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1 **Running title**: Dengue virus serotypes co-circulation

2

3 # Co-circulation of Dengue Virus Serotypes 1 and 3 during the 2019 epidemic in
4 # Dar es Salaam, Tanzania

5

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28 **Abstract**

29 **Background**

30 Dengue is an important mosquito-borne viral disease in tropical and sub-tropical
31 countries. In this study molecular characterization was carried out to determine
32 dengue viruses circulating among patients at health facilities during 2019 epidemic in
33 Dar es Salaam, Tanzania.

34 **Methods**

35 The study involved outpatients seeking care for febrile illness at four health facilities
36 in Kinondoni and Ilala Districts of Dar es Salaam City in Tanzania. A total of 45 sera
37 from the outpatients were confirmed dengue-positive for dengue virus (DENV) non-
38 structural protein 1 (NS1) antigen and/or NS1-IgG/IgM antibodies using on-site rapid
39 test. The presence of the virus was detected by reverse-transcription polymerase
40 chain reaction (RT-PCR) method. Of the 45 sera, 20 samples were selected
41 randomly for identification of specific dengue virus serotypes using RT-PCR followed
42 by evaluation of resulting amplicons on agarose gel electrophoresis.

43 **Findings and significance**

44 Both Dengue virus serotypes 1 (DENV-1) and 3 (DENV-3) were detected in the
45 samples tested with the former being dominant. We present the first evidence of
46 dengue virus co-infection of DENV-1 and DENV-3 serotypes in Tanzania. The
47 emergence of DENV-1 indicates the possibility of importation of the virus to Tanzania
48 from endemic countries. Due to DENV serotype co-circulation, there is an increased
49 risk of severe dengue in future epidemics. Our findings advocate the importance of
50 genomic-based surveillance to provide rapid evidence of dengue virus
51 emergence/re-emergence and spread.

52 **Keywords:** Dengue virus, serotype, co-circulation, epidemic, Tanzania

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53 **Author Summary**

54 Dengue viruses are the most important mosquito-borne pathogens that pose a
55 serious global health threat. Tanzania has reported several dengue virus epidemics
56 since 2010 with the majority of the epidemics occurring in Dar-es-Salaam city. Until
57 August 2019, a total of 6,859 dengue cases have been confirmed in the country. We
58 performed molecular characterization of dengue viruses (DENV) circulating during
59 the 2019 epidemic phase. It was found that DENV-1 serotype was dominant during
60 the epidemic and two samples of the tested sera were co-infected by DENV-1 and
61 DENV-3 serotypes. These findings emphasize the importance of genomic-based
62 surveillance of dengue viruses in Tanzania to guide strategies for appropriate
63 interventions.

64

65 **Introduction**

66 In recent years, Dengue has become the major mosquito-borne viral disease in
67 tropical and subtropical regions affecting people of all age groups in over 128
68 countries with 50-100 million infections annually [1]. In Africa, dengue is endemic in
69 34 countries with recent epidemics reported mostly in East Africa [2]. Recent dengue
70 outbreaks have been reported in Tanzania, Mozambique, Ghana, Benin, Ivory Coast
71 and Mauritius [3]. Population growth, increased rate and volume of movement of
72 people, uncontrolled urbanization and climate variability/change have been
73 described to be responsible for global spread of dengue virus (DENV) [4].

74 In Tanzania, dengue was first described in 1823 by Spanish sailors in the
75 southern coastal region [5]. Since 2010, four dengue outbreaks have been reported
76 in Tanzania [6–9]. To date, the 2019 epidemic seems to be the worst one in the
77 country. From January to August 2019, a total of 6,859 dengue fever cases and 13
78 deaths have been reported in the country with a large proportion from Dar es Salaam

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79 region. Other affected regions include Tanga, Pwani, Lindi, Morogoro, Kagera,
80 Arusha and Ruvuma [9,10].

81 There are five antigenic distinct serotypes of dengue virus (DENV-1 to 5)
82 [11,12] that can cause mild dengue fever (DF) to life-threatening dengue
83 haemorrhagic fever (DHF) and dengue shock syndrome (DSS) often presented with
84 atypical clinical manifestations like organ impairment [13,14]. Since 1960, four DENV
85 serotypes (DENV-1 to 4) have been reported in Africa with DENV-2 responsible for
86 most of epidemics followed by DENV-1 [15]. In Tanzania, the presence of DENV-3
87 and DENV-2 serotypes have been documented during the previous epidemics [6–
88 8,15].

89 Although dengue is endemic in Africa, molecular epidemiology of circulating
90 DENV is not well documented [17,18]. The increasing number of dengue outbreaks
91 and improved access to laboratory diagnostic tools have allowed for more effective
92 recognition of outbreaks through genomic-based surveillance [17,19]. The genomic-
93 based surveillance can help to identify the specific pathogen causing epidemics,
94 track sources and inform the public health authorities for appropriate interventions.

95 In Tanzania, few studies have reported molecular epidemiology of dengue
96 virus [6,7]. In 2014, Jaswant performed partial sequencing of core pre-membrane
97 junction region of DENV detected in patients during the 2013-2014 outbreak in Dar-
98 es-salaam region and confirmed that DENV-2 serotype genetically close to isolates
99 reported in China and Singapore was responsible for the epidemic [20]. However,
100 genomic-based data are missing for DENV detected in the country during the
101 subsequent epidemics in 2018 and 2019. The objective of this study was to perform
102 molecular characterization of dengue viruses during the 2019 epidemic phase in
103 Dar-es-Salaam, Tanzania.

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104 **Methods**

105 **Study area**

106 This cross-sectional health facility-based study was carried out in Kinondoni and Ilala
107 districts in Dar-es-Salaam, Tanzania (Fig 1). Dar-es-Salaam is usually hot and humid
108 throughout the year with the main dry season from June to September and the short
109 rainy season from October to December followed by long rainy season between
110 March and May. The average daily temperature is 26 ºC and total annual rainfall
111 averages 1100 mm with relative humidity of 100% and 60% during the night and day
112 time, respectively [21].
113

114 **Patients and sample collection**

115 Dengue-suspected patients were recruited from outpatients at four health facilities,
116 International School of Tanganyika (IST) Clinic and Premier Care Clinic in Kinondoni
117 and Doctors Plaza Hospital and Regency Medical Centre in Ilala district between
118 March and May 2019. A total of 110 dengue-suspected outpatients were enrolled in
119 the study. The inclusion criteria included patients with dengue-like illness and fever
120 (body temperature > 38 °C) prior to onset of clinical manifestations presenting with at
121 least one of the following clinical manifestations namely, retro-orbital pain, rash,
122 arthralgia, malaise, signs of haemorrhaging and organ failure. Fever patients with
123 bacterial infections and those unwilling to participate in this study were excluded.
124 Haematology data were obtained from routine blood tests performed by the
125 hospitals. All dengue positive cases were categorized clinically either as dengue
126 fever (DF), dengue haemorrhagic fever (DHF), dengue shock syndrome (DSS), or
127 expanded dengue syndrome (EDS), according to the World Health Organization
128 guidelines [22].

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129 Peripheral venous blood was collected into serum separator tubes for serum
130 separation and testing. The on-site duo dengue NS1-IgG/IgM rapid test (CTK
131 BIOTECH Inc, CA, USA) was used to detect the presence of NS1 antigen and
132 IgG/IgM antibodies against DENV NS1. Aliquots of a total of 45 positive sera
133 samples were kept in well-labeled cryotubes stored at -80 ºC. Thereafter, sera
134 samples were transported in a cool box with dry-ice to Sokoine University of
135 Agriculture (SUA) molecular biology laboratory in Morogoro and kept at -80 ºC until
136 genomic analysis.
137
138 **RNA extraction**
139 Out of 45 sera samples, we focused on 20 samples that were selected randomly. A
140 total of 60 μL viral nucleic acid (RNA) was extracted from 140 μL of each test sample
141 using QIAamp RNA mini kit (Qiagen, Hilden, Germany) following the manufacturer's
142 instructions. The extracted RNA was stored immediately in aliquots at -20 ºC to avoid
143 freeze-thawing cycles that could damage RNA stability. We also assessed the
144 quality of extracted RNA by measuring absorbance at 260 and 280 nm using the
145 NanoDrop ND1000 spectrophotometer (GE Healthcare UK Limited,
146 Buckinghamshire, UK). The A260/280 ratio of ~2.0 was accepted as pure RNA [23].
147
148 **Detection of DENV RNA**
149 Dengue virus nucleic acid (RNA) was detected by RT-PCR using SuperScript III
150 Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA) One-Step RT-PCR
151 following the manufacturer's instructions. RT-PCR amplification was performed with
152 primers as previously described [24]. We prepared a 25 μL RT-PCR reaction
153 containing 12.5 μL of 2x reaction mix, 1 μL of SuperScript III RT/Platinum *Taq* mix,

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154 0.5 µL of 10 µM sense primer (D1), 0.5 µL of 10 µM anti-sense primer (D2), 0.5 µL
155 Magnesium salt (Invitrogen, CA, USA), 6 µL of nuclease-free water and 4 µL of RNA
156 template. Reverse-transcription reaction was performed in 1 cycle at 48 ºC for 30
157 minutes, followed by one cycle of initial denaturation at 94 ºC for 2 minutes. PCR
158 amplification was done for 35 cycles of denaturation at 94 ºC for 15 seconds,
159 annealing at 55 ºC for 30 seconds and elongation at 68 ºC for 1 minute. This was
160 followed by a final extension at 68 ºC for 5 minutes. The RT-PCR products with the
161 expected band size 511 base pairs (bp) were evaluated on 1.5% agarose gel stained
162 with Gel Red (Biotium Inc, CA, USA) against 100 bp DNA ladder (Bio-Rad
163 Laboratories, Hercules, CA, USA) at 100 V for 45 minutes. The specific PCR bands
164 were visualized using Gel Doc EZ Imager system (Bio-Rad Laboratories, Hercules,
165 CA, USA).
166

167 **Identification of DENV serotypes**

168 Reverse-transcription polymerase chain reaction (RT-PCR) was performed to detect
169 DENV serotypes using serotype-specific primers. The amplification of serotype-
170 specific nucleotide sequences was done in a 25 µL RT-PCR reaction of each
171 serotype test containing 12.5 µL of 2x reaction mix, 1 µL of SuperScript III
172 RT/Platinum *Taq* mix (Invitrogen, CA, USA), 0.5 µL of 10 µM forward primer (D1),
173 0.5 µL of 10 µM reverse primer (TS1/TS2/TS3/TS4) (Table 1), 0.5 µL Magnesium
174 salt (Invitrogen, CA, USA), 6 µL of nuclease-free water and 4 µL of RNA template.
175 Reverse-transcription was performed in 1 cycle at 50 °C for 10 minutes, followed by
176 initial denaturation at 94 °C for 2 minutes. PCR was done for 35 cycles of
177 denaturation at 94 °C for 15 seconds, annealing at 55 °C for 30 seconds, elongation
178 at 72 °C 1 minute and a final extension at 72 °C for 7 minutes. The expected sizes of

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179 serotype-specific amplicons were 482 bp (DENV-1), 119 bp (DENV-2), 290 bp
180 (DENV-3) and 392 bp (DENV-4). The PCR bands were analysed on 1.5% agarose
181 gel against 100 bp DNA ladder (Bio-Rad Laboratories, Hercules, CA, USA) at 100 V
182 for 45 min and visualized using Gel Doc EZ Imager system (Bio-Rad Laboratories,
183 Hercules, CA, USA).
184
185 **Data analysis**
186 Socio-demographic data were entered in Miscrosoft Execel spreadsheet (MS-Excel
187 2010, Microsoft Corp., and Redmond, WA, USA) and descriptive statistics [counts,
188 percentages, median and interquartile range (IQR)] and relevant tables were used to
189 summarize information. RT-PCR bands were analysed using Image Lab Software
190 version 6.0.1 (Bio-Rad Laboratories, Hercules, CA, USA) and presented by gel
191 images.
192
193 **Ethical consideration**
194 The study was approved by the Medical Research Coordinating Committee of the
195 National Institute for Medical Research in Tanzania (Ref. No.
196 NIMR/HQ/R.8a/Vol.IX/2974). Witten consent was sought and obtained from all adults
197 (≥ 18 years) and assent was obtained from parents/guardians of children (<18years
198 old) for screening of dengue virus infection. Oral consent was sought from dengue-
199 positive individuals for further identification and genomic analysis and it was
200 documented through tracking forms.
201
202 **Results**
203 **Patient characteristics and dengue diagnosis**

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204 A total of 110 sera samples was collected from dengue-suspected outpatients
205 presented at health facilities with dengue-like illiness . Among them, 45 (41%) were
206 confirmed positive for dengue infection by NS1 antigen and/or DENV RNA detection
207 with RT-PCR (95.5% positivity by RT-PCR and/or NS1 and 4.5% positivity by NS1
208 only). The age of the patients ranged from 16-75 years old (median = 35, IQR= 18).
209 Two-thirds of the patients (66.7%) were males; 62.2% had post-secondary education
210 and the majority (46.7%) had formal employment (Table 2).
211

212 **Detection of DENV RNA and serotypes**

213 Dengue virus (DENV) nucleic acid (RNA) was detected in 17 of 20 tested samples
214 while three samples were negative. Serotype-specific RT-PCR test was positive for
215 DENV-1 (Fig 2A) and DENV-3 (Fig 2B) serotypes. Of the 17 positive samples, 17
216 were positive for DENV-1, of which two samples were also positive for DENV-3. RT-
217 PCR results for DENV-2 and DENV-4 serotypes were all negative. The overall
218 results for RNA quality and RT-PCR amplification of the tested samples are
219 summarized in Table 3.
220

221 **Discussion**

222 This study reports for the first time occurrence of dengue virus serotype 1 (DENV-1)
223 in Tanzania. The occurrence of DENV-2 and DENV-3 has been reported during the
224 previous outbreaks in 2010 and 2014 [6,7,25]. Our results indicate that DENV-1 was
225 dominant serotype during the 2019 outbreak in Tanzania. DENV-1 has also been
226 detected in Japan in a patient with a history of travel to Tanzania in May 2019 [26].
227 The subsequent phylogenetic analysis indicated that the DENV-1 isolate detected in
228 the Japanese traveller was genetically related to Singapore 2015 strain, suggesting

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229 that the recent DENV-1 epidemic in Tanzania may have been imported from
230 endemic countries.

231 The evidence of DENV-1 predominance in the East Africa region is
232 increasingly been reported. In a recent study in Kenya, DENV-1 was found to
233 account for 44% of all the serotypes that were detected in febrile patients [27]. The
234 results from this study demonstrated that DENV-1 isolates were genetically close to
235 isolates reported in endemic South-East Asian countries like Indonesia and India.
236 Nevertheless, the risk of DENV-1 serotype importation to Tanzania can originate
237 from countries other than South-East Asian countries due to increased movement
238 networks of people. For instance, Mauritius has recently reported DENV-1 epidemic
239 that included eleven imported cases from South-East Asia countries [9]. Circulation
240 of DENV-1 serotypes in Africa has been previously reported in Cameroon, Comoros,
241 Côte d'Ivoire, Djibouti, Gabon, Kenya, and Senegal [2,28].

242 Also, we report for the first time genomic-based evidence of dengue virus co-
243 infection with DENV-1 and DENV-3 serotypes during a single phase of outbreak in
244 Tanzania. Dengue concurrent infections have been commonly reported in endemic
245 countries including Brazil [29] and India [30]. Dengue concurrent infections have
246 serious clinical implication for patient management, as they can present with severe
247 clinical manifestations such as haemorrhagic fever syndrome [31,32]. Previous
248 findings showed that subsequent infection with a different serotype may lead to
249 increased disease severity because antibodies against the previous serotype do not
250 cross-neutralize the subsequent heterologous invading serotype, but induce
251 antibody-dependent enhancement that increases replication inside target Fc
252 receptor-bearing cells [33,34]. Co-infection with multiple DENV serotypes may
253 increase the risk of emergence of recombinant strains that have different

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254 characteristics and the areas with a circulation of more than one serotype have been
255 classified as more prone to severe DENV infections [31,35]. Co-circulation of dengue
256 virus serotypes and occurrence of serotypes co-infection in Tanzania provide alert
257 on the possibility of an increased risk of severe dengue in the country.

258 It is worth noting important limitations of this study. This was a cross-sectional
259 health facility-based study that focused on the detection of dengue viruses from
260 outpatients during an epidemic phase, thus, it was not possible to establish an
261 algorithm to classify primary and secondary dengue virus infections and sequence
262 DENV RNA genomes that could provide insights on the evolution of circulating
263 dengue viruses. Also, the health facility-based subjects included in the study may not
264 be a representative of the population parameter estimates, suggesting that
265 extrapolation of the results to unsampled locations should be made with caution.

266 In conclusion, the findings of this study indicate that DENV-1 is the dominant
267 serotype during the on-going 2019 epidemic and reveal for the first time occurrence
268 of dengue virus serotypes 1 and 3 co-concurrent infection in Tanzania. These
269 observations emphasize the need for establishing continuous genomic-based
270 surveillance of circulating dengue viruses in Tanzania. This will provide rapid
271 evidence of emerging/re-emerging and spread of dengue viruses and inform public
272 health authorities to implement effective preventive measures.

273

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284

285 **Conflicts of interest**

286 The authors declare the absence of any competing interests.

287

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291 Diseases of Humans and Animals in Tanzania.

292

293 **References**

294 1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The
295 global distribution and burden of dengue. Nature. 2013;496: 504–507.
296 doi:10.1038/nature12060

297 2. Amarasinghe A, Kuritsky JN, Letson GW, Margolis HS. Dengue Virus Infection
298 in Africa. Emerg Infect Dis. 2011;17: 1349–1354. doi:10.3201/eid1708.101515

299 3. Weekly bulletins on outbreaks and other emergencies. In: WHO | Regional
300 Office for Africa [Internet]. [cited on 23 Jul 2019]. Available:
301 https://www.afro.who.int/health-topics/disease-outbreaks/outbreaks-and-other-
302 emergencies-updates

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303 4. Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21st
304 century. Trop Med and Health. 2011;39: S3–S11. doi.org/10.2149/tmh.2011-
305 S05

306 5. Christie J. Remarks on “Kidinga Pepo”: a peculiar form of exanthematous
307 disease. Br Med J. 1872;1: 577. doi.10.1136/bmj.1.596.577

308 6. Vairo F, Nicastri E, Meschi S, Schepisi MS, Paglia MG, Bevilacqua N, et al.
309 Seroprevalence of dengue infection: A cross-sectional survey in mainland
310 Tanzania and on Pemba Island, Zanzibar. IJ Infect Dis. 2012;16: 2011–2013.
311 doi:10.1016/j.ijid.2011.09.018

312 7. Mboera LEG, Mweya CN, Rumisha SF, Tungu PK, Stanley G, Makange MR, et
313 al. The Risk of Dengue Virus Transmission in Dar es Salaam, Tanzania during
314 an Epidemic Period of 2014. PLoS Negl Trop Dis. 2016;10: 1–15.
315 doi:10.1371/journal.pntd.0004313

316 8. Gautret P, Simon F, Askling HH, Bouchaud O, Leparc-Goffart I, Ninove L, et al.
317 Dengue type 3 virus infections in European travellers returning from the
318 Comoros and Zanzibar, February-April 2010. Eurosurv. 2010;15: 19541.
319 doi.10.2807/ese.15.15.19541-en

320 9. Weekly bulletins on outbreaks and other emergencies. In: WHO | Regional
321 Office for Africa [Internet]. [cited on 7 Aug 2019]. Available:
322 https://www.afro.who.int/health-topics/disease-outbreaks/outbreaks-and-other-
323 emergencies-updates

324

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325 10. Weekly bulletins on outbreaks and other emergencies. In: WHO | Regional
326 Office for Africa [Internet]. [cited on 26 Aug 2019]. Available:
327 https://www.afro.who.int/health-topics/disease-outbreaks/outbreaks-and-other-
328 emergencies-updates

329 11. Mustafa MS, Rasotgi V, Jain S, Gupta V. Discovery of fifth serotype of dengue
330 virus (DENV-5): A new public health dilemma in dengue control. Med J Arm
331 Forc India. 2015;71: 67–70. doi.10.1016/j.mjafi.2014.09.011

332 12. WHO. World Health Organization, Special Programme for Research, Training in
333 Tropical Diseases, World Health Organization. Department of Control of
334 Neglected Tropical Diseases, World Health Organization. Epidemic, Pandemic
335 Alert. Dengue: guidelines for diagnosis, treatment, prevention and control.
336 World Health Organization 2009. pp. 139-140 [cited on 5 Jul 2019].

337 13. Nimmagadda SS, Mahabala C, Boloor A, Raghuram PM, Nayak U. A. Atypical
338 Manifestations of Dengue Fever (DF) – Where Do We Stand Today? J Clin
339 Diagn Res. 2014;8: 71–73. doi:10.7860/JCDR/2014/6885.3960

340 14. Murray NEA, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present
341 and future prospects. Clin Epidemiol. 2013;5: 299–309.
342 doi:10.2147/CLEP.S34440

343 15. Sang RC. Dengue in Africa. In Report of the scientific working group meeting on
344 dengue. Geneva: WHO Special Programme for Research and Training in
345 Tropical Diseases 2007. pp. 50-2. [Cited on 5 Jul 2019].

346

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347 16. Vairo F, Mboera LEG, de Nardo P, Oriyo NM, Meschi S, Rumisha SF, et al.
348 Clinical, virologic, and epidemiologic characteristics of dengue outbreak, Dar es
349 Salaam, Tanzania, 2014. Emerg Infect Dis. 2016;22: 895–899.
350 doi:10.3201/eid2205.151462

351 17. Hill SC, Neto de Vasconcelos J, Granja BG, Thézé J, Jandondo D, Neto Z, et al.
352 Early Genomic Detection of Cosmopolitan Genotype of Dengue Virus Serotype
353 2, Angola, 2018. Emerg Infect Dis. 2019;25: 784–787.
354 doi:10.3201/eid2504.180958

355 18. Sharp TM, Moreira R, Soares MJ, da Costa LM, Mann J, DeLorey M, et al.
356 Underrecognition of dengue during 2013 epidemic in Luanda, Angola. Emerg
357 Infect Dis. 2015;21: 1311. doi: 10.3201/eid2108.150368

358 19. Jaenisch T, Junghanss T, Wills B, Brady OJ, Eckerle I, Farlow A, et al. Dengue
359 Expansion in Africa—Not Recognized or Not Happening? Emerg Infect Dis.
360 2014;20. doi:10.3201/eid2010.140487

361 20. Jaswant G. Detection and genetic characterisation of dengue virus among
362 patients in Dar es salaam, Tanzania during the 2013-2014 outbreak. PhD
363 Thesis, Sokoine University of Agriculture. 2015. [cited on 5 Jul 2019].

364 21. Trpis M. Seasonal changes in the larvel populations of Aedes aegypti in two
365 biotopes in Dar es Salaam, Tanzania. Bulletin of the World Health Organization.
366 1972;47: 245.

367 22. World Health Organization. Comprehensive Guidelines for Prevention and
368 Control of Dengue and Dengue haemorrhagic fever 2011. pp. 31-40 [Cited on 5
369 Jul 2019].

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370 23. Desjardins P, Conklin D. NanoDrop microvolume quantitation of nucleic acids. J
371 Vis Exp. 2010; e2565. doi: 10.3791/2565.

372 24. Lanciotti RS, Calisher CH, Gubler DJ, Chang G-J, Vorndam AV. Rapid
373 detection and typing of dengue viruses from clinical samples by using reverse
374 transcriptase-polymerase chain reaction. J Clinical Microbiol. 1992;30: 545–551.

375 25. Moi ML, Takasaki T, Kotaki A, Tajima S, Lim C-K, Sakamoto M, et al.
376 Importation of dengue virus type 3 to Japan from Tanzania and Côte d'Ivoire.
377 Emerg Infect Dis. 2010;16: 1770. doi: 10.3201/eid1611.101061

378 26. Okada K, Morita R, Egawa K, Hirai Y, Kaida A, Shirano M, et al. Dengue Virus
379 Type 1 Infection in Traveler Returning from Tanzania to Japan, 2019. Emerg
380 Infect Dis. 2019;25.

381 27. Konongoi L, Ofula V, Nyunja A, Owaka S, Koka H, Makio A, et al. Detection of
382 dengue virus serotypes 1, 2 and 3 in selected regions of Kenya: 2011–2014.
383 Virol J. 2016;13: 182. doi:10.1186/s12985-016-0641-0

384 28. de Laval F, Plumet S, Simon F, Deparis X, Leparc-Goffart I. Dengue
385 surveillance among French military in Africa. Emerg infect Dis. 2012;18(2):342-
386 3. doi: 10.3201/eid1802.111333

387 29. Andrade EH, Figueiredo LB, Vilela AP, Rosa JC, Oliveira JG, Zibaoui HM, et al.
388 Spatial–temporal Co-circulation of dengue virus 1, 2, 3, and 4 associated with
389 coinfection cases in a hyperendemic area of Brazil: a 4-week survey. The Am J
390 Trop Med and Hyg. 2016;94: 1080–1084. doi.10.4269/ajtmh.15-0892

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391 30. Reddy MN, Dungdung R, Valliyott L, Pilankatta R. Occurrence of concurrent
392 infections with multiple serotypes of dengue viruses during 2013–2015 in
393 northern Kerala, India. PeerJ. 2017;5: e2970. doi:10.7717/peerj.2970

394 31. VinodKumar CS, Kalapannavar NK, Basavarajappa KG, Sanjay D, Gowli C,
395 Nadig NG, et al. Episode of coexisting infections with multiple dengue virus
396 serotypes in central Karnataka, India. J Infect and Public Health. 2013;6: 302–
397 306. doi:10.1016/j.jiph.2013.01.004

398 32. Bharaj P, Chahar HS, Pandey A, Diddi K, Dar L, Guleria R, et al. Concurrent
399 infections by all four dengue virus serotypes during an outbreak of dengue in
400 2006 in Delhi, India. Virol J. 2008;5: 1. doi:10.1186/1743-422X-5-1

401 33. Katzelnick LC, Coloma J, Harris E. Dengue: knowledge gaps, unmet needs, and
402 research priorities. Lancet Infect Dis. 2017;17: e88–e100. doi: 10.1016/S1473-
403 3099(16)30473-X

404 34. Dejnirattisai W, Jumnainsong A, Onsirisakul N, Fitton P, Vasanawathana S,
405 Limpitikul W, et al. Cross-Reacting Antibodies Enhance Dengue Virus Infection
406 in Humans. Science. 2010;328: 745–748. doi:10.1126/science.1185181

407 35. Vaddadi K, Gandikota C, Jain PK, Prasad VSV, Venkataramana M. Co-
408 circulation and co-infections of all dengue virus serotypes in Hyderabad, India
409 2014. Epidemiol Infect. 2017;145: 2563–2574.
410 doi:10.1017/S0950268817001479

411

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412 **Figure legends**

413 **Fig 1: Study location and sample collection sites.**

414 Source: This map was designed and drawn in this study

415 **Fig 2: Agarose gel electrophoresis image for RT-PCR amplified serotype-**
416 **specific DENV DNA products.**

417 (A) Dengue virus serotype 1 (482 bp), M is a 100 bp DNA ladder, 13-20 are test sera
418 samples, P1, P2 are positive controls (CDC KK0129) and N1, N2 are the negative
419 controls.

420 (B) Dengue virus serotype 3 (290 bp), M is a 100 bp DNA ladder, 1-10 are test sera
421 samples, N is the negative control and P is positive control (CDC KK0129).

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Table 1: Primers used in RT-PCR amplification

Primer	Sequence ('5->3')	Amplicon size (bp)	Ref
D1	TCAATATGCTGAAACGCGCGAGAAACCG	511	[24]
D2	TTGCACCAACAGTCAATGTCTTCAGGTTC	511	[24]
TS1	CGTCTCAGTGATCCGGGGG	482 (DENV-1)	[24]
TS2	CGCCACAAGGGCCATGAACAG	119 (DENV-2)	[24]
TS3	TAACATCATCATGAGACAGAGC	290 (DENV-3)	[24]
TS4	CTCTGTTGTCTTAAACAAGAGA	392 (DENV-4)	[24]

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Table 2: Socio-demographic characteristics of the confirmed dengue-positive patients (n=45)

Characteristic	Total participants (%)
Age group	
< 18	4 (8.9)
18-24	5 (11.1)
25-34	9 (20)
35-44	14 (31.1)
45-54	6 (13.3)
55 and above	7(15.6)
Sex	
Male	30 (66.7)
Female	15 (33.3)
Education	
None	2 (4.4)
Primary	1 (2.2)
Secondary	14 (31.1)
College/University	28 (62.2)
Occupation	
Self employed	13 (28.9)
Business	4 (8.8)
Formal employment	21 (46.7)
Students	7(15.6)

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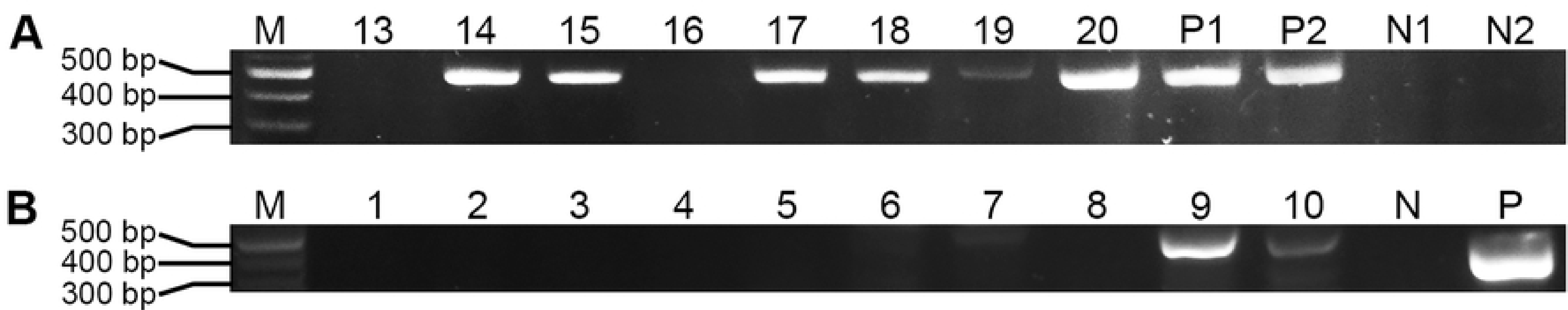
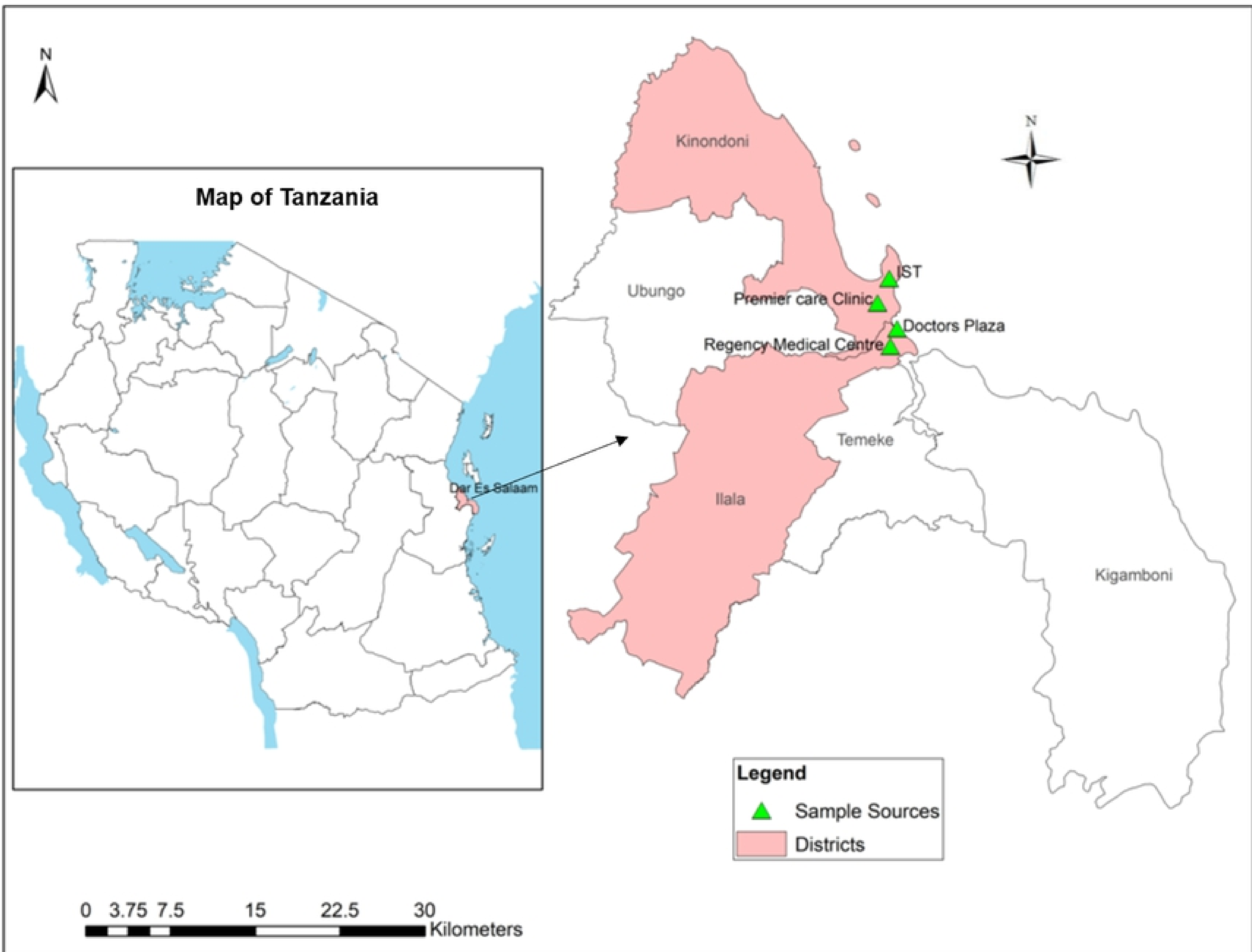
Table 3: RNA quality and RT-PCR results of the tested sera (n=20)

Sample ID	District	*Hospital	Sex	Age	RNA quality (A260/280)	DENV RNA	Serotype
1	Kinondoni	RMC	M	16	2.83	+[a]	DENV-1
2	Kinondoni	RMC	M	48	2.25	+	DENV-1
3	Ilala	DPH	M	45	1.54	+	DENV-1
4	Kinondoni	IST	F	34	2.75	+	DENV-1
5	Kinondoni	DPH	F	36	1.42	-[b]	N/A[c]
6	Temeke	RMC	M	56	2.09	+	DENV-1
7	Temeke	RMC	M	26	2.45	+	DENV-1
8	Kinondoni	DPH	M	35	2.22	+	DENV-1
9	Kinondoni	DPHC	M	44	1.57	+	DENV-1/3[d]
10	Temeke	PC	M	40	1.66	+	DENV-1/3
11	Ilala	PC	M	65	4.98	+	DENV-1
12	Kinondoni	IST	M	29	5.39	+	DENV-1
13	Ilala	RMC	M	22	1.69	-	N/A
14	Kinondoni	DPH	M	33	2.81	+	DENV-1
15	Kinondoni	DPH	M	20	2.61	+	DENV-1
16	Kinondoni	PC	M	27	1.53	-	N/A
17	Kinondoni	PC	M	21	1.95	+	DENV-1
18	Ilala	IST	F	37	2.00	+	DENV-1
19	Kinondoni	RMC	M	21	2.14	+	DENV-1
20	Kinondoni	RMC	F	31	2.43	+	DENV-1

[a]Positive for dengue virus nucleic acid (DENV RNA) reverse-transcription polymerase chain reaction test (RT-PCR); [b] Negative for DENV RNA-RT-PCR test; [c]N/A, Not applicable; [d]Positive for DENV serotypes 1 and 3 RT-PCR test; *RMC, Regency Medical Centre; DPH, Doctors Plaza Hospital; IST, International School of Tanganyika Clinic and PC, Premier Care Clinic.



Gel electrophoresisis of RT-PCR amplified DENV serotype-specifi



Study area