

# Primary and Probable Secondary Dengue Virus (DV) Infection Rates in Relation to Age among DV IgM-Positive Patients Residing in the United States Mainland Versus the Caribbean Islands

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**Dengue virus (DV) primary infection and probable secondary infection rates in relation to patient age (years) were determined for DV IgM-positive U.S. mainland residents (presumed travelers to areas of DV endemicity) and Caribbean island (area of DV endemicity) residents by evaluating IgG status and IgG avidity. Regardless of place of residence, most patients  $\leq 20$  years old exhibited primary infection and most patients  $> 60$  years old exhibited probable secondary infection. Among patients 21 to 60 years old, the primary infection rate was markedly higher in U.S. residents.**

The four serotypes of dengue virus (DV) are endemic to tropical and subtropical areas worldwide, including the Caribbean basin, where they cause illnesses of major public health concern (28). DV infections are also an important medical concern outside the tropics and subtropics, where cases in travelers returning from areas of DV endemicity have been well documented (2, 9, 12, 17, 20, 29, 31). Primary infection with any DV serotype induces an immune response that protects against later infection by that serotype; however, subsequent infection by another serotype, termed secondary DV infection, is a risk factor for dengue hemorrhagic fever, which is associated with significant morbidity and occasionally death (19, 25, 33).

DV antibody reactivity patterns serve as useful tools for classifying patients as having primary or secondary DV infection. Detection of DV IgM in the absence of DV IgG (i.e., an IgM-positive/IgG-negative [IgM<sup>+</sup>IgG<sup>-</sup>] reactivity pattern) is a clear indicator of primary DV infection (4, 11). Similarly, an IgM<sup>+</sup>IgG<sup>+</sup> pattern combined with low IgG avidity accurately identifies primary DV infection (10–12, 15, 16, 22). An IgM<sup>+</sup>IgG<sup>+</sup> reactivity pattern with high IgG avidity is an accurate marker of secondary infection among patients whose serum samples were collected within a month of symptom onset (10–12, 15, 16, 22); however, this reactivity pattern also characterizes patients with primary DV infection who were previously exposed to other flaviviruses (via infection or vaccination) (12). Further, based on IgG avidity maturation trends observed for other viral infections (5, 6, 14, 24), an IgM<sup>+</sup>IgG<sup>+</sup> pattern with high IgG avidity may occur in primary DV infection patients late in the convalescent phase (several months postinfection). Thus, in the absence of information on the timing of specimen collection in relation to symptom onset, an IgM<sup>+</sup>IgG<sup>+</sup> reactivity pattern with high avidity can only be considered a marker of probable secondary DV infection.

Epidemiological studies have shown that the likelihood of acute DV infection representing secondary infection increases with age for residents of areas of DV endemicity (18, 23, 30). However, the relationship between patient age and proportions of primary and secondary DV infections among residents of areas of nonendemicity, where DV infections are nearly always associated with foreign travel (17), has not been clearly delineated. We thus sought to employ IgM/IgG reactivity patterns and IgG avidity results to estimate the proportions of primary and probable second-

ary DV infections among different age groups of DV IgM-positive patients from geographically proximate areas of endemicity and nonendemicity, namely, the Caribbean islands and the U.S. mainland, respectively.

Sera included in this analysis were submitted to Focus Diagnostics for DV antibody testing between March 2009 and December 2010 and found to be DV IgM positive. Clinical information (e.g., time since onset of symptoms) was not supplied for any of the specimens. The DV IgM assay was a mu-capture enzyme-linked immunosorbent assay (ELISA), and the DV IgG assay was an indirect ELISA; both were performed as previously described (21, 22). Results were expressed as indexes, calculated by dividing the specimen absorbance value by the mean calibrator serum absorbance value; index values of  $> 1.10$  were considered positive. Most sera exhibiting a DV IgM<sup>+</sup>IgG<sup>+</sup> reactivity pattern were further evaluated using the DV IgG avidity ELISA, performed as previously described (22). Avidity values of  $\leq 0.39$  were considered low IgG avidity, whereas values of  $> 0.39$  were considered high avidity (22). A primary infection was defined by either an IgM<sup>+</sup>IgG<sup>-</sup> reactivity pattern or an IgM<sup>+</sup>IgG<sup>+</sup> reactivity pattern with low IgG avidity. A probable secondary infection was defined by an IgM<sup>+</sup>IgG<sup>+</sup> reactivity pattern and high IgG avidity (4, 11, 22). Differences between proportions were evaluated by chi-square analysis (MedCalc software), with significance defined by a *P* value of  $< 0.01$ .

A total of 2,609 DV IgM-positive patients were identified during the study period; 76 patients (2.9%) were excluded from further analysis because their age was unknown. Of the remaining 2,533 DV IgM-positive patients, 1,622 (64%) were also positive for DV IgG, and sera from 1,257 (77.5%) of these IgM<sup>+</sup>IgG<sup>+</sup> patients were available for IgG avidity testing. When IgM<sup>+</sup>IgG<sup>+</sup> patients not tested for avidity were compared to IgM<sup>+</sup>IgG<sup>+</sup> pa-

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**TABLE 1** Numbers of patients exhibiting various DV IgM-positive reactivity patterns and IgG avidity, segregated by age group and place of patient residence

Age group (yr)	No. of patients with indicated reactivity pattern (avidity) residing in:					
	Caribbean islands			U.S. mainland		
	IgM <sup>+</sup> IgG <sup>-</sup>	IgM <sup>+</sup> IgG <sup>+</sup> (low)	IgM <sup>+</sup> IgG <sup>+</sup> (high)	IgM <sup>+</sup> IgG <sup>-</sup>	IgM <sup>+</sup> IgG <sup>+</sup> (low)	IgM <sup>+</sup> IgG <sup>+</sup> (high)
≤20	280	34	233	95	28	54
21–40	114	8	199	155	76	140
41–60	65	10	220	132	86	211
>60	38	15	158	32	30	120
Total	497	67	810	414	220	525

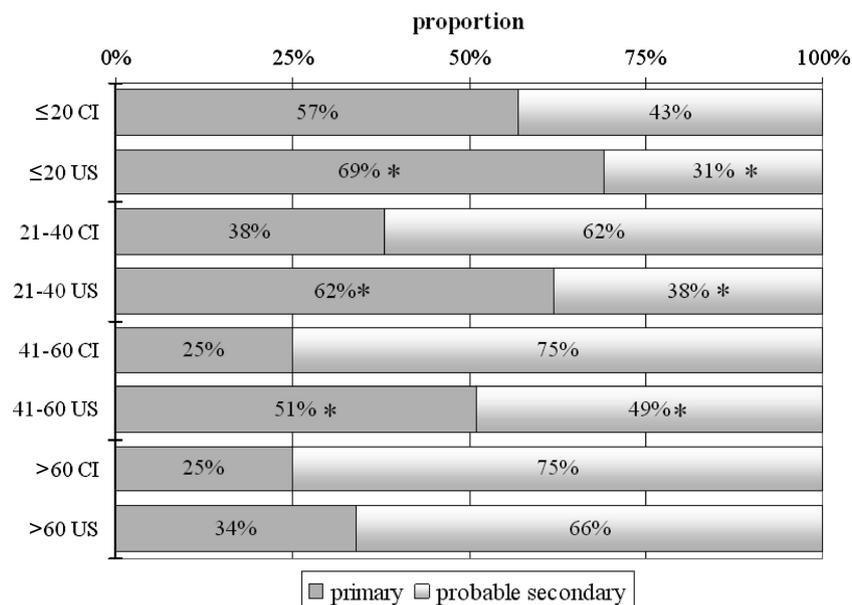
tients tested for avidity, there was no bias related to patient age or place of residence; we thus assumed that within a given age and residence category, the proportion of low IgG avidity results among IgM<sup>+</sup>IgG<sup>+</sup> sera actually tested for avidity was also applicable to IgM<sup>+</sup>IgG<sup>+</sup> sera not tested for avidity. The numbers of IgM<sup>+</sup>IgG<sup>+</sup> patients with low avidity and high avidity shown in Table 1 thus reflect the actual numbers observed for sera tested for avidity plus the calculated numbers for sera not tested for avidity.

Table 1 shows the numbers of patients within each residence group, segregated by IgM/IgG reactivity patterns, IgG avidity, and age. The overall proportion of DV IgM-positive patients exhibiting a primary infection pattern was significantly higher in U.S. mainland residents (634/1,159, 54.7%) than Caribbean island residents (564/1,374, 41.0%).

Figure 1 presents the distribution of primary and probable secondary infections among patients within a given age category in each place of residence. In patients ≤20 years old, the proportion of DV IgM-positive patients exhibiting primary infection was significantly higher (and thus the proportion exhibiting probable secondary infection was significantly lower) for U.S. mainland residents than Caribbean island residents; of particular note, how-

ever, was the observation that in both resident groups, more than 50% of patients in this age category exhibited primary infection. In patients 21 to 40 years old and patients 41 to 60 years old, the proportions of DV IgM-positive patients exhibiting primary infection were also significantly higher (and the proportions exhibiting probable secondary infection were significantly lower) in U.S. mainland residents than Caribbean island residents; the differences were more marked than the difference observed when comparing the ≤20-year-old groups by place of residence. In contrast to the observations for other age groups, the distributions of primary and probable secondary infections in patients >60 years old did not significantly differ when comparing the two resident groups; the majority of patients from both the Caribbean islands and the U.S. mainland exhibited probable secondary infections.

Our estimates of primary infection rates among DV IgM-positive individuals represent minimum estimates. Patients previously exposed to other flaviviruses via natural infection or vaccination exhibit a DV IgM/IgG reactivity pattern and IgG avidity typical of secondary infection during their primary DV infection episode (12). Further, it is unclear if DV IgG avidity matures to levels typical of secondary infection before DV IgM falls to unde-



**FIG 1** Proportional distributions of primary and probable secondary DV infections within a given age category for each place of patient residence. Age categories (years) for Caribbean island (CI) and U.S. mainland (US) residents are indicated on the vertical axis. Asterisks indicate significant differences ( $P < 0.01$ ) observed in pairwise comparisons of U.S. mainland residents and CI residents within a given age category.

tectable levels. Thus, some cases of primary DV infection were most likely misclassified by DV IgG avidity measurement and thus incorrectly placed in the probable secondary infection group. Indeed, this reasoning explains why we elected to call the IgM<sup>+</sup>IgG<sup>+</sup> reactivity pattern with high IgG avidity a “probable” secondary infection; although it is likely that most patients in this group exhibited secondary DV infection, it remains unclear how many patients were, in fact, misclassified primary infections.

Similarly, there were undoubtedly patients with recent DV infection that we did not identify because they were in the preseroconversion window and thus negative for DV IgM (1, 13, 27). Such patients would be more likely to exhibit secondary DV infections, since IgM typically appears a few days later in secondary infections than in primary infections (26); further, approximately 5% of patients with secondary infections never exhibit detectable DV IgM (13, 32). DV nonstructural protein 1 (NS1) detection is a useful test for identifying such patients (1), although NS1 detection kits are not available in the United States. DV nucleic acid detection can also identify recently infected individuals before seroconversion (27).

Our analysis of DV infection type in relation to age and place of residence revealed that, not surprisingly, the majority of DV infections among U.S. mainland residents 21 to 60 years old were primary infections, whereas the majority of infections among Caribbean residents 21 to 60 years old were secondary infections. In contrast, the majority of DV IgM-positive individuals  $\leq 20$  years old exhibited a primary infection pattern, regardless of place of residence. Based on published reports of high DV IgG prevalence rates and secondary DV infection rates among children from other areas of endemicity (3, 7, 32, 34), we expected to find a secondary infection pattern in the majority of DV IgM-positive Caribbean children and teenagers. The reasons for this unexpected finding remain unclear; possible explanations include (i) a particularly large population of never-exposed Caribbean children and young adults due to several years of relative quiescence before the 2010 Caribbean DV outbreak (8) and (ii) increased physician awareness of DV infections linked to the outbreak, leading to more testing among febrile children and teenagers. Another unexpected finding was that the majority of DV IgM-positive individuals  $>60$  years old exhibited a probable secondary infection pattern, regardless of place of residence. We expected this finding among IgM-positive Caribbean island residents  $>60$  years of age (23, 30) but not among IgM-positive U.S. mainland residents  $>60$  years old. This observation suggests that most U.S. mainland residents  $>60$  years old with recent DV infection were exposed to DV sometime in the past, probably due to prior residence in, or travel to, an area of DV endemicity. Such individuals may be at increased risk for dengue hemorrhagic fever and should thus be followed closely by their medical care providers.

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