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Research Article

Vector Competence of a Coastal Population of *Aedes aegypti* for Dengue 2 and 3 Virus Serotypes in Kenya

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Aedes aegypti is the primary vector of dengue, an arboviral disease caused by dengue virus (DENV) that exists as four distinct serotypes (DENV 1-4). While all four DENV serotypes circulate in Kenya, differential distribution of the serotypes in specific regions suggests virus transmission may differ among local vector populations. In this study, we tested the hypothesis that a coastal *Ae. aegypti* population (Rabai, Kilifi County) varies in its ability to transmit DENV-2 (predominant) and DENV-3 (less dominant) and that transmission is related to *Ae. aegypti* subspecies—domestic *Ae. aegypti aegypti* (Aaa) and sylvtic *Ae. aegypti formosus* (Aaf). We orally exposed F1 females (3-10 days old) to blood meals containing DENV-2 ($10^{5.30}$ pfu/ml) or DENV-3 ($10^{5.13}$ pfu/ml), tested them individually for infection (body), dissemination (legs), and transmission (saliva) at 7, 14, and 21 days postinfection (DPI), respectively, and compared the rates between the serotypes. We analyzed cytochrome c oxidase I gene (*cox-I*) sequences among DENV-susceptible and nonsusceptible cohorts. Of 489 mosquitoes tested (DENV-2: 240; DENV-3: 249), we found consistently higher but nonsignificant rates of infection (16% vs. 10%), dissemination (47% (18/38) vs. 35% (9/26)), and transmission (39% (7/18) vs. 11% (1/9)) for DENV-2 than DENV-3. However, DENV-2 exhibited a shorter extrinsic incubation period (EIP) for disseminated infection (7-DPI vs. 14-DPI) and transmission (14-DPI vs. 21-DPI) compared to DENV-3. Two *cox-I* lineages were recovered in phylogeny, one predominantly clustered with referenced Aaa and a minor lineage grouped with Aaf. Infected mosquitoes and those with disseminated infection were represented in both lineages; those that transmitted the viruses grouped with the Aaa-associated lineage only. We conclude that the coastal *Ae. aegypti* population is a competent vector for DENV-2 and DENV-3 likely driven by the domestic Aaa that is predominant. The shorter EIP to attain dissemination and transmission for DENV-2 could favour its transmission over DENV-3.

1. Introduction

Dengue fever (DEN) is the most prevalent and important arboviral disease that threatens about half-billion people globally [1, 2]. The disease has recently spread to new regions or countries with no prior history of outbreaks [3]. In some endemic areas, sporadic dengue outbreaks are being reported yearly. Dengue infection in humans may present with a mild flu-like illness or severe symptoms of dengue hemorrhagic fever and dengue shock syndrome [4]. The spread, reemergence, and persistence of dengue make it one of the most important diseases that require global attention.

Dengue fever is caused by dengue virus (DENV) which belongs to the genus *Flavivirus* and family *Flaviviridae*. It exists as four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) [5] and is primarily transmitted by female *Aedes aegypti* mosquitoes widely distributed in tropical and sub-Saharan countries [6, 7]. In the recent past, the epidemiology of dengue in Africa was poorly characterized as the continent experienced fewer

outbreaks and epidemics. However, incidences and outbreaks of dengue are on the rise with almost all regions reporting DENV infections involving all the known serotypes [8–10]. According to Sang et al. [11], these changes in epidemiology could be attributed to human behaviors and activities such as land-use practices, deforestation, and agricultural activities as well as urbanization, which promotes human-vector interactions and potential spillover of dengue transmissions to urban and rural settings.

The mosquito *Ae. aegypti* is largely distributed in large portions of Saharan and sub-Saharan Africa [12], and it is known to exist in two subspecies: the domestic *Ae. aegypti aegypti* and the sylvatic form *Ae. aegypti formosus* [13, 14]. *Aedes aegypti* populations from different geographical regions exhibit varying levels of susceptibility to DENV serotypes [15–17], and according to Gonçalves et al. [18], variation can also exist even among populations within a city of a country.

Kenya experienced the first DEN outbreak in 1982 [19] in which DENV-2 was involved. In 2011-2014, 2017-2018, and 2019, some areas of Mombasa and the Northeastern region experienced outbreaks from which DENV-1, DENV-2, and DENV-3 [8, 20, 21] were detected with DENV-1 and DENV-2 as the predominant serotypes and also responsible for the highest number of cases [8]. A recent study by Shah et al. [10] on children presenting with febrile illness in Kenya detected all four dengue serotypes (DENV1-4) in circulation. Entomological surveillance has detected all four DENV serotypes in field-collected *Ae. aegypti* populations from different countries [22–25]. Evidence of transovarial transmission based on detection of the virus in immature stage of both sexes of *Ae. aegypti* as well as in adult males has also been reported [26, 27]. However, information on serotype infections in wild *Ae. aegypti* mosquitoes from Kenya remain scanty.

Global studies have reported varying levels of competence of *Ae. aegypti* to all four dengue serotypes including strains/populations from selected African countries [15, 28, 29]. In Kenya, few studies have examined the vector competence of the local *Ae. aegypti* populations in transmitting the most dominant serotype DENV-2 [30, 31] but similar data for other serotypes are lacking. Given the distinct serotype distribution reported in the country in different regions [8, 10, 21], it is not known whether the local vector is preferentially adapted to transmitting one serotype over the other including the influence of vector genotypes. To date, the genetic underpinning of DENV transmission is attributed to differences in *Ae. aegypti* subspecies known to occur in sympatry in parts of Kenya including the coastal region [30, 32] which is only beginning to be appreciated.

The coastal region continues to experience low-level outbreaks and incidences, with the most recent outbreak occurring in 2021 [33]. The region remains at risk of the introduction of more virulent strains and/or serotypes of DENV [5] from other endemic parts of the world due to travel, and the presence of *Ae. aegypti* [30, 31] and favorable climatic conditions for transmission [30]. In the context of this changing epidemiological landscape, this study investigated as objectives: (1) the vector competence of the *Ae. aegypti* population from the Coastal region of Kenya to DENV-2 and 3 serotypes and (2) the potential association between the genetic forms of *Ae. aegypti* and transmission of the virus serotypes.

2. Materials and Methods

2.1. Ethical Considerations. The study was approved by the Kenya Medical Research Institute Scientific and Ethics Review Unit (KEMRI/SERU/CVR/013-2020/4210). The use of mice was reviewed and approved by the KEMRI Animal Care and Use Committee (ACUC/01.07.2021). All procedures were performed following the institution's guidelines and standards.

2.2. Study Area. Aedes aegypti eggs were collected from three nearby villages of Rabai, Kilifi County, in the coastal region of Kenya including Bengo, Chang'ombe, and Mbarakani (Figure 1). Rabai was selected due to its history with DENV-2 circulation [19]. It is located 12 miles (24.5 km) northwest of Mombasa city (latitude: 3.63° S and longitude: 39.85° E). The area experiences a moderately hot climate throughout the year, with an average temperature of about 30°C and relative humidity of 80-82% and an average rainfall of approximately 88.25 mm per month. The majority of houses in Rabai have walls that are either cemented, made of stones, or mud with roofing of either iron sheets or thatched grass. Water storage is common practice among households.

2.3. Mosquito Collections. Mosquito eggs were collected using oviposition cups in August 2019. The eggs were collected near human dwellings and in nearby forested areas. Ovicups were lined with oviposition papers, filled with water halfway, and set ~40 meters apart. After 4 days, the papers containing eggs were collected, dried, and folded into zip lock bags containing cotton wool and transported to BSL-2 Insectary at Kenya Medical Research Institute (KEMRI) in Nairobi, Kenya. The eggs were reared to adults in an insectary maintained at a temperature of 30°C, 80% relative humidity, and a 12 hr photoperiod [34] and fed with 6% glucose solution. *Aedes aegypti* females were identified using a dissecting microscope and taxonomic keys [35] and fed twice every week for up to a month, on clean laboratory-bred mice to obtain the F1 eggs which were hatched and reared into adults used in the experiment.

2.4. DENV Propagation. DENV-2 and DENV-3 serotypes were obtained from frozen isolates at the Viral Hemorrhagic Fever laboratory at KEMRI, Kenya. DENV-2 (sample number: 008/01/2012) was previously isolated from a patient in Mandera, Kenya, during an outbreak in 2012 and had been passaged 5 times [31]. DENV-3 (sample number: 110/3/2019) was isolated from a patient in Mombasa, Kenya, during an outbreak in 2019 [21] and had been passaged only once. DENV-2 was chosen because it is associated with most outbreaks [12] and is also believed to be the predominant serotype, alongside DENV-1 in human circulation. DENV-3 was chosen because it is believed to be the least predominant in human circulation [8]. The DENV serotypes were inoculated separately onto C6/36 cells grown in growth media composed

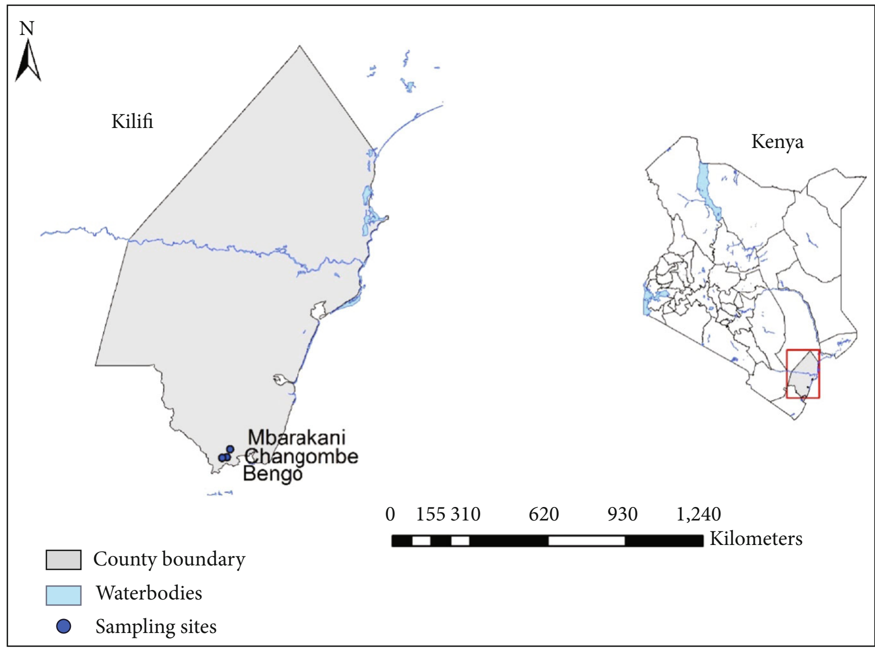

Figure 1: Map showing *Aedes aegypti* sampling sites in Kilifi County. The three sites are represented by blue dots.

of minimum essential medium (MEM) (Sigma Aldrich), 10% heat-inactivated fetal bovine serum (FBS) (Sigma Aldrich), 2% L-glutamine solution (Sigma Aldrich), 2% antimycotic/antibiotic solution (Sigma Aldrich), 0.4% of 100X MEM nonessential amino acids (Sigma Aldrich), and a reduced amount of sodium bicarbonate ($NaHCO_3$) [31]. Briefly, the viruses were thawed on wet ice and the medium aspirated from the T-25 flasks. 250 μl of each virus was gently pipetted onto a monolayer of cells in separate flasks and incubated at 28°C for 1 hour and rocked every 15 minutes. The cells were then maintained with a maintenance medium (composed of a similar concentration of reagents as above but with a 2% FBS) and incubated at 28°C in a 5% CO_2 incubator for up to 14 days. A control flask was treated using similar procedures but inoculated with a maintenance medium instead of a virus. The flasks were observed daily for any cytopathic effects (CPE) or changes in cell morphology. The flasks were frozen at -80°C for 24 hours once 70-80% CPE had been observed and the viruses harvested by thawing on wet ice and clarified by centrifugation at 1500 rpm. The clarified virus supernatant was aliquoted in cryovials and stored at -80°C [34]. DENV-2 was later passaged once on Vero cells (ATCC CCL-81) grown and maintained with media at 37°C and 5% CO_2. DENV-3 was passaged three times on C6/36 cells and one time on Vero cells. The viruses were then quantified and amplified for use in the experiment.

2.5. DENV Quantification

2.5.1. Quantification of DENV-2 by Plaque Assay. Plaque assay was performed using a protocol modified from Baer et al. [36]. Briefly, a tenfold serial dilution was prepared, and 100 μl of each dilution was inoculated onto confluent Vero cells grown 24 h earlier in 12-well plates. The plates were incubated at 37°C and 5% CO_2 for 1 hour and rocked every 15 min and maintained with 2% methylcellulose mixed with 2X MEM. The plates were incubated at 37°C in a 5% CO_2 incubator for up to 10 days while plaque formation was monitored. The media was then carefully aspirated and the cells fixed with 10% formalin solution for 24 h. The cells were then stained with 0.5% crystal violet overnight and washed gently with tap water. The plaques were counted, and the plaque-forming units (pfu) were calculated using the formula as shown below.

$$\frac{\text{pfu}}{\text{ml}} = \frac{\text{Number of Plaques}}{d \times V}, \quad (1)$$

where d represented the dilution factor while V represented the volume of the diluted virus to the wells.

2.5.2. Quantification of DENV-3 by Tissue Culture Infectious Dose-50 (TCID-50). Quantification of dengue-3 was performed using TCID-50 instead of plaque assay as the strain used could not produce visible plaques. TCID-50 was performed using a protocol modified from Li et al. [37]. Briefly, Vero cells (CCL-81) were seeded in a 96-well plate and incubated overnight at 37°C in 5% CO_2. Tenfold serial dilutions were prepared in maintenance media, and 100 μl was added to the wells. Virus adsorption was allowed to proceed for 2 hours. The wells of the last two columns of the plate were left without adding the virus dilutions as they acted as the controls. The cells were incubated under the same conditions and observed for CPE under an inverted microscope for 14 days. The 50% tissue culture infectious dose was then calculated as described by Reed and Muench [38]. The final value of TCID-50/ml was multiplied by a constant value of 0.69 to obtain the viral titer in pfu/ml for comparison to the DENV-2 titer.

2.5.3. Mosquito Oral Infection with DENV. Infections were performed on 3-10 day old F_1 field-collected female mosquitoes starved for 12 hours, on infectious blood meal using the Hemotek 6W1 membrane feeding system (Hemotek Ltd, Blackburn, UK). Briefly, a uniform mixture of infectious blood meal was prepared by mixing prewarmed defibrinated sheep blood (obtained from Kabete Veterinary Laboratories) and dengue virus (DENV-2 with a titer of $10^{5.30}$ pfu/ml and DENV-3 with a titer of $10^{5.13}$ pfu/ml) in a ratio of 1 : 1. A freshly prepared mouse skin membrane was fitted to cover the Hemotek reservoir and 2 ml of infectious blood dispensed into it. The reservoir was then screwed to the FU_1 feeder of the Hemotek system and allowed to warm at 37°C for 1 minute. The FU_1 feeder was then placed on the cages holding the female mosquitoes and allowed to feed for 30 minutes. Aliquots of the infectious blood before and after the feeding were collected and stored at -80°C until quantified using the procedures above. Fully fed mosquitoes were transferred to clean cages and provided with a 6% glucose solution. They were maintained at 30°C, 80% RH, and a 12 hour photoperiod for up to 21 days. A total of 240 female mosquitoes were exposed to blood meal mixed with DENV-2 while 249 were exposed to blood meal mixed with DENV-3.

2.5.4. Sampling of Exposed Mosquitoes for Virus Assay. Samples of the mosquitoes were picked at 7-, 14-, and 21-DPI and cold-aestheticized at -20°C for 1 minute and transferred into a glass beaker placed on wet ice. The legs and wings were removed under a dissecting microscope and placed in prelabelled Eppendorf tubes containing 350 μl of homogenization medium (HM, supplemented with 15% FBS). A single leg was also collected from each mosquito for DNA extraction and genetic analysis. The proboscis of the live immobilized bodies of the mosquitoes was inserted into a capillary tube containing 10 μl homogenization media and allowed to salivate for 30 minutes, and the contents of the capillary tube were expelled into 200 μl HM in Eppendorf tubes. The bodies of the mosquitoes were then placed in Eppendorf tubes containing 350 μl HM. The samples were stored at -80°C until screened for viruses [39].

2.6. Analysis of Sampled Mosquitoes for Infection, Dissemination, and Transmission

2.6.1. By Cell Culture. Each mosquito was screened for DENV infection, dissemination, and transmission. The bodies of the mosquitoes were first screened followed by the legs of the infected mosquitoes and, subsequently, the saliva samples for those with disseminated infections. To determine the infection and dissemination characteristics, the bodies and the leg samples were mechanically ground using copper beads (BB-caliber airgun shot) and Minibeadbeater (BioSpec Products Inc, Bartlesville, OK 74005 USA), and the samples were clarified through centrifugation at 12000 rpm for 10 minutes. 50 μl of the body, leg, and saliva samples were inoculated onto freshly grown Vero (ATCC CCL-81) cells in 24-well cell culture plates, maintained with maintenance media, incubated under similar conditions as described earlier, and monitored for CPE for up to 14 days. The presence of virus in DENV-2-positive samples was confirmed through plaque assay. DENV-3-positive samples in the wells were harvested and passaged three times on Vero cells and the presence of the virus was confirmed through RT-PCR.

2.6.2. By Reverse Transcriptase-Polymerase Chain Reaction (RT- PCR). The presence of the DENV-3 virus was confirmed by RT-PCR using a modified method described by Konongoi et al. [8]. Viral RNA was extracted using the QIAamp viral RNA (Qiagen) kit following the manufacturer's procedures. A final volume of 60 μl eluted was used as a template for cDNA synthesis and the subsequent PCR reactions. A cDNA reaction was prepared by adding a mixture of 2 μl of random hexamer (50 ng/μl) and 1 μl of dNTPs into 10 μl of the cDNA sample in a 0.2 μl PCR tube. The mixture was incubated at 65°C for 5 minutes in a thermocycler before the following components were added to the contents of the tube: 5 μl of 5X First-Strand Buffer (Invitrogen), 2 μl DTT (100 mM), 0.25 μl of RNase Out (40 U/μl), and 1 μl of Superscript III Reverse Transcriptase (200 U/μl). The mixture was then returned to the thermocycler with the following conditions set: 25°C for 5 minutes, 50°C for 40 minutes, 70°C for 15 minutes, and a hold temperature of 4°C. A total of 20 μl of cDNA was obtained. The PCR amplification targeting the DENV-3 sequences in the cDNA was performed in a 25 μl containing the following components: 12.5 μl of Amplitaq Gold PCR solution (Applied Biosystems, USA), 9.5 μl RNase-free PCR water, 0.5 μl each of forward (D1: 5′-TCAATATGCTGAAACGCGCGAGAAACCG-3′) and reverse (D3TS3: 5′-TAACATCATCATGAGACAG AGC-3′) [40] and 2 μl of cDNA sample. A DENV-3-positive control cDNA and negative control (PCR water) were included during the setting up of the PCR reaction which was performed using the following conditions: 95°C for 10 min, 35 cycles of (95°C-30 sec, 55°C-30 sec, and 72°C-20 sec), 72°C for 7 min, and 4°C hold temperature. The PCR products were electrophoresed in 1.5% agarose gel in 1% Tris-borate EDTA buffer stained with ethidium bromide and visualized using a UV transilluminator and recorded using GelDoc systems.

2.7. Analysis of Aedes aegypti Genetic Differentiation

2.7.1. DNA Extraction. To assess the possible genetic variation between the DENV positive and negative *Ae. aegypti*, genomic DNA was extracted from the legs using a modified ammonium acetate protein precipitation method as described by Adam et al. [41]. Briefly, the leg samples were homogenized using 1 copper bead (BB-caliber airgun shot) and Minibeadbeater (BioSpec Products Inc., Bartlesville, OK 74005 USA) for 20 seconds and 300 μl of cell lysate buffer (10 mM Tris-HCL, pH 8.0, 0.5% SDS and 5 mM EDTA) added and incubated for 90 minutes at 65°C. 100 μl of protein precipitate solution (8 M ammonium acetate and 1 M EDTA) was then added to each sample, vortexed for 30 seconds, and incubated in wet ice for 30 minutes. The samples were then centrifuged at 14000 rpm for 15 minutes

at 4°C, and the supernatant was transferred to new 1.5 Eppendorf tubes containing 300 μl of isopropanol and vortexed by inverting 100 times. The samples were then centrifuged at 14000 rpm for 30 minutes, the supernatant discarded, and 300 μl of ice-cold 70% molecular grade ethanol added. The samples were mixed by inversion and centrifuged at 14000 rpm for 30 minutes and ethanol discarded. The DNA pellet in the tubes was air-dried for 2 hours and resuspended in 100 μl of PCR-grade water. The genomic DNA was amplified by PCR using established primers [42] which targeted the 860 bp fragment of the Cytochrome Oxidase Subunit 1 (*cox-1*) mitochondrial gene. Briefly, a PCR master mix containing 11.6 μl of RNase-free PCR water, 4 μl of MyTaq buffer, 0.4 μl MyTaq polymerase enzyme, 1 μl of both forward and reverse *cox-1* primers, and 2 μl of the template was placed in a PCR thermocycler set at the following cycling conditions: 95°C for 3 min, 40 cycles of (95°C-20 sec, 58°C-30 sec, and 72°C-20 sec), 72°C for 7 min, and 4°C hold temperature.

The amplicons were visualized on 1.5% agarose gels stained with ethidium bromide solution. The PCR products were purified using SureClean Plus (Bioline Reagents Ltd, UK) using the manufacturer's protocol. Unidirectional sequencing (forward strand) was outsourced from Macrogen Co., Netherlands. Sequences were viewed and edited in Chromas and then analyzed in MEGA X software [43]. BlastN searches were used to identify homologous sequences from the GenBank database. The sequences were then aligned using ClustalW in the MEGA software to reference *cox-1* gene sequences for the domestic Aaa and sylvatic Aaf. The T92+G model of sequence evolution was used to infer a maximum likelihood (ML) tree in the Mega X software. Nodal support was assessed through 1000 bootstrap replications for ML.

2.8. Data Analysis. Infection rate (IR) was estimated as the number of mosquitoes with infected bodies among the total number of mosquitoes tested; dissemination rate (DR) represented the proportion of mosquitoes with DENV in legs among the total number with infected bodies; transmission rate (TR) was calculated as the proportion of mosquitoes with DENV in saliva among the total number with infected legs, and transmission efficacy (TE) estimated as the ratio of the number of mosquitoes with DENV in saliva to the number tested. The IR, DR, TR, and TE were examined at 7, 14, and 21 days postinfection and compared between DENV serotypes and across DPI for each serotype using Pearson's chi-squared test or Fisher's exact test. Bonferroni correction [44] was applied for multiple comparisons. p values of above 0.05 were considered nonsignificant. Data were entered into Microsoft Excel 2019 and analyzed statistically in R software v.4.2.1 [45] with a gtsummary package [46].

3. Results

3.1. Susceptibility to DENV Infection. Out of 489 mosquitoes tested, 64 were susceptible to either DENV-2 or DENV-3. Infection rates were 16% (38/240) and 10% (26/249) for DENV-2- and DENV-3-exposed mosquitoes, respectively (Tables 1 and 2). Each DENV serotype was detected in the abdomens of the mosquitoes across all DPI. IR for DENV-2 exposed mosquitoes was consistently higher than for the DENV-3 across the DPI (Figure 2). Higher rates were recorded at 14-DPI (DENV-2: 22% and DENV-3: 17%) while lower IR was observed at 7-DPI (DENV-2: 9.2% and DENV-3: 2.2%). A statistically, significant difference was only observed between the IR across DPI of DENV-3-exposed mosquitoes (Fisher's exact test, $p = 0.005$; Tables 1 and 2) but not between the serotypes.

3.2. Dissemination of DENV in Infected Mosquitoes. Among the DENV-infected mosquitoes, 47% (18/38) tested positive for DENV-2 in the legs compared to 35% (9/26) for DENV-3-infected mosquitoes but the difference was not statistically significant. DENV-2 was detected as early as 7-DPI while DENV-3 was first detected at 14-DPI. The DR across the DPI for the two viruses increased increasing DPI (Figure 2(s)). Higher DR was recorded for each serotype at 21-DPI (72.73% and 44.44%) for DENV-2 and DENV-3 compared to 12.5% and 0% at 7-DPI for the two viruses, respectively (Figure 2). There were no significant differences in the dissemination rates between the two viruses at each DPI (Fisher's exact test, $p \text{ at } 7-\text{DPI} = >0.9$, $p \text{ at } 14-\text{and } 21-\text{DPI} = 0.4$). However, a comparison between the DR across the DPI for DENV-2-infected mosquitoes showed a significant difference with a p value of 0.034 (Tables 1 and 2).

3.3. Transmission Potential of the Ae. aegypti Population. Mosquitoes with DENV-2 disseminations had a TR of 39% (7/18) compared to 11% (1/9) for mosquitoes with DENV-3 in their legs (Tables 1 and 2; Figure 2). DENV-2 was detected in the saliva of the mosquitoes earlier at 14-DPI compared to DENV-3 which was detected only at 21-DPI. Similarly, to the DR, the TR showed an upward trend with increasing DPI for each serotype, with higher rates observed at 21-DPI (50% and 25% for DENV-2 and DENV-3 mosquitoes with leg dissemination, respectively). However, no significant differences were detected between the mosquitoes exposed to the two DENV serotypes across the DPI (Fisher's exact test: $p \text{ at } 14-\text{and } 21-\text{DPI} = 0.3 \text{ and } 0.6$, respectively) (Tables 1 and 2).

3.4. Transmission Efficiency (TE). DENV-2-exposed mosquitoes had an overall TE of 2.9% (7/240) compared to 0.4% (1/249) for DENV-3-exposed mosquitoes. The rates for DENV-2-exposed mosquitoes were 0%, 3.5%, and 5.9% at 7-, 14-, and 21-DPI, respectively, while only 1.4% of DENV-3-exposed mosquitoes at 21-DPI had the virus in their saliva. No significant difference was observed for the TE across the DPI for each serotype as well as between the DENV serotype-infected mosquitoes ($p > 0.05$).

3.5. Cytochrome c Oxidase Subunit I (COI) Variation among DENV-Susceptible and Nonsusceptible Exposed Mosquitoes. Phylogenetic analysis based on *cox-1*gene on mosquito samples susceptible (infection, dissemination, and transmission) and nonsusceptible (infection) by both DENV-2 and DENV-3 revealed two lineages; lineage I which grouped with referenced *Ae. aegypti aegypti* in the GenBank (Accession

Table 1: Infection, dissemination, and transmission rates of mosquitoes orally exposed to dengue virus 2 and 3.

Rates	7				14				21			
	DENV-2 $N = 87$[1]	DENV-3 $N = 90$[1]	p value[2]	q value[3]	DENV-2 $N = 85$[1]	DENV-3 $N = 90$[1]	p value[4]	q value[3]	DENV-2 $N = 68$[1]	DENV-3 $N = 69$[1]	p value[4]	q value[3]
IR	8 (9.2%)	2 (2.2%)	0.055	0.11	19 (22%)	15 (17%)	0.3	>0.9	11 (16%)	9 (13%)	0.6	>0.9
DIR	1 (12%)	0 (0%)	>0.9	>0.9	9 (47%)	5 (33%)	0.4	>0.9	8 (73%)	4 (44%)	0.4	>0.9
TR	0 (0%)	0 (NA%)			3 (33%)	0 (0%)	0.3	>0.9	4 (50%)	1 (25%)	0.6	>0.9
TE	0 (0%)	0 (0%)			3 (3.5%)	0 (0%)	0.11	0.4	4 (5.9%)	1 (1.4%)	0.2	0.8

[1] n (%). [2] Fisher's exact test. [3] Bonferroni correction for multiple testing. [4] Pearson's chi-squared test. Fisher's exact test.

Table 2: Continuation of Table 1.

Rates	DENV-2						DENV-3					
	n	7-DPI[1]	14-DPI[1]	21-DPI[1]	p value[2]	q value[3]	n	7-DPI[1]	14-DPI[1]	21-DPI[1]	p value[2]	q value[3]
IR	240	8 (9.2%)	19 (22%)	11 (16%)	0.061	0.2	249	2 (2.2%)	15 (17%)	9 (13%)	**0.005**	0.019
DIR	38	1 (12%)	9 (47%)	8 (73%)	**0.034**	0.14	26	0 (0%)	5 (33%)	4 (44%)	0.7	>0.9
TR	18	0 (0%)	3 (33%)	4 (50%)	0.8	>0.9	9	0 (NA%)	0 (0%)	1 (25%)	0.4	>0.9
TE	240	0 (0%)	3 (3.5%)	4 (5.9%)	0.058	0.2	249	0 (0%)	0 (0%)	1 (1.4%)	0.3	>0.9

[1] n (%). [2] Pearson's chi-squared test; Fisher's exact test. [3] Bonferroni correction for multiple testing. IR: infection rate (number of infected bodies/number tested); DR: dissemination rate (number of infected legs/number of infected bodies); TR: transmission rate (number of infected saliva/number of infected legs); TE: transmission efficiency (number of infected saliva/total number tested).

number: AF390098.2 and MF194022.1) and lineage 2 clustered with the forest ecotype *Ae. aegypti formosus* (GenBank Accession number: AY056597.1) (Figure 3). Out of 157 sequences analyzed, 139 clustered with the domestic Aaa suggesting its dominance in the mosquito population, while only 18 mosquitoes clustered with the sylvatic Aaf (Table 3; Figure 3). A total of 17 ($n = 21$; 81%) of DENV-2 susceptible mosquitoes belonged to lineage I while 19% (4/21) belonged to lineage II. For DENV-3 susceptible mosquitoes, 93% (13/14) belonged to lineage 1 while 7.1% (1/14) belonged to lineage II (Table 3). DENV nonsusceptible samples were also represented in both lineages (Table 3; Figure 3). Susceptible mosquitoes with disseminated infections were represented in both lineages; however, only the lineage that clustered with the domestic form (lineage I) contained susceptible mosquitoes that transmitted the viruses. The haplotypes generated in this study have been deposited in GenBank under accession numbers OP854892-OP854917.

4. Discussion

Assessing the competence of local vectors is a vital step in understanding the risk of arbovirus transmission and spread. Our study was designed to assess the relative vector competency of a coastal *Ae. aegypti* population for DENV-2 and DENV-3 serotypes. The mosquito responded similarly regarding susceptibility in the bodies, disseminated and transmitted infection to both serotypes, suggesting its competence of the population for both serotypes. The competence of coastal *Ae aegypti* to DENV-2 is consistent with previous literature where similar rates in these vector competence indices were observed [30, 31]. However, our data on DENV-3 constitutes novel data and first report regarding the competence of any *Ae. aegypti* population in Kenya.

Although the IR, DR, and TR did not differ between the serotypes statistically, the values were consistently higher for DENV-2 than DENV-3. Remarkably, disseminated and transmitted infections were detected early for DENV-2 (7- and 14-DPI, respectively) compared to DENV-3 (14- and 21-DPI, respectively). The incubating temperatures and mosquito background known to affect virus replication [30, 31, 47, 48] were the same, indicating that the observed difference is inherent in the virus serotypes given similarity in infectious titers used in the experiments. On the one hand, the finding suggests differences in progression of infection between the serotypes but also that DENV-2 exhibits a shorter EIP and longer persistence in the salivary glands, potentially contributing to high rates of human infection [49]. This trend has been observed for *Aedes albopictus* and Zika virus in Florida [50]. In fact, for both serotypes, the probability of transmission potential (i.e., having infection in the saliva expectorate) increased with increasing DPI. Further time series experiments to estimate the exact EIP for each serotype and quantify the viral titers in the saliva to glean on possible infectious doses are warranted.

A reduced EIP as observed for DENV-2 could impact the vectorial capacity (VC) of the mosquito. VC measures the transmission potential of vector-borne pathogens by a competent vector and incorporates both vector competence and EIP [14, 51–53]. EIP has an exponential relation with probability of daily survival of a mosquito, both crucial determinants of VC. Indeed, a reduction in EIP by a day has a marked increase in VC assuming constancy in other factors [49, 54]. According to Salazar et al. [49], a shorter EIP for dengue virus could have important epidemiological

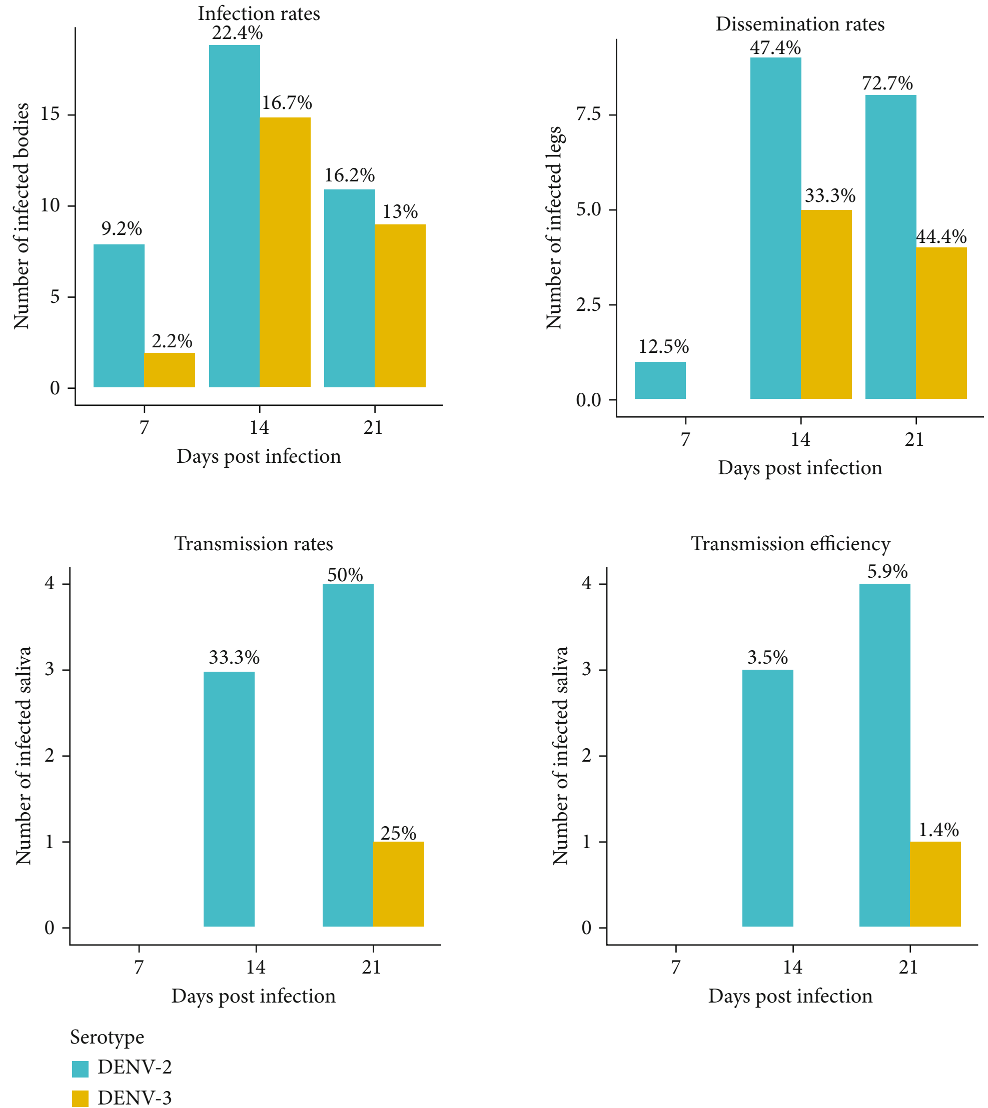

Figure 2: Bar plot showing the number of infected samples (n) and vector competence indices in percentage across DPI for *Aedes aegypti* from Coastal Kenya orally exposed with DENV-2 and DENV-3 serotypes. There were no significant differences between the challenges.

implications as there is a possibility that some mosquitoes may be able to transmit the virus to a host earlier during vector survival period. Thus, it is plausible the lower EIP could result in increased transmission potential of *Ae. aegypti* for DENV-2 over DENV-3. DENV serotypes or genotypes could be transmitted more efficiently over others through a process of competitive displacement [55, 56] through effect of higher viral titers in the saliva [57]. However, competitive displacement is only observed in coinfection studies of *Ae. aegypti* with multiple DENV serotypes, beyond the scope of the present study. Additional studies will benefit from assessing transmission efficiency of the mosquito to both serotypes in coinfection assays.

An important predictor of vector competence relates to the genotype or genetics of the local vector populations [58]. The subspecies of *Ae. aegypti* (Aaa and Aaf) are believed to exhibit differences in bionomic traits including competence for DENV [59, 60]. Our data show that susceptible mosquitoes with midgut and transmitted infections were represented in the two *cox-I* lineages containing both genetic forms that were recovered in phylogenetic analysis. Surprisingly, the susceptible *Ae. aegypti* that transmitted both viruses belonged to the *cox-I* lineage that clustered with the referenced domestic form, indicative of virus transmission associated with this genotype. This finding is inconsistent with a previous study that found equal distribution of susceptible mosquitoes among the forms in Kenya [30]. This study however was limited to infection in the midgut explaining the contrast with our findings. We note, however, that our results do not prove causality and further confirmation in experimental infection assays using established colonies of both forms are warranted. Nonetheless, our result is indicative of potential influence of genotype as an important determinant of the competence of this coastal population to dengue viruses. Increasing the risk of importation of dengue viruses through trade/international travel, vector competence

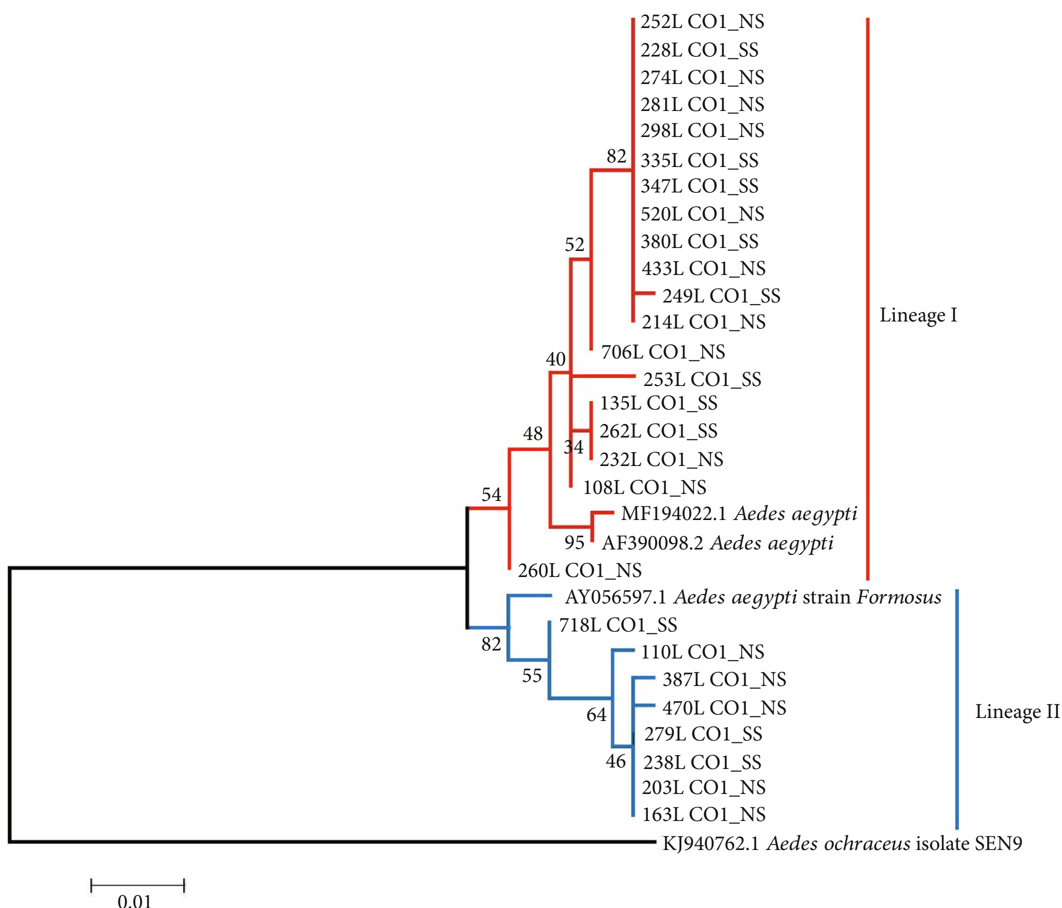

Figure 3: Maximum likelihood phylogenetic tree inferred using the T92+G as the best-fit model of evolution for COI barcode region (850 bp) of DENV-2/3 susceptible (SS) and nonsusceptible (NS) *Aedes aegypti* mosquitoes from Rabai, Kilifi, Kenya. The percentage of replicate tree in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Unique haplotypes of the 157 sequences were used during the construction. From these sequences, 139 belonged to cox-I lineage I while 18 belonged to lineage II. Referenced samples of the domestic and sylvatic forms are included. *Aedes ochraceus* was used as an outgroup.

Table 3: Summary of the genetic forms clustering with the referenced *Aedes aegypti aegypti* and *Aedes aegypti formosus*.

		DENV-2			DENV-3	
	N	Nonsusceptible	Susceptible	*N*	Nonsusceptible	Susceptible
Referenced genetic forms	83			74		
Ae. aegypti aegypti		57 (92%)	17 (81%)		52 (87%)	13 (93%)
Ae. aegypti formosus		5 (8.1%)	4 (19%)		8 (13%)	1 (7.1%)
[1]*n* (%)						

studies will be important to ascertain how well they adapt and spread by local vectors. Thus, it is important to extend similar assessments to other serotypes for potential risk in other areas.

5. Conclusion

Our findings demonstrate that the coastal *Ae. aegypti* population from Kenya is readily infected with both DENV-2 and 3 by oral route and can disseminate and transmit both viruses. With *Ae. aegypti* proven to be competent for DENV-2 in Kenya, findings on DENV-3 provide the first account of the transmission potential of the vector, with respect to other dengue serotypes in circulation. DENV-2 which could be exhibiting a shorter EIP in the vector could justify the frequency of outbreaks associated with it, though it needs to be studied further. Future studies on vector competence of the local vector for other serotypes should be undertaken in light of our findings to understand the transmission capabilities of the vector. The domestic form of the local *Ae. aegypti*, which is the most abundant in the population, could be driving dengue transmission in the region and thus further investigations with a plausible comparison between the two genotypes will provide insights into dengue outbreaks. Our findings, therefore, support the conclusion

that the local *Ae. aegypti* population in Kenya is competent to both DENV-2 and DENV-3. Going forward, continued surveillance of viral-vector interaction remains an important aspect in the development of effective vector and viral control measures.

Data Availability

The data supporting the conclusions of this article are included within the article. The haplotypes generated in this study have been deposited in GenBank under accession numbers OP854892-OP854917.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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