Characterization of dengue cases among patients with an acute illness, Central Department, Paraguay

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Background. In 2018, Paraguay experienced a large dengue virus (DENV) outbreak. The primary objective of this study was to characterize dengue cases in the Central Department, where the majority of cases occur, and identify factors associated with DENV infection. **Methods.** Patients were enrolled from January-May 2018 if they presented with a suspected arboviral illness. Acute-phase specimens (≤8 days after symptom onset) were tested using rRT-PCR, a rapid diagnostic test for DENV nonstructural protein 1 (NS1) and anti-DENV IgM and IgG, and ELISA for IgG against NS1 from Zika virus (ZIKV). **Results.** 231 patients were enrolled (95.2% adults) at two sites: emergency care and an outpatient clinical site. Patients included 119 (51.5%) dengue cases confirmed by rRT-PCR (n=115, 96.6%) and/or the detection of NS1 and anti-DENV IgM (n=4, 3.4%). DENV-1 was the predominant serotype (109/115, 94.8%). Epidemiologically, dengue cases and non-dengue cases were similar, though dengue cases were less likely to reside in a house/apartment or report a previous dengue case. Clinical and laboratory findings associated with dengue included red eyes, absence of sore throat, leucopenia and thrombocytopenia. At an emergency care site, 26% of dengue cases (26/100) required hospitalization. In univariate analysis, hospitalization was associated with increased viral load, anti-DENV IgG, and thrombocytopenia. Among dengue cases that tested positive for IgG against ZIKV NS1, the odds of DENV NS1 detection in the acute phase were decreased 10-fold (OR 0.1, 0.0-0.3). **Conclusions.** Findings from a predominantly adult population demonstrate clinical and laboratory factors associated with DENV infections and the potential severity of dengue in this group. The combination of viral load and specific IgG antibodies warrant further study

as a prognostic to identify patients at risk for severe disease.

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23   **Abstract**

24   **Background.** In 2018, Paraguay experienced a large dengue virus (DENV) outbreak. The

25   primary objective of this study was to characterize dengue cases in the Central Department,

26   where the majority of cases occur, and identify factors associated with DENV infection.

28   **Methods.** Patients were enrolled from January-May 2018 if they presented with a suspected

29   arboviral illness. Acute-phase specimens (≤8 days after symptom onset) were tested using rRT-

30   PCR, a rapid diagnostic test for DENV nonstructural protein 1 (NS1) and anti-DENV IgM and

31   IgG, and ELISA for IgG against NS1 from Zika virus (ZIKV).

33   **Results.** 231 patients were enrolled (95.2% adults) at two sites: emergency care and an

34   outpatient clinical site. Patients included 119 (51.5%) dengue cases confirmed by rRT-PCR

35   (n=115, 96.6%) and/or the detection of NS1 and anti-DENV IgM (n=4, 3.4%). DENV-1 was the

36   predominant serotype (109/115, 94.8%). Epidemiologically, dengue cases and non-dengue cases

37   were similar, though dengue cases were less likely to reside in a house/apartment or report a

38   previous dengue case. Clinical and laboratory findings associated with dengue included red eyes,

39   absence of sore throat, leucopenia and thrombocytopenia. At an emergency care site, 26% of

40   dengue cases (26/100) required hospitalization. In univariate analysis, hospitalization was

41   associated with increased viral load, anti-DENV IgG, and thrombocytopenia. Among dengue

42   cases that tested positive for IgG against ZIKV NS1, the odds of DENV NS1 detection in the

43   acute phase were decreased 10-fold (OR 0.1, 0.0-0.3).

44

45   **Conclusions.** Findings from a predominantly adult population demonstrate clinical and

46   laboratory factors associated with DENV infections and the potential severity of dengue in this

47   group. The combination of viral load and specific IgG antibodies warrant further study as a

48   prognostic to identify patients at risk for severe disease.

49 **Introduction**

50 Dengue is the commonest human arboviral disease worldwide, with an estimated 50-100 million

51 cases occurring annually throughout the tropics and subtropics (Stanaway et al. 2016; World

52 Health Organization 2009). Dengue results from human infection with one of four related

53 serotypes of dengue virus (DENV-1-4) (Guzman & Harris 2015). All four serotypes circulate in

54 the Americas and cause large outbreaks every 2-5 years (Dantes et al. 2014; Gordon et al. 2013;

55 Pan American Health Organization 2018). Paraguay reports among the highest annual incidence

56 rates of dengue in South America (Pan American Health Organization 2018). Since the

57 introduction of DENV-4 in 2012, all four serotypes have been detected in the country. Over the

58 past decade, DENV-1 has circulated in all but one year and has been predominant since 2015

59 (Dirección General de Vigilancia de la Salud & Ministerio de Salud Pública y Bienestar Social

60 2017; Pan American Health Organization 2018). Despite significant declines in dengue incidence

61 throughout the Americas following the 2015-2016 Zika virus (ZIKV) epidemic, Paraguay

62 experienced large numbers of dengue cases in 2016 and again in 2018 (Pan American Health

63 Organization 2018; Perez et al. 2019). These data suggest that arboviral epidemiology may be

64 relatively unique in Paraguay, which is located at the southern boundary of the DENV-endemic

65 region in the Americas (Bhatt et al. 2013; Stanaway et al. 2016; World Health Organization

66 2009). However, relatively little data has been published on dengue in the country, and the

67 majority of available data has come either from hospitalized pediatric cases or from international

68 studies with only a subset of patients from Paraguay (Halsey et al. 2012; Lovera et al. 2014;

69 Lovera et al. 2016; Rojas et al. 2016).

70

71 Symptomatic DENV infections classically present as an acute fever with myalgias and rash

72 (Guzman & Harris 2015; World Health Organization 2009). However, patients can develop a

73 wide array of signs and symptoms, which limits the accuracy of a clinical diagnosis based on

74 exam findings and results of routine laboratory testing (Gregory et al. 2010; Morch et al. 2017;

75 Potts & Rothman 2008; Waggoner et al. 2016b). In addition, dengue manifests differently among

76 children and adults, and factors associated with dengue cases and severe disease in a pediatric

77 population may not be applicable in older patients (Gregory et al. 2010; Hammond et al. 2005;

78 Kittigul et al. 2007). The differential diagnosis for dengue includes arboviral pathogens, such as

79 chikungunya virus (CHIKV) and Zika virus (ZIKV), and local endemic diseases, such as

80 leptospirosis, which may all cause an indistinguishable clinical picture (O. Silva et al. 2018;

81 Waggoner et al. 2016b). Accurate diagnosis in the acute phase relies upon the availability of

82 specific laboratory tests, which for DENV include molecular methods and nonstructural protein

83 1 (NS1) antigen detection. Anti-DENV IgM detection in acute-phase samples provides a

84 presumptive diagnosis (Peeling et al. 2010; World Health Organization 2009). As dengue can

85 progress to severe disease, including plasma leakage, hemorrhage, and shock, ideal testing

86 algorithms would not only detect DENV infections but also provide prognostic information.

87

88 The primary objective of the current study was to characterize DENV infections in the Central

89 Department of Paraguay and the metropolitan area of Asunción. This region annually accounts

90 for ~2/3 of dengue cases in Paraguay and also reported Zika cases in 2016. Multiplex molecular

91 testing, NS1 antigen detection and serological methods were implemented to confirm cases

92 identified using a broad clinical case definition. We then sought to evaluate factors associated

93 with dengue cases and the need for hospitalization in a predominantly adult patient population.

94

Materials & Methods

96

97 **Ethics statement.** The study protocol was reviewed and approved by the Scientific and Ethics

98 Committee of the Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de

99 Asunción (IICS-UNA, IRB00011984), and the Emory University Institutional Review Board

100 (IRB00000569). Written informed consent was obtained from all subjects. Children older than

101 six years of age provided assent.

102

103 **Patient population and clinical samples.** Patients of all ages were enrolled from January to

104 May 2018 if they presented with an acute illness (≤ 8 days) defined by 2 or more of the

105 following: fever (measured or subjective), red eyes, rash, joint pain involving more than one

106 joint, and/or diffuse muscle pain. Patients with fever and no other localizing signs or symptoms

107 were included. Day 1 was defined as the first day of symptoms. Exclusion criteria included

108 dysuria or malodorous urine, cellulitis/skin abscess, vomiting and/or a productive cough. Patients

109 were enrolled at in the Emergency Care Clinic at Hospital Villa Elisa and at IICS-UNA, both

110 located in metro Asunción. The Emergency Care Clinic serves an ambulatory urgent care patient

111 population; patients may be assigned to observation at Hospital Villa Elisa or referred to an

112 inpatient facility that can provide a higher level of care. Serum was collected during the acute

113 visit, aliquoted and stored at -80°C until use. The results from hemograms, performed as part of

114 routine care, were obtained by chart review. Data was included in this study if the hemogram

115 was obtained on the day of the study visit ± 1 day.

116

117   **Molecular detection.** RNA was extracted from 140μL of serum into 60μL of elution buffer with

118   the QIAamp Viral RNA Mini Kit (Qiagen, Germantown, MD). All samples were tested for

119   ZIKV, CHIKV and DENV by real-time RT-PCR (rRT-PCR) using a validated and published

120   multiplex assay (the ZCD assay) as previously described (Waggoner et al. 2016a). DENV

121   serotype and viral load were determined with a DENV multiplex assay using a published

122   protocol (Waggoner et al. 2013b; Waggoner et al. 2013c). Samples that tested negative in the

123   ZCD assay were tested for RNase P to confirm successful extraction and the absence of

124   inhibitors (Waggoner et al. 2013a). Arboviral rRT-PCR testing was performed at IICS-UNA.

125

126   **Serological assays.** All serum samples were test for DENV NS1 antigen and anti-DENV IgM

127   and IgG using the STANDARD Q Dengue Duo assay (SD Biosensor, Suwon, South Korea).

128   Results were read initially at 15 or up to 20 minutes, according to manufacturer

129   recommendations. One hundred fifty-six samples were tested for anti-ZIKV IgG using the

130   ZIKVG.CE kit (Diagnostic Bioprobes, Milan, Italy), which detects antibodies directed against

131   the ZIKV NS1 antigen. Given a limited supply of anti-ZIKV IgG kits, a mixture of samples was

132   selected for testing. This included dengue cases (n=76) and non-dengue cases (n=80), as well as

133   include patients with anti-DENV IgG (n=58) and without (n=98). Assays were performed

134   according to manufacturer recommendations.

135

136   **Definitions.** Dengue cases were defined by either the detection of 1) DENV RNA in serum using

137   the ZCD assay with confirmation in the DENV multiplex assay, or 2) both NS1 and anti-DENV

138   IgM. This conservative definition was used to ensure the accuracy of dengue-case calls in the

139   absence of paired acute and convalescent sera for confirmatory serological testing. This

140   definition also allowed us to evaluate the performance of the STANDARD Q DENV NS1 assay,

141   for which there was no prior published data. The sensitivity and specificity of individual

142   diagnostics were calculated in reference to positive and negative cases from this composite

143   definition.

144

145   **Statistics.** Basic statistical analyses were performed using Excel software (Microsoft, Redmond,

146   WA). Univariate analyses and multiple linear regression analyses were performed using

147   GraphPad Prism, version 8.0.1 (GraphPad, San Diego, CA). Categorical variables were

148   compared using Fisher's exact test. Age, day of illness, and continuous laboratory variables were

149   compared by t test. Viral load comparisons were performed using non-parametric tests (Mann-

150   Whitney with 2 groups; Kruskal-Wallis for 3 or more groups). Binary logistic regression analysis

151   was performed using SPSS (IBM, Armonk, NY). Model fit was assessed by comparing -2 log

152   likelihood statistics.

153

154   **Results**

155   Between January and May 2018, we enrolled 231 patients who met inclusion criteria, including

156   119 (51.5%) dengue cases and 112 (48.5%) non-dengue cases. No acute cases of ZIKV or

157   CHIKV were detected. Of the dengue cases, 115 (96.6%) tested positive by rRT-PCR and 4

158   additional cases (3.4%) were positive for DENV NS1 and anti-DENV IgM (Table 1). All cases

159   tested positive by rRT-PCR through day-of-illness 6 (n=104), with rates of detection declining

160   on days 7 (7/10, 70%) and 8 (4/5, 80%; Fig. 1). For the NS1 assay, the overall sensitivity and

161   specificity were 71.4% and 96.4%, respectively (Table 1). Although there appeared to be an

162   increase in NS1 sensitivity over the first 5 days of illness, this was not statistically significant

163   (p=0.208, day 5 vs. day 1-2; Fig. 1). The overall sensitivity and specificity of anti-DENV IgM

164   detection were 26.1% and 93.8%, respectively. The sensitivity of IgM detection increased from

165   0% on days 1-2 to 90% on day 7 (p<0.001).

166

167   DENV serotype was determined in all 115 rRT-PCR-positive cases, with DENV-1 identified in

168   109/115 (94.8%) cases and 3 cases (2.6%) each of DENV-2 and DENV-4. No co-infections were

169   detected. DENV-1 serum viral loads negatively correlated with day of illness at presentation

170   (Fig. 2A), but too few data points were available for DENV-2 and -4 to draw meaningful

171   conclusions. DENV viral load was also associated with NS1 detection: viral loads were

172   significantly higher in samples with detectable NS1 (median 7.7 $\log_{10}$ copies/mL, IQR 5.8-8.6)

173   compared to those in which NS1 was not detectable (median 5.6 $\log_{10}$ copies/mL, IQR 3.6-7.2;

174   p<0.0001; Fig. S1).

175

176   Anti-DENV and anti-ZIKV IgG results were available for 156 patients, including 76 dengue

177   cases (48.7%). 58 patients (37.2%) tested positive for anti-DENV IgG and 49 (31.4%) tested

178   positive for anti-ZIKV IgG, with 32 patients (20.5%) positive for both. The viral load among

179   dengue cases declined in a stepwise manner among patients with anti-ZIKV IgG, anti-DENV

180   IgG, or both (Fig. 2B, p < 0.0001 for the trend). In a multivariable model that included day of

181   illness at presentation and patient age, DENV serum viral load was 1.3 $\log_{10}$ copies/mL lower

182   among patients with detectable anti-DENV IgG compared to patients without anti-DENV IgG

183   (p<0.0001, Table S1). Similarly, serum viral load was 0.7 $\log_{10}$ copies/mL lower among patients

184   with anti-ZIKV IgG directed against NS1 (p=0.047).

185

186 Among dengue cases, DENV NS1 detection was also associated with IgG status. Patients with

187 anti-DENV IgG were significantly less likely to have detectable NS1 [20/36 (55.6%) vs. 65/83

188 (78.3%); OR 0.3, 95% CI 0.1-0.8]. However, when we controlled for the detection of IgG against

189 ZIKV NS1, the OR for NS1 detection among dengue cases with anti-ZIKV IgG was 0.1 (95% CI

190 0.0-0.3) and the association with anti-DENV IgG was no longer significant (OR 1.0, 95% CI 0.3-

191 3.1; Table S2).

192

193 **Epidemiologic characteristics.** The epidemiologic characteristics of the patient population are

194 shown in Table 2. This was predominantly an adult population, with only 11 participants < 18

195 years of age at study entry (4.8%). Dengue cases occurred throughout the study period (Fig. S2)

196 and were similar to non-dengue cases for the majority of epidemiological variables analyzed.

197 Most patients reported living in a house or an apartment (157/188 for which data was available,

198 83.5%), but 31 patients reported "other" for housing without providing further detail. The odds

199 of dengue in this population were significantly higher than among patients with a different living

200 arrangement (OR 2.9, 95% CI 1.3-7.0). Only 10.6% of our patients (21/199) reported having

201 screens on their windows, though 79.2% of patients had air conditioning (156/197). The

202 percentage of dengue cases among patients with neither screens nor air conditioning (19/37,

203 51.4%) was similar to that of patients with screens, air conditioning, or both (79/161, 49.1%; OR

204 1.1, 95% CI 0.5-2.2).

205

206 A subset of patients self-reported having been vaccinated against yellow fever virus (YFV). The

207 odds of having a dengue case were lower among patients who had received the YFV vaccine

208 compared to those who had not (OR 0.6; 0.4-1.2), and more time had elapsed since vaccination

209   among dengue cases. However, these trends did not reach statistical significance (p=0.15).

210   Receipt of the YFV vaccine did not increase the need for hospitalization among dengue cases.

211

212   **Clinical presentation.** Patient symptoms at presentation are shown in Table 3. The majority of

213   patients met inclusion criteria with fever plus one additional symptom in the study definition,

214   most commonly muscle pain (198/225, 88.0%) and/or joint pain (172/221, 77.8%). Only 11

215   patients (4.8%) had fever and no other localizing sign or symptom (6 dengue cases), and 8

216   patients (3.5%) were enrolled that did not have fever (1 dengue case). Patients who reported red

217   eyes were significantly more likely to have dengue (OR 2.1; 95% CI 1.2-3.6) and those with a

218   sore throat were significantly less likely to have dengue (OR 0.5; 95% CI 0.3-0.8; Table 3).

219   Although a reported headache increased the odds of having dengue, this did not reach statistical

220   significance (OR 2.3; 95% CI 1.0-5.5), and headache was very common overall. Other symptoms

221   occurred with similar frequency in the two groups, and no combination of symptoms accurately

222   differentiated between dengue and non-dengue cases.

223

224   Hemogram results are also shown in Table 3. Patients with dengue had significantly lower

225   platelet and leucocyte counts relative to non-dengue cases (Fig. 3). Thrombocytopenia (<150,000

226   per μL) and leucopenia (<4,000 cells/mm^3) were both significantly associated with DENV

227   infections (Table 3). However, patients with both findings were not at greater odds of having a

228   DENV infection (OR 8.9; 95% CI 3.4-23.0) than patients with leucopenia alone (OR 11.0, 95%

229   CI 5.1-22.2). Dengue cases had lower neutrophil and lymphocyte counts, but these occurred in

230   proportion to the decrease in leucocyte counts (data not shown).

231

232 **Hospitalization.** For the analysis of factors associated with hospitalization for dengue, we

233 focused on cases that presented to Hospital Villa Elisa, as only 1/19 dengue cases (5.3%) at

234 IICS-UNA required hospitalization. Of 100 dengue cases at Hospital Villa Elisa, 26 (26.0%)

235 were hospitalized and one patient died (Table 4). A number of clinical and laboratory findings

236 were associated with hospitalization in univariate analysis. Rash and bleeding were more

237 common among hospitalized cases. Admitted patients were significantly more likely to have

238 detectable anti-DENV IgG and IgG against both DENV and ZIKV (anti-NS1). Despite the

239 presence of anti-DENV IgG, viral load was significantly higher among admitted patients, but

240 there was no difference in NS1 detection. In multivariate analysis, the best-fit model for

241 predictors of hospitalization only included platelet count and day of illness, though the odds ratio

242 for day of illness did not reach significance (OR 1.3, 95% CI 0.9-1.8; Table S3).

243

244 **Discussion**

245 In the current study, we characterized a set of dengue cases in a primarily adult population that

246 presented to outpatient facilities in metro Asunción. Dengue is a major public health problem in

247 Paraguay, with adults accounting for a significant proportion of cases. At Hospital Villa Elisa,

248 58% of patients with an acute febrile illness were adults ≥20 years of age, and an additional 13%

249 of patients were aged 15-19. While studies have demonstrated that the clinical presentation of

250 dengue in adults may differ from that in children (Hammond et al. 2005; Kittigul et al. 2007;

251 Potts & Rothman 2008), less research has specifically evaluated factors that differentiate dengue

252 from other causes of an acute febrile illness in the adult population (Chadwick et al. 2006;

253 Gregory et al. 2010). All but one dengue case in our study presented with fever and a high

254 percentage of cases had headache, myalgia and/or arthralgia (Chadwick et al. 2006; Hammond et

255   al. 2005; Kittigul et al. 2007; Potts & Rothman 2008). However, these symptoms were common

256   among both dengue cases and non-dengue cases. The only two symptoms that were significantly

257   associated with dengue in our population were red eyes and the absence of a sore throat. Red

258   eyes have not been commonly associated with dengue (Chadwick et al. 2006), and the frequency

259   of this finding could make it difficult to differentiate dengue from Zika in regions where both

260   viruses are transmitted (Chadwick et al. 2006; O. Silva et al. 2018; Waggoner et al. 2016b). The

261   absence of a sore throat has been associated with dengue in a previous series (Gregory et al.

262   2010). However, this was only reported by 26.5% of our patients overall, which limits the utility

263   of this finding in clinical practice.

264

265   In contrast to clinical findings, the results of general laboratory studies differed significantly

266   between dengue cases and non-cases. Leucopenia and thrombocytopenia were associated with

267   dengue (ORs 11.0 and 4.0, respectively). However, patients with both findings did not have

268   higher odds of dengue that those with leucopenia alone, which may have resulted from temporal

269   differences in the development and resolution of these abnormalities (Biswas et al. 2012). The

270   nadir leucocyte counts occurred on days 5-6 after symptom onset, whereas platelet counts

271   demonstrated a consistent decline through day 8 (data not shown). Many factors were

272   significantly associated with hospitalization in univariate analyses but were also strongly

273   correlated with one another (viral load, antibody status, platelet count, day of illness). Given the

274   sample size, our ability to model all of these factors in logistic regression was limited, and

275   admission decisions were likely based on the platelet count, which may have obscured the

276   association between other factors and disease severity.

277

278 DENV infections were confirmed using a combination of methods, though all but four cases

279 were positive by rRT-PCR (115/119, 96.6%). NS1 was detected in 71.4% of infections and

280 proved specific for DENV (96.4%). Notably, the performance of this commercial NS1 kit has

281 not been published, but results appeared similar to those reported for other rapid NS1 assays

282 (Blacksell et al. 2011). Consistent with previous observations, viral loads were significantly

283 higher among NS1-positive individuals (Duong et al. 2011; Duyen et al. 2011; Erra et al. 2013;

284 Tricou et al. 2011). Both viral load and NS1 detection were significantly associated with the

285 detection of anti-DENV IgG and anti-ZIKV IgG, which in this study was directed against the

286 NS1 antigen. In an earlier study, ZIKV-specific neutralizing antibodies were not detected among

287 a subset of our patients (A. Rojas, unpublished data). As such, anti-ZIKV IgG identified by

288 ELISA in the current study is favored to represent cross-reacting anti-DENV antibodies. In the

289 subset of patients with results for both IgG assays, the presence of anti-ZIKV NS1 IgG

290 accounted for virtually all false-negative NS1 results. Although such antibodies have been

291 known to reduce NS1 detection in secondary cases (Jayathilaka et al. 2018; Lee et al. 2015; Lima

292 Mda et al. 2014), the pathophysiologic significance of anti-NS1 antibodies in human DENV

293 infections remains unclear (Glasner et al. 2018; Jayathilaka et al. 2018). We demonstrate that

294 these antibodies can be detected in the acute-phase and, in combination with anti-DENV IgG, are

295 more common among hospitalized dengue cases. These serologic findings combined with an

296 elevated DENV viral load warrant further evaluation using standardized severity criteria (World

297 Health Organization 1997; World Health Organization 2009).

298

299 Dengue cases were less likely to report living in a house or apartment (recorded as "other" in the

300 study questionnaire). This was also observed in a seroprevalence study in Mexico where these

301   patients reported a "shared" living arrangement (Pavia-Ruz et al. 2018). Other aspects of the

302   home environment evaluated in our study did not differ between dengue and non-dengue cases.

303   The absence of air conditioning and window screens did not appear to increase the risk for

304   DENV infection. However, complete screening of the home and air conditioning have been

305   associated with decreased vector indices and dengue incidence in other settings (Manrique-Saide

306   et al. 2015; Pavia-Ruz et al. 2018; Reiter et al. 2003; Waterman et al. 1985), and the addition of

307   screens has been proposed as a means of DENV control through improvements to the built

308   environment (Lindsay et al. 2017; Vazquez-Prokopec et al. 2016). Our findings may indicate that

309   patients acquired DENV outside the home or that the use of these interventions is incomplete

310   (e.g. non-intact screens, intermittent use of air conditioning). Determining the location of

311   exposure will have important implications for DENV control efforts in metro Asunción.

312

313   Vaccination against YFV is not part of the routine schedule in Paraguay, and as a result, our

314   patient population included a mixture of individuals who did or did not report receiving the

315   vaccine. There was no evidence of increased risk from YFV vaccination for either incident

316   dengue or the development of severe disease. These data are consistent with recent findings from

317   Brazil where no association was found between severe dengue and receipt of the YFV vaccine

318   (Luppe et al. 2019).

319

320   DENV-1 was the predominant serotype identified in the current study. This is consistent with

321   recent DENV epidemiology in Paraguay, but it may limit the generalizability of our findings for

322   other serotypes. An additional limitation to the study is that we were unable to evaluate the

323   performance of the clinical case definition for different arboviral infections, and in particular

324   ZIKV infections that may not present with fever (Braga et al. 2017). Finally, patients were

325   included who reported up to 8 days of symptoms prior to enrollment. Laboratory data from day 8

326   produced conflicting results and raises questions regarding the accuracy of symptom recall past

327   one week. These data support the use of earlier enrollment cut-offs with scheduled follow-up

328   visits to monitor the kinetics of certain laboratory findings.

329

330   **Conclusions**

331   In this study, we sought to characterize DENV infections in a predominantly adult population in

332   Paraguay, focusing on the region with the highest dengue incidence, metro Asunción. This work

333   highlighted clinical, epidemiologic, and laboratory factors that are associated with DENV

334   detection in the acute setting and the potential role of specific antibodies in diagnosis and the

335   progression of disease. Future directions will involve the prospective evaluation of how factors

336   identified in the current study associate with and may predict dengue severity.

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477   **Figure Legends**

478   **Figure 1.** Sensitivity of rRT-PCR, NS1, and IgM for dengue based on day of illness at

479   presentation.

480

481   **Figure 2.** DENV-1 viral load by day of illness at presentation (A). Viral loads are shown for

482   individual samples; bars display the mean and 95% CI. Six patients had infections with DENV-2

483   (n=3) or DENV-4 (n=3), which are not displayed. DENV viral load at presentation decreases in a

484   stepwise manner among individuals with anti-ZIKV IgG, anti-DENV IgG, or both (B). Results

485   were significant by ANOVA for both analyses, $p < 0.0001$.

486

487   **Figure 3.** Platelet (A) and leucocyte (B) counts at presentation among dengue cases (●) and non-

488   dengue cases (▲). Bars represent means ± 95% CI; population mean values are shown. P values

489   (t test): **** = $p < 0.0001$.

DENV diagnostic test results according to test method. DENV viral load is shown for rRT-PCR positive samples within a given category.

1 **Table 1.** DENV diagnostic test results according to test method. DENV viral load is shown for

2 rRT-PCR positive samples within a given category.

Test Results	Composite Definition		Day of Illness	Viral Load
	Positive (n = 119)	Negative (n = 112)	mean (sd)	mean (sd) [a]
Combination of Methods				
rRT-PCR	28 (23.5)	—	3.1 (1.5)	6.10 (1.69)
rRT-PCR and NS1	60 (50.4)	—	3.2 (1.4)	7.85 (1.27)
rRT-PCR, NS1, and IgM	21 (17.7)	—	5.3 (1.3)	5.40 (1.19)
rRT-PCR and IgM	6 (5.0)	—	6.7 (1.0)	3.58 (0.22)
NS1 and IgM	4 (3.4)	—	7.2 (0.1)	—
Negative	—	112	3.2 (1.6)	—
Positives according to method				
rRT-PCR	115 (96.6)	—	3.7 (1.7)	
NS1	85 (71.4)	4 (3.6) [b]	3.9 (1.8)	
IgM	31 (26.1)	7 (6.2) [b]	5.6 (1.5)	

3 [a] Reported as $\log_{10}$ copies/mL of serum

4 [b] Specificities were 96.4% (NS1) and 93.8% (IgM)

Epidemiologic data on patients presenting with an acute febrile illness who tested positive or negative for DENV.

1 **Table 2.** Epidemiologic data on patients presenting with an acute febrile illness who tested

2 positive or negative for DENV.

Factor [a]	Total	Dengue Cases	Non-Dengue	p-value
Patients	231 (100)	119 (100)	112 (100)	
Gender, female	128 (55.4)	63 (52.9)	65 (58.0)	
Age, mean (sd)	31.94 (14.3)	31.3 (15.0)	32.6 (13.6)	
Clinical Site				
Hospital Villa Elisa	185 (80.1)	100 (84.0)	85 (75.9)	
IICS-UNA	46 (19.9)	19 (16.0)	27 (24.1)	
Department				
Central	209 (90.5)	109 (91.6)	100 (89.3)	
Capital	20 (8.7)	8 (6.7)	12 (10.7)	
Residence				
House	149 (79.3)	70 (74.4)	79 (84.0)	
Apartment	8 (4.3)	2 (2.1)	6 (6.4)	
Other	31 (16.5)	22 (23.4)	9 (9.6)	0.017
Screens	21 (10.6)	10 (10.0)	11 (11.1)	
Air-conditioning	156 (79.2)	76 (78.4)	80 (80.0)	
Running water	199 (98.0)	97 (97.0)	102 (99.0)	
Water storage	18 (8.8)	8 (8.0)	10 (9.5)	
Exposures				
Travel in the last month	52 (25.0)	22 (20.8)	30 (29.4)	
Work or school outside of the home	152 (80.4)	68 (73.9)	84 (86.6)	
Work or school outdoors	24 (55.8)	11 (57.9)	13 (54.2)	
Medical History				
Received yellow fever vaccine	71 (42.0)	31 (37.3)	40 (46.5)	
Years since vaccination, mean (sd) [c]	7.6 (4.1)	8.5 (3.7)	6.8 (4.3)	

Personal history of dengue	78 (34.2)	29 (24.6)	49 (44.6) [d]	0.002

3   Abbreviations: n, number; sd, standard deviation

4   [a] Unless otherwise specific, all values presented as n (% of patients with a response recorded)

5   [b] OR for dengue for patients reporting "other", 2.9 (95% CI 1.3-7.0)

6   [c] Year of YF vaccination was available for 52 patients (24 DENV-positive, 28 DENV-negative)

7   [d] OR for dengue in patients who reported a history of dengue, 0.4 (95% CI 0.2-0.7)

Symptoms and laboratory findings among patients with and without dengue.

1   **Table 3.** Symptoms and laboratory findings among patients with and without dengue.

Factor [a]	Total	Dengue Cases	Non-Dengue	OR (95% CI) [b]	p-value
Patients	231 (100)	119 (100)	112 (100)		
Day of symptoms, mean (sd)	3.9 (2.5)	4.1 (1.9)	3.7 (3.0)		
Symptoms and signs at presentation					
Fever	221 (96.5)	117 (99.2)	104 (93.7)		
Headache	206 (89.6)	111 (93.3)	95 (85.6)	2.3 (1.0-5.5)	0.083
Retro-orbital pain	94 (40.9)	53 (44.5)	41 (36.9)		
Muscle pain	198 (88.0)	99 (86.8)	99 (89.2)		
Joint pain	172 (77.8)	92 (80.7)	80 (74.8)		
Nausea	142 (61.7)	73 (61.3)	69 (62.2)		
Malaise	119 (51.7)	62 (52.1)	57 (51.4)		
Red eyes	99 (45.0)	61 (51.5)	38 (35.8)	2.1 (1.2-3.6)	0.010
Abdominal pain	95 (41.3)	51 (42.9)	44 (39.6)		
Vomiting	73 (31.7)	41 (34.5)	32 (28.8)		
Diarrhea	66 (28.7)	31 (26.1)	35 (31.5)		
Shortness of breath	64 (27.8)	36 (30.3)	28 (25.2)		
Sore throat	61 (26.5)	23 (19.3)	38 (34.2)	0.5 (0.3-0.8)	0.011
Cough	51 (22.2)	25 (21.0)	26 (23.4)		
Rash	52 (23.1)	32 (27.8)	20 (18.2)		
Edema	37 (16.2)	17 (14.3)	20 (18.0)		
Bleeding	32 (13.9)	20 (16.8)	12 (10.8)		
Laboratory results					
Hemoglobin, g/dL, mean (sd)	13.9 (1.5)	14.0 (1.5)	13.8 (1.5)		
Platelet count, per µL, mean (sd)	217,550 (89,921)	188,227 (82,079)	252,609 (86,650)		<0.001
Thrombocytopenia, <150,000 per µL	46 (22.8)	36 (32.7)	10 (10.9)	4.0 (1. 9-8.2)	<0.001
Leucocyte count, cells per mm^3, mean (sd)	6090 (3686)	4158 (2023)	8401 (3899)		<0.001
Leucopenia, < 4,000 cells per mm^3	73 (36.1)	63 (57.3)	10 (10.9)	11.0 (5.1-22.2)	<0.001

2   Abbreviations: CI confidence interval; OR, odds ratio; sd, standard deviation

3   [a] Values presented as n (%) unless otherwise indicated, percentages were calculated based on the

4   number of patients with data recorded for a particular variable

5   [b] OR of having a dengue case versus a non-dengue case

Clinical history and test results among hospitalized and outpatient dengue cases at Hospital Villa Elisa.

1 **Table 4.** Clinical history and test results among hospitalized and outpatient dengue cases at

2 Hospital Villa Elisa.

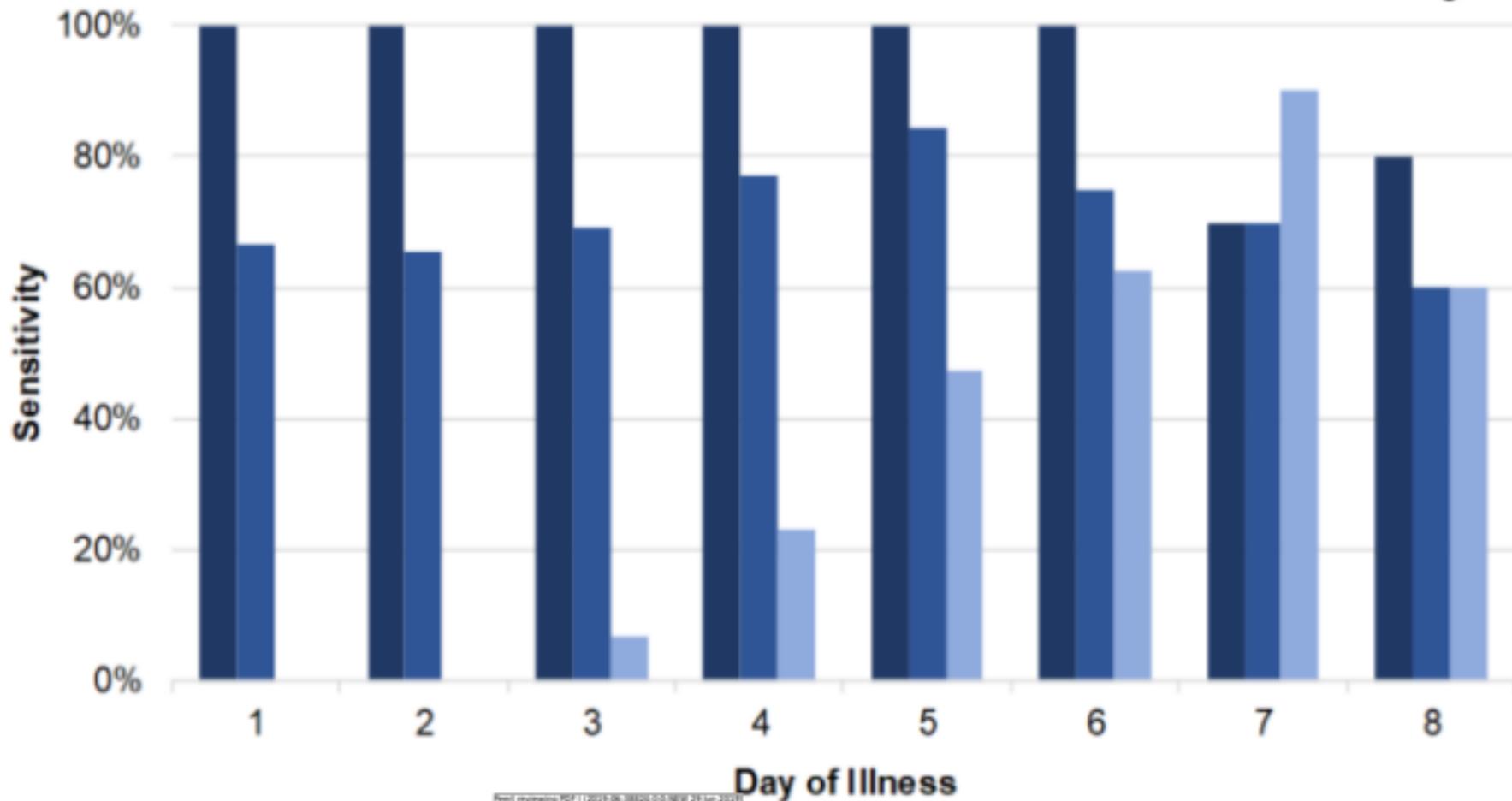
Patient Factors [a]	Total	Hospitalized	Outpatient	OR (95% CI) [b]	p-value
Patients	100 (100)	26 (100)	74 (100)		
History and Clinical findings					
Gender, female, n (%)	52 (52.0)	12 (46.2)	40 (54.1)		
Age, mean (sd)	31.6 (14.5)	36.5 (20.0)	29.9 (11.6)		0.044
Day of illness	3.81 (1.84)	5.0 (2.4)	3.4 (1.4)		<0.001
YFV vaccination	23/64 (35.9)	5/16 (31.2)	17/48 (35.4)		
Past dengue, per report	34/99 (34.3)	12/25 (48.0)	22/74 (28.6)	2.2 (0.9-5.5)	0.143
Rash	28/96 (29.2)	13/25 (52.0)	15/71 (21.1)	4.0 (1.5-10.0)	0.005
Diarrhea	27/100 (27.0)	11/26 (42.3)	16/74 (21.6)	2.7 (1.0-6.9)	0.070
Bleeding	18/100 (18.0)	10/26 (38.5)	8/74 (10.8)	5.2 (1.8-14.1)	0.006
Dengue test results					
rRT-PCR, positive	99 (99.0)	25 (96.2)	74 (100)		
Viral load, mean (sd)	6.44 (2.04)	6.76 (1.84)	5.51 (2.35)		0.028
NS1	69 (69.0)	17 (65.4)	52 (78.4)	0.8 (0.3-2.0)	0.632
IgM, anti-DENV	25 (25.0)	10 (38.5)	15 (20.3)	2.5 (1.0-6.6)	0.112
IgG, anti-DENV	28 (28.0)	14 (53.9)	14 (18.9)	5.0 (1.9-12.2)	0.002
IgG, anti-ZIKV	19/70 (27.1)	7/16 (43.8)	12/54 (22.2)	2.7 (0.9-8.1)	0.114
IgG against both DENV and ZIKV	13/67 (19.4)	7/13 (53.8)	6/54 (11.1)	9.3 (2.2 - 36.3)	0.002
Laboratory results [c]					
Hemoglobin, g/dL, mean (sd)	14.1 (1.4)	14.0 (2.0)	14.2 (1.2)		
Platelet count, per µL, mean (sd)	191,563 (85,951)	119,250 (77,402)	215,667 (74,749)		<0.001
Thrombocytopenia, <150,000 per µL	31 (32.3)	18 (75.0)	13 (18.1)	13.6 (4.5 - 43.2)	<0.001
Leucocyte count, cells per mm³, mean (sd)	4167 (2135)	4814 (3209)	3952 (1604)		0.087
Leucopenia, < 4,000 cells per mm³	55 (57.3)	13 (54.2)	42 (58.3)		

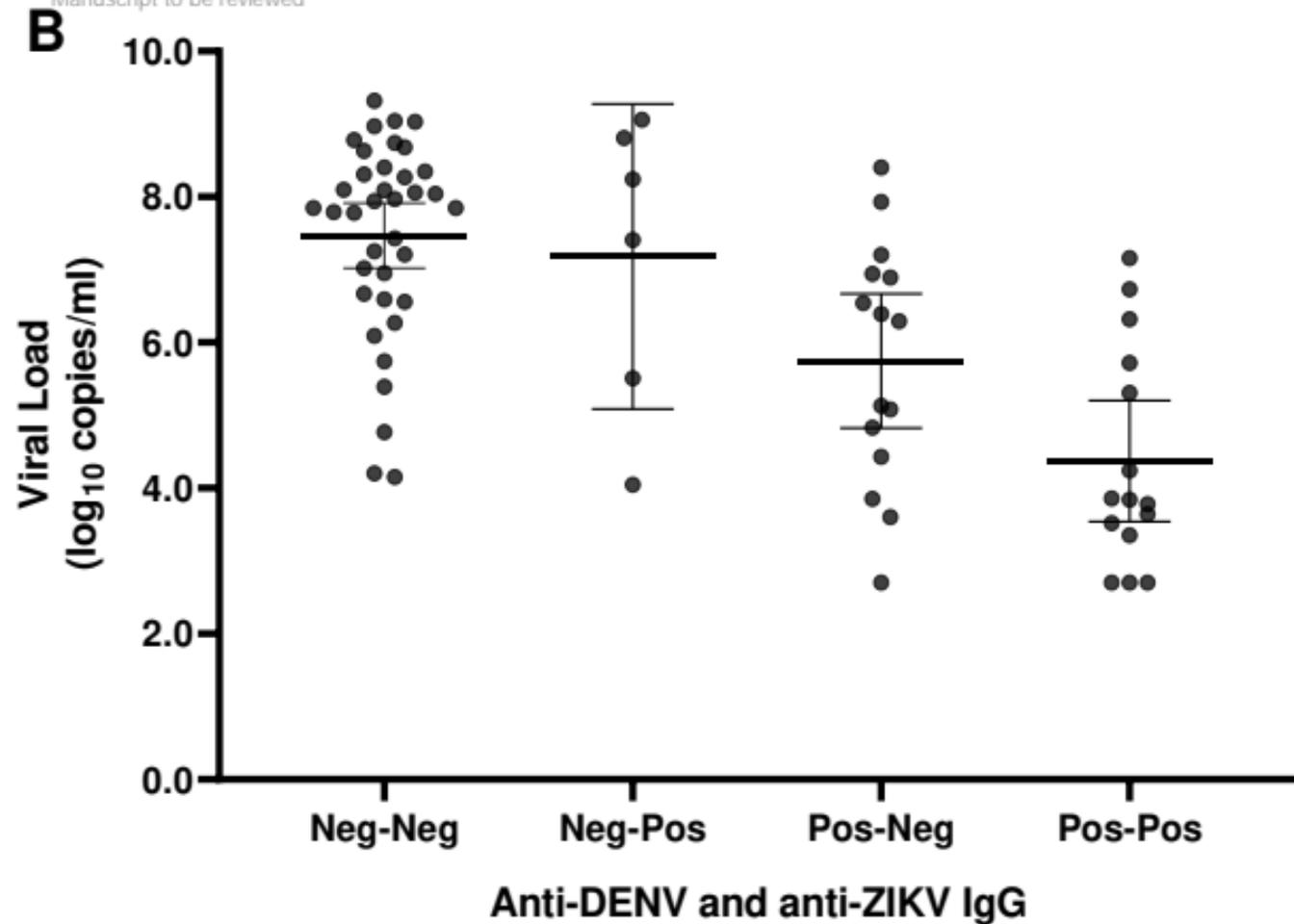
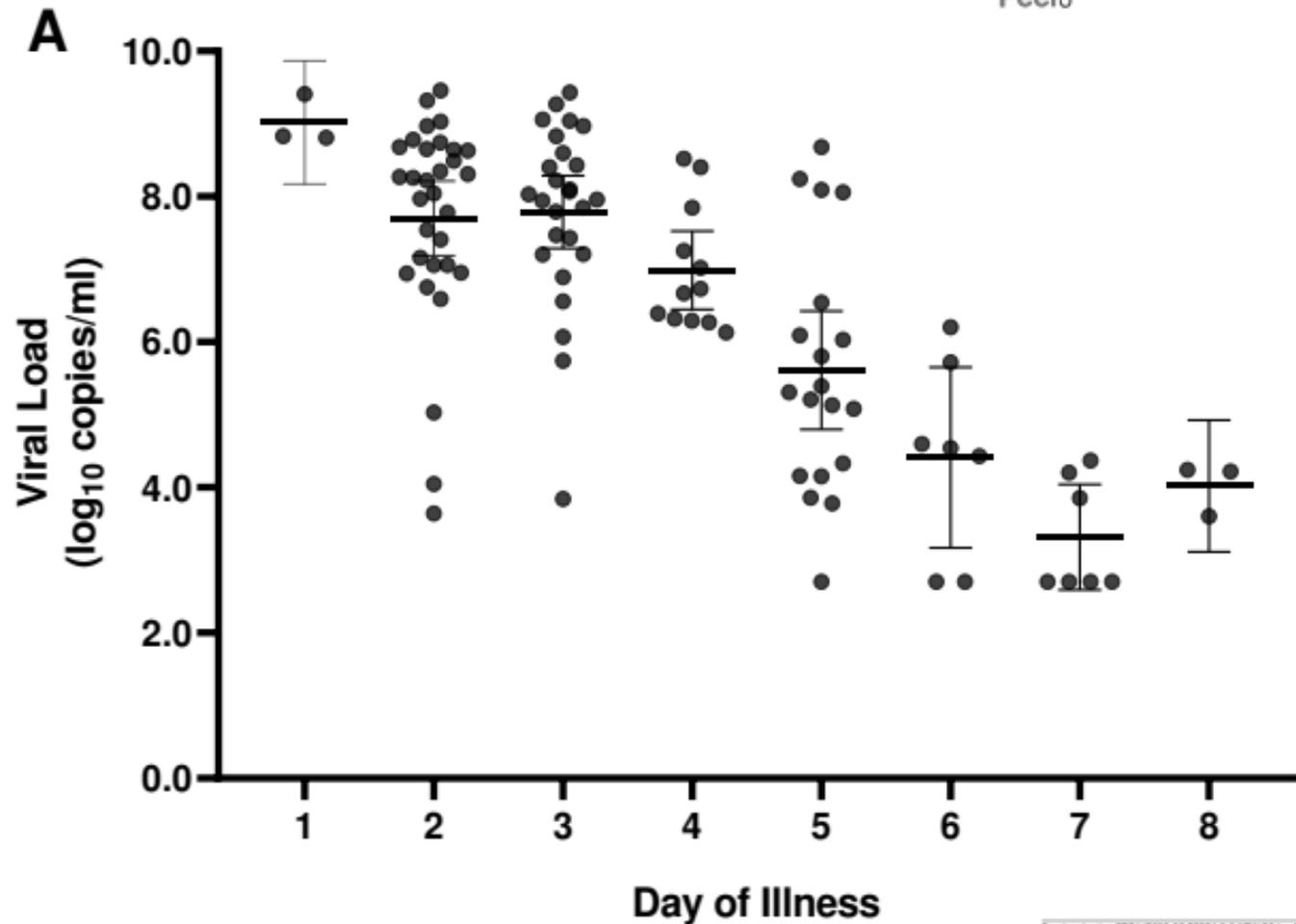
3 Abbreviations: CI confidence interval; OR, odds ratio; sd, standard deviation

4 [a] Values presented as n (%) unless otherwise indicated

5 [b] OR for hospitalization versus outpatient care

6   ^c Lab results were available for 24 and 72 hospitalized cases and outpatients, respectively.

Sensitivity of rRT-PCR, NS1, and IgM for dengue based on day of illness at presentation.

DENV viral load at presentation based on day of illness and antibody status.

DENV-1 viral load by day of illness at presentation (A). Viral loads are shown for individual samples; bars display the mean and 95% CI. Six patients had infections with DENV-2 (n=3) or DENV-4 (n=3), which are not displayed. DENV viral load at presentation decreases in a stepwise manner among individuals with anti-ZIKV IgG, anti-DENV IgG, or both (B). Results were significant by ANOVA for both analyses, p < 0.0001.

Platelet (A) and leucocyte (B) counts at presentation among dengue cases (●) and non-dengue cases (▲).

Bars represent means ± 95% CI; population mean values are shown. P values (t test): **** = p < 0.0001 .