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Tetravalent dengue vaccine reduces symptomatic and asymptomatic dengue infections in healthy children and adolescents aged 2-16 years in Asia and Latin America

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Footnote Page

Potential conflicts of interest: All named authors, except KF; are employed by Sanofi Pasteur who has developed and commercialized the quadrivalent CYD-TDV dengue vaccine (Dengvaxia®). KF is employed by Sanofi R&D, Chilly-Mazarin, France. All authors, except GOB and KF own Sanofi Pasteur shares/stock options. KF owns Sanofi shares/stock options.

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Abstract

Background: Asymptomatic dengue-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 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 two phase-III trials (NCT01373281, NCT01374516). We defined a seroconversion algorithm (≥4-fold increase in the neutralizing antibody titer and a titer ≥40 from M13 to M25) as a surrogate marker of asymptomatic infection in the vaccine and placebo groups.

Results: The algorithm detected seroconversion in 94% of individuals diagnosed with virologically-confirmed dengue between months 13 and 25, validating its discriminatory power. Among those without virologically-confirmed dengue (N=3,669) 219/2,485 (vaccine) and 157/1,184 (placebo) seroconverted between M13 and M25, giving a vaccine efficacy (VE) of 33.5% (95% CI: 17.9; 46.1) against asymptomatic infection. VE was marginally higher in subjects aged 9-16 years; 38.6% (95% CI: 22.1; 51.5). The annual incidence of asymptomatic dengue 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 VE against asymptomatic dengue infections is expected to translate into reduced dengue virus transmission, if sufficient individuals are vaccinated in endemic areas.

Introduction

Dengue is a mosquito-borne disease caused by a flavivirus, of which there are four serotypes (DENV1-4). Dengue 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 two III randomized clinical efficacy trials in Asia and Latin America showed that the quadrivalent CYD-TDV dengue vaccine can protect individuals aged from 2 to 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 dengue infections are asymptomatic. In absolute numbers, this represents 300-390 million asymptomatic dengue infections per year, worldwide [5].

Individuals with asymptomatic dengue infections may represent an important reservoir for dengue transmission to mosquitoes and subsequently to humans. Some studies have suggested that individuals with asymptomatic dengue infections are less able to transmit the virus due to a lower, or even undetectable, viral load [6-8]. However, one recent study reported that individuals with asymptomatic dengue were 5 to 10 times more likely to successfully transmit the virus, than symptomatic individuals [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 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 influenza type b, polio, pertussis, hepatitis A, pneumococcal, rotavirus, measles and mumps and rubella (MMR) vaccines [13-23]. Here we used data from the two pivotal phase III clinical trials to investigate if vaccination with CYD-TDV protected individuals from asymptomatic infection using a commonly used surrogate measure, i.e. primary, secondary or other seroconversion which for simplicity purposes we will refer to as seroconversion [24-28].

Methods

Data sources

We pooled data from two phase III clinical trials (CYD14 and CYD15: NCT01373281, NCT01374516) [2, 4]. CYD14 enrolled 10,275 participants aged 2-14 years living in five Asian countries (Indonesia, Malaysia, the Philippines, Thailand and Vietnam). CYD15 enrolled 20,869 participants aged 9-16 years living in five Latin American countries (Colombia, Brazil, Mexico, Puerto Rico, and Honduras). A total of 4,584 participants had at least one result from the plaque reduction neutralization test (PRNT) used to determine concentrations of dengue neutralizing antibodies. We analyzed data from 3,736 of these participants who had received all three doses at D0, M6 and M12 and had immunological results for M13 and M25 (Figure 1).

Virologically-confirmed dengue (VCD) episode

The full methods have been published elsewhere [2, 4]. Briefly, blood samples taken from individuals who presented acute febrile illness (i.e. temperature ≥38°C on ≥2 consecutive days) within five days of fever onset, were tested for dengue non-structural protein 1 (NS1) antigen (Platelia Biorad Laboratories, Marnes-La-Coquette, France), and with a dengue screen PCR (quantitative reverse transcription PCR), and a serotype-specific PCR (Simplexa dengue real-time PCR assay, Focus Diagnostics, CA, USA). Assays were done under masked conditions at the

sponsor's Global Clinical Immunology laboratories (Swiftwater, PA, USA) and at the Centre for Vaccine Development at Mahidol University (Bangkok, Thailand). An episode was classified as virologically-confirmed dengue if any of these tests were positive.

Plaque reduction neutralization test (PRNT)

Dengue neutralizing antibody titers were measured using the PRNT (with parental dengue virus strains of CYD dengue vaccine constructs) at the sponsor's Global Clinical Immunology laboratories [29]. The lower limit of quantification (LLOQ) or detection of the assay was 10 (1/dil). 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 intra- and inter-assay precision [29-31].

Seroconversion algorithm

A seroconversion algorithm for at least one dengue serotype was used as a proxy outcome for asymptomatic dengue infection. The result was taken to be positive if there was at least a 4-fold increase in the neutralizing antibody titer, from M13 to M25, as measured by the PRNT, and if the resulting titer at M25 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 disease [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 M25, 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 dengue infection between M13 and M25. 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 by 100. The vaccine efficacy for preventing asymptomatic infection was calculated as 100*(1-RR [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 dengue infection was also assessed (sensitivity analysis).

Results

Study population

$PRNT_{50}$ results at M13 and M25 were available from 3,736 (12.0%) participants 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 the 67 individuals who had virologically-confirmed dengue between M13 and M25, 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; 88% (95% CI: 68.8; 97.4) and 98% (95% CI: 87.4; 99.9), respectively. The characteristics of the four 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 dengue infection

A total of 3,669 individuals did not present a virologically-confirmed dengue episode between M13 and M25 (2,485 and 1,184 in the vaccine and placebo groups, respectively). Their $PRNT_{50}$ titers at M13 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 to 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. Amongst these participants, 376 (219 and 157 in the vaccine and placebo groups, respectively) seroconverted between M13 and M25. 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 be due to infection. The average and median fold-increases for the 376 individuals, who had seroconverted between M13 and M25, were 82 and 10 in the vaccine group and 104 and 16 in the placebo group, respectively.

In the individuals age ≥9 years, the efficacy of CYD-TDV against asymptomatic dengue 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 dengue infection. (Table 3).

Incidence of asymptomatic dengue infection

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

Sensitivity analysis

Varying the fold-increase threshold for seroconversion between M13 and M25 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 annual incidence of asymptomatic dengue infection was 9.2% to 17.7%.

Discussion

The efficacy of the quadrivalent CYD-TDV vaccine for up to 25 months after the first dose of a three-dose schedule has already been demonstrated in two phase III randomized clinical trials that enrolled over 31,000 participants aged 2 to 16 years from five Asian and five 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 dengue, we have shown that CYD-TDV is efficacious in preventing asymptomatic infections during 12 months post-dose 3. This efficacy was higher in participants aged 9-16 years, 38.6% (95% CI: 22.1; 51.5), compared with 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 dengue 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 to 4 symptomatic to asymptomatic dengue infections, i.e., about 80% of all dengue 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 dengue neutralizing antibody kinetics in children after symptomatic primary and post-primary dengue virus infections [6].

The assessment of the discriminatory power of our algorithm showed that it detected seroconversion in 63/67 (94%) of the participants with virologically-confirmed dengue disease, with similar results in the vaccine and placebo groups. If we had used a 3.5-fold threshold, two additional cases would have been detected (Table 2). Although PRNT is not a diagnostic test for asymptomatic dengue infection, it seems likely that the increase in neutralizing antibody titers that we observed was caused by exposure to dengue virus. 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 dengue infections has been reported to be between 1:0.9 to 1:18, with five studies reporting ratios of around 1:3 (Table 4) [24, 27, 42-47]. In the present study, which, to our knowledge, is the first multi-centric study to assess the incidence of asymptomatic dengue infections, we found that about 80% of dengue infections were asymptomatic during the 12-month observation period.

In this assessment of vaccine efficacy for asymptomatic infections we analyzed data between M13 and M25. We started the analyses at M13 i.e. 1 month post dose 3 to avoid serological interference between asymptomatic infection and vaccination. Up to M25, all symptomatic virologically-confirmed dengue cases (hospitalized and non-hospitalized) were detected; after this time, only hospitalized cases were detected. Thus during the period M13 to M25 we were able to detect all symptomatic cases and eliminate these participants from the analyses for asymptomatic infections; after M25 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 dengue 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 two years of surveillance data, for both non-hospitalized and hospitalized cases of dengue and serological data. These data will provide further insights into the duration of protection against both symptomatic and asymptomatic dengue infections.

One potential limitation of this study is that the $PRNT_{50}$ assay we used for detecting asymptomatic dengue infection could be sensitive to pre-immunity to other flaviviruses. However, when Japanese encephalitis (JE) virus or yellow fever (YF) virus immunity was induced prior to CYD vaccination in naïve animals or volunteers, it was reported to have a positive or neutral impact on CYD-induced cell-mediated immunity [48]. However the delay between YF-virus or JE-virus priming and CYD vaccination could play a role.

As expected, vaccinated and unvaccinated individuals did not have the same neutralizing antibody titers at M13 (Table 4) which could affect both the waning rates between M13 and M25 and the boosting effect associated with an asymptomatic infection. Hence, a 4-fold increase between M13 and M25 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, i.e. 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 dengue 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 post-licensure 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 endemic regions, there seems to be more asymptomatic infected individuals who may transmit dengue virus 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.

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References

1. Guzman MG, Harris E. Dengue. Lancet **2015**; 385:453-65.

2. Capeding MR, Tran NH, Hadinegoro SR, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet **2014**; 384:1358-65.

3. Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, et al. Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease. N Engl J Med **2015**; 373:1195-206.

4. Villar L, Dayan GH, Arredondo-Garcia JL, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. N Engl J Med **2015**; 372:113-23.

5. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature **2013**; 496:504-7.

6. Anders KL, Nga le H, Thuy NT, et al. Households as foci for dengue transmission in highly urban Vietnam. PLoS Negl Trop Dis **2015**; 9:e0003528.

7. Dussart P, Baril L, Petit L, et al. Clinical and virological study of dengue cases and the members of their households: the multinational DENFRAME Project. PLoS Negl Trop Dis **2012**; 6:e1482.

8. Yoon IK, Srikiatkhachorn A, Hermann L, et al. Characteristics of mild dengue virus infection in Thai children. Am J Trop Med Hyg **2013**; 89:1081-7.

9. Duong V, Lambrechts L, Paul RE, et al. Asymptomatic humans transmit dengue virus to mosquitoes. Proc Natl Acad Sci **2015**; 112:14688-93.

10. Fine P, Eames K, Heymann DL. "Herd immunity": a rough guide. Clin Infect Dis **2011**; 52:911-6.

11. Kim TH, Johnstone J, Loeb M. Vaccine herd effect. Scand J Infect Dis **2011**; 43:683-9.

12. Rashid H, Khandaker G, Booy R. Vaccination and herd immunity: what more do we know? Curr Opin Infect Dis **2012**; 25:243-9.

13. Clark HF, Lawley D, Mallette LA, DiNubile MJ, Hodinka RL. Decline in cases of rotavirus gastroenteritis presenting to The Children's Hospital of Philadelphia after introduction of a pentavalent rotavirus vaccine. Clin Vaccine Immunol **2009**; 16:382-6.

14. Kaplan EH, Wein LM. Smallpox eradication in West and Central Africa: surveillance-containment or herd immunity? Epidemiology **2003**; 14:90-2.

15. Kwong JC, Pereira JA, Quach S, et al. Randomized evaluation of live attenuated vs. inactivated influenza vaccines in schools (RELATIVES) cluster randomized trial: Pilot results from a household surveillance study to assess direct and indirect protection from influenza vaccination. Vaccine **2015**; 33:4910-5.

16. Lapinleimu K, Stenvik M. Experiences with polio vaccination and herd immunity in Finland. Dev Biol Stand **1981**; 47:241-6.

17. Lee MS, Lee LL, Chen HY, Wu YC, Horng CB. Post mass-immunization measles outbreak in Taoyuan County, Taiwan: dynamics of transmission, vaccine effectiveness, and herd immunity. Int J Infect Dis **1998**; 3:64-9.

18. Myint TT, Madhava H, Balmer P, et al. The impact of 7-valent pneumococcal conjugate vaccine on invasive pneumococcal disease: a literature review. Adv Ther **2013**; 30:127-51.

19. Peltola H, Aavitsland P, Hansen KG, Jonsdottir KE, Nokleby H, Romanus V. Perspective: a five-country analysis of the impact of four different Haemophilus influenzae type b conjugates and vaccination strategies in Scandinavia. J Infect Dis **1999**; 179:223-9.

20. Piedra PA, Gaglani MJ, Kozinetz CA, et al. Trivalent live attenuated intranasal influenza vaccine administered during the 2003-2004 influenza type A (H3N2) outbreak provided immediate, direct, and indirect protection in children. Pediatrics **2007**; 120:e553-64.

21. Plans P. New preventive strategy to eliminate measles, mumps and rubella from Europe based on the serological assessment of herd immunity levels in the population. Eur J Clin Microbiol Infect Dis **2013**; 32:961-6.

22. Reichert TA, Sugaya N, Fedson DS, Glezen WP, Simonsen L, Tashiro M. The Japanese experience with vaccinating schoolchildren against influenza. N Engl J Med **2001**; 344:889-96.

23. Dagan R, Leventhal A, Anis E, Slater P, Ashur Y, Shouval D. Incidence of hepatitis A in Israel following universal immunization of toddlers. JAMA **2005**; 294:202-10.

24. Balmaseda A, Standish K, Mercado JC, et al. Trends in patterns of dengue transmission over 4 years in a pediatric cohort study in Nicaragua. J Infect Dis **2010**; 201:5-14.

25. Corbett KS, Katzelnick L, Tissera H, Amerasinghe A, de Silva AD, de Silva AM. Preexisting neutralizing antibody responses distinguish clinically inapparent and apparent dengue virus infections in a Sri Lankan pediatric cohort. J Infect Dis **2015**; 211:590-9.

26. Endy TP, Anderson KB, Nisalak A, et al. Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand. PLoS Negl Trop Dis **2011**; 5:e975.

27. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg **1988**; 38:172-80.

28. Gordon A, Kuan G, Mercado JC, et al. The Nicaraguan pediatric dengue cohort study: incidence of inapparent and symptomatic dengue virus infections, 2004-2010. PLoS Negl Trop Dis **2013**; 7:e2462.

29. Timiryasova TM, Bonaparte MI, Luo P, Zedar R, Hu BT, Hildreth SW. Optimization and validation of a plaque reduction neutralization test for the detection of neutralizing antibodies to four serotypes of dengue virus used in support of dengue vaccine development. Am J Trop Med Hyg **2013**; 88:962-70.

30. Flipse J, Smit JM. The Complexity of a Dengue Vaccine: A Review of the Human Antibody Response. PLoS Negl Trop Dis **2015**; 9:e0003749.

31. Salje H, Rodriguez-Barraquer I, Rainwater-Lovett K, et al. Variability in dengue titer estimates from plaque reduction neutralization tests poses a challenge to epidemiological studies and vaccine development. PLoS Negl Trop Dis **2014**; 8:e2952.

32. Crill WD, Hughes HR, Delorey MJ, Chang GJ. Humoral immune responses of dengue fever patients using epitope-specific serotype-2 virus-like particle antigens. PloS One **2009**; 4:e4991.

33. de Alwis R, Beltramello M, Messer WB, et al. In-depth analysis of the antibody response of individuals exposed to primary dengue virus infection. PLoS Negl Trop Dis **2011**; 5:e1188.

34. Katzelnick LC, Montoya M, Gresh L, Balmaseda A, Harris E. Neutralizing antibody titers against dengue virus correlate with protection from symptomatic infection in a longitudinal cohort. Proc Natl Acad Sci **2016**; 113:728-33.

35. Lai CY, Williams KL, Wu YC, et al. Analysis of cross-reactive antibodies recognizing the fusion loop of envelope protein and correlation with neutralizing antibody titers in Nicaraguan dengue cases. PLoS Negl Trop Dis **2013**; 7:e2451.

36. Matheus S, Pham TB, Labeau B, et al. Kinetics of dengue non-structural protein 1 antigen and IgM and IgA antibodies in capillary blood samples from confirmed dengue patients. Am J Trop Med Hyg **2014**; 90:438-43.

37. Tsai WY, Lai CY, Wu YC, et al. High-avidity and potently neutralizing cross-reactive human monoclonal antibodies derived from secondary dengue virus infection. J Virol **2013**; 87:12562-75.

38. Guy B, Jackson N. Dengue vaccine: hypotheses to understand CYD-TDV-induced protection. Nat Rev Microbiol **2016**; 14:45-54.

39. Martinez-Vega RA, Danis-Lozano R, Diaz-Quijano FA, et al. Peridomestic Infection as a Determining Factor of Dengue Transmission. PLoS Negl Trop Dis **2015**; 9:e0004296.

40. Vikram K, Nagpal BN, Pande V, et al. An epidemiological study of dengue in Delhi, India. Acta Trop **2016**; 153:21-7.

41. Villar LA, Rojas DP, Besada-Lombana S, Sarti E. Epidemiological trends of dengue disease in Colombia (2000-2011): a systematic review. PLoS Negl Trop Dis **2015**; 9:e0003499.

42. Endy TP, Chunsuttiwat S, Nisalak A, et al. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng Phet, Thailand. Am J Epidemiol **2002**; 156:40-51.

43. Montoya M, Gresh L, Mercado JC, et al. Symptomatic versus inapparent outcome in repeat dengue virus infections is influenced by the time interval between infections and study year. PLoS Negl Trop Dis **2013**; 7:e2357.

44. Busch MP, Sabino EC, Brambilla D, et al. Duration of dengue viremia in blood donors and relationships between donor viremia, unfection incidence and clinical case reports during a large epidemic. J Infect Dis **2016**; 214:49-54.

45. Katzelnick LC, Montoya M, Gresh L, Balmaseda A, Harris E. Neutralizing antibody titers against dengue virus correlate with protection from symptomatic infection in a longitudinal cohort. Proc Natl Acad Sci U S A **2016**; 113:728-33.

46. Mammen MP, Pimgate C, Koenraadt CJ, et al. Spatial and temporal clustering of dengue virus transmission in Thai villages. PLoS Med **2008**; 5:e205.

47. Porter KR, Beckett CG, Kosasih H, et al. Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. Am J Trop Med Hyg **2005**; 72:60-6.

48. Guy B, Nougarede N, Begue S, et al. Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. Vaccine **2008**; 26:5712-21.

Figure legends

Figure 1. Disposition of participants in analysis

Figure 2. Distribution of dengue antibody titer fold-increases, in non-VCD individuals, between M13 and M25. Only individuals with increased antibody titers between M13 and M25 for at least one serotype, were included for this analysis (vaccine group: n=1,583; placebo group: n=708). For participants with increased antibody titers against more than one serotype, only the highest antibody titer ratio (M25 / M13) was included.

Figure 3. Summary of algorithm sensitivity analyses for assessing vaccine efficacy against asymptomatic dengue infection. The definition of seroconversion was varied from 3-fold to 9-fold increase in dengue antibody titer with a constant minimum titer of 40 at M25 to assess impact on the attack rate and vaccine efficacy.

Table 1: Summary of population characteristics. Among the 3,736 participants in this analysis, 67 had had virologically-confirmed dengue disease (vaccine group, n=25; placebo group, n=42) and their data were used to validate the algorithm. The data for the remaining 3,669 participants were used for the analyses of asymptomatic infections.

	Vaccine group n = 2,510	Placebo group N=1,226
CYD14 trial (n)	1,262	608
CYD15 trial (n)	1,248	618
Age (years) mean ± SD	9.9 ± 3.6	10.0 ± 3.5
Female (%)	50.8	49.5
Male (%)	49.2	50.5
Baseline seropositive[a] (%)	74.2	72.5

[a] Based on a subgroup of 2,500 and 1,220 individuals in the vaccine and placebo groups, respectively, with known dengue serological status at baseline.

Table 2: Characteristics of participants who had had virologically-confirmed dengue but who were not detected using the seroconversion algorithm (≥4-fold increase for at least one serotype and a titer ≥ 40 at M25 for the seroconverting serotype)

Study group	Participant Gender / age (years)	Baseline dengue serostatus	No. days between M13 and dengue diagnosis	Highest fold-increase between M13 and M25	Serotype (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

Table 3: CYD-TDV vaccine efficacy against both virologically-confirmed symptomatic dengue and asymptomatic infection in the immunogenicity subset (aged 2-16 years); by age group (<9 years; 9-16 years) and by baseline dengue serostatus (seropositive; seronegative).

	Virologically-confirmed symptomatic dengue	**Asymptomatic infections**	**All infections**
Overall analysis N:	**3,736**	**3,669**	**3,736**
Vaccine group n/N (attack rate, %)	25/2,510 (1.0)	219/2,485 (8.8)	244/2,510 (9.7)
Placebo group n/N (attack rate, %)	42/1,226 (3.4)	157/1,184 (13.3)	199/1,226 (16.2)
Vaccine efficacy [95% CI] (%)	70.9 [51.2; 83.0]	33.5 [17.9; 46.1]	40.1 [27.4; 50.5]
Aged ≥9 years			
Vaccine group n/N (attack rate, %)	17/1,836 (0.9)	165/1,819 (9.1)	182/1,836 (9.9)
Placebo group n/N (attack rate, %)	31/911 (3.4)	130/880 (14.8)	161/911 (17.7)
Vaccine efficacy [95% CI] (%)	72.8 [49.3; 85.9]	38.6 [22.1; 51.5]	43.9 [30.2; 54.9]
Aged < 9 years			
Vaccine group n/N (attack rate, %)	8/674 (1.2)	54/666 (8.1)	62/674 (9.2)
Placebo group n/N (attack rate, %)	11/315 (3.5)	27/304 (8.9)	38/315 (12.1)
Vaccine efficacy [95% CI] (%)	66.0 [7.2; 88.1]	8.7 [-50.7; 43.5]	23.7 [-17.4; 49.9]

	Virologically-confirmed symptomatic dengue	Asymptomatic infections	All infections
Seropositive at baseline			
Vaccine group n/N (attack rate, %)	11/1,856 (0.6)	160/1,845 (8.7)	171/1,856 (9.2)
Placebo group n/N (attack rate, %)	30/884 (3.4)	127/854 (14.9)	157/884 (17.7)
Vaccine efficacy [95% CI] (%)	82.5 [64.2; 92.1]	41.7 [25.8; 54.1]	48.1 [35.2; 58.5]
Seronegative at baseline			
Vaccine group n/N (attack rate, %)	14/644 (2.2)	59/630 (9.4)	73/644 (11.3)
Placebo group n/N (attack rate, %)	12/336 (3.6)	30/324 (9.3)	42/336 (12.5)
Vaccine efficacy [95% CI] (%)	39.1 [-44.9; 73.9]	-1.1 [-62.6; 35.9]	9.3 [-35.9; 38.8]

Table 4: Summary of the studies that assessed relative incidence of asymptomatic dengue infection and comparison with the present study

First author date	Location	Age (years)	N	Study period	Incidence ratio (symptomatic:asymptomatic)
Busch 2016 [44]	Rio de Janeiro, Brazil	16-67	16,241	2012	1:2.7
Porter 2005 [47]	West Java, Indonesia	18-66	2,536	2000-02	1:3
Balmaseda 2010 [24]	Managua, Nicaragua	2-9	3,713	2004-05	1:18
			3,689	2005-06	1:5
			3,563	2006-07	1:16
			3,676	2007-08	1:3
Montoya 2013 [43]	Managua, Nicaragua	2-14	5,541	2004-11	1:2.6 (2009-10) 1:20.4 (2006-7)
Katzelnick 2016 [45]	Managua, Nicaragua	2-14	7,547	2004-2014	1:2.6
Burke 1998 [27]	Bangkok, Thailand	4-16	1,752	1980-01	1:5.6
Endy 2002 [42]	Kamphaeng Phet, Thailand	10 (median)	2,119	1998-2000	1:0.9
Mammen 2008 [46]	Kamphaeng Phet, Thailand	0.5-15	556	2004-05	1:0.9

Present study	32 cities in 10 countries (Asia and Latin America)	2-16	3,669	2011-13	1:3.9