A case-control study to determine the effectiveness of a tetravalent dengue vaccine in the state of Paraná, Brazil

José Cássio de Moraes,[a] Irina Nastassja Riediger,[b] Fernanda Crosewski,[c] Denise Oliveira Garrett,[d] Francieli Fontana Fantinato,[e] Karina Braga Ribeiro,[a] and Expedito José de Albuquerque Luna[f]*

[a]Faculdade de Ciências Médicas da Santa Casa de São Paulo, São Paulo, Brazil
[b]Laboratório Central do Estado do Paraná, Secretaria Estadual de Saúde, São José dos Pinhais, Brazil
[c]Secretaria Estadual de Saúde do Paraná, Curitiba, Brazil
[d]Sabin Vaccine Institute, WA, USA
[e]Ministério da Saúde, Brasília, Brazil
[f]Faculdade de Medicina, Universidade de São Paulo, Av. Dr. Enéas de Carvalho Aguiar 470, São Paulo 05403-000, Brazil

Summary

Background The Brazilian state of Paraná conducted a mass vaccination campaign against dengue with the tetravalent attenuated vaccine CYD-TDV. The campaign targeted thirty endemic municipalities. The objective of this study was to assess the effectiveness of CYD-TDV in preventing symptomatic virologically confirmed dengue cases according to specific age groups in five of the municipalities.

Methods A case-control study was carried out in the five most populous municipalities targeted by the vaccination, with a vaccine uptake of 25%. Symptomatic dengue cases were identified by the municipal health departments. The age groups targeted were 15−18 and 19−27 in four municipalities and 9−14 and 28−44 in one municipality. All cases were confirmed by real time reverse transcription quantitative polymerase chain reaction (RT-qPCR). For each case, two controls were selected: a neighbourhood control and a workplace or school/college control, matched by age group. A conditional logistic regression model was used to determine the odds ratio for vaccination and the vaccine effectiveness.

Findings Study participants included 618 RT-qPCR-confirmed dengue cases and 1,236 matched controls (with a non-reactive dengue IgM serologic test). Vaccine effectiveness against dengue due to any serotype was 11·1% (95% CI: −19·0%; 33·6%). Effectiveness against DENV-1 was 33·3% (95% CI: −5·0%; 57·6%) and against DENV-2 was −56·7% (95% CI: −142·2%; −5·0%). No DENV-3 was detected. The vaccine was significantly effective in the prevention of DENV-4 cases (VE = 93·3%; 95% CI: 47·7%; 99·2%).

Interpretation CYD-TDV was effective in the prevention of symptomatic cases due to DENV-4, but not due to any serotype. The low dengue seroprevalence in the target population could possibly be related to these results.

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Introduction

Dengue is a threatening infectious disease worldwide. It is estimated that the dengue viruses infect 390 million people each year, with 96 million symptomatic cases leading to 500,000 severe cases, 12,500 deaths, and 1.1 million disability-adjusted-life-years (DALYs).[1,2]

Dengue has been endemic in Brazil since 1986, when serotype 1 (DENV-1) was introduced into the country. After two decades, three other serotypes emerged, and the mosquito-borne disease spread to all country regions.[3] From 2000 to 2016, more than 11 million cases were notified. In 2010, 2013, 2015, and 2016 more than one million cases were reported annually in

Research in context

Evidence before this study

The Brazilian state of Paraná, which experienced its largest dengue outbreak in early 2016, decided to introduce CYD-TDV as part of the state's dengue prevention program. The Brazilian National Advisory Committee on Immunization, an advisory board of experts to the National Immunization Program (PNI) recommended that although the PNI was not involved in the decision and use of CYD-TDV, it should support studies to evaluate the Paraná experience with the dengue vaccine. In joint discussions between the PNI and Paraná's State Health Secretariat it was decided to carry out studies on the effectiveness, impact and safety of the CYD-TDV.

In clinical trials, CYD-TDV showed efficacy in preventing virologically confirmed symptomatic dengue cases, severe dengue, and hospitalizations due to dengue. In a previous pooled analysis of Phase III clinical trials, the efficacy of CYD-TDV against virologically confirmed symptomatic dengue was 65·6%, with higher efficacy against serotype 4 and lower efficacy against serotype 2. Long-term follow-up of trial participants uncovered a safety signal that was not identified during the initial studies: an increased risk of hospitalization for virologically confirmed dengue was observed among vaccinated participants who were seronegative at baseline.

Added value of this study

The study presents original data about the use of the dengue vaccine in public health. This analysis finds that vaccination with the complete schedule of three doses was effective in the prevention of symptomatic virologically confirmed cases due to DENV-4 but was not effective in preventing dengue due to any serotype, nor to DENV-1, nor DENV-2. The case-control study presented here of the effectiveness of CYD-TDV in preventing symptomatic virologically dengue cases adds critical, post-marketing evidence to inform the use of this vaccine. The low dengue seroprevalence in the target population, the low vaccine coverage, and the predominant DENV serotypes circulating in the study's settings should be considered in the interpretation of the results.

Implications of all the available evidence

Health authorities deciding whether and how to introduce dengue vaccines to their communities will need to consider the available evidence, presented here and in previously published studies, of the effectiveness and safety of CYD-TDV. These findings should be weighed with local seroprevalence rates and circulating DENV serotypes. Future, similar research to this study in an area with higher seroprevalence would add value for decision-makers.

Brazil.[4,5] After a short period with lower incidence, a new outbreak struck the country in 2019, when over 1.5 million cases were reported.[6]

Sanofi Pasteur's dengue vaccine (CYD-TDV), a live-attenuated tetravalent dengue vaccine, was the first marketed dengue vaccine and licensed in over 20 countries since 2015. The vaccine showed efficacy in preventing virologically confirmed symptomatic dengue cases, and against severe dengue and hospitalizations due to dengue, in two large clinical trials.[7,8] In the pooled analysis of the two trials, the overall efficacy of CYD-TDV against virologically confirmed symptomatic dengue was 65·6% (95% CI: 60·7%; 69·9%), with higher efficacy against serotype 4 and lower against serotype 2. Efficacy analysis by serological status was performed on a subset of the trials' participants. A lower efficacy was observed among the seronegative ones. Efficacy was also lower in younger participants.[9,10]

The lower efficacy among the seronegative and an increased risk of hospitalization for virologically confirmed dengue observed among those vaccinated who were seronegative at baseline[11] lead to two World Health Organization (WHO) recommendations. The first one was to restrict the use of the vaccine to populations with dengue seroprevalence above 70% in the age groups targeted for vaccination.[12] Later, the WHO updated the recommendation to a pre-vaccination screening strategy, meaning that only people with evidence of previous dengue infection should be vaccinated based on an antibody test or a documented laboratory-confirmed dengue infection in the past.[13,14]

Before the publication of the first WHO recommendation, two initiatives using the dengue vaccine were carried out, one in the Philippines,[15] and the other one in the Brazilian state of Paraná.[16] Paraná had its largest dengue outbreak during the first quarter of 2016 and decided to introduce CYD-TDV as part of its dengue prevention program. The state introduced the dengue vaccine in 30 of 399 municipalities. These municipalities were selected using epidemiological criteria, including the occurrence and size of dengue outbreaks in the previous five years. In 28 municipalities, the target population included all residents from 15 to 27 years of age. For the remaining two municipalities, the target age group was expanded to include residents from 9 to 44 years of age. From August 13, 2016, to September 3, 2016, the vaccine was administered in a campaign approach targeting the age groups described above for the first dose. The second dose campaign was carried out from March 3, 2017, to April 7, 2017, and the third dose was from September 20, 2017, to November 11, 2017. Two additional campaigns were carried out to complete the vaccine schedule from March 20, 2018, to June 29, 2018, and from November 7, 2018, to December 14, 2018.

We conducted a case-control study to determine the effectiveness of CYD-TDV in preventing symptomatic virologically confirmed dengue cases in five municipalities in the State of Paraná. The present article analyzed cases with the complete vaccine schedule against unvaccinated cases and their matched controls.

Methods

The effectiveness of CYD-TDV was evaluated in an individually matched case-control design (age-matched dengue case and two non-dengue controls), conducted from September 2016 to December 2019. The study was conducted in five of the 30 municipalities in the Brazilian state of Paraná (2016 population 11,242,720) that had been targeted by the mass vaccination campaign: Maringá, Foz do Iguaçu, Londrina, Sarandi and Paranaguá. These municipalities were chosen because they had the largest population, accounting for 75% of the vaccine campaign target population (Table S1). The eligible subjects were in the age strata target by the vaccination campaign: 15 to 27 years-old in the five municipalities, and two additional groups, 9 to 14 and 28 to 45 years-old, in Paranaguá.

A dengue case was defined as a dengue suspected case (according to the Brazilian Ministry of Health definition)[17] with a detectable viral antigen by reverse transcription polymerase chain reaction (RT-qPCR) and identification of the serotype, in the age groups of interest. A control was defined as an asymptomatic individual in the same age group and living in the same municipality as the respective dengue case, with non-reactive immunoglobulin M (IgM) for dengue within 30 days after the onset of symptoms of the corresponding case. Controls were recruited in a 1:2 ratio matched by age strata. One of the controls was chosen in the case's neighborhood and the other in the same workplace or school/college/university. The controls were chosen from those who lived, worked, or studied at the time of the onset of the case's symptoms and who agreed to participate, signed the consent form, and provided a blood sample.

A case of dengue fever was considered vaccinated when the third dose of the vaccine was administered 15 days or more before the onset of symptoms. A control was considered vaccinated when the third dose of the vaccine had been administered up to 15 days before the symptoms' onset of the matched dengue case. The vaccination status had to be proven by documentation presented by cases and controls and/or by locating their names in the vaccine database of the State Secretariat of Health of Paraná. An exploratory analysis of the efficacy of incomplete vaccine schedules was undertaken.

The inclusion criteria for cases were: individuals who lived in one of the five municipalities during all three stages of the vaccination campaign, with virological identification of one of the dengue viruses, and ages ranging from 9 to 44 years for Paranaguá and 15 to 27 years for the other municipalities. The exclusion criterion for cases was the status of inmate of a correctional facility within 15 days before the onset of signs and symptoms.

The inclusion criteria for controls were: individuals without symptoms for dengue in the 15 days before the onset of symptoms of their matched case, and an IgM serologic test non-reactive for dengue, and who lived in the selected five municipalities during the three stages of vaccination, who belonged to the same age stratum as the corresponding case, and who resided in the same neighbourhood as the case, studied at the same institution as the case, or worked in the same company as the case for at least 15 days before the onset of the case symptoms. Enrolled cases and controls agreed to participate and signed the informed consent form. Minors under the age of 18 who agreed to participate, signed the assent form, and had the consent form signed by a parent or a legal guardian.

We generated our sample size by estimating the effectiveness of the vaccine at 50%, vaccination coverage of 25%; a two-sided hypothesis test; type I error (α) = 0·05; power of the study of (1- β) = 0·80; under null hypothesis probability ratios (OR) = 1; alternative hypothesis OR = 0.5 and a ratio of 1 case to 2 controls. Thus the calculated sample size for dengue cases was 166 cases and 332 controls for each of the study's four age strata, totalling 1992 participants for all five municipalities.

Dengue is a notifiable disease in Brazil and case detection was performed in collaboration with the municipal public health surveillance services. Cases were identified in the database of the Diseases Notification Information System (SINAN). To verify their PCR result, suspect dengue cases identified in SINAN were linked to the laboratory database (Gerenciador de Atividades Laboratoriais—GAL). During the study, as dengue cases were diagnosed, they were contacted by the field team and invited to participate, until the sample size for each age group was completed.

The field team contacted the case, usually by telephone, and scheduled a home visit. Trained interviewers administered a structured questionnaire to individuals aged $\geq$ 18 years or to the parent/ guardian for those participants < 18 years during the home visit. Ascertainment of vaccination was done by checking the vaccination card of participant during the interview. If no card was available, other written documentation was acceptable. We also confirmed the participants' vaccination status by consulting the vaccine registry dataset of the State Secretariat of Health.

The MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume kit was used for nucleic acid extraction in a MagNA Pure 96 system (Roche, Pleasanton, CA, USA). RT-qPCR tests were carried out using a FLOW system coupled with a LightCycler 480 II thermal cycler

(Roche, Pleasanton, CA, USA). RTq-PCR was carried out using previously described oligonucleotides[18] and primers and probes for dengue virus (DENV) serotype 1 (DENV-1) or DENV-2, DENV-3, or DENV-4 (sequences and concentrations as previously described).[19] Samples in which any dengue serotype was detected within 40 PCR cycles were considered positive. IgM antibodies directed towards any of the four DENV serotypes were detected using the PanBio Dengue IgM Capture ELISA (PanBio Pty Ltd., Brisbane, Australia) following the manufacturer's instructions.

The list of cases and controls included in the study was linked to the SINAN database using the Link Plus System to identify if any had been previously reported as a dengue case before 2019.

For the data analysis, exposure was defined as vaccination with dengue vaccine with three doses; effect as a case of virologically confirmed dengue fever due to any serotype.

We used conditional logistic regression modeling to generate odds ratios for matched case-control groupings, and estimated the protective effect using the formula: Vaccine Effectiveness = (1−Odds Ratio) x 100. We also conducted a stratified analysis, by age group and dengue serotype in the regression models.

The Santa Casa de São Paulo Research Ethics Committee (Resolution no. 1,817,892, on November 11, 2016) and Pan American Health Organization Ethics Review Committee−PAHOERC (Resolution PAHO 2016−11−0056) approved the study protocol. It was also approved by the appropriate authorities of the State of Paraná, including the municipalities where the study was conducted. The study was registered at clinicaltrials.gov before enrolment was initiated (NTC03960385).

Role of the funding source
The funder had no role in study design; in the collection, analysis, or interpretation of data; nor in the decision to submit the paper for publication.

Results
During the three-year study, 37,446 dengue cases were confirmed in Paraná. Of these, 8871 (23·7%) occurred in the 5 municipalities included in the study; 2239 cases were in the age groups of interest, 1232 (55·0%) of which were virologically confirmed as dengue cases. Of these, 618 (50·2%) were included in the study. Of the 614 confirmed cases not included in the study, 166 (27·2%) occurred after June 30, 2019, when the recruitment of cases in the age group 19 to 27 had been stopped because the sample size for this age group had been attained; 222 (36·1%) were not reached by the study's field teams because in the peak of the outbreak in 2019, in one of the municipalities the number of

cases exceeded the capacity of the field team; 71 (11·6%) did not live in the addresses they provided to the emergency care unit or provided a non-existent address; 63 (10·2%) lived in other municipalities, other Brazilian states, or other countries at the time of the vaccine campaigns; 39 (6·3%) had incomplete data (missing data on critical variables, such as date of birth; or the study's field teams were unable to find two controls); 34 (5·5%) had a wrong date of birth recorded in the mandatory reporting form and were out of the age range for which the vaccine was offered at the time of vaccination; and 19 (3·0%) refused to participate (Fig. 1). There were no cases excluded because of missing information regarding vaccine status.

Enrolled cases were not meaningfully different from RT-qPCR confirmed cases not included in the study (Table S2).

The majority of participants were female (53·7%), and in the 19 to 27 years age group, reflecting the age distribution of reported symptomatic dengue cases in Paraná in 2019 (Table 1).[20] The predominant serotype was DENV-1 (49·4%), followed by DENV-2 (40·8%). Most cases of DENV-4 occurred in Foz do Iguaçu (98%). Forty cases were hospitalized. Most of the hospitalized dengue cases presented with warning signs (76·9%), three required intensive care, and one presented with shock. There were no deaths due to dengue.

Of potential controls, 6·9% (91/1·327) were excluded due to a reactive dengue IgM serology, and 16·5% (15/91) of them had received three vaccine doses.

Vaccine uptake decreased from the first to the third dose. More than half (57%) of the study participants were not vaccinated with CYD-TDV (Tables 2 and S1) and the proportion of participants with a complete vaccine schedule was lower than the state's vaccine coverage. The two municipalities that provided most cases and controls had lower vaccine coverage with three doses than the remaining municipalities. 312 (50%) of the cases included in the study came from one of the municipalities, while the municipality where the largest age cohort was vaccinated contributed with just 2% of the cases (Table S3).

Most dengue cases included in the study that were vaccinated with at least one dose of CYD-TDV had received the last dose more than one year before the onset of dengue symptoms (Table S4). The median time between the date of the last vaccine dose and the onset of dengue symptoms was 19.9 months.

The effectiveness of CYD-TDV in preventing symptomatic, virologically confirmed dengue cases was 11·1% (95% CI: −19·0%; 33·6%) (Table 3). Vaccine effectiveness in the age group 15 to 18 years was −15.0% (95% CI: −77·7%; 25·5%); lower than the 19 to 27 years group, 32·1% (95% CI:−2·4%; 55·0%). Detailed results of the conditional regression analysis are presented in the supplementary materials (Tables S5−S20). It was not possible to determine the vaccine effectiveness in
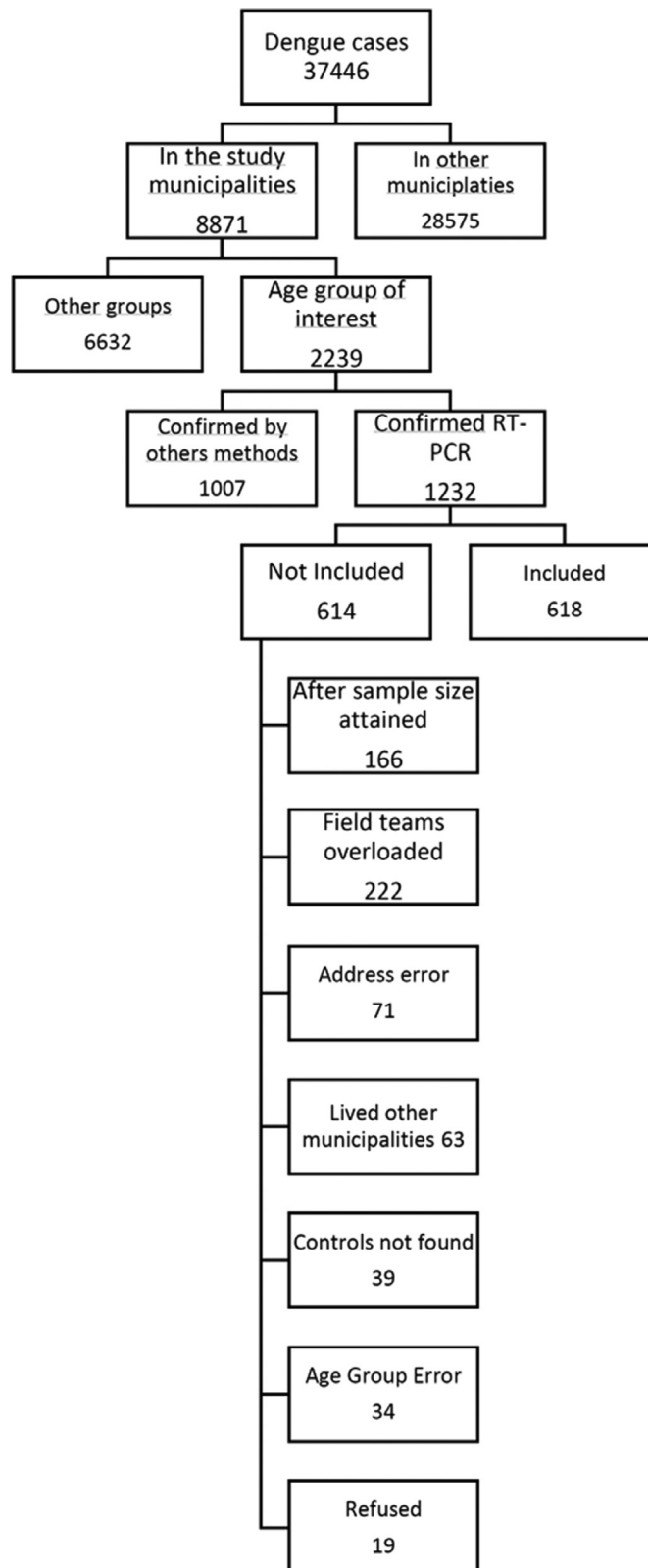
Fig. 1. Enrolment flowchart for the effectiveness of the CYD-TDV dengue vaccine case-control study, state of Paraná, Brazil, 2017—2019.

	Cases n (%)	Controls n (%)	Total n
Municipality			
Foz do Iguaçu	173 (28·0)	346 (28·0)	519
Londrina	312 (50·5)	624 (50·5)	936
Maringá	82 (13·3)	164 (13·3)	246
Paranaguá	13 (2·1)	26 (2·1)	39
Sarandi	38 (6·1)	76 (6·1)	114
Age group			
9—14	4 (0·6)	8 (0·6)	12
15—18	247 (40·0)	494 (40·0)	741
19—27	362 (58·6)	724 (58·6)	1086
28—45	5 (0·8)	10 (0·8)	15
Sex			
Female	339 (54·9)	657 (53·2)	996
Mean age (years)	20·02	19·91	19·95
Serotype			
DENV-1	305 (49·3)		
DENV-2	252 (40·8)		
DENV-4	61 (9·9)		
Previous dengue*			
NO	605 (97·9)	1153 (93·3)	
YES	13 (2·1)	83 (6·7)	

***Table 1**: Cases and controls included in the effectiveness of CYD-TDV's study, state of Paraná, Brazil, 2016—2019.*
   * This difference was statistically significant.

the age groups 9 to 14 and 27 to 45 due to the small number of virologically confirmed cases. Vaccine effectiveness was not associated with sex (Tables S6 and S7).

The vaccine's effectiveness in preventing DENV-1 cases was 33·2% (95% CI: −5·0%; 57·6%). A negative effectiveness was observed for DENV-2 cases, −56·8% (95% CI: −142·2%; −1·5%), while the effectiveness of the vaccine in preventing cases of DENV-4 was 93·2% (95% CI: 47·7%; 99·1%).

A previously reported dengue episode did not impact the results. Vaccine effectiveness was 9·3% (95% CI: −22·5%; 32·8%) for those without a past confirmation of dengue. We could not calculate the vaccine effectiveness for those reported as confirmed dengue case in the past because there was only one discordant set of cases and controls.

Vaccination with incomplete schedules showed similar results. No effectiveness against dengue was observed for any serotype. Effectiveness against DENV-4 with incomplete regimens was maintained (Tables S21 and S22).

Discussion

This study was designed to determine the effectiveness of CYD-TDV against virologically confirmed dengue cases, due to any serotype, in the four vaccinated age groups. A *post hoc* analysis was conducted to determine the effectiveness against the different serotypes and the serotypes in the distinct age groups. Our results show that vaccination with the complete schedule of CYD-TDV, the live-attenuated tetravalent dengue vaccine, was effective preventing

Vaccine status	Cases n (%)	Controls n (%)	Total n (%)
Unvaccinated	363 (58·7)	694 (56·2)	1057 (57·0)
3 doses	108 (17·5)	235 (19·0)	343 (18·5)
2 doses	74 (12·0)	160 (12·9)	234 (12·6)
1 dose	73 (11·8)	147 (11·9)	220 (11·9)
Total	618 (100·0)	1236 (100·0)	1854 (100·0)

***Table 2**: Distribution of cases and controls according to the vaccine doses across municipalities included in the effectiveness of CYD-TDV's study, state of Paraná, Brazil, 2016—2019.*

	Odds Ratio (95% CI)	Vaccine Effectiveness (95% CI)
Vaccination	0·889 (0·664; 1·190)	+ 11·1% (− 19·0%; + 33·6%)
Age 15 to 18	1·150 (0·745; 1·777)	− 15·0% (− 77·7%; − 25·5%)
Age 19 to 27	0·679 (0·450; 1·024)	+ 32·1% (− 2·4%; + 55·0%)
Sex Female	0·682 (0·418; 1·112)	+ 31·8 (− 11·2%; + 58·2%)
Sex Male	1·158 (0·647; 2·072)	− 15·8 (− 107·2%; + 35·3%)
DENV-1	0·667 (0·424; 1·050)	+ 33·3% (− 5·0%; + 57·6%)
DENV-2	1·567 (1·015; 2·422)	− 56·7% (− 142·2%; − 1·5%)
DENV-4	0·068 (0·009; 0·523)	+ 93·2% (+ 47·7%; + 99·1%)
DENV-1		
Age 15 to 18	0·796 (0·395; 1·603)	+ 20·4% (− 60·3%; + 60·5%)
DENV-1		
Age 19 to 27	0·611 (0·335; 1·114)	+ 38·9% (− 11·4%; + 66·5%)
DENV2		
Age 15 to 18	2·014 (1·053; 3·851)	− 101·4% (− 285·1%; − 5·3%)
DENV2		
Age 19 to 27	1·105 (0·593; 2·058)	− 10·5% (− 105·8%; + 40·7%)
DENV4		
Age 15 to 18	0·200 (0·023; 1·700)	+ 80·0% (− 70·0%; + 97·7%)
DENV4		
Age 19 to 27	−	−
Reported as a dengue case in the past	−	−
Not reported as a dengue case in the past	0·907 (1·225−0·672)	+ 9·3% (− 22·5%; + 32·8%)

Table 3: Effectiveness of a complete schedule (3 doses) CYD-TDV, state of Paraná, Brazil, 2016−2019.

symptomatic virologically confirmed cases due to DENV-4. However, it was not effective in preventing dengue due to any serotype, nor to DENV-1. A negative effectiveness was observed for DENV-2.

The observed effectiveness was lower than the efficacy determined in the previous trials of the vaccine.[21] This finding may be in part for the previously described impact of seroprevalence of dengue on the vaccine efficacy. CYD-TVD has shown lower efficacy in low prevalence settings. For example, trials conducted in Latin America, including Mexico, where seroprevalence was 53·1%, VE was 31·3% (95%CI: 1·3%; 51·9%) and Puerto Rico, with a seroprevalence of 56·2%, VE was 57·6% (95%CI: −2·5%; 82·2%).[7] Data on dengue seroprevalence in Brazil are scarce. In a study commissioned by the Ministry of Health in 2015 to support the decision to introduce CYD-TDV in the public vaccination program, the seroprevalence in Londrina and Foz do Iguaçu reached 20% by 16 years of age.[22] Even considering that this seroprevalence study was conducted before Paraná's largest dengue outbreak in 2016, Paraná's seroprevalence would probably be characterized as low or intermediate. On top of the possible low dengue seroprevalence in the study's settings, the vaccine uptake was also low, contributing to the small number of exposed subjects in each subset. Our results demonstrate that the vaccine was still effective despite the relatively low incidence of the DENV-4 in Paraná during

the study period. As for DENV-1, it is possible that the result, close to statistical significance, was influenced by the power of the sample (0·57). The finding of negative effectiveness for DENV-2 is in line with previous observations that vaccination with CYD-TDV may mimic a primary infection and increase the risk of a secondary infection.[11]

In addition to low prevalence, other studies have also shown a reduced efficacy among participants not previously exposed to dengue. In the Latin American trial, vaccine efficacy was low among the seronegative participants at baseline (43.2%; 95% C.I.: −61.6%; 80.0%).[9] The need for pre-vaccination screening makes the design of vaccination strategies more complex.[23] On top of that, the spatial heterogeneity of dengue transmission and seroprevalence complicates vaccine introduction decisions, even at the sub-national level.[24] In the present study, the three municipalities with the majority of cases had outbreaks with a distinct proportion of serotypes.

Among the limitations of our study, we highlight the low incidence of dengue in Paraná, in the years 2017 and 2018, and in 2019 in the municipality of Paranaguá. The low incidence in this municipality made it impossible to analyze effectiveness in the 9 to 14, and 28 to 45 age groups. Also, the small number of hospitalizations and severe cases prevented the analysis of effectiveness for these outcomes. The occurrence of DENV3

was not observed during the study period. In 2019, the dengue epidemic was more intense in one of the municipalities (Londrina) and the large number of cases concentrated in a few weeks in this municipality exceeded the field teams' capability at the peak of the outbreak. This may have introduced a selection bias. Some cases were excluded because it was not possible to find controls of the same age group in their neighborhood. As for the data on previous dengue episodes, the reported cases follow the Brazilian national dengue cases definitions, so that they may include cases confirmed by clinical-epidemiological criteria, which may introduce false-positive cases. A previous dengue infection may influence the outcome of the following infection, however, the proportion of participants with previous dengue episodes was small. To account for that, a sensitivity analysis excluding these participants was conducted and similar results were obtained (data not shown). The effect of other known or unknown confounding variables may have influenced the observed results. An additional limitation is the lack of other studies on the effectiveness of CYD-TDV to compare the results. However, this could be considered as a strength of the paper, claiming for further studies, given the vaccine has been approved in many endemic countries. Further studies in areas with high seroprevalence would add value for decision-makers.

Our study has several strengths. The probability of misclassification of cases and controls was minimized by the laboratory criteria used to ascertain their status. Dengue cases were confirmed by an RT-qPCR assay, which is highly sensitive and specific. There was no restriction in the collection and analysis of PCR for suspected cases of dengue within the study's age range who sought a health service up to the fifth day after the beginning of symptoms. Controls had a negative ELISA IgM result. In an evaluation panel conducted by TDR/WHO, the ELISA IgM brand used in the present study presented a sensitivity of 99% and a specificity of 84·4%.[25] The probability of false-negative results was low, and false-positives due to a lower specificity were excluded. A small proportion (6·9%) of potential controls were excluded for a reactive dengue IgM serology. Vaccine coverage among excluded controls was similar to the included controls, and all but two of them had received their last vaccine dose more than one year before the IgM sample collection. Therefore, their reactive IgM results are unlikely to be due to vaccination. In case-control studies for vaccine effectiveness, the key issue is to determine the vaccine status accurately.[26] The possibility of exposure misclassification (dengue vaccination) in the present study was minimal, as two sources of information were used. Vaccine coverage among the included controls was similar to the population coverage in the same age groups (excluding the municipality of Paranaguá).

In summary, the only variable significantly associated with effectiveness in the matched stratified analysis was the serotype DENV-4. However, the low dengue seroprevalence in the target population, the low vaccine coverage, and the predominant DENV serotypes circulating in the study's settings should be considered in the interpretation of the results.

CRediT authorship contribution statement
José Cássio de Moraes: Writing − original draft, Writing − review & editing, Funding acquisition, Resources, Formal analysis, Data curation, Validation. **Irina Nastassja Riediger:** Formal analysis, Data curation, Writing − review & editing, Validation. **Fernanda Crosewski:** Project administration, Data curation, Writing − review & editing, Validation. Denise Oliveira Garrett: Supervision, Formal analysis, Validation, Writing − review & editing. **Francieli Fontoura Fantinato:** Supervision, Formal analysis, Writing − review & editing, Validation. **Karina Braga Ribeiro:** Software, Writing − original draft, Writing − review & editing, Validation. **Expedito José de Albuquerque Luna:** Writing − original draft, Writing − review & editing, Funding acquisition, Resources, Formal analysis, Data curation, Validation.

Izuka, Mr. Roberto Doldan (Foz do Iguaçu); Ms. Andrea Moura (Paranaguá).

Data sharing

The study's data will be available for sharing. The data on individual participants, the data dictionary, the study's protocol, and the informed consent form will be available for sharing. In order to access the data a proposal will be required. The proposal should be made to the corresponding author. The data will be available with publication at the University of São Paulo research data repository (https://uspdigital.usp.br/repositorio/).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lana.2021.100141.

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