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Effectiveness of a single-dose mass dengue vaccination in Cebu, Philippines: A case-control study

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ABSTRACT

Background: Dengue fever is an important public health problem in the Philippines. In April 2016, the Department of Health launched a three-dose school based dengue vaccination program of nine- to fourteen-year-old children in three regions with the highest number of dengue cases using CYD-TDV (Dengvaxia, Sanofi Pasteur). In July 2017, a community-based dengue vaccination program was implemented in Cebu province. The program was discontinued in December 2017 amidst public controversy, after the first dose had been administered. We assessed the effectiveness of a single dose of CYD-TDV against hospitalized virologically confirmed dengue (VCD).

Methods: We conducted a case-control study in Cebu province following the dengue mass vaccination. Children who were nine to fourteen years of age during the mass vaccination and subsequently admitted to any of four participating public hospitals with suspected dengue were enrolled in the study as cases. Blood for RT-PCR and clinical and socio-demographic information were obtained. To estimate the level of vaccine protection, vaccination status was compared between children with hospitalized virologically confirmed dengue and controls of the same six-year age-group as the cases, matched on sex, neighborhood and time of occurrence of cases.

Findings: We enrolled 490 cases and 980 controls. Receipt of one dose of CYD-TDV was associated with 26% (95 % CI, −2 to 47%; p = 0 0675) overall protection against hospitalized virologically confirmed dengue and 51% (95 % CI, 23 to 68; p = 0 0016) protection against dengue with warning signs.

Interpretation: A single dose of CYD-TDV given to nine to fourteen-year-old children through a community-based mass vaccination program conferred protection against dengue with warning signs and severe dengue but we were unable to conclude on protection against milder illness.

1. Introduction

In 2015, the first dengue vaccine, CYD-TDV (Dengvaxia™, Sanofi Pasteur), was licensed on a three-dose schedule with an indication for nine- to forty-five-year-old individuals in dengue endemic areas. The multi-country Phase 3 trials showed a three-dose pooled vaccine efficacy against virologically-confirmed dengue (VCD) over 25 months of 66% for those nine years of age or older but an increased risk of hospitalized and severe dengue in children two to five years.[1] Since only a subset of the trial participants had dengue serostatus assessed at baseline, it could not be confirmed at that time whether the safety signal was related to age or dengue serostatus at time of vaccination. In April 2016, the World Health Organization (WHO) recommended that high dengue transmission countries consider introducing CYD-TDV vaccine in age groups with seroprevalence of 70% or greater [2].

Starting in 2016, the Philippine Department of Health (DOH) implemented a three-dose dengue vaccination program among nine- to fourteen-year-old children in Central Luzon, Calabarzon and Metro Manila. In 2017, the program was extended to Cebu pro-

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vince. Subsequently, using a novel diagnostic test that differentiates between antibodies induced by CYD-TDV and wild-type dengue infection,[3] a follow-up analysis of the CYD-TDV Phase 3 trials showed that vaccination conferred protection for at least five years among dengue-seropositive participants but resulted in an increased risk for hospitalized and severe dengue among dengue-seronegative participants.[4] Consequently the Philippine dengue vaccination program was discontinued, after the first dose had been administered in Cebu. Herein, we report the results of a case-control study to evaluate the effectiveness of a single-dose mass dengue vaccination in Cebu.

2. Methods

This is a matched case-control study.[5] The protocol was approved by the University of the Philippines Manila-Research Ethics Board. A written informed consent was provided by a parent or legal guardian and assent was documented with the participant. The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice guidelines and is reported according to the STROBE statement.

2.1. Study site and mass immunization campaign

Cebu province, as in the rest of the country, is endemic for dengue with increases in dengue cases from July to November, coinciding with the rainy season.[6] In the Philippine public health system, the Rural Health Units (RHU) are the main primary health care facilities, while hospitals provide secondary care.[7] Prior to implementation of the mass dengue vaccination in Cebu, the DOH carried-out extensive preparation and community engagement. RHU staff listed the name, *barangay* (village), birthdate, and sex of healthy children nine to fourteen years of age residing in Cebu. A total of 285,242 children were listed and invited to participate in the vaccination campaign. CYD-TDV doses given from June to August 2017 were recorded in the list and maintained as the vaccination registry. Each child received the CYD-TDV (Dengvaxia, Sanofi Pasteur) following the manufacturer's prescribing information. CYD-TDV is a live recombinant vaccine supplied as 5 doses/vial. Eligible children were vaccinated using 0.5 ml given subcutaneously in the left deltoid area. The vaccine was stored at + 2–8°C and transported using WHO recommended carriers with ice-packs.[8] A vaccination card was provided to the parents. An estimated 149,023 (52 2%) children received a single CYD-TDV dose in Cebu before the program was discontinued.[9]

2.2. Post-vaccination surveillance for dengue

We invited four government hospitals (of 13)[10] in Cebu to participate in the case-control study. Surveillance for febrile cases at the participating hospitals was started on February 15, 2018. Onset of illness was defined as the day when fever was reported to have started, whether or not documented by a temperature reading of 38 °C or higher. About five ml of blood was obtained from each patient within the first 5 days of illness (acute phase). Blood samples were collected in anticoagulant-free vacutainer tubes, processed, and aliquoted. Sera were stored at 2–8 °C and shipped within seven days to the Research Institute of Tropical Medicine in Manila for dengue RT-PCR. Total nucleic acid was extracted from the serum samples using the QIAmp Viral RNA (QIAGEN, Valencia, CA, USA) kit according to the manufacturer's protocol. Dengue detection and serotyping of samples was done using the Simplexa Dengue assay (Focus Diagnostics, Cypress, CA, USA).[11] Children hospitalized with VCD were followed until discharge and their illness classified according to WHO 2009 criteria.[12]

2.3. Case-control study

The primary research question is: Does receipt of one dose of CYD-TDV protect against hospitalized VCD? Using a case-control design, we enrolled cases from the source population and recruited two matched neighborhood controls per case. Then for both cases and controls, we ascertained whether or not they had received a dose of CYD-TDV. Information on vaccination status and other exposure variables were obtained by study staff who were unaware of how the information on vaccination status was to be used in the analysis.

All patients with suspected or probable dengue admitted at the participating hospitals were eligible to be enrolled as a case if they fulfilled the following criteria: 1) written informed consent and assent to participate in the study; 2) born between January 2003 to December 2008; 3) resided in Cebu since June 2017 and eligible to have received the dengue vaccine; 4) with fever for less than five days; and 5) submitted a blood sample within five days of fever onset. For the case to be included in the analysis, the participant should meet all the inclusion criteria and have a blood sample positive for dengue by RT-PCR. Repeat episodes meeting the criteria were included.

We aimed to recruit controls that are representative of the source population from which the cases were selected.[5] Neighborhood-matching would help ensure similar risk factors associated with vector dynamics, including the flight range of adult female *Aedes aegypti*,[13,14] the risk for clustering of dengue cases attributed to concurrent infection of nearby mosquitoes, and the *A. aegypti* behavior of taking a single blood meal from multiple hosts.[15] A systematic selection procedure was used to recruit two controls per case as soon as the RT-PCR results became available. Recruitment of controls was started on the third house to the left of the case's house (index house), up to a maximum of 500 m or 20 houses, whichever came first. If necessary, the same procedure was repeated to the right of the index house until two controls per case were recruited. Only one control was recruited per household. Sex- and neighborhood-matched controls of the same six-year age-group as the case (i.e. born between January 2003 to December 2008) were eligible as a control if they had not sought treatment for a dengue-like illness from June 2017 to the date of onset of the febrile illness of his or her matched case. Eligibility for selection also required the same informed consent, residency, and eligibility to receive the dengue vaccine, as applied to the cases. We excluded those had been previously recruited as a control.

Demographic, socio-economic, and environmental variables were ascertained through questionnaires administered to cases and controls and their families. The questionnaires did not include information on race and ethnicity (all participants were Filipino), body mass index, smoking status, medical/immunologic status and concomitant drug use. Clinical data on the cases were obtained from source documents in the hospital. Receipt of the dengue vaccine during the mass immunization program was ascertained in face-to-face interviews. The parents of cases and controls were asked to show vaccination cards distributed during the campaign and copy was kept with the case report form. For individuals who claimed to have been vaccinated but who were not in possession of a card, vaccination status was confirmed by searching the vaccination registry. Decisions about linkage to the vaccination registry were made blinded to case-control status and were based on the subject's name, *barangay*, birthdate, and sex.

2.4. Statistical analysis

The sample size was calculated based on the requirements for a matched case-control analysis. The study was designed to have a statistical power of 80% at a significance level of 0 05 to detect

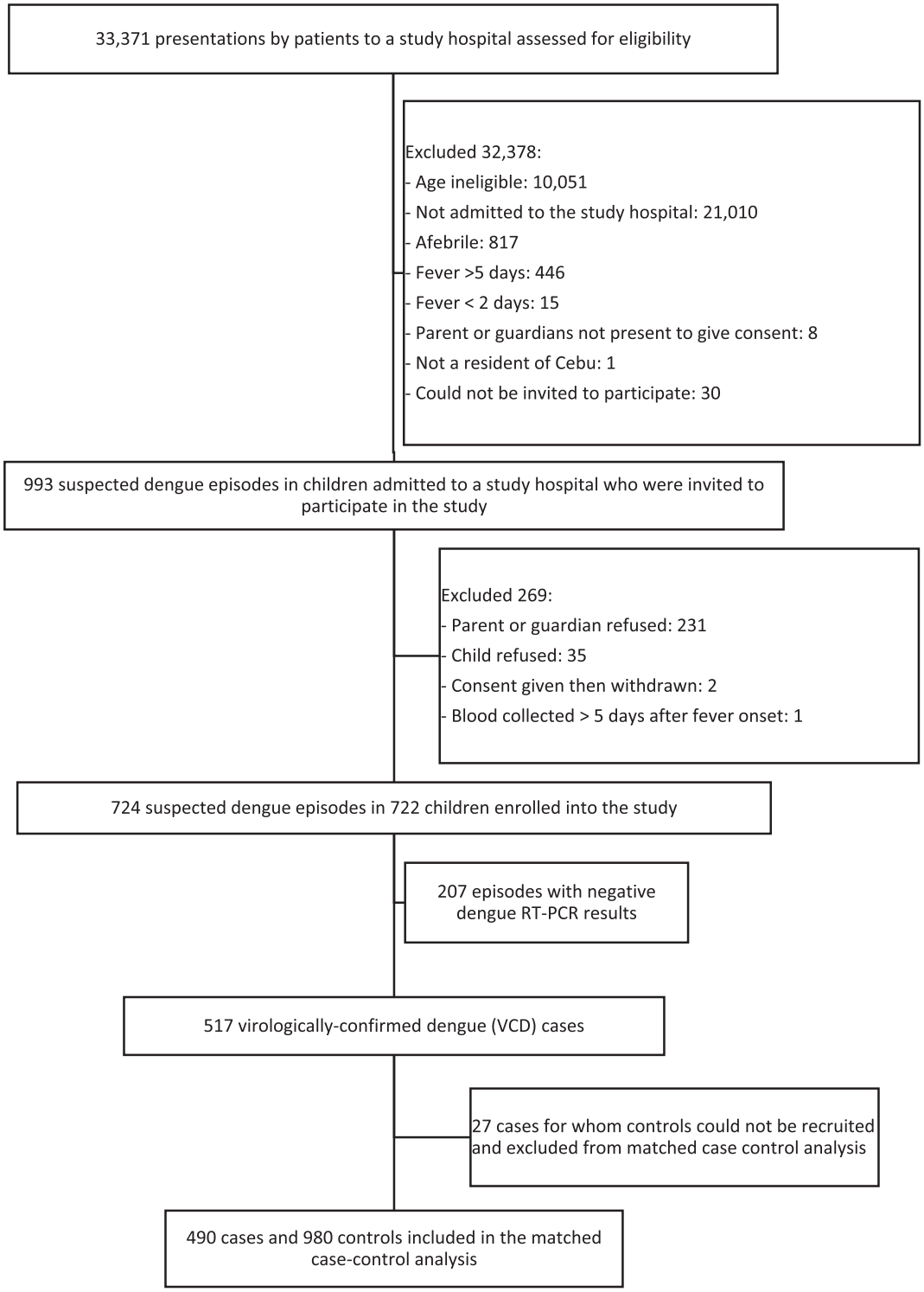

Fig. 1. Assembly of participants for the case-control study, February 15, 2018 to February 14, 2020.

a 50% vaccine effectiveness assuming a 50% vaccine coverage. Considering a 20% drop-out or subsequent non-participation, the design required at least 144 hospitalized VCD dengue cases and 288 controls. Case recruitment is continuing irrespective of numbers achieved to enable sub-analyses. Sample size was calculated using PASS 14 (Kaysville, UT, USA).

The primary analysis, formulated *a priori*, addressed the protection conferred by one dose of vaccine against VCD that was severe enough to have prompted the parents to bring the child for care at a participating hospital and for physicians to hospitalize the child. This analysis included VCD cases and their age- and sex-matched controls whose dates of enrolment were between February 15, 2018 to February 14, 2020 with vaccination defined as receipt of one dose, documented either by vaccination card or vaccination registry.

We performed crude analysis to determine the odds ratio (OR) of vaccination status by employing conditional logistic regression on the matched dataset. Selected socio-economic, environmental

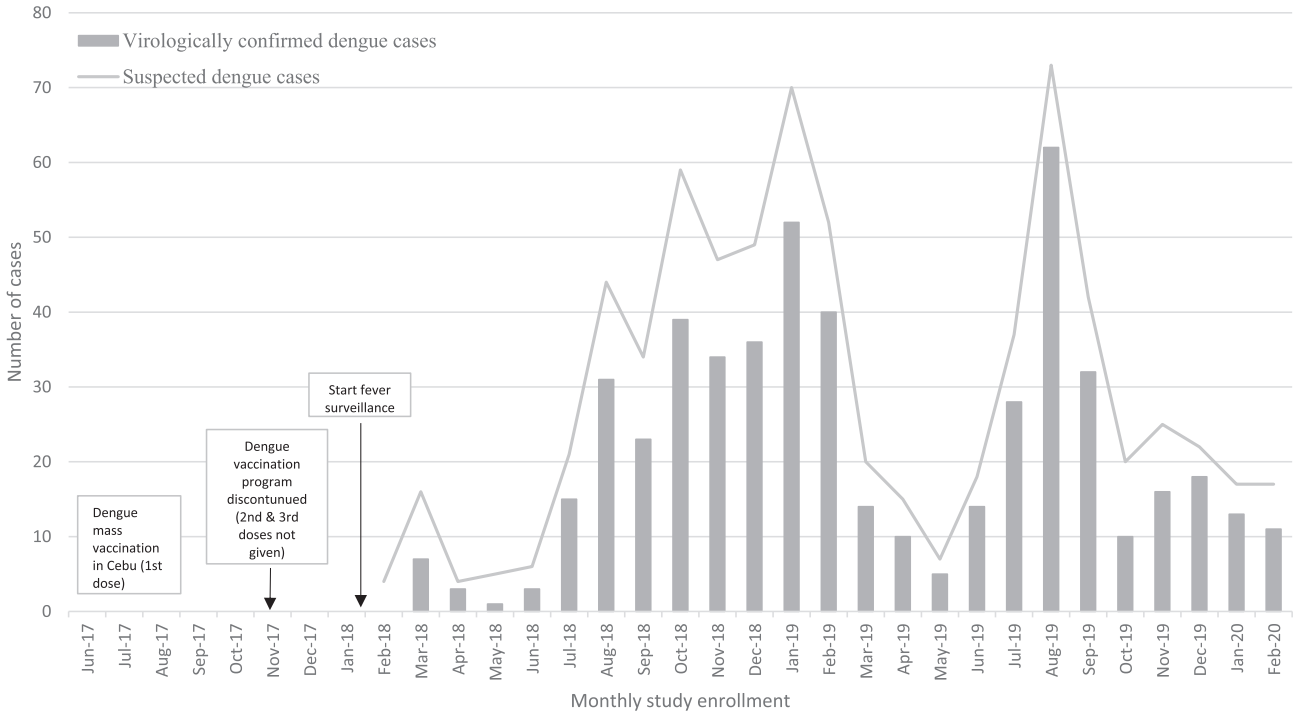

Fig. 2. Timeline of 724 enrolled febrile cases and 517 virologically-confirmed dengue cases admitted to study hospitals in Cebu, Philippines, February 15, 2018 to February 14, 2020.

and behavioral variables that were known to be associated with dengue were also compared between cases and their matched controls in bivariate analysis using conditional logistic regression to identify potential confounders. Among the variables whose associations with case-control status were statistically significant and were known correlated with each other, we selected those that had strongest association based on p-values to minimize mutual confounding among them. We fitted a logistic regression model controlling for one selected variable at a time and then performed backward stepwise regression in selecting a final model. Variables were eliminated if the tests for their corresponding coefficients did not reach statistical significance, provided their removal did not markedly change the OR for the vaccination and dengue association. Conditional likelihood estimation was used to derive estimates of the coefficients of the model. The estimated coefficient for the vaccination variable in this model was exponentiated to obtain the adjusted odds ratio. The value (1 - adjusted odds ratio) X 100 percent for the vaccination variable was computed to estimate adjusted levels of vaccine protection. We also considered case status based on dengue severity and serotype and likewise fitted conditional logistic regression models for these outcomes. All p-values and 95 percent confidence intervals were interpreted in a two-tailed fashion. The data analysis was performed using SAS software (Ver 9 4., 2016, SAS Institute Inc. Cary, NC, USA).

3. Results

From February 15, 2018 to February 14, 2020, there were 33,371 presentations by patients to a study hospital, which were assessed for eligibility (Fig. 1). Of these, 32,378 (97 0%) did not fulfil the study criteria and were excluded. Of the 993 eligible hospitalized suspected dengue episodes, 269 (27 1%) were excluded for consent refusal or withdrawal or blood collection > 5 days after fever onset. We enrolled 724 (72 9%) suspected dengue episodes in 722 children, of which 517 (71 4%) episodes were dengue RT-PCR positive. We plotted the monthly distribution of the 724 suspected dengue episodes and the 517 VCD cases (Fig. 2). Out of the 517 VCD cases, 27 (5 2%) were excluded because appropriate controls could not be recruited. A total of 490 VCD cases and 980 age- and sex-matched controls were included in this analysis.

We assessed the characteristics of the 490 cases (Table 1). Aside from fever, the most common presumptive manifestations were nausea or vomiting (349 or 71 2%), anorexia (193 or 39 4%) and myalgia (162 or 33 1%). Around two-thirds (329 or 67 1%) of cases presented one or more warning signs. The most common warning sign was abdominal pain (225 or 45 9%), followed by lethargy (125 or 25 5%) and restlessness (83 or 20 9%). Bleeding was noted in 67 (13 7%); 49 (71 0%) from the nasal mucosa, 10 (145%) from the gums and 10 (14 5%) from the lips and oral mucosa. There were four (0 8%) severe dengue cases and one death (0 2%). All four DENV serotypes were detected but over half (266 or 54 3%) were DENV 3.

We compared the clinical features of cases by vaccination status (Table 2). There was a significantly lower proportion of bleeding (p = 0 04) and less severe illness (p = 0 007) among those who had received a vaccine dose compared to the unvaccinated cases. One case who had been vaccinated died. This was an eleven-year-old girl who was admitted on the fifth day of fever and presented with nausea, vomiting, retroorbital pain, malaise and abdominal pain. She was eventually transferred to a tertiary hospital where her condition worsened, accompanied by severe bleeding. Dengue RT-PCR result was positive for DENV serotype 3.

We compared demographic and socio-behavioral variables between cases and controls (Table 3). Cases had slightly higher percentage of older children (13 years and above) than controls (p = 0 0262). Reporting a household member and neighbor diagnosed with dengue during the past seven days was significantly more common among cases than controls (p < 0 0001). Variables associated with higher socio-economic status including living in a house made of concrete (p < 0 0001), living in a screened house (p = 0 0002) and ownership of a computer (p < 0 0001), refrigerator

Table 1
Characteristics of the virologically confirmed dengue cases.

	Case (N = 490)	
	No	Percent
Hospital where case was admitted:		
Cebu Provincial Hospital – Balamban	91	18 6
Cebu Provincial Hospital – Bogo	192	39 2
Cebu Provincial Hospital – Danao	122	24 9
Eversley Child's Sanitarium and General Hospital	85	17 4
Number of days of fever on admission:		
1	1	0 2
2	68	13 9
3	127	25 9
4	222	45 3
5	72	14 7
Presumptive signs and symptoms:		
Nausea/vomiting	349	71 2
Rash	145	29 6
Headache	59	12 0
Retroorbital pain	126	25 7
Myalgia	162	33 1
Anorexia	193	39 4
Arthralgia	122	24 9
Malaise	296	60 4
Watery stools	155	31 6
Flushed skin	104	21 2
Warning signs:	329	67 1
Abdominal pain	225	45 9
Persistent vomiting	69	14 1
Lethargy	125	25 5
Restlessness	83	20 9
Bleeding	67	13 7
Enlarged liver	1	0 2
Signs of severe dengue:	4	0 8
Shock	2	0 4
Respiratory signs of fluid accumulation	2	0 4
Plasma leakage	2	0 4
Severe bleeding	2	0 2
Died	1	0 2
Serotype:		
DENV 1	36	7 3
DENV 2	158	32 2
DENV 3	266	54 3
DENV 4	22	4 5
Indeterminate (2 serotypes/RT-PCR test)	8	1 6

Table 2
Clinical features of the virologically confirmed dengue cases, by vaccination status.

	Vaccinated (n = 94)	Not vaccinated (n = 396)	X^2 test (p-value)
Presumptive signs and symptoms:			
Nausea/vomiting	63 (670)	286 (722)	1 0 (032)
Rash	28 (30)	118 (298)	0 0 (099)
Headache	7 (75)	52 (131)	2 3 (013)
Retroorbital pain	25 (266)	103 (26)	0 0 (091)
Myalgia	26 (277)	137 (346)	2 7 (025)
Anorexia	39 (415)	155 (391)	0 4 (082)
Arthralgia	19 (202)	104 (263)	2 6 (027)
Malaise	53 (564)	243 (614)	0 8 (037)
Watery stools	28 (298)	127 (321)	0 2 (090)
Flushed skin	19 (202)	86 (217)	0 1 (075)
Warning signs:			
Abdominal pain	35 (372)	191 (482)	4 0 (013)
Persistent vomiting	15 (160)	56 (141)	0 2 (065)
Lethargy	21 (223)	103 (260)	0 5 (046)
Restlessness	18 (191)	84 (212)	0 2 (066)
Bleeding	7 (74)	61 (154)	4 0 (004)
Enlarged liver	1 (11)	0 (00)	13 0 (001)
Signs of severe dengue:			
Shock	0 (00)	2 (051)	1 7 (043)
Respiratory signs of fluid accumulation	1 (11)	1 (025)	1 7 (043)
Plasma leakage	1 (11)	1 (025)	1 7 (043)
Severe bleeding	1 (11)	1 (025)	0 6 (073)
Severity of illness:			
Dengue without warning signs	42 (448)	119 (301)	7 4 (001)*
Dengue with warning sign (s)	50 (532)	275(694)	
Severe dengue	2 (21)	2 (05)	
Outcome			
Recovered	93 (989)	396 (100)	4 2 (004)
Died	1 (11)	0	

* Dengue with warning sign(s) combined with severe dengue for validity of chi-square test.

(0 0041) and car (0 0008) were significantly more common among cases than controls. The percentage reporting stagnant water in the surroundings was higher for cases than in controls (p = 0 0004). Variables associated with dengue were treated as potential confounders and were included in a logistic regression model individually and simultaneously as control variables. Using backward stepwise elimination, presence of dengue in the household and neighborhood, house with screen, stagnant water, main housing material and possession of computer were found to be independently associated with dengue (p ⟨005). The results of logistic regression analyses that controlled for these variables individually and simultaneously suggested these variables did not confound the observed effect of vaccination on dengue incidence.

We compared the odds ratios for single-dose CYD-TDV vaccination between cases and their matched controls, overall, by severity of dengue illness, and by DENV serotype (Table 4). The overall crude OR was 0 73 (95% CI: 054 to 0 99) and adjusted OR was 0 74 (95% CI: 0 53 to 1 02). This was equivalent to a vaccine effectiveness of 26% (95% CI: −2% to 47%). The crude and adjusted ORs during the first year of surveillance were 0 54 (0 35 to 0 82) and 0 51 (0 32 to 0 82), respectively. The crude and adjusted ORs during the second year of surveillance were 1 03 (0 67 to 1 60) and 1 14 (0 70 to 1 83), respectively. This was equivalent to vaccine effectiveness of 49% (19 to 68%) during the first year and −14% (-83 to 30%) during the second year. The crude OR for single-dose CYD-TDV vaccination between cases of dengue with warning signs and their matched controls was 0 52 (95% CI: 0 35 to 0 78) and the adjusted OR was 0 49 (95% CI: 0 32 to 0 77). When combined with severe dengue, the crude and adjusted ORs were 0 54 (95% CI: 0 36 to 0 80) and 0 52 (95% CI: 0 34 to 0 80), respectively. The vaccine conferred 51% (95% CI: 23% to 68%) protection against dengue with warning signs and 48% (95% CI: 20% to 66%) protection against dengue with warning signs combined with severe dengue. The crude and adjusted ORs for CYD-TDV vaccination between cases and controls and the vaccine effectiveness were calculated by DENV serotype but no conclusions could be reached due to insufficient sample size.

Table 3
Comparison of the virologically confirmed dengue cases and their controls.

	Cases (N = 490)		Controls (N = 980)		Wald X^2 test (p-value)
	No.	Percent	No.	Percent	
Sex:					
Female	238	48 6	476	48 6	Not done (matching variable
Male	252	51 4	504	51 4	
Age in years:					
9–10	77	15 7	190	19 4	
11–12	182	37 1	379	38 7	4 9 (003)
13–14	166	33 9	275	28 0	
15–17	64	13 1	136	13 9	
17	1	0 2	0	0 0	
Reported history of past dengue:					
No	468	95 5	929	94 8	0 4 (054)
Yes	22	4 5	51	5 2	
Hospital admission during previous dengue episode:					
Yes	22	4 5	41	4 2	0 01 (099)
No	0	0 0	10	1 0	
Household member diagnosed with dengue during the past 7 days	9	9 4	20	2 0	33 8 (<0 0001)
Neighbor diagnosed with dengue during the past 7 days	170	34 7	171	17 5	62 1 (<0 0001)
Household head with > 6 years of schooling	458	93 5	891	90 9	3 3 (007)
Main housing material:					
Concrete	289	59 0	440	44 9	27 1 (<0 0001)
Wood	198	40 4	528	53 9	
Thatch (*Nipa* leaves)	2	0 4	6	0 6	
Bamboo	1	0 2	5	0 51	
Galvanized iron sheet	0	0 0	1	0 1	
Environmental conditions:					
With screens	61	11 8	59	6 0	13 5 (001)
Presence of stagnant water	188	38 4	289	29 5	12 5 (001)
Ownership of specific household appliances:					
Radio	266	54 3	506	51 6	1 0 (032)
Television	423	86 3	814	83 1	2 7 (010)
Refrigerator	226	46 1	380	38 8	8 3 (001)
Bicycle	188	38 4	319	32 6	5 1 (002)
Motorcycle	258	52 7	486	49 6	1 4 (025)
Mobile phone	474	96 7	934	95 3	1 7 (019)
Desktop/handheld computer	85	17 4	90	9 2	22 2 (<0 0001)
Electricity	477	97 4	948	96 7	0 5 (050)
Car	37	7 6	35	3 6	11 2 (001)
Migrated to the current residence during the past 2 years	28	5 7	36	3 7	3 8 (005)
Household members use of topical insect repellant:					
No	407	83 1	754	76 9	8 1 (004)
<3 days/week	40	8 2	118	12 0	
3–5 days/week	19	3 9	41	4 2	
Everyday	24	4 9	67	6 8	
Burn mosquito coil* during the day:					
No	214	43 7	432	44 1	3 3 (035)
<3 days/week	88	18 0	167	17 0	
3–5 days/week	66	13 5	107	10 9	
Everyday	122	24 9	274	28 0	
Use insecticide spray at home:					
No	399	81 4	819	83 6	4 1 (025)
<3 days/week	62	12 7	114	11 6	
3–5 days/week	11	2 2	26	2 7	
Everyday	18	3 7	20	2 0	
Fogging in the neighborhood during the past month:	21	4 3	42	4 3	0 0 (100)

*Spiral made from a dried paste of pyrethrum powder, which when lit burns slowly to produce a mosquito-repellent smoke.

4. Discussion

We found that CYD-TDV conferred protection against more serious dengue illness over two dengue seasons, with protection higher during the first compared to the second year of surveillance, although the effectiveness estimate for the second year did not reach statistical significance. Our estimate of single dose effectiveness against dengue with warning signs of 51% within 30 months after vaccination is, as expected, lower than the vaccine efficacy conferred by three doses in this age group over a 25-month period (80%) against hospitalized dengue.[1]

This effectiveness study conducted under the conditions of real-life public health program incorporated several features to help ensure the validity of the results. Patients underwent systematic evaluation and confirmation of dengue by RT-PCR. Vaccination history was prospectively documented and verified by interview, vaccination card and registry. Controls were selected in a matched fashion, and extensive information about potentially confounding

Table 4
Single dose CYD-TDV vaccine effectiveness in Cebu, Philippines.

	Number (%) vaccinated among the:		Crude odds ratio (95% CI)	Adjusted* odds ratio (95% CI)	Vaccine effectiveness (95% CI)	p-value
	Cases	Matched controls				
Overall	94/490(192)	228/980(233)	0 73(0 54 to 0 99)	0 74(0 53 to 1 02)	26% (-2 to 47%)	0 07
February 15, 2018 to February 14, 2019	47/254(185)	136/508(268)	0 54(0 35 to 0 82)	0 51(0 32 to 0 82)	49% (19 to 68%)	0 01
February 15, 2019 to February 14, 2020	47/236(199)	92/472(195)	1 03(0 67 to 1 60)	1 14(0 70 to 1 83)	−14% (-83 to 30%)	0 60
According to severity of illness:						
Dengue without warning sign	42/161(261)	75/322(233)	1 21(0 74 to 1 96)	1 32(0 76 to 2 28)	−32% (-128 to 24)	0 32
Dengue with warning sign	50/326(153)	153/652(235)	0 52(0 35 to 0 78)	0 49(0 32 to 0 77)	51% (23 to 68)	0 01
Dengue with warning sign / Severe dengue	52/329(158)	153/658(233)	0 54(0 36 to 0 80)	0 52(0 34 to 0 80)	48% (20 to 66)	0 01
According to DENV serotype:						
DEN 1	4/36(111)	8/72(111)	0 48(0 13 to 1 76)	0 42(0 10 to 1 80)	58% (-80 to 90%)	0 24
DEN 2	30/158(190)	75/316(237)	0 79(0 47 to 1 30)	0 79(0 46 to 1 35)	21% (-35 to 54%)	0 39
DEN 3	55/266(207)	129/532(242)	0 73(0 48 to 1 10)	0 76(0 48 to 1 20)	24% (-20 to 52%)	0 24
DEN 4	4/22(182)	13/44(295)	0 86(0 23 to 3 25)	0 89(0 15 to 5 29)	11% (-43 to 85%)	0 89

*Adjusted for dengue in the household, dengue in the neighborhood, house with screen, stagnant water, main housing material, possession of computer after backward selection.

variables was collected and controlled for in the analyses. The analytic plan was formulated *a priori*.

Despite these measures, the study is not a double-blind, controlled, randomized trial and subject to the limitations of an observational study. Participants (and their parents) were aware of their vaccination status. The suspension of the mass dengue vaccination program occurred amidst intense media coverage and public outrage.[9] Children could be more likely to be brought and admitted to hospital if they had been vaccinated (than non-vaccinated) because they were perceived to be at an increased risk. In addition, starting in March 2018, the DOH established "dengue express lanes" for CYD-TDV recipients who present to public and private hospitals with any illness, with expenses paid by the Philippine Health Insurance Corporation. This bias would result in an underestimation of the protective effect of the vaccine. While not entirely absent, this bias was likely less important in case detection among children with more serious illness (dengue with warning signs and severe dengue) whose condition more clearly required hospital presentation and admission, regardless of vaccination status. As a result, the validity of the estimated vaccine protective effect against dengue with warning signs and severe dengue is probably less affected by health-seeking behavior, perhaps explaining the higher protection observed with these outcomes. We compared the demographic characteristics between cases of dengue without warning signs and cases of dengue with warning signs and severe dengue (Supplementary Table 1) and found that the former were more likely to have a household head with more than six years of schooling (156/161 or 96 9% versus 302/329 or 91 8%; p = 0 03). This further supports the possibility of bias between the groups; level of education may be associated with likelihood of being vaccinated,[16] risk of infection[17,18] and likelihood of presenting/being admitted to hospital, particularly in milder forms of dengue. As we are unable to determine if and to what extent the health seeking behavior of the milder dengue cases was biased by vaccination status and/or by socio-economic level, the estimated vaccine effectiveness overall and against dengue without warning signs can only be considered with caution, while we can be more confident in the validity of our estimates of effectiveness against dengue with warning signs and severe dengue. The DOH continues to monitor the recipients of CYD-TDV,[19] and the results of this study will contribute to documenting the long-term safety profile of CYD-TDV. We also noted that controls in our study were less frequently vaccinated than the overall source population, and that controls enrolled in the second year of surveillance were less frequently vaccinated than controls enrolled in the first year (**Table S1**). These differences may signal both a systematic difference in controls compared to the source population and a shift in their characteristics over the first two years of surveillance. The cause of these differences is not evident from our available data but raises the possibility they could have impacted the precision of our estimates.

As shown in the CYD-TDV clinical trials, breakthrough illness after vaccination will occur in areas such as our study sites with high dengue transmission. [20,21] Since the vaccination program in the Philippines was conducted without prior knowledge of dengue serostatus, our study measured the protective effect of the vaccine on a population of mixed serostatus at time of vaccination. In a separate longitudinal cohort study in which we enrolled 2,996 children 9 to 14 years of age in Bogo and Balamban, Cebu prior to the mass dengue vaccination, we found that nearly 90% were seropositive for dengue.[22] Thus, it is not unexpected to find that CYD-TDV demonstrated a sizeable protective effect against more serious forms of dengue.

This study is continuing for five years after vaccination so to provide longer-term effectiveness data. In the meanwhile, public health programs in areas highly-endemic for dengue will need to weigh the costs and benefits of deploying CYD-TDV. A reduction in hospitalizations by 26% (even after only one dose) for a disease of such high incidence has to be balanced against the risks to those who are seronegative at vaccination. Ideally, vaccinating only those with prior dengue infection would be the preferred strategy but depends on the development of sensitive and specific point-of-care tests [20]. Since 2018 the WHO now recommends that countries considering CYD-TDV vaccination as part of their dengue control program should include pre-vaccination screening, so that only dengue-seropositive persons are vaccinated but that the limitations of such screening should be clearly communicated to those offered vaccination.[23]

In summary, we found that one dose of the CYD-TDV vaccine conferred protection against dengue with warning signs and severe dengue. While our analysis did not find any statistically significant protective effect overall and against dengue of lesser severity, potential biases preclude drawing any conclusion about this outcome. We plan to continue the case-control study for five years after the mass dengue vaccination campaign.

5. Role of the funding source

The study sponsor is the University of the Philippines – Manila and its staff developed the protocol, implemented the study, managed and analyzed the data and wrote the manuscript. The Philippine government paid for the vaccine and the DOH implemented

the dengue mass vaccination program. The University of the Philippines - Manila received funding from Sanofi Pasteur to undertake the assessment of vaccine effectiveness. Sanofi Pasteur was involved in discussions of the trial design and contributed to the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.07.042.

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