2019, IBM SPSS Statistics, and GraphPad Prism version 9. Excel was used for data entry and basic calculations, SPSS for advanced statistical analyses, and Prism version 7.0 for data visualization.

3. Results

3.1. Distinctive characteristics of *Ae. albopictus* and *Ae. aegypti* observed in the laboratory

Our study focused on the observation of adult mosquitoes to identify the species *Ae. albopictus* and *Ae. aegypti*. Several morphological criteria allowed us to differentiate between these two species (Figure 2).

Regarding the coloration and patterns, *Ae. albopictus* is characterized by a black body with distinct white stripes on the legs, particularly on the femur of the midleg, where the stripes are broader and more contrasted. A prominent longitudinal white stripe runs along the scutum. Whereas *Ae. aegypti* exhibits a darker overall coloration with thinner and less contrasted white stripes. The white band on the scutum forms a lyre-shaped pattern extending from the head to the thorax.

Wings and Antennae are also distinctive. The wings of *Ae. albopictus* are bordered with a fringe of scales at the base of the alula. The antennae are longer and featherier, with well-developed segments, giving them a lighter, silky appearance. Sexual dimorphism is evident in this species, with males possessing longer, more plume-like antennae covered in numerous sensory hairs that give them a characteristic feathery look. The wings of *Ae. aegypti* feature a posterior position of the apex of the vein. The antennae are shorter and less feathery.

The Abdomen of *Ae. albopictus* is adorned with white spots, including a distinct white band visible on the second abdominal segment. While the abdomen of *Ae. aegypti* also features white spots, they are less pronounced compared to those of *Ae. albopictus*.

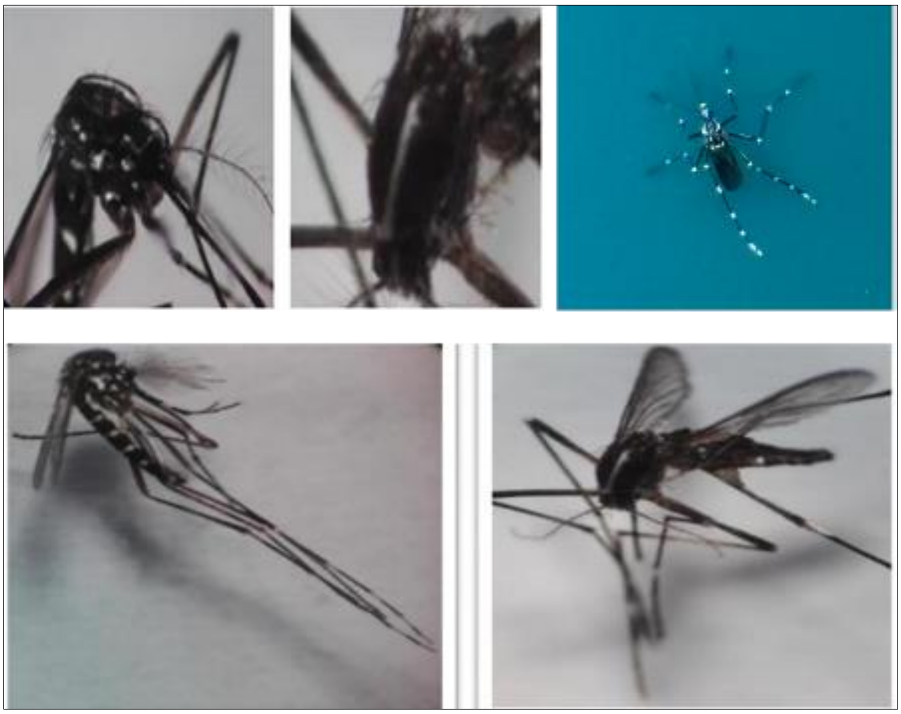

Figure 2 The three upper images display the general morphological features of *Aedes* mosquitoes. The lower images show respectively, a slenderer male (left) and a female (right) *Aedes*, with antennae characteristic of each sex

3.2. Repartition of mosquito's species

Mosquitoes were collected in nine of twenty-five neighborhoods of Abéché namely Digueri, Djamtata, Taradona, Kamina 1, Goz-Amir, Gabartou, Hilele, Garamandji, and Zongo. The total number of collected mosquitoes was 370, where 318 (85.95%) were identified as *Aedes* and 52 (14.05%) unidentified mosquitoes' species. Out of the 318 identified mosquitoes, 305 (95.91%) were *Ae. albopictus*, and 13 (4.09%) were *Ae. aegypti* (Figure 3).

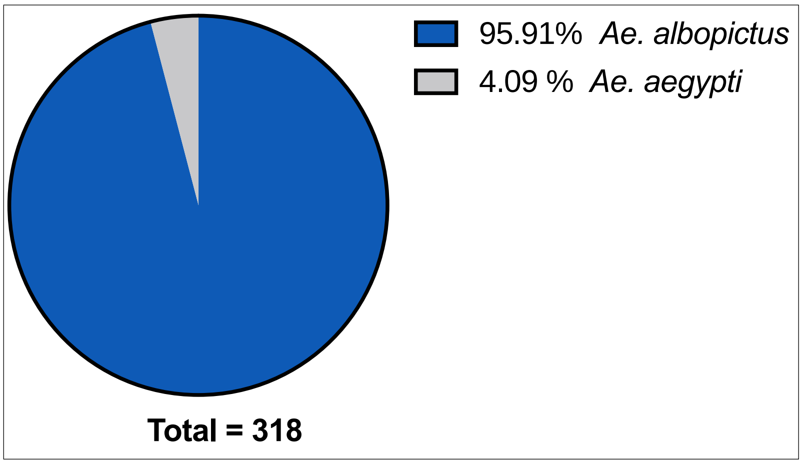

Figure 3 Repartition of mosquito's species

3.3. Sex-Based Sorting of Captured Mosquitoes

Table 2 presents the distribution of sorted mosquitoes by sex. A predominance of females was observed in both identified species; 69.18% for *Ae. albopictus* and 4.09% for *Ae. aegypti*. Males were underrepresented, accounting for 26.73% of *Ae. albopictus*, with no males identified for *Ae. aegypti*.

Table 2 Mosquito species distribution and sex ratio

Species	Sex	Count (%)	95% Confidence Interval
Ae. albopictus	Female	220 (69.18)	(63.88%, 74.48%)
Ae. albopictus	Male	85 (26.73)	(21.93%, 31.53%)
Ae. aegypti	Female	13 (4.09)	(0.60%, 7.58%)
Ae. aegypti	Male	0 (0)	(0%, 0%)

3.4. Distribution of *Aedes* by Neighborhood

Figure 4 illustrates the distribution of *Aedes* mosquitoes across various neighborhoods in Abéché, comparing the prevalence of *Ae. albopictus* and *Ae. aegypti*.

Aedes albopictus is significantly more abundant in all neighborhoods, with the highest count recorded in Diguéri. In other neighborhoods, such as Zongo, Taradona, Djamtata, Kamina 1, Goz-Amir, Gabartou, Garamandji, and Hilélé, moderate to high numbers of *Ae. albopictus* are observed. In contrast, *Ae. aegypti* is nearly absent or found in very low numbers, particularly in Diguéri, Kamina 1 and Djamtata, where its presence is slightly noticeable.

This distribution underscores the dominance of *Ae. albopictus* across all surveyed neighborhoods, with *Ae. aegypti* showing minimal dispersion.

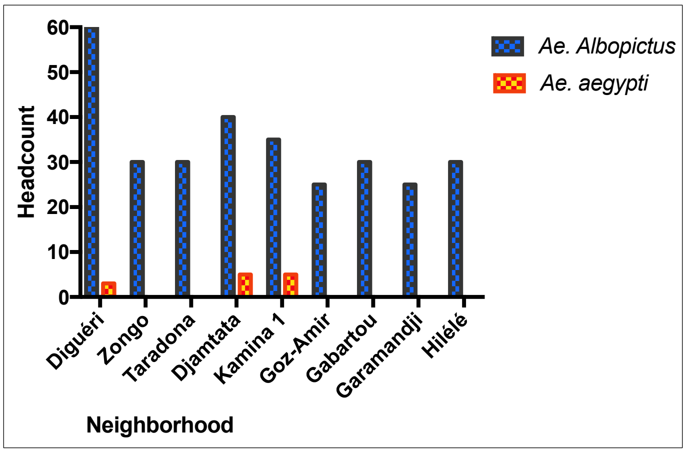

Figure 4 Distribution of *Aedes* Mosquitoes by Neighborhood in Abéché

3.5. Distribution of Mosquitoes by Species, Sex, and Neighborhood in Abéché

3.5.1. *Aedes albopictus*

The following bar chart illustrates the distribution of *Ae. albopictus* mosquitoes by gender (female and male) across neighborhoods in Abéché, revealing a higher prevalence of females in all areas (Figure 5). Diguéri stands out with the highest number of *Ae. albopictus*, where females significantly outnumber males. In other neighborhoods, such as Zongo, Taradona, Kamina 1, Goz-Amir, Gabartou, Garamandji, and Hilélé, moderate to low counts are observed, but females consistently dominate over males, except in Djamtata where male and female were equal. This gender imbalance, with females being more abundant across nearly all neighborhoods, is particularly notable since female *Ae. albopictus* are the primary vectors for arboviruses such as dengue virus, emphasizing their importance in disease transmission dynamics in Abéché.

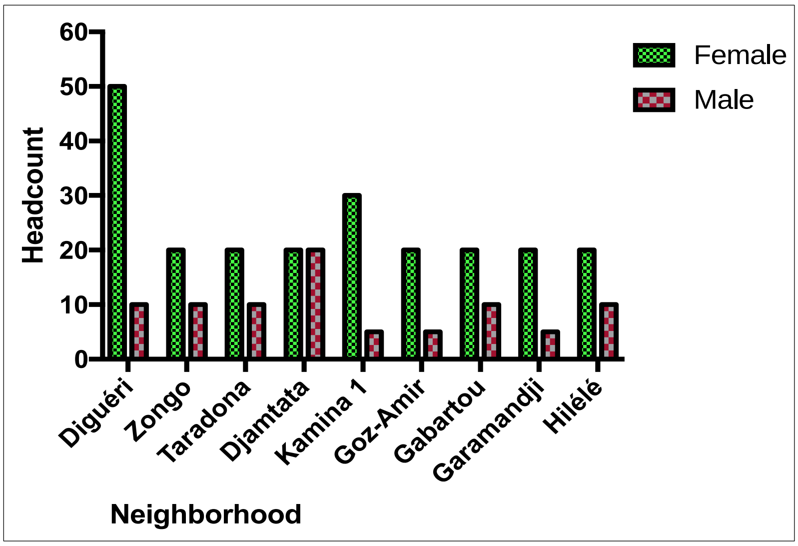

Figure 5 Distribution of *Ae. albopictus* populations by Sex (females in green, males in red) across different neighborhoods in Abéché

3.5.2. *Aedes aegypti*

While *Ae. albopictus* is present in many neighborhoods, *Ae. aegypti* is restricted to only three: Diguéri, Djamtata, and Kamina 1. In these neighborhoods, the captured mosquito population consists exclusively of females (Figure 6).

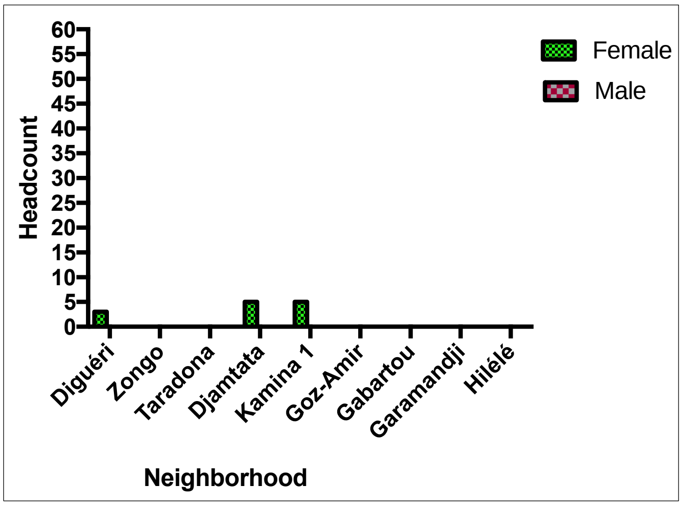

Figure 6 Distribution of *Ae. aegypti* populations by sex (females in green, males in red) across different neighborhoods in Abéché

3.6. Virus detection using the RT-PCR method

RT-PCR was performed on 48 samples, organized into pools, each containing 15 mosquitoes. The complete results, presented in graphical form, were obtained after 127 minutes of amplification (Figure 7).

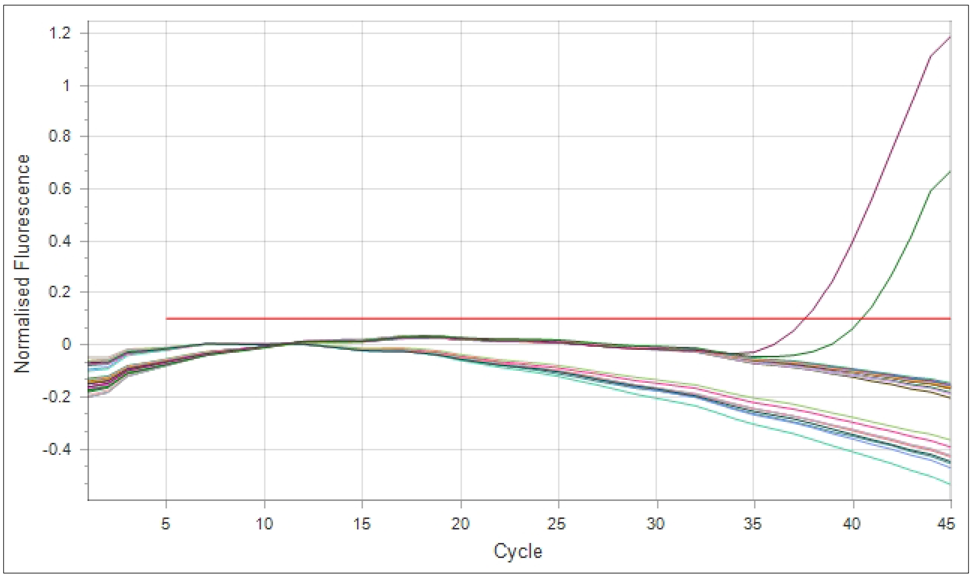

Figure 7 Real-Time RT-PCR amplification curves for Dengue in mosquitoes from selected neighborhoods in Abéché

All tested samples were negative for dengue virus. However, the positive controls diluted to 1/100 and 1/1000 showed positive results with respective values of 37.59 and 40.47. These findings confirm the validity of the amplification process, indicating that the captured mosquitoes were not infected with dengue virus.

4. Discussion

This study conducted in Abéché focused on the distribution and identification of mosquito vectors of the dengue virus and provided valuable insights into the presence of Dengue virus and dynamics of *Aedes* species, particularly *Aedes albopictus* and *Aedes aegypti*. The findings of this study contribute to a better understanding of dengue control efforts in the region, while simultaneously emphasizing the challenges presented by the expanding populations of these mosquito vectors.

4.1. Morphological characteristics of *Ae. albopictus* and *Ae. aegypti*

The morphological traits observed in this study allow clear differentiation between *Ae. albopictus* and *Ae. aegypti*. These differences are essential for entomologists and public health authorities involved in vector surveillance. The black coloration with marked white stripes on the legs and a longitudinal white stripe on the scutum were distinctive features of *Ae. albopictus*, corroborating previous studies [11], [14]. In contrast, *Ae. aegypti* displayed a darker coloration with fine stripes and a lyre-shaped white band on the scutum [11], [15].

Additional features, such as the fringed scales at the base of the alula in *Ae. albopictus* and its longer, plume-like antennae in males, were also significant for identification. These characteristics emphasize the pronounced sexual dimorphism in this species [16]. Conversely, *Ae. aegypti* lacked fringed scales and had less conspicuous antennae [17].

The striped abdomen of *Ae. albopictus* more pronounced than in *Ae. aegypti*, further aids in species differentiation. Similar differences in urban environments, confirming the reliability of these traits was observed. However geographical, environmental, and genetic factors may lead to variations [18], underscoring the importance of additional tools like genetic analysis for more detailed identification.

4.2. Unidentified species of mosquitoes in Abéché

The presence of unidentified *Aedes* mosquito species in Abéché, as observed in our study (52 (14.05%)), indicates a potential gap in entomological knowledge. Divers species have been reported in other studies conducted in rural and semi-urban settings across sub-Saharan Africa [19], where unidentified or poorly characterized mosquito species have been detected. These highlight the limitations of available identification tools, insufficient taxonomic expertise, or the presence of cryptic species complexes [20], [21]. Furthermore, emerging research suggests that some of these species might play overlooked roles in disease transmission or ecological balance [22]. Addressing these knowledge gaps through targeted entomological surveys, advanced molecular identification techniques, and improved surveillance systems is essential for a more comprehensive understanding of mosquito biodiversity and its implications for public health.

4.3. Species composition and distribution

The study revealed a significant dominance of *Ae. albopictus* in Abéché, comprising 95.91% of the identified mosquito population, while *Ae. aegypti* accounted for only 4.09%. This dominance aligns with global observations where *Ae. albopictus* adapts remarkably to anthropized environments [2], [4], [23]. Its invasion is facilitated by environmental factors such as arid climates, cross-border trade, and human displacement, creating favorable conditions for its proliferation.

Interestingly, *Ae. aegypti* was absent in some neighborhoods, such as Zongo and Taradona, and sparsely present in others like Diguéri and Djamtata. This distribution may be influenced by ecological factors, including stagnant water availability, temperature, and humidity, which favor one species over another [24].

4.4. Sex ratio and implications for vector control

Females accounted for 69.18% of the *Ae. albopictus* population, while males comprised 26.73%. This sex ratio aligns with other studies where females, responsible for disease transmission dominate [16]. Understanding such dynamics is critical for vector control strategies, as targeting females could significantly reduce transmission risks.

4.5. Absence of Dengue Virus Detection

Although there was a significant presence of *Aedes* mosquitoes, known vectors of the dengue virus, no genetic material of this virus was detected in the analyzed samples. Several factors could explain this absence: the lack of active viral circulation within the human population during the study period, viremia in infected individuals being too low or short-lived to allow effective transmission to mosquitoes, or potential issues with sample handling and transport conditions that may have affected the results. These findings align with other studies in dengue-endemic areas, where little to no evidence of dengue virus has been found in *Aedes* mosquito populations [25], [26]. Furthermore, no cases of dengue outbreaks were reported in Chad in 2024, supporting the hypothesis of an absence of viral circulation.

4.6. Study Limitations and Future Directions

The study’s short duration (July–October 2024) and the relatively small sample size limit the generalizability of the findings, particularly regarding temporal dynamics. Longer studies with larger samples are needed to capture seasonal and spatial trends.

4.7. Implications for Public Health

The data provide a strong foundation for Chad’s health authorities to implement effective mosquito surveillance and control strategies. Understanding the local vector species and their biology is essential for designing targeted interventions.

The semi-arid region of Abéché with its seasonal variations plays a key role in the intensification of mosquito populations and requires close monitoring. This situation underscores the necessity of strengthening entomological surveillance and developing appropriate prevention and control strategies. Tools such as BG-Sentinel traps and oviposition traps can be used to monitor local mosquito populations and identify high-risk areas. The combination of traditional methods (morphological identification) with modern techniques such as PCR is essential for generating precise data. It is also crucial to intensify efforts to eliminate larval habitats and promote the use of targeted insecticide spraying for *Aedes* mosquitoes. In the long term, innovative strategies such as the introduction of genetically modified mosquitoes or the release of sterile males could be considered to reduce mosquito populations.

Community engagement is also indispensable in the fight against dengue, particularly through awareness campaigns on preventive behaviors, such as eliminating stagnant water reservoirs. Health authorities must strengthen international partnerships to secure technical and financial support for conducting in-depth studies and implementing effective prevention programs.

This study provides a foundation for future actions aimed at mitigating the risk of dengue outbreaks in Abéché and similar regions.

5. Conclusion

Although no dengue virus was detected in this study, the high prevalence of *Ae. albopictus* and *Ae. aegypti* in Abéché raises concerns about potential future transmission risks. These findings underscore the need for continuous surveillance and proactive measures to prevent dengue and other arboviruses outbreaks. The study contributes to understanding vector dynamics in Chad and highlights the importance of integrated approaches combining morphological, environmental, and genetic data for effective vector management.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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