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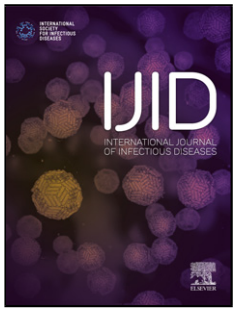

A prospective, multicentre, cohort study to assess the incidence of dengue illness in households from selected communities in Brazil (2014−2018)

Daniele Fernandes de Aguiar, Eliana Nogueira C de Barros, Guilherme de Sousa Ribeiro, Patricia Brasil, Maria Paula Gomes Mourao, Kleber Luz, Francisco Hideo Aoki, Andre Ricardo Ribas Freitas, Guilherme Amaral Calvet, Eduardo Oliveira, Bianca F Branco, Ariane Abreu, Brigitte Cheuvart, Adrienne Guignard, Melanie de Boer, Ana Claudia Duarte, Maria Beatriz Borges, Tatiana Guimarães de Noronha

PII: S1201-9712(21)00374-X

DOI: https://doi.org/10.1016/j.ijid.2021.04.062

Reference: IJID 5357

To appear in: *International Journal of Infectious Diseases*

Received Date: 15 January 2021

Revised Date: 16 April 2021

Accepted Date: 17 April 2021

Please cite this article as: de Aguiar DF, de Barros ENC, Ribeiro GdS, Brasil P, Mourao MPG, Luz K, Aoki FH, Freitas ARR, Calvet GA, Oliveira E, Branco BF, Abreu A, Cheuvart B, Guignard A, de Boer M, Duarte AC, Borges MB, de Noronha TG, A prospective, multicentre, cohort study to assess the incidence of dengue illness in households from selected communities in Brazil (2014−2018), *International Journal of Infectious Diseases* (2021), doi: https://doi.org/10.1016/j.ijid.2021.04.062

Title page

Manuscript Title:

A prospective, multicentre, cohort study to assess the incidence of dengue illness in households from selected communities in Brazil (2014−2018)

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Running/short title: Dengue incidence in Brazil

Word count: 3563 excluding in-text references (3975 with references; max 3500)

Previous congress activities: None

Clinical Trial Registration: NCT01751139

Highlights

- This was a prospective surveillance study of dengue in Brazil from 2014 to 2018.
- Dengue seroprevalence was 76%; 23% of participants reported dengue history.
- Incidence of laboratory-confirmed symptomatic infection was 6.1/1000 person-years.
- For each symptomatic infection, there were ~7 inapparent primary dengue infections.
- Study highlights underestimation of dengue infection in Brazil.

Abstract

Objectives

To estimate the incidence of dengue infection across geographically distinct areas of Brazil.

Methods

This prospective, household-based, cohort study enrolled participants in five areas and followed them up for up to 4 years (2014−2018). Dengue seroprevalence was assessed at each scheduled visit. Suspected dengue cases were identified through enhanced passive and active surveillance. Acute symptomatic dengue infection was confirmed through reverse-transcriptase quantitative polymerase chain reaction in combination with an antigenic assay (NS1) and serology.

Results

Among 3300 participants enrolled, baseline seroprevalence was 76.2%, although only 23.3% of participants reported a history of dengue. Of 1284 suspected symptomatic dengue cases detected, 50 (3.9%) were laboratory-confirmed. Based on 8166.5 person-years (PY) of follow-up, the incidence of laboratory-confirmed symptomatic infection (primary endpoint) was 6.1 per 1000 PY (95% confidence interval [CI]: 4.5, 8.1). Incidence varied substantially in different years (1.8−7.4 per 1000 PY). The incidence of inapparent primary dengue infection was substantially higher: 41.7 per 1000 PY (95% CI: 31.1, 54.6).

Conclusions

Our findings, highlighting that the incidence of dengue infection is underestimated in Brazil, will inform the design and implementation of future dengue vaccine trials.

Keywords: dengue, incidence, seroprevalence, epidemiology, multicentre cohort study, Brazil (max 6)

Introduction

Dengue is a viral disease primarily transmitted by *Aedes aegypti*, a mosquito highly adapted to urban environments (WHO, 2020a). Approximately half of the global population lives in areas suitable for dengue transmission (Messina et al., 2019; Bhatt et al., 2013; Brady et al., 2012). Rising temperatures attributed to climate change, alongside intensifying urbanisation and globalisation, are expected to increase dengue transmission in areas that are already endemic and allow the virus to spread through areas that are currently at low risk (Gubler, 2011; Murray et al., 2013; Kraemer et al., 2019). The Global Burden of Disease Study estimated approximately 58.4 million symptomatic dengue infections in 2013, with approximately 10,000 deaths per year (Stanaway et al., 2016). The study also reported that the number of symptomatic infections more than doubled every 10 years between 1990 and 2013 (Stanaway et al., 2016). Another study estimated 390 million dengue infections per year, of which approximately 96 million were symptomatic (Bhatt et al., 2013). Several studies have found that the incidence of dengue infection is grossly underestimated because so many infections are asymptomatic and illness is under-reported (Undurraga et al., 2013; Shepard et al., 2014; Standish et al., 2010; Wichmann et al., 2011). Four serotypes of the virus currently circulate, dengue virus (DENV) types 1, 2, 3 and 4 (Andrioli et al., 2020).

Dengue has become a major public health problem in Brazil (Nunes et al., 2019; Salles et al., 2018). In 2019, the Pan American Health Organization (PAHO) reported 3,140,872 dengue cases in the World Health Organization (WHO) region of the Americas (PAHO, 2020), the highest number of cases in history (WHO, 2019). Of these, 2,226,914 (>70%) cases occurred in Brazil (PAHO, 2020). Brazil succeeded in eradicating the *Aedes aegypti* mosquito during the 1950s but it returned during the 1980s (Silva et al., 2016). The first dengue outbreak following reintroduction was reported in 1982 (PAHO, 1982), during which serotypes DENV1 and DENV4 were isolated (Silva et al., 2016). A 1986 epidemic starting in Rio de Janeiro was largely associated with DENV1, and DENV2 was introduced in Rio de

Janeiro in 1990. During 1998, more than half a million cases were reported in Brazil. DENV3 was first isolated in 2000, followed by an epidemic between 2001 and 2003. A pattern of outbreaks every 3−5 years was observed in Brazil until 2010, after which the frequency increased to every 2 years (Silva et al. 2016).

In Brazil, dengue fever has been a mandatorily reportable disease since 1986. The surveillance system depends upon passive reporting from health care services, with data entered into the National Reportable Disease Information System (SINAN). It is well recognized that passive surveillance systems often lead to under-reporting of dengue. A study in Thailand and Cambodia detected 1.4-fold and 2.6-fold more dengue hospitalizations, respectively, with active versus passive surveillance (Wichmann et al., 2011). In Brazil, under-reporting of hospitalized dengue via the mandatory system has been estimated at 37% (Duarte et al., 2006), and another study suggested that dengue hospitalizations recorded in the public health system database were 33% higher than those recorded in SINAN (Coelho et al., 2016). Under-reporting of dengue appears to be even more pronounced for non-hospitalized cases, ranging from 4-fold to 29-fold in Cambodia and 14-fold to 28-fold in Nicaragua (Wichmann et al., 2011; Standish et al., 2010). The higher rate of under-reporting in non-hospitalized patients is likely because people with non-severe dengue do not seek medical care. It is noteworthy that the true incidence of dengue in Brazil is believed to be underestimated (Silva et al., 2016).

The WHO Global Strategy for Dengue Prevention and Control advocates improving surveillance to enhance reporting, prevention and control of dengue, and prioritises vaccine development (WHO, 2020b). The incidence of dengue varies widely between geographical areas and from year to year within the same geographical area. Estimation of dengue incidence is critical when designing a vaccine trial as making a valid assumption of the average incidence over time and across study centres is important. The present study was conducted to estimate the incidence of dengue infection and disease across geographically distinct areas of Brazil with the aim of validating infection rate assumptions for future vaccine

trials.

Study objectives

The primary objective was to estimate the incidence of laboratory-confirmed symptomatic dengue infection by year. Secondary objectives were to estimate: (1) incidence of virologically-confirmed symptomatic dengue infection; (2) incidence of symptomatic dengue infection (including laboratory-confirmed and probable cases) overall and by study site, gender and age group (≤17 years, 18-49 years and ≥50 years at enrolment); (3) incidence of inapparent primary dengue infection overall and by study site, gender and age group.

Methods

Study design, communities and participants

This was a prospective, multicentre, community-based, household (cluster) sampling, cohort study. The first participant was enrolled in February 2014 and the last study visit took place in December 2018. The study was originally planned to last for 1 year, with three study centres, but it was extended by 3 years to include more dengue seasons at the same centres and to add two study centres (expansion cohort) (Figure 1). The study was terminated earlier than expected because of the sponsor's decision not to proceed with development of the dengue vaccine given the associated technical challenges. Therefore, not all analyses planned in the protocol were conducted.

Five centres covering different areas of Brazil (Rio de Janeiro, Manaus, Salvador, Natal and Campinas) were included (Figure 2). Centres had support from the Family Health Strategy (FHS) or the Larval Index Rapid Assay (LIRA) programmes (Brazil Ministry of Health, 2020a; Brazil Ministry of Health, 2020b), or had field research experience in the community. Centres began enrolment at different years. Further inclusion criteria for centres and participants are described in the Supplement.

Participants of at least 6 months of age were recruited outside of the peak dengue season over approximately 3 months. Participants from the initial cohort (Year 1) were invited to extend participation for another 3 years. Replacement participants were enrolled to substitute for those who did not wish to extend, discontinued the study or were lost to follow-up. The number of active participants at each centre was evaluated yearly and replacements were enrolled if required until the end of Year 3. The aim was to maintain at least 500 participants per centre at the start of each dengue season. For an individual participant, study duration ranged from 1 to 4 years. A review of the demographics of participants enrolled into the initial cohort showed that children were under-represented compared with

the Brazilian population, and adults ≥50 years of age were over-represented. Therefore, an age stratification was employed for participants enrolled in the expansion cohort or as replacements, so that a sample distribution of ≥30% of participants <18 years of age and ≤20% of participants ≥50 years of age could be maintained.

Three serological surveys performed during home visits were scheduled for the initial cohort at approximately 6-month intervals (Figure 1). Thereafter, one serological survey was scheduled per year at a period of low dengue transmission for the remaining 3 years of the study. Participants in the expansion cohort had one serological survey scheduled per year; the number of surveys depended on the study year in which they were enrolled (range 2−4) (Figure 1). Study visit procedures are described in the Supplement.

Dengue case detection and definitions

Suspected cases were identified through enhanced passive and active surveillance. For enhanced passive surveillance, participants were instructed to contact study staff if they experienced fever (temperature ≥38°C for ≥2 days and <14 days) at any time, or to contact the designated hospital or clinic in an emergency or if they experienced warning signs (Supplement). For active surveillance, study staff used a structured script to enquire about dengue symptoms since the previous contact, which occurred monthly. Early presenters were defined as those presenting within 5 days following the onset of fever and late presenters were defined as those presenting ≥6 days after the onset of fever. Further details are provided in the Supplement.

Suspected dengue cases had acute and convalescent (~21 days later) blood samples collected for dengue laboratory diagnosis. Samples from early presenters were tested by reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR). In all suspected dengue cases, enzyme-linked immunosorbent assay (ELISA) methodology was used to detect dengue virus NS1 antigen and IgG and IgM antibodies to dengue virus. Further details are given in the Supplement.

Suspected symptomatic dengue was defined as temperature ≥38°C by any route on ≥2 consecutive days and <14 days, with or without other dengue symptoms, without obvious aetiology unrelated to dengue (Table 1). Suspected cases were classified according to laboratory results as laboratory-confirmed, virologically-confirmed, probable, negative, or indeterminate (Table 1). Laboratory-confirmed symptomatic dengue cases were further stratified as primary dengue if they did not have IgG antibodies at the previous scheduled visit and as secondary dengue if they did. Cases were defined as probable based on a strong clinical suspicion or diagnosis in a late presenter, with IgM or IgG positivity on the acute or convalescent sample, NS1-negative on the acute sample and no IgM seroconversion between the acute and convalescent samples (Table 1).

In addition, inapparent primary infection was detected during the sequential serological surveys. Participants presenting IgG seroconversion between two sequential study visits who did not have a clinical suspicion of dengue during the corresponding interval between the surveys were defined as having an inapparent primary dengue infection (Table 1).

For all patients meeting the case definition of suspected dengue, the physician was asked to express his or her opinion on the diagnosis based on clinical symptoms only, before the laboratory results were available. Cases which the physician diagnosed as dengue in this scenario were classified as clinically diagnosed cases.

Statistical analysis

Descriptive statistics (mean, standard deviation, frequency) were calculated for sociodemographic characteristics. The seroprevalence of dengue infection at baseline was calculated as a proportion of participants who were seropositive for dengue IgG antibodies divided by the total number of participants whose dengue IgG serostatus was known. The estimated annual incidence rate of dengue infection over a calendar year was computed with 95% confidence intervals (CI). For the annual incidence of laboratory-confirmed or probable symptomatic dengue infection, the CI was obtained from a log-linear model with the number

of dengue events (all cases) as the dependent variable and the total participant-months as the offset. The model included the study centre, year and month as independent categorical fixed effects. The annual incidence rate at a study centre for each year was estimated as the average of the incidence rate over months. For the annual incidence of other dengue case definitions (laboratory-confirmed symptomatic, virologically-confirmed symptomatic, clinically diagnosed and inapparent primary), and for subgroup analyses by gender or age, the CI was computed using the exact CI based on a Poisson Distribution.

For the sample size calculation, the average incidence of dengue was assumed to be approximately 1% per year (or 10 cases per 1000 person-years [PY]). This assumption was based on an average incidence of reported dengue cases in the Brazilian population of approximately 0.3% per year between 2007 and 2010, with an under-reporting factor of 3 as estimated by the Brazilian Ministry of Health (Brazil Ministry of Health, 2020c). Based on an overall minimum sample size of approximately 3,600 participants enrolled and a follow-up period of 1 year, the 95% CI for an expected incidence of 9 dengue cases per 1000 person-years using a cluster design was 4.7−13.3. With 3600 participants enrolled, 4 years of follow-up and new enrolments to keep the number of participants above 3000 at the beginning of each subsequent season, the 95% CI was 6.3−11.7. To maintain ≥500 participants per centre, assuming a discontinuation rate of 15−20%, 600 participants were planned to be enrolled per centre.

The primary and secondary analyses were performed on the according-to-protocol (ATP) cohort which included all participants who met all inclusion and exclusion criteria and had recorded seroprevalence status at baseline.

Ethics and registration

The study was conducted in accordance with Good Clinical Practice and all applicable regulatory requirements including the Declaration of Helsinki and was approved by national and local ethics committees (Supplement). Participants (or for those <18 years old, their

parents or legally acceptable representatives) provided written or thumb printed informed consent. The study was registered at clinicaltrials.gov (NCT01751139).

Results

A total of 3300 participants were enrolled from 1353 households, of whom 3264 from 1348 households were included in the ATP cohort (Figure 3). Between one and 11 participants per household were enrolled (not all occupants of a household were necessarily enrolled in the study): one participant in 34.4% of households, two in 25.7%, three in 18.0%, four in 12.8% and five or more in 9.1%.

Baseline characteristics

Approximately half of the participants were adults 18−49 years of age (49.1%), the mean age was 31.9 years and most participants were female (60.4%) (Table 2). The number of adults per household was 1−12 and the number of children and adolescents 5−17 years of age was 0−10 (Table 2). A total of 61.7% of households had an income of US$168−502 (Table 2).

Seroprevalence at baseline was 76.2%, although only 23.3% of the participants self-reported a history of dengue (Table 3). Campinas had lower seroprevalence than other centres (Table 3). Seroprevalence was higher in adults than children (Table 3) and similar between males and females (72.1% in males and 78.9% in females).

Participant follow-up

Two centres completed the initially planned 4-year follow-up (Rio de Janeiro and Manaus); three centres did not complete because of early study termination (Salvador, Natal and Campinas, which had 3, 2, and 1 years of follow-up, respectively). The planned 4-year follow-up was completed by 1033 participants (31.4%); most of the participants who did not complete the study did not do so due to the early study termination (Figure 3). The total number of PY of follow-up for the 3264 participants was 8166.5.

Incidence of symptomatic dengue infection

A total of 1284 suspected symptomatic dengue cases was detected, which 50 were laboratory-confirmed, 249 were classified as probable dengue, 738 were classified as negative and 247 were classified as indeterminate (Figure 4). Of the 299 laboratory-confirmed or probable symptomatic cases, 96.0% were secondary symptomatic infections and 57.9% occurred in adults 18−49 years of age (Figure 4). The clinician established a dengue diagnosis based on clinical signs in 28.8% of the cases that were later considered as laboratory-confirmed or probable when testing results became available (Figure 4). Of the 738 cases classified as negative, 117 (15.9%) received a clinician-established diagnosis of dengue. Only one case (classified as probable) was considered to be severe, based on development of severe bleeding.

The overall incidence of laboratory-confirmed symptomatic dengue infection was 6.1 per 1000 PY (95% CI: 4.5, 8.1), based on 8166.5 PY of follow-up. The incidence varied substantially in different years: 1.8 in 2014, 5.1 in 2015, 7.4 in 2016, 5.8 in 2017 and 7.1 in 2018 (Figure 5A). Nine cases were identified by RT-qPCR, of which five were positive for DENV serotype 1 and two were positive for DENV serotype 4; the other two cases were non-typeable but were confirmed as dengue by real-time PCR. The overall incidence of virologically-confirmed symptomatic dengue infection was 1.1 per 1000 PY (95% CI: 0.5, 2.1) (Figure 5B).

A total of 299 cases of laboratory-confirmed or probable symptomatic dengue cases were identified, corresponding to an incidence of 20.1 per 1000 PY (95% CI: 13.2, 30.6) (Table 4). The lowest incidence was reported in 2018 (13.1 per 1000 PY) and the highest in 2016 (27.9 per 1000 PY). Salvador experienced the highest incidence (59.1 per 1000 PY), while Campinas experienced only a single case (incidence 1.9 per 1000 PY) (Table 4). Incidence varied according to age, with participants 18−49 years of age experiencing the highest and those ≥50 years of age the lowest (Table 4). Females experienced a higher incidence than

males (Table 4).

Incidence of inapparent primary dengue infection

A total of 777 participants who were seronegative at baseline were followed for 1248.3 PY. Of these, 52 participants experienced an inapparent primary dengue infection during the follow-up period, corresponding to an incidence of 41.7 per 1000 PY (95% CI: 31.1, 54.6) (Table 5). Incidence varied by year and study centre but was similar in females and males. It was also similar in children and the 18−49 years age group (42.7 and 48.1 per 1000 PY, respectively). The point estimate was lower in participants ≥50 years of age (17.6 per 1000 PY), although there was no statistically significant difference versus younger age groups (Table 5).

Discussion

This large, multicentre cohort study conducted in five Brazilian cities and including up to 4 years of follow-up showed that the overall incidence of laboratory-confirmed symptomatic dengue (the primary endpoint of the study) was 6.1 per 1000 PY. Substantial year-on-year variation was reported, ranging from 1.8 per 1000 PY in 2014 to 7.4 per 1000 PY in 2016.

Few reports of prospective studies of the incidence of dengue in Brazil using active surveillance are available, and data in adults are particularly sparse. In a prospective study with active surveillance for fever among 3000 children 9−16 years of age, the incidence of laboratory-confirmed dengue per 1000 PY ranged from 0.4 in Natal to 9.0 in Campo Grande during 2010, and from 1.1 in Goiania to 6.6 in Fortaleza during 2011 (Dayan et al., 2015). A further prospective, school-based study with active and enhanced passive surveillance for dengue among children 5−13 years of age in Fortaleza identified the incidence of laboratory-confirmed dengue as 11.0, 18.1 and 10.2 per 1000 PY in 2012, 2013 and 2014, respectively (Coelho et al., 2020).

Data on the incidence of dengue in adults in Latin America as a whole are also rare, but several studies report data in children. A study conducted between 1999 and 2005 among approximately 2400 schoolchildren and some adult family members in the city of Iquitos, Peru, prospectively monitored for serological evidence of dengue infection, and reported an incidence of 20−30 per 1000 PY during the early part of the study when DENV1 and DENV2 were co-circulating; the incidence rose substantially to 890 infections per 1000 PY at its peak following the introduction of DENV3 in 2001 (Morrison et al., 2010). In a study of approximately 3800 children 2−9 years of age in Managua, Nicaragua, from 2004−2008, incidence of confirmed symptomatic dengue ranged from 0.4% to 1.9% (Balmaseda et al., 2010). Another study in Managua among 5545 children 2−14 years from 2004 to 2010 reported an incidence of symptomatic dengue of 16.1 per 1000 PY and an incidence of dengue infection of 90.2 per 1000 PY (Gordon et al., 2013). A prospective, school-based

cohort study of 767 families in Yucatan, Mexico, reported an incidence of all dengue infection of 33.9 per 1000 PY and of symptomatic infection of 3.5 per 1000 PY during the first year of follow-up (2015−2016) (Rojas et al., 2018).

The overall incidence of laboratory-confirmed or probable symptomatic dengue was 20.1 per 1000 PY, with considerable variation over the different study years. The incidence also varied between study centres, ranging from 1.9 per 1000 PY in Campinas to 59.1 per 1000 PY in Salvador. Of note, the city of Campinas was included in the study in 2016 after a major epidemic in 2014 and 2015. This must have contributed to the low incidence in the years studied, considering the multi-annual periodicity of dengue in this location (Yang et al., 2016). A community-based enhanced surveillance conducted in the same community of Salvador where we performed the current study estimated the risk of symptomatic laboratory-confirmed or probable dengue as 2.1 and 7.0 cases per 1000 inhabitants ≥5 years of age in 2009 and 2010, respectively (Kikuti et al., 2015). These much lower incidences most likely reflect differences in the study design, rather than annual or seasonal variation in dengue occurrence and highlight the importance of active detection of suspected cases during follow-up to provide a more accurate measure of disease incidence.

Numerous factors might have contributed to regional differences, including sociodemographic factors, infrastructure and water supply, history and timing of dengue introduction, and transmission of different serotypes between areas. Seasonality and climate also have considerable impact on dengue transmission and vary substantially between the areas covered by our study (Churakov et al., 2019; Stolerman et al., 2019). Differences between centres in duration of follow-up might also have influenced the results, and it is noteworthy that Campinas had the shortest follow-up of the centres. The co-circulation of the Zika and chikungunya viruses during the study (Brasil et al., 2016; Cardoso et al., 2017; Silva et al., 2019; Cardoso et al., 2015) might also have differentially impacted dengue incidence across areas, as discussed in more detail later.

The study identified high overall seroprevalence for dengue at baseline (>80%, except for Campinas, which had 31% seroprevalence), confirming that dengue is common in four of the five communities studied. Of the cities included in the study, Campinas has the smallest population, is the only city that is not a capital, and is the only city with a Tropical Altitude climate (cooler and with less rainfall than the Tropical Atlantic or Equatorial climates of the other cities of the study); these factors could explain the lower baseline seroprevalence compared with the other communities, despite the 2014−2015 epidemic in Campinas. The high overall seroprevalence across the study contrasts with self-reported history of dengue (23%), which might indicate that most dengue infections are asymptomatic (inapparent) or subclinical.

Furthermore, 52 participants out of 777 who were seronegative at baseline experienced inapparent primary dengue infection during the follow-up period. The overall incidence of inapparent primary dengue was double that of laboratory-confirmed or probable symptomatic dengue; the difference between inapparent and symptomatic infections is likely to be an underestimate because primary infections are expected to be much less frequent than secondary infections in settings where >80% of the population have experienced a previous infection. Although individuals with inapparent infection do not directly impact upon health services since they do not seek medical care, they may be important in the transmission cycle, serving as potential source of infection to mosquitoes. Further studies to determine the contribution of inapparent infections to the dynamics of DENV transmission should be conducted.

Notably, only 28.8% of confirmed or probable cases in our study received a clinically based dengue diagnosis; conversely, 15.9% of cases classified as laboratory-negative received a clinically based dengue diagnosis. This highlights the difficulty in differentiating true dengue from dengue-like illness based on clinical signs only. In Brazil, dengue surveillance depends upon passive reporting from outpatient and hospital facilities and is likely to substantially underestimate incidence (Siqueira et al., 2005). Indeed, a study using enhanced surveillance

conducted in Salvador estimated that there are 12 patients with symptomatic dengue seeking medical care per case reported in the community to Brazil's Notifiable Diseases Information System (Silva et al., 2016). This emphasises the importance of cohort studies such as our own to better understand the dynamics of dengue infection in the field.

Study limitations included the logistical challenges in implementing surveillance in some areas, including risk of violence towards study staff, resulting in variable adherence to study procedures and missed contacts. An important limitation was that the suspected symptomatic dengue definition was based on ≥2 days of fever (temperature ≥38°C), with or without other dengue symptoms. This definition is associated with two difficulties. First, few participants would undergo ≥2 days of fever without self-administering antipyretics, and parents would almost certainly administer them to children. However, the protocol did allow investigators to consider a suspected dengue case if the participant presented on the first day of fever or illness. Second, some clinical dengue cases are now presenting without fever or with low fever (Tukasan et al., 2017). This has the effect of lowering the sensitivity for identification of dengue cases. There are other limitations regarding definitions of dengue cases used in the study.

For inapparent infections, since the exact date of the infection was not known, the calculated person-years used in the incidence estimate likely included post-exposure calendar time, resulting in an underestimation of the actual incidence. Probable cases were defined based on a strong clinical suspicion in the judgement of the physician, IgM or IgG positivity, NS1 negativity and no IgM seroconversion. It is likely that participants who were seropositive at baseline and presented with dengue-like symptoms would have been diagnosed as a probable case; given that seroprevalence was 76% at baseline, this is likely to represent an overestimation.

Other important limitations of the study resulted from the unexpected concomitant transmission of Zika and chikungunya viruses during the study (Brasil et al., 2016; Cardoso

et al., 2017; Silva et al., 2019; Cardoso et al., 2015). The clinical characteristics and the transmission vector associated with these three viruses are similar and might have hampered an accurate clinical diagnosis of dengue (Silva et al., 2019). In addition, because antibodies produced against the Zika virus can cross-react and be detected by dengue virus serological tests (Landry and St George, 2017), and we did not rule out a Zika diagnosis, some of the cases identified using serological tests might have been caused by the Zika virus. Thus, we might have overestimated the number of suspected dengue virus infections (both inapparent and symptomatic) because some probable cases were, in part, defined by dengue IgM or IgG detection.

On the other hand, it is likely that the dengue incidence estimated in our study was decreased as a consequence of cross-reactive immunity against the Zika virus. Collective immunity against Zika was estimated to reach high levels in certain Brazilian settings, such as Salvador, where 63%–73% of the population was exposed by the end of the 2015 outbreak (Rodriguez-Barraquer et al., 2019; Netto et al., 2017). Experimental and epidemiological evidence have suggested that high exposure of the population to Zika virus might elicit cross-protective herd immunity to dengue infections (Pérez-Guzmán et al., 2019; Ribeiro et al., 2020) and possibly modulates dengue virus transmission (Borchering et al., 2019). In Salvador, for example, a large reduction in dengue detection following Zika virus introduction was identified during longitudinal surveillance for acute febrile illnesses (AFI) (Ribeiro et al., 2018a; Ribeiro et al., 2018b). Before the Zika epidemic, 25% of patients with AFI were diagnosed with dengue infection by RT-PCR, but this proportion fell to 3% after the epidemic (Ribeiro et al., 2018a; Ribeiro et al., 2018b). A decrease in dengue transmission following Zika epidemics was also observed throughout Latin America (Ribeiro et al., 2020; Mugabe et al., 2020) and the most acceptable explanation for this reduction is the existence of cross-reactive herd immunity to dengue virus (Perez et al., 2019).

In conclusion, our study contributes to current knowledge of the epidemiology of dengue infection in different areas of Brazil. Prospective studies of dengue incidence such as our

own will help to evaluate surveillance strategies and the impact of new technologies on dengue control, as well as inform the design of dengue vaccine trials. As dengue virus was reintroduced into Brazil in 1986, and 30 years later the four serotypes circulate widely in the country, there is a natural shift in the age of susceptible population towards the youngest, who would be the target of new prevention research. Figure 6 provides a lay summary of the findings of this study.

Disclosures

Funding

This study (NCT01751139) was co-funded by Bio-Manguinhos/Fiocruz and GlaxoSmithKline Biologicals SA. Bio-Manguinhos/Fiocruz and GlaxoSmithKline Biologicals SA were involved in all stages of study conduct, including analysis of the data. GlaxoSmithKline Biologicals SA also covered all costs associated with the development and publication of this manuscript.

Contributorship

All authors participated in the design or implementation or analysis, and interpretation of the study; and the development of this manuscript. All authors had full access to the data and gave final approval before submission.

Disclosures

PB, MPGM, FHA, KL, and GAC have no financial and non-financial relationships to disclose. PB is also supported by FAPERJ (research support foundation of Rio de Janeiro). ARRF received fees from the GSK group of companies to contribute to the design of the study, he also received personal fees from the Faculdade de Medicina São Leopoldo Mandic, outside of the submitted work. EO, BFB, BC, AG and MdB are employees of the GSK group of companies; EO, BC, AG and MdB hold shares in the GSK group of companies. AA was a third-party employee of the GSK group of companies during the conduct of the study. EdB was a GSK employee during the conduct of the study. ACD, DA, MBB and TGN received financial support via their institution (Bio-Manguinhos / Fiocruz) through the research fund for vaccine co-development from the GSK group of companies for the conduct of the study.

Declaration of interests

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

Acknowledgements

The authors would like to thank the participants and their legal representatives for their participation to the study. The authors thank all the field and laboratory technical staff who helped with participants' recruitment and follow-up, and with sample processing and testing, as well as the regulatory and administrative staff who helped during study conduct and the Clinical Operations teams from GSK, Bio-Manguinhos/Fiocruz and PPD. The authors also thank the Business & Decision Life Sciences platform for editorial assistance and manuscript coordination, on behalf of GSK. Janne Tys coordinated the manuscript development and editorial support. Mary Greenacre provided medical writing support.

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Tables and figures

Table 1. Case definitions of dengue used in the study

Case definition	Description
Suspected symptomatic	Temperature ≥38°C by any route on ≥2 consecutive days and <14 days, with or without other dengue symptoms,[1] without obvious aetiology unrelated to dengue, based on the investigator's judgement
Laboratory-confirmed	At least one of the following: DENV identification on an acute[2] serum sample by RT-qPCR (early presenters only)[3] DENV NS1 positive on an acute[2] serum sample by ELISA (early presenters only) [3] Anti-dengue IgM seroconversion between acute and convalescent[2] serum samples by ELISA (early and late presenters) [3]
Virologically-confirmed	DENV identification by RT-qPCR
Probable	A suspected dengue case, based on strong clinical suspicion or a clinical diagnosis, in a late presenter (ie RT-qPCR not performed),[3] and: Anti-dengue IgM or anti-dengue IgG* positivity on at least one sample (either the acute or convalescent sample[2]) and NS1 negative on the acute sample[2] and No evidence of anti-dengue IgM seroconversion between the acute and the convalescent sample[2] *The ELISA test allows the detection of dengue IgG levels characteristic of acute secondary infections.
Negative	Negative result on all laboratory tests on both acute and convalescent samples[2]
Indeterminate	Evaluated as a suspected dengue case, but not classified as a laboratory-confirmed, probable or negative case (insufficient data to classify)
Inapparent primary infection	Seroconversion for anti-dengue IgG antibodies between two sequential serum samples obtained during scheduled visits without clinical suspicion of dengue

[1]Other symptoms of dengue, associated with fever, included but were not limited to fatigue, headache, pain behind the eyes, abdominal pain, nausea, vomiting, muscle ache, joint ache, diffuse rash on the trunk, photophobia and pruritis.

[2]Acute and convalescent serum samples were taken at the first and follow-up visits, respectively.

[3]Early presenters were defined as presenting at the health care facility within 5 days following the

onset of fever; late presenters were defined as presenting ≥6 days after the onset of fever.

DENV: dengue virus; ELISA: enzyme-linked immunosorbent assay; IgG/M: immunoglobulin G/M; NS1: non-structural protein 1; RT-qPCR: reverse transcriptase quantitative polymerase chain reaction

Table 2. Participant demographic and household socioeconomic characteristics at baseline (ATP cohort)

Characteristic	Number (%)[1]
Demographic characteristic	**Participants N=3264**
Age, years, mean (SD)	31.9 (20.2)
Female	1972 (60.4)
Socioeconomic characteristic	**Households N=1348**
Number of adults (≥18 years) in household	
1	141 (10.5)
2	591 (43.9)
3	315 (23.4)
4	163 (12.1)
5−12	136 (10.1)
Missing	2
Number of children (<18 years) in household	
0	383 (28.6)
1	398 (29.7)
2	319 (23.8)
3	142 (10.6)
4−10	98 (7.3)
Missing	8
Monthly family income (US$)[2]	
≤167	133 (12.3)
168−335	419 (38.7)
336−502	249 (23.0)
503−837	176 (16.3)
838−1600	92 (8.5)
≥1661	14 (1.3)
Missing	265
Running water from public system inside home	1340 (99.6)
Available 6−7 days per week	1303 (97.5)
Health insurance	1345 (99.8)
Visited by FHS programme	671 (52.0)
Mean (SD) no. times per year	6.2 (4.9)
Visited by LIRA programme	476 (39.3)

Mean (SD) no. times per year	1.6 (1.3)

[1]Number (%) participants or households shown unless otherwise stated

[2]Based on conversion from Brazilian reals using an exchange rate at 1 July 2016 (the mid-point of the first enrolment into the study [February 2014] and the last study visit [December 2018]): 1 Brazilian real equals 0.31 US dollars (https://www.exchangerates.org.uk/BRL-USD-spot-exchange-rates-history-2016.html)

ATP: according to protocol; FHSP: Family Health Strategy; LIRA: Larval Index Rapid Assay; SD: standard deviation

Table 3. Seroprevalence and self-reported dengue medical history at baseline by study centre and age group (ATP cohort)

	Rio	Manaus	Salvador	Natal	Campinas	Total
All ages	**N=737**	**N=727**	**N=605**	**N=598**	**N=597**	**N=3264**
Seroprevalence, n (%)	604 (82.0)	583 (80.2)	580 (95.9)	534 (89.3)	186 (31.2)	2487 (76.2)
Dengue medical history, n (%)	180 (24.4)	231 (31.8)	69 (11.4)	172 (28.8)	109 (18.3)	761 (23.3)
Age ≤17 years	**N=211**	**N=203**	**N=192**	**N=178**	**N=180**	**N=964**
Seroprevalence, n (%)	95 (45.0)	85 (41.9)	171 (89.1)	117 (65.7)	22 (12.2)	490 (50.8)
Dengue medical history, n (%)	9 (4.3)	10 (4.9)	10 (5.2)	34 (19.1)	9 (5.0)	72 (7.5)
Age 18−49 years	**N=358**	**N=329**	**N=324**	**N=300**	**N=291**	**N=1602**
Seroprevalence, n (%)	344 (96.1)	304 (92.4)	320 (98.8)	297 (99.0)	121 (41.6)	1386 (86.5)
Dengue medical history, n (%)	110 (30.7)	130 (39.5)	42 (13.0)	94 (31.3)	73 (25.1)	449 (28.0)
Age ≥50 years	**N=168**	**N=195**	**N=89**	**N=120**	**N=126**	**N=698**
Seroprevalence, n (%)	165 (98.2)	194 (99.5)	89 (100)	120 (100)	43 (34.1)	611 (87.5)
Dengue medical history, n (%)	61 (36.3)	91 (46.7)	17 (19.1)	44 (36.7)	27 (21.4)	240 (34.4)

ATP: according to protocol; N: number of participants at risk; n: total number of cases

Table 4. Incidence rate per 1000 person-years of laboratory-confirmed or probable symptomatic dengue infection by calendar year, study centre, age and gender (ATP cohort)

	N	n	PY	Incidence per 1000 PY (95% CI)
Overall	3264	299	8166.5	20.1 (13.2, 30.6)
Year				
2014	1192	10	562.5	18.7 (8.7, 40.0)
2015	1823	43	1180.4	26.3 (15.8, 43.7)
2016	2343	104	1884.8	27.9 (17.9, 43.5)
2017	2924	87	2419.9	18.1 (11.6, 28.3)
2018	2804	55	2118.9	13.1 (8.2, 20.8)
Study centre				
Rio de Janeiro	737	53	2291.8	19.6 (14.8, 26.0)
Manaus	727	77	2290.2	29.0 (22.6, 37.3)
Salvador	605	109	1636.9	59.1 (46.2, 75.5)
Natal	598	59	1243.2	51.7 (37.9, 70.5)
Campinas	597	1	704.5	1.9 (0.3, 13.4)
Age group (years)				
≤17	964	69	2092.8	33.0 (25.6, 41.7)
18−49	1602	173	4117.5	42.0 (36.0, 48.8)
≥50	698	57	1956.2	29.1 (22.1, 37.8)
Gender				
Female	1972	202	5006.3	40.3 (35.0, 46.3)
Male	1292	97	3160.2	30.7 (24.9, 37.4)

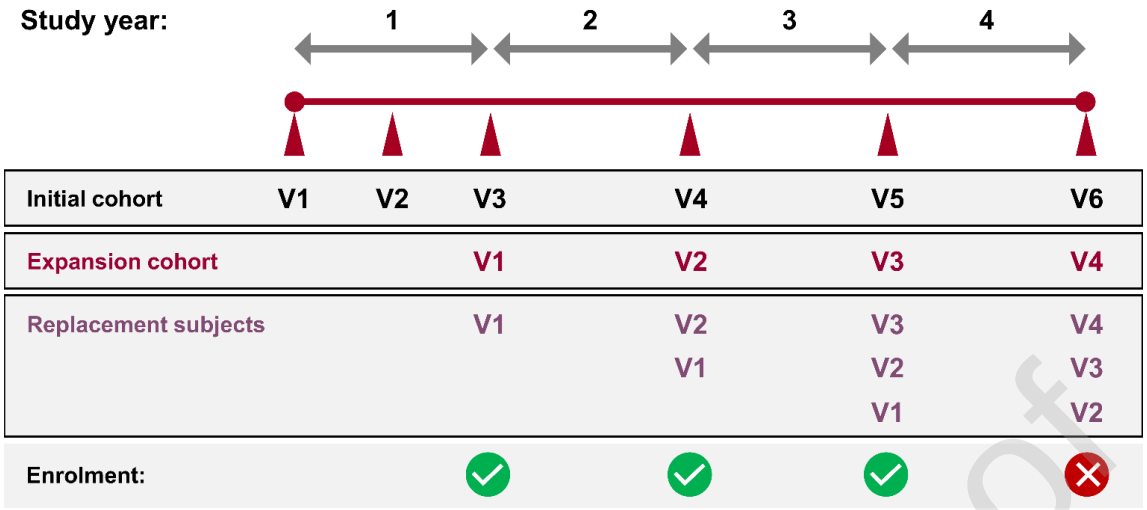
ATP: according-to-protocol; CI: confidence interval; N: number of participants at risk; n: total number of cases; PY: person-years

Table 5. Incidence rate per 1000 person-years of inapparent primary dengue infection by calendar year, study centre, age and gender in participants who were seronegative at baseline (ATP cohort)

	N	n	PY	Incidence per 1000 PY (95% CI)
Overall	777	52	1248.3	41.7 (31.1, 54.6)
Year				
2014	119	0	55.6	0 (0, 66.3)
2015	205	7	108.0	64.8 (26.1, 133.5)
2016	250	14	180.5	77.6 (42.4, 130.2)
2017	719	8	386.2	20.7 (8.9, 40.8)
2018	707	23	518.1	44.4 (28.1, 66.6)
Study centre				
Rio de Janeiro	133	13	338.6	38.4 (20.4, 65.7)
Manaus	144	21	242.3	86.7 (53.7, 132.5)
Salvador	25	5	51.9	96.3 (31.3, 224.8)
Natal	64	9	128.9	69.8 (31.9, 132.6)
Campinas	411	4	486.7	8.2 (2.2, 21.0)
Age group (years)				
≤17	474	36	843.3	42.7 (29.9, 59.1)
18−49	216	14	291.3	48.1 (26.3, 80.6)
≥50	87	2	113.8	17.6 (2.1, 63.5)
Gender				
Female	416	27	647.1	41.7 (27.5, 60.7)
Male	361	25	601.3	41.6 (26.9, 61.4)

ATP: according-to-protocol; CI: confidence interval; N: number of participants at risk; n: total number of cases; PY: person-years

Figure 1. Study design

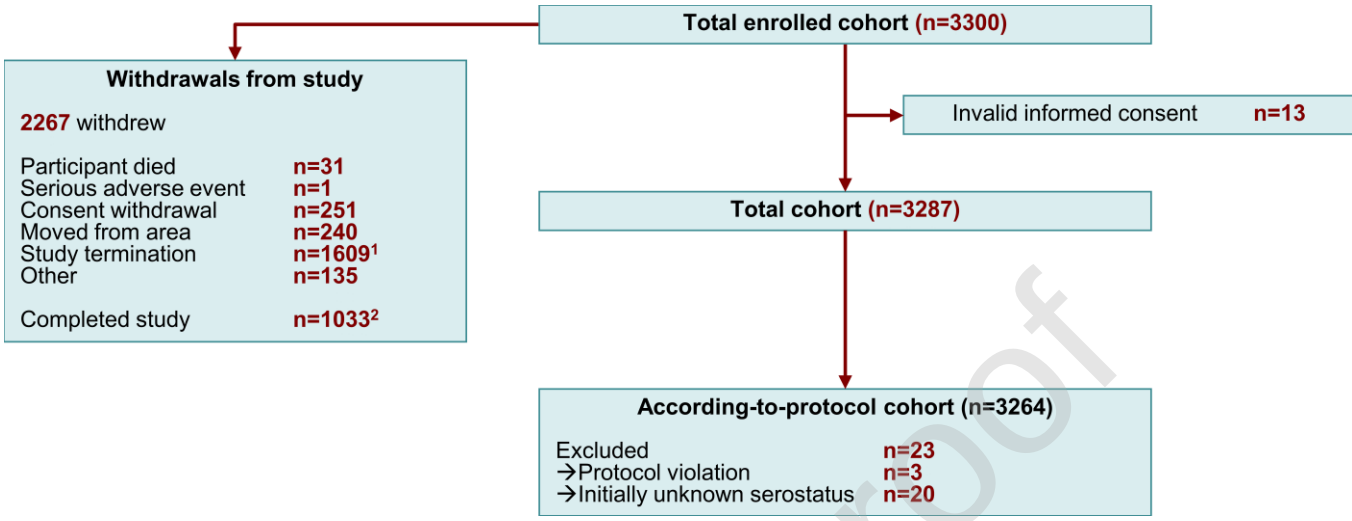


Not all visits took place because of early study termination.

V: visit

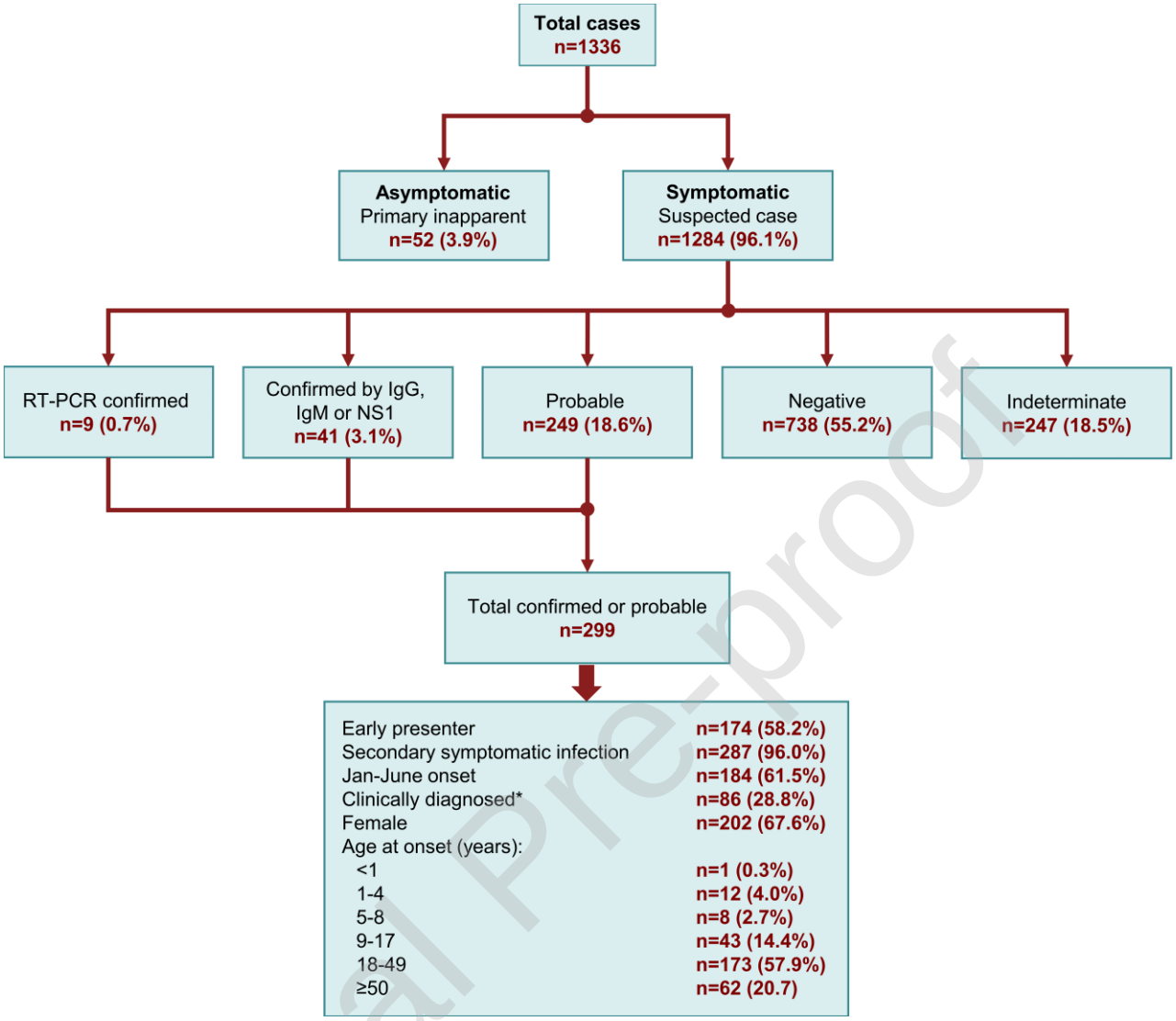
Figure 2. Map of Brazil showing study centres

Figure 3. Participant flow



[1]1609 participants discontinued the study before completing the follow-up visits because of the early study termination; [2]1033 participants completed the planned 4 years of follow-up.

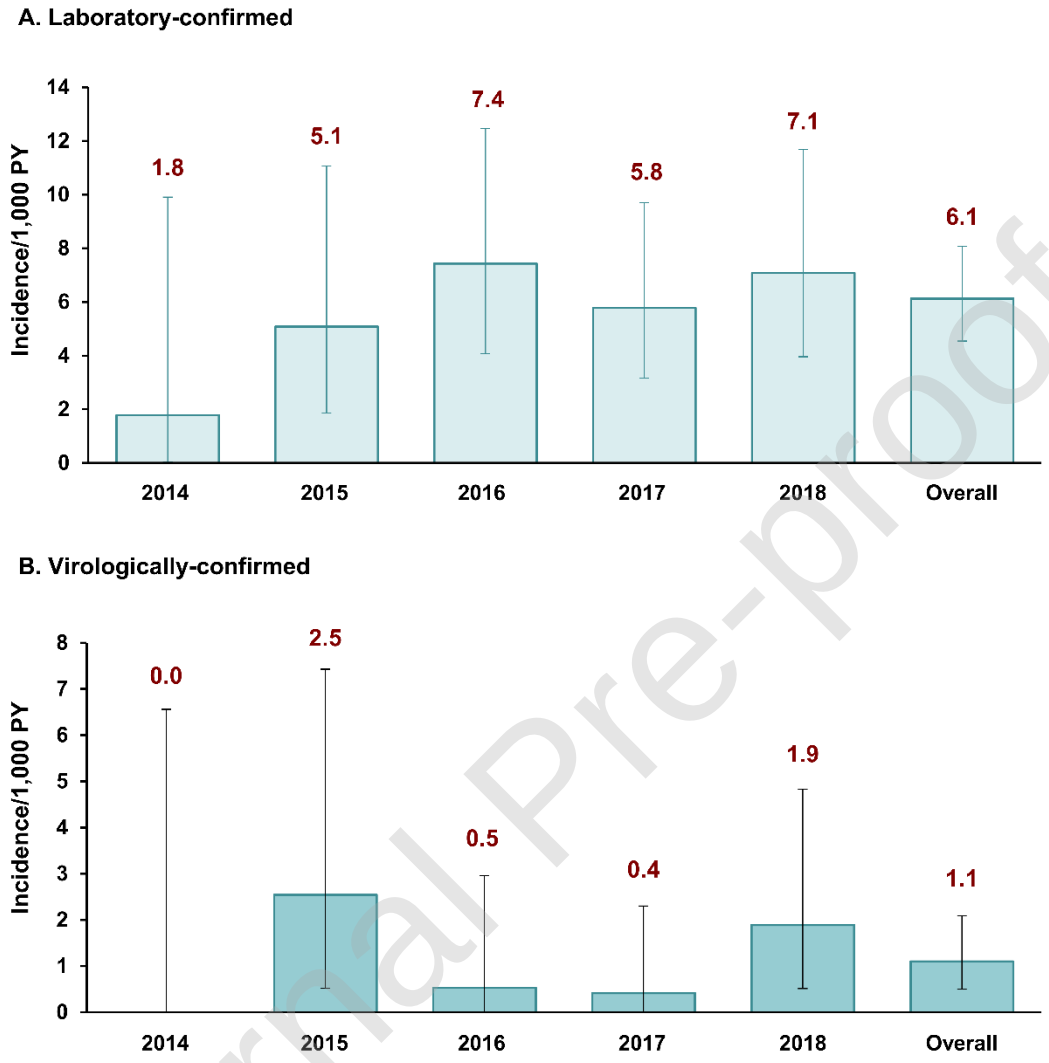
Figure 4. Classification of dengue cases (ATP cohort)



*For all patients meeting the case definition of suspected dengue, the physician was asked to express his or her opinion on the diagnosis based on clinical symptoms only, before the laboratory results were available. Cases which the physician diagnosed as dengue in this scenario were classified as clinically diagnosed cases

ATP: according-to-protocol; IgG/M: immunoglobulin G/M; n: number of cases; NS1: non-structural protein 1; RT-PCR: reverse-transcriptase polymerase chain reaction

Figure 5. Incidence rate per 1000 person-years of laboratory-confirmed (A) and virologically-confirmed (B) symptomatic dengue infection by calendar year (ATP cohort)



Error bars: 95% confidence interval

ATP: according-to-protocol; PY: person-years

Figure 6. Plain Language Summary