Vaccine 37 (2019) 5891–5898

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Feasibility of case-control and test-negative designs to evaluate dengue vaccine effectiveness in Malaysia

Joshua Nealon [a,*], Wei-Yin Lim [b,#], Annick Moureau [c], Sharon Linus Lojikip [b], Suria Junus [b], Suresh Kumar [d], Jeyaseelan P. Nachiappan [e], Shamala Devi Sekaran [g], Cedric Radigue [a], Benjamin J. Cowling [h], R. Leon Ochiai [c], Amar-Singh HSS [b]

[a] Sanofi Pasteur Asia and JPAC Region, 38 Beach Road # 18-11, Singapore
[b] Clinical Research Centre Perak, Hospital Raja Permaisuri Bainun, Ministry of Health Malaysia, Raja Ashman Shah Road, 30450 Ipoh, Perak, Malaysia
[c] Sanofi Pasteur, Lyon, France
[d] Department of General Medicine, Hospital Sungai Buloh, Ministry of Health Malaysia, Hospital Road, 47000 Sungai Buloh, Selangor, Malaysia
[e] Department of Paediatrics, Hospital Raja Permaisuri Bainun, Ministry of Health Malaysia, Raja Ashman Shah Road, 30450 Ipoh, Perak, Malaysia
[g] Department of Microbiology, Faculty of Medicine, MAHSA University, Bandar Saujana Putra, 42610 Jenjarom, Selangor, Malaysia
[h] School of Public Health, The University of Hong Kong, Hong Kong Special Administrative Region, China

ARTICLE INFO

Article history:
Received 24 January 2019
Received in revised form 9 July 2019
Accepted 24 July 2019
Available online 21 August 2019

Keywords:
Dengue
Vaccine effectiveness
Malaysia
Case-control

ABSTRACT

Background: The world's first dengue vaccine [Dengvaxia; Sanofi Pasteur] was licensed in 2015 and others are in development. Real-world evaluations of dengue vaccines will therefore soon be needed. We assessed feasibility of case control (CC) and test-negative (TN) design studies for dengue vaccine effectiveness by measuring associations between socio-demographic risk factors, and hospitalized dengue outcomes, in Malaysia.

Methods: Following ethical approval, we conducted hospital-based dengue surveillance for one year in three referral hospitals. Suspected cases aged 9–25 years underwent dengue virological confirmation by RT-PCR and/or NS1 Ag ELISA at a central laboratory. Two age- and geography-matched hospitalized non-dengue case-controls were recruited for a traditional CC study. Suspected cases testing negative were test-negative controls. Socio-demographic, risk factor and routine laboratory data were collected. Logistic regression models were used to estimate associations between confirmed dengue and risk factors.

Results: We recruited 327 subjects; 155 were suspected of dengue. The planned sample size was not met. 124 (80%) of suspected cases were dengue-confirmed; seven were assessed as severe. Three had missing RT-PCR results; the study recruited 28 test-negative controls. Only 172 matched controls could be recruited; 90 cases were matched with ≥1 controls. Characteristics of cases and controls were mostly similar. By CC design, two variables were significant risk factors for hospitalized dengue: recent household dengue contact (OR: 54, 95% CI: 7.3–397) and recent neighbourhood insecticidal fogging (OR: 2.1; 95% CI: 1.3–3.6). In the TN design, no risk factors were identified. In comparison with gold-standard diagnostics, routine tests performed poorly.

Conclusions: The CC design may be more appropriate than the TN design for hospitalized dengue vaccine effectiveness studies. Selection bias in case control selection could be minimized by protocol changes more easily than increasing TN design control numbers, because early-stage dengue diagnosis in endemic countries is highly specific. MREC study approval: (39)KKM/NIHSEC/P16-1334.

1. Background

Dengue, a mosquito-borne flavivirus, causes around 100 million clinical episodes, and likely results in 10.5 million hospitalizations annually, mostly in Asia [1–3]. The disease has a wide and unpredictable range of clinical presentations, from mild/asymptomatic flu-like illness, progressing to acute, febrile, and severe/haemorrhagic disease and rarely, death [4,5]. Risk factors for severe outcomes may include the presence of heterologous antibodies from a previous infection, viral characteristics, and the age and genetic background of the infected human host [6]. Population-level risk

* Corresponding author.
E-mail address: Joshua.nealon@sanofi.com (J. Nealon).
Current affiliation: Centre for Clinical Epidemiology, Institute for Clinical Research, National Institutes of Health, Ministry of Health Malaysia.

https://doi.org/10.1016/j.vaccine.2019.07.083
0264-410X/

factors include urbanization, high population density, and presence of *Aedes* mosquito vector breeding sites [7].

The world's first dengue vaccine [Dengvaxia; Sanofi Pasteur] was licensed in 2015 and has since been introduced in Asia and Latin America [8]. A number of other dengue vaccines are in clinical development and evaluations of the real-world performance of dengue vaccines will therefore soon be needed [8–10]. A workshop of international experts took place in 2014 to discuss the underlying principles; participants agreed that case-control (CC) and test-negative (TN) designs should be considered for this purpose [11].

CC studies are established methodologies for assessing associations between vaccine exposure and infectious disease outcomes including for influenza [12]; Japanese encephalitis [13]; whooping cough [14], and pneumococcal pneumonia [15]. For dengue, CC studies have been used to evaluate individual- and population-level risks factors [16,17]. The TN design is a variant of the CC study whereby suspected cases with negative laboratory results – and who are therefore considered absent of the outcome of interest – are used as controls, and has been used extensively for evaluating the effectiveness of influenza vaccines [18–21] and other vaccines [22–24]. The TN design has advantages in reducing bias in control recruitment, and has been used to understand dengue risk factors [25].

Post-licensure dengue vaccine effectiveness studies have not been published and, given the clinical and epidemiological specificities of dengue, challenges may be expected which warrant preparatory study [26]. These include the ability to recruit dengue patients satisfying relevant case definitions; laboratory capacity to confirm infection with adequate specificity and sensitivity; and the practical infrastructure to identify and recruit suitable control subjects.

In Malaysia, dengue outbreaks occur nationwide with increased risk in urban and *peri*-urban areas. Peaks in transmission often coincide with rainfall but cases occur year-round and reported cases have doubled since 2010 [27,28]. Although cases showed some reduction in 2016, the disease was highlighted in the Eleventh Malaysia plan of 2016–2020 to expand health promotion programmes for communicable diseases, which aims primarily to mitigate dengue risk [29]. Dengvaxia was granted a two-year conditional registration in October 2016 by the Drug Control Authority of Malaysia for post-registration study, with conditions to monitor long term risks, safety and efficacy over a wider population [30].

To prepare for vaccine introduction, we assessed the feasibility of conducting traditional CC and TN studies for dengue vaccine effectiveness evaluation, in Malaysia, by measuring associations between socio-demographic and environmental risk factors and dengue outcomes. We considered hospitalized/severe dengue as policy-relevant and specific endpoints, so we conducted hospital-based dengue surveillance for a period of one year, matching cases to control subjects who were hospitalized for a non-dengue condition. We assessed feasibility of recruitment, logistics, and laboratory confirmation as well as likely biases and potential remedies to minimize them to improve the design of future dengue vaccine effectiveness studies.

2. Methods

2.1. Ethical approval for study

This study was conducted in accordance with the Declaration of Helsinki [31], the Guidelines for Good Epidemiology Practices [32] and local regulatory requirements. Before subjects were enrolled the protocol and study documents were approved by the Medical Research and Ethics Committee of the Ministry of Health, Malaysia (study approval: (39)KKM/NIHSEC/P16-1334). Institutional approval was obtained from each Hospital Director and relevant Head of Department before data collection commenced.

2.2. Study design

Prospective, hospital-based enhanced surveillance. Suspected dengue cases who were laboratory-confirmed were enrolled as cases and matched to two hospitalized non-dengue, age- and geography-matched controls, to conduct the traditional CC study. Laboratory-confirmed dengue cases were considered test-positive cases for the TN study; suspected cases testing negative were considered as the TN controls.

2.3. Study sites and population

Surveillance for suspected dengue cases starting from October 2016, over a period of 12 months, among hospitalized patients at three Malaysian study sites: (1) Raja Permaisuri Bainun Hospital, Ipoh, Perak; (2) Selayang Hospital, Selangor; and (3) Sungai Buloh Hospital, Selangor. Study sites are large, tertiary care hospitals operating within the Malaysian Ministry of Health system. Two (Selayang and Sungai Buloh Hospitals) are located within large urban areas and one (Ipoh Hospital) is located in a smaller, more *peri*-urban city. The hospitals accept referrals from health districts within their catchment areas, ranging from 16 km to 76 km. Nonetheless, patients living outside of the catchment areas (in regional and rural areas) may be referred for tertiary care services. An estimated 1000–2000 febrile cases are seen in these hospitals each month. All hospitals are centres of excellence for dengue, treating several thousand hospitalized dengue cases, annually.

Study subjects were classified according to the following case definitions:

- Suspected dengue: patients on whom the attending clinician makes a diagnosis of probable dengue according to clinical history, physical examination and results of routine diagnostic tests which may have been used.
- Virologically-confirmed dengue (VCD): suspected dengue cases that are virologically confirmed by the central laboratory by dengue RT-PCR and/or NS1 antigen (Ag) ELISA.
- Severe dengue: a patient presenting with fever of 2–7 days plus any of the following: severe plasma leakage, severe haemorrhage or severe organ impairment, as derived from raw clinical data, based on WHO 2009 definitions [4].
- Case-controls: non-dengue patients, age- and geographically-matched to VCD cases.
- TN controls: suspected dengue cases who tested negative for dengue by both RT-PCR and NS1 Ag.

2.4. Inclusion criteria for cases and controls

Inclusion criteria for suspected dengue were: age 9–25 years; acutely ill and suspected of dengue infection; admitted to the study hospital within 5 days of fever onset; resident of the hospital catchment area. Due to low case enrolment, a protocol amendment was approved on 31st July 2017, extending the recruitment window to within 7 days of fever onset.

For each laboratory-confirmed dengue case, study teams attempted to identify two hospitalized, matched case-controls. Inclusion criteria were: hospitalized in the same hospital as cases; with no suspicion of dengue infection; with a final diagnosis other than dengue; admission within one month (before or after) of the laboratory confirmation of the case. The last control subject was enrolled on 3rd December 2017. Controls were age-matched to cases in three age groups: 9–12 years; 13–17 years; and 18–25 years; and geographically-matched based on the catchment areas of district health offices (*Pejabat Kesihatan Daerah*).

2.5. Subject screening and enrolment

Screening from medical and paediatric wards was performed during weekdays and within working hours by the study coordinators. Eligibility was assessed based on clinical history, physical examination and following discussions with attending physicians. Typically in Malaysia, individuals are suspected of dengue based on clinical signs and symptoms and, at these referral centres, it is likely that most subjects already received either IgM/IgG and/or NS1 Ag rapid diagnostic tests (RDT) and/or a previous clinical diagnosis of dengue at primary care clinics or hospital emergency departments. Children suspected of dengue are typically admitted, whereas adults will be hospitalized following a poor or worsening clinical condition.

Suspected dengue cases were screened by study coordinators and principal investigators for other inclusion criteria before being invited to join the study. Informed consent and assent forms, available in English, Malay, Tamil, and Chinese languages, were reviewed and signed by subjects and parents of subjects aged <18 years. Subjects' identification cards (18 years and above) and birth certificates (below 18 years old) were collected to verify legal relationships, as required by the Medical Research and Ethics Committee.

2.6. Data collection and laboratory analysis

Following enrolment, a standardized questionnaire was administered by study staff which collected socio-demographic information, reported dengue histories of subjects and household contacts, other risk factor data (e.g., household and neighbourhood vector control practices; time spent outdoors) and flavivirus vaccination history. Final discharge diagnoses, made by attending physicians based on routine clinical practice, were retrieved from electronic medical records upon discharge, verified by the investigator and recorded.

For suspected cases, during routine blood sampling in the wards, an additional aliquot of 5 mL venous blood was collected. Blood was kept at room temperature for 30–60 min (or refrigerated at 2–8 °C for $\leq$24 h) before centrifugation. Serum was transferred into two 650 μL aliquots, frozen at −20 °C and shipped in dry ice to the central laboratory, the Department of Medical Microbiology, University of Malaya Medical Center in Kuala Lumpur. Virological confirmation of dengue was by RT-PCR and NS1 Ag ELISA. RNA extraction was performed using Roche High Pure viral RNA extraction kit; RNA purity and concentration were assessed by spectrophotometry. One step real-time Sybr Green RT-PCR was performed using Bio-rad iTaq universal one step Sybr Green premix and in-house designed primers [33]. The SD Dengue NS1 ELISA kit was used according to the manufacturer's instruction.

The results of routinely-performed dengue diagnostic testing, which could include RDTs and ELISAs detecting IgM, IgG and NS1 Ag, before or during hospitalization, were recorded.

All data were entered into an electronic database by study teams and verified through computerized logic and consistency checks to detect errors or omissions.

2.7. Sample size

In the context of vaccine effectiveness study preparation the sample size was based on a hypothetical effectiveness objective comparing the odds ratio (OR) of having a virologically confirmed, hospitalized dengue episode between vaccinees and non-vaccinees, assuming a power of 80%, a two-sided alpha of 5%, vaccine coverage of 50%, and an expected vaccine effectiveness of 50%. Assuming 70% of suspected cases test positive, the TN design would require 223 cases and 96 TN controls. A CC design would require 88 cases and 352 controls (with a case:control ratio of 1:4) or 110 cases and 220 controls (with a 1:2 ratio). Expecting a minimum of 20% non-evaluable cases, a target of 300 confirmed cases (meaning 400 suspected cases) and 600 controls was planned. Targets were provided for each site to enrol equal numbers, stratified into age categories, resulting in a total of 100 suspected cases aged 9–12 years, 100 aged 13–17 years, and 200 aged 18–25 years.

2.8. Statistical analysis

We compared socio-demographic characteristics of VCD cases and controls enrolled for both designs. Univariate logistic regression models were used to estimate associations between confirmed dengue and risk factors using the CC (in which only subjects with at least 1 matched control was included, by conditional logistic regression) and TN study designs. Variables with a P-value <0.2 on univariate analysis were included in a final multivariable model and were backward-selected to retain in the model at a P-value of <0.05.

Dengue discharge diagnoses were compared with WHO 2009 case definitions, including severity assessment, as derived from subjects' clinical data [4]. The sensitivity and specificity of each diagnostic test used in routine practice were calculated using RT-PCR and/or NS1 Ag ELISA positive test results as the reference standard, with confidence intervals computed using the normal approximation method.

All statistical analyses were performed with SAS 9.4 using Enterprise Guide 5.1 software or later.

3. Results

3.1. Characteristics of study subjects

Fig. 1 is a study flow chart. The study recruited 327 subjects; the mean age was 18 (SD 4.2) years for VCD cases, 18 (SD 3.7) for TN controls, and 19 (SD 4.3) for case-controls. There were 155 subjects suspected of dengue within 5 days of fever of whom 18 were aged 9–12 years; 48 were aged 13–17 years, and 89 were aged 18–25 years. The planned sample size was therefore not met in any age group. Many suspected cases were ineligible to participate because they were not aged 9–25 years old; had experienced onset of fever >5 days previously; parents were unavailable to provide informed consent and/or birth certificates. Following protocol amendment, ten suspected dengue cases were enrolled, admitted between 5 and 7 days of fever, two of whom were VCD. Due to the low impact on overall results, these subjects were not considered in further analyses. Table 1 summarises the socio-demographic characteristics of study subjects. Of the 155 suspected dengue cases, 124 (80%) were VCD. Three subjects had missing RT-PCR results and the study therefore recruited 28 TN controls. To match 124 confirmed dengue cases in a 1:2 ratio, 248 controls were required. A total of 172 matched controls were recruited and some cases therefore lacked controls: 90 cases were matched with 1 or 2 controls. Time between case and matched control recruitment was on average 74 days.

3.2. Dengue risk factors – univariate analysis

The characteristics of cases and controls were similar in terms of most baseline clinical characteristics, individual dengue history and educational and socio-demographic dengue risk factors (Table 1; complete table of risk-factors in supplementary Table S1). Differences were observed in the sex distribution: of VCD cases, 43 (34.7%) were female in comparison with 98

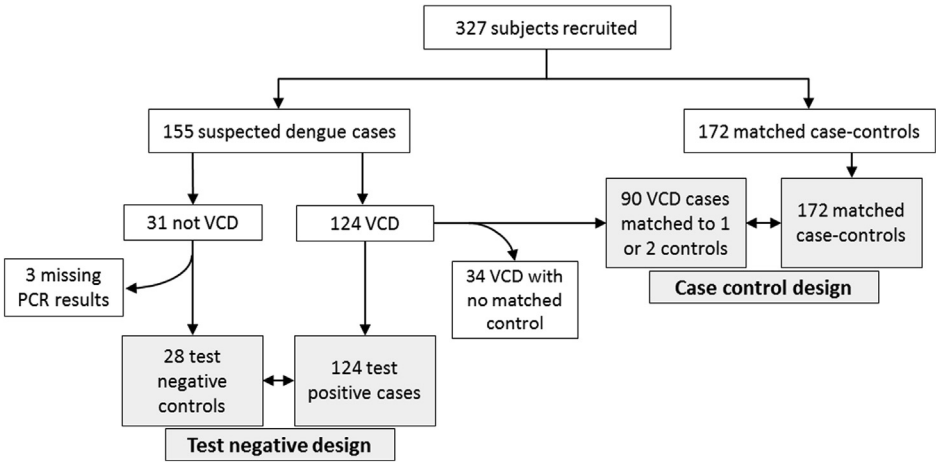

Fig. 1. Study flow chart. VCD = virologically confirmed dengue.

Table 1
Numbers (%; SD for Mean age) of subjects with different socio-demographic characteristics and risk factors recruited as VCD cases and case- or test-negative controls. P-values are in bold font with ORs below, vs the reference category.

	Case-control design			**Test-negative design**		
	VCD cases[^]	**Case-controls**	**P-value** OR (95% CI)	**VCD cases**	**Test-negative controls**	**P-value** OR (95% CI)
N	90	172		124	28	
Mean age, years (SD)	18 (4.3)	19 (4.3)		18 (4.2)	18 (3.7)	
Sex			**0.003**			**0.181**
M	57 (63)	74 (43)	Ref	81 (65)	22 (79)	Ref
F	33 (37)	98 (57)	0.4 (0.3; 0.8)	43 (35)	6 (21)	1.9 (0.7; 5.2)
Site[#]			–			**0.1697**[*]
Ipoh, Perak	31 (34)	59 (34)	–	45 (36)	5 (18)	Ref
Selayang, Selangor	32 (36)	62 (36)	–	44 (36)	14 (50)	0.3 (0.1; 1.1)
Sungai Buloh, Selangor	27 (30)	51 (30)	–	35 (28)	9 (32)	0.4 (0.1; 1.4)
Education level			**0.270**			**0.110**[*]
No formal or primary	5 (5.6)	14 (8.1)	Ref	10 (8)	2 (7.1)	Ref
Secondary	58 (64.4)	114 (66)	4.4 (0.5; 37)	76 (61)	23 (82)	0.7 (0.1; 3.2)
Tertiary	27 (30.0)	44 (26)	5.6 (0.6; 50)	38 (31)	3 (101)	2.5 (0.4; 17)
Type of dwelling			**0.135**[*]			**0.589**
Individual house	58 (64)	127 (74)	Ref	82 (66)	17 (61)	Ref
Apartment/flat/others	32 (36)	45 (26)	1.5 (0.9; 2.6)	42 (34)	11 (39)	0.8 (0.3; 1.8)
Number of family members in household			**0.596**			**0.224**
≤3	11 (12)	31 (18)	Ref	14 (11)	7 (25.0)	Ref
4–5	32 (36)	54 (31)	1.8 (0.8; 4.2)	44 (36)	11 (39)	2 (0.7; 6.1)
6–7	31 (34)	59 (34)	1.6 (0.7; 3.7)	45 (36)	7 (25)	3.2 (1; 10.7)
≥8	16 (18)	28 (16)	1.7 (0.7; 4.3)	21 (17)	3 (10.7)	3.5 (0.8; 16)
Household member diagnosed with dengue within the past month?			**<0.001**[*,z]			**0.630**
No	62 (69)	171 (99)	Ref	92 (74)	22 (79)	Ref
Yes	28 (31)	1 (0.6)	54 (7.3397)	32 (26)	6 (21)	1.3 (0.5; 3.4)
Subject previously diagnosed with dengue?			**0.589**			**0.202**
Yes	12 (13)	19 (11)	Ref	15 (12)	6 (21)	Ref
No	78 (87)	153 (89)	0.8 (0.4; 1.8)	109 (88)	22 (79)	2 (0.7; 5.7)
Average time spent outdoors, daily (hours)			**0.213**			**0.280**
<4 h	17 (19)	19 (11)	Ref	23 (19)	3 (11)	Ref
4 h ≤ time < 8 h	57 (63)	122 (71)	0.5 (0.2; 1.1)	77 (62)	16 (57)	0.6 (0.2–2.3)
≥8 h	16 (18)	31 (18)	0.6 (0.2–1.4)	24 (19)	9 (32)	0.3 (0.1–1.4)
Insecticidal fogging in neighbourhood in the past month?			**0.0049**[*,z]			**0.174**[*]
No	39 (43)	108 (63)	Ref	55 (45)	16 (59)	Ref
Yes	51 (57)	64 (37)	2.1 (1.3–3.6)	68 (55)	11 (41)	1.8 (0.8–4.2)

[#] Study site not included in CC design because controls were matched to cases based on site.
[^] Includes only cases with ≥1 matched control.
[*] Variables included in multivariate model.
[z] Variables retained in final multivariate model.
Ref = reference category.
Columns totals may vary due to lack of responses; or not equal 100% due to rounding.

(57.0%) case-controls and 6 (21.4%) TN controls. There were also differences in reported recent dengue history in the household (32 [25.8%] cases; 6 [21.4%] TN controls and one [0.6%] case control) and reports of recent neighbourhood fogging (68 [55.3%] VCD; 11 [40.7%] TN controls and 64 [37.2%] case-controls). Previous flavivirus vaccination was rare: only one subject reported having received a yellow fever vaccine.

3.3. Utility of case-control and test-negative design for risk factor identification

In the CC study, only the 90 VCD cases with at least one matched control were included in the analysis. Two variables remained significant in the final model: respondents who reported a recent household dengue contact (OR: 54; 95% CI: 7.3–397; P < 0.001) and those reporting neighbourhood insecticidal fogging in the last month (OR: 2.1; 95% CI: 1.3–3.6; P = 0.005) were associated with an increased risk of hospitalized dengue as compared to subjects without household dengue contacts or neighbouring fogging. In the TN analysis, no risk factors were identified. This might be partially a result of the number of controls (n = 28), resulting in imprecise estimates. No risk factors associated with severe VCD could be calculated as the number of severe dengue cases was too small (n = 7).

3.4. Dengue severity

Of the 124 hospitalized VCD cases, according to discharge diagnoses 69 (55.6%) were dengue fever (clinically/serologically diagnosed); one (0.8%) was dengue fever (virologically confirmed); 53 (42.7%) were dengue with warning signs and one (0.8%) was severe dengue. According to WHO 2009 criteria, classified from clinical data, seven (5.6%) were severe, and 117 were non-severe. The seven cases classified as severe presented with severe bleeding, mainly epistaxis and gum bleeding, either at admission (4 subjects) and/or during the hospitalization (3 subjects). The one case who was additionally diagnosed as severe also presented with severe plasma leakage. No severe organ impairment was observed.

3.5. Routine laboratory diagnosis of dengue

The most commonly-used dengue confirmatory test in routine practice was the NS1 Ag RDT, in 148 (95.5%) of 155 suspected cases, followed by the IgM RDT (110; 71.0%) and the IgG RDT (109; 70.3%). The IgM ELISA, IgG ELISA and NS1 ELISA were used in 45 (29.0%), 31 (20.0%) and 2 (1.3%) subjects, respectively. The NS1 Ag RDT correctly identified 108 of the 118 VCD cases on which the test was used, a sensitivity of 91.5% (95% CI 86.5–96.6%). However, 14 of 27 negative samples were incorrectly classified as positive, giving a specificity of 48.1% (29.3–67.0%). IgM rapid tests correctly identified 8 out of 87 VCD cases, a sensitivity of 9.2% (3.1–15.3%) and specificity of 81.0% (64.2–97.7%; correctly identifying 17 of 21 negative cases). The IgM ELISA had a sensitivity of 47.2% (30.9–63.2%; 17/36 VCD cases positive) and specificity of 25.0% (0–55.0%; 2/8 negative cases correctly identified). The NS1 Ag ELISA misclassified both VCD cases on which it was used as dengue negative (Table 2).

4. Discussion

We aimed to assess feasibility in recruitment, logistics and laboratory confirmation of a traditional CC or TN design to evaluate dengue vaccine effectiveness in Malaysia. The study also aimed to assess biases, stemming primarily from the methods of control recruitment and misclassification of disease and vaccine status [34]. We considered that such an assessment was needed because many of these aspects depend on the characteristics of specific pathogens and healthcare systems and will therefore be different for dengue than for other vaccine-preventable disease studies in the past [26]. Primarily due to low levels of TN design control recruitment and selection bias resulting in unbalanced case and control populations in the CC study, it is likely that protocol changes would be required before embarking on a hospitalized dengue effectiveness evaluation. Selection bias in case control selection could potentially be minimized whereas low recruitment of TN design controls will likely persist in current healthcare settings where dengue diagnoses prior to hospitalization are specific. Key challenges and possible solutions are provided in Table 3.

4.1. Identified dengue risk factors

The exposures under assessment were a selection of sociodemographic and behavioural risk factors which were generally well-matched between cases and controls, and were therefore not identified as risk factors in multivariable models. Two risk factors were identified with the CC method: living with household members recently diagnosed with dengue (OR: 54), and neighbourhood insecticidal fogging conducted in the last month (OR: 2.1). Biologically plausible explanations could explain these findings: case-contacts may be more likely than other individuals to become infected with dengue due to geographical clustering of cases; [35] and it may be reasonable to suggest that insecticidal fogging is directed towards outbreak-prone areas. Alternatively, recall or reporting bias may be responsible: perhaps hospitalized

Table 2
Results (number of subjects) of diagnostic tests used in routine practice and confirmed VCD using the gold standard of PCR and/or NS1 ELISA; and resulting sensitivity and specificities. RDT = rapid diagnostic test. ND = not done.

Test	Result	VCD Positive	Negative	Sensitivity, %	Specificity, %
NS1 Ag, RDT	Positive	108	14	91.5 (86.5; 96.6)	48.1 (29.3; 67.0)
	Negative	10	13		
	ND	6	1		
IgM, RDT	Positive	8	4	9.2 (3.1; 15.3)	81 (64.2; 97.7)
	Negative	79	17		
	ND	37	7		
NS1 Ag, ELISA	Positive	0	0	–	–
	Negative	2	0		
	ND	122	28		
IgM, ELISA	Positive	17	6	47.2 (30.9; 63.5)	25 (0; 55)
	Negative	19	2		
	ND	88	20		

Table 3
Requirements for a dengue vaccine effectiveness study; challenges encountered and potential remedies.

Study requirement	Challenge encountered	Potential remedies
Sufficient sample size and characteristics of cases	Few hospitalized suspected dengue cases	- Increase number and/or range of study sites (e.g., include emergency department) - Assess and improve enrolment mechanisms - Assess local ethics administrative requirements and incorporate mechanisms to ease enrolment
	Few severe dengue cases	- Recruit retrospectively using stored serum samples and/or medical records - Assess and improve enrolment mechanisms
Sufficient number of case-controls and test-negative controls	Few case-controls recruited	- Consider community-based control recruitment (family members; neighbours; etc.) - Assess logistics of hospital-based recruitment during site selection - Relax matching criteria based on expected exposure status
	Few test-negative controls recruited due to high confirmation rates in suspected cases	- Enrol suspected cases prior to use of rapid tests - Recruit from primary health centres or otherwise earlier in the patient pathway - Recruit TN controls separately from routine clinical practice with a follow-up to assess severity/hospitalization
Exposure history (e.g., exposure to risk factors under study) of controls representative of source population of cases	Duration between case and control recruitment may introduce bias in exposure (during a vaccination campaign; or if vaccination increases during an outbreak)	- Enrol controls immediately after identification of suspected cases - Consider community-based control recruitment (family members; neighbours; etc.) - Improve laboratory test turnaround time
	Females over-represented as controls in CC design which could bias results if vaccination rates are unequal	- Match controls on sex - Recruit from alternative hospital wards
Controls have similar outcome risk (e.g., reporting to study site with hospitalized dengue) as cases	Severity of conditions suffered by case-controls may have differed from hospitalized dengue	- Assess impact of using different control populations - Make changes to study enabling test-negative design after assessing misclassification bias arising from imperfect confirmatory diagnostics

dengue cases preferentially recall dengue episodes in household contacts; are more likely to report fevers and thus become hospitalized because of recent dengue cases at home; and recall vector control activities having been conducted in their communities more readily than non-dengue controls. Reported rates of household dengue/recent fogging were higher in TN than case-controls, providing evidence for reporting bias.

4.2. Recruitment challenges

We recruited a lower-than-expected number of suspected dengue cases and controls. This is partially associated with epidemiology: Malaysia reported ~20,000 fewer dengue cases in 2017 than in preceding years [36]. But even those hospitalized dengue cases were often ineligible for study inclusion, for a number of interrelated reasons associated with local care-seeking and hospitalization practices. Some suspected cases were monitored as outpatients within the emergency department but were never admitted; others were admitted after >5 days of fever due to late care-seeking or hospital referral; and a proportion of subjects and/or their parents declined to participate in the study. Local ethical committee regulations stating that parents must provide birth certificates at study enrolment were particularly challenging to satisfy. Scheduled laboratory operating hours resulted in loss of potential cases, particularly on Friday afternoons or weekends when clinical samples could not be processed. To remedy this, we relaxed inclusion criteria, enrolling subjects with onset of fever $\leq$7 days. This change is not aligned with WHO guidance on dengue confirmation; [4] it was included for exploratory purposes only, and yielded few additional cases during the short period in which it was implemented.

For each VCD case we also failed to recruit two matched case-controls. Our study enrolled adolescents, teenagers and young adults, a healthy demographic unlikely to be hospitalized in Malaysia. Additionally, the logistics of identifying suitable controls within large, complex hospitals was challenging, resulting in over-sampling from some wards in which eligible controls were likely to be found (e.g., gynaecology/orthopaedic surgery). Perhaps the age- and geographical matching used here should be relaxed in the future; or alternative methods of control selection, including recruiting community-based controls, could be considered. Such an approach may facilitate age-matching but would be labour-intensive for study teams. Because virological confirmation rates were high and also to reduce potential bias, it may be beneficial to recruit controls immediately following suspected case enrolment to better-match on exposure risk which may vary over time.

For the TN study, recruitment of controls was low because a higher-than expected (80% vs. 70%) proportion of suspected cases was VCD. This may be due to clinical expertise and familiarity with dengue in Malaysia and/or frequent use of RDTs in Malaysian clinics and emergency departments, and subsequent decisions to admit based on their results. Indeed, 95.2% of VCD cases had received an NS1 Ag RDT as part of their routine care; and 87% of VCD cases had a positive NS1 Ag RDT result. The frequency of pre-admission testing and subsequent hospitalization are likely influenced by epidemic activity, availability of RDTs at health facilities and hospital congestion, effects which have been shown to introduce bias to TN studies of influenza vaccines.[37] The proportion of suspected cases testing negative is also likely to vary across time and study setting, requiring conservative sample size estimates in future studies. Probably, a TN study would only be efficient if a higher proportion of suspected cases tested negative, perhaps by using a less specific case definition, and/or enrolment at an earlier stage of the treatment pathway and before full clinical assessment, for example in the clinic before RDTs are used, with a follow-up to assess severity and hospitalization at a later timepoint. This approach would be less specific and require a larger sample to capture the same number of outcomes.

4.3. Impact of disease severity and routine clinical practice

The efficacy of dengue vaccination varies according to disease severity, and vaccination has been shown to increase the risk of hospitalized dengue in seronegative vaccine recipients [8,38]. It is therefore likely that effectiveness studies should capture severe disease outcomes and we considered this an indicator of study

feasibility. Here, only seven cases had symptoms of severe dengue. This may be associated with changing dengue epidemiology in Malaysia, the cyclical nature of outbreaks or, perhaps more likely, due to challenges in recruiting subjects from intensive care units or who are otherwise clinically severe. This represents an important study bias, confining analysis to milder cases and prohibiting effectiveness estimation against severe outcomes which may be of particular relevance for policymakers and in whom vaccine performance may differ. A study design should consider this bias – perhaps by retrospective testing of stored biological specimens after recovery or death of severe cases, for example, or by designing streamlined methods of enrolment of severe patients.

Rates of confirmatory diagnostics used in routine clinical practice were variable and of inadequate sensitivity/specificity to conclude on infection status. This was most concerning for the NS1 Ag RDT which is most-commonly used in Malaysia and displayed specificity much lower than reported elsewhere (many false-positive results) [39]. This low specificity could be caused by false-negative results in the reference assays but we have no evidence of operational failings in sampling, specimen collection and shipment. RT-PCR is considered the gold-standard. This study was not designed specifically to assess diagnostic test performance and subjects are not representative of the full spectrum of suspected dengue cases in Malaysia. Nonetheless, the observation deserves additional investigation, for example via clinical assessment of discordant cases; or programmatic evaluation of RDTs in the field.

4.4. Potential biases identified

CC studies are vulnerable to a number of biases, most notably due to challenges in control selection [34,40]. Our approach was to use hospitalized controls, matched to cases and recruited within a similar time window. Hospitalized dengue is a rare outcome and in this scenario, resulting ORs approximate the rate/risk ratio [40]. To minimize bias, controls should represent the population at risk; and should be selected independent of the exposure of interest [41]. Important biases may therefore arise if family dengue history or community fogging – rates of which were elevated in cases over controls – led to the decision to vaccinate. In such a scenario the case-control population would have lower vaccination exposure rates than cases, under-estimating the protective effects of vaccination. Consideration of this and other related biases deserves further assessment when patterns of dengue vaccine distribution after launch are better-understood, including by verifying the accuracy of patient-reported data with family members or public health authorities to limit recall bias. We similarly observed gender differences between cases and controls, perhaps caused by the wards used for control identification. This may constitute a bias because the sex-distribution of dengue in Malaysia is not equal [27]. Matching controls to cases based on sex may be advisable in the future.

We considered virological, rather than serological confirmation essential to avoid misclassifying vaccinated controls as cases due to false-positive serological test results [42]. However we cannot exclude misclassification of cases as non-cases due to lack of sensitivity of PCR/NS1 Ag ELISA, which we considered the gold-standard assays. We also only enrolled subjects reporting $\leq$5 days' fever in whom viremia and NS1 Ag circulation is most likely [4]. Modelling experiments indicate that misclassification in outcome can constitute a significant source of bias, particularly in TN studies and under relatively extreme diagnostic test sensitivity/specificity scenarios, depending on vaccination coverage rate and other parameters. It may therefore be prudent in future, if practical, to minimize this bias by restricting TN control enrolment to those with confirmed alternative discharge diagnoses. We have no data on exposure misclassification because dengue vaccination is not practiced in Malaysia, but we expect recollection of dengue vaccination history to be good and the potential bias to be modest.

4.5. Limitations

This study was conducted in three sites in Malaysia over only one year. Results should be generalized only in the context of local epidemiology and treatment practices. Lower-than expected recruitment led to frozen samples being stored for $\leq$6 weeks for shipment but we do not anticipate an adverse impact on results. Our difficulty in recruiting matched hospitalised controls resulted in low statistical power which may have prevented identification of risk factors, an effect difficult to describe because strong socio-demographic risk factors for hospitalized dengue are unknown. Practical limitations also led to lower-than-possible recruitment.

5. Conclusions

It is likely the TN design would not be efficient for a dengue vaccine effectiveness study in Malaysia unless a less-specific endpoint were used to recruit subjects, enabling recruitment of higher numbers of TN controls. The CC method, with adjustments to methods of control recruitment, may be feasible: we recruited 124 confirmed dengue cases, an approximate minimum sample size. However, there is a risk of significant bias and a full bias assessment after vaccination patterns are better-understood would be needed. Case-based methods with retrospective ascertainment of vaccination status have limitations. The feasibility of population-based/community evaluation methods should be explored to assess VE according to serostatus prior to vaccination; and measure herd immunity.

Declaration of Competing Interest

- JN, CR, RLO, AM are employed by and own stocks in Sanofi Pasteur, a company producing a dengue vaccine.
- BJC has received honoraria from Sanofi Pasteur and Roche.
- JPN has been involved with an Industry Sponsored Research with Sanofi Pasteur.
- SJ, WYL, SLL, SK, JPN, AR and Amar-Singh declare no conflict of interest.

Acknowledgments

Dr Azmillah Rosman was the site PI, responsible for subject recruitment, at Hospital Selayang. Study sub-investigators: Ker Hong Bee, Cheng Joo Thye, Arvinder Singh HS, Haema Shunmugarajo, Wan Jazilah Wan Ismail, Marina Md Sham, Lau Ing Soo, Lee Yoong Wah, Annie Liau Fui Vun, Jamaluddin Hj. Mohamed, Amita Vivehanantham, Nor Arisah Misnan, Sharimila a/p Shanmugam, Mohd Aizuddin Abdul Rahman. Study coordinators: Lina Hashim, Nur Fatin Zulaikha Kharudin, Hasni Adha Ibrahim, Nur Azwin Ismail, Anusha d/o T Ganesapillai, Citra d/o Palanisamy. Laboratory staff from the Department of Medical Microbiology, UMMC. The following team contributed to data collection, management and coordination: Karina Abalos, Emily De Jesus, Yong-Ho Oh, Saranyaporn Phrutisunakon, Céline Zocchetti. Jean-Sebastien Persico coordinated the publication process. The study was funded by Sanofi Pasteur.

Contributions

RLO led development of the study protocol with inputs from SJ, WYL, SLL, AM, JN and AS.

SK, JPN and AR were responsible for data collection, site management and study management.

CR, SJ, WYL were responsible for study management.
SDS was responsible for laboratory analyses and interpretation.
AM was responsible for the statistical analysis.
JN, SK, JPN and AR coordinated writing of the research report.
JN drafted the manuscript outline with support from SK.
All authors revised the article and approved the final publication and are accountable for the final paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.07.083.

References

[1] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature 2013;496:504–7. https://doi.org/10.1038/nature12060.
[2] Shepard DS, Undurraga EA, Halasa YA, Stanaway JD. The global economic burden of dengue: a systematic analysis. Lancet Infect Dis 2016;16:935–41. https://doi.org/10.1016/S1473-3099(16)00146-8.
[3] World Health Organization. Global strategy for dengue prevention and control 2012–2020. Geneva: World Health Organization; 2012.
[4] World Health Organization. Dengue: Guidelines for Diagnosis, Treatment, Prevention, and Control. Geneva: World Health Organization; 2009.
[5] World Health Organization. Dengue and severe dengue, WHO Factsheet No 117. Geneva: 2017.
[6] Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. Arch Virol 2013;158:1445–59. https://doi.org/10.1007/s00705-013-1645-3.
[7] Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol 2002;10:100–3. https://doi.org/10.1016/S0966-842X(01)02288-0.
[8] Wichmann O, Vannice K, Asturias EJ, de Albuquerque Luna EJ, Longini I, Lopez AL, et al. Live-attenuated tetravalent dengue vaccines: The needs and challenges of post-licensure evaluation of vaccine safety and effectiveness. Vaccine 2017;35:5535–42. https://doi.org/10.1016/j.vaccine.2017.08.066.
[9] Vannice KS, Durbin A, Hombach J. Status of vaccine research and development of vaccines for dengue. Vaccine 2016;34:2934–8. https://doi.org/10.1016/j.vaccine.2015.12.073.
[10] World Health Organization. Guidelines for the clinical evaluation of dengue vaccines in endemic areas. Geneva: WHO Document Production Services, Switzerland; 2008.
[11] Sabin vaccine institute. Principles for the evaluation of the effectiveness of dengue vaccines workshop; 2014 https://www.sabin.org/updates/events/principles-evaluation-effectiveness-dengue-vaccines-workshop [accessed June 8, 2018].
[12] Kimiya T, Shinjoh M, Anzo M, Takahashi H, Sekiguchi S, Sugaya N, et al. Effectiveness of inactivated quadrivalent influenza vaccine in the 2015/2016 season as assessed in both a test-negative case-control study design and a traditional case-control study design. Eur J Pediatr 2018;177:1009–17. https://doi.org/10.1007/s00431-018-3145-7.
[13] Hennessy S, Liu Z, Tsai TF, Strom BL, Wan CM, Liu HL, et al. Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2): a case-control study. Lancet (Lond, Eng) 1996;347:1583–6. https://doi.org/10.1016/S0140-6736(96)91075-2.
[14] Saul N, Wang K, Bag S, Baldwin H, Alexander K, Chandra M, et al. Effectiveness of maternal pertussis vaccination in preventing infection and disease in infants: the NSW Public health network case-control study. Vaccine 2018;36:1887–92. https://doi.org/10.1016/j.vaccine.2018.02.047.
[15] Diaz J, Terrazas S, Bierrenbach AL, Toscano CM, Alencar GP, Alvarez A, et al. Effectiveness of the 10-Valent Pneumococcal conjugate vaccine (PCV-10) in children in Chile: a nested case-control study using nationwide pneumonia morbidity and mortality surveillance data. PLoS ONE 2016;11:e0153141. https://doi.org/10.1371/journal.pone.0153141.
[16] Pang J, Salim A, Lee VJ, Hibberd ML, Chia KS, Leo YS, et al. Diabetes with hypertension as risk factors for adult dengue hemorrhagic fever in a predominantly dengue serotype 2 epidemic: a case control study. PLoS Negl Trop Dis 2012;6:e1641. https://doi.org/10.1371/journal.pntd.0001641.
[17] Gibson G, Souza-Santos R, Honório NA, Pacheco AG, Moraes MO, Kubelka C, et al. Conditions of the household and peridomicile and severe dengue: a case-control study in Brazil. Infect Ecol Epidemiol 2014;4:1–7. https://doi.org/10.3402/iee.v4.22110.
[18] Puig-Barberà J, Burtseva E, Yu H, Cowling BJ, Badur S, Kyncl J, et al. Influenza epidemiology and influenza vaccine effectiveness during the 2014–2015 season: annual report from the Global Influenza Hospital Surveillance Network. BMC Public Health 2016;16(Suppl 1):757. https://doi.org/10.1186/s12889-016-3378-1.
[19] Sullivan SG, Tchetgen Tchetgen EJ, Cowling BJ. Theoretical basis of the test-negative study design for assessment of influenza vaccine effectiveness. Am J Epidemiol 2016;184:345–53. https://doi.org/10.1093/aje/kww064.
[20] Chiu SS, Kwan MYW, Feng S, Wong JSC, Leung C-W, Chan ELY, et al. Interim estimate of influenza vaccine effectiveness in hospitalised children, Hong Kong, 2017/18. Euro Surveill 2018;23:4–8. https://doi.org/10.2807/1560-7917.ES.2018.23.8.18-00062.
[21] Jackson ML, Chung JR, Jackson LA, Phillips CH, Benoit J, Monto AS, et al. Influenza vaccine effectiveness in the United States during the 2015–2016 Season. N Engl J Med 2017;377:534–43. https://doi.org/10.1056/NEJMoa1700153.
[22] Fujii Y, Noguchi A, Miura S, Ishii H, Nakagomi T, Nakagomi O, et al. Effectiveness of rotavirus vaccines against hospitalisations in Japan. BMC Pediatr 2017;17:1–7. https://doi.org/10.1186/s12887-017-0916-7.
[23] McLaughlin JM, Jiang Q, Isturiz RE, Sings HL, Swerdlow DL, Gessner BD, et al. Effectiveness of 13-Valent Pneumococcal Conjugate Vaccine Against Hospitalization for Community-Acquired Pneumonia in Older US Adults: A Test-Negative Design. Clin Infect Dis 2018;67:1498–506. https://doi.org/10.1093/cid/ciy312.
[24] Franke MF, Jerome JG, Matias WR, Ternier R, Hilaire IJ, Harris JB, et al. Comparison of two control groups for estimation of oral cholera vaccine effectiveness using a case-control study design. Vaccine 2017;35:5819–27. https://doi.org/10.1016/j.vaccine.2017.09.025.
[25] Yung CF, Chan SP, Thein TL, Chai SC, Leo YS. Epidemiological risk factors for adult dengue in Singapore: an 8-year nested test negative case control study. BMC Infect Dis 2016;16:323. https://doi.org/10.1186/s12879-016-1662-4.
[26] Verani JR, Baqui AH, Broome CV, Cherian T, Cohen C, Farrar JL, et al. Case-control vaccine effectiveness studies: Preparation, design, and enrollment of cases and controls. Vaccine 2017;35:3295–302. https://doi.org/10.1016/j.vaccine.2017.04.037.
[27] Mohd-Zaki AH, Brett J, Ismail E, L'Azou M. Epidemiology of dengue disease in Malaysia (2000–2012): a systematic literature review. PLoS Negl Trop Dis 2014;8:e3159. https://doi.org/10.1371/journal.pntd.0003159.
[28] Malaysia administrative modernization and management planning unit. Malaysia government open data portal; 2018 <http://www.mampu.gov.my/en/> [accessed August 15, 2018].
[29] Economic Planning Unit of the Malaysian Prime Minister's office. Eleventh Malaysia Plan, 2016–2020. Chapter 4, 2016, p. 4–6.
[30] NPRA Malaysia. Conditional registration of a dengue vaccine for use in Malaysians 9–45 years of age; 2017 <http://npra.moh.gov.my/index.php/about-npcb/drug-control-authority-dca/dca-news/dca-annoucement/1154-conditional-registration-of-a-dengue-vaccine-for-use-in-malaysians-9-45-years-of-age> [accessed April 6, 2017].
[31] World medical association. Declaration of Helsinki – ethical principles for medical research involving human subjects. Fortaleza, Brazil; 2013.
[32] International epidemiological association. Guidelines for good conduct in epidemiological practice; 2007.
[33] Yong YK, Thayan R, Chong HT, Tan CT, Sekaran SD. Rapid detection and serotyping of dengue virus by multiplex RT-PCR and real-time SYBR green RT-PCR. Singapore Med J 2007;48:662–8.
[34] Wacholder S, McLaughlin JK, Silverman DT, Mandel JS. Selection of controls in case-control studies. I. Principles. Am J Epidemiol 1992;135:1019–28. https://doi.org/10.1016/S1047-2797(00)00134-4.
[35] Mammen MP, Pimgate C, Koenraadt CJM, Rothman AL, Aldstadt J, Nisalak A, et al. Spatial and temporal clustering of dengue virus transmission in Thai villages. PLoS Med 2008;5:e205. https://doi.org/10.1371/journal.pmed.0050205.
[36] WHO western Pacific regional office. WPRO Dengue situation updates 2018: update: 29 March 2018 <http://www.wpro.who.int/emerging_diseases/DengueSituationUpdates/en/>.
[37] Fukushima W, Hirota Y. Basic principles of test-negative design in evaluating influenza vaccine effectiveness. Vaccine 2017;35:4796–800. https://doi.org/10.1016/j.vaccine.2017.07.003.
[38] Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med 2015;373:1195–206. https://doi.org/10.1056/NEJMoa1506223.
[39] Pal S, Dauner AL, Mitra I, Forshey BM, Garcia P, Morrison AC, et al. Evaluation of dengue NS1 antigen rapid tests and ELISA kits using clinical samples. PLoS One 2014;9:e113411. https://doi.org/10.1371/journal.pone.0113411.
[40] Rodrigues L, Kirkwood BR. Case-control designs in the study of common diseases: updates on the demise of the rare disease assumption and the choice of sampling scheme for controls. Int J Epidemiol 1990;19:205–13. https://doi.org/10.1093/ije/19.1.205.
[41] Grimes DA, Schulz KF. Compared to what? Finding controls for case-control studies. Lancet 2005;365:1429–33. https://doi.org/10.1016/S0140-6736(05)66379-9.
[42] Plennevaux E, Sabchareon A, Limkittikul K, Chanthavanich P, Sirivichayakul C, Moureau A, et al. Detection of dengue cases by serological testing in a dengue vaccine efficacy trial: utility for efficacy evaluation and impact of future vaccine introduction. Vaccine 2016;34:2707–12. https://doi.org/10.1016/j.vaccine.2016.04.028.