Les infections asymptomatiques dans la transmission de la dengue, mythe ou réalité ? Une étude épidémiologique à La Réunion

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LES INFECTIONS ASYMPTOMATIQUES DANS LA TRANSMISSION DE LA DENGUE, MYTHE OU REALITE ?

UNE ETUDE EPIDEMIOLOGIQUE A LA REUNION

Thèse
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par

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Knowledge, gain perspective for strategic vision, in short, to gain wisdom.

LIST OF PUBLICATIONS AND ORAL COMMUNICATIONS

Publications

- **De Santis O**, Pothin E, Bouscaren N, Irish SR, Jaffar-Bandjee MC, Menudier L, et al. Investigation of Dengue Infection in Asymptomatic Individuals during a Recent Outbreak in La Réunion. Viruses. 14 mars 2023;15(3):742.
- **De Santis O**, Bouscaren N, Flahault A. Asymptomatic dengue infection rate: A systematic literature review. Heliyon. 1 sept 2023;9(9):e20069.
- Lamaurt F, **De Santis O**, Ramis J, Schultz C, Rivadeneyra A, Waelli M, et al. Knowledge, Attitudes, Beliefs, and Practices Regarding Dengue in La Réunion Island, France. Int J Environ Res Public Health. 6 avr 2022;19(7):4390.

Oral Communications

- Présentation de l'étude DEMARE à l'Institut Pasteur de Madagascar et séminaire sur l'épidémiologie de la dengue en Afrique australe, Antananarivo, IPM, 01 juillet 2017
- Formation pour les médecins de centre de santé à Tamatave: aspects cliniques et épidémiologique de la dengue, Tamatave, IPM, 08 février 2019
- Cours Faculté de médecine, Université de Lausanne, "*Parcours d'un jeune chercheur*", Lausanne, UNIL, 29 mars 2019
- Présentation des résultats de l'étude DEMARE à Santé Publique France, Saint-Denis, La Réunion, 07 octobre 2021
- Conférence de l'Institut Pasteur de Madagascar, "*Déterminants influençant la transmission de la dengue dans les quartiers : une étude basée à la Réunion*". Dr Olga DE SANTIS, 19 mai 2022

ABSTRACT IN ENGLISH

Background and Rationale

Dengue is the most widespread arboviral disease in the world. Its incidence is increasing due to urbanization, population growth, air traffic, and climate change. The transmission of dengue in the Indian Ocean region is poorly understood. *Aedes albopictus*, a vector present in La Réunion, is generally considered a secondary vector of the dengue virus (DENV). The clinical presentation of dengue in this emerging site is still poorly defined, and the role of asymptomatic forms in transmission is also unclear.

Objectives

- To estimate the prevalence of dengue infections across all clinical forms encompassing asymptomatic, pauci-symptomatic, undifferentiated fever, dengue-like, and severe cases, confirmed by blood laboratory tests, among members of the same household as well as in the neighbourhood of a dengue detected case
- To assess the proportion of asymptomatic cases in dengue epidemics worldwide
- To assess the Knowledge, Beliefs, Attitudes, and Practices (KBAP) of the Reunion population regarding dengue

Design

In La Réunion, from January 2019 to July 2020, 605 community participants were recruited into geographical clusters around dengue index cases. Demographic and clinical data, as well as biological samples, were collected and laboratory analyses performed such as DENV RT-PCR and serology.

Some of the recruited community participants will undergo semi-structured interviews.

A systematic literature review will be conducted to assess the proportion of asymptomatic infection during worldwide dengue outbreaks.

Impact

The data collected in this study will provide an estimation of the prevalence of DENV infections across all disease patterns in La Réunion. Refining the clinical presentation of dengue and gaining insights into knowledge and attitude of local population in this specific tropical, high-income setting, where dengue is emerging, could significantly enhance public health surveillance measures.

Investigating dengue transmission in an environment where the primary vector is *A. albopictus* is highly relevant, given that this vector is spreading almost worldwide.

It is assumed that half or more of DENV infections are asymptomatic, making it essential to obtain a more comprehensive understanding of asymptomatic infections to evaluate their role in transmission and determine whether public health institutions should focus on it or not.

ABSTRACT IN FRENCH

Contexte et justification

La dengue est la maladie arbovirale la plus répandue dans le monde. Son incidence augmente en raison de l'augmentation de l'urbanisation, de la taille de la population, du trafic aérien et du changement climatique. La transmission de la dengue dans la région de l'océan Indien est mal connue. *Aedes albopictus*, vecteur présent à La Réunion, est généralement considéré comme un vecteur secondaire du virus de la dengue (DENV). La présentation clinique de la dengue dans ce site émergent est encore mal définie et le rôle des formes asymptomatiques dans la transmission également.

Objectifs

- Estimer la prévalence des infections par le virus de la dengue dans toutes les formes cliniques, y compris les cas asymptomatiques, parmi les membres du même foyer ainsi que dans le voisinage d'un cas de dengue
- Évaluer la proportion des infections asymptomatiques de dengue dans le monde
- Évaluer les connaissances, les croyances, les attitudes et les pratiques de la population réunionnaise concernant la dengue

Méthodologie

À La Réunion, de janvier 2019 à juillet 2020, 605 participants communautaires sont recrutés dans des clusters géographiques autour de cas index de dengue. Des données démographiques et cliniques, ainsi que des échantillons biologiques, sont collectés pour des investigations telles que la RT-PCR du DENV et la sérologie. Certains des participants communautaires recrutés seront soumis à des entretiens semi-structurés. Une revue systématique de la littérature est menée pour évaluer la proportion d'infections asymptomatiques lors des épidémies de dengue dans le monde.

Impact

Les données collectées dans cette étude permettront d'estimer la prévalence des infections par le DENV dans toutes les formes de la maladie à La Réunion. Affiner la présentation clinique de la dengue et comprendre les connaissances et les attitudes de la population locale dans ce contexte tropical spécifique à revenu élevé, où la dengue émerge, pourrait considérablement améliorer les mesures de surveillance de la santé publique. Étudier la transmission de la dengue dans un

environnement où le principal vecteur est *A. albopictus* est particulièrement pertinent, étant donné que ce vecteur se propage pratiquement dans le monde entier. On suppose que la moitié ou plus des infections par le DENV sont asymptomatiques, ce qui souligne l'importance d'obtenir une compréhension plus complète des infections asymptomatiques pour évaluer leur rôle dans la transmission et déterminer s'ils devraient être au centre des efforts de santé publique.

OUTLINE

GENERAL INTRODUCTION

> Note to the reader :

In the following introductory pages, the text is formatted to allow you to skip the blue boxes if you already have a good understanding of dengue and infectious diseases in general or if you have limited interest in technical aspects. You can exclusively read the text between the boxes to obtain a narrative overview of the story of this thesis.

Enjoy your reading!

Dengue is a global health challenge that encompasses a multifaceted landscape ranging from the subtleties of virology to the epidemiological dynamics governing its spread. The role of asymptomatic infections in the transmission of the disease remains enigmatic but essential.

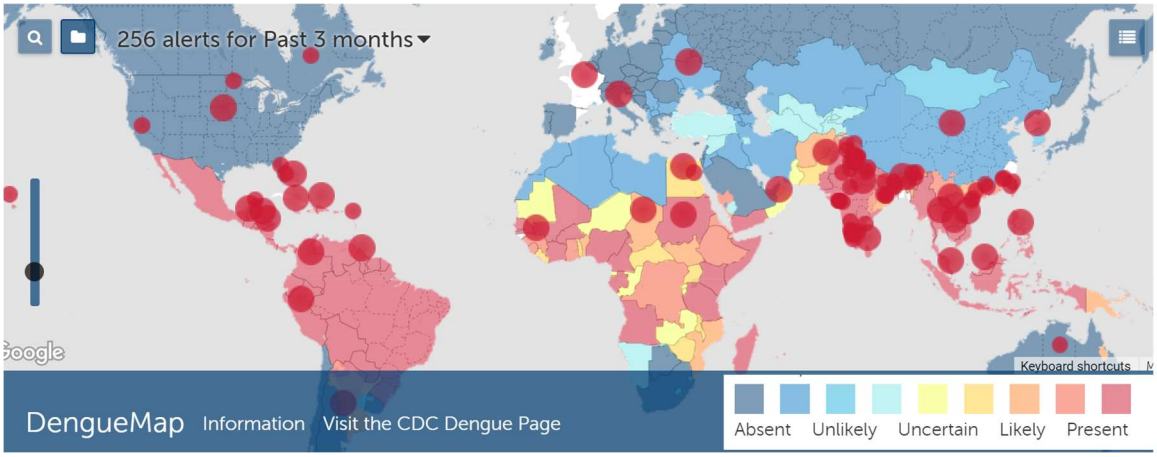
During the last two decades, humanity had to confront many emerging viral threats(1) :

- 2002 - Severe acute respiratory syndrome corona virus infection (SARS CoV1), approximately 800 deaths ;
- 2009 - Influenza A H1N1, hundreds of thousands deaths ;
- 2012 - Middle East respiratory syndrome CoV infection (MERS), hundreds of deaths ;
- 2013 - Ebola, 11'000 deaths, mainly in West Africa ;
- 2015 - Zika, several birth defects in newborns, mainly in Brazil and Central America.

Dengue: the most prevalent arboviral disease globally

Dengue is the **most prevalent arboviral disease** globally(2). Up to 3.97 billion people (half of the world) living in 128 countries are at risk of contracting dengue virus (DENV) infections(3). The incidence of dengue has increased thirty times during the last fifty years with about 100 million new infections, 500 000 hospitalizations for severe cases and approximatively 20 000 deaths each year (4–6). This rise is expected to continue in the future due to the increase in urbanization, increase in population size (i.e. the worldwide population doubled since the sixties), high interconnectivity (e.g. currently there are about 3 billion flights every year) and climate change (7–9). The situation of dengue in Africa and in the Indian Ocean region is poorly known (10). These regions seem to be less affected by the disease than the Americas or Asia, but data are conflicting and scarce. For example, serologic data from febrile travelers returning from these different regions, showed that dengue seems to be less prevalent in Africa than in Asia or in the Americas (11,12). *Bhatt et al 2013* estimated that in 2010, 15.7 (10.5-22.5) million symptomatic dengue infections occurred in Africa (including

Madagascar and La Réunion) and that Africa was the region with the second highest prevalence rate after Asia (5). In some eastern and western African countries, some outbreaks recently occurred with strains coming from Asia (11,12).



Dengue map: Red spots represent recent alert of dengue outbreaks in the past three months https://www.healthmap.org/dengue/en/.

In 2016, when the idea of this study on asymptomatic forms of dengue in Réunion Island first emerged, the dengue epidemic in La Réunion had not started yet. The dengue situation on the island was both intriguing and unique. All the predisposing factors for the emergence of an epidemic were present: a high population density living in urban areas close to wetlands and vegetation, the known presence of the dengue vector *Aedes albopictus* (13,14), and occasional virus detections each year indicating the presence of the virus into the territory (15). Cases remained sporadic despite these conditions, around 20 to 40 cases per year, with only occasional clusters being detected(15).

This specific context raised several questions about why this low-level circulation persisted: Was it due to the lower vector competence of *A. albopictus* compared to *A. aegypti*? Were the people of La Réunion genetically resistant to the disease? And were the few cases observed during the summer season a result of virus introductions from endemic countries such as the neighboring Seychelles or more distant nations like Thailand or Sri Lanka? All these questions highlighted the central inquiry of this thesis work: **Is there a silent transmission due to asymptomatic forms of infection, thus creating a kind of human reservoir for the disease?**

Dengue virus, diagnosis, immunology and vaccine

The **Dengue Virus (DENV) is a single-stranded RNA virus**, a member of the *Flaviviridae* family, a lineage shared with other notorious pathogens like Yellow Fever, West Nile, Zika, and Japanese Encephalitis viruses (16). DENV is characterized by the presence of four antigenically and phylogenetically distinct serotypes: DENV1 and DENV2, discovered in 1944, and DENV3 and DENV4, discovered in 1954(17,18). All four serotypes can inflict a wide spectrum of diseases, ranging from mild dengue fever to severe and often fatal conditions such as dengue hemorrhagic fever and dengue shock syndrome. The dengue-like syndrome is defined as an acute fever disease that has two or more of the following signs or symptoms: nausea, vomiting, rash, headache, retro-orbital pain, myalgia, arthralgia and hemorrhagic signs(6). The World Health Organization (WHO) recommends several **methods for diagnosing** dengue, including the detection of dengue-specific IgM antibodies using the enzyme-linked immunosorbent assay (ELISA) or a ≥ 4-fold increase in the titer of total antibodies to DENV in paired acute and convalescent sera. Additionally, the diagnosis can involve the detection of DENV using Reverse Transcription–Polymerase Chain Reaction (RT-PCR) in plasma, serum, or whole blood (16). It is also possible to diagnose dengue through RT-PCR or serology using samples collected on dried-blood spot filter papers(19,20). **A Rapid Diagnostic Test (RDT),** which relies on the detection of a DENV antigen (Non-Structural protein 1 or NS1) and an anti-DENV antibody (IgM), is available and can achieve a sensitivity of 87.3% and a specificity of 86.8% (21). At the time of the study, dengue RDTs were not adopted in La Réunion or in Madagascar.

The study project submitted for funding to the Swiss National Science Foundation included two study sites in the Indian Ocean: La Réunion Island and Madagascar. The Madagascar site had to meet the same objectives of researching asymptomatic dengue infections in three towns in Madagascar. An additional objective was to search for dengue RNA on malaria RDTs. The field studies, which included more than 1000 participants, were carried out thanks to our collaboration with the Institut Pasteur de Madagascar (IPM). Due to the Covid-19 pandemic, the study in Madagascar had to be suspended for some

months. In view of the long delay, these results are not included in this thesis work. However, analyses are currently being carried out by the IPM team and a publication is expected next year.

Considering their user-friendly nature, rapid results, and cost-effectiveness, implementing RDTs in clinical practice during General Practitioners (GP) consultations on La Réunion could be highly beneficial. However, before being implemented, their performance should be assessed in low transmission settings. Interestingly, studies have demonstrated that dengue RNA can be detected by RT-PCR in the sample pad of the dengue RDT(22). Similar findings have been observed in the detection of malaria DNA using malaria RDTs(23). Malaria RDTs are increasingly being used, even in remote areas, for detecting malaria in endemic regions like Madagascar(24). The exploration of DENV RNA presence on malaria RDTs offers a novel approach for estimating dengue prevalence in a febrile target population. Furthermore, non-invasive diagnostic tools based on the detection of viral RNA or NS1 in the saliva of symptomatic dengue patients have been investigated(25–29). However, their sensitivity remains limited. Due to its various serotypes, **dengue's immunology** is intricate. Infection with one serotype can provide complete cross-protection for two months and partial cross-protection for two years against the other serotypes(30). Conversely, after an infection, the immune response may facilitate subsequent infections through an immunological reaction known as "antibody-dependent enhancement," thereby increasing the severity of secondary infections(31). The situation in the case of tertiary or quaternary infection is not fully understood. Immunological cross-reactions with other Flaviviruses are currently under discussion but remain controversial. It is unclear whether a prior infection with DENV would offer protection or exacerbate a Zika virus (ZIKV) infection, for example(32,33). A **dengue vaccine** was licensed in 2016, but it is recommended by the World Health Organization (WHO) only for areas with high transmission rates and for individuals aged 9 years and above due to safety concerns(34). Consequently, in low transmission areas like La Réunion, prevention primarily relies on vector control and personal protective measures against mosquito bites(35).

One of the secondary objective of the study was to evaluate the performance of RDTs in the context of La Réunion. Unfortunately, this objective had to be abandoned for budgetary reasons. As far as I know, RDTs are now used in emergency departments of the hospital but have not yet been adopted by GPs in everyday practice.

A.albopictus

Transmission of DENV to humans is predominantly mediated by **mosquitoes**, with *Aedes aegypti* and *Aedes albopictus* serving as the primary vectors. Traditionally, *Aedes albopictus*, commonly known as the *Asian tiger mosquito*, was considered a secondary vector for dengue. This species has a high adaptability and can therefore survive in more temperate and cooler regions of Europe. Its spread is due to its tolerance to temperatures below 0°C through egg diapause, its ability to hibernate and its ability to breed in microhabitats(36). Its relevance during CHIKV outbreaks has been demonstrated during the La Réunion 2006 outbreak. However, it is poorly understood to what extent *A.albopictus* can transmit dengue, as many dengue outbreaks are allocated to it but most of them included only a few cases(37–45). History then showed us, in La Réunion, with almost 60 000 cases of infection in 2021, that *A. albopictus* is perfectly capable of transmitting dengue.

After an initial unsuccessful attempt to secure funding for studying dengue transmission in La Réunion, the funding needed to kick-start the study was eventually obtained through the *Swiss National Fund for Scientific Research* in 2018. Meanwhile, the dengue situation in La Réunion underwent significant changes with an epidemic breaking out. This altered context, which ultimately did not affect the project's fundamental question – that is understanding the role of asymptomatic infections in epidemic transmission – allowed the field study to commence in 2019.

La Réunion Island

La Réunion is a French island of 2 503 km2 located in the Indian Ocean with approximatively 860 000 inhabitants. The island is characterized by a tropical environment and industrialized high-income development. Among the twelve species of mosquitoes present on the island, *A.albopictus* is the most widespread(13,14). La Réunion dengue chronicle is punctuated by significant outbreaks. A significant outbreak of chikungunya (CHIKV) in 2005-2006 had an attack rate of 35%(46). A first likely dengue outbreak was described in 1851(47). In 1977-78 a massive DENV2 outbreak originating from the Seychelles took place on the island with an attack rate of 30%(48–50). After an outbreak with 200 cases in 2004, only sporadic cases were detected on the island until 2012(51). After 2012, new cases emerged with a mean of thirty dengue cases per year and an outbreak of 231 cases in 2016. In 2017, 69 cases have been notified. The island typically experiences dengue peaks between
April and May, coinciding with the conclusion of the rainy season. Dengue has shown varying levels of morbidity and mortality in recent years. The following data provides insight into the estimated cases, confirmed cases, and fatalities associated with dengue on the island(15). The prevalence of dengue on Réunion Island has reached 3.5%. The dengue-related mortality rate is of 0.11%.

Date	Estimated Cases	Confirmed Cases	Deaths
2018	15,460	6,770	6
2019	42,420	18,217	14
2020	30,580	16,414	22
2021	59,230	29,577	33

Our hypothesis was that a substantial portion of dengue infections elude routine surveillance due to their asymptomatic nature or limited interactions with the healthcare system. These "silent" infections may wield significant influence over disease transmission. To validate our hypothesis, we have formulated the following research questions:

1. *What is the true prevalence of dengue infections, including asymptomatic cases, within a defined radius around a detected index case?*
2. *What diverse clinical presentations manifest within these communities?*

3. *What proportion of cases remain asymptomatic, and how many mild infections evade healthcare-seeking behavior?*

4. *What is the prevalence of dengue IgG immunity in regions where dengue is emerging, such as La Réunion ?*

Why study asymptomatic cases ?

Understanding the prevalence of asymptomatic and pauci-symptomatic cases is pivotal for devising public health strategies, particularly for blood donor screenings and vaccine trial designs. Estimates suggest that asymptomatic cases can account for 50% to 90% of infections during outbreaks(30,52,53). Studies collecting data on the occurrence of DENV infections taking into account all types of disease, including asymptomatic, have been carried out in Latin America and South-East Asia(54), data on these silent infections in the Indian Ocean region remain scant. Asymptomatic and pauci-symptomatic people could constitute a reservoir of the virus, and it is necessary to know their prevalence in order to establish public health policies for selecting blood donors and setting up clinical trials of vaccines. The role of asymptomatic infections in cross-immunity is not fully understood, and the course and severity of a secondary infection when the previous infection was asymptomatic cannot be predicted. Finally, studying the genotype of asymptomatic cases is important for understanding which gene may have an impact on the course of the disease. Although it was assumed that asymptomatic individuals could transmit DENV to *Aedes* species during dengue inoculation experiments with soldiers and monkeys by *Simmons et al* in 1931(55), this was only demonstrated in 2015 by *Duong et al*, for *A.aegypti*(56).

Paper 1 : Investigation of Dengue Infection in Asymptomatic Individuals during a Recent Outbreak in La Réunion

To address our research questions and study the role of asymptomatic infections in the transmission of dengue, we decided to undertake an **epidemiological field study** with the following objective :

- To estimate the prevalence of dengue infections across all clinical forms encompassing asymptomatic, pauci-symptomatic, undifferentiated fever, dengue-like, and severe cases, detected by RT-PCR or IgM/IgG ELISA serology among members of the same household as well as in the neighborhood of a dengue detected case.

Studies conducted on dengue transmission patterns have consistently emphasized the **focal nature** of outbreaks, characterized by clusters of cases concentrated in specific regions. These studies recruiting participants in geographical clusters around index cases (IC) have been carried out in South east Asia and Latin America in areas of high transmission(57–60). They have demonstrated an initial higher rate of dengue infections in clusters around IC that vanishes over time. We therefore opted for a cluster design for this initial study, given the focal nature of dengue. This design was expected to provide us with a better chance of detecting dengue circulation.

Following this field study, in order to corroborate our findings, we conducted a **systematic literature review** to assess the proportion of asymptomatic cases in dengue epidemics worldwide. A focused literature review had been conducted prior to drafting the field study protocol. However, as our results did not align with the expected outcomes, we decided to delve back into the literature systematically and analytically this time to gain a more detailed understanding of the published data.

Paper 2 : Asymptomatic dengue infection rate : a systematic literature review

What is called an asymptomatic infection in literature?

The proportion of asymptomatic dengue infections is a topic of interest but can be misleading due to inconsistent terminology and definitions in the literature. While it is commonly accepted that a significant percentage of dengue cases are asymptomatic, the term *'asymptomatic'* is often used interchangeably with *'mild'* or *'under-reported'* infections(61). These variations in terminology create challenges when assessing risk factors and conducting epidemiological studies. For epidemiological purposes, infections with no symptoms are often grouped together with *'unapparent symptomatic'* infections(62). This grouping can lead to confusion because a symptomatic infection, even if mild, can be detected by the patient or a healthcare practitioner, whereas truly asymptomatic infections go completely unnoticed.

This distinction is crucial when making decisions about blood donor screening policies or responding to new outbreaks and communicating preventive measures to the population.

Furthermore, the heterogeneity in reported rates of asymptomatic dengue cases across studies is partly due to the lack of standardized terminology and study designs. To address this issue, it is essential to clearly differentiate between infections with no symptoms and *'unapparent'* infections in order to better understand the epidemiology of dengue and its associated risk factors.

Given that dengue was an emerging, or rather re-emerging, disease in La Réunion, a region that had recently experienced a significant outbreak of chikungunya, we deemed it necessary to complement our work with a **qualitative study** to gain insight into the population's perception of the disease and their healthcare attitudes. Our primary research question for this qualitative aspect was:

- *Can the endemicity of dengue in La Réunion be partially attributed to the knowledge, beliefs, attitudes, and practices of the population ?*

Paper 3 : Knowledge, Attitudes, Beliefs, and Practices Regarding Dengue in La Réunion, France

Given the prevailing epidemic conditions, and in light of the numerous prevention campaigns led by healthcare institutions, it is interesting to evaluate the population's awareness, local perceptions of the disease, risk perception, and its perceived severity. As far as we are aware, no studies pertaining to dengue in La Réunion have been undertaken. The most recent studies published in France primarily centered on the Chikungunya epidemic or populations in the Caribbean. The objective of this qualitative study was :

- To assess the Knowledge, Beliefs, Attitudes, and Practices (KBAP) of the Reunion population regarding dengue

In order to gain a better understanding of the concerns of La Réunion residents during the semi-structured interviews, it is essential to have a clear

grasp of the existing prevention policies in La Réunion, especially the surveillance system. The following section provides a description of the surveillance system currently in place in La Réunion.

Surveillance system

La Réunion's proactive stance against dengue has led to the establishment of a robust surveillance system, a response triggered by the chikungunya outbreak of 2006. The *Cellule Inter-Régionale d'Epidémiologie* (CIRE) (currently renamed as *Santé Publique France*) is at the forefront of this system, promptly informed of any laboratory-confirmed cases of arbovirus diseases. Vector control measures are swiftly implemented, including targeted insecticide spraying around the residences of confirmed cases. The surveillance system is an essential pillar of the island's defense against dengue fever.

The fight against vectors has been ongoing since 1914 in Réunion. Led by the "prophylaxis service," it primarily targeted the anopheles mosquito, responsible for transmitting malaria, which caused around 2,000 deaths annually on the island in the mid-20th century. The late 1970s saw the eradication of malaria, and the following years witnessed a decline in vigilance until the chikungunya epidemic in 2005. On that occasion, the various stakeholders of the Vector Control Program developed a specific response system, which is currently being implemented in the context of the dengue epidemic(63). The actions carried out by the Vector Control Program rely on surveillance work conducted by the CIRE. At the start of an epidemic, healthcare professionals are asked to report each suspected case and systematically prescribe a biological confirmation to monitor the epidemic's onset and document the viral serotype. The reported cases are investigated to determine the scope of intervention for the Vector Control teams in the field. The Vector Control strategy is based on several parallel actions, including the elimination of larval breeding sites with mechanical treatment and larvicidal treatment ; systematic interventions for daytime adulticidal treatment around biologically confirmed cases and emerging hotspots or nighttime spatial spraying in established transmission areas using deltamethrin ; mobilization of the public through communication campaigns ; communal sanitation actions in the public domain(64).

METHODS

The research methods employed by the candidate in this thesis encompassed several approaches:

- An observational epidemiological study involving cross-sectional household surveys and blood sample collection in La Réunion, detailed in the first article. The association between determinants and outcomes was statistically measured using odds ratios and logistic regression models for multivariate analysis.
- The second article comprised a systematic literature review, estimating proportions of asymptomatic infections and confidence intervals by compiling frequencies of asymptomatic infections and sample sizes from included studies.
- Additionally, a Knowledge, Attitudes, Beliefs, and Practices (KABP) study was conducted, consisting of a quantitative section analyzing cross-relationships between socio-demographic characteristics, levels of knowledge, and perception. A qualitative section was carried out through semi-structured interviews.

Role of the candidate in the thesis work

The candidate conceptualized this thesis project during a visit to Réunion in 2016, engaging in discussions with various local stakeholders about the prevalent cases of dengue on the island. An initial letter of intent focusing on studying asymptomatic forms of dengue in Réunion was submitted to the Swiss National Fund for Scientific Research in 2016 but was not accepted. Subsequently, while based in La Réunion, the candidate reached out to the Institut Pasteur in Madagascar (IPM) to explore this topic further. A new letter of intent, broadening the scope to study asymptomatic forms of dengue in both La Réunion and Madagascar, was submitted to the same funding body and this time was accepted.

The candidate then took charge of establishing the entire research framework in La Réunion, which involved drafting the study protocol, creating informed consent documentation and writing the study questionnaire, recruiting nurses, formulating standardized operating procedures for the research, seeking approval from French, Malagasy, and Swiss ethics committees, and developing collaboration agreements between the University of Geneva, the University Hospital Center of Réunion, and the IPM.

Field surveys in Réunion were conducted by the candidate alongside a team comprising three nurses and a Creole-speaking investigator.

Study documents in Madagascar were jointly developed with the IPM team and translated into Malagasy by an IPM coordinator. Field surveys in Madagascar were conducted by six Malagasy nurses engaged in the project, trained and supervised by the candidate and a Malagasy medical coordinator from the IPM.

As mentioned earlier, the articles forming this thesis only encompass the Réunion part of the study. Due to the COVID-19 pandemic, delays in the surveys in Madagascar prevented the finalization of analyses and presentation of the Malagasy results on time. This work is ongoing, led by the IPM, and the data will be disseminated and discussed during 2024.

For each article, the role of the candidate is detailed below.

De Santis O, Pothin E, Bouscaren N, Irish SR, Jaffar-Bandjee MC, Menudier L, et al. Investigation of Dengue Infection in Asymptomatic Individuals during a Recent Outbreak in La Réunion. Viruses. 14 mars 2023;15(3):742.

- Writing proposal for funding demand, protocol elaboration, screening and inclusion of participants, field data collection, data analysis, paper writing

De Santis O, Bouscaren N, Flahault A. Asymptomatic dengue infection rate: A systematic literature review. Heliyon. 1 sept 2023;9(9):e20069.

- Systematic literature search, data extraction, data analysis, paper writing

Lamaurt F, De Santis O, Ramis J, Schultz C, Rivadeneyra A, Waelli M, et al. Knowledge, Attitudes, Beliefs, and Practices Regarding Dengue in La Réunion Island, France. Int J Environ Res Public Health. 6 avr 2022;19(7):4390.

- Protocol co-elaboration with F.Lamaurt, screening and inclusion of participants, F.Lamaurt did the interviews and transcription, reading and analyzing the transcript, revising the quantitative data analysis, paper co-writing

RESULTS

First article

De Santis O, Pothin E, Bouscaren N, Irish SR, Jaffar-Bandjee MC, Menudier L, et al. Investigation of Dengue Infection in Asymptomatic Individuals during a Recent Outbreak in La Réunion. Viruses. 14 mars 2023;15(3):742.

The study, conducted from October 2019 to August 2020, identified 17 dengue index cases (IC) and recruited 605 participants from 368 households within a 100 to 200 meter radius of the IC's homes. Clusters were formed based on these cases, totaling 19 clusters distributed across the island of La Réunion. While no active asymptomatic infections were confirmed by RT-PCR, 32 participants (5.3%) had recent or active dengue infections, occurring within three months preceding the survey. Most recent infections were detected before the study team's visit.

Dengue infections were found in 10 of the 19 investigated clusters, with active infections limited to only two clusters (in the south and east regions). Serotyping of the active infections revealed DEN-1 and DEN-3. Dengue infections were symptomatic, with fever and fatigue as primary symptoms, although some cases were pauci-symptomatic. Dengue-like symptoms were common, including anorexia, headache, myalgia, and arthralgia.

Anti-dengue IgM was detected in 8% of participants with negative RT-PCR results, often accompanied by IgG. Notably, 42% of those with IgG had no history of dengue, indicating past undiagnosed infections or potential assay specificity issues. IgG prevalence varied widely among clusters.

Households often had multiple members with IgG antibodies (primarily in the west and south regions), suggesting potential clustering of past infections. Environmental factors like housing type and waste presence showed significant associations with dengue infection, while sociodemographic criteria did not exhibit such associations.

Second article

De Santis O, Bouscaren N, Flahault A. Asymptomatic dengue infection rate: A systematic literature review. Heliyon. 1 sept 2023;9(9):e20069.

The literature review encompassed 465 papers, with 74 included in the analysis. Among these, 50 studies presented asymptomatic rates of dengue infections, while 24 studies detailed the prevalence of dengue infections among asymptomatic populations.

Geographical distribution indicated that the vast majority (96%) of studies occurred in Asia and Latin America, with only one study in Africa. Asymptomatic rates (AR) varied widely across regions, with Latin America showing rates from 16% to 97%, Southeast Asia from 0% to 96%.

Most studies (73%) were published after 2006, marking a shift from serosurveys and studies on travelers before 2000 to more recent cohort and cluster studies. Three categories defined asymptomatic dengue infections: those with no symptoms (AR 0-42%), subclinical cases (AR 0-100%), and unapparent cases (AR 50-97%).

Studies were classified based on recruitment design: cluster studies (7.4% to 92% AR), cohort studies (60% to 92% AR), serosurveys (6.6% to 97% AR), studies on travelers (0% to 27% AR), and studies on blood donors (0% to 5.5% prevalence). These studies presented various risk of biases, age groups, and detection methods.

Analysis by age group indicated a higher AR for children (20-87%, median 66%) compared to adults (0-96%, median 33%). Serotype association with AR was inconclusive due to limited data. Studies suggested that primary infections tended to be more symptomatic, while secondary infections were milder or asymptomatic, especially with shorter intervals between infections.

Overall, the review revealed a wide range of asymptomatic rates across different regions, recruitment designs, age groups, and detection methods, highlighting the complex nature of asymptomatic dengue infections.

Third article

*Lamaurt F, **De Santis O**, Ramis J, Schultz C, Rivadeneyra A, Waelli M, et al. Knowledge, Attitudes, Beliefs, and Practices Regarding Dengue in La Réunion Island, France. Int J Environ Res Public Health. 6 avr 2022;19(7):4390.*

The study combined quantitative data collected through a questionnaire administered to a representative sample of 622 people to assess the use of protective measures and the perception of severity and risk of dengue, and a sample of 336 people to assess the level of

knowledge and concern about dengue, as well as qualitative data collected through semi-structured interviews among 11 individuals who had previously completed the questionnaire. The study results show that 63% of the surveyed population had a good level of knowledge associated with age, education, and socio-professional category variables—78% considered dengue to be a serious threat, and concern was estimated at 6/10, while 71% were likely to use protective measures. The interviews revealed contradictory behaviors in the implementation of recommended actions, in conflict with personal beliefs regarding respect of human body and nature. The study also revealed a loss of confidence in public authorities.

PUBLICATIONS

Investigation of Dengue Infections in Asymptomatic Individuals during a Recent outbreak in La Réunion

Citation

***De Santis O**, Pothin E, Bouscaren N, Irish SR, Jaffar-Bandjee MC, Menudier L, et al. Investigation of Dengue Infection in Asymptomatic Individuals during a Recent Outbreak in La Réunion. Viruses. 14 mars 2023;15(3):742.*

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Article

Investigation of Dengue Infection in Asymptomatic Individuals during a Recent Outbreak in La Réunion

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Abstract: The number of dengue cases has increased dramatically over the past 20 years and is an important concern, particularly as the trends toward urbanization continue. While the majority of dengue cases are thought to be asymptomatic, it is unknown to what extent these contribute to transmission. A better understanding of their importance would help to guide control efforts. In 2019, a dengue outbreak in La Reunion resulted in more than 18,000 confirmed cases. Between October 2019 and August 2020, 19 clusters were investigated in the south, west, and east of the island, enabling the recruitment of 605 participants from 368 households within a 200 m radius of the home of the index cases (ICs). No active asymptomatic infections confirmed by RT-PCR were detected. Only 15% were possible asymptomatic dengue infections detected by the presence of anti-dengue IgM antibodies. Only 5.3% of the participants had a recent dengue infection confirmed by RT-PCR. Although the resurgence of dengue in La Réunion is very recent (2016), the rate of anti-dengue IgG positivity, a marker of past infections, was already high at 43% in this study. Dengue transmission was focal in time and space, as most cases were detected within a 100-m radius of the ICs, and within a time interval of less than 7 days between infections detected in a same cluster. No particular demographic or socio-cultural characteristics were associated with dengue infections. On the other hand, environmental risk factors such as type of housing or presence of rubbish in the streets were associated with dengue infections.

Keywords: dengue; asymptomatic infections; La Réunion; cluster study; dengue outbreak

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1. Introduction

Dengue is the most common arboviral disease in the world [1]. Half the world's population lives in areas at risk of dengue virus (DENV) infection [2]. The burden is increasing due to climate change and increases in urbanization, population, and air traffic [3–5]. Globally, there are an estimated 100 million new infections per year, including 500,000 hospitalizations for severe cases and 10,000 to 15,000 deaths (2021 estimate) [1,6,7]. The epidemiological situation of dengue in Africa and the Indian Ocean region is poorly understood [8]. Seroprevalence studies of febrile travelers returning to Europe have estimated a lower prevalence of dengue in travelers from Africa than from Asia or the Americas [9,10]. However, it has been estimated

that 15.7 (10.5–22.5) million symptomatic dengue infections occurred in Africa (including Madagascar and La Réunion), making Africa the second most affected continent after Asia [6].

La Réunion is a French overseas department of 2503 km^2 with nearly 860,000 inhabitants [11]. It is located in the southwest Indian Ocean, near the east coast of Madagascar. A tropical environment and a high-income industrial development characterize this island. Dengue is transmitted by the bite of a mosquito and has no animal reservoir in La Réunion. *Aedes albopictus* is the main mosquito vector of dengue found in La Reunion [12]. Four serotypes for dengue (DENV1–DENV4) have been identified worldwide. The infection with one serotype confers lifelong immunity for it and only some cross-immunity for the other during the first months after infection. The island faced a major chikungunya epidemic in 2005–2006 with an attack rate of 38% [5]. In 1977–1978, a massive DENV2 epidemic occurred on the island with an attack rate of 30% [13–15]. Thereafter, only sporadic dengue cases or small outbreaks of approximatively 200 cases had been detected on the island. In 2017, the surveillance system noted an unusual persistence of dengue cases during the austral winter (June to September) likely due to warmer temperatures [16] and then an intensification of the circulation of the virus that has continued until the 2021. Most dengue cases have been detected in April and May [17]. Three serotypes have been detected, DENV1, DENV2, DENV3. The most affected sectors were the south and west of the island [17]. Table 1 provides the number of estimated and confirmed dengue cases and deaths from 2018 to 2021 in La Réunion. The mortality rate reached 0.11% in 2021 [17].

Table 1. Morbidity and mortality data due to dengue from 2018 to 2021 in La Réunion [17].

Date	Estimated Cases	Confirmed Cases	Death
2018	15,460	6770	6
2019	42,420	18,217	14
2020	30,580	16,414	22
2021	59,230	29,577	33

In La Réunion, following the chikungunya epidemic in 2006, a surveillance system was created in order to rapidly identify the first cases of arbovirus infections [18]. Santé Publique France is directly informed of any laboratory-confirmed case of arbovirus infections. Vector control measures are provided by the Agence Régionale de Santé (ARS) to limit the spread of the virus by spraying with insecticide and eliminating breeding sites within a defined perimeter around the residence of the identified case.

Despite very suitable access to care, a sustained surveillance system as well as dengue prevention and control policies that have been in place for more than 10 years, the dengue epidemic in La Réunion continues to be rampant. One hypothesis that could explain the persistence of the epidemic is that part of the transmission is due to asymptomatic forms of the disease thus not detected by the surveillance system. The estimated percentage of asymptomatic cases during DENV outbreaks varies between 50% and 90% [19–23]. Studies collecting data on the presence of DENV infections considering all disease patterns including asymptomatic people have been performed in Latin America and Southeast Asia [19,24–26] but not in the Indian Ocean region. Asymptomatic individuals could act as a reservoir of the virus. Knowing their prevalence is necessary for establishing public health policies for blood donor screenings, for conducting dengue vaccine clinical trials and has implications for vector control strategies.

This study aimed to assess the proportion of asymptomatic dengue infections by an active search around identified dengue index cases, households, and neighborhoods and to identify the main risk factors related to recent and past dengue infections.

2. Materials and Methods

2.1. Study Design

This cross-sectional observational study included a household-based survey covering the whole island of La Réunion. The strategy to detect dengue infections, according to a

geographical cluster recruitment study design, consisted in an active search in a group of participants selected within a fixed radius of the home or workplace of an index case (IC).

2.2. Clinical Definitions and Laboratory Diagnosis

Dengue-like syndrome is defined as an acute fever associated with two or more of the following signs or symptoms: nausea, vomiting, rash, headache, retro-orbital pain, myalgia, arthralgia, and bleeding [27]. No standard definitions exist for "pauci-symptomatic" and "asymptomatic". In the present study, "asymptomatic" infections are defined as the complete absence of symptoms after a follow-up period of 14 days and the "pauci-symptomatic" include the symptomatic infections that do not meet the criteria for a dengue-like syndrome.

2.3. Laboratory Diagnosis

The World Health Organization (WHO) recommendations for the diagnosis of dengue include enzyme-linked immunosorbent assay (ELISA)-based detection of dengue-specific IgM antibodies or a $\geq$4-fold increase in the titer of total antibodies to DENV in paired acute and convalescent sera; or detection of DENV by reverse transcription polymerase chain reaction (RT-PCR) in plasma, serum, or whole blood [28]. According to the kinetics of DENV infection markers in the blood, anti-dengue IgM antibodies are detectable in the blood approximately three months after the infection, and anti-dengue IgG antibodies are lifelong markers [29]. Molecular detection of the virus through RT-PCR is possible during the first five days of the disease. The plaque reduction neutralization test (PRNT) is a serological test, more sensitive and specific than the ELISA method for dengue diagnosis. Moreover, PRNT can be used to identify the infecting serotype in primary infection. Furthermore, this technic was too expensive and labor consuming to be retained for our needs [30]. In the present study, the three markers (IgM, IgG (ELISA), and RT-PCR) were measured in the blood to detect recent or past dengue infections among people living in the neighborhood of dengue cases.

2.4. Identification of Index Cases

The ICs were recruited among patients who consulted the emergency rooms of the North or South University Hospital or their general practitioners with dengue-like symptoms and in whom dengue was confirmed by biological examination in hospital or city laboratories. The selection criteria for ICs were individuals $\geq$ 12 months old, with a positive dengue RT-PCR or a positive IgM, and with a dengue-like syndrome. An IC could not be located within 400 m of an already included IC to avoid overlap. IC was notified to the principal investigator of the study in the 14 days following their lab-confirmed infection and then included in the study by the field team as soon as possible according to the team capacity. The rationale for this time interval was to try to be close to the infection day of the IC while being constrained by logistical considerations of field recruitment. The date of inclusion of the IC was the date of commencement of screening and inclusion of study participants in the IC household and neighborhood forming a cluster. The duration for including participants in a cluster was of two weeks after the date of inclusion of the IC.

2.5. Sample Size

The sample size was targeted according to the capacity of the study team and laboratory and estimated a population density of approximately 100 people within a 100-m radius of the household of the IC. Estimating that 40% of individuals would be absent at the time of the survey and that 20% of individuals would refuse, a target of 40 participants enrolled per cluster seemed realistic. Considering an estimated prevalence of dengue in La Reunion of 3% and that in similar studies using a geographical cluster design around ICs, between 4% and 27% of the participants screened had asymptomatic dengue infection [26,31]. A sample size of 600 participants would likely provide enough power (with a confidence level of 1.96 (95%) and an error of 5%), i.e., a minimum of 15 ICs were required.

2.6. Cluster Definitions and Data Collection

The residence of an IC represented the center of a geographic cluster. The participants were recruited from households that were within 100 m of the house of an index case. The rationale behind this 100 m was that *Ae. albopictus* mosquitoes have been shown to have limited flight ranges in Reunion [32]. This radius could be increased to 200 m if the targeted sample size was not achieved due to absences or refusal. All houses in the cluster were identified with *Google Earth* and *OpenStreetMaps* and visited for inclusion in the study. Data collection started with the household of the IC and then continued in the neighboring houses. In each household, all consenting individuals were eligible to participate, with the intent to include approximately 40 participants in each cluster. The exclusion criteria were age under one year old and any contraindication to proceeding to blood sampling.

A complete medical history including a collection of signs and symptoms by organic system was conducted.

Blood samples were collected in dry and with anticoagulant (EDTA) tubes: 1 dry tube of 4 mL for anti-DENV IgM/IgG serology (*Panbio* ^{TM} *Dengue IgG, IgM Capture ELISA*); 1 EDTA tube of 4 mL for DENV RT-PCR (in-house technique [33]). Urine samples were also collected and frozen for further search of the excretion of dengue virus by RT-PCR. All analyses were conducted at the laboratory of the North University Hospital. Serotypes were determined with a second RT-PCR (in-house technique of the *Centre National de Référence (CNR) Arbovirus de Marseille*). Participants reporting a dengue diagnosis in the weeks preceding the study were asked to provide their lab analysis report to confirm RT-PCR positivity for DENV. If they did not have the report, the study team requested it directly from the laboratory or from the general practitioner. Due to the lack of specificity of anti-dengue IgM for asymptomatic cases, a follow-up visit was held 15 to 21 days after the first home visit for participants with positive IgM to provide paired serology and look for IgG seroconversion or an increase in total antibody titers. At this visit, blood was only collected for anti-DENV IgM/IgG serology.

By going in the field and recruiting participants who lived in the neighborhood of identified dengue cases, the study team had the opportunity to collect, in addition to clinical data, data on socio-demographic characteristics of households as well as environmental potential risk factors present in the neighborhoods and to evaluate their possible impact on the transmission of dengue.

2.7. Statistical Methods

The proportions of the sampled population with DENV infections confirmed by RT-PCR and with IgG DENV-positive results were selected respectively as primary and secondary outcomes. Signs and symptoms were recorded in DENV RT-PCR-positive participants and ICs; proportions were computed and cases were classified accordingly as symptomatic (presence of fever only or with one or more other symptoms), pauci-symptomatic (absence of fever but presence of one or more other symptoms) or asymptomatic (absence of symptoms) infections. The comparison between groups of categorical variables were made using the chi-square or Fisher exact test according to the sample size. The association between explanatory variables and outcomes (positive DENV RT-PCR, positive anti-dengue IgG) was estimated using bivariate analyses and multivariate logistic regression models. The multivariate logistic regression models were constructed with the variables that showed significant association in the bivariate analysis and the best models were selected following a backward stepwise procedure of selection of variables. All analyses were performed with R Statistical Software (v.4.2.2 R Core Team 2022).

2.8. Ethics and Confidentiality

This study was carried out in accordance with the law n 2012-300 of 5 March 2012 relating to research involving the human person, as well as in accordance with the Good Clinical Practices [34] and the Helsinki declaration, and the participants or the parents of minors participating in the study provided written informed consent before inclusion. The

study was accepted by the Comité de Protection des Personnes of the University Hospital of Saint-Etienne (CPP SUD-EST I), France (n ID RCB:2018-A02357-48).

3. Results

The study was conducted between October 2019 and August 2020. A total of 17 dengue index cases (IC) were identified, and 605 participants were recruited from 368 households within a 100 to 200 m radius of the IC's homes (Figure 1).

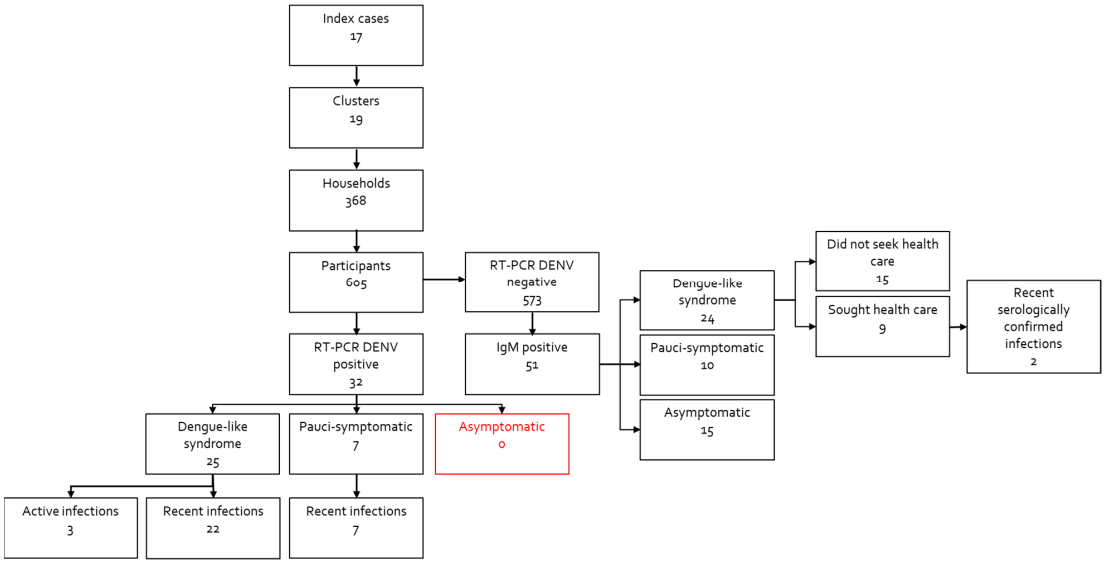

Figure 1. Flowchart describing the included study population and the dengue RT-PCR and anti-dengue IgM results.

3.1. Cluster Investigation

Seventeen ICs formed the basis for 17 clusters. Additionally, the place of work of one IC was used to create an additional cluster, and one cluster was formed for an IC who did not return the consent form, so while the data of the IC were not used, the cluster was kept. This resulted in 19 clusters. The clusters, distributed throughout the whole island, are described in Table 2, with the prevalence of dengue infections detected by RT-PCR per cluster and the seroprevalence of IgM and IgG. The clusters are mapped in Figure 2.

Table 2. Locations of the clusters and number of participants included with DENV RT-PCR and anti-dengue IgM- and IgG-positive results (results of IC are not included). In clusters where no DENV RT-PCR-positive participants were detected, the serotype of the IC is displayed.

Cluster	Season [Month]	Location	Number of Participants	DENV RT-PCR+	IgM +	IgG +	Serotype
				n (%)	*n* (%)	*n* (%)	
1	Winter [10]	South	17	0	0	2 (12%)	DEN-2
2	Summer [11]	South	33	0	5 (15%)	14 (42%)	DEN-2
3	Summer [11]	South	43	0	2 (5%)	21 (49%)	DEN-1
4	Summer [02]	South	39	3 (8%)	6 (15%)	19 (49%)	DEN-1
5	Summer [02]	South	39	1 (3%)	1 (3%)	11 (28%)	DEN-2
6	Summer [02]	South	46	0	3 (7%)	19 (41%)	DEN-1
7	Summer [03]	East	33	6 (18%)	5 (15%)	15 (45%)	DEN-3
8	Summer [03]	East	27	0	3 (11%)	7 (26%)	NA

Table 2. *Cont.*

Cluster	Season [Month]	Location	Number of Participants	DENV RT-PCR+	IgM +	IgG +	Serotype
				n (%)	*n* (%)	*n* (%)	
9	Summer [04]	South	3	0	0	1 (33%)	DEN-1
10	Winter [05]	South	31	2 (6%)	3 (10%)	5 (16%)	DEN-1
11	Winter [06]	South	45	5 (11%)	14 (31%)	32 (71%)	NA
12	Winter [06]	West	16	2 (12%)	7 (44%)	8 (50%)	DEN-1
13	Winter [06]	West	13	0	0	2 (15%)	DEN-1
14	Winter [07]	West	12	2 (17%)	3 (25%)	6 (50%)	NA
15	Winter [07]	West	49	1 (2%)	4 (8%)	28 (57%)	NA
16	Winter [07]	West	56	7 (13%)	6 (11%)	29 (52%)	DEN-1
17	Winter [08]	West	28	0	1 (4%)	6 (21%)	DEN-1
18	Winter [08]	West	12	0	2 (17%)	6 (50%)	NA
19	Winter [08]	West	63	3 (5%)	1 (2%)	27 (43%)	NA
Total			605	32 (5.3%)	66 (11%)	258 (43%)	

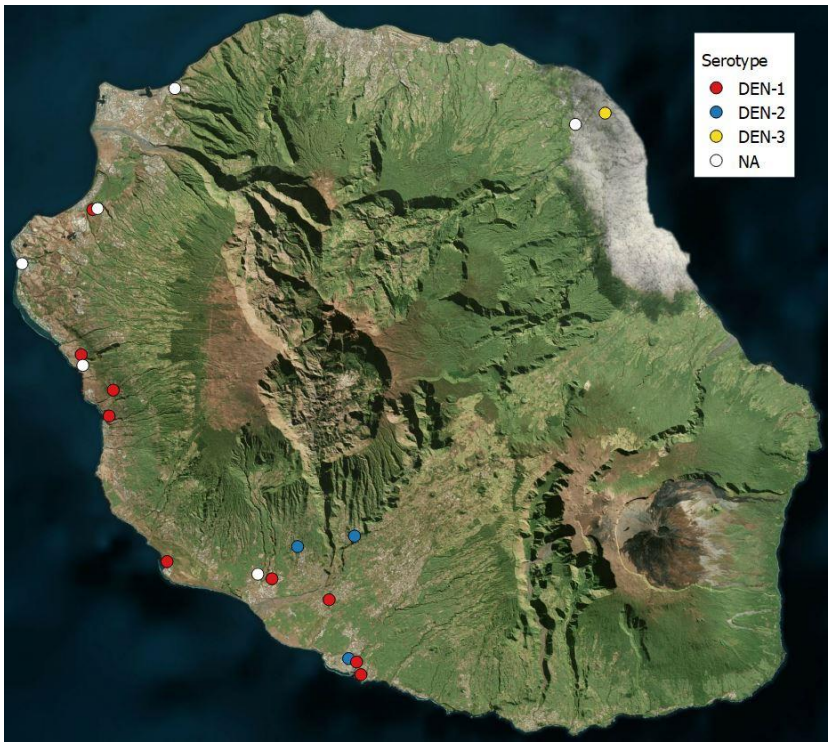

Figure 2. Map of the 19 clusters throughout the island. The dots represent the locations of clusters. The colors represent the serotype found in the cluster, as listed in Table 2.

3.2. Demographic Characteristics

Table 3 shows the demographic and health characteristics of all the study subjects. Eighty-six percent were adults, and the mean age was 46 years old. Thirty percent were more than 60 years old. Fifty-seven percent were female.

Table 3. Demographic characteristics.

Demographic Characteristics	Index Cases N = 17	Participants N = 605
Sex		
Female	11 (65%)	346 (57%)
Male	6 (35%)	259 (43%)
Age mean (SD); median [IQR]	36 (21); 36 [25]	46 (20); 48 [30]
Age categories (years)		
<5	1 (6%)	5 (1%)
5–11	3 (18%)	36 (6%)
12–17	0	39 (6%)
18–59	11 (65%)	340 (56%)
≥60	2 (12%)	185 (31%)
Activity		
Housewife/husband or unemployed	1 (6%)	135 (22%)
Retired/Disabled	1 (6%)	160 (26%)
Student/in training	4 (24%)	93 (15%)
Worker	11 (65%)	205 (34%)
NA	0	12 (2%)

SD: standard deviation; IQR: interquartile range; NA: not available.

3.3. Detection of Dengue Virus by RT-PCR

No active asymptomatic infections confirmed by RT-PCR were detected. Out of the 605 participants recruited around the index cases, only 5.3% (32/605) presented a recent or active dengue infection confirmed by RT-PCR: only 3 were detected during the field survey, and the other 29 corresponded to confirmed recent infections reported by the participants and for which the study team could retrieve the lab results. These recent infections occurred in the 3 months preceding the survey, corresponding to the duration of IgM persistence in blood after infection. Participants with recent dengue infections were found in 10 of the 19 clusters investigated. The active dengue infections were found in only two clusters (in the south in February and in the East in March). The prevalence of recent dengue infections confirmed by molecular analysis (RT-PCR) in each cluster varied from 0% to 18% (Table 2).

3.4. Dengue-Positive RT-PCR in Households

The 622 study subjects (IC and participants) were distributed in 368 households. The 17 ICs and the 32 RT-PCR-positive participants were distributed across 44 households. In 17/44 households, only one study subject was included in the study, and in 27/44 households, multiple study subjects were included per house (range 2 to 5). Among these 27 households, only 2 contained more than one detected dengue infection.

3.5. Time Interval between Dengue Infections within Clusters

Figure 3 shows the timelines of dengue infection onset and the inclusion study periods for each cluster where dengue cases were detected. Dengue recent infections are clustered close in time and generally just after the onset of IC infection. The study team's inclusion

period occurred after the clustered dengue cases. Table 4 shows the time intervals between the date of laboratory confirmation of dengue infection and the date of the study team visit to include the IC and screen and include the participants in the neighborhood. For clusters 14, 16, and 19, the time interval for inclusion of the IC was not respected due to inaccurate information received at the time of screening. These data were not excluded as the deviation did not prevent from interpreting them. In clusters 4, 7, and 9, active dengue infections were detected during the field visit to recruit participants. This explains the time interval of '0' days. For all the other clusters, participants with recent dengue infections, already diagnosed by general practitioners before our field visit, were included. The date of these infections could precede the infection of the ICs. These findings suggest that, for most of the clusters, the dengue virus circulated before the infection of the IC. Moreover, the infections detected in a cluster occurred in a limited period of time. Looking at the dates of these recent dengue infections, it resulted that, in the majority of the clusters, for each infection, another occurred within 7 days.

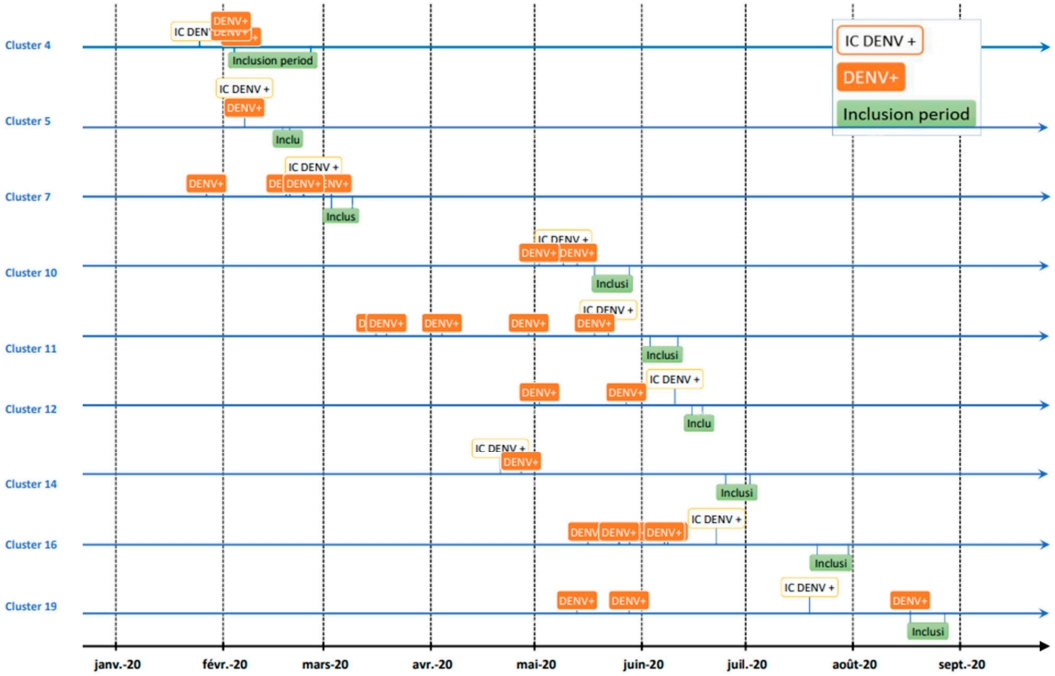

Figure 3. Timelines displaying the occurrence of dengue-detected infections in clusters and the inclusion period.

Table 4. Time interval in days between the date of laboratory confirmation of dengue infections and the date of the study team field visits to screen and include ICs and participants.

Cluster no	Time Interval between the Date of Dengue Infection Confirmation and the Date of Inclusion in the Study [days]		Minimum Time Interval between Dengue Infections Dates in Each Cluster [days]
	IC	Participants	
Cluster 01	4	-	-
Cluster 02	8	-	-
Cluster 03	7	-	-
Cluster 04	10	0, 7, 9	0–9
Cluster 05	12	13	0
Cluster 06	11	-	-
Cluster 07	13 *	0, 12, 0, 10, 13, 36	0–23

Table 4. *Cont.*

Cluster no	Time Interval between the Date of Dengue Infection Confirmation and the Date of Inclusion in the Study [days]		Minimum Time Interval between Dengue Infections Dates in Each Cluster [days]
	IC	Participants	
Cluster 08	14	-	-
Cluster 09	0 [&]	-	-
Cluster 10	9	8, 25	4–7
Cluster 11	13	22, 86, 84, 43, 68	3–19
Cluster 12	5	22, 46	14–25
Cluster 13	12	-	-
Cluster 14	71	65	6
Cluster 15	NA [µ]	36	-
Cluster 16	31	57, 54, 54, 44, 45, 73, 64	1–14
Cluster 17	13	-	-
Cluster 18	NA [#]	-	-
Cluster 19	30	81, 105, 9	15–29

* all diagnostics were confirmed by RT-PCR except for the IC of cluster 07, which was confirmed by the presence of IgM. [&] the dengue confirmation by RT-PCR was made by the study team on the day of inclusion, [µ] the IC could not be included in the study because the signed informed consent was not returned. [#] there is no IC for cluster 18, the center of the cluster was the working place of IC 17.

3.6. Dengue Serotypes

For the three active dengue infections detected within the study, serotyping was performed and resulted in one DEN-1 in cluster 4 (south) and two DEN-3 in cluster 7 (east). For ICs and participants with recent infections detected prior to the recruitment team's visit, serotyping results were not always available. Some could be performed on residual blood samples held by the diagnosing laboratories, and for seven cases, dengue virus could be detected in a urine sample, and serotyping was performed. In the end, serotypes were available for 16/48 dengue infections. As presented in the last column of Table 2, DEN-1, DEN-2, and DEN-3 were detected, with a strong majority of DEN-1. DEN-3 was only detected in the eastern region, and DEN-2 was only found in the south. In clusters 4, 7, and 12, several serotype results were available, but only one serotype was present per cluster. Unfortunately, serotype results from the two households with multiple dengue infections were not available.

3.7. Clinical Presentation of Dengue RT-PCR Confirmed Infections

By gathering the 16 ICs diagnosed with RT-PCR (excluding one case detected with serology), the 27 recent dengue infections included in the study, and the 3 active dengue infections, the total number of dengue infections confirmed by RT-PCR was 48. Among these, 30 (83%) presented a dengue-like syndrome, and 8 (17%) were pauci-symptomatic, which means that symptoms were declared but not meeting the definition of a dengue-like syndrome (described in the method). Overall, the pauci-symptomatic participants did not present fever but other symptoms. Of the three cases detected by the study team, all had typical dengue presentations, and one was pre-symptomatic at the time of recruitment, reporting only intense fatigue but declared a fever the day after.

The symptoms of the 48 dengue infections are summarized in Table S1 of the supplementary material. The accuracy of these results may suffer from some memory bias as the infections of some included participants could go back 3 months. The main symptoms of dengue infections were a severe fever and severe and prolonged fatigue. The other most frequent symptoms were anorexia, headache, myalgia, and arthralgia. All these symptoms, except for anorexia, are part of the dengue-like syndrome definition. Less than 10% of

dengue-confirmed study subjects complained of bleeding signs. The other pathognomonic symptoms of dengue, which are rash and retro-orbital pain, were present in 42% and 33% of study subjects, respectively. Besides fatigue, which had a mean duration of 11 days, all other symptoms had mean durations of 3 to 5 days.

3.8. Detection of Anti-Dengue IgM and IgG Antibodies in Blood Samples

As shown in Figure 1, anti-dengue IgM was detected in 8% (51/573) of participants with negative dengue RT-PCR results. Among these, 42 (81%) also had anti-dengue IgG antibodies. Additionally, 24 of them (47%) presented dengue-like symptoms, half of which (15/24) did not seek dengue laboratory confirmation (likely to be undiagnosed dengue infections). Ten of those with IgM (19%) were pauci-symptomatic, and 15 (29%) did not report any symptoms in the 3 months preceding the blood collection. Among the symptomatic cases, all were IgG-positive either at the first visit (37) or had seroconverted by the second visit 15 days later (2). Among the 15 cases that did not present any symptoms, 6 of them had no IgG at the first visit. Among these, three participated in a second visit: one was seroconverted, and two remained IgG negative, implying that the presence of IgM at the first visit might not be due to dengue infection. The proportion of possible asymptomatic dengue infections detected by IgM (15) accounts for 15% of all the 100 possible dengue infections (including ICs (17), recent infections (29), active infections (3), and IgM-positive participants (51)). However, this result is overestimated as it is likely that not all of the asymptomatic participants with positive IgM results were true dengue cases, as there is some lack of specificity in the dengue IgM assay performed with the ELISA technique [30]. Moreover, considering that IgM could persist for more than 3 months in some individuals, these results are difficult to interpret [35].

A total of 43% of participants had the presence of anti-dengue IgG, 42% (109/258) of which were not associated with a history of dengue, which means that 18% (109/605) of the study population had IgG with no history of dengue.

As shown in Table 1, the proportion of the population with IgG anti-dengue antibodies varies greatly between clusters, ranging between 12% and 71% of participants investigated with positive anti-dengue IgG in a cluster. The clusters with the highest prevalence of IgG-positive participants were located in the west part of the island, in the municipality of Saint-Paul, and in the south, in the municipality of Saint-Louis.

3.9. IgG Anti-Dengue Antibodies in Households

In 165/368 households, more than one study subject was tested (range 2 to 7). In 106/165 (64%), at least one subject had positive IgG anti-dengue antibodies. In 45/106 (42%) households, more than 50% of the study subjects living in the household were IgG-positive. In 35/106 (33%), all study subjects included were IgG-positive.

3.10. Risk Factors for Dengue Infection Confirmed by RT-PCR and Parameters Associated with Past Dengue Infection Detected by the Presence of Anti-Dengue IgG

Table 5 lists the explanatory variables that were first cross-analyzed with two outcomes: RT-PCR DENV-positive and IgG anti-dengue-positive. "History of dengue", "smoking", "earthen courtyard floor", "presence of windows with glasses", "presence of rubbish in the surrounding area", and "farm in the surrounding area" showed a significant association with a positive RT-PCR DENV result. The variable "History of dengue" was not introduced in the multivariable model as the significance was due to a recruitment bias since most RT-PCR dengue-positive study subjects knew their diagnosis at the time of their study inclusion. The multivariate logistic regression model identified three explanatory variables significantly associated with the outcome of RT-PCR dengue-positive, which are listed in Table 6 with their adjusted odds ratios. Several variables were associated with the outcome of IgG anti-dengue-positive in the bivariate cross-analysis (Table 5). However, many of them were linked to the "age" of the study subjects. Indeed, when adjusting them in the multivariate logistic regression model, only three variables remained significantly

associated with the outcome (Table 6). The bivariate analysis and the multivariate logistic regression models did not show any socio-demographic criteria (neither activity, nor level of education, nor comorbidity) as risk factors for dengue infections. Only environmental factors, such as the type of housing and the presence of waste in the environment, were risk factors associated with dengue infection.

Table 5. List of explanatory variables cross-analyzed with the outcomes RT-PCR DENV-positive and IgG-positive and the associated *p*-value (Wilcoxon rank sum test; Fisher's exact test; Pearson's chi-squared test).

Explanatory Variables	**Outcome**					
	RT-PCR DENV-Positive			**IgG-Positive**		
	OR [#]	**95%CI**	***p*-Value**	**OR** [#]	**95%CI**	***p*-Value**
Age categories			0.3			>0.9
Sex			>0.9			0.7
Body mass index (BMI) categories			0.3			0.3
Duration of stay home $\geq$ 20 h a day			0.5	**2.78**	1.7-4.7	**<0.001**
Activity: student/in training			0.2	**0.3**	0.2–0.5	**<0.001**
History of dengue	**28**	8.8–144	**<0.001**	**7.5**	5–11.5	**<0.001**
History of chikungunya			0.8	**3.7**	2.6–5.5	**<0.001**
Level of education						
Never attended school			0.5	**5.2**	1.5–23.9	**0.015**
Primary school			>0.9	**2**	1.2–3.4	**0.005**
Secondary school			0.5	**1.6**	1.1–2.5	**0.031**
High school			0.13	0.8		0.2
Yellow fever vaccination			0.6			0.11
Smoking	**0.09**	0.002–0.6	**0.001**			0.8
Risky drinking [$]			0.6	**1.2**	0.4–4.2	**0.017**
Chronic disease			0.11	**1.4**	1–2	**0.04**
Chronic medication			0.7	**1.5**	1.1–2.1	**0.017**
Sense of severity			0.2			0.7
Sense of risk [&]			0.5			0.2
Mosquito protection use [¥]			0.8			0.8
Mosquito bites [α]			0.4			0.11
Mosquito presence			0.2			0.074
Season			0.2			0.2
House with courtyard			0.2	**4.6**	2.4–9.9	**<0.001**
Type of courtyard			>0.9			0.2–0.9
Surface of courtyard						
100–500 square meters			0.5	**1.8**	1.2–2.6	**0.004**
>500 square meters			0.5	**2**	1.3–3	**0.003**
Earthen courtyard floor	**2.1**	1–4.4	**0.032**	**1.8**	1.2–2.9	**0.009**
Presence of grass			0.3	**1.5**	1–2.1	**0.04**
Presence of windows with glasses	**0.5**	0.2–1	**0.032**	**0.5**	0.3–0.9	**0.007**
Presence of air conditioning			0.6	**0.6**	0.5–1	**0.032**
Presence of swimming-pool			>0.9	**0.3**	0.1–0.8	**0.007**
Presence of stagnant water			0.7			0.6
Presence of animals			0.8	**1.9**	1–3.4	**0.028**
Presence of poultry			0.076	**2**	1.3–3.2	**0.001**
Presence of rubbish in the courtyard			>0.9			0.12
Presence of rubbish in the surrounding area	**1.8**	0.9–3.5	**0.043**	**1.8**	1.2–2.6	**0.002**
Orchard in the surrounding area			0.12			0.8

Table 5. *Cont.*

Explanatory Variables	Outcome					
	RT-PCR DENV-Positive			IgG-Positive		
	OR [#]	95%CI	*p*-Value	OR [#]	95%CI	*p*-Value
Banana plantation in the surrounding area			>0.9	**0**	0–0.4	**0.002**
Sugarcane plantation in the surrounding area			0.4			0.14
Other agriculture in the surrounding area			0.2			0.12
Farm in the surrounding area	**5.6**	1.5–17.9	**0.006**			0.051
Seaside			0.6			0.2
Industrial area			>0.9			0.4
Neighborhood of houses			0.8	**1.7**	1.2–2.6	**0.003**
Neighborhood of buildings			0.6	**0.5**	0.3–0.8	**0.003**

[#] OR: odd ratios are displayed only for explanatory variables that presented a *p*-value < 0.05. [$] A risky drinking is defined as more than two drinks per day. [&] The question behind the "sense of risk" variable was the estimated probability (low, high, very high) of contracting the disease in the next five years. [α] Feeling like you are frequently bitten by mosquitoes. [¥] Mosquito-repellent body lotion

Table 6. Significant ($p < 0.05$) adjusted ORs resulting from the multivariate logistic regression models associating the significant explanatory variables from the bivariate cross-analysis to the outcomes RT-PCR DENV-positive and IgG anti-dengue-positive.

Explanatory Variables	aOR	95%CI	*p*-Value
	Outcome: RT-PCR DENV-positive		
Smoking	**0.08**	**0.0–0.4**	**0.02**
Earthen courtyard floor	**2.1**	**1–4.3**	**0.046**
Farm in the surrounding area	**5.5**	**1.6–16.9**	**0.005**
Presence of rubbish in the surrounding area	1.6	0.3–1.12	0.20
Presence of windows with glasses	0.5	0.8–3.1	0.09
	Outcome: IgG anti-dengue-positive		
History of chikungunya	**2.5**	**1.6–3.9**	**<0.001**
Type of housing: house with courtyard	**5.3**	**2.2–14**	**<0.001**
Presence of rubbish in the surrounding area	**1.6**	**1.1–2.6**	**0.04**
Duration of stay home $\geq$ 20 h a day	1.7	0.9–3.3	0.15
Activity: student/in training	0.4	0.1–1.7	0.30
Level of education			0.07
Never attended school	2.2	0.5–15.9	
Primary school	1.6	0.7–3.6	
Secondary school	0.9	0.6–1.8	
High school	0.6	0.3–1.1	
Surface of courtyard			0.26
100–500 square meters	1.02	0.6–1.8	
>500 square meters	1.5	0.8–2.9	
Earthen courtyard floor	1.4	0.8–2.5	0.21
Presence of windows with glasses	1.04	0.6–1.9	0.89
Presence of swimming-pool	0.7	0.3–1.7	0.44
Presence of poultry	0.9	0.5–1.6	0.81
Neighborhood of houses	0.8	0.4–1.3	0.36

4. Discussion

In 2019, the dengue outbreak in La Réunion had more than 18,000 confirmed cases. Among these, 17 were included as index cases for the study, and the study team surveyed a perimeter of 100–200 m around their households or working places looking for identified, undiagnosed, or asymptomatic dengue infections. The findings suggest that a very small proportion of the population presented asymptomatic forms of dengue. Indeed, confirmed dengue cases (PCR or serology) were only detected in 5.3% of the study population during the survey or 3 months prior to the survey, and all were symptomatic cases. These results should be interpreted with caution because our study design allowed us to obtain only part of the picture. We were able to collect data over a short period of time for each cluster, and the inhabitants of the cluster area were only partially represented. Therefore, we may have missed some cases. The value of IgM testing is that it provides a broader picture of dengue circulation. The fact that we found only a maximum of 15% of possible asymptomatic recent dengue infections detected by the presence of anti-dengue IgM supports the idea that asymptomatic cases do not appear to be numerous. Although the re-emergence of dengue in La Réunion is very recent (2016), the rate of IgG anti-dengue positivity, markers of past infections, has already reached 43%. In the literature, the frequency of DENV infections detected around an IC using a geographic cluster design ranges from 4% to 27% [26,31]. With the 5.3% prevalence found in this study, La Réunion is located in the lower part of this range. The prevalence of anti-dengue IgG was high, and 42% of the IgG-positive participants could not recall a past dengue infection. Moreover, the multivariate analysis identified an association between a past infection of chikungunya and the presence of anti-dengue IgG. Some past dengue infections could have been classified as "chikungunya" cases as the latter was well known by the population after the important outbreak of 2005. On the one hand, 30% of participants with no history of dengue infection had IgG-positive results, and on the other hand, 24% of participants with a history of dengue infection did not have any IgG, which is a lifelong marker of past disease. These discordant results reflect the likely memory bias and that dengue clinical presentation is not specific and challenging to confirm without testing.

Because of the four serotypes of dengue, no herd immunity could be expected, mainly as only dengue 1 and dengue 2 have circulated in the whole island, whereas dengue 3 only circulated on the eastern coast, and no dengue 4 serotype was identified yet [17].

We detected only 15 (15%) undiagnosed probable dengue infections, defined as participants who presented a dengue-like syndrome in the 3 months preceding our visit and the presence of IgM but who did not seek health care. La Réunion is a high-income island with very suitable access to health. Universal health care is largely financed by government national health insurance, and people, therefore, easily seek care to obtain medicine or time off work. In fact, we found many confirmed dengue infections that occurred before our visit.

Within cities such as Saint-Pierre, for example, the proportion of IgG across clusters differed widely. This illustrates that the dengue virus seems to circulate most often in the same places and that the geographical range of transmission could be very limited (less than 200 m of radius).

To the best of our knowledge, the usual transmission rate in a household is not known. In our study, 20% of the households visited contained only one inhabitant, and in 44% of the households that contained more than one inhabitant, only one member agreed to be included in the study. Although we were not able to include all members of every household visited, we have data on 27 households containing dengue infections in which more than one participant was included. Only 2 households contained more than one infection, and in 25 households with multiple inclusions, only one household member had a recent dengue infection. These data suggest that the rate of transmission in households is probably not that high.

In the present study, we follow a geographic cluster recruitment design. We needed an initial case to allow us to identify in which neighborhoods it would be worthwhile to

include asymptomatic participants to look for infection. This first case was called an index case because it represented an approximation of dengue circulation in a neighborhood, but in reality, it did not mean that it was the index case of an epidemic. This is well illustrated in Figure 3, where the timeline highlighted that the IC for the study was not the first case in a neighborhood transmission outbreak. Interestingly, this timeline shows that the inclusion period of the study team occurred mostly after the occurrence of a few cases of clustered dengue. Moreover, the discovery of active infections during the inclusion period was rare; only three active infections were detected during the entire study period. This underscores the need for very reactive action to detect circulating cases.

Although none of the demographic nor socio-economic variables studied in our participants were found to be associated with dengue infection, environmental risk factors such as the type of housing or the presence of rubbish in the streets were associated with dengue infections. Considering that dengue is a vector-borne disease, thus transmitted by the bite of a mosquito, it seems reasonable that the environment has an impact on transmission, as observed in our results. As shown in Table 6, the presence of a courtyard, farm, or rubbish in the neighborhood is a predictor of dengue infections. This may be simply because these locations provide ample breeding opportunities for mosquitoes.

Our main result, the proportion of dengue infection found in the clusters, is consistent with previous studies. However, unlike most observational epidemiological studies on dengue, we did not find a large proportion of asymptomatic cases. We can put forward several hypotheses to try to explain this important difference between the proportion of asymptomatic cases generally described in the literature and our results. First, our recruitment method with a single inclusion visit with blood sampling (and a second visit only for IgM-positive cases) provided us only a limited view of the potential viremia of our participants; a design with repeated sampling to look for the presence of the virus would have been more likely to detect cases (but hardly acceptable to the participants). Furthermore, the serology provided a larger vision in time and could have signaled the presence of asymptomatic infections that occurred before our venue. Only 15% of possible recent asymptomatic dengue infections detected thanks to positive anti-dengue IgM were found, which is still far below the expected 50% to 90%. Second, we included only 7% of children, and according to the literature, the main victims of dengue in endemic areas are children. The data to establish a 50–90% asymptomatic rate come mainly from studies in children [36–42]. We can hypothesize here that asymptomatic forms are rarer in non-immune adults. Moreover, on Reunion Island, the re-emergence of dengue is relatively recent (2016), and only two serotypes have been widely circulating on the island. The level of immunity in the population, 43% IgG-positive, should be essentially monotypic. The role of immunity in the clinical presentation of the disease is widely discussed in the literature with no absolute consensus except for secondary infections that may be likely more severe [19]. Finally, the high rate of asymptomatic infections in the literature may rather correspond to undiagnosed infections as in endemic countries, people accustomed to the occurrence of dengue do not necessarily seek care. Moreover, most observational studies on dengue transmission were conducted in low- and middle-income countries where dengue was highly endemic. Our results are interesting as it is one of the first studies on dengue transmission in a high-income country where dengue is emergent. The external validity of our results should be evaluated in countries with similar characteristics of high income, suitable access to health care, and the emergence of the disease.

The implications of these results for public health are that, firstly, as the proportion of asymptomatic dengue infections and undiagnosed infections in La Réunion appears to be low, the data provided by the health authorities are likely a suitable estimation of reality. Second, the same neighborhoods seemed more affected both by a high prevalence of anti-dengue IgG and by active infections, suggesting that known areas of dengue transmission could benefit from targeted prevention actions ahead of the outbreak. Third, the focal nature of dengue in place and time, illustrated by the short time interval between detected infections within clusters, as already described in previous studies, reinforces

the need for very rapid and localized actions. Studied clusters in the same city showed different levels of IgG prevalence; therefore, prevention actions should be considered by neighborhood and not by city. Fourth, the analysis failed to demonstrate any association between dengue infection risk and education level or other demographic characteristics. Only environmental variables were significant, which shows the importance of cleaning work in the neighborhoods in addition to prevention messages. Moreover, the knowledge of the population about dengue is very suitable, as demonstrated in a previous qualitative study [43].

The design of recruitment had several limitations in detecting asymptomatic dengue infections. The proportion of houses within 200 m of the IC from which participants were included in the study was low, less than 50% across all the clusters. Many houses visited were empty during usual working hours. Indeed, the proportion of women and retired people is high in our study population. This reflects a high proportion of non-working women in La Réunion [11]. However, the proportion of workers on the island was 55% in 2019, according to INSEE data [44]. In the present study, the proportion of workers among the adult population reached 39% despite the constraint of recruitment during weekday working hours. In the houses where people were present at the time of our visit, the acceptability was globally suitable, and the local population showed great willingness to participate in the research. However, refusal was common for children as our study procedures included venipuncture. Despite the low proportion of houses included in the study, dengue cases confirmed with RT-PCR were detected in half of the clusters investigated.

According to the study protocol, the interval between the confirmation of the dengue infection and the study visit was meant to be within 15 days. However, due to challenges faced during the field recruitment work, this interval of 15 days was not respected for three index cases and ranged between 30 and 71 days. This increased delay may have reduced our capability to detect dengue infections.

Due to the COVID-19 pandemic that occurred during the recruitment period, we had to restrict the study procedures for safety reasons and were not able to proceed to all of the initially planned second visits for participants who had a positive anti-dengue IgM result. These IgM results had to be interpreted with caution as they could not be correlated to a clinical presentation nor to an IgG seroconversion.

Further research is needed as detecting active asymptomatic dengue infections is a real challenge given the likely short duration of viremia and the very focal duration of dengue outbreaks in neighborhoods. Testing would need to take place much more widely and repeatedly, which may not be acceptable to the participants, especially if they do not suffer from any symptoms. It could be interesting to consider urine testing to detect dengue infection by molecular analysis. This is a non-invasive test and, thus, more acceptable. We demonstrated the possibility of detecting the virus in urine a few days after infection [data not yet published]. Further research will be helpful to complete and refine these results to improve public health policies for dengue control in countries in the Indian Ocean region.

5. Conclusions

The proportions of asymptomatic and undiagnosed dengue infections are very low in La Réunion. Dengue transmission is focal in time and place, and environmental factors arising from human life are the principal risk factors. Public health prevention actions should be highly targeted.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v15030742/s1, Table S1: Description and frequency of symptoms declared by the 48 dengue RT-PCR confirmed subjects.

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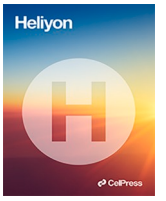

Review article

Asymptomatic dengue infection rate: A systematic literature review

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ABSTRACT

Objectives: Dengue infection is spreading worldwide. The clinical spectrum is broad and includes asymptomatic infections. This review provides an overview of the different proportions of asymptomatic infections described in epidemiological studies according to definitions, study designs, and detection methods.
Methods: Medline and Embase databases were searched without restriction of date or language. Studies were included if they reported data on the incidence or prevalence of asymptomatic dengue infections. The data were summarized and classified according to the definitions of the term 'asymptomatic'.
Results: A total of 74 studies were included. The mean proportion of asymptomatic infections among dengue-infected persons was 54% in 50 included studies. The prevalence of dengue infections detected in healthy persons was 0.2% in 24 included studies. The term 'asymptomatic' has been used to refer to 'clinically undetectable infection', but also to 'undiagnosed infection' or 'mild infection'. Only 8% were clinically undetectable laboratory-confirmed dengue infections.
Conclusion: The proportion of asymptomatic dengue infections varied greatly. Studies proving data on clinically undetectable laboratory-confirmed dengue infections were very few, but provided consistent results of low proportions of asymptomatic infections. These data challenge the assumption that the majority of dengue cases are asymptomatic.

1. Introduction

Dengue is the most globally prevalent arboviral disease [1]. Up to 3.97 billion people is at risk of contracting dengue virus infection [2]. Incidence of dengue has increased thirty times during the last fifty years. 50 to 100 million new infections are estimated to occur annually [1,3,4]. This increasing trend is expected to continue due to the increase in urbanization, population size, air traffic and climate change [5,6].

The symptomatic disease spectrum ranges from mild fever to severe and deadly haemorrhagic fever and shock syndrome [1]. The dengue-like syndrome is defined, according to the World Health Organization, as an acute fever disease with two or more of the following signs or symptoms: nausea, vomiting, rash, headache, retro-orbital pain, myalgia, arthralgia, and haemorrhagic signs [1].

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A dengue virus infection can also present without clinically detectable symptoms. *Duong* et al. *in 2015* demonstrated that symptomless infected humans transmitted the virus to *Aedes aegypti* [7], which could have a significant impact in spreading the disease. It is commonly accepted that the higher proportion of dengue infections are '*asymptomatic*'. The factsheet about dengue of the European Centre for Disease Prevention and Control mentioned: "*up to 40–80% of all dengue infections are asymptomatic*" [8]. However, data on the proportion of dengue infection referred to as 'asymptomatic' are highly heterogeneous in literature, which could be partly explained by a lack of clarity in the wording and on the meaning of the word 'asymptomatic'. As illustrated by the WHO Key facts of the May 10, 2021 [8] "*A vast majority of cases are asymptomatic or mild and self-managed, and hence the actual numbers of dengue cases are under-reported*", the clinical term 'asymptomatic' is frequently associated to 'mild' infections or 'under-reported' infections. Clinical terms such as 'asymptomatic' defined as the absence of symptoms, or 'mild' presentations are often gathered with the epidemiological consideration of 'under-reported' or 'unapparent' infections which correspond to undetected cases regardless of the presence or absence of symptoms. In fact, there are many reasons why dengue infection remains 'unapparent' for health authorities, apart from infections without symptoms, including mild symptoms, easily self-managed symptoms by an accustomed population, a lack of access to health care or misdiagnoses with other febrile diseases.

For epidemiological needs, such as mathematical modelling, infections presenting no symptoms are frequently pooled with 'unapparent symptomatic' infections as in *Clapham* et al. *2017* meta-analysis [9], where the word 'asymptomatic' gathers 'clinically asymptomatic' plus 'unapparent symptomatic' resulting in a proportion of 82% 'asymptomatic' for primary dengue infections and 59% for secondary infections in cohort studies and 78% and 43% respectively in cluster studies. These data are used for a mathematical modelling study [10] that concludes, "*more than 80% of dengue virus infections are attributable to individuals with mild to no symptoms who do not seek treatment from a physician*". As described in these studies, for epidemiological and modelling purposes, dengue infections could be divided into infections with no symptoms, detected symptomatic infections and undetected symptomatic infections. Grange et al., in 2014 [11] published a comprehensive review of the epidemiological factors associated with the frequency of unapparent dengue virus infections. They concluded that the epidemiological evidence suggested that the majority of infections were unapparent in endemic regions and highlighted the important role of short-term immunity. In their review, they grouped together under the term "unapparent" infections that were clinically undetectable, mild and not detected by surveillance systems. In this review, we aimed to provide an update as many studies have been published since 2014, but also to offer a different perspective by trying to demonstrate that part of the large heterogeneity in rates of asymptomatic dengue between studies is due to a lack of standardisation of terminology and study designs, which jeopardises the assessment of associated risk factors.

The proportion of infections with no symptoms needs to be known and clearly distinguished from 'unapparent' grouped data, because a symptomatic infection, even a 'mild' one, can be detected by the patient him/herself or by a healthcare practitioner, using a questionnaire, whereas infections without any symptoms are completely hidden. So, when deciding on screening policies for blood donors or at the start of new outbreaks and on the preventive message to be communicated to the population, it is essential to distinguish between infections whose symptoms are not clinically detectable and 'unapparent' infections, both of which are described as 'asymptomatic'.

The authors of this review recently conducted an observational study in the Southwest Indian Ocean islands [12], where dengue is emerging, to estimate the proportion of the infections with no symptoms through active screening for dengue infections in the community and, surprisingly, none could be found. Given the wide variation in data and the lack of clarity in clinical and epidemiological formulation in the literature, we decided to undertake this literature review to extract the proportions of 'asymptomatic' infections in epidemiological studies and to provide data with a higher granularity based on a specific definition of 'asymptomatic' and the detection method used.

2. Methods

2.1. Eligibility criteria

All observational studies reporting proportion, prevalence or incidence of asymptomatic dengue infections were included. No language, publication date, or publication status restrictions were imposed. Participants with any age with a diagnosis of dengue were considered.

A confirmed infection of dengue was defined by the detection of the virus with one of the following assays: virus culture and isolation, real-time polymerase chain reaction (RT-PCR), transcription-mediated amplification (TMA), detection of non-structural protein 1 antigen (NS1) by ELISA or rapid diagnostic tests (RDT); or, detection of an antibody response as a seroconversion or a 4-fold increase in total antibodies with one of the following assays: IgM/IgG ELISA or RDT, haemagglutination inhibition assays, plaque reduction neutralisation test (PRNT).

We did not restrict to a pre-defined definition of 'asymptomatic infection' nor to a pre-defined term used.

2.2. Information sources

Studies were identified by searching electronic databases and scanning references lists of published literature reviews on the topic. No limits were applied for language and other than English or French languages' paper were translated. This search was applied to the National Library of MEDicine's MEDLINE (1966-Present) by PubMed and Embase (1980-present) by ODS and NB. The last search was run on December 01, 2020. ODS and NB conducted the search in blind each other. In addition to searching databases, authors used i)

https://connectedpapers.com/, ii) search in Google Scholar and iii) backward citation tracking to identify articles not retrieved by electronic searches.

2.3. Search

The following search strategies were used: Pubmed: ((dengue[MeSH Terms]) AND ((asymptomatic infection[MeSH Terms]) OR (asymptomatic disease[MeSH Terms]) OR (infection, subclinical[MeSH Terms]))); Embase: 'dengue' AND ('asymptomatic infection' OR 'asymptomatic disease').

Observational studies found through backward citation tracking were included if they contained data on the proportion of dengue asymptomatic infections.

2.4. Study selection

First, eligibility assessment was performed independently in an unblinded standardized manner by two reviewers (ODS and NB) by screening first title and abstract. If title and/or abstract provided insufficient information to assess the relevance or if a final decision could not be made, the full article was assessed. Second, full texts of articles selected in the first stage were independently reviewed for final inclusion. Disagreements were resolved by discussion between the two reviewers (ODS and NB). All duplicates' articles were removed. When more than one published manuscript concerned the same study, these manuscripts were pooled, and data were extracted only once.

2.5. Data collection process

We developed a data extraction sheet containing the data items listed below. ODS extracted the data from included studies and NB checked the extracted data. Disagreements were resolved by discussion.

2.6. Data items

Data extracted concerned the following items: publication year, study site, recruitment design, age of participants, term used to describe 'asymptomatic infections', definition of 'asymptomatic infection', dengue diagnostic test, proportion of asymptomatic infections on total dengue infection, asymptomatic/symptomatic ratio, percent of dengue infection among asymptomatic participants, follow-up of dengue infection if any.

2.7. Risk of bias in individual studies

Risk of bias in individual studies were discussed considering the recruitment design and the diagnostic test used. We attempted to minimize selection, publication, and language bias through a comprehensive search strategy without language restrictions and by employing a transparent methodology. However, some biases remain due to the lack of standardization regarding the definition of "asymptomatic" infections and variations in recruitment designs among the included studies. The main bias encountered in this review, as explained later, is recall bias in serosurveys, particularly in children. In fact, as discussed later, the clinical presentation of dengue is similar to that of other common viral diseases, making it highly likely that children and parents may not remember the specific occurrence of a dengue infection.

2.8. Data analysis

R Core Team (2021) was used to calculate the summary statistics. The estimated proportions of asymptomatic infections and confidence intervals were obtained by compiling the frequencies of asymptomatic infections and the sample sizes of the populations of the different selected studies.

3. Results

3.1. Search strategy and PRISMA flow diagram

We identified 465 papers. After an automatic removal of 42 duplicates by a reference management tool (Endnote), 423 papers were screened by title, of which 178 were selected, retrieved and assessed for eligibility, with abstract and full text reading. One hundred and four papers were excluded (reasons listed in Fig. 1: PRISMA flow diagram), and 74 were included, listed in Table S1 (supplementary material). Table 1 lists the 50 studies that provided asymptomatic rates of dengue infections by calculating the number of asymptomatic infection among the total dengue infections detected and the associated study characteristics and epidemiological risk factors. The 24 remaining studies, presented in Supplementary Table S1, provided the prevalence of dengue infections among asymptomatic populations.

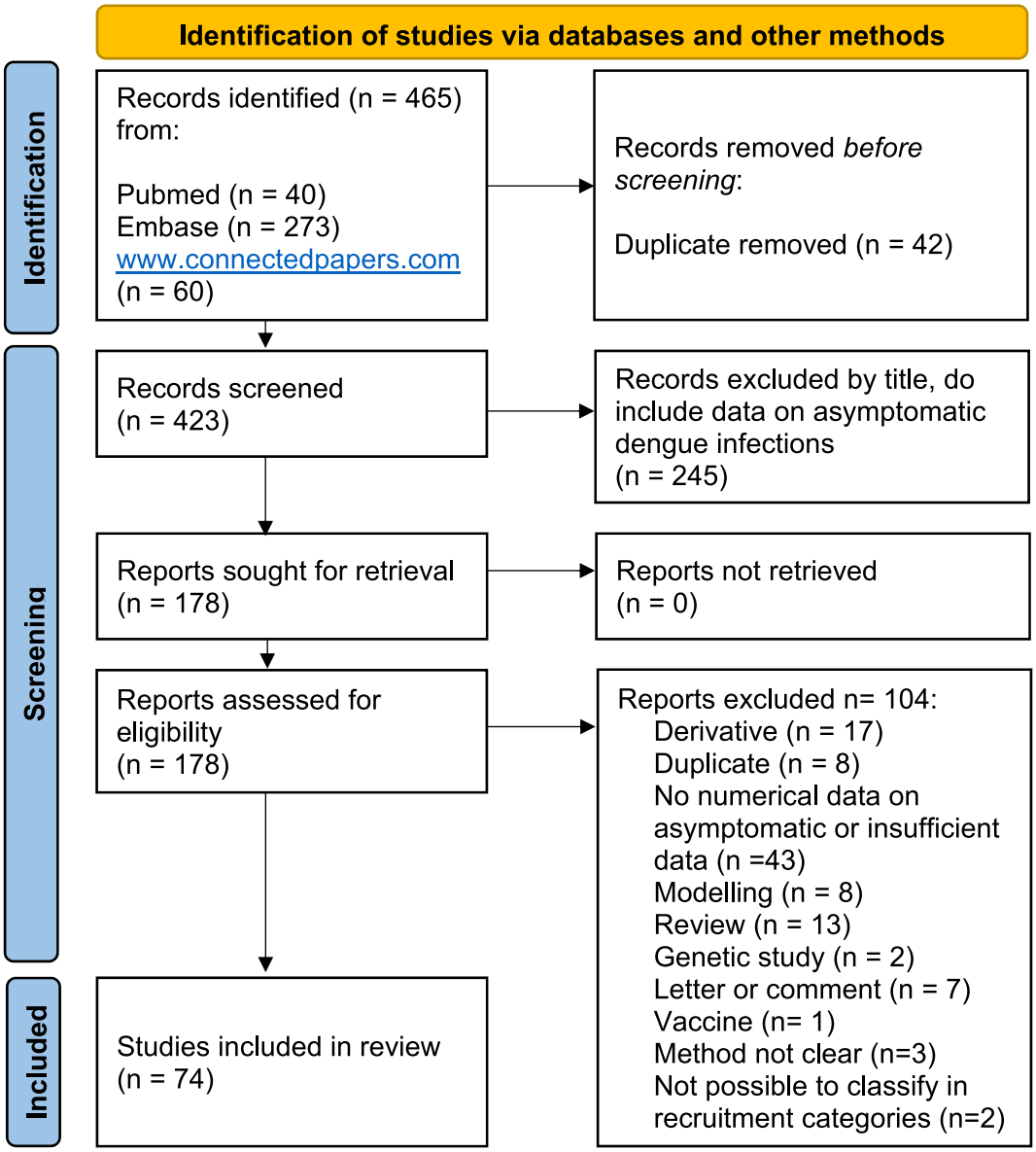

Fig. 1. PRISMA Flow diagram.
From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021; 372:n71. https://doi.org/10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

3.2. Study sites

Excluding three studies in travellers in multiples regions, 96% of studies took place in Asia and Latin America, half in each continent. Only one study was conducted in Africa. In supplementary material. As presented in Table 1, the Asymptomatic Rate (AR) per region ranged from 16 to 97% (19 studies, median 58%) for Latin America; 0–96% (19 studies, median 75%) for South-east Asia; 63–100% (4 studies) for East Asia; 0% (2 studies) for America, Caribbean; 16% (1 study) for the Horn of Africa and 11.5% (1 study) for Oceania.

3.3. Publication dates

Most of the studies (73%) were published since 2006. Before 2000, only some serosurveys were conducted in endemic areas or after outbreaks, and some studies on travellers returning from endemic countries. In the last fifteen years, cohort or cluster studies were mostly conducted. In the last five years, many studies on blood donors were published. (See Fig. S1 in Supplementary material).

Table 1
List of 50 observational studies providing data on the asymptomatic rate of dengue infections.

Definition	Reference	Publication Year	Study Type	Region	**Age (years)**	Diagnostic Test	Serotype	Sero-prevalence	Incidence*	**Dengue infections** N	**Asympto-matic Rate** % (N)
No symptom	[29]	2020	Cluster	South-East Asia	11–63	RT-PCR	Dengue virus 1 (1/13) Dengue virus 2 (2/13) Dengue virus 3 (7/13) Dengue virus 4 (5/13)	9%	NA	175	7.4% (13)
No symptom	[38]	2000	Travellers	America, Caribbean	≥ 14	IgM/IgG ELISA, PRNT	NA	69%	NA	22	0% (0)
No symptoms	[31,39,40]	2008	Cluster	South-East Asia	0.5–15	RT-PCR, IgM/IgG ELISA	All, mainly Dengue virus 1, Dengue virus 4	8%	NA	119	20% (24)
No symptoms	[41]	2010	Cluster	Latin America	All	IgM/IgG ELISA, HI titersx4, RT-PCR	Dengue virus 2	4%	NA	12	42% (5)
No symptoms	[14]	2018	Cluster	Latin America	All	RT-PCR, NS1	NA	13%	NA	50	32% (16)
No symptoms	[30,42]	2019	Cluster	South-East Asia	0.5–40	RT-PCR	Dengue virus 1 (80.8%) Dengue virus 2 (7.7%) Dengue virus 4 (11.5%) Dengue virus 3 (1.1%)	4%	NA	346	7.5% (26)
Subclinical	[43]	2015	Cluster	South-East Asia	All (mainly <15)	IgM/IgG ELISA	NA	6%	NA	113	92% (104)
Subclinical	[44]	2005	Cluster	South-East Asia	All	HI titersx4, RT-PCR	Dengue virus 1, Dengue virus 2	2%	NA	17	47% (8)
Subclinical	[45]	2012	Cluster	South-East Asa, Latin America	>2	RT-PCR	Dengue virus 1, Dengue virus 2, Dengue virus 3	10%	NA	101	29% (29)
Subclinical	[46]	2016	Cluster	South Asia	All	RDT NS1,IgM/IgG	NA	11%	NA	226	63% (142)
Subclinical	[17]	2015	Cluster	Latin America	≥ 5	IgM/IgG ELISA seronconversion or ab titers x4	NA	22%	NA	253	60% (151)
Subclinical	[47]	2015	Cluster	East Asia	All	IgM/igG ELISA	NA	5%	NA	41	68% (28)
Subclinical	[48]	2011	Cohort	South-East Asia	0–8	PRNTx4	NA	NA	11%	NA	75% (NA)
Subclinical	[15]	2016	Cohort	South-East Asia	All	HI titersx4	NA	NA	9%	77	79% (61)
Subclinical	[35]	2009	Cohort	South-East Asia	<1	IgM/IgG ELISA	NA	NA	1%	10	90% (9)
Subclinical	[49]	2010	Cohort	South-East Asia	2–15	IgG ELISA	Dengue virus 2, Dengue virus 1	NA	3%	953	80% (764)
Subclinical	[50]	2013	Cohort	Latin America	≥ 5	IgM/IgG ELISA, PRNT	Dengue virus 3, Dengue virus 4	NA	11%	2286	90% (2074)
Subclinical	[51]	1973	Serosurvey	Latin America	All	HI titersx4	NA	45%	NA	148	43% (63)
Subclinical	[52]	1967	Serosurvey	Latin America	>4	HI titers x4	NA	38%	NA	25	16% (4)
Subclinical	[53]	1985	Serosurvey	Latin America	All	HI titersx4	Dengue virus 4	7%	NA	56	45% (25)
Subclinical	[54]	2009	Serosurvey	Latin America	1–79	IgM/IgG ELISA	NA	10%	NA	33	70% (23)
Subclinical	[55]	1998	Serosurvey	Latin America	NA	HI	Dengue virus 2	44%	NA	588	41% (243)
Subclinical	[56]	1998	Serosurvey	Oceania	14–50	HI, IgG ELISA, PRNT	Dengue virus 2	26%	NA	139	11.5% (16)
Subclinical	[57]	1990	Serosurvey	Latin America	All	PRNT	Dengue virus 1, Dengue virus 2	17%	NA	219	76% (167)
Subclinical	[58]	2013	Serosurvey	South-East Asia	7–85	IgM/IgG ELISA	NA	7%	NA	NA	78% (NA)
Subclinical	[59]	2006	Serosurvey	East Asia	≥ 18	IgG ELISA	Dengue virus 2	NA	NA	55	78% (43)
Subclinical	[60]	2006	Serosurvey	Latin America	7–20	Viral isolation	Dengue virus 1, Dengue virus 2, Dengue virus 3, Dengue virus 4	7%	NA	215	86% (185)

(*continued on next page*)

Table 1 (*continued*)

Definition	Reference	Publication Year	Study Type	Region	**Age (years)**	Diagnostic Test	Serotype	Sero-prevalence	Incidence*	**Dengue infections** N	**Asympto-matic Rate** % (N)
Subclinical	[61]	2002	Serosurvey	Latin America	≥ 14	IgM/IgG ELISA	Dengue virus 1	21%	NA	42	33% (14)
Subclinical	[62]	2011	Travellers	America, Caribbean	≥ 18	RT-PCR, IgM ELISA	Dengue virus 1	33%	NA	7	0% (0)
Subclinical	[63]	2011	Travellers	NA	≥ 18	IgM/IgG ELISA	NA	1%	NA	14	64% (9)
Subclinical	[64]	2002	Travellers	NA	≥ 18	IgM/IgG ELISA	NA	3%	NA	NA	77% (NA)
Subclinical	[65]	1999	Travellers	NA	≥ 18	IgM/IgG ELISA	NA	7%	NA	7	42% (3)
Subclinical	[21]	2012	Travellers	Asia	≥ 16	IgG ELISA	NA	1%	NA	4	100% (4)
Subclinical	[66]	2005	Travellers	South-East Asia	≥ 18	Serology	NA	10%	NA	27	11% (3)
Subclinical	[26]	1969	Travellers	South-East Asia	All	Serology	NA	NA	NA	NA	0% (NA)
Subclinical	[67]	1995	Travellers	Horn of Africa	≥ 18	IgM ELISA	NA	9%	NA	44	16% (7)
Unapparent	[68]	1988	Cohort	South-East Asia	4–16	HI titers x4	Dengue virus 1, Dengue virus 2, Dengue virus 4	NA	12%	103	87% (90)
Unapparent	[69,70]	2005	Cohort	South-East Asia	18–66	HI titersx4	All, mainly Dengue virus 4	NA	1%	NA	72% (NA)
Unapparent	[16,34,71,72]	2002	Cohort	South-East Asia	7–16	HI titers x4	All, mainly Dengue virus 3	NA	7%	615	66% (406)
Unapparent	[73]	2006	Cohort	Latin America	4–16	HI titersx5	Dengue virus 1, Dengue virus 2	NA	5%	NA	85–92% (NA)
Unapparent	[74,75]	2010	Cohort	Latin America	2–14	Total antibodies Inhibition ELISA titersx4	Dengue virus 1, Dengue virus 2	NA	1%	NA	60–95% (NA)
Unapparent	[76]	2010	Cohort	South-East Asia	6 and 18 weeks	HI titersx4	NA	NA	1%	NA	85% (NA)
Unapparent	[77]	2015	Cohort	Latin America	10–18	IgG ELISA	NA	NA	6%	19	61% (10)
Unapparent	[78]	2010	Cohort	Latin America	5–60	PRNT	Dengue virus 1, Dengue virus 2, Dengue virus 3	NA	7%	NA	50–84% (NA)
Unapparent	[79]	2014	Cohort	South-East Asia	≤ 12	IgG ELISA	NA	NA	4%	67	60% (40)
Unapparent	[80]	1995	Serosurvey	Latin America	5–19	HI	Dengue virus 1, Dengue virus 2	62%	NA	277	58% (160)
Unapparent	[81]	1995	Serosurvey	Latin America	All	IgM/IgG ELISA	NA	17%	NA	59	53% (28)
Unapparent	[82]	2000	Serosurvey	Latin America	All	IgM ELISA, PRNT	Dengue virus 1, Dengue virus 2	41%	NA	NA	97% (NA)
Unapparent	[83]	2006	Serosurvey	South-East Asia	All (mainly >18)	IgM ELISA	NA	NA	21%	NA	82% (NA)
Unapparent	[84]	2009	Serosurvey	South-East Asia	18–74	IgM/IgG ELISA	NA	3%	NA	NA	96% (NA)

Different meanings of 'asymptomatic' dengue infection.

We defined the three following categories to classify with more precision the group of dengue infections referred to as 'asymptomatic' detected by the included studies.

- the *"no symptoms"*: detected laboratory confirmed infections with absolutely no symptoms declared during a follow-up; 6 studies corresponds to this definition and 3/6 were published after the Grange et al., 2014 [11], previous review. The AR ranged from 0 to 42% (median 7.5%)
- the *"subclinical"*: mild or aspecific infections: presence of symptoms but that do not fit with the WHO definition of clinical dengue; 30 studies included with this definition. The AR ranged form 0–100% (median 64%)
- the *"unapparent"*: infections not detected by the health care system or by any surveillance system regardless of symptomatology; 14 studies. The AR ranged from 50 to 97% (median 72%)

The term 'asymptomatic' is kept as a container including all the above definitions and corresponds to the proportions of cases extracted in the studies because referred to as 'asymptomatic'.

3.4. Proportion of asymptomatic participants among dengue infections (asymptomatic rate) versus prevalence of dengue infections among asymptomatic participants

In the majority of the included studies (50), the results extracted for this review were the proportions of asymptomatic participants among dengue infections. Studies were classified according to the definitions of 'asymptomatic', the diagnostic tests and the age group as shown in the summary Table 2. In the remaining 24 studies, including all the studies on blood donors (16), 2 cohorts and 6 serosurveys, the results presented were the prevalence of dengue infections among healthy participants. We presented these results separately in Table 2, classified according to the viral or antibody detection method. Indeed, in these 24 studies, the denominator is a population of healthy persons and not dengue infected cases. The 6 serosurveys studies presented in Table 2 presented a proportion of participants with traces of old dengue infections (i.e. presence of IgG in blood) but no dengue history. We decided to include these studies in the review, as they are another way to detect the presence of possible dengue infections with no symptoms, in the population. The studies on blood donors are important to evaluate the risk of dengue transmission through blood transfusion.

3.5. Asymptomatic rate according to the categories of recruitment and detection methods

Five categories according to the participants' recruitment design were identified among the included studies: 1) Cluster studies; 2) Cohort studies; 3) Serosurveys; 4) Studies on travellers; 5) Studies on blood donors. Median (and interquartile range) proportions of asymptomatic infections according to the categories of recruitment for 50 studies providing data on the proportion of asymptomatic infection among dengue infections were calculated. Studies on travellers show the lower proportions of asymptomatic infections but with a high variability and cohort studies the higher with the shorter.

Each recruitment design presented different characteristics, risk of bias and distribution of dengue infections prevalence or incidence. Table 3 present the results of proportion of asymptomatic infections according to the clinical definition of 'asymptomatic' and to the detection method (viral or antibody). In cluster studies (number of studies = 11), the recruitment took place in geographical areas of a predefined radius, among people living in the neighbourhood of a dengue index case. Dengue prevalence in this kind of studies ranged from 2.2% to 21.5% (median 7.9%) and the AR ranged 7.4–92% (median 42%). Five studies provided proportions of asymptomatic infections corresponding to a strict clinical definition of 'No symptom' and detected with virus detection methods (RT-PCR or viral isolation), the mean proportion was of 8%.

In cohort studies (n = 14), the recruitment took place in a predefined group of persons, as children of a primary school. Participants gave a blood sample at inclusion, and were followed by annual blood samples for serology. Detection of clinical apparent dengue

Table 2
Prevalence of dengue infection among asymptomatic participants per study category and detection method.

Recruitment method	Virus detection[b]					Antibody detection				
	Number of studies	References	I/A[a]	Prevalence of dengue infection (CI)	Range	Number of studies	References	I/A[a]	Prevalence of dengue infections (CI)	Range
Blood donors	14	[13, 85–97]	125/ 54333	0,2% (0-0,7%)	[0–5.5%]	2	[98,99]	15/ 573	2,60% (0–6,8%)	[0–4.2%]
Cohort	1	[100]	NA	12,70%		1	[32]	NA	5–20%	
Serosurvey [c]						6	[33, 101–105]	NA	28%	[7–48%]
Total	15					9				

[a] I/A: number of dengue infections (I) among healthy asymptomatic people (A).
[b] Virus detection thourgh RT-PCR, Transcription Mediated Amplification (TMA) or NS1.
[c] Presence of IgG and no history of dengue.

Table 3
Summary table providing the proportions expressed in % of asymptomatic infections among dengue infections classified by the definition of 'asymptomatic' and the diagnostic test (the detailed tables are provided in supplementary material).

Recruitment category	Asymptomatic definition	Virus detection				Antibody detection			
		Number of studies	References	A/I[d]	Proportion of 'asymptomatic' infections (CI)	Number of studies	References	A/I[d]	Proportion of 'asymptomatic' infections (CI)
Cluster	No symptoms	5[a]	[14,29,31, 41,42]	59/ 702	8% (5–12%)	2[a]	[31,41]	25/ 131	19% (10–28%)
	Subclinical	2[a]	[44,45]	31/ 118	26% (17–35%)	5[a]	[17,43,44, 46,47]	433/ 650	67% (63–71%)
	Unapparent								
Cohort	No symptoms								
	Subclinical					5	[15,35, 48–50]	2908/ 3326	87% (86–89%)
	Unapparent					9	[68,69,71, 73,74, 76–79]	546/ 804[b]	68% (64–71%)
Serosurvey	No symptoms								
	Subclinical	1	[60]	185/ 215	86% (79–93%)	10	[51–59,61]	598/ 1305	46% (43–49%)
	Unapparent					5	[80–84]	188/ 336[c]	56% (50–61%)
Travellers	No symptoms					1	[38]	0/22	0% (0–21%)
	Subclinical	1	[62]	0/7	0% (0–38%)	7	[21,26, 63–67]	26/96	27% (17–37%)
	Unapparent								

[a] Ref 39, 47 and 53 used both detection methods: virus or antibody detection, results have been splitted for the table.
[b] Data extracted from 4/9 studies that provided detailed data.
[c] Data extracted from 2/5 studies that provied detailed data.
[d] Number of 'asymptomatic' dengue infection/total number of dengue infections, aggregated results extracted from the publications.

infection was possible thanks to school absenteeism surveillance and with the collaboration of surrounding health centres. Dengue incidence ranged from 0.8% to 12.7% (median 6%) with an AR that ranged from 60 to 92% (median 78%). All cohort studies used antibody detection methods. By refining the results according to asymptomatic definition, only subclinical or unapparent infections were described with high proportion of 87 and 68% respectively (Table 2). The risk of recall bias was high mainly in children who might present aspecific clinical forms and would not have consulted a general practitioner for mild symptoms.

Serosurveys (n = 22) recruited participants in general population of an administrative region to know the attack rate and herd immunity of the population. Dengue prevalence showed a large distribution ranging from 2.6% to 61.6% (median 19.3%) with an AR range of 6.6–97% (median 53%). Sixteen serosurveys provided data on asymptomatic infections among dengue infections presented in Table 2. The proportion of asymptomatic infections according to clinical definition varies among 46–86%, only subclinical and unapparent infections were considered. Six serosurveys provided a mean of 28% of healthy participants who had IgG positive but no history of dengue infections (Table 1).The studies were retrospective with a high risk of recall bias. Interpretation of lab results differed among studies and the WHO recommendation to collect paired sera was not always respected.

Studies on travellers or migrants (n = 9) included adult participants, not immune for dengue virus, and not used to this disease. Sample sizes were small. Dengue prevalence showed also a large distribution among studies, between 1% and 68.8% (median 7.8%), the AR was of 0% for one study using virus detection method and between 0 and 27% for studies using antibody detection methods. The risk of recall bias was low as a symptomatic episode during a short holiday or mission was generally a key fact for participants. Moreover, the small size of the sample sizes and the very aggregated and defined group of participants allowed a better follow-up and accurate clinical data. Studies on travellers included almost only adult participants (one study >14 years old and another >16 years old), only one included also children. On seven studies including adult participants with available data, we gathered 125 adult participants. Twenty-six participants had subclinical symptoms. Thus 99/125 (79%) of adult travellers had typically dengue-like syndrome. Travellers all came from countries without dengue. In all but one study, the diagnostic was based on a seroconversion, which mean that all were primary infections.

Studies on blood donors (n = 16) only included adult 'asymptomatic' participants due to the eligibility criteria for blood donation. The prevalence of asymptomatic infections was among all the included study population and was equivalent to the prevalence of dengue infections that ranged between 0 and 5.5% (median 0.07%). As presented in Table 1, most of the studies (14/16) used viral detection methods and the prevalence of dengue were very low for all except for one study (5.5%) [13]. These studies were undertaken to assess the risk of dengue transmission through blood donation during outbreaks or in endemic countries but the methodology was not elaborated to detected and evaluate asymptomatic infections. Indeed, no questionnaire on signs and symptoms was submitted to the participants besides the basic eligibility criteria for blood donation. As no follow-up was undertaken, it was not possible to determine if a dengue-positive participant had an asymptomatic or a pre-symptomatic infection.

3.6. Asymptomatic rate according to the age group

By classifying the studies according to the age group (the age is mentioned in Table 1), it resulted that the AR for adults defined as, more than 14 years or more than 18 years old, depending on studies, ranged 0–96% (13 studies, median 33%). The AR for the children group ranged 20–87% (11 studies, median 66%) and two studies concerned infants (less than one year old) with an AR of 85–90%. The AR in 23 studies including participants of all ages ranged 0–97% (median 63%). The AR seems then be higher for children than for adults but with a high variability.

3.7. Asymptomatic rate according to the serotype

In Table 1 are listed the serotypes detected during the studies. Data were not available for 26 on 50 studies and when available, the data were mostly aggregated and did not provide sufficient detail to be able to calculate any association between the AR and the serotype circulated. Moreover, for many studies many or even all four serotypes were circulating concomitantly. We extracted the AR for the studies were one serotype was dominant: Dengue virus 1: AR 0–33% (4 studies); Dengue virus 2: AR 11.5–78% (4 studies); Dengue virus 3: AR 7.4–66% (2 studies); Dengue virus 4: AR 20–72% (3 studies). But here again, the variability is high and the number of studies low.

3.8. Asymptomatic rate according to primary versus secondary infections

Since the Grange et al. review [11], some new studies provided information concerning the association between the severity of symptoms and the immunity. Most of the recent studies suggest that primary infections are more likely to be overt symptomatic and milder or asymptomatic in secondary or repeated infections especially if the time between the infections is short [14–17]. Inversely, Sun et Luo, China, 2018 [18], suggested that secondary or repeated infections are less likely to be asymptomatic.

4. Discussion

4.1. Main results

The mean proportion of ‘asymptomatic’ in the broad sense, including mild and unapparent dengue infections among identified dengue infections, was 54% overall in the 50 studies included in this review. By extracting data from studies with a precise definition of the word ‘asymptomatic’, meaning a clinical absence of symptoms, this proportion is equal to 18%. A proportion of 8% is obtained by combining the definition of no symptoms with a viral detection method using molecular detection, antigen detection or viral isolation. What is sometimes referred to as the ‘*majority*’ [19] or ‘*40–80%*’ [8,20] of asymptomatic dengue infections includes both purely no symptoms infections and subclinical or unapparent infections which could be confirmed infections detected by molecular biology but also suspected infection diagnosed by antibody detection.

The prevalence of dengue infections detected in apparent healthy participants in endemic countries was 14% in the 24 studies included. Including only blood donors and using viral detection methods yielded 0.2% of detected infections. If we exclude one study which presented particularly high results of 5.5% [13], the prevalence of dengue infections among blood donors, using the viral detection method, is 0.1%.

4.2. High heterogeneity in data

Our results showed that there was considerable heterogeneity in the proportion of dengue infections classified as ‘asymptomatic’ in the studies, which ranged from 0% to 100%. Methodological differences could explain this heterogeneity. The following parameters differed from one study to another: recruitment designs, the definitions considered for ‘asymptomatic’ infections, the age of the participants included and the diagnostic tests used to detect dengue infection. The extreme differences between 0 and 100% observed in the studies of travellers are also due to the small sample sizes and different interpretations of what was considered an ‘asymptomatic’ infection. For example, the study with 100% asymptomatic infections [21] included four dengue-infected travellers whose symptoms did not meet the WHO clinical definition of a dengue-like syndrome and who were therefore classified as ‘asymptomatic. In attempting to identify epidemiological risk factors – such as age, serotype, immunity, study location - associated to the AR of dengue infections, we were confronted with considerable heterogeneity in the results preventing to identify trends. This heterogeneity can be explained by study parameters that are not standardized, such as the definition considered for ‘asymptomatic’, the study recruitment design or the detection method. To be able to evaluate the risk factors more accurately, these parameter would have to be fixed, but there have not been enough studies published to have a reliable sample with fixed parameters. Up to now, only five studies shared the same definition of “no symptoms”, used a cluster recruitment design and an RT-PCR detection method.

4.3. Study sites

The study sites of almost all the included studies are located in Asia and in Latin America. Historically, the burden of dengue concerned essentially these two continents, sharing the same vector, *A. aegypti*. However, the epidemiology of dengue has changed, partly because of the spread of a second vector *A. albopictus* due to international trade [22,23]. *A. albopictus* invaded Africa since 1989

and was responsible for several dengue outbreaks [24]. Unfortunately, research on dengue has been completely neglected in this continent [25]. Although several studies were published on the epidemiology of dengue, the resulting knowledge is partial due to the exclusivity of study sites, which share a high level of endemicity and the same vector.

4.4. History of recruitment designs and asymptomatic detection throughout the years

This review includes 74 studies published between 1964 and 2020. Most of the studies (80%) were published in the last 20 years with an increasing trend over the last 5 years. Five categories of recruitment methods were identified: *Serosurveys in general population*, *Cohort studies*, *Cluster studies*, *Surveys on travellers or migrants* and *Surveys on blood donors*. Before 2001, all studies were serological surveys carried out in endemic countries or following outbreaks. Some were carried out on travellers returning from tropical area in European or US countries. The design of the studies subsequently changed to adapt and keep pace with the growing threat of dengue. The need for precise data on disease transmission and clinical presentations, led researchers to refine their methodologies by adopting cohort and cluster studies. Finally, recent years have seen an increase in studies of blood donors, reflecting the fear of this emerging disease and the need for data for policy makers.

The hypothesis that infections with no symptoms could play a role in the transmission of the disease appeared in the literature around 2000. Prior to this, a few sporadic detections of "*not overt diseases*" [26,27] had been described in large serosurveys. The increasing spread of the disease and the development of new vaccines [28] compelling to a better knowledge of the prevalence and herd immunity may explain the growing interest in studying infections without clinical presentation or not detected by surveillance systems.

4.5. Definitions of 'asymptomatic' in literature

No consensus has been reached on a standard definition or terminology for 'asymptomatic' dengue infections. The literature provided different terms such as: "*asymptomatic*"; "*subclinical*"; "*unapparent*" or "*inapparent*"; "*mild*"; "*not overt disease*". These terms were not always defined in the same way and sometimes were used as synonyms. By refining the definitions and classifying the studies as *no symptoms*, *subclinical* and *unapparent*, the proportions of asymptomatic fall into opposite trends (18, 55 and 75% respectively).

The studies estimating the proportion of dengue infections with no symptoms detected by the presence of the virus (RT-PCR, TMA, virus isolation or NS1) were only five in number and resulted in a proportion of 8% of infections with no symptoms by aggregating the data. All five used a cluster recruitment design, which appears to be the most suitable for finding infections without any clinical presentation. The two studies with the largest sample sizes [7,29] were from Asia (Cambodia and Thailand) and both reached a same result of 7.5%.

4.6. Clinical presentation according to age

The high and prolonged endemicity meant that it was mainly children who fell victims to dengue and that by adulthood, they had already acquired partial or complete immunity. Most dengue infections were mild and rarely led to a medical consultation. The WHO has provided a clinical definition for a dengue-like syndrome but no definition for a "mild" dengue infection. In this review, the publications considered mild infections as infections without fever [30,31], or presenting low-grade dengue symptoms (headache, muscular and articular pain, rash, pruritus, fatigue) [32,33] or, mainly in children, as undifferentiated fevers presenting as other febrile childhood diseases [34–37]. No typical symptoms that would help diagnose mild dengue infection emerged from the papers included in this review.

If we consider the studies carried out on travellers, 80% of these adult travellers with a primary dengue infection presented with a typical dengue-like syndrome. Twenty percent were not compatible with the WHO definition of dengue, which does not mean that they presented no symptoms. Only one study of travellers also included children and concluded that these children did not have typical dengue symptoms. In addition to travellers, most studies included participants with multiple dengue infections.

4.7. Parameters that contribute to explain the extent of asymptomatic infection in studies

By classifying the studies and extracting the data according to the following parameters: *recruitment design - definition of 'asymptomatic' - age of participants - diagnostic tests* - it emerged that certain parameters appeared to influence the proportion of asymptomatic infections (see Table 2). This interpretation must be taken with caution, as the results could not be perfectly compared due to the lack of standardization in the methods used to collect the data. Nevertheless, it is possible to identify a trend in these factors that is medically and epidemiologically plausible. The following parameters appear to increase the proportion of asymptomatic infections: *"children"*, *"serosurveys"* and *"cohort studies"*, *"dengue diagnosed with antibodies detection"*. Rather, *"adults"*, *"cluster studies"*, *"studies on travellers"* and *"dengue diagnosed with viral detection methods"* would decrease this proportion.

The multiplication of study parameters such as methods of detection, study designs, and the clinical definition of "asymptomatic" makes it difficult or impossible to interpret the impact of other variables such as serotype or age on the rate of asymptomatic infections. However, the number of studies using a strict definition of the absence of symptoms, a similar design and a similar method of detection can be counted on the fingers of one hand (5). Further studies with fixed parameters are needed to determine the association of serotype, primary or secondary infection, and age on the proportion of asymptomatic cases.

4.8. Limitations and futures prospects

While the systematic review provides an overview of the proportions of asymptomatic dengue infections, it is subject to limitations mainly as heterogeneity of definitions, and variability in study designs. The lack of standardized criteria for defining asymptomatic dengue infections makes it challenging to compare and synthesize the results accurately. To address these limitations, future research should focus on establishing standardized definitions, conducting large-scale prospective studies, and strengthening global surveillance efforts. These steps will contribute to a more robust understanding of asymptomatic dengue infections and inform public health strategies to control and prevent the spread of the disease.

5. Conclusion

This literature review provides a more detailed understanding of the proportion of asymptomatic dengue infections. By carefully examining the available data and considering the context and design of the studies, it becomes evident that infections with no symptoms were rare. Most of the so-called 'asymptomatic' dengue infections were actually mild or nonspecific infections that could easily go undetected by public health surveillance. These considerations are particularly valuable for areas with high endemicity, as there is very little data available for geographic areas where dengue is emerging.

Dengue is rapidly spreading worldwide, reaching countries without herd immunity and where the population has limited knowledge about the disease. The transition to endemicity can occur swiftly, as demonstrated by the sustained transmission of the virus on La Reunion since 2016. Therefore, there is a critical need for data to model the disease and forecast its evolution; interpret surveillance data with caution, and discuss the possibility of introducing a vaccination. In areas recently affected by dengue, the proportion of infections with no symptoms appears to be quite low, as well as the proportion of unapparent cases, assuming adequate surveillance systems and the access to healthcare. However, if the disease become endemic, the proportion of asymptomatic infections, the persons most affected by the disease and the disease presentation may change.

Furthermore, this literature review highlights the lack of research and knowledge on the epidemiology of dengue in Africa and on the clinical presentation of dengue in countries where the disease is emerging. Most of the published data on clinical presentation focused on children, while information regarding the clinical presentation in the adult population was scarce. These data are crucial for assisting public health authorities in adapting policies related to dengue surveillance and blood donors, as well as determining the need introducing dengue vaccines.

Ethical approval statement

Ethics approval was not required for this literature review.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20069.

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Article

Knowledge, Attitudes, Beliefs, and Practices Regarding Dengue in La Réunion Island, France

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Abstract: Since 2017, La Réunion island has been facing a major epidemic of dengue. Despite actions carried out by the anti-vector control department, public authorities have failed to contain this epidemic. As individual involvement is key to success in vector control, we carried out a mixed-methods study on population knowledge, attitudes, beliefs, and practices (KABP) regarding dengue infection risk in La Réunion. The study combined quantitative data collected through a questionnaire administered to a representative sample of 622 people to assess the use of protective measures and the perception of severity and risk of dengue, and a sample of 336 people to assess the level of knowledge and concern about dengue, as well as qualitative data collected through semi-structured interviews among 11 individuals who had previously completed the questionnaire. The study results show that 63% of the surveyed population had a good level of knowledge associated with age, education, and socio-professional category variables—78% considered dengue to be a serious threat, and concern was estimated at 6/10, while 71% were likely to use protective measures. The interviews revealed contradictory behaviors in the implementation of recommended actions, in conflict with personal beliefs regarding respect of human body and nature. The study also revealed a loss of confidence in public authorities.

Keywords: KABP; dengue; La Reunion; mixed-methods study

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1. Introduction

Dengue is the most prevalent arboviral disease in the world. The virus infects up to 390 million people living in tropical and sub-tropical areas [1]. The incidence of dengue has increased by 30-fold in the last 50 years due to global warming, increased urbanization, population growth, and the development of international travel [2].

The dengue virus is transmitted to humans by two species of *Aedes* mosquitoes: *Aedes aegypti* and *Aedes albopictus*. *A. aegypti* is the principal vector of transmission worldwide, while *A.albopictus* settles where *A. aegypti* is not very present, like in Indian Ocean islands [3].

The first documented outbreak of dengue in La Réunion took place in 1977–1978. The estimated attack rate was 30%. Between 2004 and 2017, only sporadic autochthonous cases

were reported [4]. The most significant outbreak reached 200 cases in 2016. In March 2018, a new dengue epidemic broke out in the island and local authorities launched level 3 of the ORSEC plan (Organisation de la Réponse de Sécurité Civile), which is a program for organizing assistance at a departmental level in the event of a disaster. It allows for the rapid and effective implementation of all necessary resources under the authority of the prefect. This plan is aimed at efficiently organizing the response to limit the spread of the virus.

Vector control measures have existed in La Réunion since 1914, for the prevention of malaria, which was eradicated at the end of the 1970s. After a major outbreak of chikungunya in 2005, which infected 38% of the population, a specific vector control system was set up [5]. However, cases of dengue infection are currently reported from almost all municipalities, and three serotypes (DEN-1, DEN-2, and DEN-3) co-circulate on the island. In 2021, 29,222 confirmed cases and 19 deaths directly linked to dengue were reported [6].

To the best of our knowledge, no KABP study has been carried out on dengue in La Réunion. In 2008, one study focused on social, environmental, and behavioral factors during the outbreak of chikungunya [7]. One KABP study about dengue fever was set in Martinique (another French island) in 2008 [8]. Only a few studies have looked at the level of knowledge of the population and their perception of the disease [9–11]. Most were interested in the level of knowledge of schoolchildren or students, in order to improve school prevention programs [12–15].

According to the World Health Organization (WHO), a communities' involvement is key to the success of vector control [16]. In addition, many studies have shown the importance of taking into account local knowledge and representations of vector-borne diseases when developing vector control strategies [8,17,18]. The aim of this study is to describe the knowledge, attitudes, beliefs, and practices (KABP) related to dengue transmission and prevention among the population living on La Réunion Island and to understand the impact of reported KABP on dengue's shift to endemicity.

2. Materials and Methods

2.1. Study Sites and Population

This study was conducted as a part of a larger epidemiological research work, the DEMARE study, an observational cross-sectional epidemiological study conducted in La Réunion and Madagascar in 2019–2020. The main objective of the DEMARE study was to estimate the prevalence of dengue infections of all clinical forms, including asymptomatic cases in the community, according to a clustered geographical design based on dengue index cases.

Our study was carried out in 19 geographical clusters following the field design of the DEMARE study. Clusters were defined by a 200-m radius around a dengue index case. These clusters were mainly located in the west coast of La Réunion, where the epidemic was still raging during the study recruitment period. Participants were first contacted at their homes following a door to door recruiting schedule in order to arrange an inclusion appointment. This meeting allowed for collecting informed consent, administering the study questionnaire, and contacting participants who subsequently accepted being interviewed.

2.2. Study Design

This mixed-methods study combined quantitative and qualitative data collection. First, quantitative data were obtained from a questionnaire including socio-demographic variables, perception of danger related to dengue, and use of protective measures against mosquito bites. The calculation of the sample size was based on a known probability of good level of knowledge about dengue rate, estimated at 80% [8], a confidence level of 1.96 (95%), and an error of 5%. The resulting sample size was of 246 participants.

The DEMARE study included 622 participants. The KABP study was nested in the DEMARE study and questionnaires were administered to 336 participants. However, as part of the questions of the KABP study were asked in the DEMARE case report form,

for some variables, data for 622 participants were available. Data were collected from 28 October 2019 to 27 August 2020. Data analyses were carried out using the statistical package R, version 3.4.4.

Second, qualitative data were obtained from semi-structured interviews using an interview guide following five main dimensions of disease representation: identity, temporality, causes, consequences, and controllability (Leventhal, 1980). A convenience sample was selected based on the principle of maximum variation on a number of variables: gender, level of education, socio-professional category, type of dwelling, place of birth, and data from the quantitative survey questionnaires (self-reported use of protective measures, feeling frequently being bitten or not by mosquitoes). The sample size was defined by data saturation when the interviews did not provide any new information.

A convenience sample of 11 interviewees was composed according to their age, sex, level of education, protective measures declared in the questionnaires, and housing conditions. Interviews were conducted at the participants' home, in a calm environment and according to their availability. Each interview was recorded and completely transcribed. A thematic analysis of the collected data was carried out by two investigators using an individual open coding, and then confronting analyses and creating a new thematic.

3. Results

3.1. Results of the Quantitative Study

3.1.1. Level of Knowledge about Dengue

Knowledge about dengue was rated by six questions concerning disease classification, vector, transmission, severity, symptoms, and immunology. The questions could be answered by "yes", "no", or "I do not know". Here are the questions with the correct answer rate in brackets (CAR): (1) Is dengue a disease caused by bacteria? (CAR = 53%); (2) Can all mosquitoes transmit dengue? (CAR = 82%); (3) Can dengue be transmitted through saliva? (CAR = 75%); (4) Can you get sick without having any symptoms? (CAR = 79%); (5) Can dengue be a deadly disease? (CAR = 97%); and (6) Can you get dengue more than once? (CAR = 86%). The individual CAR oscillated between 17% and 100%. The mean of individual CAR was 79%.

The level of knowledge was defined as "poor" for CAR $\leq$ 25%, "insufficient" for CAR > 25 $\leq$ 50%, "medium" for CAR > 50 $\leq$ 70%, and "good" for CAR > 70%. Figure 1 shows the repartition of level of knowledge about dengue.

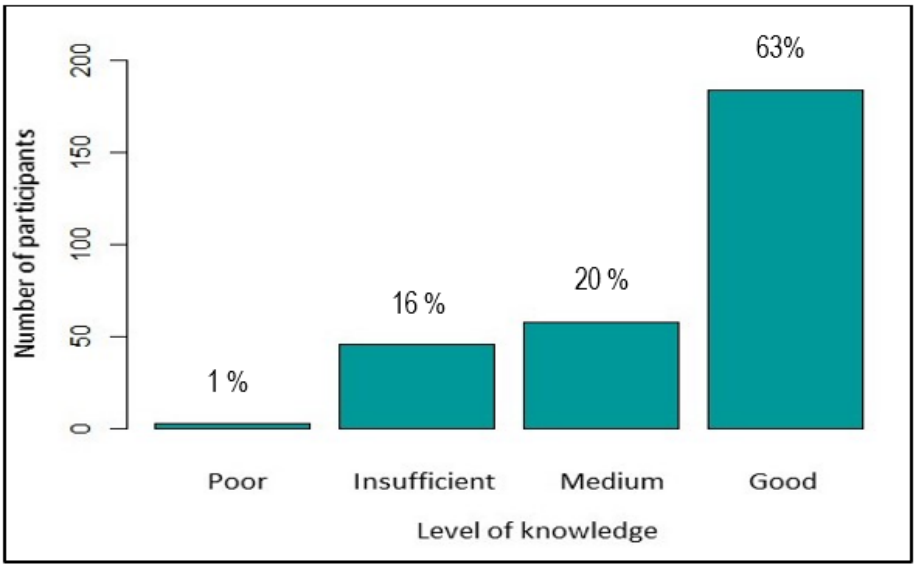

Figure 1. Level of knowledge about dengue.

The cross-analysis between the level of knowledge and the other study variables is detailed in Table 1. Advanced age ($p < 0.05$), none or primary education ($p < 0.05$), and professional inactivity (unemployed, retired, or disabled) ($p < 0.05$) are associated with a poor level of knowledge. These variables were adjusted in a logistic multivariate model that showed interactions between age and professional inactivity, and between age and a poor level of education. These determinants were linked and concerned the same subset of the population.

Table 1. Cross analysis between demographic characteristics or the experience of a dengue-like symptoms and the level of knowledge.

	LEVEL OF KNOWLEDGE						
	Poor or insufficient		Medium		Good		*p*
	n	%	n	%	n	%	
GENDER							0.2
Female	33	18	29	16	118	66	
Male	16	14	29	26	66	60	
AGE							<0.005
Mean	61		52		49		
IC95%	57—66		48—57		47—51		
LEVEL OF EDUCATION							<0.005
None or primary	24	46	14	26	15	28	
Secondary 1	20	21	25	27	49	52	
Secondary 2	3	6	12	23	37	71	
Bac + 2	1	4	3	11	22	85	
Bac > +2	1	2	4	6	61	92	
SOCIOPROFESSIONNAL CATEGORY							<0.005
Schoolchild or student	0	0	3	18	14	82	
Housewife	5	17	7	24	17	59	
State employee	4	10	4	10	32	80	
Private employee	3	7	6	13	37	80	
Independant	1	4	7	25	20	71	
Unemployed	12	24	17	35	20	41	
Retired	24	30	14	18	41	52	
USE OF PROTECTIVE MEASURE							0.5
Yes	32	15	44	21	134	64	
No	17	21	14	17	50	62	

3.1.2. Perception of Danger Related to Dengue

The perception of danger was rated by two questions: "What about the seriousness of having dengue fever?" and "Do you think you are at risk of contracting dengue in the next five years?". Figures 2 and 3 show the distribution of answers.

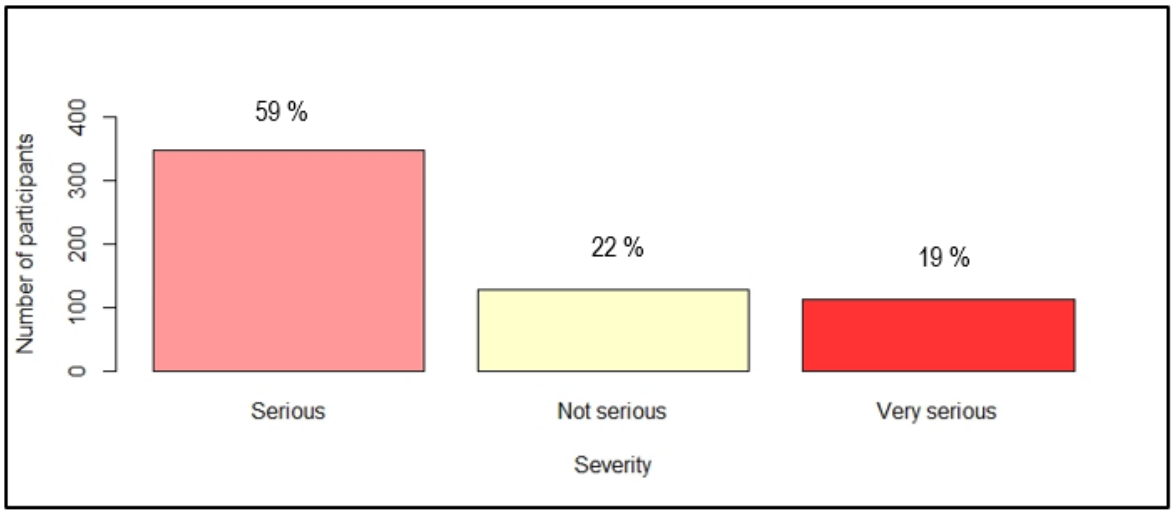

Figure 2. Distribution of participants according to their perception of severity of dengue (n = 587).

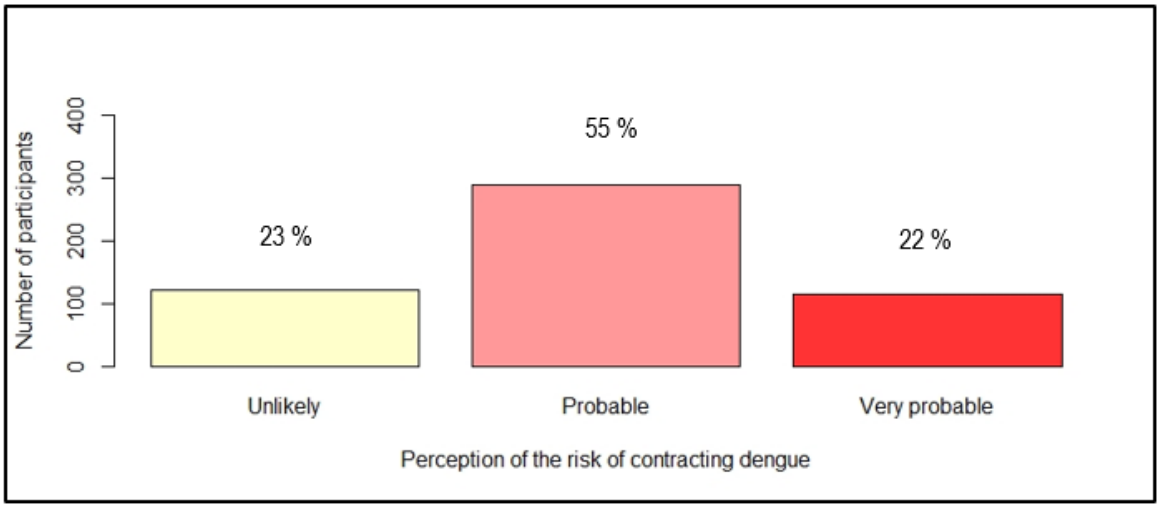

Figure 3. Distribution of participants according to their perception of the risk of contracting dengue in the coming five years (n = 525).

The cross-analysis between the perception of severity and other studies variables is detailed in Table 2. Gender, age, level of education, and socioprofessional categories were associated with the perception of severity of dengue fever. There was no association between the knowledge about dengue and the perception of severity.

The cross-analysis between the perception of risk and other studied variables is detailed in Table 3. School children or students were the less concerned with the risk of contracting dengue, contrary to state employees who were the most concerned. People who had already experienced dengue-like symptoms were more susceptible to perceive a high risk of contracting the disease. Unsurprisingly, participants who declared never having been bitten by mosquitos were more susceptible to perceive a low risk of contracting dengue. The cross-analysis also highlighted an association between the level of knowledge about dengue and the perception of risk (p = 0.01).

Table 2. Cross analysis between socio-demographic factors, previous experience of a dengue-like symptoms, level of knowledge about dengue, and perception of severity.

	PERCEPTION OF SEVERITY						
	Not serious		Serious		Very serious		*p*
	n	%	*n*	%	*n*	%	
GENDER							0.01
Female	59	17	210	62	69	20	
Male	68	27	138	56	42	17	
AGE							0.01
Mean	46		49		50		
IC95%	42—49		47—51		57—54		
LEVEL OF EDUCATION							0.02
None or primary	15	15	68	67	19	19	
Secondary 1	32	18	112	62	36	20	
Secondary 2	23	18	76	60	27	21	
Bac + 2	14	27	29	58	9	17	
Bac > +2	42	34	61	50	20	16	
SOCIOPROFESSIONNAL CATEGORY							0.004
Schoolchild or student	16	21	51	67	9	12	
Housewife	10	17	38	64	11	19	
State employee	21	27	36	46	21	27	
Private employee	23	29	47	59	10	13	
Independant	20	37	21	39	13	24	
Unemployed	12	16	48	65	14	19	
Retired	23	15	101	65	31	20	
ANTECEDENT OF DENGUE-LIKE SYNDROM							0.6
Yes	51	24	126	59	38	18	
No	76	20	221	60	74	20	
FEELING OF BEING BITTEN BY MOSQUITOES							0.6
Often	49	22	130	58	47	21	
Occasionnaly	37	26	78	56	25	18	
Rarely	29	17	107	64	31	19	
Never	12	23	32	60	9	17	
LEVEL OF KNOWLEDGE ABOUT DENGUE							0.3
Poor or insufficient	32	27	65	55	22	18	
Medium	61	21	174	61	51	18	
Good	22	19	63	56	28	25	

Participants were also asked to express their level of personal concern about five other health risks by giving them a score from 0 to 10 in the same way, namely: road accidents, seasonal flu, cyclones, diabetes, and chemicals in food. This technique made it possible to situate the concern about dengue in a broader context of multiple health risks to which the people of Reunion are exposed. The results are presented in Figure 4. The median of the dengue-related concern was of 6/10 (range 0–10). Dengue was less worrying than road accidents (8/10), use of pesticides (7/10), and diabetes (7/10), but more worrying than cyclone (5/10) and seasonal flu (5/10).

Table 3. Cross analysis between socio-demographic factors, previous experience of a dengue-like symptoms, level of knowledge about dengue, and perception of risk.

	PERCEPTION OF RISK						
	Unlikely		Probable		Very probable		*p*
	n	%	*n*	%	*n*	%	
GENDER							0.2
Female	59	19	175	58	69	23	
Male	62	28	113	51	46	21	
AGE							0.4
Mean	42		49		46		
IC95%	43—51		47—51		43—49		
LEVEL OF EDUCATION							0.2
None or primary	21	27	38	49	19	24	
Secondary 1	35	23	93	60	26	17	
Secondary 2	29	25	65	57	21	18	
Bac + 2	10	20	29	59	10	20	
Bac > +2	24	19	62	50	39	31	
SOCIOPROFESSIONNAL CATEGORY							0.0006
Schoolchild or student	25	40	28	45	9	15	
Housewife	12	22	29	54	13	24	
State employee	10	13	41	53	27	35	
Private employee	11	14	46	61	19	25	
Independant	6	13	26	54	16	33	
Unemployed	16	25	37	57	12	18	
Retired	38	29	75	57	19	14	
ANTECEDENT OF DENGUE-LIKE SYNDROM							0.006
Yes	30	16	108	57	51	27	
No	90	27	181	54	64	19	
FEELING OF BEING BITTEN BY MOSQUITOES							0.0002
Often	35	17	109	53	60	30	
Occasionnaly	24	19	79	62	24	19	
Rarely	44	30	76	51	28	19	
Never	18	40	25	56	2	4	
LEVEL OF KNOWLEDGE ABOUT DENGUE							0.01
Poor or insufficient	9	25	20	56	7	19	
Medium	15	28	32	61	6	11	
Good	22	13	101	58	51	29	

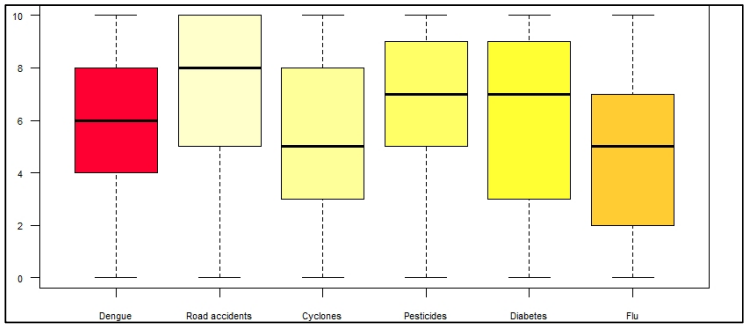

Figure 4. Degree of concern about dengue regarding other potential risks.

3.1.3. Use of protective Measures against Mosquito Bites

Here, 71% of participants reported using protective measures against mosquito bites, where 59% used it "sometimes" and 35% "daily". Only 6% reported using protective measures "very rarely".

Reported protectives measures are shown in Figure 5. The most frequent answer was "other measures of protection", including essential oils (lemongrass and geranium), UV lamps, mosquito nets on windows, insecticides use in gardens, fishponds, and smoke (traditional method of burning herbs and leaves, while cleaning gardens to keep mosquitoes away).

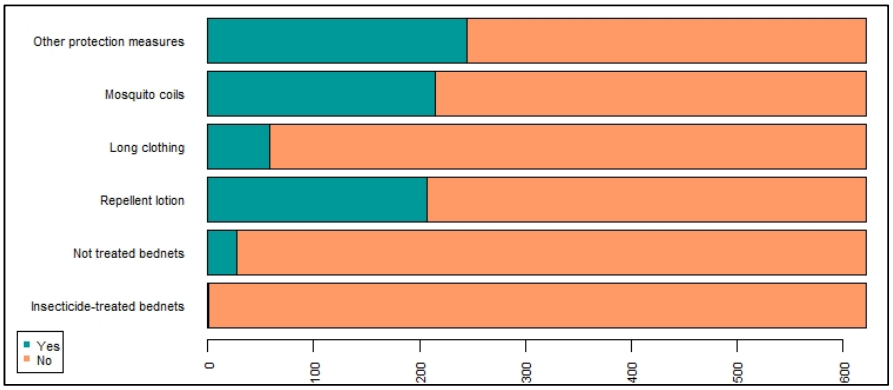

Figure 5. Repartition of the different protective measures reported by study participants.

Participants were also asked about their perception of the efficiency of different protective, individual, and collective measures, by rating them from 0 (not efficient) to 10 (extremely efficient). The results are presented in Figure 6. The measure considered the most efficient was the elimination of breeding sites in gardens or courtyards (with a median of 9/10 and a low dispersion). The other measures rated by the participants included, in decreasing order, maintenance of gullies by municipalities, mosquito nets, skin repellent lotions, mosquito control program, and indoor repellents. These results have to be considered with caution, as answering this question was complex for participants for two reasons: a moral dilemma between efficiency and environmental harmfulness, and between theoretical and real efficiency.

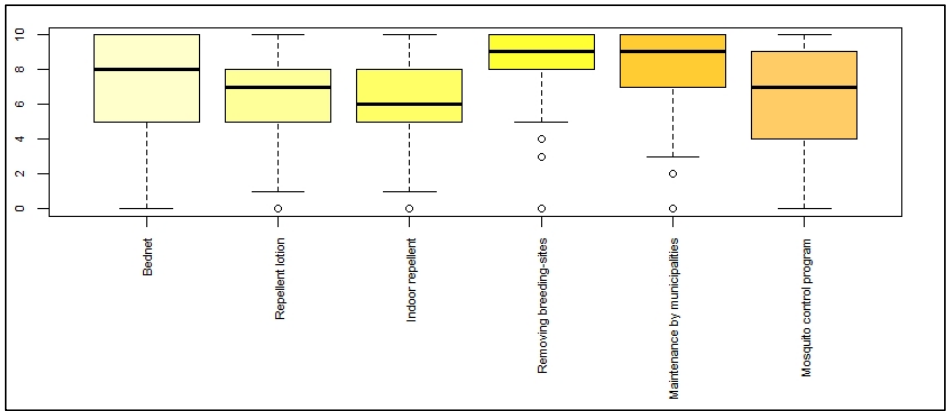

Figure 6. Perception of efficiency of different protective measures against mosquito bites.

The cross-analysis between the use of protective measures and other studied variables is detailed in Table 4. This analysis shows that respondents' sex, the level of education, the professional activity, and having the idea of being bitten by mosquitos would significantly influence the use of protection measures. Indeed, the variables associated with the use of protection measures were "women", "high level of education", and "impression to be frequently bitten by mosquitos". On the contrary, variables associated with a decreased use

of protection measures included "schoolchildren or students" and "impression to be never bitten by mosquitos". The perception of danger did not significantly influence the use of protective measures. Moreover, the level of knowledge about dengue did not significantly influence the use of protective measures.

Table 4. Cross analysis between use of protective measures, socio-demographic characteristics, experience of a dengue-like symptoms, and perception of severity or risk.

	USE OF PROTECTIVE MEASURES AGAINST MOSQUITO'BITES				
	Yes		No		*p*
	n	%	*n*	%	
GENDER					0.003
Female	268	75	87	25	
Male	170	64	94	36	
AGE					0.0002
Mean	49			42	
IC95%	48—51			36—49	
LEVEL OF EDUCATION					0.05
None or primary	79	64	45	36	
Secondary 1	133	73	49	27	
Secondary 2	83	65	45	35	
Bac + 2	41	77	12	23	
Bac > +2	99	77	29	23	
SOCIOPROFESSIONNAL CATEGORY					0.001
Schoolchild or student	50	52	46	48	
Housewife	45	76	14	24	
State employee	64	79	17	21	
Private employee	59	73	22	27	
Independant	40	74	14	26	
Unemployed	52	68	25	32	
Retired	121	76	39	24	
ANTECEDENT OF DENGUE-LIKE SYNDROM					0.1
Yes	166	74	57	26	
No	271	68	125	32	
FEELING OF BEING BITTEN BY MOSQUITOES					0.004
Often	175	75	57	25	
Occasionnaly	113	75	38	25	
Rarely	118	67	58	33	
Never	31	53	27	47	
PERCEPTION OF SEVERITY					0.2
Not serious	83	65	44	35	
Serious	255	73	93	27	
Very serious	84	75	28	25	
PERCEPTION OF RISK					0.1
Unlikely	86	71	35	29	
Probable	206	68	83	32	
Very probable	93	73	22	27	
LEVEL OF KNOWLEDGE ABOUT DENGUE					0.4
Poor or insufficient	32	65	17	35	
Medium	44	76	14	24	
Good	134	73	50	27	

3.2. Results of the Qualitative Study

The sample was composed of five men and six women, between 30 and 70 years old, with an average age of 55. One was unemployed, five worked in public or private firms, and five were retired. Five were born in Metropolitan France, one living in La Réunion for 4 years and four for more than 5 years. Six were born in La Réunion. Four of them had already contracted dengue, biologically confirmed.

3.2.1. Knowledge and Beliefs about Dengue

The overall knowledge about dengue was good among interviewees. They compared dengue with chikungunya or seasonal flu, because of their similar symptoms. The 2005–2006 chikungunya epidemic was a vivid memory, whether people got sick or not. The symptomatology of dengue was known by all interviewees, but descriptions varied from one participant to another. They all insisted on the suddenness and the long duration of the disease.

Interviews showed different interpretations of the word "dengue". Firstly, for many interviewees, dengue was a vector-borne disease transmitted by the "tiger mosquito". However, for elderly people, the word dengue was designated an important flu syndrome not related to mosquitoes. They estimated that dengue was an airborne disease, and that the "tiger mosquito" had disappeared from the island following the chikungunya epidemic.

Interviewees also showed a lack of knowledge about immunology and the different serotypes. They often did not know if dengue could be contracted more than once. Some of them thought that one serotype was more dangerous than another, and had no idea about secondary infection and the related risk of increased severity.

3.2.2. Perception of Danger

The concept of risk was present among all interviewees, and it was linked to the geographical and social proximity of the epidemic. As a result of its presence on the island for several years, dengue appeared to be a common disease: people got used to it because they, or their relatives, had contracted it. Most of interviewees expressed concern for others: the elderly, people with chronic pathologies, or young children.

The severity and mortality of dengue were also discussed with interviewees. They felt concerned as the disease seemed to get more severe from year to year, and often mentioned number of deaths and possible comorbidities, and frequently compared the number of deaths to that associated to chikungunya or COVID-19 (the COVID-19 crisis was widely covered by the media over the study period). Interviewees often considered dengue as a less lethal disease than chikungunya or COVID-19, and pointed out a lack of information about its severity. Sometimes, they assumed dengue victims presented comorbidities.

3.2.3. Perception of Local Ecology

Interviewees who had grown up in La Réunion shared the idea that the island was naturally protected due its remoteness. Nevertheless, they also thought that La Réunion had been subjected to critical changes in recent years, including growing urbanization and increasing international travel and tourism, that disrupted its natural protection. Within this context, dengue was considered as an imported disease.

All interviewees thought that mosquitoes were part of La Réunion historical fauna and for many of them, they were not responsible for any disease in the past (although malaria was once endemic before it was eradicated in the late 1970s). Finally, they were well informed of risks factors for dengue transmission. Having been exposed to malaria and chikungunya epidemics, they were aware of the risks and knew how to protect themselves against vector-borne diseases.

3.2.4. Responsibility for Prevention

Three levels of responsibility in the fight against dengue were identified: individual responsibility, responsibility of municipalities, and responsibility of the Health Regional Agency (Agence Régionale de Santé, ARS).

Regarding individual responsibility, prevention campaigns seemed to have achieved their objective: all interviewees mentioned cleaning their garden and removing stagnant water tanks. Field observations confirmed this practice, even among participants who refuted the role of mosquitoes in disease transmission. Most of the interviewees mentioned the use of individual prevention measures such as body sprays, spirals, long clothes, mosquito repellent plugs, air blowers, air conditioning, and essential oils. These protecting measures were used to protect oneself or to protect others, as a civic duty, especially when one was contagious. However, such practices were only occasional, when mosquitoes were visible or during high-risk periods.

The responsibility of municipalities was involved in the various means deployed to clean up public areas and to stimulate individual responsibility. Environmental pollution was identified as a risk factor leading to mosquito proliferation. Garbage, car wrecks, and any other material found on waysides are reservoirs for mosquitoes on the island as soon as it rains. Acting on fly-tipping was repeatedly mentioned during the interviews as an important lever for action in vector control. Verbalizing polluters seemed to be a necessary solution in response to the lack of civic mindedness, and the state of cleanliness of cities was criticized. While interviewees felt their individual responsibility was constantly challenged through prevention campaigns, they considered that public areas were not as clean as their own places.

The role of the ARS was frequently mentioned in relation to the vector control strategy based on door-to-door or night trucks campaigns. The interviewees considered that there is a lack of clarity regarding the organization of these actions. Some interviewees mentioned that they were still waiting for the ARS to pass by their homes, and one explicitly declared that it passed very seldomly. Others felt that the employed methods were inconsistent: spraying the top of piles of leaves, next to piles of grass, or not treating ponds. The treatment of ravines was questioned by most of the interviewees. According to them, while the prophylaxis service acting against malaria was used to disinfect the ravines, and this prevention measure was no longer carried out. Nobody knew for sure why this prophylaxis had stopped, and some interviewees evoked potential reasons such as banning related to ecological risks (ocean pollution) or difficulty of access. All participants considered ravines as the largest mosquito reservoir and estimated that they should be a priority target for the ARS, which should act where individuals and municipalities cannot gain access.

3.2.5. Individual Beliefs: A Barrier to Prevention?

Our analysis reveals some beliefs that could impact dengue prevention behaviors. Individual or collective protection was considered necessary, in accordance with individual beliefs and two main imperatives: respect for one's body and respect of nature. The use of anti-mosquito sprays was occasional because of limited knowledge about chemicals and related long-term effects. Interviewees were more willing to use individual protection measures if they were natural. Those who chose to protect themselves did so in accordance with their own health-related values.

The role of insecticides in the mosquito control measures carried out by the ARS was strongly contested in the interviews. Few people were totally opposed to them, but they had many questions about the dangerousness and the effectiveness of the products spread. The impact of insecticides on other animals was highlighted: wasps, lizards, birds, chameleons, bees, fish, spiders, etc. A vicious circle was pointed out: the disappearance of the species regulating the populations of mosquitoes. Not totally opposed to insecticides, several interviewees were eager to change the products currently used by the ARS, on the one hand, to achieve more efficacy, because mosquitoes had developed resistance to the

product used for years and, on the other hand, to use insecticides specifically targeting against mosquitoes and not killing other harmless species.

3.2.6. Motivation and Confidence in Public Authorities

Public authorities were strongly criticized by almost all of the interviewees, evidencing a significant loss of confidence in public authorities. They felt that many preventive actions were conducted without any results and the lack of feedback was clearly condemned.

In addition, prevention campaigns were described as oppressive by some interviewees. They expressed the feeling of being constantly blamed by public authorities, while many other actions could be carried out by the municipalities or the regional authorities.

3.2.7. Vision of the Future

Opinions were divided among interviewees. Those more fatalistic thought dengue would subsist because mosquitoes would not be extinct in La Réunion and preventive actions were thus done in vain. Those more optimistic believed actions could be conducted to control the spread of the epidemic. Nevertheless, some of them thought the existing vector control strategy and the resulting actions were not suitable and they had some proposals to define new strategies. Others believed that advances in biology and medicine research would help find a solution.

4. Discussion

The main findings of our study concern the high level of knowledge about dengue among the population, the link between the perception of risk and the adoption of individual protection measures, and the existence of constraints to social mobilization.

More than 97% of participants were informed about dengue. Two thirds of the participants had a good level of knowledge about dengue; a similar result was found in the West Indies [8]. The level of knowledge was related to the age, the education level, and the socio-professional category, which was consistent with the literature on the determinants of health knowledge [19]. We did not find a link between the level of knowledge and antecedents of dengue, which has been highlighted in other studies [11].

Knowledge on dengue vectors and its mode of transmission was also well integrated: 82% of interviews knew that not all mosquito species can transmit dengue and 75% knew that dengue was not transmitted through saliva. Some secular beliefs persisted, especially linked to the different meanings of the word "dengue" in La Réunion. Indeed, the word dengue represented "mosquito bite", but also a set of symptoms similar to dengue-like symptoms or flu-like symptoms. Seasonal influenza had long been referred by doctors as dengue, a synonym widely accepted on the island [5]. A previous study already highlighted this point [20]. Dengue was associated with vector transmission only in 2012. The actual vector control service was created after the chikungunya epidemic and the first prevention campaign against dengue was conducted in 2012 [5,20].

The existence of several serotypes of dengue, as well as the concept of secondary dengue was very poorly known and understood. The result to the question "Can you have dengue more than once?" was certainly overestimated, because the recruitment in the field allowed for a first exchange with the interviewees and answering some of preliminary questions. Many of them believed that there were several types of dengue, one of which was more serious than the others. Providing information about the existence of four different dengue serotypes would allow individuals to be more aware of the risk of new infection and be more involved in the application of prevention measures, particularly in a context of potential low recourse to healthcare. In fact, dengue diagnosis has not been confirmed in the laboratory for more than a third of people who declared having contracted the disease. However, these findings must be taken with caution due to the COVID-19 pandemic, which might have introduced a bias. Indeed, the lock-down might have discouraged some people to seek laboratory tests.

Dengue seemed to be considered as a relatively serious disease for almost 80% of the surveyed population. More than 75% considered that they would likely contract dengue in the coming five years. Dengue was a moderate source of health concern (median score of 6/10). On a health risk scale, dengue ranked fourth after road accidents, use of pesticides, and diabetes, but ahead of cyclones or flu. This paradox was highlighted in a similar study carried out in 2008 in Martinique [8]. Our qualitative study showed that dengue severity was often compared to chikungunya, which killed around 150 people in 2005–2006. The death rate of dengue was generally not well known, and the existence of associated comorbidities was still suspected. Interviewees felt more concern for others, especially for the elderly and children, than for themselves. Cross-analysis showed associations between the perception of dengue risk and the knowledge on the vector, as shown in other studies [10].

More than 70% of the surveyed population used a protective measure against mosquito bites. This represents a higher frequency compared to various studies carried out on the same subject in La Réunion [8,21]. Skin repellents were relatively barely used, while they are recommended as one of the most effective methods (BEH, 2012). The use of mosquito coils (spirales), a less efficient protection (BEH, 2012), was significant even though it decreased compared to 2014. However, the perceived effectiveness of these devices (sprays and mosquito coils) slightly increased in La Réunion since 2014 [21]. Protective measures were not used daily, but on an occasional basis.

The level of individual protection was not significantly linked to the perception of severity or risk. This surprising observation was already described in a qualitative study carried out during the chikungunya epidemic in La Réunion [7]. This result contradicts that of another study underlining that these two variables are critical in the adoption of protective measures (Slovic, 1999). Our results showed no significant link between the experience of dengue and the attitude towards dengue control, which contradicts other studies [10]. In addition, the use of protective measures did not reflect the level of individual knowledge, while many other studies stressed the importance of knowledge to the perception of risk and the implementation of protective measures [11,22]. The reasons people did or did not use individual protection measures can only be understood through qualitative studies, which should be used more often. The use of an alternative protection method was frequently cited. Its effectiveness was not scientifically proven, but acceptability was considered more important. Scoring the efficiency of the listed protection measures was difficult for the study population. Protective measures were a source of moral dilemma when balancing the benefits and health risks. The need for a benefit–risk approach taking into account safety and efficiency of protective measures has been mentioned in other studies [5,21].

After the chikungunya epidemic, the anticipation of epidemics became a central point of health policies. To date, the lack of confidence in the health authorities seems to be a barrier to social mobilization. The place of insecticides in vector control and its consequences on the environment in the short or long term take an important place in the public debate. Beyond questioning the dangerousness of chemical products, which is mainly due to a lack of clear and easily accessible information or convincing studies, it is above all a lack of organization, and results in the general fight against dengue that is pointed out by the participants of the study.

The qualitative analysis defined three levels of responsibility in the fight against mosquitoes: individuals, municipalities, and the ARS. Prevention campaigns are still mainly focused on populations only, as underlined by the ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) report on the vector control strategy in 2018 [5], and as also pointed out by several interviewees. By linking the spread of mosquitoes to human practices, authorities held the population responsible for mosquito proliferation, and thereby for dengue proliferation. This was frequently used to hide the difficulties of the public authorities in dealing with the epidemic [20]. Convinced of doing their best at home, interviewees naturally shifted responsibility to public authorities.

Strongly committed from the start of the epidemic in 2017, municipalities initially stepped up their actions of waste collection, road cleaning, and elimination of fly-tipping, in particular by hiring jobs. As the years passed by, it appeared to lose some strength [5]. A remobilization of the municipal level seems to be essential for a collective vector control strategy and to re-engage the community.

Regarding the action of the ARS, this study showed a perceived lack of organization and training of workers for peri-domiciliary mosquito control. There were doubts about the effectiveness of the strategy employed, in particular due to the total lack of information on the results obtained. The adequacy between the means implemented regarding the objectives and the feedback on the effectiveness of the measures was regularly challenged. Effectiveness studies on the impact of the measures taken would be necessary to regain the confidence of the population. The ultra-vertical system and the complexity of the administrative organization in the governing bodies are often criticized [5]. In 2005–2006, the low-risk perception of chikungunya in La Réunion resulted in poor adherence to the vector control strategy that was put in place to tackle the epidemic [7]. Today, faced with a higher perception of risk and a strong perception of danger, communication campaigns should be part of a more horizontal approach by opening a dialogue with the population and by moving away from standardized messages sometimes badly perceived.

Finally, the results of the interviews suggest the emergence of a "dengue culture" linked to the proximity of the vector and the disease. This observation was also made in Martinique, where the epidemic context was similar [8]. It was found from our interviews that the population of La Réunion was used to living with mosquitoes. The elderly said mosquitoes have always been there, unlike diseases. Even the youngest or the most recently arrived on the island said that living with mosquitoes in La Réunion was usual. Mosquitoes occupy an important place in the local ecology. Directing control action solely on the eradication of the vector seemed to raise doubts on the efficiency.

Strengths and Limits

This study is an original attempt to identify the level of knowledge, beliefs, attitudes, and practices of the population living in La Réunion considering the dengue epidemic that began in 2017. It was carried out on a statistically representative sample of 622 people (with some questions addressed to a sub-group of 336 people), well beyond what was recommended by the sample size calculation in the study protocol. The quantitative study was based on a protocol and an analysis plan defined upstream of the study (internal validity). Some variables used came from previous studies carried out in La Réunion or in other DROM (Département et Régions d'Outre-Mer) on dengue or other arboviruses, in order to allow for external validity of the study. The use of a qualitative approach has shown its relevance to complement the quantitative approach and to understand different representations of the disease.

The main limitation of the study is the short time allowed to implement it. Fieldwork remains a complex issue, especially in the context of the COVID-19 crisis. For the qualitative study, interviews could be conducted only after the lifting the lockdown (11 May 2020). The health protocol imposed a strict procedure for disinfecting the equipment of the DEMARE study (serology test) during which this study was conducted, between each participant as well as the fitting out of a vehicle for the needs of this procedure.

The possible biases of our study are the following:

(i) Quantitative recruitment bias: field recruitment took place during the week, in normal working hours. Indeed, the sample showed a high proportion of retired people, who were more available at this time slot.

(ii) Information biases: with certain variables being declarative, it is relevant to think that estimates are over or underestimated. For example, the frequency of use of protective measures may have been overestimated, as participants were able to respond by compliance with the obligations conveyed in prevention campaigns.

(iii) Confounding factors: the variable "type of environment" was not used to conclude on the results of the study. Indeed, this data cannot be used as such and should be subjected to additional analysis relating to the social level with additional data that were not available.

5. Conclusions

This study indicated that the population of La Réunion is very well informed about the dengue virus, its severity, and the current individual risk on the island. This population is used to living with mosquitoes, and it seems utopian for them to think of the island without mosquitoes. This leads to poor adherence to vector control programs. The concerns about toxic products used for individual or collective protection measures is real and must be taken into account by the public authorities to allow for effective social mobilization. Finally, interviewees asked for more feedback and transparency on the prevention actions conducted by health authorities.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data will be publicly available in a data repository (https://yareta.unige.ch/#/home, accessed on 31 March 2022) as soon as all the manuscripts referring to the DEMARE study will be ready for publication.

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CONCLUSIONS AND PERSPECTIVES

Hypothesis not confirmed

The three studies conducted during this doctoral research have not substantiated our initial hypothesis that a significant portion of dengue infections remains undetected by routine surveillance due to their asymptomatic nature or limited engagement with the healthcare system. The notion of these "silent" infections exerting a substantial impact on disease transmission has been challenged. On the contrary, our findings suggest a different perspective: **asymptomatic infections likely play only a marginal role in dengue transmission**.

This research endeavor has unveiled several noteworthy and valuable insights into the dengue epidemic in La Réunion. These findings hold particular significance as dengue epidemics are typically studied in contexts of high endemicity within middle or low-income countries. Investigations into re-emergent epidemics in high-income industrialized nations are relatively scarce. However, such studies are essential due to the global spread of dengue.

Limitations

The limitations associated with conducting field epidemiological studies are multifaceted. They stem from the intricate logistics required for their implementation, the high cost involved, and the inherent challenges posed by such endeavors in high-income countries. In such settings, individuals are often less available to respond and participate due to their busy schedules. Moreover, the time constraints are substantial, adding to the complexity of these studies. Additionally, the invasiveness of the research, involving blood samples, can deter participation. This can result in the study being able to capture only a partial picture of the overall situation. Therefore, while the insights gained from such studies are

undoubtedly crucial, the ongoing discussion regarding their cost-effectiveness remains imperative.

In our case, our study design does not allow us to assert that asymptomatic infections are exceedingly rare, even though we did not identify such cases, as our participant recruitment capabilities were subject to several biases. However, our results, when considered alongside the extensive and detailed literature review we conducted, give us a reasonable degree of confidence in our findings. Field studies, despite their challenges in terms of logistics, cost, and duration, remain the only means to truly observe the circulation within neighborhoods and, where applicable, detect asymptomatic cases.

Key Findings

- ✓ The **proportion of asymptomatic** and undiagnosed dengue infections in La Réunion is notably **low**
- ✓ Dengue transmission is **focal in time and place**
- ✓ **Human-associated environmental factors** emerge as the primary risk contributors, with no significant socio-demographic factors identified
- ✓ The proportion of asymptomatic dengue infections exhibits considerable **variation in the existing literature**
- ✓ Few studies provide data on clinically undetectable, laboratory-confirmed dengue infections, consistently reporting **low rates of asymptomatic cases**
- ✓ The population of La Réunion demonstrates a **high level of knowledge** and concern regarding the dengue virus, its severity, and individual risk
- ✓ **Used to coexisting with mosquitoes**, the population finds it unrealistic to envision an existence without these insects, leading to limited compliance with vector control programs.

- ✓ Legitimate concerns about the **use of toxic products** for individual or collective protection measures are prevalent

Recommendations

The findings presented in this study challenge the long-held assumption that the majority of dengue cases are asymptomatic. If the proportion of asymptomatic infections is very low, as well as the undetected infections, it can therefore be suggested that **the surveillance data is reliable** for La Réunion and provides an accurate reflection of reality.

Targeting asymptomatic infections as a preventive measure to reduce transmission does not appear to be an effective approach considering our field study results.

The highly localized nature of dengue transmission underscores one of the key recommendations arising from this research, highlighting the imperative need for **precision-targeted public health prevention measures**, both in terms of timing and geographical specificity.

Furthermore, there is a pressing need for **increased feedback and transparency** regarding the prevention actions conducted by health authorities. This will not only foster greater trust between the public and health agencies but also enable communities to actively participate in and support these initiatives.

Lastly, the concerns raised about the use of **toxic products** in vector control and individual protection measures should not be overlooked by public authorities. It is imperative that these concerns are addressed promptly and comprehensively to ensure the success of dengue prevention efforts. This entails not only adopting safer alternatives but also engaging in open dialogue with the public to build consensus and ensure effective social mobilization.

The **dynamic evolution towards endemicity** is a complex phenomenon influenced by various factors. Dengue becomes a persistent part of the local health landscape in La Réunion. As **immunity levels** in the population rise due to repeated exposures to dengue, the dynamics of the disease may shift. Continuous monitoring and adaptation of prevention strategies will be necessary in such a scenario. Moreover, the ongoing **changes in climate patterns** have a direct impact on the distribution and prevalence of diseases like dengue. As climate conditions evolve, so does the suitability of areas for vector breeding and disease transmission. In light of these factors, it is imperative to **reevaluate our results periodically** to account for changing conditions and trends. Additionally, understanding the evolving demographics and habits of the population is crucial for effective dengue control. Changes in lifestyle and habits among the population can significantly impact dengue transmission. These shifts may include factors such as travel patterns, housing conditions, and water storage practices. **Qualitative studies** can provide valuable insights into these evolving behaviors.

The question arises whether the **responsibility for dengue prevention and surveillance should be partially or fully transferred to neighborhood associations**. While such a shift could promote community engagement and ownership of prevention efforts, it also raises questions about resources, training, and the ability of these associations to effectively carry out such responsibilities. Careful consideration and further research may be needed to explore the feasibility and effectiveness of such an approach.

The Final Word: Shifting from Asymptomatic Research to Climate Change Health Adaptation Research

In the end, dengue transmission, which had been latent for several years in Réunion, surged in 2019, and an endemic circulation pattern was established. While our hypothesis regarding the role of asymptomatic infections could not explain this epidemiological shift, an increase in temperature during the austral winter, leading to continued transmission during the winter months, could offer a partial explanation.

This epidemic is yet another example of the impact of climate changes on our health. While I was initially focused on investigating asymptomatic forms of dengue as the hidden widespread phenomenon that was driving transmission, my study has shown that I was targeting the wrong aspect. We are living in an unsettling era with a rising number of epidemic outbreaks, either emerging or resurging, largely facilitated by climate change. It is therefore high time to redirect our research towards understanding how to adapt to these new challenges.

The End

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