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VICTORIA CRUZ PARANÁ

RISK FACTORS ASSOCIATED WITH SEVERE DENGUE IN LATIN AMERICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

SALVADOR – BA

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Trabalho de Conclusão de Curso apresentado à Escola Bahiana de Medicina e Saúde Pública, como parte dos requisitos para obtenção do título de Bacharel em Biomedicina.

Orientador: Prof. Dr. Luciane Amorim Santos

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Este Trabalho de Conclusão de Curso foi julgado adequado à obtenção do grau de Bacharel em Biomedicina e aprovada em sua forma final pelo Curso de Biomedicina da Escola Bahiana de Medicina e Saúde Pública.

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Prof. Dra. Luciane Amorim Santos
Escola Bahiana de Medicina e Saúde Pública

Prof. Dra. Luana Leandro Góis
Escola Bahiana de Medicina e Saúde Pública

MSc. Rafael Teixeira Tibúrcio dos Santos
Instituto Gonçalo Moniz - LIB

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RESUMO

O vírus da dengue pode produzir um espectro de manifestações clínicas que vão desde uma infecção assintomática até uma doença grave. Na América Latina, a dengue grave é uma importante causa de morbimortalidade, especialmente entre crianças. Esse estudo tem como objetivo revisar sistematicamente a literatura para identificar, por meta-análise, fatores de risco associados à dengue grave na América Latina. As bases de dados PubMed, SciELO, LILACS e EMBASE foram utilizadas para a busca dos artigos científicos elegíveis para o estudo. Foram considerados como desfechos: sintomas de dengue grave, hospitalização e óbitos por dengue. Os dados foram analisados usando o software STATA v 13.0. Para meta-análise os gráficos foram apresentados através de *forest plots*. O grau de heterogeneidade entre os estudos foi quantificado pela medida I^2, e os resultados com valores de $p < 0,05$ foram considerados estatisticamente significantes. Após a aplicação dos critérios de inclusão e exclusão, 43 artigos foram incluídos na revisão sistemática e 41 foram analisados por meta-análise. Identificou-se como fatores de risco associados à dengue grave a infecção secundária por dengue, gênero feminino, raça branca ou caucasiana, cefaleia, mialgia e/ou artralgia, vômitos/náuseas, dor ou sensibilidade abdominal, diarreia, prostração, letargia, fadiga ou similares. Para o desfecho óbito, vômitos/náuseas e idade < 18 anos foram identificados como fatores de risco, enquanto o gênero feminino, teste do torniquete +, plaquetas < 100.000 por μL e erupção cutânea, petéquias, exantema, hematomas e/ou equimoses indicaram menores chances de morrer por dengue. Esse resultado ajudará a definir estratégias, manejo e grupos de risco para a doença, evitando complicações com risco de morte. Este estudo foi capaz de demonstrar a importância do diagnóstico precoce e interpretação correta de alguns exames e sinais e sintomas, devido as menores chances de irem a óbito pessoas com teste do torniquete +, plaquetas < 100.000 por μL e sintomas de fragilidade capilar. Estudos futuros são necessários para garantir a confirmação de alguns fatores de risco e padronização para futuras diretrizes, levando assim a um melhor manejo dos pacientes em risco.

Palavras-chave: Dengue, Fatores de risco, América Latina, Revisão sistemática ou meta-análise

SUMÁRIO

1. Artigo Científico

Risk factors associated with severe dengue in Latin America: A systematic review and meta-analysis

Victoria Cruz Paraná[1]; Greice Carolina Santos da Silva[2]; Caroline Alves Feitosa[1]; Luciane Amorim Santos[1,2]

[1] Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brasil

[2] Instituto Gonçalo Muniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brasil

Corresponding author:

Luciane Amorim Santos

lucianeamorim@bahiana.edu.br

Street Silveira Martins, 100 - Cabula, Salvador - BA, 41150-100

ABSTRACT

Background:

DENV can produce a wide spectrum of clinical manifestations ranging from asymptomatic infection to a serious illness. In Latin America, severe dengue is one of the leading causes of serious illness and death, especially among children. Thus, the study aims to systematically review the literature to identify, through meta-analysis, risk factors related to severe dengue in Latin America.

Methodology/Principal Findings:

PubMed, SciELO, LILACS and EMBASE databases were used to search for scientific articles eligible for the study. Symptoms of severe dengue, hospitalization and deaths were considered as an outcome. Data were analyzed using STATA v 13.0 software. For the meta-analysis, the graphs were presented through forest plots. The degree of heterogeneity between studies was quantified by the I^2 measure, and results with p values < 0.05 were considered statistically significant.

After applying eligibility criteria, 43 articles were included in the systematic review and 41 were analyzed through meta-analysis. The main risk factors associated with severe dengue were secondary dengue infection, female gender, white or Caucasian ethnicity, headache, myalgia and/or arthralgia, vomiting/nausea, abdominal pain or tenderness, diarrhea, prostration, lethargy, fatigue or similar. For the death outcome, vomiting/nausea and < 18 years old were identified as risk factors, while females, tourniquet test +, platlet count <100,000 per µL and rash, petechiae, exanthema, hematomas and/or ecchymoses had lower chances of dying from dengue.

Conclusions/Significance:

The results will help to define strategies, management, and risk groups for the disease, because will help in the formulation of future guidelines. This study was able to demonstrate the importance of early diagnosis and correct interpretation of some

tests and signs and symptoms. Future studies are needed to ensure confirmation of certain risk factors and standardization for future guidelines, thus leading to better management of patients at risk**.**

Key words: Dengue, Risk factors, Latin America, Systematic review or meta-analysis

International Prospective Register of Systematic Reviews (PROSPERO) registration number: CRD42021283608.

AUTHOR SUMMARY

The dengue virus can cause asymptomatic infections to serious illness. In Latin America it is one of the leading causes of serious illness and death, especially among children. In this study, we systematically reviewed the literature to identify, through meta-analysis, the risk factors associated with severe dengue in Latin America. We found that secondary dengue infection, female gender, white or Caucasian ethnicity, headache, myalgia and/or arthralgia, vomiting/nausea, abdominal pain or tenderness, diarrhea, prostration, lethargy, fatigue or similar as risk factors for severe dengue. In addition, vomiting/nausea and < 18 years old were identified as risk factors for death due to dengue, while females, tourniquet test +, platlet count < 100,000 per µL and rash, petechiae, exanthema, hematomas and/or ecchymoses had lower chances of dying from dengue. In this way, this result will help to define strategies, management, and risk groups for the disease, avoiding complications with life-threatening.

INTRODUCTION

Dengue virus (DENV) is a single-stranded RNA virus belonging to the Flaviviridae family and the Flavivirus genus [1, 2]. This virus is transmitted by the bite of the female mosquito of the genus *Aedes spp.,* mainly by *Ae. aegypti* and less frequently by *Ae. Albopictus* [3]. There are four known serotypes of the virus (DENV 1, DENV 2, DENV 3 and DENV 4), and each one is antigenically different. All four of these serotypes circulate in Asia, Africa, and the Americas [4, 5].

Dengue appears as explosive outbreaks, affecting urban centers, as urban environments favor the dispersion and increase in the density of *Ae. Aegypti* [6, 7]. The probability of dengue occurrence was associated with areas of tropical and subtropical zones, commonly found in Latin American regions [8, 9]. Nearly half of the world's population, around 4 billion people, live in areas at risk of infection [10].

Each year, up to 400 million people get infected with dengue and approximately 100 million people get sick from infection [11]. Globally, an average of nine thousand dengue deaths occurs annually [12]. Specifically, in the Americas, the number of dengue cases has increased in the last four decades, from 1.5 million cases in the 1980s to 16.2 million in the 2010-2019 decade [9]. Previous study has shown that the combined incidence of dengue in Latin America was 72.1 cases per 100,000 population from 1995 to 2010 [13].

A major challenge of DENV surveillance and diagnosis is that the virus can produce asymptomatic infections and a spectrum of clinical illnesses that range from mild febrile illness to fatal hemorrhagic illness [14]. Symptoms appear after an incubation period of 4 to 10 days from the bite of an infected mosquito and usually last for 2 to 7 days [3]. The World Health Organization (WHO) classifies dengue into two broad categories: dengue (with/without warning signs) and severe dengue [3]. In dengue, the first manifestation is fever, usually high (39ºC to 40ºC), associated with

103 headache, adynamia, myalgias, arthralgias and/or retroorbital pain [15]. In severe
104 dengue, severe plasma leakage leads to shock and/or fluid accumulation with
105 respiratory distress, severe hemorrhage evaluated by the clinician, or severe organ
106 involvement are observed [16]. Severe forms of the disease can also be known as
107 dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [17].

108 Circulation of several DENV serotypes at the same site can cause co-infections
109 with different serotypes in patients in subsequent or simultaneous infections and this
110 is associated with an increased risk of severe dengue [18, 19]. In addition, it has been
111 reported that the white race, people of younger age, especially children, and some
112 chronic diseases, such as diabetes, kidney disease, and hypertension, may be
113 possible risk factors for the severity of the disease [18, 20-24]. The presence of warning
114 signs (ie, increased hematocrit with a concomitant decrease in platelet count,
115 abdominal pain, lethargy, vomiting, hepatomegaly, ascites, pleural effusion, and
116 melena) can also be considered predictors of the development of the disease severe
117 form [24].

118 Because dengue infection can progress to severe cases or lead to death, it is
119 necessary to identify risk factors associated with the severe forms of the disease, so
120 that the risk group for the disease can be identified as soon as possible, and care
121 actions and interventions can be taken to avoid complications such as the risk of death.
122 In addition, according to the Pan American Health Organization (PAHO) in some Latin
123 American countries, severe dengue is one of the main causes of serious illness and
124 death, especially among children [9]. It is important to study Latin America due to the
125 regions of tropical and subtropical climate, which contributes to the largest dispersion
126 of the mosquito vector [9]. Therefore, the aim of this study is to systematically review
127 the literature to identify, through meta-analysis, risk factors associated with severe
128 dengue in Latin America.

METHODS

This study is a systematic review and meta-analysis which was conducted following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [25]. The study followed the Population Intervention Comparison Outcome Study Design (PICOS) strategy to construct the research question “What are the risk factors associated with severe dengue in Latin America?”. This review protocol was registered in PROSPERO with the number: CRD42021283608.

The National Library of Medicine (PubMed), Scientific Electronic Library Online (SciELO), Latin American and Caribbean Literature in Health Sciences (LILACS), and Excerpta Medica Database (EMBASE) databases were used to search for scientific articles eligible for the study. Descriptors were selected using the Medical Subject Headings (MeSH) tool combined with Boolean connectors to form the search algorithm: ((DENGUE OR DENV*) AND (“RISK FACTORS” OR COMORBIDITY) AND (“LATIN AMERICA*” OR “SOUTH AMERICA*” OR “CENTRAL AMERICA*” OR CARIBBEAN* OR CHILE* OR COLOMBIA* OR ECUADOR* OR “FRENCH GUIAN*” OR GUYAN* OR PARAGUAY* OR PERU* OR SURINAME* OR URUGUAY* OR “TRINIDAD AND TOBAGO” OR “TRINIDADIAN AND TOBAGONIAN” OR TRINBAGONIAN OR TRINI OR VENEZUELA* OR BOLIVIA* OR BRAZIL* OR ARGENTIN* OR BARBAD* OR BAHAM* MEXIC* OR BELIZE* OR “COSTA RICA*” OR “EL SALVADOR” OR SALVADOR* OR GUANAC* OR GUATEMALA* OR CHAPÍN OR HONDURAS* OR NICARAGUA* OR PANAMA* OR CUBA* OR “DOMINICAN REPUBLIC” OR DOMINICAN OR QUISQUEYAN OR HAITI* OR “ANTIGUA AND BARBUDA” OR “ANTIGUAN BARBUDAN” OR JAMAICA* OR GRENAD* OR “SAINT

VINCENT AND THE GRENADINES" OR "SAINT VINCENTIAN" OR VINCENTIAN OR "SAINT LUCIA*" OR "SAINT KITTS AND NEVIS" OR KITTITIAN OR NEVISIAN OR DOMINICA* OR GUADELOUPE* OR MARTINIQUE OR MARTINI* OR "PUERTO RIC*" OR BURICUA OR SAINT-MARTIN OR ST. MARTIN* OR ST. MAARTENER OR SAINT-BARTHÉLEMY OR BARTHÉLEMOIS OR SAINT-BARTH)).

First, the inclusion criteria were applied for the selection of studies: (I) patients infected with dengue, (II) studies performed in humans, (III) studies that analyze risk factors and/or comorbidities associated with severe dengue, death and/or hospitalization due to dengue (IV) studies carried out in Latin American countries, (V) study that used Odds ratio (OR), Relative risk (RR), Hazard ratio (HR) or Prevalence ratio (PR) as measures of association. There were no restrictions related to the study language. Then, the following exclusion criteria were used: (I) literature reviews, (II) case reports, (III) editorial, (IV) research protocol, and (V) comments. The search for scientific articles was carried out on November 16, 2021. The selection of these articles was reviewed twice by two independent authors (PARANÁ, V.C and SILVA, G.C.S) and the final selection was reviewed by all authors.

WHO 2009 guideline was mainly followed for the definition of severe dengue, which includes patients with severe plasma leakage leading to shock and/or fluid accumulation with respiratory distress, severe hemorrhage, or severe organ involvement, but articles published before 2009 use the WHO 1997 guideline, which includes DHF and DSS as severe cases. [16, 17]. In addition, the Brazilian Ministry of Health created the intermediate classification of Complicated dengue due to the difficulty of classifying severe cases and the classification of classic dengue is unsatisfactory, the presence of one of the following findings characterizes this clinical condition: severe alterations of the nervous system; cardiorespiratory dysfunction; liver

failure; thrombocytopenia ≤ 20,000/mm^3; digestive bleeding; cavity spills; global leukometry ≤ 1,000/mm^3; suspected case of dengue with evolution to death, but without all the criteria for DHF [15]. This classification was included in severe cases because studies carried out in Brazil consider these cases as severe. In addition, outcomes such as severe manifestations, deaths, and hospitalization due to dengue were also considered severe cases.

The Joanna Briggs Institute Critical Appraisal Checklist for Analytical Cross-Sectional Studies, Case-Control Studies, Cohort Studies, Quasi-Experimental Studies (non-randomized experimental studies), and Randomized Controlled Trials was used to assess the quality of the studies [26]. Studies were classified as low, medium, and high risk of bias if 70% or more, 50-69%, and 50% or less of checklist responses were "yes", respectively.

The selected articles were read, and the data of interest were collected, such as the year of publication, country of study, number of the study population, number of deaths, frequency of cases, signs and symptoms presented, reported comorbidities, frequency of secondary dengue infection, gender, age, and risk factors reported. In addition, to perform the meta-analysis, the type of study, population number, analyzed outcome, risk factor related to the outcome, values of the association measures that the article used (OR, RR, HR or PR) and their confidence intervals (CI) were collected from each article. The information was organized in an Excel version 16.61.1 spreadsheet.

For the meta-analysis, the extracted data were pooled using STATA/MP version 13.0 software. The degree of heterogeneity between studies was quantified using the I^2 measure, values ≤ 25% were considered as low heterogeneity, 26-74% as medium heterogeneity and values ≥ 75% as high heterogeneity. The calculation of pooled

measures of association was performed, with their corresponding 95% CI to identify risk factors for severe dengue, death, and hospitalization due to dengue based on study variables, according to data availability, and the results were presented in forest plots. All results with p values < 0.05 were considered statistically significant. After testing all the variables, those forest plots that did not show statistical significance, that had less than three studies and that the value of I^2 > 75% were excluded. Relevant numbers, descriptive statistics and narrative synthesis were used for information where statistical clustering was not possible.

RESULTS

Selection of articles

From the search algorithm applied to the databases, we obtained 1646 articles, of which 186 were duplicates and in five articles the full text was not available. Of the 1455 articles that were screened for title and abstract, 1249 were not included. Thus, 206 articles were considered eligible and had the full text screened for exclusion criteria, of which 163 were excluded after the complete reading of the article. Finally, forty-three articles were included in the systematic review, and forty-one articles were used for the meta-analysis **(Fig 1)**. Two articles could not be used for meta-analysis due to interpretation of results and association measure used.

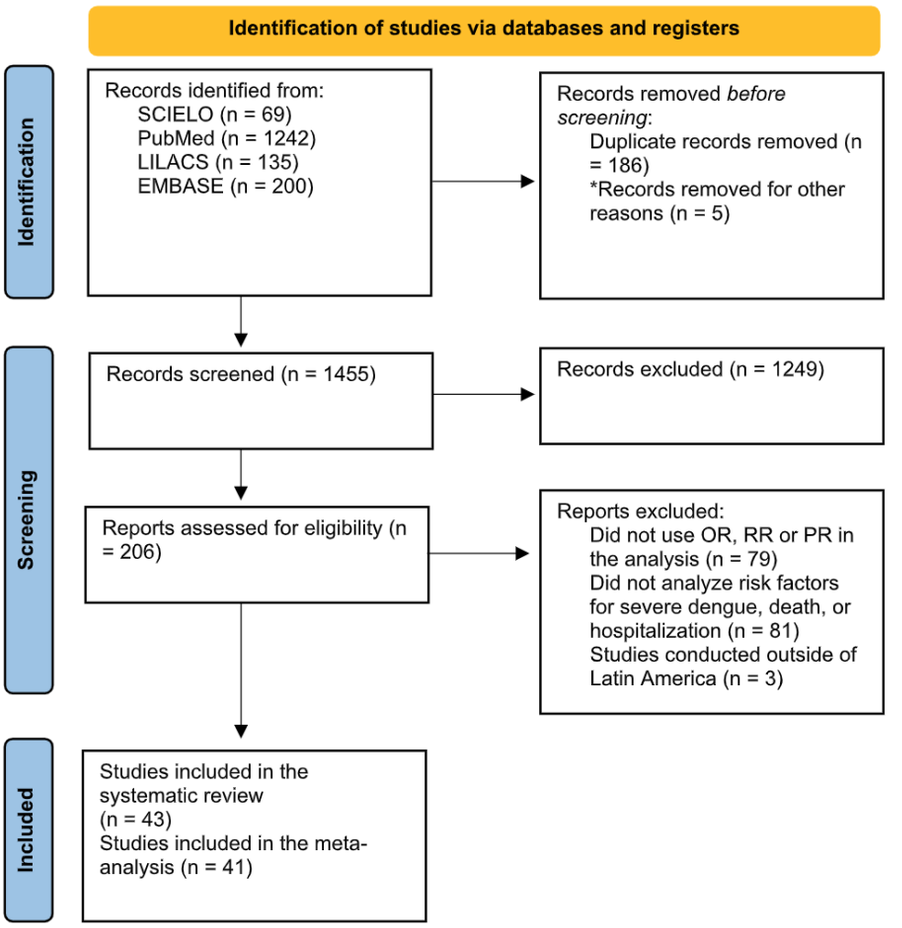


Fig 1. Flow-diagram of articles´ selection and screening for inclusion in the systematic review and meta-analyses. * Articles not found

Evaluation of study quality

Thirty-seven (86%; 37/43) of the selected studies had a low risk of bias, while four (9%; 4/43) and two (5%; 2/43) had a medium and high risk of bias, respectively **(Table 1)**.

Main characteristic of the included articles

Thirty-seven of the articles included were conducted in South American countries and two studies were carried out in more than one country [63, 66]. More than half of the studies are from Brazil (56%; 24/43), followed by studies from Paraguay (16%; 7/43) and Colombia (9%; 4/43) **(Table 1)**.

The study period of the included articles ranged from 1986 to 2020, while one article did not provide this information. However, it is possible to verify that most (86%; 37/43) of the studies were carried out after the 2000's **(Table 1)**.

Among the forty-three articles included, there was a variety of population types that each article studied. In addition, eleven articles studied only the pediatric population (< 18 years), and Lugo S et al. (2015) studied only babies up to 12 months. Elenga N et al. (2020) included only hospitalized children with sickle cell disease, being the only article to study a population in which all cases were already diagnosed with an underlying disease. Furthermore, the study by Machado CR et al. (2013) included only women of childbearing age with complete information about pregnancy, which is the only article to study this specific population **(Table 1)**.

When it comes to the population of all articles that was classified as severe dengue, the percentage ranged from 1% to 81%, with a mean of 36%. When adding up all the populations of the studies, it is possible to verify that severe dengue cases represented 2% of the total population (n = 99,063/6,476,330). Six articles did not report the population classified as severe dengue, and in these articles the outcome analyzed was death or hospitalization due to dengue **(Table 1).**

Among the twenty-seven articles that reported the number of deaths due to dengue in the population, the percentage ranged from 0.06% to 23.64%, with a mean of 5% of deaths. When adding the population number of each study that bring death data, it is observed that 0.09% of the total cases died (n = 6,051/6,476,330). Two studies stated that there were zero deaths in the population. **(Table 1)**.

Nineteen articles did not report gender data in the population classified as severe dengue. Of the studies that reported the male gender, the percentage ranged

from 31% to 63%, with a mean of 46%. When it comes to the female gender, the percentage ranged from 27% to 64%, with a mean of 55%, being higher than the male gender **(Table 1)**. Eleven articles provided information about the gender of patients who died, the mean number of deaths among the studies analyzed was 50% for males, ranging from 33% to 75%, while the mean for females was 52%, ranging from 32% to 67%. Only three articles reported the gender of the hospitalized population, the mean number of hospitalized males was 46%, ranging from 39% to 50%, while the female gender was 55%, ranging from 50% to 60%.

Thirty articles presented the age data, eight analyzed only children and/or adolescents, and severe dengue infection was more frequent in children over four years old. The other twenty-one articles showed that most of or the mean of the population with severe dengue was in the age group of 15 to 60 years, only four articles brought most of or the mean of the severe population being children from 1 to 15 years old, it was not found a study that brought most of the severe population being elderly (> 60 years). Macias AE et al. (2021) revealed that the population between 9 - 45 years old is the majority, while population ≥ 60 years is the minority of the cases. (**Table 1**). Twelve articles presented data about the age of patients who died, six articles presented most of the population or the mean being between 15 - 51 years and two studies presented most of the population or the mean age being >51 years. Four articles studied only children, with the mean or most of the patients being > 1 year old. Only three articles provided information about the age of hospitalized patients, two studies presented most participants aged between 15 - 60 years, and one study showed most of the hospitalized population being children aged between 5 and 10 years old.

Table 1. Main characteristic of the population of each study.

Author, year	Country	Study period	Type of study	Type of Population	Total population (n)	Severe dengue cases n (%)	Deaths n (%)	*Male gender n (%)	*Female gender n (%)	* Age group	Risk of bias
Casali CG et al. (2004) [27]	Brazil	2001 – 2002	Ecological	Notified cases	82.277	958 (1%) - DHF	60 (0.07%)	NR	NR	Mean: 32.8 years old	Low (71%)
Hammond SN et al. (2005) [28]	Nicaragua	1999 - 2011	Cohort	Patients presenting to hospitals	3.173	869 (27%) - Severe clinical manifestations	13 (0.41%)	NR	NR	0 – 11 months: 73 (8%) 1 – 14 years: 666 (77%) ≥ 15 years: 130 (15%)	Low (87.5%)
Navarrete-Espinosa J et a. (2005) [29]	Mexico	1995 – 2003	Case control	Cases diagnosed as DF or DHF	1.415	898 (63%) – DHF	79 (5.58%)	50 (6%)	50 (6%)	Mean: 26.9 years old	Low (78%)
Acioli-Santos B et al. (2008) [30]	Brazil	NR	Cohort	Patients with dengue symptoms	110	[a] 79 (72%) – DFT	NR	NR	NR	5 – 15 years: 6 (8%) 16 – 25 years: 15 (19%) 26 – 50 years: 45 (57%) 51 – 76 years: 13 (16%)	Low (78%)
González AL et al. (2008) [31]	Colombia	2006 - 2007	Cohort	Hospitalized patients	328	116 (35%) – DHF	1 (0.3%)	36 (31%)	46 (40%)	Mean: 20,06 years old	Low (100%)
Rubio DG et al. (2008) [32]	Cuba	2001 – 2002	Case control	Adult patients	228	94 (41%) – DHF/DSS	NR	51 (54%)	25 (27%)	< 20 years: 1 (1%) 20 – 59 years: 72 (77%) ≥ 60 years: 3 (3%)	Medium (60%)
Cavalcanti LPG et al. (2010) [33]	Brazil	2003	Ecological	DHF cases	291	291 (100%) – DHF	20 (6.87%)	130 (47%)	161 (55%)	Mean (range): 33 years old (2 – 88)	Low (86%)
Figueiredo MAA et al. (2010) [23]	Brazil	2002 – 2005	Case control	Cases and controls that tested positive for IgG	1.345	170 (13%) – DHF	0 (0%)	75 (44%)	95 (56%)	≤ 15 years: 35 (21%) ≥ 16 years: 135 (79%)	Low (90%)
Thomas L et al. (2010) [34]	Martinique	2005 – 2008	Cohort	Patients admitted to the adult emergency department	560	95 (17%) – DHF/DSS	7 (1.25%)	NR	NR	Median (range): DHF = 43(17-73); DSS = 42 (16-75); [C]DHF/DSS incompleto = 45 (16-83)	Low (100%)

Giraldo D et al. (2011) [35]	Brazil	2007 – 2008	Cohort	Children up to 15 years old admitted to the hospital	181	30 (17%) – Severe dengue	0 (0%)	16 (53%)	14 (47%)	Mean: 104 months	Medium (55%)
Suárez-Ognio L et al. (2011) [36]	Peru	2010 – 2011	Case control	Inpatients	226	73 (32%) – Severe dengue	NR	31 (42%)	42 (57%)	= 15 years: 38 (53%) >15 years: 34 (47%)	Low (89%)
Monteiro SP et al. (2012) [37]	Brazil	2002 – 2008	Case control	Outpatients and inpatients	109	42 (39%) – DHF	NR	19 (45%)	23 (55%)	Mean (range): 35.02 (12 – 66)	Low (100%)
Moraes GH et al. (2013) [38]	Brazil	2000 – 2005	Case control	Severe Dengue cases	12.321	12.321 (100%) – Severe dengue	1.062 (8.62%)	5.059 (41%)	7.262 (59%)	0 - 14 years =1.903 (15%) 15 - 49 years = 8.596 (70%) ≥50 yrs = 1.815 (15%)	Low (90%)
Machado CR et al. (2013) [39]	Brazil	2007 – 2008	Ecological	Women of childbearing age with information about pregnancy	546	129 (24%) – DHF/DSS	8 (1.47%)	0 (0%)	129 (100%)	Mean (range): 25.5 (15 – 49)	Low (75%)
Lora AJM et al. (2014) [40]	Dominican Republic	2008 - 2009	Cross-sectional	Pediatric population aged less than 1 year to 16 years old	796	207 (26%) – Severe dengue	41 (5.15%)	100 (48%)	107 (52%)	< 1 year = 38 (18%) ≥ 1 year = 169 (82%)	Low (75%)
Branco MRFC et al. (2014) [41]	Brazil	2006 – 2007	Case control	Age under 13 years and hospital admission	95	77 (81%) – Severe dengue	18 (18.95%)	34 (44%)	43 (56%)	Mean (range): 4.04 (0 – 12)	Low (90%)
Gibson G et al. (2014) [42]	Brazil	2008	Ecological	Confirmed cases of severe dengue fever	18.341	5.721 (31%) – Severe dengue	NR	NR	NR	≤5 years = 410 (7%) 6 – 15 years = 2632 (46%) 16 – 20 = 365 (6%) 21 – 60 = 1927 (34%) >60 years = 387 (7%)	Low (75%)
Campos KB et al. (2015) [43]	Brazil	2008 – 2010	Cohort	DHF, DSS and complicated dengue cases	2.214	2.214 (100%) – Severe dengue	156 (7.05%)	1070 (48%)	1144 (52%)	0 – 5 years = 141 (6%) 6 – 14 years = 366 (17%) 15 – 49 years = 979 (44%) 50 – 60 years = 428 (19%) >60 years = 300 (14%)	Medium (55%)
Teixeira MG et al. (2015) [44]	Brazil	2009 – 2012	Case control	Recruited in Hospitals	1.806	490 (27%) – DHF	NR	211 (43%)	279 (57%)	≥ 15 years = 316 (64%) ≤ 15 years = 174 (36%)	Low (100%)
Amâncio FF et al. (2015) [45]	Brazil	2008 – 2013	multicenter case series	adult (≥ 15 years) admitted to ICU	97	68 (70%) – Severe dengue	19 (19.59%)	NR	NR	NR	Low (80%)

Lugo S et al. (2015) [46]	Paraguay	2012	Case control	Pediatric hospital population up to 18 years old	217	57 (26%) – Severe dengue	NR	25 (44%)	32 (56%)	Mean: 133 months	Low (100%)
Lugo S et al. (2015) [47]	Paraguay	2013	Cohort	Hospitalized children from 1 week to 12 months of age	60	15 (25%) – Severe dengue	NR	6 (40%)	9 (60%)	≤ 6 months = 15 (100%)	Low (87.5%)
Negrete AFA et al. (2015) [48]	Paraguay	2012 – 2013	Case control	Adults over 18 years old who died from dengue	258	NR	61 (23.64%)	NR	NR	NR	Low (78%)
Lovera D et al. (2016) [49]	Paraguay	2011 – 2013	Case control	Children under 15 years old	471	354 (75%) – DSS	6 (1.27%)	183 (52%)	171 (48%)	< 24 months = 26 (7%) 2 - 5 years = 25 (7%) >5 years = 303 (86%)	Low (90%)
Burattini MN et al. (2016) [50]	Brazil	2000 – 2014	Ecological	Notified cases	5.444.285	NR	NR	NR	NR	NR	Low (71%)
Pinto RC et al. (2016) [51]	Brazil	2001 – 2013	Cohort	Severe dengue cases and dengue-related deaths	105.459	1.605 (2%) – Severe dengue	62 (0.06%)	NR	NR	NR	Low (100%)
Dias Júnior JJ et al. (2017) [52]	Brazil	2002 – 2011	Ecological	Notified cases	14.780	1.229 (8%) – Severe dengue	58 (0.39%)	570 (46%)	660 (54%)	<15 years = 812 (66%) ≥15 years = 417 (34%)	Low (71%)
Tukasan C et al. (2017) [53]	Brazil	1998 – 2012	Ecological	Confirmed cases of Dengue	14.756	368 (2%) – Severe dengue	NR	NR	NR	NR	Low (100%)
Teixeira LAS et al. (2017) [54]	Brazil	2012 – 2015	Cohort	Patients older than 14 years hospitalized and/or receiving outpatient care	113	368 (5%) – Severe dengue	NR	NR	NR	Mean: 55,3	Low (89%)
Cuellar CM et al. (2017) [55]	Paraguay	2010 – 2013	Ecological	Patients younger than 15 years	57.483	NR	35 (0.06%)	NR	NR	NR	Low (71%)
Ferreira RAX et al. (2018) [56]	Brazil	2008	Cross-sectional	Hospitalized patients under then 16 years old	419	296 (71%) – DHF	6 (1.43%)	NR	NR	< 5 years = 27 (9%) ≥5 years = 270 (91%)	Low (75%)
Nunes PCG et al. (2018) [57]	Brazil	1986 – 2015	Cross-sectional	Fatal cases of dengue	5.391	NR	1047 (19.42%)	NR	NR	NR	High (43%)

Silva NS et al. (2018) [58]	Brazil	2002 - 2012	Ecological	Dengue cases	7.613	NR	28 (0.37%)	NR	NR	NR	High (37.5%)
Delgado-Enciso I et al. (2018) [59]	Mexico	2007 – 2008	Cross-sectional	Patients with fever who consulted the Health Services	31	6 (19%) – Severe dengue	1 (3.23%)	NR	NR	NR	Low (71%)
Lovera D et al. (2019) [60]	Paraguay	2007 – 2018	Cross-sectional	Patients ≤ 15 years hospitalized	784	361 (46%) - DSS	5 (0.64%)	NR	NR	NR	Low (71%)
Luppe MJ et al. (2019) [61]	Brazil	1998 – 2006	Case control	Inpatients and outpatients	11.448	4.268 (37%) – Severe dengue	NR	1526 (36%)	2742 (64%)	>15 years = 4033 (94%) 0 – 15 years = 244 (6%)	Low (100%)
Kumar A et al. (2020) [62]	Barbados	2006 – 2015	Cohort	Confirmed cases of dengue	4.344	190 (4%) – Severe dengue	18 (0.41%)	102 (54%)	88 (46%)	NR	Low (89%)
Elenga N et al. (2020) [63]	Martinique, Guadeloupe and French Guiana	2005 – 2013	Cohort	Children hospitalized with sickle cell disease under then 15 years old	106	33 (31%) – Severe dengue	6 (5.66%)	15 (45%)	18 (55%)	Median (interquartile range): 10.5 (5; 15)	Low (78%)
Barry MR et al. (2020) [64]	Colombia	2015	Cross-sectional	Children up to 15 years and adults	2.446	NR	NR	NR	NR	NR	Medium (62.5%)
Hernández JPR et al. (2020) [65]	Colombia	2016	Case control	Patients younger than 18 years admitted to a pediatric ICU	200	[b] 24 (12%) – Dengue with warning signs or severe dengue	3 (1.50%)	15 (63%)	9 (38%)	NR	Low (80%)
Macias AE et al. (2021) [66]	Mexico, Brazil, Colombia	2008 – 2017	Ecological	Hospitalized cases	678.836	65.203 (10%) – Severe dengue	3.225 (0.48%)	NR	NR	0 – 8 years = 12318 (19%) 9 – 45 years = 42072 (65%) 46 – 60 yearas = 6763 (10%) ≥60 years = 4050 (6%)	Low (75%)
Solórzano VEF et al. (2021) [67]	Brazil	2013	Cohort	Adults with suspected dengue infection	225	[b] 63 (28%) - Dengue with warning signs or severe dengue	NR	25 (40%)	38 (60%)	Median (interquartile range): 36 (23 – 50)	Low (77%)
Mosqueira R et al. (2021) [68]	Paraguay	2019 – 2020	Case control	Hospitalized patients over then 18 years old	146	43 (29%) – Severe dengue	6 (4.11%)	19 (44%)	24 (56%)	18 – 30 years = 13 (30%) 31 – 60 years = 21 (49%) ≥60 years = 9 (21%)	Low (89%)

*Gender and age from the severe dengue population

[a] Data from patients with dengue hemorrhagic fever and dengue with complications, both with manifestation of thrombocytopenia, were analyzed together as the “Dengue fever with thrombocytopenia” (DFT) group.
[b] Dengue with warning signs and severe dengue were analyzed together, it was not possible to distinguish these two groups
[c] Incomplete DHF/DSS means missing bleeding or thrombocytopenia data
NR = Not reported
DF = Dengue fever
DHF = Dengue hemorrhagic fever
ICU = Intensive care units
DSS = Dengue shock syndrome
Note: Numbers may vary depending on the availability of data in the articles

Signs and symptoms, comorbidities, and number of secondary dengue infections according to the outcome

Seventeen articles presented signs and symptoms in the population classified as severe dengue. The occurrence of fever was more common, and in one study 100% of the population had this symptom [32]. Then, headache and vomiting/nausea were frequent, with a mean of 72% and 65% among patients with these symptoms, respectively, but in one study 100% of the population experienced vomiting/nausea [46], and the myalgia symptom also stood out with a mean of 69%. Six articles brought up the population's comorbidities, but they were not so frequent. In all studies, less than 18% of the population had some comorbidity, but patients with hypertension were more common. In addition, a mean of 34% of the severe dengue had a secondary infection **(Table 2)**.

In the death outcome, vomiting/nausea was more common, with a mean of 81%, followed by abdominal pain or tenderness and shock, and in one study 100% of the population that died had shock [41]. Only two studies reported comorbidities, but hypertension was also the most common [43, 45]. A mean of 42% of the population that died had a secondary infection, in one study 100% of the population had a secondary infection **(Table 2)**.

Lastly, few articles brought information about patients hospitalized. Among these studies, the manifestations of bleeding/hemorrhagic and vomiting/nausea were in common between the two articles that brought the data of signs and symptoms [54, 58], and the mean of the population of articles with secondary infection was 26% **(Table 2)**.

Table 2. Main signs and symptoms, comorbidities and number of secondary dengue infections reported by the studies according to the outcome

OUTCOME		[c]Population (N)	Mean % (min-max)
SEVERE DENGUE			
***Signals and symptons (17 articles)**			
	Fever	5581	82% (11-100%)
	Headache	5849	72% (10-95%)
	Myalgia	5492	69% (10-94%)
	Vomiting/nausea	4950	65% (15-100%)
	Abdominal pain or tenderness	3647	58% (7-79%)
[#]**Comorbidities (6 articles)**			
	Hypertension	503	11.2% (1-17%)
	Diabetes	540	4.4% (1-12%)
	Asthma or lung disease	297	3.7% (0-6%)
Secondary infection (12 articles)			
	Yes	776	34% (2-71%)
DEATH			
[a]**Signals and symptons (9 articles)**			
	Vomiting/nausea	141	81% (65-97%)
	Abdominal pain or tenderness	219	55% (9-88%)
	Shock	167	49% (13-100%)
	Abdominal pain or tenderness	219	55% (9-88%)
[#]**Comorbidities (2 articles)**			
	Hypertension	20	27% (7-47%)
	Chronic kidney disease	6	9% (2-16%)
	Diabetes	8	7% (4-11%)
Secondary infection (3 articles)			
	Yes	151	42% (12-100%)
HOSPITALIZATION			
[b]**Signals and symptons (2 articles)**			
	Bleeding/hemorrhagic manifestations	680	74% (71-77%)
	Vomiting/náusea	364	29% (14-43%)
Comorbidities (0 articles)	NR	NR	NR
Secondary infection (3 articles)			
	Yes	50797	26% (4-44%)

* Top 5 most frequently cited signs and symptoms in the articles included
# Top 3 most frequent cited comorbidities in the articles included
[a] Top 4 most frequently cited signs and symptoms in the articles included
[b] Top 3 most frequently cited signs and symptoms in the articles included
[c] Number of the population that presented this symptom
NR = Not reported
Note: The main signs and symptoms vary due to the availability of data in the articles.
Note: Numbers may vary depending on the availability of data in the articles.
Note: Only articles that had at least one of the variables were included in the calculation.

Type of analysis of the included articles

Twenty-nine articles analyzed the outcome of severe dengue, of which nineteen (66%) performed multivariate analysis, six articles (21%) performed exclusively univariate analysis, while four (14%) studies did not present this information. When it comes to death due to dengue, fourteen studies analyzed this outcome, with seven (50%) using multivariate analysis, four (29%) using univariate analysis, and three (21%) did not report this information. Finally, eight studies analyzed the hospitalization outcome, six (75%) used multivariate analysis and only two (25%) did not report this information.

Risk factors for severe dengue

The clinical characteristic with the highest number of articles with statistically significant association for the outcome of severe dengue was having some comorbidity or underlying disease but being in a secondary dengue infection draws attention. However, three articles showed an association, and three articles did not **(Fig 2)**. In relation to sociodemographic characteristics, < 18 years old was shown to be a frequent risk factor in the articles, although the article by Marina Jolli Luppe et al. (2019) presented this variable as a protective factor. In this same category, being white or Caucasian was shown to be a risk factor in all articles that studied this variable **(Fig 2)**. Most studies analyzed signs and symptoms associated with severe dengue, among which abdominal pain or tenderness, bleeding/hemorrhagic manifestation, hepatomegaly, splenomegaly, or similar and altered hematocrit or hemoglobin were the most frequently associated with severe dengue. In addition, prostration, lethargy, fatigue, or similar symptoms were significantly associated with severe dengue in all articles that studied this variable. Other signs and symptoms also stood out, but many

347 articles were also not significant, such as rash, petechiae, exanthema,
348 hematomas and/or ecchymoses, and vomiting/nausea **(Fig 2).**

349

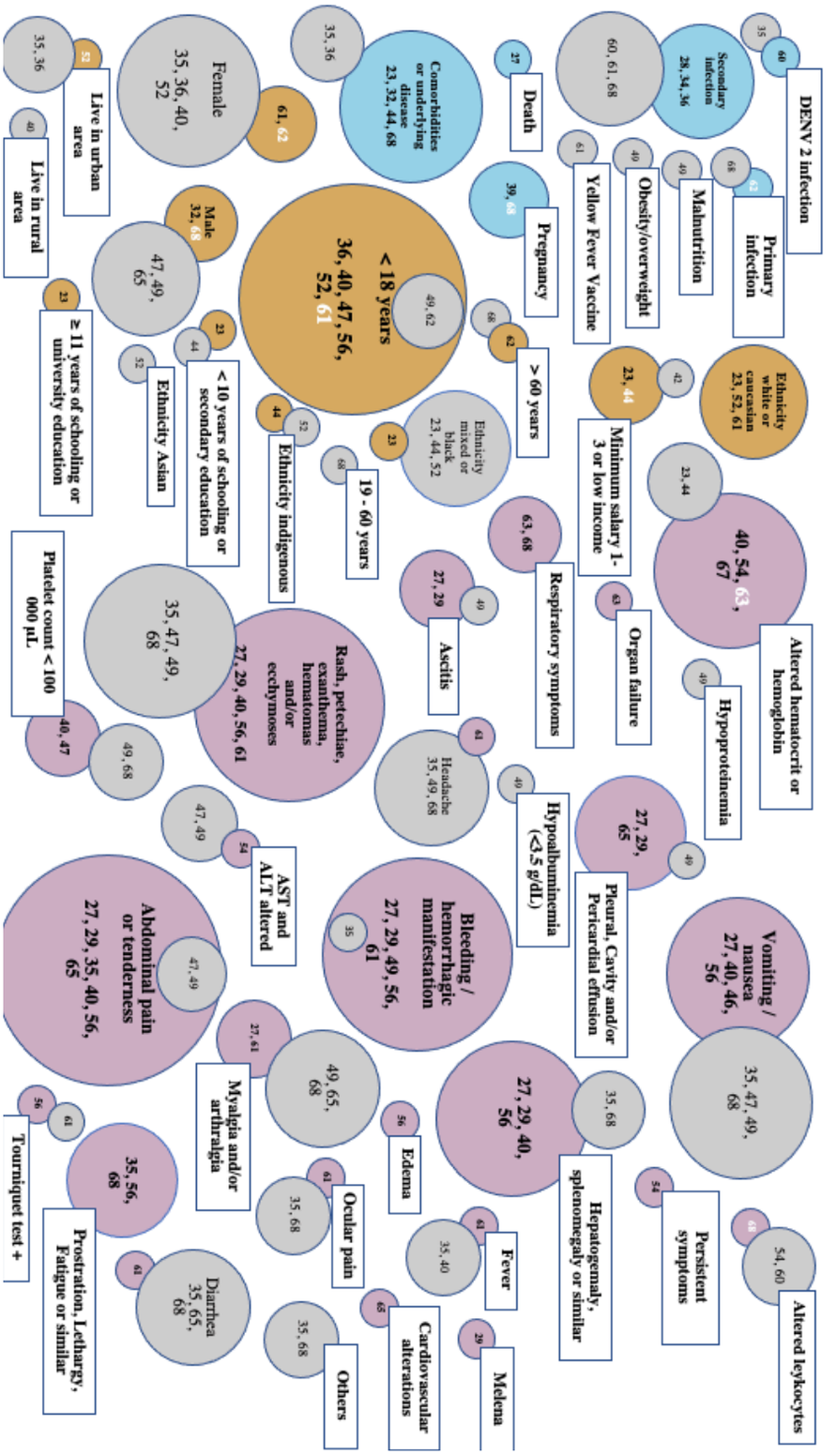
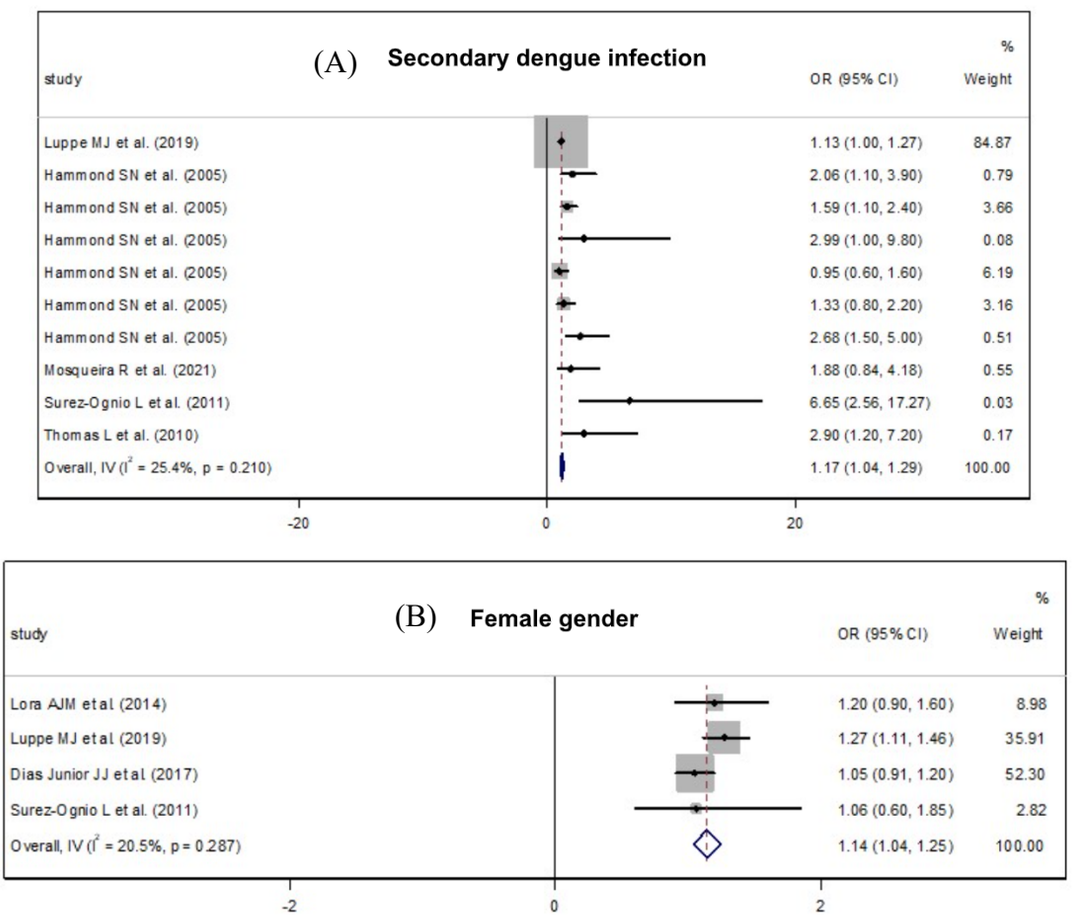


Fig 2. Risk factors for the outcome of Severe Dengue: sociodemographic characteristics (brown), signs and symptoms (pink), clinical characteristics (blue), non-statistically significant (grey). Risk factors are displayed as circles. The digits inside the circles are the relevant reference numbers.
Note: In white are articles that were statistically significant, but as a protective factor.
Note: if the article analyzed the same variable or variables in the same group more than once, if one of the analyzes was statistically significant, the entire article was marked as significant.
Note: The sizes of each circle is proportional to the amount of articles that analyzed the risk factor.

Meta-analysis results showed that secondary dengue infection is a risk factor for disease severity (OR: 1.17, 95% CI: 1.04 - 1.29), as female gender (OR: 1.14, 95 % CI: 1.04 - 1.25) and white or Caucasian ethnicity (OR: 1.28, 95% CI: 1.14 - 1.42) **(Fig 3)**. However, male gender also showed a statistically significant association, but as a protective factor (OR: 0.67, 95% CI: 0.45 – 0.90) **(Fig 3)**. In addition, the variables of signs and symptoms had a statistically significant association with severe dengue, such as headache (OR: 1.20, 95% CI: 1.07 - 1.34), myalgia and/or arthralgia (OR: 1.30, 95% CI: 1.19). - 1.41), vomiting/nausea (OR: 1.48, 95% CI: 126 - 1.70), abdominal pain or tenderness (OR: 1.64, 95% CI: 1.40 - 1.89), diarrhea (OR: 1.79, 95% CI: 1.45 - 2.14) and prostration, lethargy, fatigue, or similar symptoms (OR: 2.46, 95% CI: 1.33 - 3.58) **(Fig 4)**. In cases of abdominal pain or tenderness, the analysis with the RR/RP measures showed a similar result.



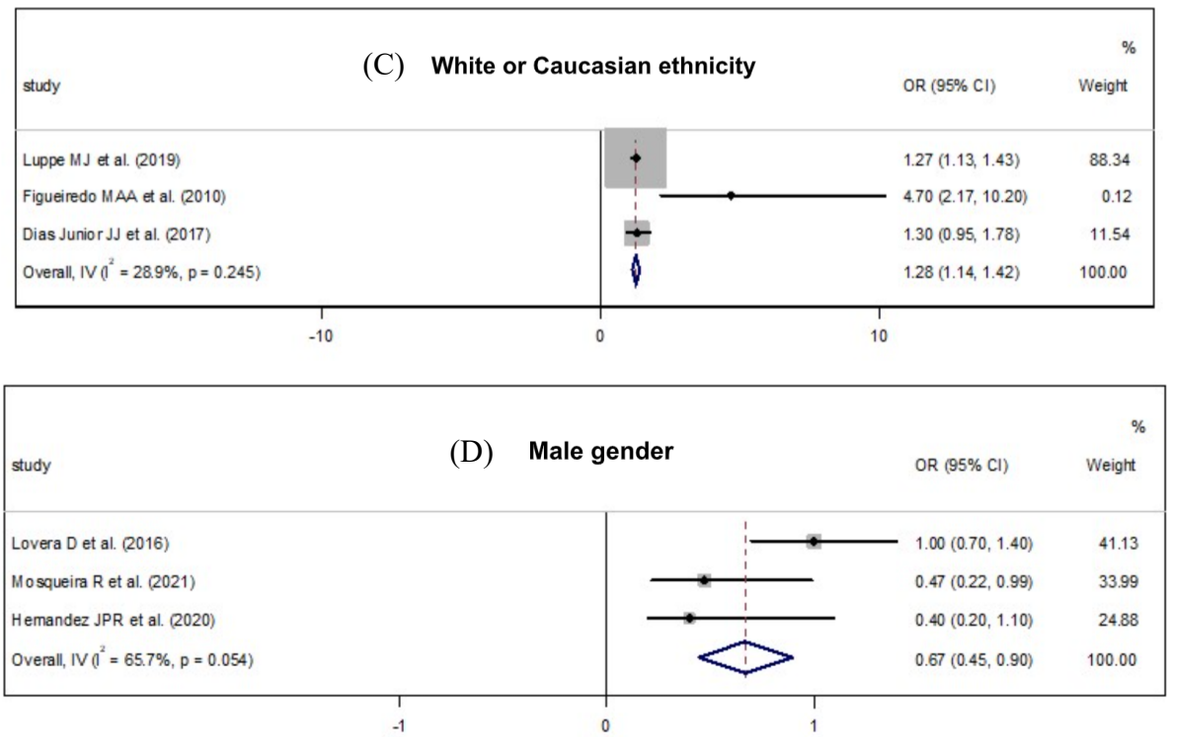
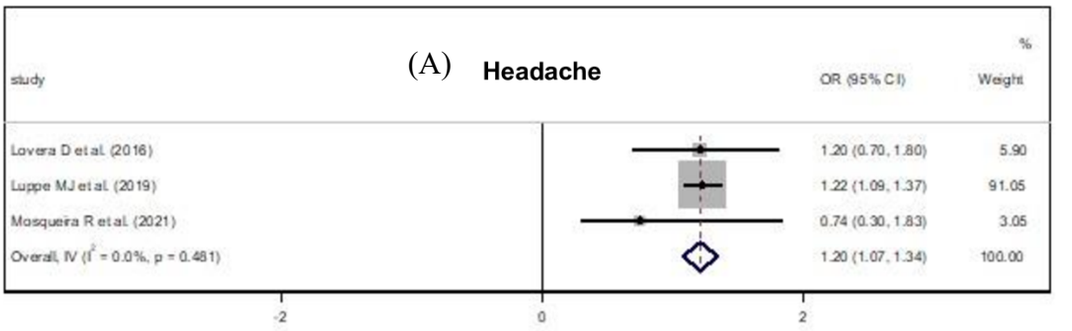
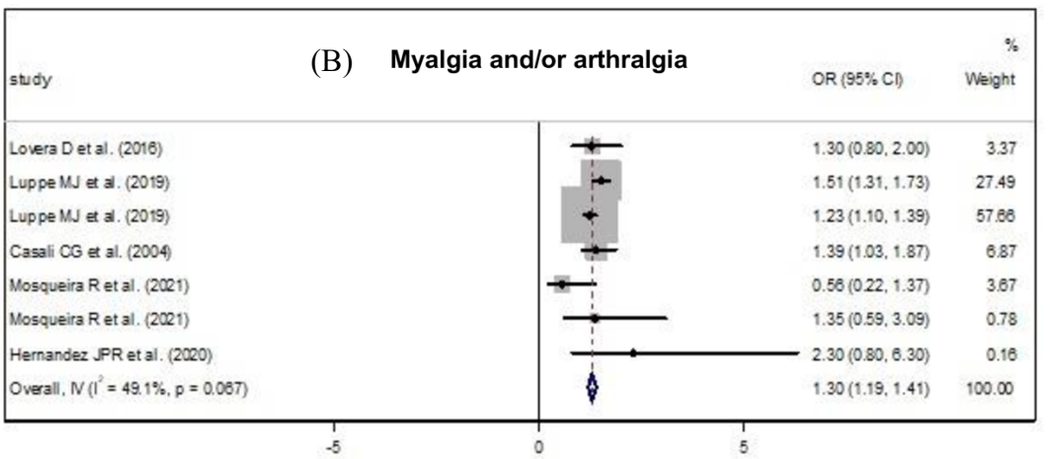
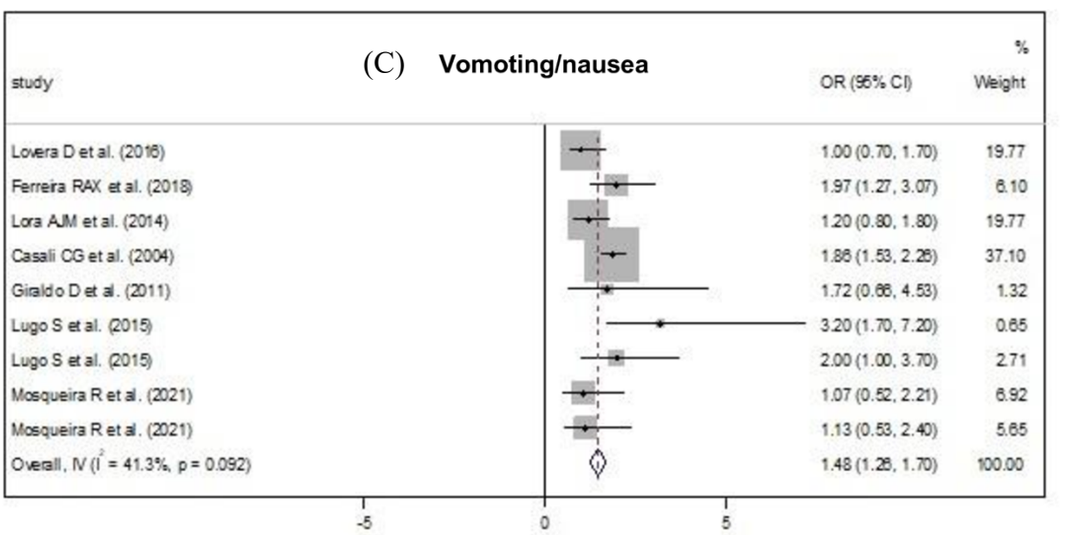


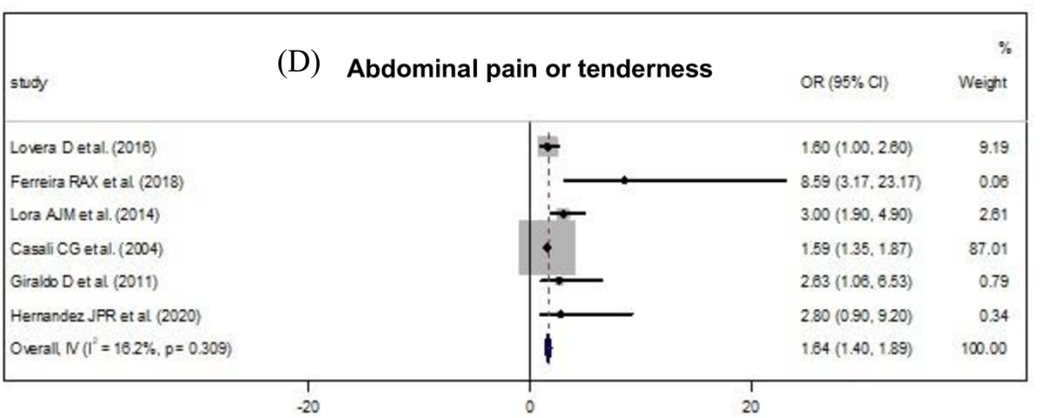
Fig 3. Meta-analysis: forest plot of the associations between clinical and sociodemographic characteristics and severe dengue.
OR = odds ratio
CI = confidence interval


(A) Headache
study
OR (95% CI)
%
Weight
Lovera D et al. (2016) 1.20 (0.70, 1.80) 5.90
Luppe MJ et al. (2019) 1.22 (1.09, 1.37) 91.05
Mosqueira R et al. (2021) 0.74 (0.30, 1.83) 3.05
Overall, IV (I² = 0.0%, p = 0.481) 1.20 (1.07, 1.34) 100.00
-2
0
2


(B) Myalgia and/or arthralgia
study
OR (95% CI)
%
Weight
Lovera D et al. (2016) 1.30 (0.80, 2.00) 3.37
Luppe MJ et al. (2019) 1.51 (1.31, 1.73) 27.49
Luppe MJ et al. (2019) 1.23 (1.10, 1.39) 57.66
Casali CG et al. (2004) 1.39 (1.03, 1.87) 6.87
Mosqueira R et al. (2021) 0.56 (0.22, 1.37) 3.67
Mosqueira R et al. (2021) 1.35 (0.59, 3.09) 0.78
Hernandez JPR et al. (2020) 2.30 (0.80, 6.30) 0.16
Overall, IV (I² = 49.1%, p = 0.067) 1.30 (1.19, 1.41) 100.00
-5
0
5


(C) Vomoting/nausea
study
OR (95% CI)
%
Weight
Lovera D et al. (2016) 1.00 (0.70, 1.70) 19.77
Ferreira RAX et al. (2018) 1.97 (1.27, 3.07) 6.10
Lora AJM et al. (2014) 1.20 (0.80, 1.80) 19.77
Casali CG et al. (2004) 1.86 (1.53, 2.26) 37.10
Giraldo D et al. (2011) 1.72 (0.66, 4.53) 1.32
Lugo S et al. (2015) 3.20 (1.70, 7.20) 0.65
Lugo S et al. (2015) 2.00 (1.00, 3.70) 2.71
Mosqueira R et al. (2021) 1.07 (0.52, 2.21) 6.92
Mosqueira R et al. (2021) 1.13 (0.53, 2.40) 5.65
Overall, IV (I² = 41.3%, p = 0.092) 1.48 (1.28, 1.70) 100.00
-5
0
5

370


(D) Abdominal pain or tenderness
study
OR (95% CI)
%
Weight
Lovera D et al. (2016) 1.60 (1.00, 2.60) 9.19
Ferreira RAX et al. (2018) 8.59 (3.17, 23.17) 0.06
Lora AJM et al. (2014) 3.00 (1.90, 4.90) 2.61
Casali CG et al. (2004) 1.59 (1.35, 1.87) 87.01
Giraldo D et al. (2011) 2.63 (1.06, 6.53) 0.79
Hernandez JPR et al. (2020) 2.80 (0.90, 9.20) 0.34
Overall, IV (I² = 16.2%, p = 0.309) 1.64 (1.40, 1.89) 100.00
-20
0
20

371

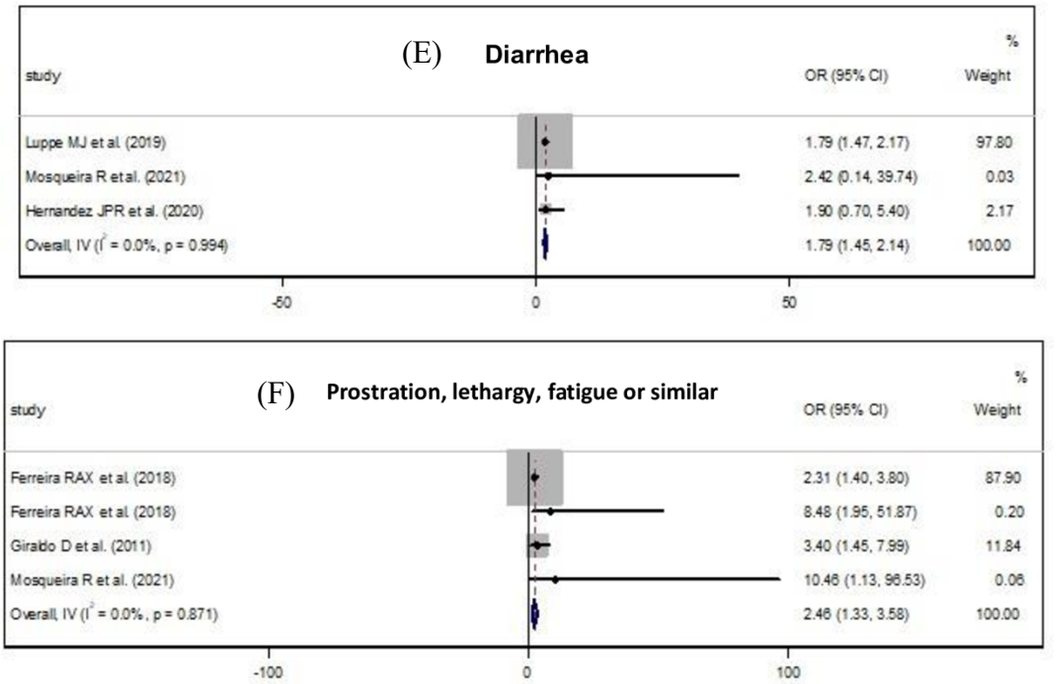


Fig 4. Meta-analysis: Meta-analysis: forest plot of the associations between signs and symptoms and severe dengue
OR = odds ratio
CI = confidence interval

Risk factors for death due to dengue

When it comes to the outcome of death due to dengue having either a comorbidity or underlying diseases was the clinical characteristic most often associated with death in the studies **(Fig 5)**. In addition, age > 60 years old was statistically significant in all articles that analyzed this variable, while age < 18 years old in half of the studies showed an association and the other half did not **(Fig 5)**. The most prominent signs and symptoms associated with death were bleeding/hemorrhagic manifestation, shock, warning signs or severe dengue and vomiting/nausea, and the articles by Moraes GH et al. (2013) and Arnaldo Fabián Negrete A et al. (2015) presented bleeding/hemorrhagic manifestation as a protective factor **(Fig 5)**.

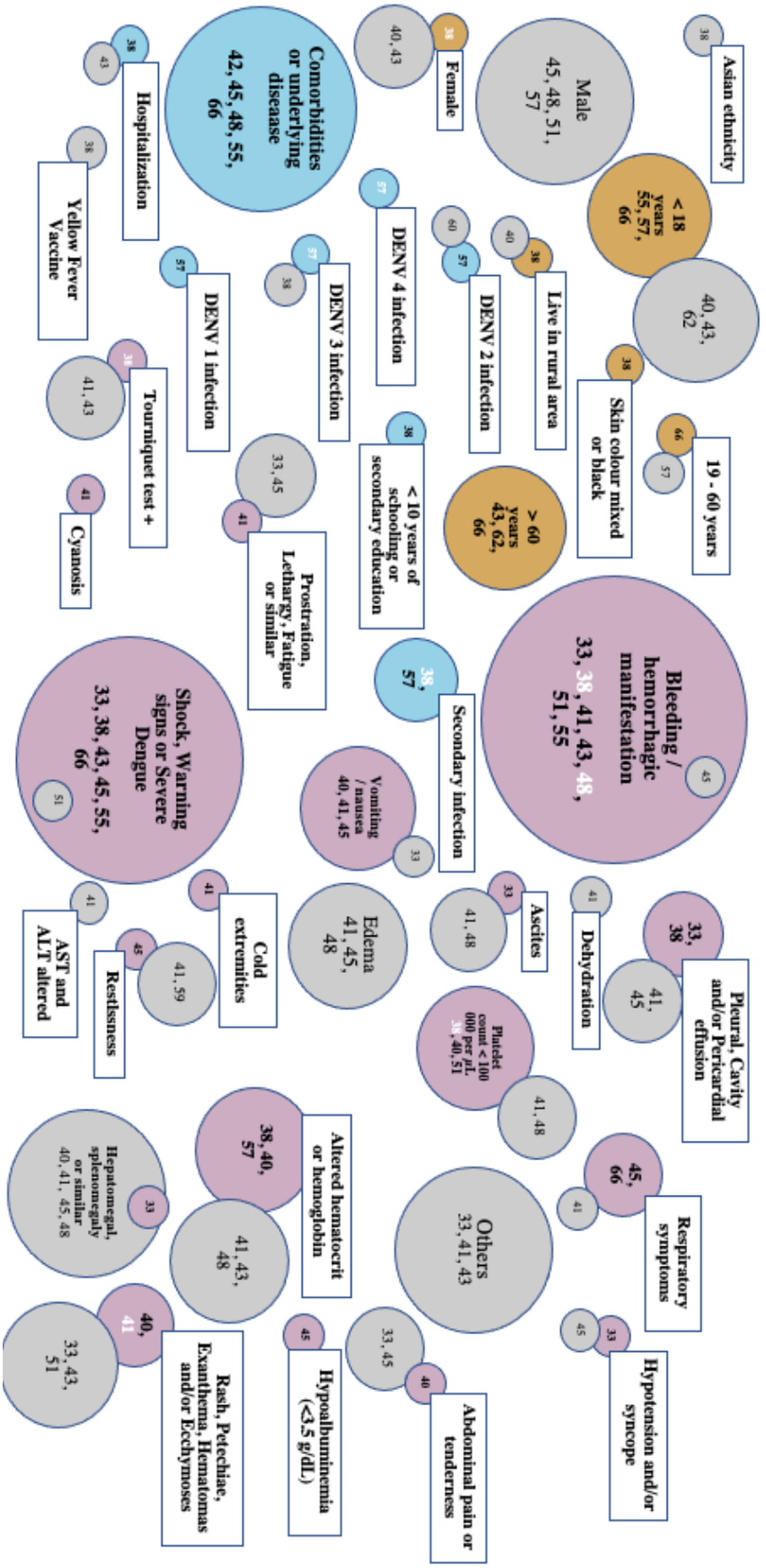


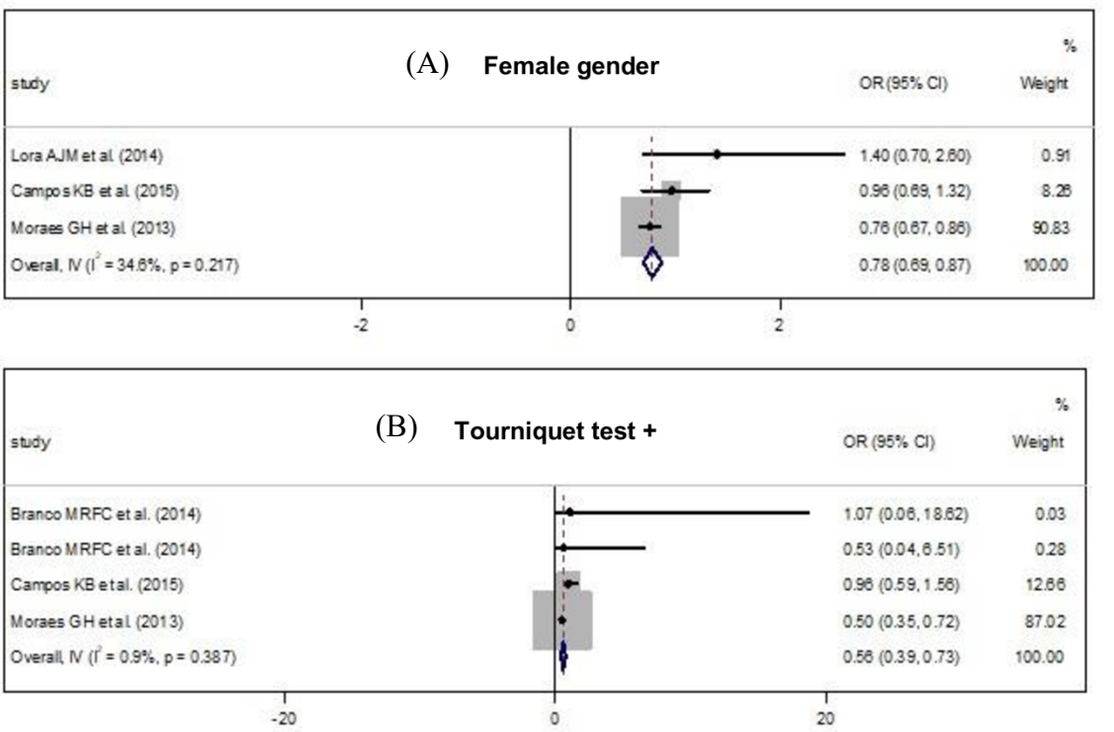
Fig 5. Risk factors for the outcome of death due to dengue: sociodemographic characteristics (brown), signs and symptoms (pink), clinical characteristics (blue), non-statistically significant (grey). Risk factors are displayed as circles. The digits inside the circles are the relevant reference numbers.

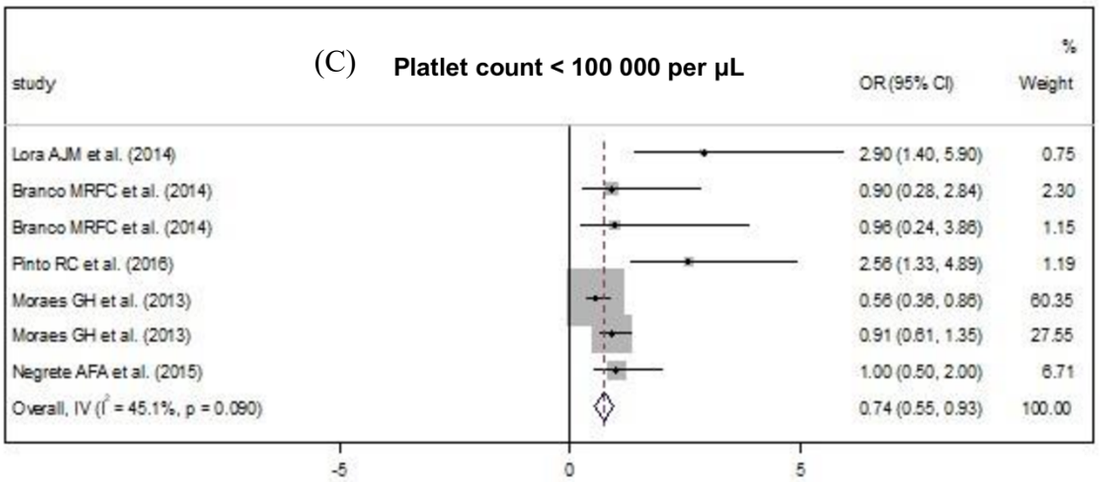
Note: In white are articles that were statistically significant, but as a protective factor.

Note: if the article analyzed the same variable or variables in the same group more than once, if one of the analyzes was statistically significant, the entire article was marked as significant.

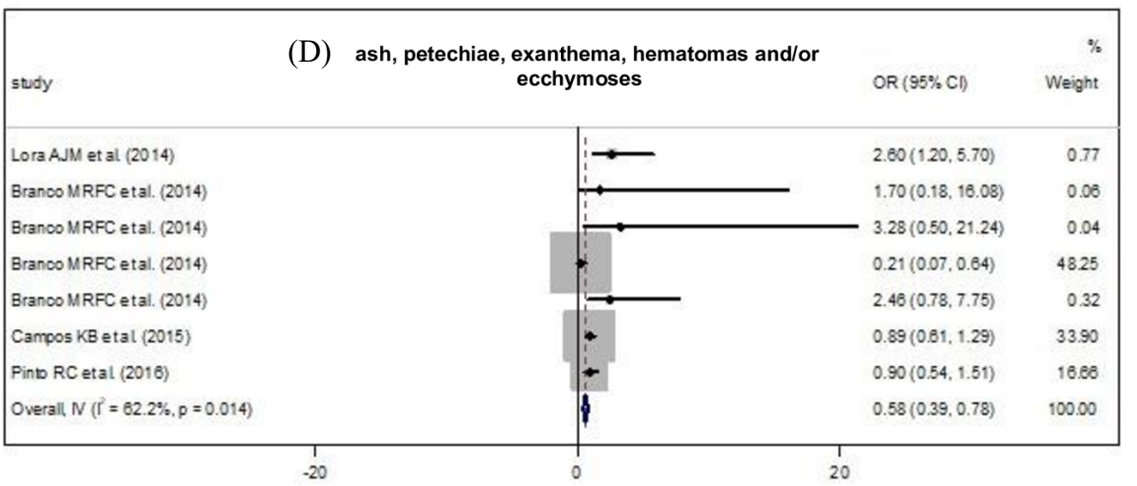
Note: The sizes of each circle is proportional to the amount of articles that analyzed the risk factor.

The meta-analysis for the death outcome showed that the female gender had a statistically significant association, but with a sense of protection (OR: 0.78, 95% CI: 0.69 - 0.87), as well as the positive tourniquet test (OR: 0.56, 95% CI: 0.39 - 0.76), platelet count < 100 000 µL (OR: 0.74, 95% CI: 0.55 - 0.93) and rash, petechiae, exanthema, hematomas and/or ecchymoses (OR: 0.58, 95% CI: 0.39 – 0.78) **(Fig 6)**. Few signs and symptoms showed significance for the death outcome or showed high heterogeneity, however, vomiting/nausea had a statistically significant association in this outcome (OR: 4.10, 95% CI: 1.10 - 7.10) **(Fig 6)**. For the RR/PR association measure, being < 18 years old was shown to have a statistically significant association with death as a risk factor (OR: 3.31, 95% CI: 1.70 – 4.93) **(Fig 6)**.

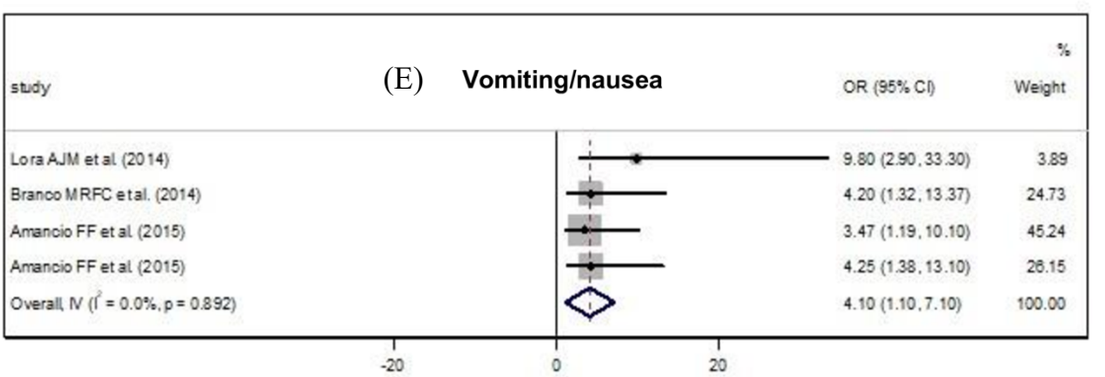



(C) Platlet count < 100 000 per µL
study
OR (95% CI)
%
Weight
Lora AJM et al. (2014) 2.90 (1.40, 5.90) 0.75
Branco MRFC et al. (2014) 0.90 (0.28, 2.84) 2.30
Branco MRFC et al. (2014) 0.96 (0.24, 3.86) 1.15
Pinto RC et al. (2016) 2.56 (1.33, 4.89) 1.19
Moraes GH et al. (2013) 0.56 (0.36, 0.86) 60.35
Moraes GH et al. (2013) 0.91 (0.61, 1.35) 27.55
Negrete AFA et al. (2015) 1.00 (0.50, 2.00) 6.71
Overall, IV (I² = 45.1%, p = 0.090) 0.74 (0.55, 0.93) 100.00
-5
0
5

401


(D) ash, petechiae, exanthema, hematomas and/or ecchymoses
study
OR (95% CI)
%
Weight
Lora AJM et al. (2014) 2.60 (1.20, 5.70) 0.77
Branco MRFC et al. (2014) 1.70 (0.18, 16.08) 0.06
Branco MRFC et al. (2014) 3.28 (0.50, 21.24) 0.04
Branco MRFC et al. (2014) 0.21 (0.07, 0.64) 48.25
Branco MRFC et al. (2014) 2.46 (0.78, 7.75) 0.32
Campos KB et al. (2015) 0.89 (0.61, 1.29) 33.90
Pinto RC et al. (2016) 0.90 (0.54, 1.51) 16.66
Overall, IV (I² = 62.2%, p = 0.014) 0.58 (0.39, 0.78) 100.00
-20
0
20

402


(E) Vomiting/nausea
study
OR (95% CI)
%
Weight
Lora AJM et al. (2014) 9.80 (2.90, 33.30) 3.89
Branco MRFC et al. (2014) 4.20 (1.32, 13.37) 24.73
Amancio FF et al. (2015) 3.47 (1.19, 10.10) 45.24
Amancio FF et al. (2015) 4.25 (1.38, 13.10) 26.15
Overall, IV (I² = 0.0%, p = 0.892) 4.10 (1.10, 7.10) 100.00
-20
0
20

403

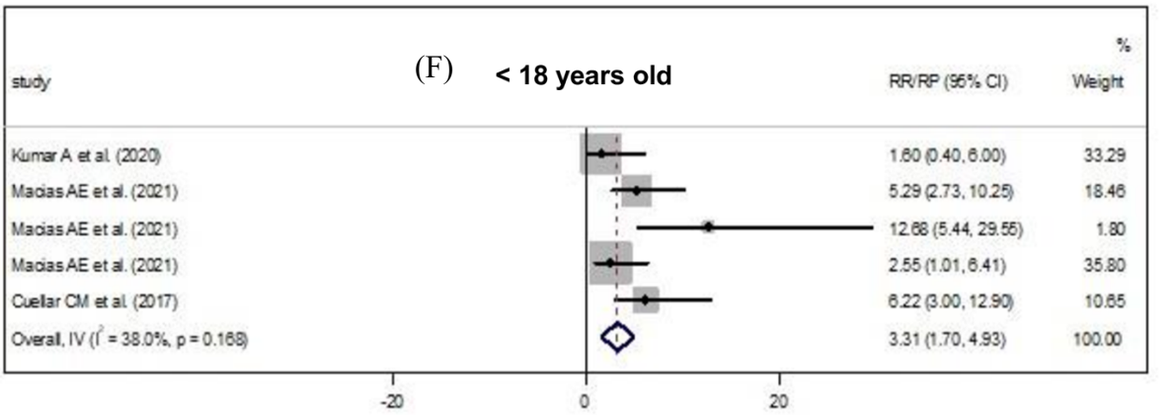


Fig 6. Meta-analysis: forest plot of the associated variables with death due to dengue.
OR = odds ratio
RR/RP = risk relative/prevalence ratio
CI = confidence interval

Risk factors for hospitalization due to dengue

Few studies analyzed the outcome of hospitalization due to dengue, among which the most frequent were < 18 years old and > 60 years old **(Fig 7)**.

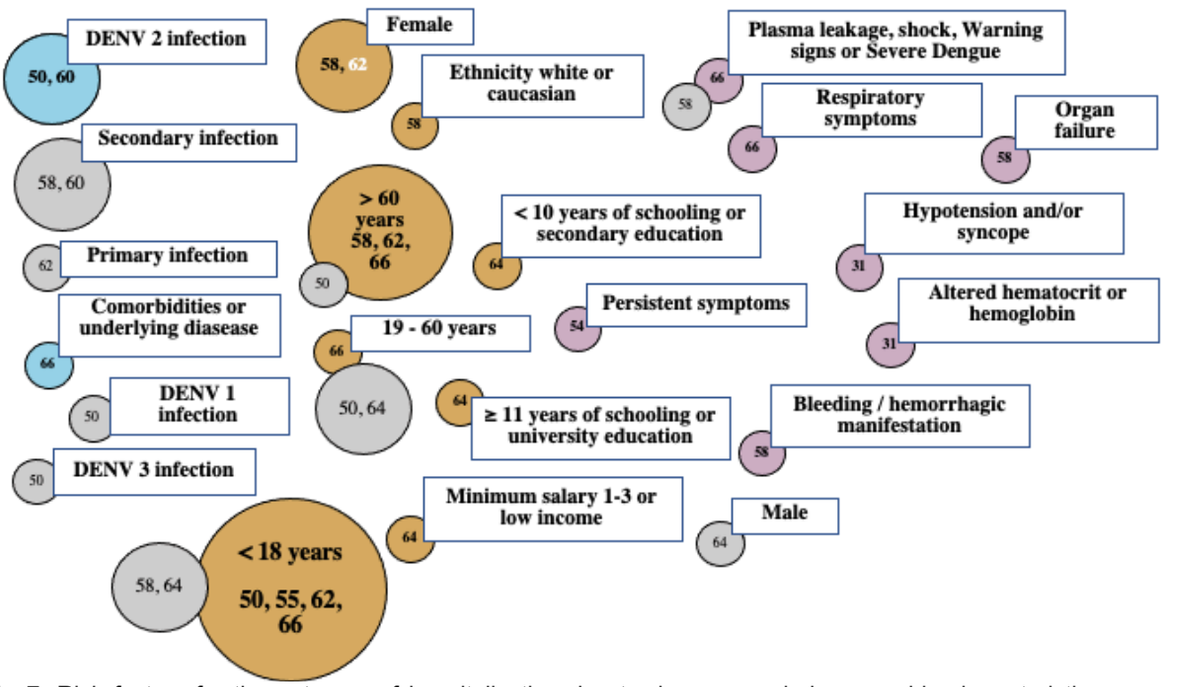
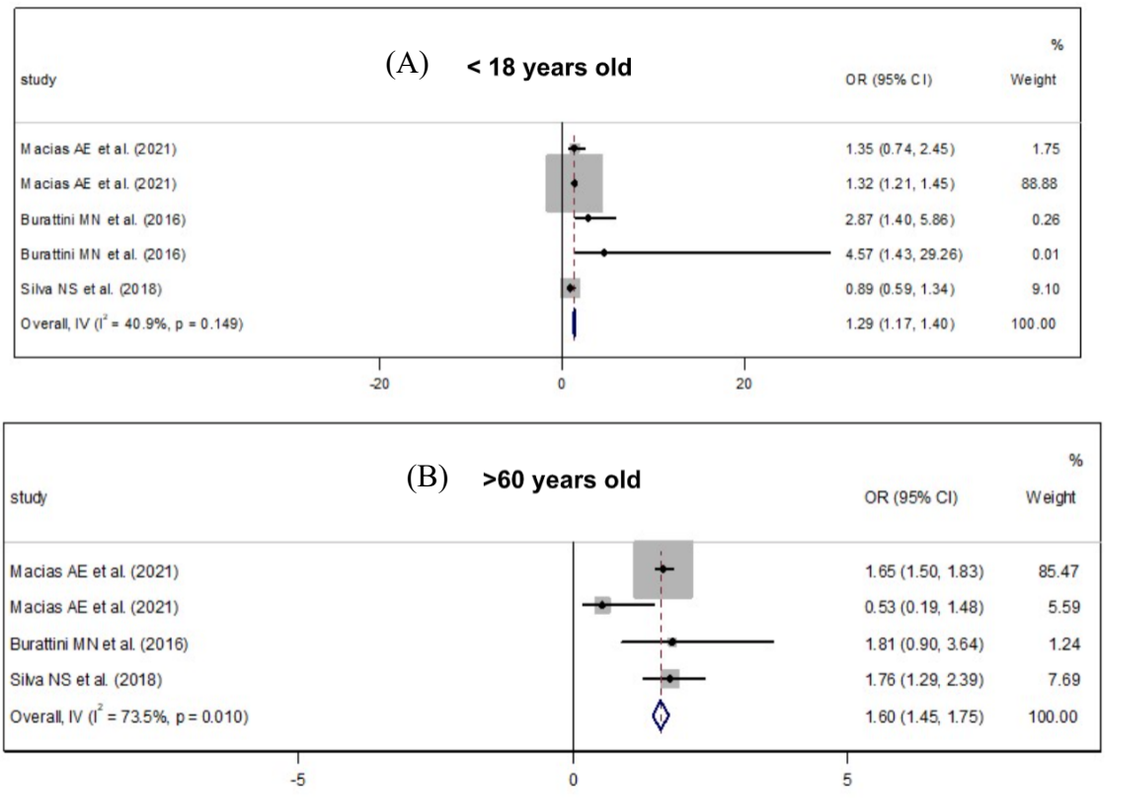


Fig 7. Risk factors for the outcome of hospitalization due to dengue: sociodemographic characteristics (brown), signs and symptoms (pink), clinical characteristics (blue), non-statistically significant (grey). Risk factors are displayed as circles. The digits inside the circles are the relevant reference numbers.
Note: In white are articles that were statistically significant, but as a protective factor.
Note: if the article analyzed the same variable or variables in the same group more than once, if one of the analyzes was statistically significant, the entire article was marked as significant.
Note: The sizes of each circle is proportional to the amount of articles that analyzed the risk factor.

Few articles analyzed the hospitalization outcome, because of this and the other criteria used, only the variable < 18 years old (OR: 1.29, 95% CI: 1.17 - 1.40) and > 60 years old (OR: 1.60, 95% CI: 1.45 – 1.75) presented statistically significant association (**Fig 8).**



426

427 **Fig 8.** Meta-analysis: Meta-analysis: forest plot of the associated variables with hospitalization.
428 OR = odds ratio
429 CI = confidence interval

DISCUSSION

This systematic review and meta-analysis evaluated risk factors for severe dengue, death, and hospitalization due to dengue infection. Because dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome, it is necessary to identify risk factors associated with such outcomes [16].

The risk factors for severe dengue found in this meta-analysis were secondary dengue infection, female gender, white or Caucasian ethnicity and some signs and symptoms. The literature already describes that secondary dengue infection is associated with a higher chance of developing severe dengue [3, 19, 69, 70]. The increased severity in cases of secondary infection is because T cells and antibodies generated during a primary DENV infection can respond to variant peptides during secondary infection [71]. The selective expansion of lower avidity cross-reactive memory T cells from the primary infection may out-compete the naive T cells with higher avidity for the current infecting serotype, thus altering the repertoire of responding T cells in a secondary infection due to cross reaction [71]. Other meta-analyses showed that female gender was not associated with the development of severe dengue [72, 73]. However, other studies show a positive association, the reason for this is not yet fully known, but it can be explained by the difference between genders in the search for health services and by the different immune responses [74-77]. These facts would also justify why the male gender proved to be a protective factor. In addition, in relation to white or Caucasian ethnicity, a study carried out in Cuba found a greater risk of whites to develop severe dengue, whites showed a more vigorous dengue-virus-specific cellular immune response, with a high cross-reactivity to heterologous dengue antigens when compared to Blacks [22]. However, more studies are needed to understand this mechanism in other Latin American countries.

Signs of symptoms such as headache, myalgia and/or arthralgia, vomiting/nausea, abdominal pain or tenderness, diarrhea and prostration, lethargy, fatigue or similar have been identified as risk factors for severe dengue. Persistent vomiting and severe abdominal pain are considered warning signs, and in these cases, it can progress to severe cases and even lead to death [3, 16]. And in this meta-analysis, vomiting/nausea was also a risk factor for death. A study was found headache as a protective factor for severe dengue [73]. However, an *in vitro* study showed that DENV 1 strains were neurotopic in infected mouse, which may justify headache as a risk factor [78]. In addition, aches and pains are typical symptoms of dengue, especially in the initial phase of the disease, but neuromuscular complications can occur in the disease and even lead to the development of Guillain-Barré syndrome [16, 79]. One study reported diarrhea as the second most common gastrointestinal symptom in dengue, but diarrhea is a nonspecific symptom that may be present in several febrile illnesses [80]. The presence of gastrointestinal symptoms was associated with the hemorrhagic type of dengue [81]. In addition, symptoms related to physical and mental tiredness were positively associated with severe dengue. According to the WHO, lethargy and fatigue are warning signs, which can lead to the development of severe dengue [3, 16]. Studies demonstrated that lethargy or restlessness are associated with the development of severe dengue and are the most common symptoms in severe patients [72, 82].

For the outcome of death, women were less likely to die, and in this analysis, the article with the greatest weight was that of Moraes GH et al. (2013), a study carried out in Brazil. Statistics and analyses already indicate that Brazilian women use the health service more than Brazilian men, which may justify women being less likely to die from dengue [83]. However, the variables positive tourniquet test, platelet count <

100 00 µL and symptoms of capillary fragility also reduced the chances of death in this meta-analysis, which may indicate an opportunity for early diagnosis and adequate patient management, avoiding death [38]. One study showed that not using the tourniquet test impacts the diagnosis of severe dengue, which makes proper management difficult [84]. In addition, studies show that tourniquet test, leukopenia, and symptoms of capillary fragility are associated with increased odds of identifying and diagnosing dengue cases [85, 86]. Which would reduce the mortality rate to below 1% [9].

In addition, < 18 years old was also a risk factor for death and hospitalization, which corroborates with information from the WHO and PAHO that state that dengue is one of the main causes of death and hospitalization in children in some parts of Asia and Latin America [9, 16].

The variable age > 60 years has a statistically significant association with the outcome of hospitalization. A previous study found that older people have been shown to stay longer in the hospital [87].

This systematic review found that a mean of 36% of dengue cases can progress to severe dengue, which can also be represented as 2% of the total population. One estimate showed that 390 million dengue infections occur per year (95% CI 284–528 million) of which 96 million (95% CI 67-136 million) manifests apparently with any level of clinical severity, which would correspond to 25 % of cases [88]. PAHO reported that in 2021 there were 1,267,151 cases of dengue in the Americas, of which 3,273 progressed to severe dengue, representing less than 0.3% of cases [89]. The numbers may vary according to countries and regions, which can be explained by the frequency of outbreaks in each country and the risk factors of each population.

It was found that a mean of 5% of dengue cases died, also representing 0.09% of the total population of the included studies. A study showed that of 1,018 severe cases in São Luis, Maranhão, Brazil, 74 died, which would be equivalent to 7% of severe cases, a global estimate found that in 2013 there were a total of 58.40 million symptomatic dengue virus infections worldwide, including 13,586 fatal cases, equivalent to 0.02% of cases [90, 91]. It is possible to observe a constancy in the number of deaths due to dengue, being necessary to implement measures to avoid the risk of life and reduce the number of deaths.

A mean of 46% (31% - 63%) of the participants are male and 55% (27% - 64%) are female in the severe dengue outcome, with no noticeable difference between genders. These data on the outcome of death were similar. A study carried out in seven dengue endemic countries in Latin America and Asia found that 18% of the population with severe dengue was female, while 14% was male [92]. Two other studies carried out in Asian countries found similar data, with the female gender being slightly more prevalent in cases of severe dengue [74, 93]. In addition, studies also showed that more women die from dengue, but there are also studies that report the opposite, the study by Thein Tl et al. (2013) found that 68% of the cases that died were male [74, 90, 94, 95]. Although the female gender shows a slightly higher prevalence in cases of severe dengue, there is little difference from the male gender, but this may indicate a greater frequency of women evolving into severe dengue than men. Which was also confirmed as a risk factor in this study. However, this meta-analysis showed that women are less likely to die, which can be explained by the difference between countries in access to health systems.

Of the included articles that analyzed only children and adolescents, the occurrence of severe dengue in children older than four years was more common.

Other studies show that the occurrence of severe dengue is more frequent in older children, especially from four years old, few studies bring data about babies younger than one-year-old, because of this it becomes difficult to characterize this population in cases of severe dengue [40, 47, 96-98]. It is already well described in the literature that children are a risk factor for severe dengue, and there are already many studies that characterize this population [24, 69, 99]. However, it is important to study babies for better management and diagnosis. The other studies included in this systematic review showed most of the population between 15 and 60 years old, it is an extremely wide range, which makes it difficult to say whether it is a population at risk for severe dengue.

In relation to cases of death due to dengue, it was more common among adults aged 15 – 51 years, despite occurring in people > 51 years. Studies show that death from dengue can occur at all ages, but it has a certain frequency in children and the elderly [2, 94, 100-102]. Other studies show different results about if age is a risk factor for death, however, the study by Karunakaran et al. (2014) and Chagas GCL et al. (2017) show that death is associated with adults over 40 years old [103-105]. Thus, it is necessary to evaluate other factors of the individual that could have led to death.

When it comes to the most frequent signs and symptoms of each outcome, according to the mean taken, fever, headache, myalgia, vomiting/nausea and abdominal pain or tenderness were the most common in severe dengue. Abdominal pain or tenderness and vomiting/nausea show a statistically significant association for severe dengue in this study. Just as they were also frequent in the outcome of death and hospitalization. As discussed, abdominal pain or tenderness and persistent vomiting are considered warning signs for dengue, which may justify patients presenting these symptoms in severe cases and death [16]. In addition, other

554 systematic reviews and meta-analyses have reported abdominal pain or tenderness,
555 vomiting, and headache as the most frequent symptoms in cases of severe dengue
556 [24, 73]. One study showed that abdominal pain or tenderness may be associated with
557 acute hepatitis, acalculus cholecystitis, acute pancreatitis, appendicitis, and
558 spontaneous bacterial peritonitis, i.e [106]. Myalgia, headache, nausea, and vomiting,
559 with high fever, are characteristic symptoms of dengue and observed during the initial
560 phase of the disease, not necessarily associated with severe outcomes [3, 107].

561 Shock was a frequent symptom in the outcome of death. It is already well
562 described that it occurs in severe cases and is associated with mortality, as it results
563 from an abnormal and exaggerated host immune response that increases the severity
564 of the infection [108, 109].

565 More than half of the hospitalized patients presented bleeding/hemorrhagic
566 manifestations, other articles show the tendency of nausea/vomiting symptoms to be
567 more common and bleeding/hemorrhagic manifestations proved to be less frequent,
568 but it is present in some hospitalized patients. [87, 110, 111].

569 Although comorbidities or underlying diseases have a low frequency among
570 cases of severe dengue and death in this study, the one that stood out the most was
571 hypertension, according to the mean taken. Another systematic review showed that
572 the most frequent comorbidity in the articles was diabetes, followed by hypertension
573 [24], in this study diabetes was the second most common in severe dengue according
574 to the mean. Other studies have shown that diabetes is a risk factor for the
575 development of DSS and severe dengue [112, 113]. It should be remembered that
576 diabetes increases the severity of several endemic diseases, such as dengue, as viral
577 infections can increase inflammation, or internal swelling, in people with diabetes which
578 can contribute to more serious complications [114, 115]. In addition, the association of

579 hypertension and diabetes has been described as a possible risk factor for dengue
580 severity, however, a meta-analysis found no association between hypertension and
581 severe dengue [24, 116]. Hypertension may be associated with the severity of dengue
582 because there is still endothelial and vascular dysfunction, which leads to inflammation
583 of the endothelium, altering the regulation of vascular tone and flow, but this
584 mechanism is not fully understood [44, 117]. It is already well known that cases of
585 severe dengue can lead to death [16, 17, 44, 117, 118].
586
587 This systematic review and meta-analysis have some limitations. First, it
588 included papers published in journals indexed in main research platforms. Such
589 selection could be susceptible to publication bias, as it is known that positive results
590 tend to be more published than negative ones, which can overestimate false results
591 [119]. Furthermore, this systematic review did not include studies published in
592 conference proceedings, epidemiological bulletins, and technical reports, which may
593 have reduced the number of studies. Other studies could be available in other
594 databases besides those used in this systematic review, which is also considered a
595 limitation. Finally, our meta-analysis showed high heterogeneity among some results,
596 indicating a large discrepancy between the measures of association reported by some
597 of the included studies. This heterogeneity hinders a clear understanding of whether
598 the risk factor is associated with the outcome as well as the direction of this association,
599 either risk or protection. Another problem was the variability in terms of confounders
600 among the studies using multivariable analysis. Further studies using meta-regression
601 could contribute to a better comprehension of the impact of other variables in the
602 association between the risk factors and dengue outcomes.
603 Our study aimed to identify the main risk factors associated with severe dengue
604 outcomes in Latin America, an area with high dengue incidence levels and comprising

605 countries with major social inequalities, as well as different strategies for dengue
606 management, control, and prevention. We identified that secondary dengue infection,
607 female gender, white or Caucasian ethnicity, headache, myalgia and/or arthralgia,
608 vomiting/nausea, abdominal pain or tenderness, diarrhea and prostration, lethargy,
609 fatigue or similar were risk factors for severe dengue. This result will help to define
610 strategies, management, and risk groups for the disease, avoiding complications such
611 as risk of life. And will help the formulation of future guidelines for the disease in Latin
612 America countries. This study was able to demonstrate the importance of early
613 diagnosis and correct interpretation of the tourniquet test, platelet count and the
614 identification of certain symptoms such as rash, petechiae, exanthema, hematomas
615 and/or ecchymoses to avoid death due to dengue. In addition, this study confirmed
616 WHO and PAHO information about children being risk factors for hospitalization and
617 death. Future studies are needed to ensure confirmation of certain risk factors and
618 identify other risk factors that this meta-analysis failed, to standardization for future
619 guidelines, thus leading to better management of patients at risk**.**
620

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625

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2.Proposta de submissão

2.1 Revista: PLOS *NEGLECTED TROPICAL DISEASES*
(https://journals.plos.org/plosntds/)

2.2 Regras para Submissão:

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The writing style should be concise and accessible, avoiding jargon so that the paper is understandable for readers outside a specialty or those whose first language is not English. Editors will make

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About the Journal

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Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
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1.1.2.1

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The Abstract is conceptually divided into the following three sections with these headings: Background, Methodology/Principal Findings, and Conclusions/Significance.

Do not include any citations. Avoid specialist abbreviations.

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1.1.10

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The introduction should put the focus of the manuscript into a broader context. As you compose the Introduction, think of readers who are not experts in this field. Include a brief review of the key literature. If there are relevant controversies or disagreements in the field, they should be mentioned so that a non-expert reader can delve into these issues further. The Introduction should conclude with a brief statement of the overall aim of the experiments and a comment about whether that aim was achieved.

1.1.12 Methods

This section should provide enough detail for reproduction of the findings. Protocols for new methods should be included, but well-established protocols may simply be referenced. Detailed methodology or supporting information relevant to the methodology can be published on our web site.

This section should also include a section with descriptions of any statistical methods employed. These should conform to the criteria outlined by the Uniform Requirements, as follows:

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to judge its appropriateness for the study and to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as P values, which fail to convey important information about effect size and precision of estimates. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the statistical software package(s) and versions used. Distinguish prespecified from exploratory analyses, including subgroup analyses.

Submit detailed protocols for newer or less established methods. Well-established protocols may simply be referenced. Protocol documents for clinical trials, observational studies, and other **non-laboratory** investigations may be uploaded as supporting information.

We recommend and encourage you to deposit **laboratory protocols** in protocols.io, where protocols can be assigned their own persistent digital object identifiers (DOIs).

To include a link to a protocol in your article:

1. Describe your step-by-step protocol on protocols.io
2. Select **Get DOI** to issue your protocol a persistent digital object identifier (DOI)
3. Include the DOI link in the Methods section of your manuscript using the following format provided by protocols.io: http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]

At this stage, your protocol is only visible to those with the link. This allows editors and reviewers to consult your protocol when evaluating the manuscript. You can make your protocols public at any time

by selecting **Publish** on the protocols.io site. Any referenced protocol(s) will automatically be made public when your article is published.

PLOS ONE offers an option for publishing peer-reviewed Lab Protocol articles, which describe protocols hosted on protocols.io articles. Read more information on Lab Protocol articles.

1.1.13 **Results**

The Results section should include all relevant positive and negative findings. The section may be divided into subsections, each with a concise subheading. The Results section should be written in past tense.

PLOS journals require authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception.

Large data sets, including raw data, may be deposited in an appropriate public repository. See our list of recommended repositories.

For smaller data sets and certain data types, authors may provide their data within supporting information files accompanying the manuscript. Authors should take care to maximize the accessibility and reusability of the data by selecting a file format from which data can be efficiently extracted (for example, spreadsheets or flat files should be provided rather than PDFs when providing tabulated data).

For more information on how best to provide data, read our policy on data availability. PLOS does not accept references to "data not shown."

As outlined in the Uniform Requirements, authors that present statistical data in the Results section should do the following:

Give numeric results not only as derivatives (for example, percentages) but also as the absolute numbers from which the derivatives were calculated, and specify the statistical significance attached to them, if any. Restrict tables and figures to those needed to explain the argument of the paper and to assess supporting data. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. Avoid nontechnical uses of technical terms in statistics, such as "random" (which implies a randomizing device), "normal," "significant," "correlations," and "sample."

1.1.14 **Discussion**

The Discussion should be concise and tightly argued. It should start with a brief summary of the main findings. It should include paragraphs on the generalizability, clinical relevance, strengths, and limitations of your study.

You may wish to discuss the following points also:

- How do the conclusions affect the existing knowledge in the field?
- How can future research build on these observations and what are the key experiments that must be done?

1.1.15 **Acknowledgments**

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

PLOS journals publicly acknowledge the indispensable efforts of our editors and reviewers on an annual basis. To ensure equitable recognition and avoid any appearance of partiality, do not include editors or peer reviewers—named or unnamed—in the Acknowledgments.

Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

1.1.16

1.1.17 References

Any and all available works can be cited in the reference list. Acceptable sources include:

- Published or accepted manuscripts
- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., "unpublished work," "data not shown"). Instead, include those data as supplementary material or deposit the data in a publicly available database.
- Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)
- Submitted research should not rely upon retracted research. You should avoid citing retracted articles unless you need to discuss retracted work to provide historical context for your submitted research. If it is necessary to discuss retracted work, state the article's retracted status in your article's text and reference list.

Ensure that your reference list includes full and current bibliography details for every cited work at the time of your article's submission (and publication, if accepted). If cited work is corrected, retracted, or marked with an expression of concern before your article is published, and if you feel it is appropriate to cite the work even in light of the post-publication notice, include in your manuscript citations and full references for both the affected article and the post-publication notice. Email the journal office if you have questions.

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.
Accepted, unpublished articles	Same as published articles, but substitute "Forthcoming" for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1 Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

1.1.18 Supporting information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

Supporting information files are published exactly as provided, and are not copyedited.

1.1.18.1 Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

Example caption

S1 Text. Title is strongly recommended. Legend is optional.

1.1.18.2 n-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the supporting information guidelines for more details about submitting supporting information and multimedia files.

1.1.19 Figures and Tables

1.1.19.1 Figures

You can include figures in the main manuscript file at initial submission. If the manuscript reaches the revise stage, prepare and submit each figure as an individual file.

Cite figures in ascending numeric order at first appearance in the manuscript file.

The instructions on this page pertain to figures included in the main article.

***PLOS Neglected Tropical Diseases* waives all formatting requirements until your manuscript has received a provisional Editorial Accept decision.** In order to proceed to publication, your figures must meet the requirements on this page. The more closely your figures adhere to these specifications, the more quickly your manuscript can be published once accepted.

Figures as Supporting Information

Supporting information is auxiliary to the main content of the article. Supporting information figures are held to the requirements of all supporting information files. They have fewer requirements than figures that are included in the main article, and they need to be uploaded separately.

For full instructions, follow the supporting information guidelines.

Figure Preparation Checklist

- Read our figure policies on **depictions of humans**, **licenses and copyright**, and **image manipulation**.
- Read the **figure file requirements** for the full list of technical specifications, and ensure your figures comply.
- Read **how to format and submit your figures and captions** for peer review.
- **Use PACE** before submitting to check your figures and convert to our accepted formats.

Figure File Requirements

The list below is an abbreviated summary of the figure specifications. Read the full details of the requirements in the corresponding sections on this page.

File Format	TIFF or EPS
Dimensions	Width: 789 – 2250 pixels (at 300 dpi). Height maximum: 2625 pixels (at 300 dpi).
Resolution	300 – 600 dpi
File Size	<10 MB
Text within Figures	Arial, Times, or Symbol font only in 8-12 point
Figure Files	Fig1.tif, Fig2.eps, and so on. Match file name to caption label and citation.
Captions	In the manuscript, not in the figure file.

Read the guidelines for figures.

1.1.19.2

1.1.19.3 Figure captions

Insert figure captions in manuscript text, immediately following the paragraph where the figure is first cited (read order). Don't include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

- A figure label with Arabic numerals, and "Figure" abbreviated to "Fig" (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of "Fig 1" must refer to a figure file named "Fig1.tif").
- A concise, descriptive title

The caption may also include a legend as needed.

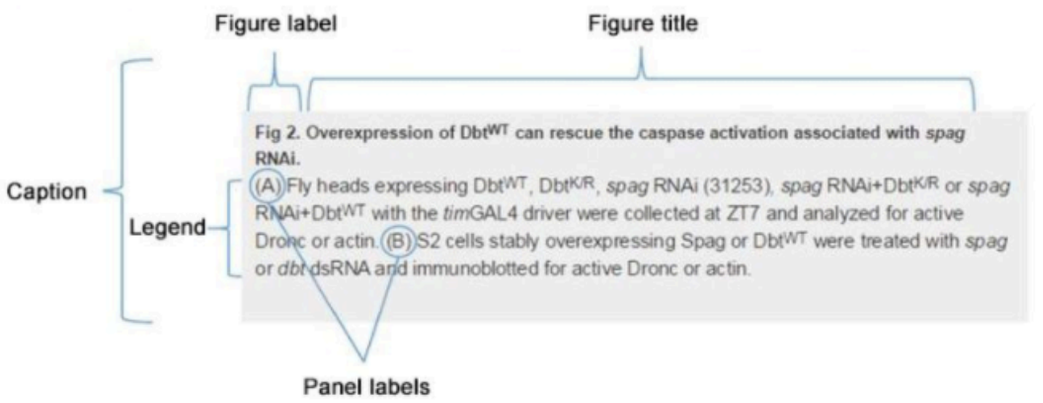
- **Place** figure captions in the manuscript text in read order, immediately following the paragraph where the figure is first cited. Do not include captions as part of the figure files or submit them in a separate document.

- **Format** your figure captions. There are two required elements: figure label and figure title. Legends are optional.

  Label. Name figure labels using Arabic numerals, and abbreviate the word "Figure" to "Fig" (e.g., Fig 1, Fig 2, Fig 3, etc.).

  Title. The title should be concise and descriptive. Restrict it to 15 words or less.

  Legend. Place the legend directly after the title of the figure to which it belongs. Place any figure credits in the last sentence of the legend.



Caption credit: Means JC, Venkatesan A, Gerdes B, Fan J-Y, Bjes ES, Price JL (2015) Drosophila Spaghetti and Doubletime Link the Circadian Clock and Light to Caspases, Apoptosis and Tauopathy. PLoS Genet 11(5): e1005171. https://doi.org/10.1371/journal.pgen.1005171

1.1.19.4 Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., "Table 1") and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Read the guidelines for tables.

1.1.20

1.1.21 Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

Read our policy on data availability.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories

that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

See our list of recommended repositories.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

- Deposit data in the integrated repository of choice.
- Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.
- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

1.1.22 Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

Accession numbers (and version numbers, if appropriate) should be provided in the Data Availability Statement. Accession numbers or a citation to the DOI should also be provided when the data set is mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at full submission.

1.1.22.1 Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
- PubChem

Identifiers should be provided in parentheses after the entity on first use.

1.1.23 Small and macromolecule crystal data

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of recommended repositories).

1.1.23.1 Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

1.1.23.2 Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

1.1.24 Striking image

You can upload a visually striking image alongside your submission, which we may use to showcase your article through PLOS' online channels. We choose the monthly issue image from the striking images submitted with articles scheduled for publication.

1.1.24.1 Submission Criteria

- Choose an image that represents the article in a striking and eye-catching way.
- It can be derived from a figure or supporting information file from the paper, and it may be a cropped portion of an image or the entire image.
- Alternatively, you can create or source an image, as long as it adheres to our CC BY license.
- High resolution: between 300-600 dpi
- Single panel
- Ideally avoid added details like text, scale bars, and arrows.

1.1.24.2 How to Submit

1. Submit your striking image to the submission system using the file type "Striking Image".
2. Upload a separate file with the corresponding caption.

If no striking image is uploaded, a member of the journal team will choose an appropriate image, which may be a figure from the submission or a separately sourced CC BY image.

Striking images should not contain potentially identifying images of people. Read our policy on identifying information.

The PLOS licenses and copyright policy also applies to striking images.

1.2 ADDITIONAL INFORMATION REQUESTED AT SUBMISSION

1.2.1 **Financial Disclosure Statement**

This information should describe sources of funding that have supported the work. If your manuscript is published, your statement will appear in the Funding section of the article.

Include your statement in the Financial Disclosure section of the initial submission form.

The statement should include:

- Specific grant numbers
- Initials of authors who received each award
- URLs to sponsors' websites

Also state whether any sponsors or funders (other than the named authors) played any role in:

- Study design
- Data collection and analysis
- Decision to publish
- Preparation of the manuscript

If they had no role in the research, include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript."

If the study was unfunded, include this sentence as the Financial Disclosure statement: "The author(s) received no specific funding for this work."

Read our policy on disclosure of funding sources.

1.2.2

1.2.3 **Competing interests**

The corresponding author is asked at submission to declare, on behalf of all authors, whether there are any financial, personal, or professional interests that could be construed to have influenced the work.

Any relevant competing interests of authors must be available to editors and reviewers during the review process and will be stated in published articles.

Read our policy on competing interests.

1.2.4

1.2.5 **Related manuscripts**

When submitting a manuscript, all authors are asked to indicate that they do not have a related or duplicate manuscript under consideration (or accepted) for publication elsewhere. If related work has been or will be submitted elsewhere or is in press elsewhere, then a copy must be uploaded with the article submitted to PLOS. Reviewers will be asked to comment on the overlap between related submissions.

Read our policies on related manuscripts.

1.2.6

1.2.7 **Preprints**

PLOS encourages authors to post preprints to accelerate the dissemination of research. Posting a manuscript on a preprint server does not impact consideration of the manuscript at any PLOS journal.

Authors posting preprints on bioRxiv or medRxiv can choose to concurrently submit their manuscripts to relevant PLOS journals through the direct transfer service.

Authors submitting manuscripts in the life and health sciences to *PLOS Neglected Tropical Diseases* may choose to have PLOS forward their submission to bioRxiv or medRxiv, depending on the scope of the paper, for consideration for posting as a preprint.

Read more about preprints.

Learn how to post a preprint to bioRxiv or medRxiv at *PLOS Neglected Tropical Diseases*.

1.2.8

1.2.9 Reviewer and editor suggestions

We ask authors to suggest suitable editors and at least four potential reviewers when submitting their manuscript. Bear in mind any potential competing interests when making these suggestions. It is not appropriate to suggest recent collaborators or other researchers at your institution. See our policy on competing interests for more information.

1.3 GUIDELINES FOR SPECIFIC STUDY TYPES

1.3.1 Systematic reviews and meta-analyses

Submissions with systematic reviews and meta-analyses are considered research articles. Submit these manuscripts with the "Research Article" type in the submission system.

Reports of systematic reviews and meta-analyses must adhere to the PRISMA Statement or alternative guidelines appropriate to the study design, and include the completed checklist and flow diagram to accompany the main text. Authors must complete the appropriate reporting checklist not only with page references, but also with sufficient text excerpted from the manuscript to explain how they accomplished all applicable items.

Download blank templates of the checklist and flow diagram from the EQUATOR web site.

Abstracts should follow PRISMA for Abstracts, using the PLOS abstract format. Authors must also state within the Methods section of their paper whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information.

The journal supports the prospective registration of systematic reviews. Authors whose systematic review was prospectively registered (e.g., in a registry such as PROSPERO) should provide the registry number in their abstract. Registry details and protocols will be made available to editors and reviewers, and included with the paper if the report is ultimately published.

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ANEXO

ANEXO A – Author contributions

All authors contributed to the study design. VCP and GCSS did the systematic literature search, drafting and selection of articles included in this study, GCSS participated in the preliminary data analysis, while VCP participated in the entire analysis process. LAS and CAF guided the development of the article and reviewed all the information collected and analyzed. All authors participated in the interpretation and discussion of the results, as well as in the writing of the article.

ANEXO B – Cover Letter

To the Editor-in-Chief,

Please find attached our manuscript entitled **"RISK FACTORS ASSOCIATED WITH SEVERE DENGUE IN LATIN AMERICA: A SYSTEMATIC REVIEW AND META-ANALYSIS"**. In this manuscript we performed a systematic literature review and meta-analysis to identify the risk factors for severe dengue and others severe outcomes, such as death and hospitalization due to dengue. A major challenge of DENV surveillance and diagnosis is that the virus can produce asymptomatic infections and a spectrum of clinical illnesses that range from mild febrile illness to fatal illness. Dengue is one of the main causes of hospitalization and death in some Latin American countries, especially among children. Dengue is a neglected disease, and its occurrence is associated with tropical and subtropical climates. Considering this, it is important to identify risk factors associated with severe dengue, death, and hospitalization so that measures can be taken to avoid life-threatening complications. We identified secondary dengue infection, female gender, white or Caucasian ethnicity, headache, myalgia and/or arthralgia, vomiting/nausea, abdominal pain or tenderness, diarrhea, prostration,

lethargy, fatigue or similar as risk factors associated with the outcome of severe dengue. For the death outcome, vomiting/nausea and < 18 years old were identified as risk factors, while females, tourniquet test +, platlet count < 100,000 μL and rash, petechiae, exanthema, hematomas and/or ecchymoses had lower chances of dying from dengue. The risk factors for the hospitalization outcome were < 18 years old and > 60 years old. In this way, we believe that these results will help to define strategies and management of patients infected with dengue in Latin America, a region greatly affected by the disease, and affirm the importance of interpreting certain tests, signs and symptoms to reduce the chances of death.

We would be grateful if you would consider it for publication in the **P*LOS Neglected Tropical Diseases***. We confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere. The authors declare no conflicts of interest.

Best regards.

ANEXO C - Funding

The authors did not receive financial support for the research.

ANEXO D - Competing interests

The authors declare have no conflicts of interest.

ANEXO F – PRISMA checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	7
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	8
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	10 - 11
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	11
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	13
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	12 – 13
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	12 – 13
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	13 – 14
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	13 – 14
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	13 – 14
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	13 – 14
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	14
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	13 – 14

Section and Topic	Item #	Checklist item	Location where item is reported
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	14
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	14 – 15
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	14 – 15
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	14
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	14
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	14 – 15
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	14 – 15
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	14 – 15
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Fig 1; 15
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Fig 1
Study characteristics	17	Cite each included study and present its characteristics.	Table 1; 16 - 18
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Table 1; 16
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Fig 3, 4, 6, 8; 27 – 36
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Table 1; 16 – 18
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Fig 3, 4, 6, 8; 27 - 36
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	42 – 43
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Fig 3, 4, 6, 8; 16

Section and Topic	Item #	Checklist item	Location where item is reported
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Table 1; 16
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Fig 3, 4, 6, 8; 27 - 36
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	36 – 43
	23b	Discuss any limitations of the evidence included in the review.	42 – 43
	23c	Discuss any limitations of the review processes used.	42 – 43
	23d	Discuss implications of the results for practice, policy, and future research.	43
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	9, 12
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	12
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	12
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	80
Competing interests	26	Declare any competing interests of review authors.	80
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Not applicable

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