EFFICACY AND SAFETY OF SINGLE DOSE, LIVE ATTENUATED, TETRAVALENT BUTANTAN DENGUE VACCINE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract:-

Objectives: Dengue remains an expanding global health burden with no vaccine offering protection regardless of serotype status across wide age groups. Thus, this meta-analysis focuses on the efficacy and safety of Butantan-DV, a single dose, live attenuated, tetravalent vaccine

Methods: We searched Cochrane, PubMed, Science Direct, and Clinicaltrials.gov for studies published anytime from inception till July 2026. Studies were included if they were RCTs having healthy individuals ranging between 2-60 years receiving Butantan-DV. ROB2 was used to assess risk of bias among RCTs. Primary efficacy end point included virologically confirmed dengue virus post vaccination and safety outcomes included solicited, unsolicited and serious adverse events. Protocol of the review was registered on PROSPERO

Results: 5 RCTs were considered eligible out of which 4 had some concerns and one had low risk of bias on ROB2 assessment. Significant reduction in virologically confirmed dengue virus was observed with Butantan-DV in regardless of serostatus (RR 0.30; 95% CI [0.23, 0.39], $p < 0.00001$, $I2 = 69\%$) and similar results were observed for seropositive and dengue naïve individuals. For safety profile, Butantan-DV had a slightly more risk of solicited (RR 1.24; 95% CI [1.21, 1.28], $p < 0.00001$, $I2=0$), unsolicited (RR 1.09; 95% CI [0.97, 1.22], $p = 0.14$, $I2=60\%$) and serious adverse events (RR 1.14; 95% CI [1.11, 1.17], $p < 0.00001$, $I2=0$).

Conclusion: Although Butantan-DV is a potential single dose option for preventing dengue, however, establishment of long-term safety and efficacy against all four serotypes requires future trials with longer follow ups.

Keywords - Dengue Vaccine; Dengue Virus; Butantan-DV; Tetravalent Vaccine

I. INTRODUCTION

Dengue is not only an emerging viral disease caused by dengue virus DENV, but also represents a global health burden. This virus is predominantly transmitted by Aedes aegypti mosquitoes. Dengue virus is a single-stranded RNA virus from the genus Flavivirus and has four antigenically distinct serotypes (DENV1- DENV4). All of these serotypes co-circulate in endemic regions [1,2]. Approximately half of world's population resides in areas at risk for transmission of dengue [1]. According to cartographic model, it was estimated that 100 to 400 million dengue infections occurring per year across more than 100 endemic countries annually [3,4]. The clinical spectrum of dengue ranges from a mild, self-limiting illness to severe forms including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). A unique and critical characteristic of dengue immunopathology is phenomenon of antibody dependent enhancement (ADE), where non-neutralizing antibodies from primary infection can exacerbate heterotypic infections, thereby increasing the risk of the severe disease [5,6]. In absence of specific antiviral treatment, vaccination constitutes the cornerstone of dengue prevention strategies [4]. Currently, two live-attenuated vaccines are licensed worldwide. First one, Dengvaxia (CYD-TVD), a chimeric vaccine made on yellow fever virus backbone. While efficacious in seropositive individuals, it's use is constrained by an elevated risk of severe dengue in seronegative individuals, enforcing restrictive recommendations from WHO [7,8]. More recently, TAK003 (Qdenga), a vaccine utilizing a DENV2 backbone has received regulatory approval in many countries for use irrespective of baseline serostatus. Although this vaccine offers robust protection against DENV-1 and -2, extended follow up data have revealed variable efficacy against DENV-3 and -4, especially in seronegative individuals. This led to cautious recommendations from WHO's Strategic Advisory Group on Immunization [9,10,11].

Butantan dengue vaccine (Butantan-DV) represents a single-dose, live-attenuated, tetravalent vaccine candidate analogous to the NIH-developed TV003 formulation [12]. The vaccine composition includes

three full length DENV genomes (rDENV1Δ30, rDENV3Δ30/31 and rDENV4Δ30) representing serotypes 1,3 and 4, alongside a chimeric virus (rDENV2/4Δ30[ME]) containing the prM and E genes of DENV-2 on DENV-4 genetic backbone for serotype 2 [12,13]. This formulation is made to elicit a balanced and humoral and cellular immune response throughout all four serotypes while mitigating risk of ADE [13]. Single dose of Butantan-DV offers a significant advantage over multi dose schedules required for currently licensed vaccines, facilitating the vaccination coverage in endemic regions.

In 2016, they initiated a phase 3 trial of single dose Butantan-DV in the participants of aged 5 to 29 years in Brazil met its primary efficacy endpoint, demonstrating an overall efficacy of 79.6% against virologically Confirmed dengue (VCD) during first two years follow up [14]. Extended follow up data through minimum of two years and up to five years have further characterized the efficacy and safety profile of vaccine. This meta-analysis aims to synthesize evidence from randomized controlled trials to provide comprehensive evaluation of overall and serotype specific efficacy and also the safety profile of Butantan-DV.

II. MATERIALS AND METHODOLOGY

This meta-analysis was carried out in accordance with PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020 guidelines in order to analyze the efficacy and safety of novel Butantan-DV, and the review protocol was registered on PROSPERO (CRD420261451310) [15].

Search Strategy

We mainly searched four data bases including PubMed, Cochrane Library, and ClinicalTrials.gov and Science Direct. We used a combination of following keywords to develop a search strategy for each data base using (AND and OR) Boolean operators: Dengue Virus, Dengue Vaccine, Dengue, Live Attenuated Dengue Vaccine and Butantan Dengue Vaccine. Our search strategy for each data base is given below

- PubMed: (((((Dengue Virus) OR (Dengue)) AND (Dengue Vaccine)) OR (Live attenuated dengue vaccine)) AND (Butantan)) OR (butantan dengue vaccine)
- Cochrane: ("dengue virus"):ti,ab,kw OR ("dengue fever"):ti,ab,kw AND (Live attenuated vaccine):ti,ab,kw OR (dengue vaccine):ti,ab,kw AND (Butantan Dengue Vaccine):ti,ab,kw
- Science Direct: (((((Dengue Virus) OR (Dengue)) AND (Dengue Vaccine)) OR (Live attenuated dengue vaccine)) AND (Butantan)) OR (butantan dengue vaccine)
- Clinicaltrials.gov: (Condition/Disease: Dengue OR Dengue Virus; Intervention/Treatment: Live Attenuated Vaccine OR Butantan Dengue Vaccine)

The articles were searched from inception to July 2026 without any language or article type restriction. Additionally, we searched reference lists of the included articles in order to retrieve any relevant article.

Selection Criteria and Outcomes

A specified eligibility criteria was used to screen the articles. Studies were included if they involved healthy participants aged between 2 to 60 years or participants with previously controlled disease. Only randomized controlled trials published in English were included. The intervention had to be Butantan Dengue vaccine compared to placebo. Studies were included if they reported on one of the safety or efficacy outcomes.

We excluded articles if they were not published even if the RCT had been completed. Studies having pregnant and lactating women, participants with immunocompromising or uncontrolled disease and history of allergy to vaccine or its components were excluded. Additionally, studies were excluded if the live attenuated tetravalent Butantan dengue vaccine was not used or if the studies did not report on any of the efficacy and safety outcomes. Cohorts, case reports, cross sectional studies, reviews, editorials and conference abstracts were excluded.

Our efficacy outcomes included virologically confirmed dengue virus after vaccination either regardless of previous exposure, with previous exposure or based on any serotype. Similarly, our safety outcomes focused on solicited, unsolicited and serious adverse events. Studies had to report on at least one of these to be included in the final analysis.

Screening, Selection and Data Extraction

The retrieved articles were independently reviewed two reviewers by removing duplicates then removing the irrelevant articles based on the titles and abstracts. However, the article proceeded to screening against selection criteria, if a decision could not be made, in which we retrieved the full texts and applied the selection criteria for the remaining studies. Studies not fulfilling the eligibility criteria were removed and any dispute regarding selection of a study was dealt with the help of another reviewer.

The extracted data from the included studies included author name, trial number, study design and population, sample size, mean age, numbers of males and females, study duration, details of intervention, comparator, and outcomes and was pooled into excel sheet.

Quality Assessment and Data Synthesis

ROB2 developed by The Cochrane Library was used to assess the risk of bias in the included RCTs. The tool consists of five domains and an overall bias. High risk of bias was given to a study If it had high risk of bias in anyone of the five domains or more than one domain showed some concerns. Some concerns were given to a study if one domain had some concerns while remaining had low risk. Studies were awarded an overall low risk of bias if all the domains showed a low risk of bias [16].

RevMan 5.4. was used to analyze the available data for pre-defined outcomes. Risk ratios were used as a measure for dichotomous variables. The Mantel–Haenszel random-effects model was used in the conduct of this meta-analysis. A 95% confidence interval was used for the analysis. I2 was used as a measure of heterogeneity. I2 was considered significant and worth exploring if it was above 40% as per Cochranes Handbook. I2 below 25% was considered low, and mild for 26% to 50%. Moderate heterogeneity was considered for 51% to 75% and high for >75%. P values below 0.05 were considered significant. A subgroup analysis was also planned based on risk of various dengue virus serotypes and age. A leave one out sensitivity analysis was also conducted, if an outcome had more than 3 studies, to assess the robustness of our findings. Publication bias could not be assessed due to limited number of studies. Forest plots were used to represent the results for each outcome.

III. RESULTS

Our search on various databases yielded a total of 745 articles. The articles obtained from each database separately are mentioned in the PRISMA flowchart. 8 duplicate studies were identified using EndNote and were removed after reviewing them manually. We screened the titles and abstracts of 737 articles, and removed 698 articles for being either irrelevant to the scope of the study. From the remaining 39 articles, full text of 1 article could not be found and thus only 38 articles were screened against our selection criteria out of which 33 were removed and only 5 were part of the final study [14, 17, 18, 19, 20, 21]. The whole screening and selection process has been summarized below in Figure 1.

Characteristics of Studies

The analysis included five randomized controlled studies, four of which were carried out in Brazil and one in India. The studies' sample sizes ranged from 100 to 16,235 participants. The stated mean or median ages of the enrolled populations, which comprised both adults and children, ranged from 12 to 36.5 years. While one experiment had more male participants, the majority of trials had a higher percentage of female participants. The intervention groups ranged from 75 to 10,259 participants, while comparator groups ranged from 25 to 5,976 participants. Characteristics of the included studies are summarized below in Table 1.

Quality Assessment

The risk of bias assessed using ROB2 showed that 4 out of 5 studies had an overall some concern in the risk of bias while 1 study was low risk. All 4 studies with some concerns had some issues in the measurement of the outcome with Mohanty L et al., 2022 using their own sponsors laboratory for confirmation of dengue other analysis and other 3 studies although confirmed dengue virus virologically using objective tests but mainly relied on self-presentation of participants with complaints of fever or dengue signs or symptoms. The risk of bias among our studies is summarized below in Figure 2.

Efficacy End Point

The primary efficacy outcome was virologically confirmed dengue virus and 3 studies reported this outcome. Our analysis showed that dengue virus post-vaccination was significantly lower in the vaccine group compared to placebo (RR 0.29; 95% CI [0.24, 0.35], $p < 0.00001$), with moderate heterogeneity observed $I2 = 73\%$. For any serotype, the risk was significantly lower in the vaccine group compared to placebo (RR 0.30; 95% CI [0.23, 0.39], $p < 0.00001$) but with moderate heterogeneity I2= 69%. For participants with previous exposures (RR 0.38; 95% CI [0.31, 0.47], $p < 0.00001$) and without previous exposure (RR 0.21; 95% CI [0.15, 0.29], $p < 0.00001$), the risk was lower in the vaccine group compared to placebo with $I2 = 35\%$ and $I2 = 46\%$ respectively. The results are given below in the forest plot in Figure 3.

Safety End Point

In contrast to the efficacy end point, all five studies reported the safety of Butantan DV except for solicited adverse events. For serious adverse events, our analysis showed that the risk was slightly more in the vaccine group compared to placebo (RR 1.14; 95% CI [1.11, 1.17], $p < 0.00001$) and the results were significant with no heterogeneity. For unsolicited adverse events, the results showed a slightly increased risk with Butantan, but the results were not significant, and moderate statistical heterogeneity ($I2 = 60\%$) was observed (RR 1.09; 95% CI [0.97, 1.22], $p = 0.14$). Similarly, risk was significantly more in the participants receiving Butantan DV compared to placebo for solicited adverse events (RR 1.24; 95% CI [1.21, 1.28], $p < 0.00001$) with zero heterogeneity. The results are shown below in the forest plot in Figure 4.

Subgroup Analysis

We conducted a subgroup analysis for efficacy end point based on DENV1 and DENV2. Our subgroup

analysis showed risk of dengue virus was reduced in the vaccine group compared to placebo when analyzed regardless of serotype status (RR 0.21; 95% CI [0.14, 0.32], $p < 0.00001$), with previous exposure (RR 0.17; 95% CI [0.10, 0.31], $p < 0.00001$) and without previous exposure (RR 0.26; 95% CI [0.19, 0.34], $p < 0.00001$) with I2 being 67%, 38%, and 25% respectively.

Similarly, for DENV2, the results showed less risk in Butantan group compared to placebo for all groups i.e., regardless of serotype (RR 0.41; 95% CI [0.34, 0.48], $p < 0.00001$) $I2 = 0$, with previous exposure (RR 0.2; 95% CI [0.18, 0.32], $p < 0.00001$) $I2 = 0$, and without previous exposure (RR 0.56; 95% CI [0.45, 0.72], $p < 0.00001$) $I2 = 0$. However, the risk reduction was less in DENV2 than in DENV1, but no heterogeneity was observed in the DENV2 group, contrary to DENV1. The forest plots for subgroup analysis are given in supplementary figures.

Sensitivity Analysis

Sensitivity Analysis could only be conducted for unsolicited adverse events. The results of leave-one-out sensitivity analysis did not change the results except for the removal of the study by Nogueria LM et al., 2024. The removal of this study removed all the heterogeneity ($I2 = 0$) and the results became statistically significant (RR 1.14; 95% CI [1.05, 1.23], $p = 0.002$).

IV. DISCUSSION

This meta-analysis analyzed the efficacy and safety live attenuated single dose Butantan Dengue vaccine using 5 RCTs. The results were significantly in favor of Butantan vaccine in terms of risk reduction for virologically confirmed dengue virus post vaccination. Significant risk reductions were observed regardless of baseline serotype status, with both seropositive and seronegative individuals benefiting from vaccination. While participants with previous dengue exposure showed a greater risk reduction of VCD than dengue naive individuals, significant protection was observed in all groups. Although varying heterogeneity was observed in efficacy analysis, the overall findings favored the use of Butantan DV. Subgroup analysis showed risk reduction in the efficacy end point was less in DENV2 than in DENV1 but no heterogeneity was observed in DENV2 group contrary to DENV1. Contrary to the efficacy outcome, risk adverse events was slightly more with Butantan compared to placebo.

The favorable efficacy of Butantan regardless of baseline serostatus in our results could be due to the immunological characteristics of Butantan-DV. As a live attenuated tetravalent vaccine, Butantan-DV contains attenuated genomes for DENV-1, DENV-3, and DENV-4, plus a DENV-2 chimera designed to induce broad immune responses against dengue virus serotypes through controlled viral replication and stimulation of both humoral and cellular immunity [14, 21]. The higher efficacy observed among participants with previous dengue exposure may be explained by the fact that their immune system already has memory B and T cells primed from the earlier natural infection, so the vaccine triggers a more rapid and effective response across serotypes consistent with the study by Sridhar S et al., 2018 which showed that baseline dengue serostatus is an important determinant of vaccine performance, with several dengue vaccines showing stronger protection among individuals with prior infection [7].

The significant efficacy of Butantan DV against dengue virus in dengue naïve individuals in our meta-analysis warrants particular attention since the principal reason for limited use of CYD-TDV was reduced protection and an increased risk of severe dengue among dengue-naïve individuals [22]. The reason could be that Butantan DV retains nearly full length dengue genomes for three of its four components, exposing recipients to a considerably more comprehensive dengue antigenic profile than CYD-TDV, which uses a yellow fever backbone with only the dengue surface genes put in [14 ,23]. The results of our meta-analysis showed 71% risk reduction for the efficacy end point with Butantan DV which is more than the 44% efficacy reported in a meta-analysis of studies on CYD-TDV. Similar patterns were observed with TAK-003, another live attenuated tetravalent dengue vaccine, in which protection was observed regardless of serotype status but was generally favorable among individuals with previous dengue exposure. However, Butantan DV could be more favorable compared to TAK-003 as Butantan is a single dose vaccine and showed efficacy across a wide age group [24]. Our subgroup analysis further showed protection against DENV-1 appeared somewhat stronger than against DENV-2, consistent with the phase III Butantan trial, however, DENV-1 results were associated with greater heterogeneity, whereas no heterogeneity was observed for DENV-2, suggesting that although protection against DENV-1 may be stronger suggesting variability across trials either due to age distribution, duration of follow up, immune status, and varying viral genotypes [14].

Regarding safety, our results showed that risk of solicited adverse events was slightly more in the Butantan-DV participants compared to participants receiving placebo, while unsolicited adverse events were not statistically significant in the initial analysis. These findings are consistent with the phase III Butantan trial [14]. The occurrence of solicited reactions might be due to the mechanism of live attenuated vaccines, where transient viral replication

stimulates innate and adaptive immune activation. Similar safety patterns were observed with TAK-003 and CYD-TDV [22, 24]. Sensitivity analysis for unsolicited adverse events did not change the direction of effect but removed heterogeneity, and results became significant and could be explained by variation between studies instead of any inconsistency in the vaccine's original effect. Although serious adverse events were significantly more in the Butantan-DV group, the overall number of adverse events was generally low and could suggest an acceptable safety profile for Butantan-DV.

V. IMPLICATIONS

The results of this meta-analysis highlight the potential role of Butantan-DV in dengue prevention, particularly in endemic regions and in regions where screening prior to vaccination is not possible. is challenging. Compared to previous dengue vaccines, Butantan's effectiveness for both seropositive and seronegative individuals may lead to wider vaccine implementation. Furthermore, Butantan is a one dose vaccine which may ease logistic and economic considerations, simplify vaccine scheduling, and increase uptake. Minimal DENV-3 and DENV-4 circulated throughout the included studies, hence we couldn't evaluate Butantan's efficacy against these serotypes. This gap highlights the need for future studies to include all 4 serotypes in future trials.

VI. LIMITATIONS

Although this meta-analysis produced beneficial results, there were a few limitations. 1) Majority of the included studies were centered around one region, thus limiting generalizability globally. 2) Although individual studies showed better results across different age groups, subgroup analysis based on various age grouping could not be conducted due to limited data available to be used. 3) This meta-analysis, does not report on DENV3 AND DENV4 serotypes as the trials did not extensively report on those, thus future trials should focus on all serotypes. 4) Publication bias could not be conducted due to limited number of studies. 5) Heterogeneity was observed across all primary efficacy outcomes and sensitivity analysis for the efficacy outcome could not be conducted due to limited number of studies. 6) We did extensive search to include all the relevant literature available, and did not leave any relevant trial. However, some trials might have been missed due to human error.

VII. CONCLUSION

This review highlights the significant effect of Butantan-DV in reducing the risk of virologically confirmed dengue virus across seropositive and seronegative individuals as well as dengue naïve and dengue exposed individuals. The efficacy of Butantan-DV extended across DENV-1 and DENV-2. This live attenuated tetravalent vaccine exhibited an acceptable safety profile with no evidence of significant harm in dengue naïve individuals. Butantan-DV with its single dose regimen, effectiveness and safety could contribute substantially to reducing the global burden of dengue disease. However, due to the limited number of trials, heterogeneity, and limited data on DENV-3 AND DENV-4, further large-scale trials with longer follow-up durations are needed before definitive conclusions can be made regarding its efficacy and safety.

REFERENCES

[1] World Health Organization. Dengue and severe dengue. 2024. Available from: https://www.who.int/news-room/questions-and-answers/item/dengue-and-severe-dengue. Accessed 2 Oct 2024.

[2] Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6(8):e1760. doi:10.1371/journal.pntd.0001760.

[3] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504-7. doi:10.1038/nature12060.

[4] Wilder-Smith A, Ooi EE, Horstick O, Wills B. Dengue. Lancet. 2019;393(10169):350-63. doi:10.1016/S0140-6736(18)32560-1.

[5] Halstead SB. Dengue virus-mosquito interactions. Annu Rev Entomol. 2008;53:273-91. doi:10.1146/annurev.ento.53.103106.093326.

[6] Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, Gordon A, et al. Antibody-dependent enhancement of severe dengue disease in humans. Science. 2017;358(6365):929-32. doi:10.1126/science.aan6836.

[7] Sridhar S, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T, et al. Effect of dengue serostatus on dengue vaccine safety and efficacy. N Engl J Med. 2018;379(4):327-40. doi:10.1056/NEJMoa1800820.

[8] Dengue vaccine: WHO position paper, September 2018—Recommendations. Vaccine. 2019;37(35):4848-9. doi:10.1016/j.vaccine.2018.09.063.

[9] Tricou V, Sáez-Llorens X, Yu D, Rivera L, Jimeno J, Villarreal AC, et al. Safety and immunogenicity of a tetravalent dengue vaccine in children aged 2–17 years: a randomised, placebo-controlled, phase 2 trial. Lancet. 2020;395(10234):1434-43. doi:10.1016/S0140-6736(20)30556-0.

[10] Rivera L, Biswal S, Sáez-Llorens X, Reynales H, López-Medina E, Borja-Tabora C, et al. Three-year efficacy and safety of Takeda's dengue vaccine candidate (TAK-003). Clin Infect Dis. 2022;75(1):107-17. doi:10.1093/cid/ciab864.

[11] World Health Organization. Meeting of the Strategic Advisory Group of Experts on Immunization, September 2023: conclusions and recommendations. Wkly Epidemiol Rec. 2023;98(47):599-620. Available from: https://www.who.int/publications/i/item/WER-9847-599-620.

[12] Durbin AP, Kirkpatrick BD, Pierce KK, Elwood D, Larsson CJ, Lindow JC, et al. A single dose of any of four different live attenuated tetravalent dengue vaccines is safe and immunogenic in flavivirus-naive adults: a randomized, double-blind clinical trial. J Infect Dis. 2013;207(6):957-65. doi:10.1093/infdis/jis936.

[13] Kallás EG, Precioso AR, Palacios R, Thomé B, Braga PE, Vanni T, et al. Safety and immunogenicity of the tetravalent, live-attenuated dengue vaccine Butantan-DV in adults in

Brazil: a two-step, double-blind, randomised placebo-controlled phase 2 trial. Lancet Infect Dis. 2020;20(7):839-50. doi:10.1016/S1473-3099(20)30023-2.

[14] Kallás EG, Cintra MAT, Moreira JA, Patiño EG, Braga PE, Tenório JCV, et al. Live, attenuated, tetravalent Butantan-dengue vaccine in children and adults. N Engl J Med. 2024;390(5):397-408. doi:10.1056/NEJMoa2301790.

[15] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi:10.1136/bmj.n71.

[16] Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898. doi:10.1136/bmj.l4898.

[17] Kallás EG, Moreira JA, Patiño EG, Braga PE, Tenório JCV, Alves LBO, et al. Long-term efficacy and safety of the single-dose tetravalent Butantan dengue vaccine. Nat Med. 2026;32(4):1393-400. doi:10.1038/s41591-026-04255-3.

[18] Nogueira ML, Cintra MAT, Moreira JA, Patiño EG, Braga PE, Tenório JCV, et al. Efficacy and safety of Butantan-DV in participants aged 2-59 years through an extended follow-up: results from a double-blind, randomised, placebo-controlled, phase 3, multicentre trial in Brazil. Lancet Infect Dis. 2024;24(11):1234-44. doi:10.1016/S1473-3099(24)00376-1.

[19] Mohanty L, Prabhu M, Kumar Mishra A, Purty AJ, Kanungo R, Ghosh G, et al. Safety and immunogenicity of a single dose, live-attenuated tetravalent dengue vaccine in healthy Indian adults: a randomized, double-blind, placebo-controlled phase I/II trial. Vaccine X. 2022;10:100142. doi:10.1016/j.jvacx.2022.100142.

[20] Peixoto de Miranda EJF, de Sousa Moreira JA, da Silva Braga R, Silveira DHR, Infante V, de Oliveira Alves LB, et al. Randomized, double-blind, placebo-controlled, phase 3 trial to demonstrate lot-to-lot consistency of three lots of the simplified formulation of Butantan-dengue vaccine. Vaccine. 2025;66:127836. doi:10.1016/j.vaccine.2025.127836.

[21] Kirkpatrick BD, Durbin AP, Pierce KK, Carmolli MP, Tibery CM, Grier PL, et al. Robust and balanced immune responses to all four dengue virus serotypes following administration of a single dose of a live attenuated tetravalent dengue vaccine to healthy, flavivirus-naive adults. J Infect Dis. 2015;212(5):702-10. doi:10.1093/infdis/jiv082.

[22] Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med. 2015;373(13):1195-206. doi:10.1056/NEJMoa1506223.

[23] Blaney JE Jr, Durbin AP, Murphy BR, Whitehead SS. Development of a live attenuated dengue virus vaccine using reverse genetics. Viral Immunol. 2006;19(1):10-32. doi:10.1089/vim.2006.19.10.

[24] Biswal S, Borja-Tabora C, Martinez Vargas L, Velásquez H, Theresa Alera M, Sierra V, et al. Efficacy of a tetravalent dengue vaccine in healthy children aged 4-16 years: a randomised, placebo-controlled, phase 3 trial. Lancet. 2020;395(10234):1423-33. doi:10.1016/S0140-6736(20)30414-1.

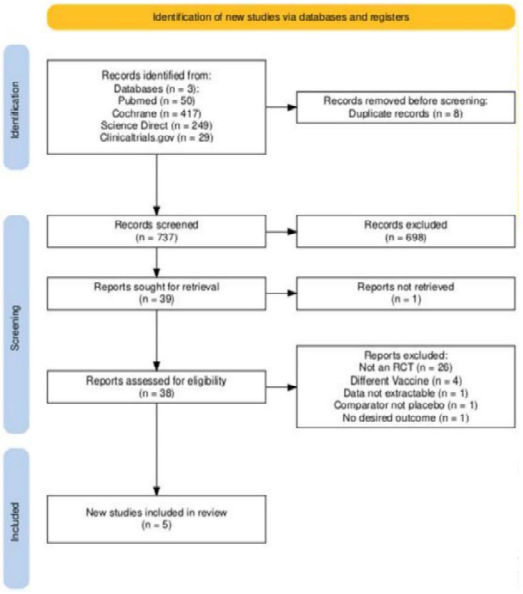


Figure 1. PRISMA flowchart for screening and selection

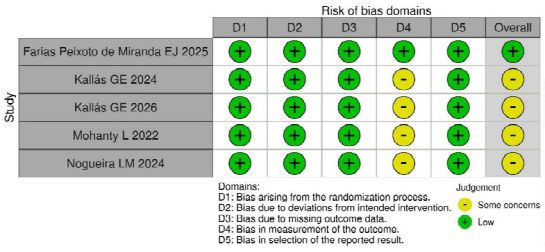


Figure 2. Summary of risk of bias

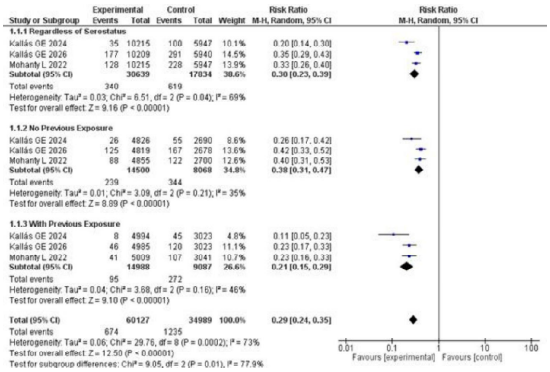


Figure 3. Forest plot presenting the efficacy outcomes

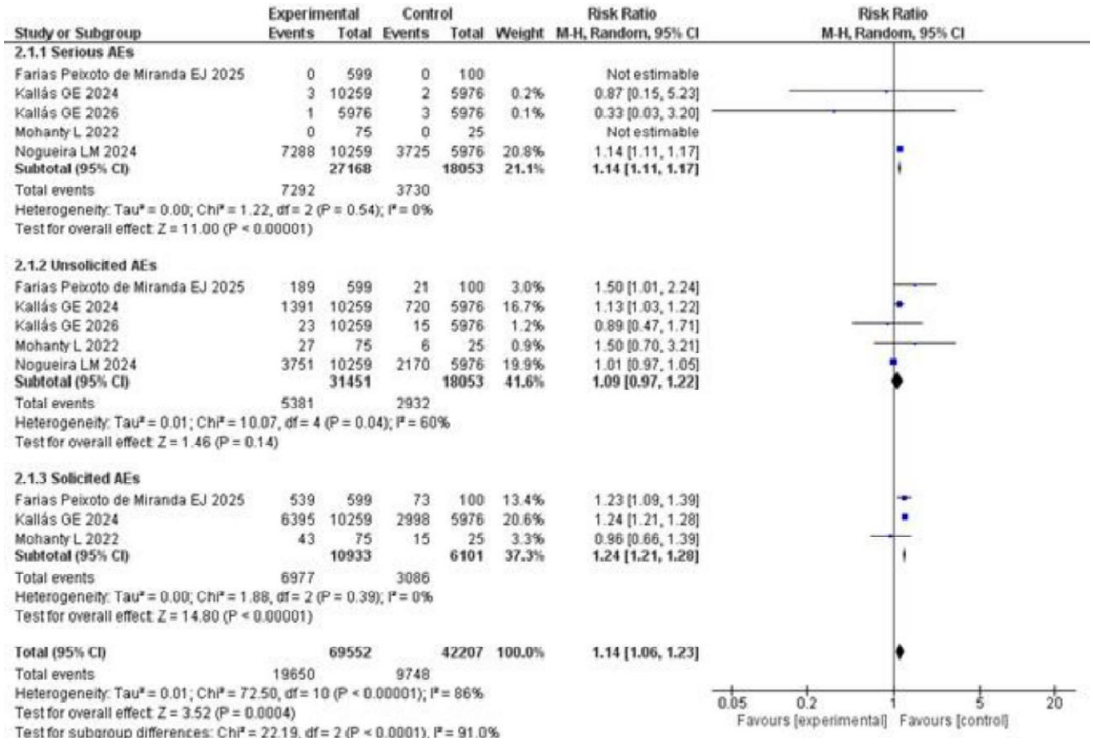


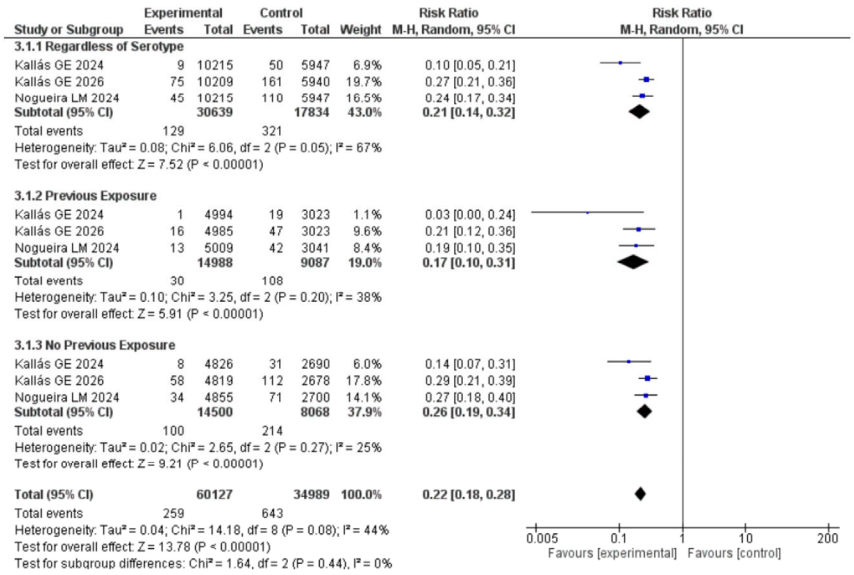
Figure 4. Forest plot representing the effect of Butantan-DV on safety endpoints

Study ID	Location	Sample size	Age	Male: Female	Experimental (n)	Comparator (n)	Outcome
Farias Peixoto de Miranda EJ., et al 2025	Brazil	700 randomized; 699 safety population; 607 per-protocol	Mean 36.5 ± 11.4 years (range 18.2–59.9)	222: 477	Butantan-DV (599)	Placebo: 100	GMT lot-to-lot consistency (VRNT60) by serotype, seroconversion, solicited/unsolicited AEs
Nogueira LM et al., 2024	Brazil	16,235 randomized; 16,162 per-protocol	Median 12 (IQR 6–33) years	7,464 (46.0%): 8,771 (54.0%)	Butantan-DV: 10,259 (10,215 per-protocol)	Placebo: 5,976 (5,947 per-protocol)	VE against VCD at 3.7 years; safety through extended follow-up
Kallás GE et al., 2026	Brazil	16,235 randomized; 16,149 per-protocol	Median 12 (IQR 6–33) years	7,468: 8,767	Butantan-DV: 10,259 (10,209 per-protocol)	Placebo: 5,976 (5,940 per-protocol)	VE against VCD at 5 years; VE against severe dengue/warning signs; AEs/SAEs
Mohanty L et al., 2022	India	100 randomized; 92 completed all visits	Mean 33.41 ± 9.36 years (range 18–57)	61: 39	TDV: 75	Placebo: 25	Seroconversion/seropositivity (PRNT50), solicited/unsolicited AEs; SAEs; viremia
Kallás GE et al., 2024	Brazil	16,235 randomized; 16,162 per-protocol	Median 12 (IQR 6–33) years	7,464: 8,771	Butantan-DV: 10,259 (10,215 per-protocol)	Placebo: 5,976 (5,947 per-protocol)	VE against VCD at 2 years; solicited/unsolicited AEs

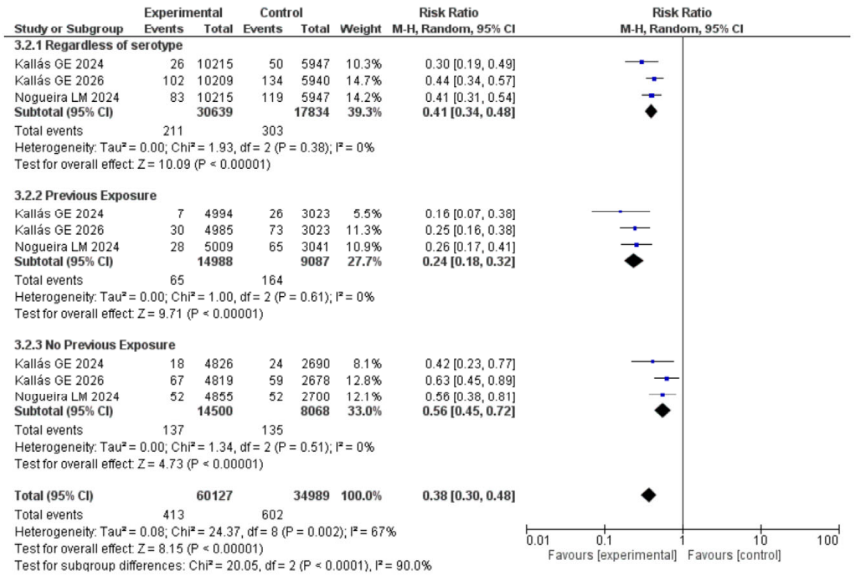
Table 1. Characteristics of studies

Supplementary Figures

Supplementary Figure 1



Supplementary Figure 2



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