

Detection of dengue virus from suspected yellow fever samples in Ghana

ABSTRACT

Background: Dengue fever remains a serious public health treat throughout the world. Ghana shares borders with countries that have reported dengue cases yet no case has been reported in Ghana. Dengue infections with its broad range of clinical presentations makes it potentially unrecognized by clinicians. In this study, serological tests were used to detect antigens and antibodies and molecular tests were used to detect viral RNA specific to dengue viruses in archived human serum specimens in Ghana.

Methods: Blood samples of 360 patients aged 6 months to 82 years old with suspected yellow fever in hospitals across Ghana were tested for dengue virus exposure. Samples were screened using SD Dengue NS1 Ag ELISA and the SD Dengue IgM/ IgG capture ELISA test, which detects dengue virus antigen and antibodies to dengue virus in human blood.

Results: A total of 360 serum samples were tested, 8% (29/360) were found positive by the antigen/antibody ELISA test, 2.5% (9/360) were positive by NS1 ELISA with 1.9% (7/360) and 3.6% (13/360) being positive by IgM and IgG ELISA respectively. None of the specimens tested positive by real time RT-PCR. 0.6% (2/360) of cases tested positive for both NS1 and IgG. Proportion of NS1 antigen positivity is highest in the Brong Ahafo region 0.8% (3/9) followed by Ashanti and Upper West regions with 0.6% (2/9) respectively. IgM positivity was however highest in Upper West 0.4% (3/7).

21 **Conclusion:** Introduction of dengue virus surveillance with routine diagnosis will serve to alert
22 the health system of possible outbreak and also minimize spread in the event of an outbreak in
23 the country.

24

25 Running title: Dengue infections in Ghana

26

27 Key words: Dengue virus, Yellow fever virus, Sero-prevalence, ELISA, Ghana

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29

30 **BACKGROUND**

31 Dengue is an acute viral illness caused by the dengue virus which is made up of four closely
32 related but antigenically distinct serotypes (Dengue 1-4) [1]. All four Dengue virus (DENVs)
33 serotypes circulate in South America, where almost all countries are hyper-endemic for DENV
34 transmission [2]. In 2014, the World health Organization (WHO) estimated that 50-100 million
35 human DENV infections occur annually in tropical and sub-tropical regions, posing considerable
36 public health problems in over 100 countries [3]. In Africa DENV was first isolated in Ibadan,
37 Nigeria in 1960 [4] and since then, the disease has been reported in Ivory Coast, Burkina Faso
38 and Nigeria [5]. Recently in 2009, a large outbreak of dengue involving over 17,000 cases was
39 documented in the Cape Verde islands [6]. DENV importation from West African countries to
40 European countries have also been documented [7, 8, 9]

41 Dengue fever is self-limiting though debilitating febrile illness whose symptoms include fever,
42 headache, muscle and joint pains, and a characteristic skin rash that is similar to measles
43 [Kalayanarooj et al, 1997]. In some cases, disease develops into life-threatening dengue
44 hemorrhagic fever or Dengue shock syndrome and death [10, 3].

45 The DENV is a single-stranded positive-sense RNA virus belonging to the *Flaviviridae* family
46 and are transmitted to humans by virus-carrying *Aedes* mosquitoes; the same type of mosquitoes
47 implicated in yellow fever transmission [3, 11]. The *Aedes spp* of mosquitoes is found in close
48 association with human settlements throughout the tropics, breeding mainly in containers in and
49 around buildings, car tyres and water filled tree-holes [12, 13].

50 Dengue fever is rarely fatal and survivors appear to have lifelong immunity to the homologous
51 serotype. The mortality from DHF can exceed 30% if appropriate care is unavailable. The most
52 significant risk factor for DHF is when secondary infection with a different serotype occurs in
53 people who have already had, and recovered from, a primary dengue infection. It is suggested
54 that virus infection is enhanced by the presence of pre-existing heterotypic antibodies [14, 15].

55 There is no cure for Dengue fever or for DHF. Currently, the only treatment is the management
56 of symptoms which is known to reduce mortality from DHF to less than 1% [16].

57 The non-structural protein 1 (NS1) encoded in the viral genome has been shown to be useful as a
58 tool for the diagnosis of acute dengue infections [17]. Dengue NS1 antigen has been detected in
59 the serum of DENV infected patients as early as 1 day post onset of symptoms (DPO), and up to
60 18 DPO. The NS1 antigen is useful for differential diagnosis between flaviviruses because it is
61 highly specific for dengue [18]. Conversely dengue IgM and IgG antibodies which are used to
62 determine recent and past dengue infections, are prone to cross-reactivity with other members of

63 the *Flavivirus* genus such as West Nile Virus, Japanese Encephalitis Virus and Yellow Fever
64 Virus [19, 20].

65 There has been no reported case of dengue virus infection in Ghana although Ghana shares
66 common borders with Ivory Coast and Burkina Faso, where Dengue cases have been detected
67 [21]. **Research has also shown a high potential for the transmission of Dengue in Ghana**
68 Research making in 218 children positive for malaria revealed previous dengue exposure in
69 21.6% of and recent exposure in 3.2% of children[22]. An international expert conference on
70 Dengue in Africa in Accra, Ghana, in 2013 to consider why dengue in Africa does not cause a
71 disease incidence of a magnitude similar to that of Latin America, or dengue fever is not
72 spreading to Africa for epidemiological reasons still not understood? The report suggests, among
73 other research, retrospective studies the test for dengue virus in serum samples stored in
74 repositories tested for yellow fever. These results can be used to clarify the extent of dengue
75 transmission in Africa [23].

76 Currently, there are surveillance activities on yellow fever in Ghana but very little is being done
77 on dengue. Although many samples of suspected yellow fever cases when screened turn out to be
78 negative in laboratory, such samples are not investigated for other likely endemic arboviruses
79 such as Dengue and Chikungunya virus. This study investigated the presence of antigens and
80 antibodies specific to dengue viruses in archived human serum specimens of suspected yellow
81 fever cases in Ghana, using both serological and molecular methods. We also matched these
82 diagnostic markers of Dengue infection to areas where they might be significantly prevalent.

83

84 MATERIALS AND METHODS

85 **Study population and sample collection**

86 This was a laboratory based cross-sectional study which involved the use of archived samples
87 from patients suspected of yellow fever infection at the National Public Health and Reference
88 Laboratory (NPHRL) in Accra. The NPHRL of the Ghana Health Service is a World Health
89 Organization (WHO) designated and accredited laboratory for the testing of all suspected yellow
90 fever cases in Ghana. The laboratory receives clinical specimens of serum or plasma from
91 suspected yellow fever cases across the country through the Disease Surveillance Department
92 (DSD) for epidemic prone diseases or diseases of public health importance. We tested 360
93 archived serum samples collected between January and December 2013 from all the ten regions
94 of Ghana, stored at -80°C for the presence of Dengue NS1 antigen as well as dengue IgM/ IgG
95 using anti-Dengue specific IgM, anti-Dengue specific IgG ELISA test kits. These archived
96 samples had previously tested negative for anti-yellow fever specific IgM antibodies using the
97 sandwich ELISA protocol developed by WHO. Subsequently, all ELISA positive Dengue
98 samples were sent to the Noguchi Memorial Institute for Medical Research for molecular testing.
99 Demographic and epidemiological data was obtained from the case-based forms that
100 accompanied the clinical specimens. .

101

102 **Serologic Testing for Dengue Infection**

103 Enzyme-Linked Immunosorbent Assay (ELISA) for the qualitative determination of antigens and
104 antibodies specific to dengue virus was performed using the SD Dengue NS1 Ag ELISA and the
105 SD Dengue IgM/IgG capture ELISA as described by the manufacturer, SD Bioline.

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106 Subsequently all positive samples were serologically confirmed using SD Bioline Dengue Duo
107 rapid test kit with NS1, IgM and IgG combo test.

108

109 **Nucleic acid isolation and purification**

110 Twenty-five (25) clinical specimens that tested positive with SD Dengue NS1 Ag ELISA and or
111 the SD Dengue IgM/IgG capture ELISA were selected for molecular analysis. Ribonucleic acids
112 (RNA) were extracted and purified from serum and/or plasma specimens with the QIAamp viral
113 RNA mini kit (QIAGEN, Hilden Germany) in accordance with the manufacturer's instruction
114 with slight modifications. Briefly, 140µl and 70µl of sera/plasma from each pool was separately
115 mixed with 560µl of a lysis buffer, AVL from the kit. After an incubation of 10 min, 500µl non-
116 denatured absolute ethanol (100%) was added and the RNA was affinity-purified as described in
117 the manufacturer's instructions of the kit. The purified RNA was eluted in 60µl of buffer AVE
118 equilibrated to room temperature from the kit.

119

120 **Detection by Real time Reverse Transcription-Polymerase Chain Reaction**

121 Volumes each of 5µl of the extracted and purified RNA duplicates from the pooled clinical sera
122 or plasma were used in a TaqMan assay real time reverse transcription-polymerase chain
123 reaction (rRT-PCR) assay using the Applied Biosystems® 7300 RT-PCR system (Life
124 Technologies, Grand Island, NY, USA). In singleplex reaction mixtures, the RNA was combined
125 with 10pmol each of Dengue serotype specific primer and probe sets with targets describe by
126 Johnson B.W *et al.*, 2005 in a 25-µl reaction. Each reaction mixture contained a single dengue

127 serotype primer pair and probe – making eight separate reactions for the isolated and purified
128 RNAs for each clinical specimen. The reagent master mix was prepared according to the number
129 of reactions required for each test run using the AgPath-ID™ One-Step RT-PCR kit (#AM1005,
130 Thermo Fisher Scientific, NY, USA). The master mix was divided and dispensed into portions of
131 20 µl each into an optical-ready 96-well plate (MicroAmp® Optical 96-well reaction plate,
132 Thermo Fisher Scientific, NY, USA). Then a final addition of 5µl of individual RNA to each
133 appropriately labeled well. Included in each test run were ‘no RNA’ or water reagent and dengue
134 positive RNA controls.

135 Result interpretation was in accordance with the instructions of the software (SDS version 1.4
136 patch 2) used with the Applied Biosystems® 7300 RT-PCR system (Life Technologies, Grand
137 Island, NY, USA). Test runs that exhibited fluorescence growth curves that cross or did not cross
138 the threshold line for ‘no RNA’ or positive control reagent reactions respectively were
139 invalidated and repeated. A presumptive positive for Dengue serotype specific virus was
140 considered for a clinical specimen when the controls met stated requirements and the growth
141 curves crossed the threshold line within 40 cycles with the inverse true for negatively considered
142 clinical specimens for dengue serotype specific virus. Test runs were valid, however none of the
143 pooled extracts was detected to be positive - had growth curves that crossed the threshold line
144 with the exception of the positive controls.

145

146 **Conventional RT-PCR**

147 Published primers by Lanciotti et al., 1992 were used to perform a conventional RT-PCR on the
148 pooled clinical specimens with slight modifications with the use of the thermal cycler, (Aeris G-

96 well by Esco Micro Pte Ltd, Singapore). Total reaction volumes of 25µl including 5µl of the target RNA from the Qiagen viral RNA mini kit (QIAGEN, Hilden Germany). The RT-PCR (45 cycles) contained QIAGEN OneStep RT-PCR reagents with the sense primer Duc 5'-TTGCACCAACAGTCAATGTCTTCAGGTTC-3' homologous to the genomic RNA of the four serotypes and the antisense primer Dus 5'-TCAATATGCTGAAACGCGGAGAAACCG-3', as described in detail by Lanciotti et al., 1992. The reactions were proceeded for 30 minutes at 50°C and then 95°C for 15 minutes for initial denaturation followed by 40 cycles of denaturation [(94°C for 30 seconds); primer annealing (55°C for 30 seconds); and primer extension (72°C for 1 minute) along with final extension (72°C for 10 minutes)]. The amplification products (expected size, 511 base pairs) were electrophoresed on a 2% Agarose gel (peqlab Biotechnologie, Erlangen, Germany), stained with Ethidium bromide, and viewed under UV light.

161

162 **Data Analysis**

163 Data was double entered and cleaned in Excel and the analyses done with RStudio
164 (Version 0.99.878) and Epi Info software package version 3.5.1. Appropriate measures of central
165 tendency for mean, median, frequency distributions, percentages, and standard deviation were
166 calculated.

167

168 **Ethical considerations**

169 Human sera samples analysed in the study were initially collected with the national ethical
170 guidelines as part of the National yellow fever surveillance programme in Ghana and sent to the

171 National Public Health and Reference Laboratory in Accra, Ghana. This does not require ethical
172 clearance. Since yellow fever was negative, the lab wanted to ascertain whether Dengue virus is
173 the etiologic agent of the diagnosed yellow fever. We sought permission from the Disease
174 Surveillance Department to use their data. We protected the confidentiality of participants
175 through use of codes. However, ethical committee review did not apply as this was a public
176 health response to surveillance. The study does not involve the use of animals or animal samples.
177

178 **RESULTS**

179 Of the 360 achieved samples processed, 44% (159/360) and 56% (201/360) were from females
180 and males respectively. The age of the population ranges from seven months to 82 years with
181 median age of 16 years, mean age of 19.2 and standard deviation of 16.1. One percent of the
182 study sample (4/360) was positive for yellow fever (Table 1).

183 The proportion of cases that were positive for Dengue NS1 antigen was 2.5% (9/360). For
184 Dengue IgM, 1.9% of cases were positive while 3.6% of cases were positive for Dengue IgG
185 (TableTable 1).

186 The age of the population ranges from seven months to 82 years with median age of 16 years,
187 mean age of 19.2 and standard deviation of 16.1. The median age for females was 16 years and
188 that for males was 15 years. Median age for cases that were positive for dengue NS1 antigen,
189 dengue IgM and dengue IgG was 27 years, 22 years and 15 years respectively. TableTable 12
190 presents summary statistics on age grouped by variables of interest.

Comment [S2]: According to the statement in Lines 87-89, the samples were negative for Yellow Fever. Their results showed 4 (1%) positive samples. Discuss this result.

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191 All the samplesclinical specimens that were found positive by NS1 antigen test and capture
192 ELISA for both anti-IgM and anti-IgG antibodies to Dengue were further subjected to molecular
193 testing and were found to be negative by RT-PCR. But all results were negative.

194 Most study patients, 174 (48.3%), were 15 years or younger. The results show that dengue NS1
195 antigen was relatively more prevalent (N=4) aged 16-30 years. In addition, 6 out of the 9 positive
196 cases for Dengue NS1 antigen had 30 years or younger.

197 Of the 7 positive cases for dengue IgM antibodies, 6 were detected among the age groups of 45
198 years or less.

199 Although no Dengue NS1 was detected in cases older than 75 years, anti-IgM detected 1 case in
200 this age group.

201 The positive Dengue IgG was relatively more prevalent in the range of 15 or less (N = 7).

202 Neither anti-IgM nor anti-IgG were detected in cases between 61-75 years, although the NS1
203 antigen has been detected in this age group (Table 2).

204 The results presented in TableTable 3 indicate that none of the samples included in this study
205 was positive for both Dengue NS1 antigen and Dengue IgM. However, 2 of 360 cases examines
206 were positive for both NS1 and IgG. In additionally, 7/360 of the samples that were positive for
207 Dengue IgM were negative for the NS1 antigen and 11/360 were positive for Dengue IgG and
208 negative for NS1 antigen.

209 As shown in Table 4, most of the samples in this study, 21% (74/360), came from the Brong
210 Ahafo region while the smaller sample came from the Northern region, 2% (8/360). The largest
211 proportion of the samples was NS1 antigen positivity also in the Brong Ahafo region (0.8%)

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212 followed by Ashanti and Upper West regions with 0.6% respectively. The positive IgM was
213 higher in Upper West (0.8%). The IgM test detected no cases in Brong Ahafo (Table Table 4).
214 On the other hand, the Eastern region recorded the highest IgG positivity index (1.1%). Both
215 Ashanti and Volta region recorded relatively high proportions of IgG, 0.8% and 0.6%
216 respectively (Figure 1).

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219 DISCUSSION

220 Dengue has long been thought to be widespread in Africa but case reporting remains very poor.
221 In the absence of laboratory test facilities, it is likely that Dengue fever, as opposed to DHF,
222 would often be mistaken for other diseases such as malaria [23,24,25]. In this study we
223 employed serological and molecular assays for the identification of probable dengue cases in
224 yellow fever negative samples. A strategy aimed at increasing the capacity for surveillance and
225 outbreak response, changing behaviours and reducing the disease burden using integrated vector
226 management in conjunction with early and accurate diagnosis was adopted. We were able to
227 identify NS1 Ag in 2.5%, anti-IgM antibodies in 1.9% and anti-IgG antibodies in 3.6% of all the
228 study samples with the serological assay but none was confirmed with the molecular tools of RT-
229 PCR used in the present study. Even though the overall sensitivity based on this test is quite low,
230 it is consistent with a study in Pakistan [25] but different from another study to evaluate an
231 enzyme immunoassay for detection of Dengue virus [26] that recorded higher sensitivity. The
232 low positivity could be that the samples were collected from patients with suspected yellow fever
233 which may have other infections that clinically resemble Dengue and may not meet the case

234 definition for dengue [27]. Those patients whose samples tested positive for anti-Dengue specific
235 IgM antibodies by IgM antibody capture ELISA assay but were negative by RT-PCR result in
236 the acute phase specimen, may be classified as having had recent probable Dengue infection.
237 This is due to the fact that IgM antibodies for Dengue may remain elevated in serum for 2 to 3
238 months after the illness [28].

239 In the present study, we found Dengue to be more in males than females and infection was found
240 in all the age groups except for those 75 years and above. The highest number of positive cases
241 of NS1 were between 16 and 30 year of age observed correlated with the results of a study
242 conducted by Halstead [10, 29], Neeraja in India [30] and Khan in the Indo-Myanmar border
243 area [31]. Although tests which detect antibodies as markers of Dengue infection may cross-react
244 with other members of the Flavivirus family, the detection of the NS1 antigen in some of the
245 study samples is a sure sign of Dengue infection. This is because the NS1 antigen is highly
246 specific for Dengue [32]. According to our results some NS1 negative cases were found positive
247 for IgM antibodies and some IgM negative cases were found positive for NS1 antigen; these
248 results are in agreement with studies conducted by Dussart et al., 2006 [27] and Peeling et al.,
249 2010 [33]. Furthermore, 2 cases tested positive for both NS1 antigen and IgG antibodies.
250 Therefore, these reciprocal results from both assays (NS1 and IgM ELISA) showed that the
251 overall diagnostic capability to identify DENV infections can be increased by using a
252 combination of these tests. Judging from the dynamics of immune responses to Dengue
253 infection, we can speculate that the two samples which tested positive for both dengue NS1
254 antigen and Dengue IgG antibodies, had been exposed to a secondary dengue infection.

255 Although the Brong Ahafo and Northern regions are contiguous regions and the two largest
256 regions by land size, one (Brong Ahafo) presented the largest number of suspected cases while
257 the Northern region presented the least. Similarly the greater proportion of NS1 antigen
258 positivity came from the Brong Ahafo region while the Northern region showed zero positivity.

259 Our study could not confirm the ELISA positive cases by RT-PCR, hence could not distinguish
260 the Dengue serotypes. This is the study limitation. However, the detection of NS1 antigen and
261 IgM antibodies is an indication of exposure and possible circulation of Dengue virus. Dengue is
262 likely being missed in Ghana because of low awareness by health care providers, prevalent
263 febrile illnesses, and lack of diagnostic testing and systematic surveillance. Other hypotheses to
264 explain low reported numbers of cases include cross-protection from other endemic flavivirus
265 infections, genetic host factors protecting against infection or disease, and low vector
266 competence and transmission efficiency. However, it is still unclear why the circulation of
267 dengue virus in Africa and this includes Ghana, does not result in serious cases such as in Asia
268 and Latin America [23]. The correct detection of the circulation of dengue virus may pave the
269 way for studies that indicate solutions how to deal with the severe manifestations of dengue in
270 other regions of the world. It is noteworthy positive tests for yellow fever virus in samples
271 archived as negative for this virus. This result underscores the need to invest and provide more
272 sophisticated diagnostic tools for health services.

273

274 CONCLUSION

275 | Dengue disease appear to be in Ghana at least since 2013. Silent circulation of different Dengue
276 serotypes may predispose infected individuals to Dengue hemorrhagic fever in infection by a
277 different serotype. Active surveillance with closely monitoring of cases, couple with training of
278 clinicians on case detection in the health facilities in Ghana is essential to understand the
279 dynamics and disease burden of Dengue. Further studies are needed to elucidate the transmission
280 and non-detection of of dengue in Ghana and the possible epidemiological implications for the
281 population of Ghana and visitors.

282

283 LIST OF ABBREVIATIONS

284 IgM = Immunoglobulin M; IgG – Immunoglobulin G; ELISA – Enzyme-Linked Immunosorbent
285 assay; Ag – Antigen; YF = Yellow Fever; DENV = Dengue virus; DHF = Dengue hemorrhagic
286 fever; DPO = Days Post Onset; NPHRL – National Public Health and Reference Laboratory;
287 WHO = World Health Organization; RNA = Ribonucleic acid; RT-PCR = Reverse Transcriptase
288 Polymerase Chain Reaction.

289

290 DECLARATIONS

291 We retrieved stored sera samples and analysed for Dengue virus in the study according to the
292 national ethical guidelines as part of the National yellow fever surveillance programme in Ghana
293 and sent to the National Public Health and Reference Laboratory in Accra, Ghana to ascertain
294 whether Dengue virus is the etiologic agent of the diagnosed yellow fever. We sought

295 permission from the Disease Surveillance Department to use their data. We protected the
296 confidentiality of participants through use of codes. However, ethical committee review did not
297 apply as this was a public health response to surveillance. The study does not involve the use of
298 animals or animal samples

299

300 **Ethical approval and consent to participate;** this section is not applicable

301 **Consent for publication;** this section is not applicable

302

303 **Competing interests**

304 None declared.

305

306 **Statement of Consent for publication:** Not applicable

307 **Funding:** No funding was provided. Reagents and consumables were provided by the National
308 Public Health and Reference Laboratory

309 **Author's contributions**

310 LHOA participated in the study design, sample processing, analysis of results and manuscript
311 writing, RK was involved in analysis of results and writing of manuscript, BA was involved in
312 sample processing and analysis of results, JHKB was involved in sample processing, data
313 interpretation and editing of the manuscript, GB participated in data analysis and editing of the

314 manuscript, RBA was involved in data analysis and manuscript writing, DO was involved in the
315 writing of manuscript, JKO supervised the study protocol, analysis, editing and interpretation of
316 data and manuscript writing. All authors have read and approved for submission.

317

318

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332 **References**

- 333 1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farkow AW, Moyers CL, et al. The global
334 distribution and burden of dengue. Nature 2013; 496:504-7.

2. Isturiz RE, Gubler DJ, Brea del Castillo J. Dengue and dengue hemorrhagic fever in Latin America and the Caribbean. *Infect Dis Clin North Am* 2000;14:121–40
3. Simmons, C.P., Farrar, J.J., Nguyen, V., Wills, B., 2012. Dengue. *N. Engl. J. Med.* 366, 1423-1432.
4. Ayukekbong JA. Dengue Virus in Nigeria: Current Status and Future Perspective. *Bri J Virol.* 2015 1(4) 106-111. DOI: 10.13140/RG.2.1.4784.7520
5. Franco L, Di Caro A, Carletti F, Vapalahti O, Renaudat C, Zeller H, Tenorio A. Recent expansion of dengue virus serotype 3 in West Africa. *Euro Surveill.* 2010;15(7):pii=19490. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19490>
6. Amarasinghe A, Kuritsky JN, Letson GN, Margolis HS. Dengue virus infection in Africa. *Emerg. Infect. Dis.* 2011; 17:1349-1354.
7. Institut de Veille Sanitaire, France. *Bulletin Hebdomadaire International.* 2009; N°151 (Accessed 17 November 2016). Available from: http://www.invs.sante.fr/international/bhi/bhi_120808.pdf. French.
8. Wichmann O, Muhlberger N, Jelinek T. Dengue-the underestimated risk in travellers. *Dengue Bull.* 2003; 27:126-37.
9. Tarantola A, Quatresous I, Ledrans M, Lassel L, Krastinova E, Cordel H, et al. [Imported cases of dengue fever diagnosed in metropolitan France, from January 2001 to December 2006]. *Med Mal Infect.* 2009;39(1):41-7. French.
10. Halstead SB. Dengue. *Lancet.* 2007; 370, 1644-1652
11. Raghwani, J., Rambaut, A., Holmes, E.C., Hang, V.T., Hien, T.T., Farrar, J., Wills, B., Lennon, N.J., Birren, B.W., Henn, M.R., 2011. Endemic dengue associated with the co-

circulation of multiple viral lineages and localized density-dependent transmission. PLoS Pathog. 7, e1002064.

12. Baba, M.M., Talle, M., 2011. The Effect of Climate on Dengue Virus Infections in Nigeria. New York Science Journal 4(1), 28-33.

13. Anosike JC, Nwoke1 BEB, Okere AN, Oku EE, Asor JE, Emmy-Egbe IO, Adimike DA. Epidemiology of tree-hole breeding mosquitoes in the tropical rainforest of Imo state, south-east Nigeria. Ann Agric Environ Med 2007, 14, 31-38.

14. Scott B. Halstead. Pathogenesis of Dengue: Dawn of a New Era. 2015 Nov 25;4, F1000Research 2015, 4(F1000 Faculty Rev):1353 Last updated: 25 DEC 2016. doi: 10.12688/f1000research.7024.1. eCollection 2015.

15. Halstead SB. Immunological parameters of Togavirus disease syndromes. In: Schlesinger RW, ed. The Togaviruses, Biology, Structure, Replication. New York: Academic Press, 1980.

16. Low JG, Ooi EE, Vasudevan SG. 2017. Current status of dengue therapeutics research and development. Journal of Infectious Diseases 215(suppl_2):S96–S102 DOI 10.1093/infdis/jiw423.

17. Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J Clin Microbiol 2005; 43(10): 4977-4983.

18. Moi, M.L., Omatsu, T., Tajima, S., Lim, C., Kotaki, A., 2013. Detection of Dengue virus nonstructural protein 1 (NS1) by using ELISA as a laboratory diagnostic method for Dengue virus infection of international travelers. J. Travel Med. 20 (30), 185–193.

19. Centers for Disease Control and Prevention Branch. Laboratory guidance and diagnostic testing; 2012. Available from: <http://www.cdc.gov/dengue/clinicalLab/Laboratory.html> (accessed 25.04.17).
20. Houghton-Trivino N, Montana D, Castellanos J. Dengue-yellow sera cross-reactivity; challenges for diagnosis. *Rev Saude Publica* 2088; 8:S30-8
21. Ridde V, Agier I, Bonnet E, Carabali M, Dabiré KR, Fournet F, Ly A, Meda IB and Parra B. Presence of three dengue serotypes in Ouagadougou (Burkina Faso): research and public health implications. *Infect. Dis. Pov.* 2016; 5:23
22. Stoler J, Delimini RK, Bonney JHK, Oduro AR, Owusu-Agyei S, Fobil JN and Awandare GA. Evidence of Recent Dengue Exposure among Malaria Parasite-Positive Children in Three Urban Centers in Ghana. *Am. J. Trop. Med. Hyg.*, 92(3), 2015, pp. 497–500
- 22,23. Jaenisch T et al. Dengue Expansion in Africa—Not Recognized or Not Happening? *Emerging Infectious Diseases*. 2014;20(10):e140487. doi:10.3201/eid2010.140487
- 23,24. Oyer O and Ayukekbong JA. High dengue NS1 antigenemia in febrile patients in Ibadan, Nigeria. *Virus Res.* 2014;191:59–61.
- 24,25. Schoepp R et al. Undiagnosed acute viral febrile illnesses, Sierra Leone. *Emerg Infect Dis.* 2014;20:1176–1182.
- 25,26. Ali A, Rehman HU, Nisar M, Rafique S, Ali S, Hussain A, et al. Seroepidemiology of dengue fever in Khyber Pakhtunkhawa, Pakistan. *Int J Infect Dis* 2013; 17(7): e518-e523

- 26,27. Suleman M, Faryal R, Alam MM, Sharif S, Shaukat S, Aamir UB, Khurshid A, Ang M. NS1 antigen: A new beam of light in the early diagnosis of dengue infection Asian Pacific J Trop Med 2016; 9(12): 1212–1214.
- 27,28. Dussart P, Labeau B, Lagathu G, Louis P, Nunes MR, Rodrigues SG, et al. Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. Clin Vaccine Immunol 2006; 13(11): 1185-1189.
- 28,29. Shu PY, Huang JH. Current advances in dengue diagnosis. Clin Diagn Lab Immunol 2004; 11(4): 642-650
- 29,30. Plennevaux E, Sabchareon A, Limkittikul K, Chanthavanich P, Sirivichayakul C, Moureau A, Boaz M, Wartel TA, Saville M and Bouckennooghe A. Detection of dengue cases by serological testing in a dengue vaccine efficacy trial: Utility for efficacy evaluation and impact of the future vaccine introduction. Vaccine, 2016; 34:2707-2712
- 30,31. Neeraja M, Lakshmi V, Dash PK, Parida MM, Rao PVL. The clinical, serological and molecular diagnosis of emerging dengue infection at a tertiary care institute in Southern, India. Journal of Clinical and Diagnostic Research. 2013; 7: 457-461.
- 31,32. Khan SA, Dutta P, Borah J, Chowdhury P, Doloi PK, Mahanta J. Dengue outbreak in an Indo-Myanmar border area: epidemiological aspects and risk factors. Trop Biomed. 2013; 30: 451–58.
- 32,33. Chaterji S, Allen JC, Chow A, Leo Y-S, Ooi E-E. Evaluation of the NS1 Rapid Test and the WHO Dengue Classification Schemes for Use as Bedside Diagnosis of Acute Dengue Fever in Adults. The American Journal of Tropical Medicine and Hygiene. 2011;84(2):224-228. doi:10.4269/ajtmh.2011.10-0316.
- 33,34. Peeling RW, Artsob H, Pelegriño JL, Buchy P, Cardoso MJ, Devi S, et al. Evaluation of diagnostic tests: dengue. Nat Rev Microbiol 2010; 8(12 Suppl): S30-S38.

Durand JP, Vallée L, de Pina JJ, Tolou H. Isolation of a dengue type 1 virus from a soldier in West Africa (Côte d'Ivoire). *Emerging Infectious Diseases*. 2000;6(1):83-84.

C. G. Akoua-Koffi, V. Akran, O. Faye, M. Grandadam, E. Ekaza, K. S. Kouassi, D. Coulibaly, A. Sall, and M. Dosso, *Yellow Fever and Dengue Fever Serotype 3 Viruses Cocirculation in Côte d'Ivoire in 2008*, *African Journal of Pathology and Microbiology*, 3 (2014), art235834. doi:10.4303/ajpm/235834

S. Kalayanaroj, D. W. Vaughn, S. Nimmannitya, S. Green, S. Suntayakorn, N. Kunentrasai, W. Viramitrachai, S. Ratanachu-ek, S. Kiatpolpoj, B. L. Innis, A. L. Rothman, A. Nisalak, F. A. Ennis; Early Clinical and Laboratory Indicators of Acute Dengue Illness, *The Journal of Infectious Diseases*, Volume 176, Issue 2, 1 August 1997, Pages 313–321, <https://doi.org/10.1086/514047>

Solanke VN, Karmarkar MG, Mehta PR. Early dengue diagnosis: Role of rapid NS1 antigen, NS1 early ELISA, and PCR assay. *Trop J Med Res* 2015;18:95-9

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- 446
1. Table 1: Statistical summary of results of interest
- 447
2. Table 2: Distribution of Dengue indicators by age groups
- 448
3. Table 3: Cross tabulation of dengue NS1 antigen results against dengue IgM and IgG
- 449
- results respectively
- 450
4. Table 4: Proportional distribution of indicators of dengue infection by region
- 451
5. Figure 1: Geographical distribution of Dengue a) NS1 antigen, b) Dengue-specific
- 452
- IgM antibodies and c) Dengue-specific IgG antibodies in Ghana

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Table1: Descriptive statistics of results according to gender and test performed on 360 blood samples

Variable	N	N%	Min	Max	CV%
Sex					
Female	159	44.2	0.58	76	81.1
Male	201	55.8	1	82	87.2
Dengue NS1.Ag					
Negative	351	97.5	0.58	82	85.6
Positive	9	2.5	7	61	62.3
Dengue IgM					
Negative	353	98.1	0.58	82	84.7
Positive	7	1.9	2	77	85.4
Dengue IgG					
Negative	347	96.4	0.58	82	85.0
Positive	13	3.6	0.58	51	86.8
YF IgM results					
Positive	4	1.1	3.83	42	94.1
Negative	352	97.8	0.58	82	85.3
Equivocal	1	0.3	37	37	NA

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463 **Table2:** Distribution of number of negative and positive results by age group

Age Categories							
Variables	0-15yr	16-30yr	31-45yr	46-60yr	61-75yr	Above 75yr	Total
Dengue NS1 Results							
Negative	172	98	54	16	6	5	351
Positive	2	4	1	1	1	0	9
Dengue IgM Results							
Negative	172	100	53	17	7	4	353
Positive	2	2	2	0	0	1	7
Dengue IgG Results							
Negative	167	99	53	16	7	5	347
Positive	7	3	2	1	0	0	13
Total	174	102	55	17	7	5	360

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466 **Table3:** Cross tabulation of dengue NS1 antigen results against dengue IgM and IgG results respectively

Dengue NS1 Results			
	Negative	Positive	Total
Dengue IgM Results			
Negative	344	9	353
Positive	7	0	7
Dengue IgG Results			

Negative	340	7	347
Positive	11	2	13
Total	351	9	360

Table4: Distribution of positive and negative numbers by test and region in Ghana

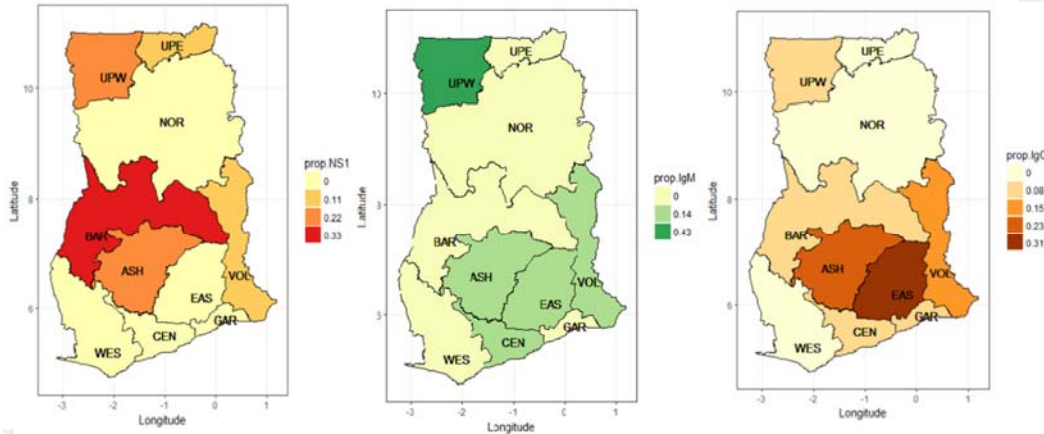
Region	Dengue NS1 Results		Dengue IgM Results		Dengue IgG Results		Total	Positive
	Negative	Positive	Negative	Positive	Negative	Positive		
Ashanti	48	2 (0.22)*	49	1 (0.14)*	47	3 (0.23)*	5006	
Brong Ahafo	71	3 (0.33)	74	0	73	1 (0.08)	7404	
Central	9	0	8	1 (0.14)	8	1 (0.08)	902	
Eastern	60	0	59	1 (0.14)	56	4 (0.31)	6005	
Greater Accra	11	0	11	0	10	1 (0.08)	1101	
Northern	8	0	8	0	8	0	800	
Upper East	29	1 (0.11)	30	0	30	0	3001	
Upper West	60	2 (0.22)	59	3 (0.43)	61	1 (0.08)	6206	
Volta	35	1 (0.11)	35 (9.7)	1 (0.14)	34	2 (0.15)	3604	
Western	20	0	20 (5.6)	0	20	0	2000	
Total	351	9	353	7	347	13	36029	

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b

c

472 **Figure1: Geographical distribution of positive results for dengue second test in Ghana: a) NS1 antigen, b)**
473 **specific dengue IgM antibodies and c) specific IgG antibodies.**

