Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Mosquito-borne pathogens and entomological outcomes in a high-risk setting: evidence from BG-Sentinel FTA surveillance in Djibouti

Sara Manzi
Istituto Zooprofilattico Sperimentale delle Venezie
Eleonora Perugini
Sapienza University of Rome
Michele Pazienza
Comando Tecnico del Comando Logistico dell'Esercito
Onofrio Zaccaria
Stato Maggiore della Difesa
Vincenzo Abbate
Stato Maggiore della Difesa
Luca Nelli
University of Glasgow
Francesca Romana Di Maio
Sapienza University of Rome
Filippo Molinari
Defense Institute for Biomedical Sciences
Riccardo De Santis
Defense Institute for Biomedical Sciences
Andrea Ciammaruconi
Defense Institute for Biomedical Sciences
Cristiana Poggi
Sapienza University of Rome
Ilaria Bernardini
Istituto Superiore di Sanità
Fabrizio Montarsi
Istituto Zooprofilattico Sperimentale delle Venezie
Ferdinando Spagnolo
Defense Institute for Biomedical Sciences
Verena Pichler
Saint Camillus International University of Health and Medical Sciences
Florigio Lista
Defense Institute for Biomedical Sciences
Marco Pombi
marco.pombi@uniroma1.it

Sapienza University of Rome

Article

Keywords: Entomological surveillance, Arbovirus detection, Insecticide resistance

Posted Date: June 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9690015/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Djibouti is highly vulnerable to several mosquito-borne diseases, although entomological surveillance data remain limited. In this study, BG-Sentinel traps equipped with FTA cards were employed in Djibouti City for the first time to investigate arbovirus circulation and target-site insecticide resistance in mosquito vectors. Weekly samplings were conducted over two months in 2020 and 2022 within an Italian military base in Djibouti City subjected to periodic mosquito control program with pyrethroids. Collected mosquitoes were morphologically identified and genotyped for insecticide resistance mutations, while FTA-cards were screened for arboviruses released by mosquito females during sugar feeding. A serological survey for dengue virus (DENV) seroconversion was also conducted among military personnel in 2022. A total of 19,362 mosquitoes were collected, with *Culex quinquefasciatus* accounting for > 94% of specimens. Resistance-associated alleles were detected at high frequencies in *Cx. quinquefasciatus* (1014F) and *Aedes aegypti* (1534C), whereas *Anopheles stephensi* showed lower 1014F frequencies (< 25%). Three FTA cards tested positive for DENV and West Nile virus in 2020, and one DENV human seroconversion was detected. These findings support the applicability of BG-FTA surveillance in an African context, integrating entomological and serological approaches, and highlight arbovirus circulation in the area and exposure risk to human personnel.

Introduction

The Republic of Djibouti is highly vulnerable to the emergence and re-emergence of mosquito-borne diseases (MBDs) due to a combination of ecological and socio-economic factors that promote both pathogens introduction and sustain high vector population density. MBDs outbreaks reported in this area were historically associated to human movements, especially from inner and endemic countries of Africa, as suggested for malaria (1–4). Indeed, due to its strategic position, the country is an important human migration route with overseas countries, as well as a crossroad for goods and livestock trade. Concerning malaria, its transmission in Djibouti has been considered low and unstable since the early 20th century, with most cases imported from neighbouring countries (2–4). During the last decades, the rise in human cases has also been linked to the recent establishment of *Anopheles stephensi*, an invasive malaria vector probably introduced from Pakistan through marine cargo traffic (4–8). Other than malaria, several arboviral diseases have been reported in this country including dengue (DENV), chikungunya (CHIKV), West Nile (WNV) and Rift Valley Fever virus (RVFV). The most recurrent outbreaks were related to dengue fever with the first cases dating back to 1991 (DENV) (1). Since 1991, dengue has circulated with an endemic–epidemic pattern, and three serotypes (DENV-1, -2, and – 3) have been detected (1, 9–10). In 2010, a sero-epidemiological study on arboviral infections, showed a high prevalence of antibodies against dengue virus and, for the first time, also CHKV was reported (11). Subsequent serological evidence has been documented over the time, with a major CHIKV outbreak occurred between 2019 and 2020 (12–13). Regarding RVFV, its circulation in livestock and human has been sporadically reported in the region, however Djibouti was not recognised as a regional hot spot for transmission (11). WNV circulation has been also described, with the virus detected both in vertebrate (ruminants, horses, human) and invertebrate hosts (*Cx. quinquefasciatus, Cx. pipiens*), highlighting a local transmission cycle (11, 14–16).

Overall, entomological surveillance data for Djibouti are exceptionally limited, as few studies investigated species composition of mosquito population as well as pathogen circulation in their vectors (4, 17–18). Additionally, information on insecticide resistance is limited to a single report on *An. stephensi*, with no details on the underlying mechanisms (19) and no data for other major vector species. Nevertheless, the presence of insecticide resistance mechanisms can significantly affect vector control efforts and, in turn, influence outbreak dynamics, highlighting the critical need to monitor and characterize resistance for effective disease prevention and control strategies. This study aims to provide an entomological assessment of arbovirus circulation in mosquito populations within a military area subjected to pyrethroid-based control. To this aim, we employed a novel entomological surveillance approach based on BG-Sentinel traps with FTA cards, specifically developed for an early detection of infective vectors, which has been previously validated and applied in other endemic settings (20). Secondly, given the endemicity of DENV, serological analyses were conducted on military personnel employed in Djibouti City, to complement the entomological investigations related to the virus. Finally, we assessed target-site resistance mechanisms in major vector species, focusing on mutations in the voltage-gated sodium channel (*vgsc*) gene linked to reduced effectiveness of commonly used pyrethroids.

Methods

Study area

The Republic of Djibouti is in the Horn of Africa, where the Rift Valley meets the Gulf of Aden and the Red Sea. Positioned on a primary shipping lane, the economy of this country is based on service activities connected with the country's strategic location and status as free trade zone in the Horn of Africa. The country has a population of 1.106 million and more than half live in the growing capital city (Djibouti City). The climate is marked by two distinct seasons with low and irregular precipitation patterns (< 130 mm annually). The cold season (October-April) has mild temperatures ranging between 22°C and 30°C with relatively high humidity and sea winds. The hot and dry season (May to June and September to October) has high temperatures, ranging between 30°C and 40°C with often violent, hot, and dry sand wind

(khamsin). The wettest months are April, July, and August, with a monthly average of 30 mm. January, June and December are the driest months, with average rainfall of 10 mm or less (climateknowledgeportal.worldbank.org).

Sample collection

Mosquito samplings were carried out in Djibouti City from military personnel deployed in the Italian Military Support Base (BMIS). The BMIS is a strategic installation, built outskirts of the city centre that provides logistics, maintenance, and infrastructure for external operations. The entomological collections took place outdoors from January to February 2020 and from February to March 2022 using modified BG-Sentinel traps (hereafter BG-FTA) worked once a week for three-to-four consecutive days. Eleven BG-FTA were placed inside the BMIS area and one in the city centre (Caritas) about two kilometres far from the base (Fig. 1). In the BMIS area, weekly outdoor pyrethroid spraying was conducted as mosquito control activity. The traps used in this study were equipped with BG-lure as attractant and hold a sugar delivery system based on the use of an FTA card soaked in a blue sugar-feeding solution as previously described (20). The modification was applied to collect and preserve at environmental temperature viral RNA present in mosquito saliva and released from infective specimens during sugar feeding on the FTA card. Formal permission was obtained from the relevant military/base authorities to conduct mosquito collection activities, which were performed by authorized military personnel.

In 2022, Italian military personnel were voluntarily enrolled in a serological screening for DENV, conducted both at the beginning (i.e., within 48 hours after arrival in Djibouti) and at the end of their deployment. During the initial blood draw, personal data were collected, and any travel to or from endemic areas within the preceding 6 months was documented. Military personnel included in the screening were selected based on a minimum required stay of 14 days in the study area.

Sample processing and analysis

Mosquitoes

All mosquitoes collected were morphologically examined to identify species (21–22) and sex. The evidence of sugar feeding on the FTA cards was assessed for all female specimens by looking for the presence of blue dye (the blue sugar-based solution used to soak the FTA cards) in mosquito bodies. All samples were stored at room temperature in tubes containing silica gel until further analysis.

DNA extraction was performed according to DNAzol® (Molecular Research Center, USA) protocol (23) on a mosquito subsample processed for i) the molecular identification of species and ii) the genotyping of pyrethroid target-site resistance. Indeed, a DNA barcoding approach based on the use of cytochrome oxidase subunit I of was applied to confirm species identification and clarify doubtful classification for some specimens, including the majority of *Anopheles spp.* (24).

Genotyping of pyrethroid target-site resistance mutations was performed on a *Culicinae* and *Anophelinae* subsample. Specimens belonging to the *Culex* and *Anopheles* genera were processed to detect L1014F (TTA◊ TTT) and L1014S (TTA◊TCA) substitutions in the domain II of the voltage gated sodium channel (*vgsc*) gene. Primers Cdg1-Cdg2 (25) and KdrF-VGS1R (26) were used to target the locus 1014 in *Culex sp.* and *Anopheles sp.*, respectively. The forward primers Cdg1 and KDRF were used as sequencing primers to confirm allelic variants. *Aedes spp.* specimens were genotyped for the F1534C (TTC◊TGC) and V1016I (GTA-◊ATA) substitutions (domain III and II of *vgsc*) following Kasai et al. (27) and Fan et al. (28), respectively. A subset of *Aedes* specimens was further genotyped for additional, less common *vgsc* mutations at loci 982, 989, 1011, and 1014 in domain II (28). Primers E-20F and E21R were used for both amplification and sequencing of domain II loci (28), while for the domain III, the primer pairs aegSCF7-SCR7 were used for amplification and aegSCR8 (27) as sequencing primer.

FTA cards

All FTA cards were stored separately in tubes containing silica gel, sorted by trap number and collection date. All samples were preserved at room temperature until the delivery to the reference laboratory (no later than one month after the collection) and then stored at -20°C. RNA was extracted from each FTA card using the QIAamp® Viral RNA Kit (Qiagen, Germany) as previously described (20).

Three different PCR protocols completed by sequencing were used to identify pathogens in RNA extracted from FTA cards. *Othoflavivirus* detection was carried out using a pan-flavivirus hemi-nested PCR targeting the non-structural protein 5 (NS5) with the primer set MAMD, cFD2 and FS788 designed by Scaramozzino et al. (29). PCR products resulting from the cFD2-FS778 pairs were loaded on a 2.5% agarose gel to confirm positive samples (bands visible at approximately 220 bp). Positive PCR products were sequenced using the inner primers to discriminate among *Orthoflavivirus* species.

The presence of Chikungunya and Rift Valley Fever Virus RNA was assessed using two different real-time (TaqMan®) reverse transcription-PCR (real time RT-PCR). Specifically, a one-step real time RT-PCR targeting the E1 gene was applied for CHIKV detection (30), whilst primers and probe designed by Drosten et al. (31) were used to target the G2 region of RVFV.

Sequence processing and analysis

DNA amplified from mosquito and FTA card samples were purified according to SureClean Plus protocol (Bioline, Meridian Bioscience, UK) and sequenced at the Eurofins Genomics Center (GmbH, Ebersberg, Germany). Electropherograms were inspected by Chromas Lite (Technelysium Pty. Ltd., Tewantin, Queensland). Sequences were edited and aligned with MEGA-X (32) and then compared with representative sequences available on GenBank database using Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Human sera

Blood samples were collected from each volunteer, and serum samples were analysed for the presence of IgG antibodies against DENV serotypes 1–4 using an enzyme-linked immunosorbent assay (ELISA) method. The presence of IgG antibodies against dengue was identified through Dengue Virus IgG ELISA TECAN (IBL International GmbH, Hamburg, D). The assay was performed as a semi-quantitative assay with positive, negative, and cut-off controls run in duplicate. Each sample diluted 1:100 was considered positive if the absorbance value (at 450 nm with a 620 nm reference) was 10% higher than the specific IgG cut-off, as recommended by the manufacturer. According to the manufacturer's instructions, results were expressed in Units (U) relative to the run-specific cut-off. Samples were classified as negative (< 9 U), indeterminate (9–11 U), or positive (> 11 U).

All methods were carried out in accordance with relevant guidelines and regulations. The experimental protocols were approved by the "Celio" Ethical Committee (Prot.n. CE/2019u/03/a-/09/10/2019). Informed consent was obtained from all subjects.

Statistical analysis

Entomological data were summarised to describe community structure of mosquito populations. For each sampling year, relative frequencies, and mean abundance with 95% confidence intervals (95% CI) were defined for all detected species. Ecological diversity was assessed using the Shannon-Weaver diversity index (33), calculated for each trap and collection date, and averaged for each mosquito-sampling period.

The overall sugar-feeding rate of female mosquitoes was determined as the proportion of sugar-fed specimens by species per sampling year. This is calculated as the mean percentage of blue (sugar-fed) females per species, trap, and collection date.

Comparisons of H values, female mosquito abundance and sugar feeding rates between sampling years was performed using the Wilcoxon–Mann–Whitney (WMW) test.

Allele frequencies at the VGSC gene locus were estimated for each analysed species. Temporal differences in allele distribution across years were evaluated using a chi-square test.

Data manipulation, statistical analyses and graphical representations were generated using the statistical software R (version 4.3.3) and the RStudio integrated development environment with the following packages: *vegan 2.6–10* (34) and *mixIndependR* 1.0.0 (35).

Results

Species composition, gonotrophic stage and feeding rate on FTA card

A total of 19,362 mosquitoes were collected over the two sampling years: 13,248 in 2020 and 6,114 in 2022. Overall, a significantly higher number of mosquitoes was collected in 2020 ($W = 3,699.5$, $p < 0.0001$) with mean abundance of 184 (95% CI: 146.77-221.23), compared with 86.11 (95%CI: 62.33-109.90) in 2022. Conversely Shannon diversity (H) index was significantly higher in 2022 than in 2020 ($W = 1522$, $p < 0.0001$), with mean H values of 0.23 (95% CI: 0.19-0.28) and 0.11 (95% CI: 0.07-0.15), respectively (Figure 2).

A total of 13,166 and 5,988 specimens were successfully identified at species level for the year 2020 and 2022, respectively (Table 1). *Culex quinquefasciatus* was the dominant species in both years, accounting for 96.6% of collections in 2020 and 94.1% in 2022. Other species were found at lower frequencies: *Aedes aegypti* (2.7% in 2020; 1.3% in 2022), *Anopheles stephensi* (0.5% in 2020; 3.3% in 2022), *Culex sitiens* (0.1% in 2020; 1.2% in 2022) and *Anopheles dthali* (0.1% in 2020; 0.1% in 2022). Additionally, in 2022, two specimens were identified as *Aedes vexans* (Table 1).

Table 1. Descriptive statistics (counts and mean number) and Wilcoxon–Mann–Whitney (WMW) test output for female mosquitoes by species/genus and collection year (2020 and 2022). n. total specimens: Total number of mosquitoes collected including male and female specimens; n. females: number of female mosquitoes; mean: mean number of mosquitoes; 95% CI: confidence intervals. The W and p-value columns show the statistics for the Wilcoxon–Mann–Whitney test.

	2020				**2022**				**WMW test**	
	n. total specimens	**n. females**	**mean**	**95% CI**	**n. total specimens**	**n. female**	**mean**	**95% CI**	**W**	**p-value**
Species	**13166**	**7045**	**97.85**	**76.72-118.98**	**5988**	**4335**	**61.06**	**43.99-78.12**	**3459.5**	2.66e-04
Ae. aegypti	357	154	2.14	0.63-3.65	77	43	0.61	0.35-0.86	2938.5	0.08
Ae. vexans	0	0	-	-	2	2	-	-	-	-
An. dthali	12	6	0.08	0.02-0.15	5	4	0.06	0.00-0.11	2625	0.53
An. stephensi	64	22	0.17	0.17-0.45	197	59	0.83	0.51-1.15	2114.5	0.03
Cx. quinquefasciatus	12719	6857	95.24	74.40-116.07	5636	4187	58.97	42.39-75.56	3475	2.08e-04
Cx. sitiens	14	6	0.08	0.02-0.15	70	39	0.55	0.28-0.82	1920	2.46e-04
Genus	**82**	**82**	**1.14**	**0.72-1.56**	**127**	**123**	**1.72**	**1.09-2.34**	**2263**	0.20
Culex sp.	82	82	1.14	0.72-1.56	123	122	1.70	1.08-2.33	2266	0.21
Anopheles sp.	0	0	-	-	4	1	-	-	-	-

Consistently with the overall trends observed for mosquito densities, the number of *Cx. quinquefasciatus* and *Ae. aegypti* females decreased from 2020 to 2022 sampling. Conversely, a significant increase in *An. stephensi* and *Cx. sitiens* females was observed between the two years of collection, while no differences were reported for *An. dthali* (Table 1).

For all female mosquitoes, the sugar meal on FTA cards was analysed by assessing evidence of blue dye in mosquito body. The overall feeding rate was significantly higher in 2020 as compared to 2022, with some variability reported in relation to the species (Table 2).

Table 2. Mean sugar feeding rate of female mosquitoes by species and sampling year. For each species the mean proportion of sugar-fed female and the corresponding 95% confidence interval (CI) are reported for 2020 and 2022.The W statistics and the associated p-value derive from the Wilcoxon–Mann–Whitney (WMW) test comparing sugar-feeding rate between years.

Sugar feeding rate	2020		2022		WMW test	
	mean	95% CI	mean	95% CI	W	p-value
Species	0.49	0.44-0.54	0.18	0.14-0.22	4279	3.57e-15
Ae. aegypti	0. 34	0.25-0.44	0.03	0.00-0.06	516	1.65e-03
Ae. vexans	-	-	0.00	0.00-0.00	-	-
An. dthali	0.83	0.74-0.92	0.50	0.35-0.64	16	0.35
An. stephensi	0.32	0.22-0.43	0.01	0.00-0.02	295	2.14e-03
Cx. sitiens	0.17	0.07-0.26	0.02	0.00-0.03	71	0.34
Cx. quinquefasciatus	0.49	0.44-0.54	0.19	0.14-0.23	4223.5	2.25e-21
Genus	**0.18**	**0.11-0.25**	**0.02**	**0.00-0.06**	**870**	2.00e-03
Culex sp.	0.18	0.11-0.25	0.02	0.00-0.05	883	5.00e-04
Anopheles sp.	-	-	1.00	-	-	-

Insecticide resistance in main vector species

A total of 140 *Ae. aegypti,* 231 *Cx. quinquefasciatus* and 227 *An. stephensi* specimens were successfully genotyped for the most common and widespread insecticide target-site mutations. The number of specimens analysed, and their corresponding genotypes are summarized in Table 3 for each species and sampling year. A significant increase in the frequency of the 1534C resistant allele was detected in *Ae. aegypti*, from 71% in 2020 to 99% in 2022 ($\chi^2 = 41.3$, $p < 0.0001$). **N**one of the *Ae. aegypti* specimens carried the V1016I substitution in domain II, and no other target-site mutations were detected at the additional domain II loci (989, 982, 1011, and 1014) genotyped in a subset of 33 *Ae. aegypti* specimens. In *Cx. quinquefasciatus* the 1014F resistant allele was detected at consistently high frequency across both years of sampling: 89% in 2020 and 94% in 2022 ($\chi^2 = 1.57$, $p = 0.21$). Conversely, in *An. stephensi* the 1014F mutation was observed at low frequency with no significant differences among the years: 25% in 2020 and 22% in 2022 ($\chi^2 = 0.38$, $p = 0.53$). The 1014S resistant allele was not detected at locus 1014 in any of the *Cx. quinquefasciatus* or *An. stephensi* specimens genotyped for insecticide resistance.

Table 3. Genotyping of pyrethroid target site mutations divided per years and vector species. Total specimens analysed (n.), genotype at each locus and resistant allele frequencies are shown. +/+: double mutant; +/-: heterozygous; -/-: wild type

Year	Species	*n.*	*substitution*	Genotype +/+	Genotype +/-	Genotype -/-	Resistant allele frequency
2020	*An. stephensi*	57	L1014F	7	15	35	0.25
	Ae. aegypti	71	F1534C	35	31	5	0.71
	Ae. aegypti	69	V1016I	0	0	69	0.00
	Cx. quinquefasciatus	113	L1014F	95	14	4	0.89
2022	*An. stephensi*	170	L1014F	8	59	103	0.22
	Ae. aegypti	69	F1534C	68	1	0	0.99
	Ae. aegypti	68	V1016I	0	0	68	0.00
	Cx. quinquefasciatus	118	L1014F	105	12	1	0.94

Arbovirus circulation

FTA cards

Over the two sampling years, FTA cards detected different *Orthoflavivirus* species, while no cards were found positive for CHIKV and RVFV. All positive traps were placed inside the BMIS area during both years of monitoring. Out of 71 FTA-cards collected in the year 2020, two were positive for WNV and one for DENV viruses. DENV-2 was detected on week 6 (9th February), while the two positive cards for WNV were detected on weeks 7 and 9 (14th and 24th February, respectively). In the DENV- positive trap, no evidence of blue dye was reported in *Ae. aegypti* while in WNV positive traps the 47% and 60% of *Cx. quinquefasciatus* specimens had a visible sugar meal.

In 2022 only 1 FTA card out of the 71 collected, tested positive for an *Insect-specific flavivirus* (ISFV; not related to vertebrate infection), during week 11 (10th March). No evidence of blue dye was reported in female mosquitoes collected in the corresponding trap. The sequence analysis showed the 97.2% identity with ISFVs isolated from *Ae. aegypti* collected in different locations (i.e., Mosquito flavivirus GenBank accession OR531688.1 and *Phlebotomus*-associated *Orthoflavivirus*, GenBank accession numbers MN294942.1 and OR636213.1). However, the low-quality of the sequence data did not allow the identification of this virus with confidence (Supplementary material, Table S1). Although all cards were analysed, two traps set in weeks 9 (February 24) and 10 (3rdMarch) did not collect mosquitoes. An overview of detected viruses for each week and for each sampling year, together with the mean number of female vectors species collected per trap (female/trap), was reported in Figure 3.

Human sera

Serological screening was conducted on a total of 52 volunteers of military personnel, all men residing in Italy, who were deployed in Djibouti during the season 2022. The mean interval between baseline and follow-up serum sampling was 40 days and varied according to each volunteer's deployment duration (Supplementary material figure S1). Only one volunteer showed evidence of DENV seroconversion (from 7.99U to 11.70 U) between the first and second blood draws, collected 40 days apart. The individual declared no travel or missions in other Dengue endemic areas in the six months preceding the survey suggesting exposure during his deployment at the BMIS in Djibouti. In addition, two individuals had IgG response above the positivity cut-off in both blood draws, suggesting possible previous exposure. Just one of them reported travelling to Niger in the six months preceding the survey. Another volunteer showed a slight positive response (11.31 U) at the first blood sampling but a value 1.3 times lower at the second draw (8.80 U) 22 days later, suggesting an intermediate inconclusive result. Among the remaining participants who tested negative in both blood draws (N=48), 15 reported travels outside Italy in the preceding six months, and only 7 of these visited non-European countries (Djibouti, Niger, Afghanistan, United Arab Emirates, Turkey) (Supplementary file S2).

Discussion

This study provides an overview of arbovirus circulation within mosquito populations in the urban military area of Djibouti City, demonstrating the applicability of the BG-FTA system for entomological surveillance in a tropical endemic context, building on its original validation.

This surveillance revealed a mosquito community with low species diversity, highlighting a scenario of ongoing or potential transmission of different MBDs. The low values of H index observed throughout the sampling period (0.11 in 2020 and 0.23 in 2022) indicate a simplified mosquito community structure, which is common in urban environments (36). However, periodic adulticidal vector control measures (based on outdoor pyrethroid spraying) could have affected both species community and densities reported. Regarding population size, an overall higher mean abundance was reported in 2020 compared to 2022 (184 in 2020 vs 86.11 in 2022), while the significant increase of H diversity in 2022 suggests a broader species occurrence and changes in species distribution. These variations can likely reflect natural population dynamics, with partially overlapping sampling periods, driven by climatic and environmental changes across sampling years.

Vector species were mainly represented by *Cx. quinquefasciatus*, followed by *Ae. aegypti* and *An. stephensi*, while *An. dthali* and *Ae. vexans* were only sporadically detected at low densities.

Consistently with previous studies conducted in Djibouti (17, 19), *Cx. quinquefasciatus* was the dominant species in the study area, representing most of the total sample in both collections (96.6% in 2020 and 94.1% in 2022). This species is well adapted to urban and semi-urban environments (37) although it can also exploit rural habitats. Its overdominance at the study site is likely sustained by widespread insecticide resistance mechanisms, as indicated by the high frequency of the 1014F allele observed during both sampling years (89% in 2020 and 94% in 2022). The L1014F substitution in the *vgsc* gene is one of the main and well-documented mechanism associated with pyrethroid resistance in different mosquitoes of *Anopheles* and *Culex* genera (38–39). A high prevalence of this mutation was reported in resistant *Cx. quinquefasciatus* population from different countries (40–42), although metabolic mechanisms may act synergistically and need to be considered (40, 43–44). The high frequency of the 1014F allele observed in the study area may be linked to selective pressure exerted by insecticide, including the routine spraying of pyrethroids within the BMIS, but also to other activities, such as agricultural pest management and malaria vector control measures implemented in the country over the last decade, as suggested in other part of Africa (2, 45).

A similar insecticide pressure may also affect *Ae. aegypti*, particularly within the urban area. In fact, the species is a typical urban vector and shares numerous larval habitats with *Cx. quinquefasciatus* in urban and peri-urban settings, including manholes, ditches, plastic drums, and water storage tanks (5). *Aedes aegypti* showed a higher, though not statistically significant, average abundance in 2020 compared to 2022 (2.14 vs 0.61), with densities consistent with those reported in in 2019 in a French military camp of Djibouti (5). The observed frequency of the 1534C target-site resistance allele, suggests a growing insecticide selective pressure, leading almost to the fixation of this mutation in 2022. The F1534C substitution is strongly associated with pyrethroid resistance in *Aedes* spp. (46) and is the most widespread mutation in *Ae. aegypti* populations across Africa. Other mutations in domains II and III of the *vgsc* (i.e., loci 1016, 982, 982, 1011, 1014) were not detected in

this species, consistently with their low frequency or absence reported in *Aedes* populations across the African continent (47). To the best of our knowledge, this study represents the first investigation on pyrethroid resistance in *Cx. quinquefasciatus* and *Ae. aegypti* from Djibouti.

Concerning the main malaria vectors, *An. stephensi*, was poorly represented during the sampling period, although a slight but significant increase was observed in 2022 (0.17 in 2020 vs 0.83 in 2022). The low numbers of specimens collected is comparable to those reported in previous studies (5, 8, 19) but it cannot be excluded the impact of adulticidal control.

Insecticide resistance appears to spread in *An. stephensi*, indeed studies conducted in other countries reported resistance to all four major insecticide classes in this species (48). However, the low frequency of 1014F and the absence of the 1014S alleles according to density reported, suggest low level of pyrethroids resistance and/or the presence of alternative mechanisms, as reported in countries neighbouring Djibouti (49–50). Further studies are highly needed to comprehensively characterize the resistance profile of this malaria vector and to support targeted and effective vector control strategies.

The BG-FTA system detected different arboviruses highlighting the suitability of the BG-FTA system for arbovirus surveillance in remote areas and for long-term sample storage and extending previous evidence (20) in a different epidemiological setting. The detection of WNV and DENV RNA on 2 and 1 FTA cards, respectively, provided evidence of virus circulation during the year 2020. The two WNV-positive traps were recorded within a short time window, the first occurring before the marked increase in mosquito abundance observed toward the end of the monitoring period (Fig. 2). Since FTA cards detect viral RNA from mosquito saliva released during sugar feeding, these findings suggest that infective mosquitoes were already present in the study area from the first half of February 2020 (week 7). Notably, visible evidence of sugar feeding (blue dye) was observed in 47% and 60% of *Cx. quinquefasciatus* collected in WNV-positive traps, consistent with trap positivity and vector sugar-feeding behaviour.

Results also indicated the circulation of infective mosquitoes for DENV during the first half of February 2020 (week 6). In the same year, two other studies (13, 18) reported dengue transmission among American and French military personnel deployed in Djibouti, with peaks of human cases occurring in January and May 2020. According to Javelle et al. (13) the first outbreak was recorded between the week 4 and 5 of the year and was linked to DENV-1 in 36 of 56 dengue fever confirmed cases. The virus detection in the present study showed the highest similarity with DENV-2, suggesting possible co-circulation of different serotypes, as previously reported (10). In line with DENV detection the positive trap collected two specimens of *Ae. aegypti*, which is recognized as the primary vector in the country. However, none of the specimens collected in the positive trap showed visible evidence of sugar feeding. This may be partially explained by the fact that the BG-FTA system worked continuously for four consecutive days. Therefore, mosquitoes may have digested the sugar meals prior to trap collection. It is also possible that the pathogen was released in the saliva during probing, even in the absence of an actual sugar-feeding event. In addition, detection of the blue dye can be particularly challenging in dry mosquitoes with dark-coloured abdomens, such as *Ae. aegypti* (20). It should also be noted that the detection of DENV, in a context where insecticide control was implemented and vector densities were low, is also indicative of a good efficiency of BG-FTA system in mosquito surveillance.

The absence of WNV- and DENV-positive traps in 2022 does not rule out viruses' circulation, although the significantly lower abundance of vector species compared to 2020 (2020 vs 2022: *Cx. quinquefasciatus* 95.2 vs 58.97; *Ae. aegypti* 2.14 vs 0.61) may suggest reduced transmission in the study area. Additionally, a marked decrease in feeding rates - approximately two-fold for *Cx. quinquefasciatus* and eight-fold for *Ae. aegypti* - could have further affected the detection capability of the BG-FTA system. These aspects may be linked to possible climatic variations between sampling years. Microclimatic factors, such as changes in humidity and temperature, can strongly affect vector population density as well as mosquito hydration and sugar-starved status, thereby influencing their propensity to feed on the FTA cards and, consequently, the virus release. However, the single case of seroconversion observed in 2022 among Italian military personnel, together with the absence of reported DENV cases from January to March 2022 in another study (18), suggests low circulation in the study area, partially explaining the failed detection with the BG-FTA. In contrast, no data are available for WNV, as infections remain poorly investigated in Djibouti, as in other parts of Africa.

The only virus identified during 2022 sampling belong to ISFVs, a large group of viruses belonging to *Orthoflavivirus* genus which naturally infect only insects. Over the past two decades, an increasing number of ISFVs was reported from different mosquito species, however their physiological and transmission dynamics remain unknown (51). The virus detected in this study revealed highest similarity with a mosquito *Flavivirus* reported in Nigeria (GenBank accession OR531688.1) and a *Phlebotomus* associated *Flavivirus* (PAFV) isolated from mosquitoes in Saudi Arabia (Accession number MN294940.1). The PAFVs was described both in sand flies (52) and mosquitoes (53–54), suggesting a wide host range and a probably common source of infection. According to current knowledge, the ISFVs have no relevance for human and animal health, but the detection from FTA card further indicates the proper functioning and sensitivity of the surveillance system.

In contrast to *Orthoflavivirus* positivity, no FTA cards tested positive for RVFV or CHIKV in either year of sampling. For RVFV, this finding is consistent with the lack of evidence for endemic transmission and supports the hypothesis of sporadic exposure through contaminated aerosols, as previously suggested (11). Although different mosquito species can potentially transmit the virus, *Ae. vexans*, is among the

primary vectors in Africa and middle east (55–56), while *Culex* mosquitoes (such as *Cx. quinquefasciatus*) could act as secondary vectors during outbreaks (57). *Aedes vexans* was reported for the first time in Djibouti in October 2019 at low densities (58) and was observed only sporadically in the present study, thus partially explaining the absence of RFVF-positive FTA cards. However, the potential impact of adulticides interventions within the study site must be considered, and further research in another area not under vector control interventions is needed to determine the actual density and dynamics of this species in the country.

CHIKV circulation remains poorly documented in Djibouti, with exception of a seroprevalence study of 2010 describing a low level of immunity (11) and confirmed autochthonous cases reported in 2019 and 2020 among military personnel living in Djibouti city (12–13). However, during the present study, there were no evidence of CHIKV circulation in mosquitoes, according to the drop in human cases from the first week of January 2020 documented by French military personnel deployed in the area (13). Although these observations could explain negative results for RVFV and CHIKV in this study, there are no published entomological surveillance available for these viruses in Djibouti, and more intensive monitoring is needed to better understand the epidemiology of these arboviral diseases in the area.

This study provides an integrated framework of pathogen circulation and insecticide resistance in mosquito vectors in an urban tropical endemic setting of Djibouti City. The entomological surveillance using the BG-FTA system confirmed the presence of key vector species—*Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*—and provided evidence of ongoing West Nile and Dengue virus transmission at the study site, as further supported by DENV seroconversion in a member of the military personnel. The successful detection of viral RNA on FTA confirmed the BG-FTA system effectiveness for arbovirus surveillance and extended its applicability in tropical endemic contexts characterised by operational and ecological constraints and insecticide pressure affecting vector communities and densities. For the first time in Djibouti, this study reported the frequency of main pyrethroid target-site resistance alleles in *Cx. quinquefasciatus* and *Ae. aegypti*, indicating almost fixation of the 1014F and 1534C mutations, respectively in the two species. Overall, this study confirms the vulnerability of Djibouti to MBDs and underscores the importance of strengthening the entomological surveillance in the country, adopting an early warning framework that integrates vector and serological data. It is also provided a solid baseline to guide future risk assessments and support the development of targeted, evidence-based vector control strategies.

Declarations

Author contributions statement

M.Po., M.Pa, S.M., F.L. conceived the study. M.Po. and S.M. organized and supervised field collections. M.P., O.Z., V.A., carried out field collection. S.M, M.Po, E.P. performed morphological identification of entomological samples. S.M., E.P., V.P., performed molecular analyses. A.C., F.M. performed serological analyses. S.M., M.Po., C.P., E.P, analysed the entomological data. S.M., E.P., M.Po., C.P. drafted the manuscript. All authors read and approved the final manuscript.

Data availability statement

Data supporting the conclusions of this article are included within the article and its additional files. The datasets used and analysed during the present study are available from the corresponding author upon request.

Funding

This study was supported by: Italian Ministry of Defence, Programma Nazionale per la Ricerca Militare (PNRM) 2017, project “SENSOR”, n. a2016.140 (P.I. Marco Pombi), and PNRM 2018, project C3-LAB”, n. A2018.057 (P.I. Marco Pombi).

Acknowledgements

The authors are grateful to Paola Serini for her continuous support in laboratory activities.

Additional information

Supplementary files 1 and 2.

Competing interests

The authors declare no competing interests.

References

1. Kodier G.R., Gubler D.J., Cope S.E., Cropp C.B., Soliman A.K., Polycarpe D., et al. Epidemic dengue 2 in the city of Djibouti 1991–1992. *Trans. R. Soc. Trop. Med. Hyg*. **90**, 237–240 (1996).
2. Ollivier L., Nevin R. L., Darar H. Y., Bougère J., Saleh M., Gidenne S., et al. Malaria in the Republic of Djibouti, 1998–2009. *Am. J. Trop. Med. Hyg*. **85**, 554–559 (2011). https://doi.org/10.4269/ajtmh.2011.11-0122
3. Khaireh, B.A., Briolant, S., Pascual, A. Mokrane M., Machault V., Travaillé C., et al. Plasmodium vivax and Plasmodium falciparum infections in the Republic of Djibouti: evaluation of their prevalence and potential determinants. *Malar. J*. **11**, 395 (2012). https://doi.org/10.1186/1475-2875-11-395
4. Faulde M. K., Rueda L. M., Khaireh B. A. First record of the Asian malaria vector Anopheles stephensi and its possible role in the resurgence of malaria in Djibouti, Horn of Africa. *Acta Trop*. **139**, 39–43 (2014). https://doi.org/10.1016/j.actatropica.2014.06.016
5. de Santi V.P., Khaireh B.A., Chiniard. T., Pradines B., Taudon N., Larréché S. et al. Role of Anopheles stephensi Mosquitoes in Malaria Outbreak, Djibouti, 2019. *Emerg. Infect. Dis*. **27**, 1697–1700 (2021). https://doi.org/10.3201/eid2706.204557
6. Ahn, J., Sinka, M., Irish, S., Zohdy, S. Modeling marine cargo traffic to identify countries in Africa with greatest risk of invasion by Anopheles stephensi. *Sci. Rep*. **13**, 1–12 (2023).
7. Dennis T. P. W., Sulieman J. E., Abdin M, Ashine T, Asmamaw Y, Eyasu A, et al. The origin, invasion history and resistance architecture of Anopheles stephensi in Africa. *bioRxiv* (2025). https://doi.org/10.1101/2025.03.24.644828
8. Abebe W., Sisay A., Mihret Y., Setegn A., Asmare Z., Woldesenbet D., et. al. Prevalence of Anopheles stephensi in Horn of Africa: a systematic review and meta-analysis. *BMC Infect. Dis*. **25**, 614 (2025). https://doi.org/10.1186/s12879-025-11022-1
9. de Laval F., Dia A., Plumet S., Decam C., Leparc Goffart I., Deparis X. Dengue surveillance in the French armed forces : a dengue sentinel surveillance system in countries without efficient local epidemiological surveillance. *J. Travel Med.* **20**, 259–261 (2013).
10. Le Gonidec E., Maquart M., Duron S., Savini H., Cazajous G., Vidal P.O., et al. Clinical Survey of Dengue Virus Circulation in the Republic of Djibouti between 2011 and 2014 Identifies Serotype 3 Epidemic and Recommends Clinical Diagnosis Guidelines for Resource Limited Settings. *PLoS Negl Trop Dis*. **10**, e0004755 (2016). https://doi.org/10.1371/journal.pntd.0004755
11. Andayi F., Charrel R. N., Kieffer A., Richet H., Pastorino B., Leparc-Goffart I., et al. A seroepidemiological study of arboviral fevers in Djibouti, Horn of Africa. PLoS Negl. Trop. Dis. **8**, e3299 (2014). https://doi.org/10.1371/journal.pntd.0003299
12. Fourié T., Dia A., Savreux Q., Pommier de Santi V., de Lamballerie X., Leparc-Goffart I., et al. Emergence of Indian lineage of ECSA chikungunya virus in Djibouti, 2019. *Int. J. Infect. Dis*. **106**, 93–95 (2021). https://doi.org/10.1016/j.ijid.2021.03.090
13. Javelle E., de Laval F., Durand G. A., Dia A., Ficko C., Bousquet A., et al. Chikungunya outbreak in country with multiple vectorborne diseases, Djibouti, 2019–2020. *Emerg. Infect. Dis*. **29**, 826–830 (2023). https://doi.org/10.3201/eid2904.221850
14. Cabre O., Grandadam M., Marié J.L., Gravier P., Prangé A., Santinelli Y., et al. West Nile Virus in horses, sub-Saharan Africa. *Emerg. Infect. Dis*. **12**, 1958–1960 (2006). https://doi.org/10.3201/eid1212.060042
15. Faulde M.K., Spiesberger M., Abbas B. Sentinel site-enhanced near-real time surveillance documenting West Nile virus circulation in two *Culex* mosquito species indicating different transmission characteristics, Djibouti City, Djibouti. *J. Egypt. Soc. Parasitol*. **42**, 461–74 (2012). https://doi.org/10.12816/0006332
16. Eybpoosh S., Fazlalipour M., Baniasadi V., Pouriayevali M.H., Sadeghi F., Ahmadi Vasmehjani A., et al. Epidemiology of West Nile Virus in the Eastern Mediterranean region: A systematic review. *PLoS. Negl. Trop. Dis*. **13**, e0007081 (2019). https://doi.org/10.1371/journal.pntd.0007081.
17. Faulde M.K., Ahmed A.A. Haematophageous vector monitoring in Djibouti city from 2008 to 2009: first records of Culex pipiens ssp. torridus (IGLISCH), and Anopheles sergentii (theobald). *J. Egypt. Soc. Parasitol.* **40**, 281–294 (2010). https://doi.org/10.48327/mtsi.v4i1.2024.365
18. Harwood J. F., Noundou W., Tageldin R. A., Zayed A. M., Fahmy N. T., Omar K., et al. Improving the vector-borne disease risk assessment to deployed forces in Djibouti: military operational entomology in the Horn of Africa, 2018–2022. U.S. Army Med. Dep. (2025).
19. Govoetchan R., Ibrahim M., Sovi A., Omar H., Boulhan A., Darar H. Y. Anopheles stephensi: the emerging vector of malaria in the Republic of Djibouti, Horn of Africa. *Int. J. Biosci*. **22**, 8–17 (2023). https://doi.org/10.12692/ijb/22.1.8-17
20. Manzi S., Nelli L., Fortuna C., Severini F., Toma L., Di Luca M., et al. A modified BG-Sentinel trap equipped with FTA card as a novel tool for mosquito-borne disease surveillance: a field test for flavivirus detection *Sci. Rep.* **13**, 12840 (2023). https://doi.org/10.1038/s41598-023-39857-1.
21. Rueda L.M. *Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission*. *Zootaxa*, **589,** 1–60 (2004).
22. Coetzee M. Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae). *Malar. J.* **19**, 1–20 (2020).
23. Rider M.A., Byrd B.D., Keating J., Wesson D.M., Caillouet, K.A. PCR detection of malaria parasites in desiccated Anopheles mosquitoes is uninhibited by storage time and temperature. *Malar. J.* **11**, 1–6 (2012). https://doi.org/10.1186/1475-2875-11-193.

24. Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*. **3**, 294–299 (1994)
25. Martinez-Torres D., Chevillon C., Brun-Barale A., Bergé J., Pasteur, N., Pauron, D. Voltage-dependent Na^+ channels in pyrethroid-resistant *Culex pipiens* L mosquitoes†. *Pestic. Sci.* **55**,1012–1020 (1999). https://doi.org/10.1002/(SICI)1096-9063(199910)55:10<1012::AID-PS39>3.0.CO;2-5
26. Singh O.P., Dykes C.L., Lather M., Agrawal O.P., Adak T. Knockdown resistance (kdr)-like mutations in the voltage-gated sodium channel of a malaria vector Anopheles stephensi and PCR assays for their detection. *Malar. J.* **10**, 59 (2011). https://doi.org/10.1186/1475-2875-10-59
27. Kasai S., Ng L.C., Lam-Phua S.G., Tang C.S., Itokawa K., Komagata O., et al. First detection of a putative knockdown resistance gene in major mosquito vector, Aedes albopictus. *Jpn. J. Infect. Dis*. **64**, 217–221 (2011). https://doi.org/10.7883/yoken.64.217
28. Fan Y., O'Grady P., Yoshimizu M., Ponlawat A., Kaufman P. E., Scott J. G. Evidence for both sequential mutations and recombination in the evolution of kdr alleles in Aedes aegypti. *PLoS Negl. Trop. Dis.* **14**, e0008154 (2020). https://doi.org/10.1371/journal.pntd.0008154
29. Scaramozzino N., Crance J.M. Jouan A., DeBriel D.A., Stoll F.,Garin D. Comparison of Flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. *J. Clin. Microbiol.* **39**, 1922–1927 (2001). https://doi.org/10.1128/jcm.39.5.1922-1927.2001
30. Pastorino B., Bessaud M., Grandadam M., Murri S., Tolou H.J., Peyrefitte C.N. Development of a TaqMan® RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses. *J. Virol. Methods* **124**, 65–71 (2005). https://doi.org/10.1016/j.jviromet.2004.11.002
31. Drosten C., Göttig S., Schilling S., Asper M., Panning M., Schmitz H., Günter S. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *J. Clin. Microbiol.***40**, 2323–2330 (2002). https://doi.org/10.1128/jcm.40.7.2323-2330.2002
32. Kumar S., Stecher G., Li M., Knyaz C., Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol*. **6**, 1547–1549 (2018). https://doi.org/10.1093/molbev/msy096.
33. Shannon, C.E. A Mathematical Theory of Communication. *Bell Syst. Tecj. J.* **27**, 379–423, 623–656 (1948).
34. Oksanen J., Simpson G., Blanchet F. G., Kindt R., Legendre P., Minchin P. R., et al. vegan: community ecology package. R package version 2.6-2 (2022).
35. Song B., Woerner A. E., Planz J. mixIndependR: a R package for statistical independence testing of loci in database of multilocus genotypes. *BMC Bioinformatics* **22**, 12 (2021). https://doi.org/10.1186/s12859-020-03945-0
36. Perrin A., Glaizot O., Christe P. Worldwide impacts of landscape anthropization on mosquito abundance and diversity: a meta-analysis. *Glob. Change Biol*. **28**, 6857–6871 (2022). https://doi.org/10.1111/gcb.16406
37. Farajollahi A., Fonseca D. M., Kramer L. D., Kilpatrick A. M. "Bird biting" mosquitoes and human disease: a review of the role of Culex pipiens complex mosquitoes in epidemiology. *Infect. Genet. Evol.* **11**, 1577–1585 (2011). https://doi.org/10.1016/j.meegid.2011.08.013
38. Scott J.G., Yoshimizu M.H., Kasai S. Pyrethroid resistance in Culex pipiens mosquitoes. *Pestic Biochem Physiol*. **120**, 68–76 (2015). https://doi.org/10.1016/j.pestbp.2014.12.018.
39. Clarkson C.S., Miles A., Harding N.J., O'Reilly A.O., Weetman D., Kwiatkowski D., Donnelly M.J. Anopheles gambiae 1000 Genomes Consortium. The genetic architecture of target-site resistance to pyrethroid insecticides in the African malaria vectors Anopheles gambiae and Anopheles coluzzii. *Mol. Ecol.* **21**, 5303–5317 (2021). https://doi.org/10.1111/mec.15845.
40. Pocquet N., Milesi P., Makoundou P., Unal S., Zumbo B., Atyame C. et al. Multiple Insecticide Resistances in the Disease Vector *Culex p. Quinquefasciatus* from Western Indian Ocean. *PLoS one* **10,** e077855 (2013). https://doi.org/10.1371/journal.pone.0077855
41. Yadouléton A., Badirou K., Agbanrin R., Jöst H., Attolou R., Srinivasan R. et al. Insecticide resistance status in Culex quinquefasciatus in Benin. *Parasit. Vectors*. **8**, 17 (2015). https://doi.org/10.1186/s13071-015-0638-3
42. Okafor M.A., Ekpo N.D., Opara K.N. Udoidung N.I., Ataya F.S., Yaro C.A. *et al.* Pyrethroid insecticides susceptibility profiles and evaluation of L1014F kdr mutant alleles in *Culex quinquefasciatus* from lymphatic filariasis endemic communities. *Sci. Rep.* **13**, 18716 (2023). https://doi.org/10.1038/s41598-023-44962-2
43. Talipouo A., Mavridis K., Nchoutpouen E., Djiappi-Tchamen B., Fotakis E.A., Kpya E. et al. High insecticide resistance mediated by different mechanisms in Culex quinquefasciatus populations from the city of Yaoundé, Cameroon. *Sci. Rep.* **11**, 7322 (2021). https://doi.org/10.1038/s41598-021-86850-7
44. Chamnanya S., Kiddela B., Saingamsook J., Nachaiwieng W., Lumjuan N., Somboon P., Yanola J. Overexpression of multiple cytochrome P450 genes with and without knockdown resistance mutations confers high resistance to deltamethrin in Culex quinquefasciatus. *Infect. Dis. Poverty.* **14**, 2 (2025). https://doi.org/10.1186/s40249-024-01269-2

45. Ministère de la Santé, République de Djibouti. Plan stratégique national de lutte contre le paludisme 2020–2024 (PNLP 2020–2024) (Ministère de la Santé, Djibouti, 2020). https://extranet.who.int/cpcd/sites/default/files/public_file_repository/DJI_Djibouti_National-Malaria-Strategic-Plan_2020-2024.pdf
46. Fan Y., Scott J.G. The F1534C voltage-sensitive sodium channel mutation confers 7- to 16-fold resistance to pyrethroid insecticides in Aedes aegypti. *Pest. Manag. Sci*. **76,** 2251–2259 (2020). https://doi.org/10.1002/ps.5763
47. Bello S.O.T., Zoure A.A., Ouattara A.K., Somda D., Nadembega C., Obiri-Yeboah D., et al. Geographical Distribution of Arboviruses, Aedes aegypti and Aedes albopictus Vectors and Their Resistance to Insecticides in Africa: A systematic Review. *Adv. Entomol.* **12**, 249–274 (2024). https://doi.org/10.4236/ae.2024.124019.
48. Enayati A., Hanafi-Bojd A.A., Sedaghat M.M. Zaim M., Hemingway J. Evolution of insecticide resistance and its mechanisms in Anopheles stephensi in the WHO Eastern Mediterranean Region. *Malar. J.* **19**, 258 (2020). https://doi.org/10.1186/s12936-020-03335-0
49. Samake J.N., Yared S., Getachew D., Mumba P., Dengela D., Yohannes G. et al. Detection and population genetic analysis of kdr L1014F variant in eastern Ethiopian Anopheles stephensi. *Infect. Genet. Evol.***99**, 105235 (2022). https://doi.org/10.1016/j.meegid.2022.105235
50. Carter T. E., Gebresilassie A., Hansel S., Damodaran L., Montgomery C., Bonnell V., et al. Analysis of the knockdown resistance locus (kdr) in Anopheles stephensi, An. arabiensis and Culex pipiens s.l. for insight into the evolution of target-site pyrethroid resistance in eastern Ethiopia. *Am. J. Trop. Med. Hyg.* **106**, 632–638 (2022). https://doi.org/10.4269/ajtmh.20-1357
51. Carvalho V.L., Long M.T. Insect-Specific Viruses: An overview and their relationship to arboviruses of concern to humans and animals. *Virology*. **557**, 34–43 (2021). https://doi.org/10.1016/j.virol.2021.01.007.
52. Moureau G., Ninove L., Izri A., Cook S., De Lamballerie X., Charrel R.N. Flavivirus RNA in phlebotomine sandflies. *Vector Borne Zoonotic Dis.* **10**, 195–197 (2010). https://doi.org/10.1089/vbz.2008.0216.
53. Hamel R., Vargas R.E.M., Rajonhson D.M., Yamanaka A., Jaroenpool J., Wichit S. et al. "Identification of the Tembusu virus in mosquitoes in Northern Thailand." *Viruses* **15**, 1447 (2023). https://doi.org/10.3390/v15071447
54. Ogunlusi O.S., Opayele A.V., Motayo B.O., Faneye, A.O. Detection of Flaviviruses in Mosquitoes from a Livestock Farm in Ibadan, Nigeria. *Res. Sq.* (2025). https://doi.org/10.21203/rs.3.rs-8271914/v1
55. Fontenille D., Traore-Lamizana M., Zeller H., Mondo M., Diallo M., Digoutte J.P. Rift Valley fever in Western Africa: isolations from Aedes mosquitoes during an interepizootic period. *Am. J. Trop. Med. Hyg.* **52**, 403–404 (1995). https://doi.org/10.4269/ajtmh.1995.52.403
56. Jupp P.G., Kemp A., Grobbelaar A., Leman P., Burt F.J., Alahmed, A.M., et al. The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Med. Vet. Entomol.* **16**, 245–252 (2002). https://doi.org/10.1046/j.1365-2915.2002.00371.x
57. Lumley S., Horton D.L., Hernandez-Triana L.L.M., Johnson N., Fooks A.R., Hewson R. Rift valley fever virus: Strategies for maintenance, survival and vertical transmission in mosquitoes. *J. Gen. Virol.* **98**, 875–887 (2017). https://doi.org/10.1099/jgv.0.000765
58. Armed Forces Pest Management Board, Office of the Assistant Secretary of Defense for Sustainment. Djibouti disease vector ecology profile (DVEP) (2021). https://www.acq.osd.mil/eie/afpmb/docs/dveps/Djibouti.pdf

Figures

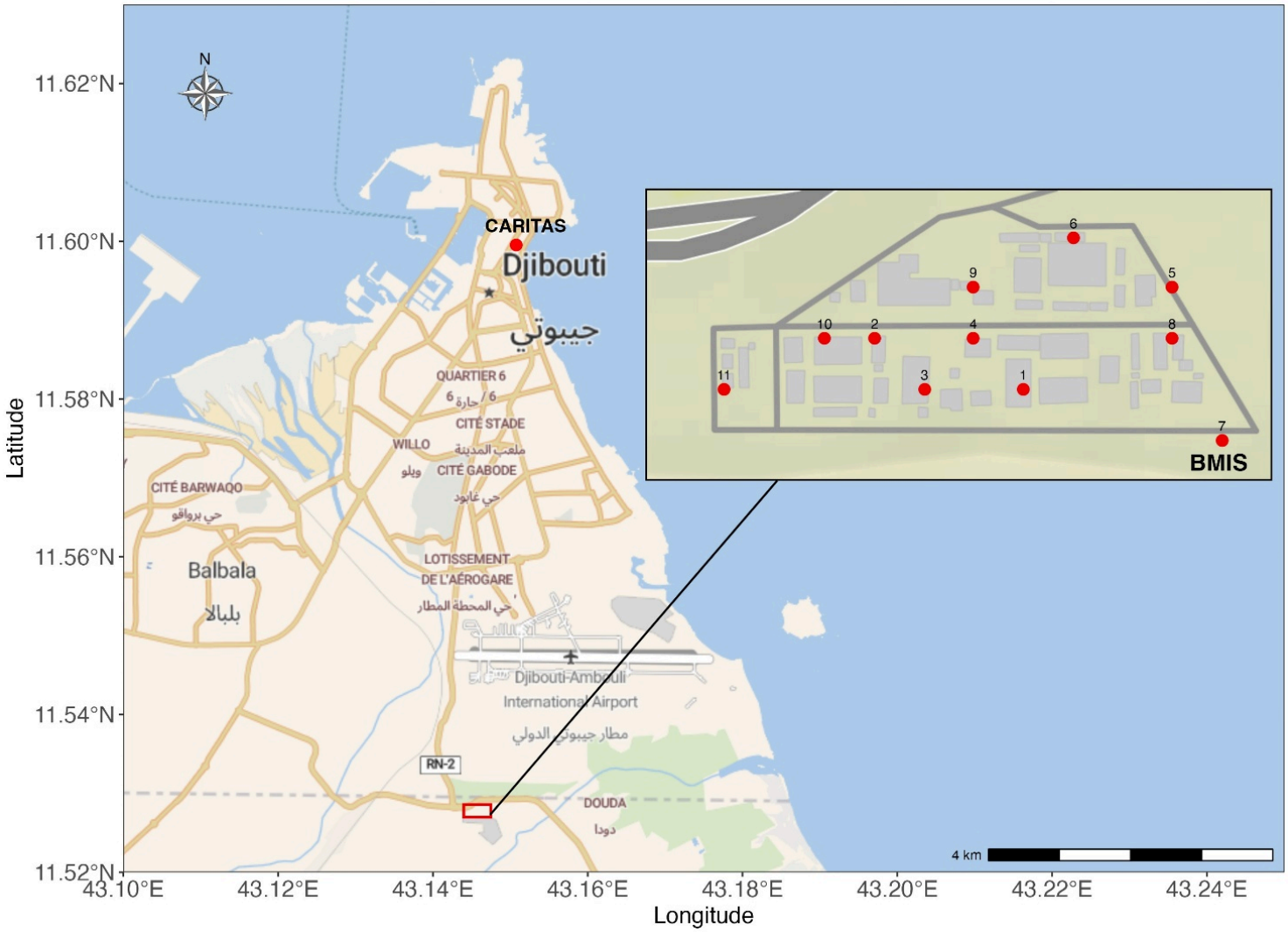


Figure 1

Study area showing sampling locations at the Italian Military Support Base (BMIS) and the city centre (CARITAS). Red dots represent trap locations.

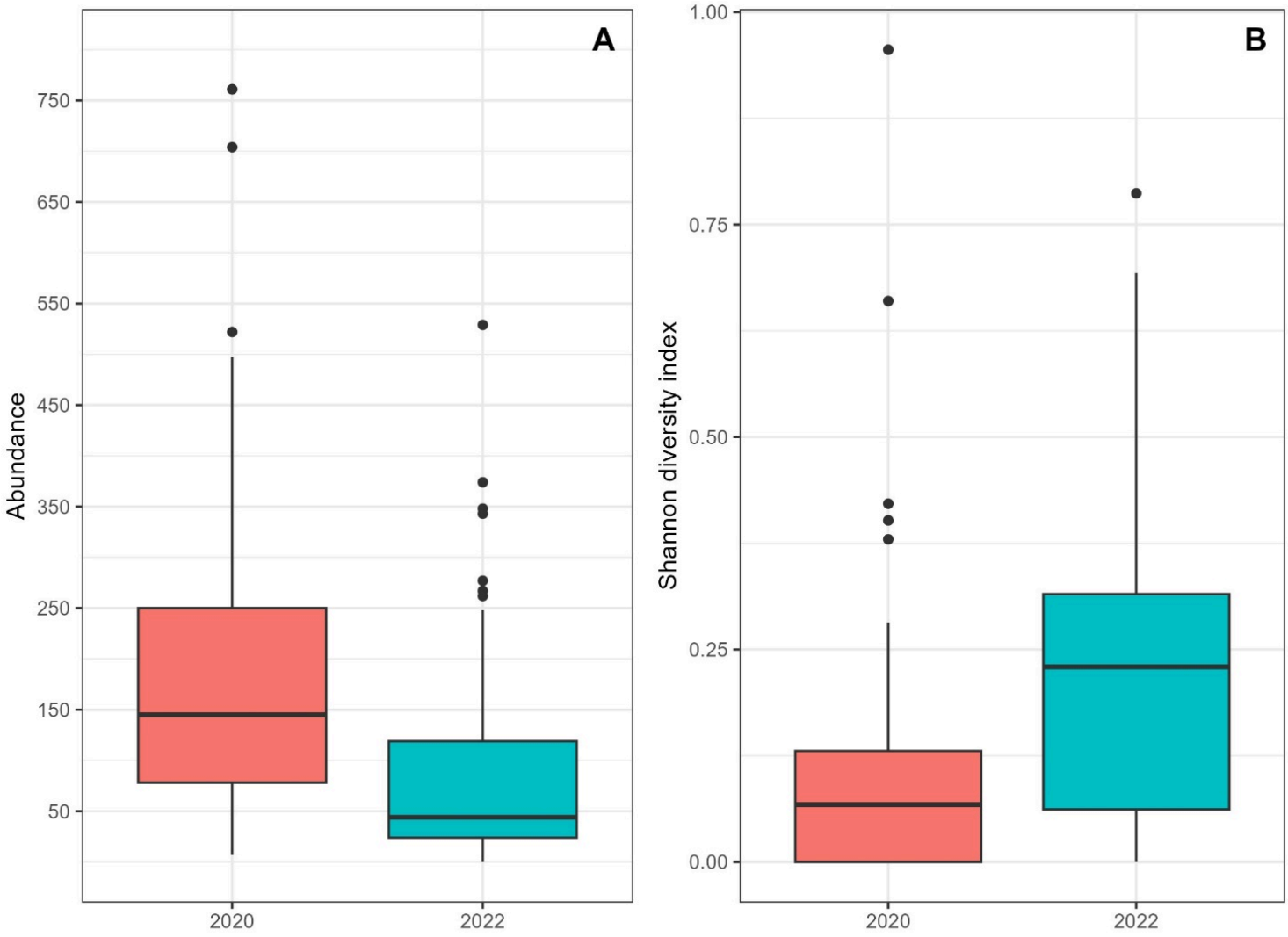


Figure 2

Ecological metrics reported for the mosquito population in the study area. The x-axis shows the year of collection, while the y-axis reports a) abundance expressed as the total number of mosquitoes, including both female and male identified at least at the genus level b) species diversity measured using the Shannon-Weaver index (H), with higher values indicating an increase in the number of species and higher values of evenness. The central line of Box plots represents mean values; whiskers show the minimum and maximum observed values; points represent outliers.

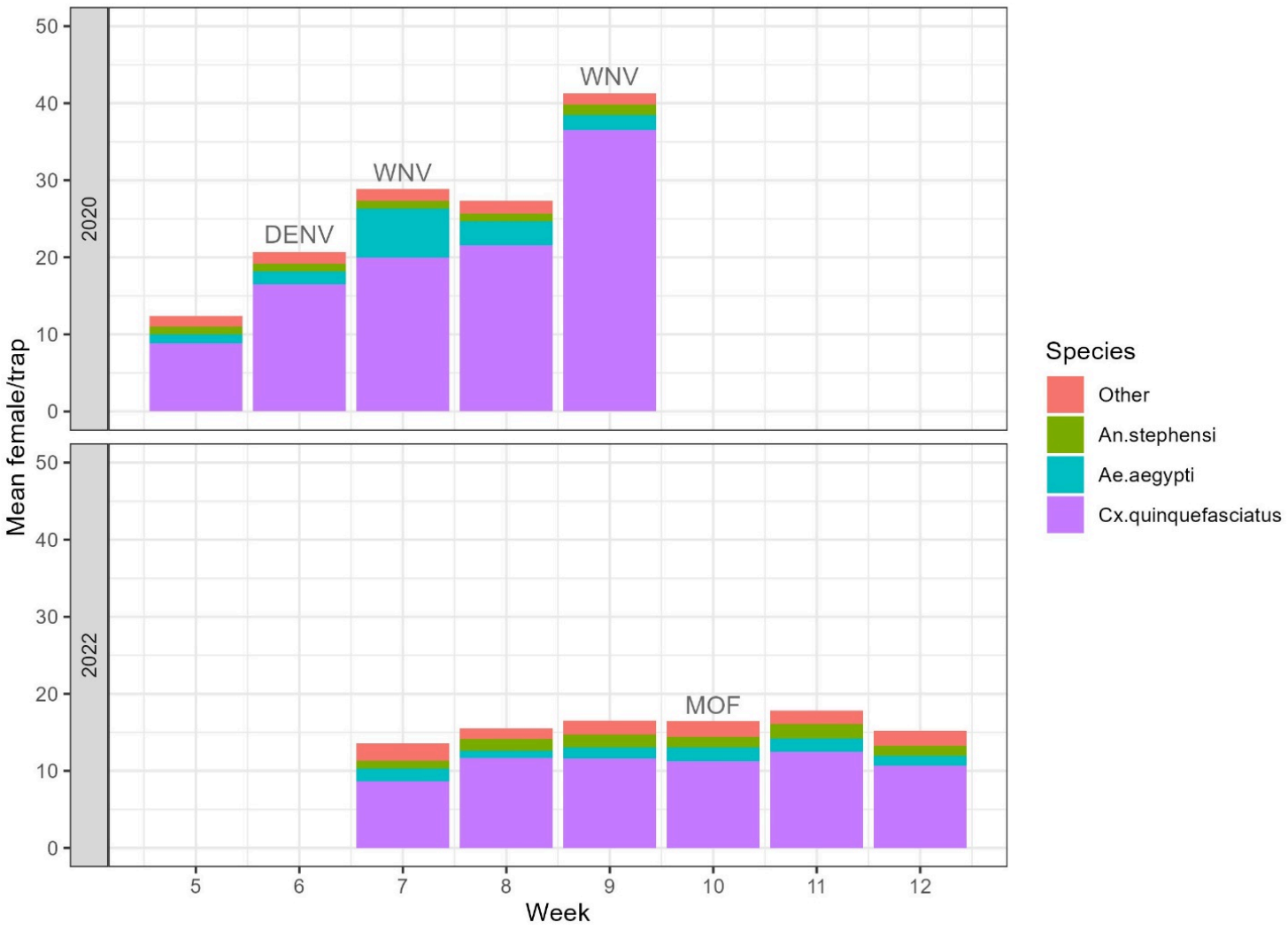


Figure 3

Mean number of female mosquito vector species collected per week during each sampling year. Virus detected from FTA cards is reported on the top of the bars (DENV: Dengue; WNV: West Nile; ISFV: mosquito Orthoflavivirus).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryfileS2.xlsx
- SupplementaryS1.docx