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MAJOR ARTICLE

Tetravalent Dengue Vaccine Reduces Symptomatic and Asymptomatic Dengue Virus Infections in Healthy Children and Adolescents Aged 2–16 Years in Asia and Latin America

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Background. Asymptomatic dengue virus–infected individuals are thought to play a major role in dengue virus transmission. The efficacy of the recently approved quadrivalent CYD-TDV dengue vaccine against asymptomatic dengue virus infection has not been previously assessed.

Methods. We pooled data for 3 736 individuals who received either CYD-TDV or placebo at 0, 6, and 12 months in the immunogenicity subsets of 2 phase 3 trials (clinical trials registration NCT01373281 and NCT01374516). We defined a seroconversion algorithm (ie, a ≥4-fold increase in the neutralizing antibody titer and a titer of ≥40 from month 13 to month 25) as a surrogate marker of asymptomatic infection in the vaccine and placebo groups.

Results. The algorithm detected seroconversion in 94% of individuals with a diagnosis of virologically confirmed dengue between months 13 and 25, validating its discriminatory power. Among those without virologically confirmed dengue (n = 3 669), 219 of 2 485 in the vaccine group and 157 of 1 184 in the placebo group seroconverted between months 13 and 25, giving a vaccine efficacy of 33.5% (95% confidence interval [CI], 17.9%–46.1%) against asymptomatic infection. Vaccine efficacy was marginally higher in subjects aged 9–16 years (38.6%; 95% CI, 22.1%–51.5%). The annual incidence of asymptomatic dengue virus infection in this age group was 14.8%, which was 4.4 times higher than the incidence for symptomatic dengue (3.4%).

Conclusions. The observed vaccine efficacy against asymptomatic dengue virus infections is expected to translate into reduced dengue virus transmission if sufficient individuals are vaccinated in dengue-endemic areas.

Keywords. dengue vaccine; symptomatic dengue virus infection; asymptomatic dengue virus infection; children; adolescents; Asia; Latin America.

Dengue is a mosquito-borne disease caused by a flavivirus, of which there are 4 serotypes (dengue virus [DENV] 1–4). DENV infections can be asymptomatic or symptomatic, with symptoms ranging from mild febrile illness to severe dengue, which can lead to shock and death if not treated appropriately [1].

Results from 2 phase 3 randomized clinical efficacy trials in Asia and Latin America showed that the quadrivalent CYD-TDV dengue vaccine can protect individuals aged 2–16 years against virologically confirmed symptomatic disease [2–4]. In addition to protection against symptomatic infection, it is also important to assess protection against asymptomatic infection, since an estimated 80% of all DENV infections are asymptomatic. In absolute numbers, this represents 300–390 million asymptomatic DENV infections per year, worldwide [5].

Individuals with asymptomatic DENV infections may represent an important reservoir for DENV transmission to mosquitoes and subsequently to humans. Some studies have suggested that individuals with asymptomatic DENV infections are less able to transmit the virus, owing to a lower, or even undetectable, viral load [6–8]. However, one recent study reported that individuals with asymptomatic dengue were 5–10 times more likely than symptomatic individuals to successfully transmit the virus [9].

Vaccines generally confer direct protection that reduces the risk of infection, disease and possible disease complications. Vaccines that reduce the ability of vaccinated individuals to transmit the infectious agent also confer indirect protection, commonly referred to as herd immunity. The extent of indirect protection is related to the speed with which the infectious agent can spread through a population, the proportion of vaccinated individuals, and the vaccine efficacy against infection (both symptomatic and asymptomatic) [10–12]. Indirect

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protection can ultimately lead to the interruption of disease transmission if the proportion of protected individuals is large enough to generate herd immunity. Examples of vaccines that have been reported to confer indirect protection include smallpox, influenza, *Haemophilus influenzae* type b, polio, pertussis, hepatitis A, pneumococcal, rotavirus, and measles, mumps, and rubella [13–23].

Here we used data from the 2 pivotal phase 3 clinical trials to investigate whether vaccination with CYD-TDV protected individuals from asymptomatic infection, using a commonly used surrogate measure, primary, secondary, or other seroconversion, which, for simplicity, we will refer to as seroconversion [24–28].

METHODS

Data Sources

We pooled data from 2 phase 3 clinical trials (CYD14 and CYD15; clinical trials registration NCT01373281 and NCT01374516, respectively) [2, 4]. CYD14 enrolled 10 275 participants aged 2–14 years living in 5 Asian countries (Indonesia, Malaysia, the Philippines, Thailand, and Vietnam). CYD15 enrolled 20 869 participants aged 9–16 years living in 5 Latin American countries (Colombia, Brazil, Mexico, Puerto Rico, and Honduras). A total of 4584 participants had at least 1 result from a plaque reduction neutralization test (PRNT) used to determine concentrations of DENV neutralizing antibodies. We analyzed data from 3736 of these participants who had received all 3 doses, at day 0, month 6, and month 12, and had immunological results for months 13 and 25 (Figure 1).

Virologically Confirmed Dengue Episode

The full methods have been published elsewhere [2, 4]. Briefly, blood samples collected from individuals who presented with acute febrile illness (ie, a temperature of ≥38°C on ≥2 consecutive days) within 5 days of fever onset were tested for DENV nonstructural protein 1 (NS1) antigen (Platelia Biorad Laboratories, Marnes-La-Coquette, France) and were screened for DENV by a quantitative reverse transcription–polymerase chain reaction (PCR) and a serotype-specific PCR (Simplexa dengue real-time PCR assay, Focus Diagnostics, California). Assays were done under masked conditions at the sponsor's Global Clinical Immunology laboratories (Swiftwater, Pennsylvania) and at the Center for Vaccine Development at Mahidol University (Bangkok, Thailand). An episode was classified as virologically confirmed dengue if results of any of these tests were positive.

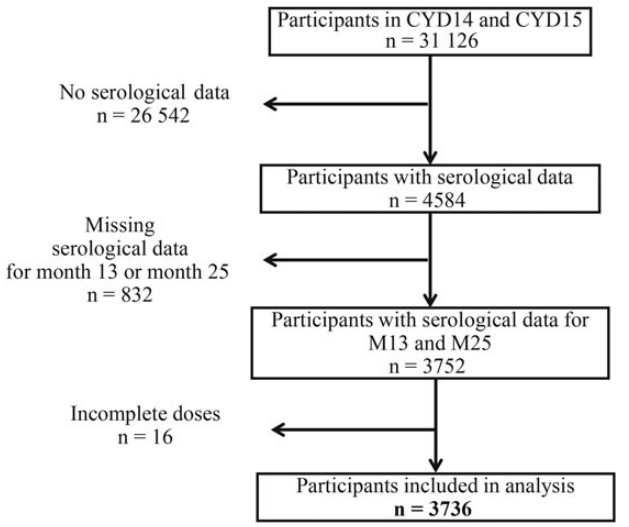

Figure 1. Disposition of participants in the analysis.

Plaque Reduction Neutralization Test (PRNT)

DENV neutralizing antibody titers were measured using the PRNT (with parental DENV strains of CYD dengue vaccine constructs) at the sponsor's Global Clinical Immunology laboratories [29]. The lower limit of quantification or detection of the assay was 10 (1/dilution). Among the observed results, ≥90% were within a 3-fold difference of the median titer for 80% of the positive samples tested, which shows acceptable intraassay and interassay precision [29–31].

Seroconversion Algorithm

A seroconversion algorithm for at least 1 DENV serotype was used as a proxy outcome for asymptomatic DENV infection. The result was taken to be positive if there was at least a 4-fold increase in the neutralizing antibody titer from months 13 to 25, as measured by the PRNT, and if the resulting titer at month 25 was at least 40. The 4-fold increase threshold was used because this is above the known, inherent variability of the PRNT [29–31]. Seroconversion can be considered as a good proxy for asymptomatic infection, in the absence of clinically apparent dengue [25, 32–37].

The discriminatory power of the seroconversion algorithm was assessed using data from participants who had symptomatic, virologically confirmed dengue of any severity up to month 25, which was the primary outcome in the trials. These individuals were then excluded from the study population, before using the seroconversion algorithm as a surrogate outcome to assess vaccine efficacy against asymptomatic DENV infection between months 13 and 25. The attack rate for asymptomatic infections was calculated by dividing the number of individuals who seroconverted by the number of individuals who were analyzed and multiplying the value by 100. The vaccine efficacy for preventing asymptomatic infection was calculated as 100 × [1 − relative risk] between the vaccine group and the placebo group.

The impact of varying the fold-increase threshold used in the seroconversion algorithm on the estimate of vaccine efficacy against asymptomatic DENV infection was also assessed (sensitivity analysis).

Table 1. Summary of Population Characteristics

Variable	Vaccine Group n = 2510	Placebo Group N = 1226
CYD14 trial, subjects, no.	1262	608
CYD15 trial, subjects, no.	1248	618
Age, y, mean ± SD	9.9 ± 3.6	10.0 ± 3.5
Female sex, %	50.8	49.5
Male sex, %	49.2	50.5
Baseline seropositivity,[a] %	74.2	72.5

Among the 3736 participants in this analysis, 67 had had virologically confirmed dengue virus infection (vaccine group, n = 25; placebo group, n = 42), and their data were used to validate the algorithm. The data for the remaining 3669 participants were used for the analyses of asymptomatic infections.

[a] Based on a subgroup of 2500 and 1220 individuals in the vaccine and placebo groups, respectively, with known dengue virus serological status at baseline.

RESULTS

Study Population

$PRNT_{50}$ results at months 13 and 25 were available from 3736 participants (12.0%) in the CYD14 and CYD15 clinical trials (Figure 1). Their characteristics are summarized in Table 1.

Discriminatory Power of the Seroconversion Algorithm

The seroconversion algorithm detected seroconversion in 63 of 67 individuals who had virologically confirmed dengue between months 13 and 25 in both the vaccine and placebo groups, giving an overall sensitivity of 94%. In the vaccine and placebo groups, the sensitivities were not statistically significantly different, with values of 88% (95% CI, 68.8%–97.4%) and 98% (95% CI, 87.4%–99.9%), respectively. The characteristics of the 4 participants who had had virologically confirmed dengue but who were not found to have seroconverted with the algorithm are summarized in Table 2.

Vaccine Efficacy Against Asymptomatic DENV Infection

A total of 3669 individuals did not present a virologically confirmed dengue episode between months 13 and 25 (2485 and 1184 in the vaccine and placebo groups, respectively). Their $PRNT_{50}$ values at month 13 in the vaccine group were 263.2 (95% CI, 243.3–284.8), 463.7 (95% CI, 436.5–482.5), 332.2 (95% CI, 310.9–354.9), and 193.2 (95% CI, 183.7–203.2) for serotypes 1–4, respectively, in the vaccine group and 78.5 (95% CI, 68.3–90.1), 94.4 (95% CI, 82.7–107.6), 74.5 (95% CI, 65.5–84.6), and 35.0 (95% CI, 31.5–38.8), respectively, in the placebo group. Among these participants, 376 (219 and 157 in the vaccine and placebo groups, respectively) seroconverted between months 13 and 25. Thus, the estimated vaccine efficacy of CYD-TDV against asymptomatic infection in the overall population was 33.5% (95% CI, 17.9%–46.1%; Table 3).

Figure 2 shows that, among the individuals who had a ≥4-fold antibody titer increase but who did not have virologically confirmed dengue, the majority had a >10 fold increase, both in the vaccine and the placebo group. Below the ≥4-fold antibody titer increase limit, the increases are more likely to be due to the intrinsic variability of the $PRNT_{50}$ results than to infection. The average and median fold increases for the 376 individuals who had seroconverted between months 13 and 25 were 82 and 10, respectively, in the vaccine group and 104 and 16, respectively, in the placebo group.

In the individuals aged ≥9 years, the efficacy of CYD-TDV against asymptomatic DENV infection was 38.6% (95% CI, 22.1%–51.5%), which is approximately half the vaccine efficacy observed for symptomatic dengue. Taken together, these results represent a vaccine efficacy of 43.9% (95% CI, 30.2%–54.9%) against symptomatic and asymptomatic DENV infection (Table 3).

Incidence of Asymptomatic DENV Infection

In the overall population, the ratio of attack rates in the placebo group between asymptomatic and symptomatic DENV infection was 3.9 (13.3%/3.4%), which means that, for each symptomatic dengue case detected, it is likely that there are 4 individuals with asymptomatic infection who can potentially transmit the virus. In the individuals aged ≥9 years, the observed annual incidence for all types of DENV infection was 17.7% (Table 3). This value was significantly higher than the annual incidence of 12.1% for all types of DENV infections among individuals aged <9 years.

Sensitivity Analysis

Varying the fold-increase threshold for seroconversion between months 13 and 25 from 3 to 9 in the overall population gave estimates for vaccine efficacy against asymptomatic infection ranging from 31.7% to 50.4% (Figure 3). From the same analysis, the corresponding range for the annual incidence of asymptomatic DENV infection was 9.2% to 17.7%.

Table 2. Characteristics of Participants With Dengue Virus (DENV) Infection Confirmed Virologically but Not Detected Using the Seroconversion Algorithm

Study Group	Participant Sex; Age, y	Baseline DENV Serostatus	Time Between Month 13 and Dengue Diagnosis, d	Highest Fold Increase Between Months 13 and 25	Serotype(s) (for Highest Fold Increase)
Vaccine	Male; 6	Seronegative	13	3.6	DENV-1
Vaccine	Male; 10	Seronegative	15	2.7	DENV-3
Vaccine	Male; 10	Seronegative	128	1.7	DENV-3, -4
Placebo	Female; 12	Seropositive	71	3.9	DENV-2

The seroconversion algorithm was as follows: a ≥4-fold increase in the neutralizing antibody titer and a titer of ≥40 from month 13 to month 25.

Table 3. CYD-TDV Vaccine Efficacy Against Both Virologically Confirmed Symptomatic Dengue and Asymptomatic Infection in the Immunogenicity Subset Among Individuals Aged 2–16 Years, by Age Group and Baseline Dengue Virus Serostatus

Variable	Virologically Confirmed Symptomatic Dengue	Asymptomatic Infection	All Infections
Overall analysis, no.	3736	3669	3736
Vaccine group	25/2510 (1.0)	219/2485 (8.8)	244/2510 (9.7)
Placebo group	42/1226 (3.4)	157/1184 (13.3)	199/1226 (16.2)
Vaccine efficacy	70.9 (51.2–83.0)	33.5 (17.9–46.1)	40.1 (27.4–50.5)
Aged ≥9 y			
Vaccine group	17/1836 (0.9)	165/1819 (9.1)	182/1836 (9.9)
Placebo group	31/911 (3.4)	130/880 (14.8)	161/911 (17.7)
Vaccine efficacy	72.8 (49.3–85.9)	38.6 (22.1–51.5)	43.9 (30.2–54.9)
Aged <9 y			
Vaccine group	8/674 (1.2)	54/666 (8.1)	62/674 (9.2)
Placebo group	11/315 (3.5)	27/304 (8.9)	38/315 (12.1)
Vaccine efficacy	66.0 (7.2–88.1)	8.7 (−50.7–43.5)	23.7 (−17.4–49.9)
Seropositive at baseline			
Vaccine group	11/1856 (0.6)	160/1845 (8.7)	171/1856 (9.2)
Placebo group	30/884 (3.4)	127/854 (14.9)	157/884 (17.7)
Vaccine efficacy	82.5 (64.2–92.1)	41.7 (25.8–54.1)	48.1 (35.2–58.5)
Seronegative at baseline			
Vaccine group	14/644 (2.2)	59/630 (9.4)	73/644 (11.3)
Placebo group	12/336 (3.6)	30/324 (9.3)	42/336 (12.5)
Vaccine efficacy	39.1 (−44.9–73.9)	−1.1 (−62.6–35.9)	9.3 (−35.9–38.8)

Data are no. of subjects with the characteristic/no. evaluated (attack rate, %) or % (95% confidence interval).

DISCUSSION

The efficacy of the quadrivalent CYD-TDV vaccine for up to 25 months after the first dose of a 3-dose schedule has already been demonstrated in 2 phase 3 randomized clinical trials that enrolled >31 000 participants aged 2–16 years from 5 Asian and 5 Latin American countries [2, 4]. In the present analysis, using seroconversion as a surrogate outcome for asymptomatic infection in the absence of virologically confirmed symptomatic

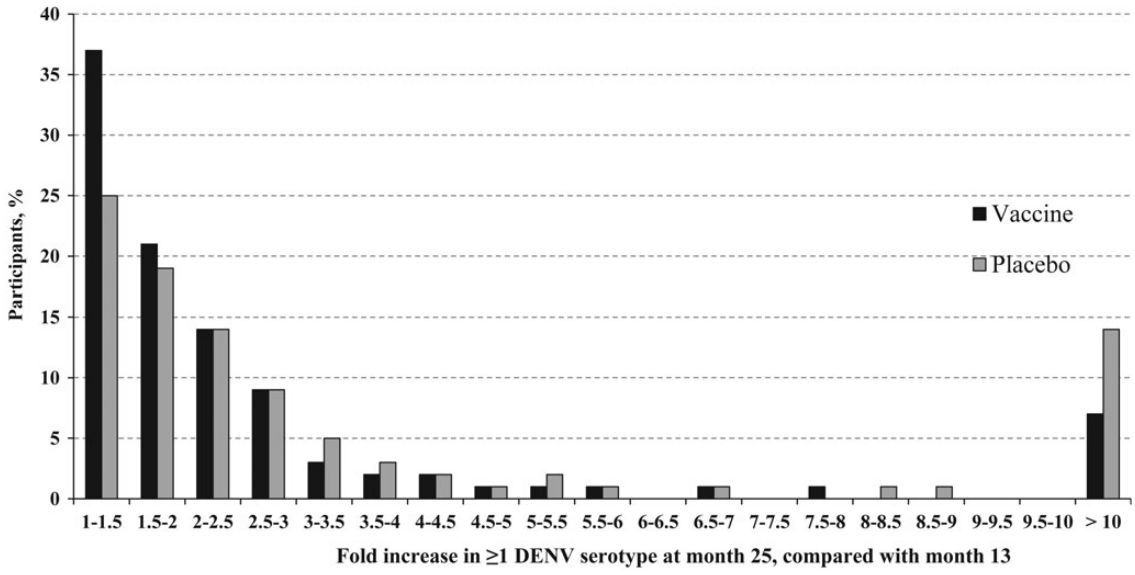

Figure 2. Distribution of dengue virus (DENV) antibody titer fold increases in individuals without virologically confirmed dengue between months 13 and 25. Only individuals with increased antibody titers between months 13 and 25 for at least 1 serotype were included for this analysis (vaccine group, n = 1583; placebo group, n = 708). For participants with increased antibody titers against >1 serotype, only the highest antibody titer ratio (titer a month 25/titer at month 13) was included.

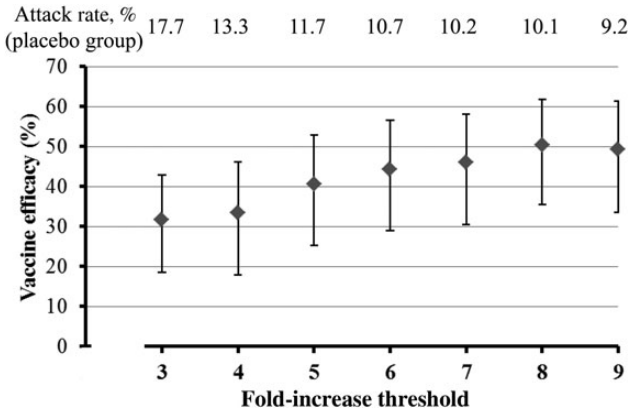

Figure 3. Summary of algorithm sensitivity analyses for assessing vaccine efficacy against asymptomatic dengue virus (DENV) infection. The definition of seroconversion was varied from 3-fold to 9-fold increases in DENV antibody titer, with a constant minimum titer of 40 at month 25, to assess the impact on the attack rate and vaccine efficacy.

dengue, we have shown that CYD-TDV is efficacious in preventing asymptomatic infections for 12 months after dose 3. This efficacy was higher in participants aged 9–16 years (38.6%; 95% CI, 22.1%–51.5%), compared with the efficacy for those aged 2–8 years (8.7%; 95% CI, −50.7%–43.5%), although the 95% CI for the younger age group was wide and included 0. For the subgroup analyses based on baseline DENV serological status, vaccine efficacy was 41.7% (95% CI, 25.8%–54.1%) for the baseline seropositive subgroup, compared with −1.1% (95% CI, −62.6%–35.9%) in the baseline seronegative subgroup; here again, the 95% CI for the younger age group was wide and included 0. This difference in vaccine efficacy observed between seropositive and seronegative individuals is consistent with that reported for vaccine efficacy against symptomatic dengue [3]. Currently, these differences remain unexplained, but several hypotheses have been suggested, such as the possible induction of stronger immune responses in seropositive individuals due to a boosting effect or the fact that younger subjects have a less mature innate and adaptive immune system, with narrower B-cell and T-cell repertoires and therefore immune responses of a relative lesser quality. These hypotheses have been discussed elsewhere [38]. Additionally, in agreement with previous estimates, the present study showed that there is a ratio of approximately 1–4 symptomatic to asymptomatic DENV infections (ie, about 80% of all DENV infections are asymptomatic) [9, 39–41].

The efficacy results here presented may mean that the immune responses elicited by the CYD-TDV vaccine could confer sterilizing immunity, which, in some cases, could prevent the peripheral and central immune systems from seeing the virus delivered by an infected mosquito and thus preventing a new response being mounted. A possible association between high antibody titers and sterilizing immunity was suggested in a recent study that assessed DENV neutralizing antibody kinetics in children after symptomatic primary and postprimary DENV infections [6].

The assessment of the discriminatory power of our algorithm showed that it detected seroconversion in 63 of 67 participants (94%) with virologically confirmed dengue, with similar results in the vaccine and placebo groups. If we had used a 3.5-fold threshold, 2 additional cases would have been detected (Table 2). Although PRNT is not a diagnostic test for asymptomatic DENV infection, it seems likely that the increase in neutralizing antibody titers that we observed was caused by exposure to DENV. In other studies, a 4-fold increase in neutralizing antibodies in the absence of clinically apparent disease has been used to detect asymptomatic infections [24–28, 42, 43]. In these studies, the ratio of symptomatic to asymptomatic DENV infections has been reported to be between 1:0.9 and 1:18, with 5 studies reporting ratios of around 1:3 (Table 4) [24, 27, 34, 42–46]. In the present study, which, to our knowledge, is the first

Table 4. Summary of the Studies That Assessed Relative Incidence of Asymptomatic Dengue Virus Infection and Comparison With the Present Study

Reference	Location	Age, y	Subjects, No.	Study Period	Incidence Ratio (Symptomatic:Asymptomatic)
Busch et al [44]	Rio de Janeiro, Brazil	16–67	16 241	2012	1:2.7
Porter et al [45]	West Java, Indonesia	18–66	2536	2000–2002	1:3
Balmaseda et al [24]	Managua, Nicaragua	2–9	3713	2004–2005	1:18
			3689	2005–2006	1:5
			3563	2006–2007	1:16
			3676	2007–2008	1:3
Montoya et al [43]	Managua, Nicaragua	2–14	5541	2004–2011	1:2.6 (2009–2010); 1:20.4 (2006–2007)
Katzelnick et al [34]	Managua, Nicaragua	2–14	7547	2004–2014	1:2.6
Burke et al [27]	Bangkok, Thailand	4–16	1752	1980–2001	1:5.6
Endy et al [42]	Kamphaeng Phet, Thailand	10 (median)	2119	1998–2000	1:0.9
Mammen et al [46]	Kamphaeng Phet, Thailand	0.5–15	556	2004–2005	1:0.9
Present study	32 cities in 10 countries (Asia and Latin America)	2–16	3669	2011–2013	1:3.9

multicenter study to assess the incidence of asymptomatic DENV infections, we found that about 80% of DENV infections were asymptomatic during the 12-month observation period.

In this assessment of vaccine efficacy for asymptomatic infections, we analyzed data between months 13 and 25. We started the analyses at month 13 (ie, 1 month after dose 3), to avoid serological interference between asymptomatic infection and vaccination. Up to month 25, all symptomatic virologically confirmed dengue cases (hospitalized and nonhospitalized) were detected; after this time, only hospitalized cases were detected. Thus, during months 13–25, we were able to detect all symptomatic cases and eliminate these participants from the analyses for asymptomatic infections; after month 25, we would not have been able to eliminate symptomatic cases that were not hospitalized (and therefore had not been serologically tested for confirmation of a DENV infection). Recently, the long-term follow-up protocols for both the CYD-14 and CYD-15 studies have been amended to include the collection of an additional 2 years of surveillance data, for both nonhospitalized and hospitalized cases of dengue, and serological data. These data will provide further insights into the duration of protection against both symptomatic and asymptomatic DENV infections.

One potential limitation of this study is that the $PRNT_{50}$ assay we used for detecting asymptomatic DENV infection could be sensitive to preimmunity to other flaviviruses. However, when Japanese encephalitis virus or yellow fever virus immunity was induced prior to CYD vaccination in naive animals or volunteers, it was reported to have a positive or neutral impact on CYD-induced cell-mediated immunity [47]. However, the delay between yellow fever virus or Japanese encephalitis virus priming and CYD vaccination could play a role.

As expected, vaccinated and unvaccinated individuals did not have the same neutralizing antibody titers at month 13 (Table 4), which could affect both the waning rates between months 13 and 25 and the boosting effect associated with an asymptomatic infection. Hence, a 4-fold increase between months 13 and 25 would require a larger absolute change in the vaccine group than in the placebo group, which could lead to some asymptomatic infections being missed in the vaccine group and, therefore, to an overestimation of vaccine efficacy against asymptomatic infections. However, the impact of this potential bias is limited since the distribution of the fold increases observed in the vaccine and placebo groups were similar (Figure 2). Moreover, the median fold increases observed in the 376 subjects who did not have virologically confirmed symptomatic dengue but who had seroconverted were much higher than the 4-fold threshold we used (ie, 10 in the vaccine group and 16 in the placebo group).

In the context of this study, it was not possible to analyze for serotype-specific vaccine efficacy, since serological cross-reactions made it impossible to identify the serotype responsible for the asymptomatic infections.

Dengue vaccination that prevents symptomatic infection contributes to reducing viral transmission, but vaccination may also prevent transmission by decreasing asymptomatic infections. Since about 80% of DENV infections are asymptomatic, it is likely that they contribute significantly to viral transmission to mosquitoes and thus to other human hosts. Consequently, providing simultaneous protection against both asymptomatic and symptomatic infections could contribute to reduced transmission and thus to indirect protection if the vaccine coverage rates are sufficient. The data reported here will be useful for the development of mathematical models to predict disease reduction associated with vaccine implementation with different levels of vaccine coverage rates. However, ultimately, large-scale postlicensure effectiveness or impact studies will be required to demonstrate the benefits of indirect protection in unvaccinated individuals.

The public-health impact that dengue vaccination will have on at-risk populations will largely depend on the reduction of virus transmission. In DENV-endemic regions, there seems to be more asymptomatic infected individuals who may transmit DENV than there are symptomatic individuals. Here, for the first time, we provide evidence that the recently approved quadrivalent CYD-TDV dengue vaccine can prevent asymptomatic infection.

STUDY GROUP MEMBERS

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Notes

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Potential conflicts of interest. G. O.-B., L. Coudeville, B. G., L. Chambonneau, F. N., and N. J. are employed by Sanofi Pasteur, which has developed and commercialized the quadrivalent CYD-TDV dengue vaccine (Dengvaxia). L. Coudeville, B. G., L. Chambonneau, F. N., and N. J. own Sanofi Pasteur shares/stock options. K. F. owns Sanofi shares/stock options.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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