



Australian
National
University

Epidemiology of dengue in Bhutan

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Doctor of Philosophy
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Declaration

This thesis is composed of original works on account of research undertaken during academic program between February 2019 and April 2023 towards the degree of Doctor of Philosophy of the Australian National University. All previously published materials included in this thesis were dully acknowledged and appropriately cited in the text. Contributions of each author in jointly-authored works were clearly stated. This is a thesis by compilation and all the papers included have been approved by all co-authors.

I hereby certify that the work included in this thesis has not been previously submitted to qualify for the award of any other degree in any university or tertiary institution.

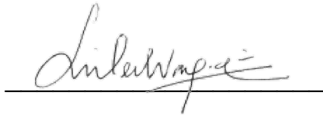
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25 April 2023

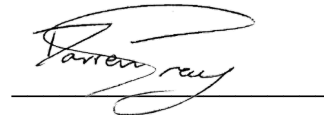
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Abstract

Dengue has been rapidly spreading worldwide, resulting in higher morbidity than other vector-borne viral diseases. The majority of this burden is borne by the South-East Asia Region of the World Health Organization (WHO-SEAR). Bhutan is a lower-middle-income country located in the WHO-SEAR. After the first documented outbreak in 2004, dengue has gradually expanded its geographical range with a concomitant increase in incidence and severity. The aim of my research was to describe the epidemiological characteristics of dengue in Bhutan.

Member countries in the WHO-SEAR have become hyper-endemic with the co-circulation of multiple dengue virus (DENV) serotypes. Although case fatalities have decreased steadily, dengue incidence has increased dramatically over the past decade in the region. As 0.5%–5% of the infection progresses to severe dengue including deaths, a meta-analysis was conducted to identify the risk factors of the disease. Children (odds ratio [OR]: 1.96; 95% CI: 1.22, 3.13), patients with secondary infection (OR: 3.23; 95% CI: 2.28, 4.57), and patients with pre-existing co-morbidities such as diabetes (OR: 2.88; 95% CI: 1.72, 4.81) and renal disease (OR: 4.54; 95% CI: 1.55, 13.31) were shown to have an increased risk for severe dengue. The study also corroborated the WHO's recommendation to monitor seven warning signs of severe dengue. However, the current surveillance system in Bhutan and other countries in the WHO-SEAR does not provide adequate information for early case detection and for effective management of dengue due to inadequate reporting. Additionally, vector surveillance was predominantly carried out using larval indices without routinely employing adult mosquito sampling.

To explore the spatio-temporal pattern of dengue incidence, an ecological study was conducted in a Bayesian framework. Individuals aged >14 years (relative risk [RR]: 0.471, 95% confidence interval [CI]: 0.378–0.582) and temperature (RR: 1.625, 95% CI: 1.493–1.772) were positively associated with dengue incidence, while normalised difference vegetation index [NDVI] (RR: 0.515, 95% CI: 0.353–0.748) was negatively associated with dengue incidence. Bhutan experienced the worst dengue epidemic in 2019, which resulted in cases greater than the combined total cases in all the previous years. Dengue expanded from six to 44 sub-districts across 19 districts. The effective reproduction number was <3 for most of the epidemic period, except for ~1 month period of explosive growth, coinciding with a period of monsoon season, where the effective reproduction number peaked >30.

A multicriteria decision-making analysis (MCDA) was used to develop a vulnerability map of dengue at a fine spatial resolution. Interestingly, this study pointed out new geographical locations including the nation's capital, as being at risk of dengue.

An online survey revealed critical gaps in the clinical management of dengue amongst medical practitioners (MPs). Major gaps included limited knowledge about the peak activity of feeding time of the dengue vector, the use of clinical warning signs of severe dengue for monitoring dengue patients, the use of NS1 antigen for early diagnosis, and the clinical significance of hematological parameters.

This thesis illustrates the contemporary epidemiology of dengue in Bhutan and identified ecological, clinical, and demographic risk factors of dengue transmission. This information will be crucial for forward planning of dengue prevention and control by the public health authorities in the country.

Keywords: Bhutan; dengue; epidemiology; vulnerability; clinical management

Abbreviations

<i>Ae. aegypti</i>	<i>Aedes aegypti</i>
<i>Ae. albopictus</i>	<i>Aedes albopictus</i>
AHP	Analytical Hierarchy Process
AIC	Akaike Information Criterion
ANU	Australian National University
AUC	Area Under the Curve
BG Sentinel	BioGents Sentinel
BHU	Basic Health Unit
BI	Breteau Index
BIC	Bayesian Information Criteria
BMHC	Bhutan Medical and Health Council
CAR	Conditional Autoregressive
CFR	Case Fatality Rate
CI	Container Index
CO	Clinical Officers
CrI	Credible Interval
CYD-TDV	Chimeric Yellow Fever Virus—DENV Tetravalent Dengue Vaccine
DENV	Dengue Virus
DHF	Dengue Haemorrhagic Fever
DIC	Deviance Information Criterion
DSS	Dengue Shock Syndrome
ELISA	Enzyme-Linked Immunosorbent Assay
	Earth Resources Observation and Science Moderate Resolution
eMODIS	Imaging Spectroradiometer
GDMO	General Duty Medical Officer
GI	Gastrointestinal
GIS	Geographical Information System
HA	Health Assistant
HI	House Index
IHR	International Health Regulation

IRR	Incidence Rate Ratio
MCDA	Multicriteria Decision Making Analysis
MF	Membership Function
MoH	Ministry of Health
MP	Medical Practitioner
NDVI	Normalised Difference Vegetation Index
NEWARS	National Early Warning, Alert, Response and Surveillance
NS1 antigen	Non-structural protein 1
OI	Ovitrap Index
PHC	Primary Health Care
PHEIC	Public Health Emergency of International Concern
RCDC	Royal Centre for Disease Control
REBH	Research Ethics Board of Health
ROC	Receiver Operating Characteristic Curve
R_t	Effective Reproductive Number
SMR	Standardised Morbidity Ratios
USGS	U.S. Geological Survey's
VDCP	Vector-borne Disease Control Program
VIF	Variance Inflation Factor
WHA	World Health Assembly
WHO-SEAR	WHO-South East Asia Region
WLC	Weighted Linear Combination
ZIP	Zero-inflated Poisson

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Introduction

1.1 Research motivation

Dengue is the most rapidly spreading vector-borne disease with serious public health consequences, including high morbidity and mortality (1). There has been a dramatic increase in the frequency of disease incidence over the recent decades, associated with an explosive number of outbreaks and expansion of dengue-affected areas (2). Although dengue is primarily a tropical and sub-tropical disease, it has also become a major concern in countries with temperate climates. This is a result of an increasing number of imported cases in recent years, brought about by the increased frequency of human travel and the introduction of exotic vectors adapted to a cold climate (3).

The dramatic rise and geographical expansion of dengue is an indication of population growth, unplanned and dense urbanization, globalization and environmental change. Rapid industrial and economic growth has brought about massive infrastructure and commercial development, increased trade and travel, and congestion of urban cities/centres. This unplanned economic development and urbanization creates breeding habitats suitable for *Aedes* mosquitoes, which are the vectors of dengue. Recently, globalization related to air travel was associated with the transportation of both dengue virus (DENV) and *Aedes* mosquitoes, leading to the introduction and establishment of dengue in previously unaffected areas. Previously confined to urban regions, dengue has now penetrated the countryside in Bhutan to rural setups due to improved road networks and socio-economic conditions (4). Even the highly resourced and well-implemented dengue control programs have not been able to prevent dengue epidemics (5). The continuous occurrence of dengue and increasing incidence of severe cases has raised the need to have a proper understanding of the determinants of dengue, so as to develop more effective surveillance and control measures (6). The control methods need to be closely aligned with existing knowledge of epidemiology and the challenges associated with both the disease and vector control.

World Health Assembly Resolution WHA55.17 in 2002 urged greater commitment to dengue among member states and the World Health Organization (WHO) because of the disease's

rapidly increasing public health importance. Subsequently the international health regulation (IHR) 1969 was revised in 2005 (WHA58.3), to include dengue as a disease which may constitute a public health emergency of international concern (PHEIC), which mandated member countries to report to WHO on the detection of such event. Following this, Dengue Scientific Working Group, consisting of 60 experts from 20 different countries and WHO staff from four regions and headquarters was formed: the group met in 2006 to review the current knowledge and establish priorities for future research in order to improve dengue management, prevention and control. One of the key research areas identified is related to reducing disease severity and fatality, which includes methods and guidelines for triage and out-patient care, capacity building, and best practices for the treatment of dengue. The other research areas included the development and utilization of early warning and response systems, host factors that determine the severity of the disease, transmission dynamics, the control of dengue transmission through improved vector management by using effective surveillance and vector control, tools for decision making, and adequate prioritization of dengue (7).

In Bhutan, dengue transmission occurs every year, with a recent rise in the reports of dengue outbreaks. DENV has propagated to new areas and is associated with the widespread distribution and expansion of dengue vectors (8). The intensity of transmission of dengue in Bhutan is evident from the detection of DENV-1, DENV-2, and DENV-3 in the past outbreaks. Outbreaks have occurred after every 3–5 years on average, and these were predominantly associated with a shift in the DENV serotypes and genotypes. Despite increasing incidence and the geographical expansion of dengue, there is limited research on the epidemiological characteristics of dengue. To have an extensive impact, the control strategies for dengue must rely on the sound evidence of the contemporary epidemiology of dengue. Without appropriate control strategies in place, dengue virus will find its way to expand further, causing greater incidence and case fatalities with subsequent substantial health service disruption and burden to a fragile economy.

1.2 Aim of thesis

This thesis aims to understand the epidemiology of dengue in Bhutan. The evidence generated from the research will be crucial for planning intervention strategies for dengue control in Bhutan. It is expected to contribute towards developing an evidence-based model of surveillance and an effective response plan for dengue.

1.3 Objectives

The research objectives of my thesis were to:

1.3.1 Describe the contemporary epidemiology and critically analyse the existing surveillance strategies for responding to dengue.

1.3.2 Identify the clinical predictors of severe dengue.

1.3.3 Investigate the spatial and temporal pattern of dengue, and quantify the factors driving the transmission.

1.3.4 Describe the spatiotemporal characteristics of the epidemic and estimate the transmissibility of the epidemic.

1.3.5 Visualise the dengue vulnerability of different areas for prioritizing public health resources.

1.3.6 Characterise dengue-related knowledge and clinical management practices among medical practitioners (MP).

The above research questions are addressed as published research articles in the respective thesis chapters as outlined below.

1.4 Thesis structure

The thesis is organised into seven chapters including this introduction (**Figure 1**).

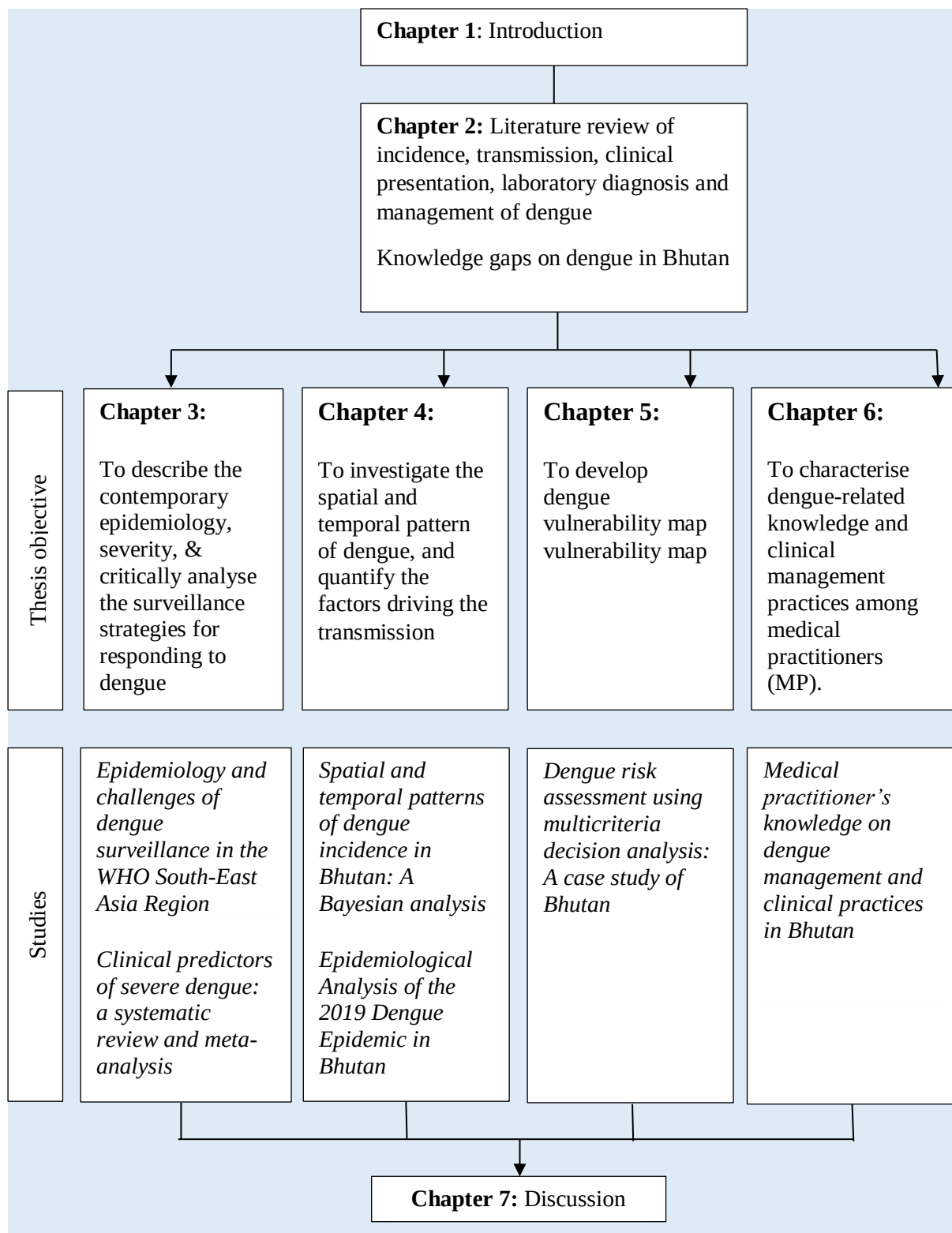


Figure 1. Thesis structure

Chapter 1 is an introduction to the thesis. It briefly outlines the research motivation, aim, objectives, and structure of the thesis.

Chapter 2 provides an updated literature review on the epidemiology and clinical characteristics of dengue. It also highlights the gaps and the need to better understand the epidemiology of dengue in Bhutan, which warrants further research.

Chapter 3 embodies a published study on the epidemiology, severity, and surveillance challenges of dengue in Bhutan and other countries in the WHO South-East Asia Region (WHO-SEAR). Using a narrative review, I describe the circulating DENV serotypes/genotypes and how the region remains at continued risk for dengue. Strength and weaknesses of the existing surveillance strategies are described with some practical recommendations. Pooling together 143 studies enabled me to conduct a meta-analysis and identify demographic and clinical predictors of severe dengue. The pooled odds ratios with 95% confidence intervals were estimated using the inverse variance heterogeneity (IVhet) model.

Chapter 4 describes the spatial and temporal pattern of dengue in Bhutan using surveillance data reported by health centres across the country. A multivariable zero-inflated Poisson model using Bayesian framework was used to quantify the climatic and environmental drivers of dengue and identify the residual clustering. Next, I presented the spatial and temporal progression of the largest epidemic ever recorded in Bhutan since dengue was first reported in 2004. I used a geographical information system (GIS) to present the geographical expansion of dengue and reproduction number to estimate the intensity of the epidemic.

Chapter 5 shows the vulnerability map of dengue in Bhutan. I applied a Multicriteria Decision Analysis (MCDA) model to determine areas that are vulnerable to dengue. I used weighted linear combination (WLC) to combine different risk factors including temperature, rainfall, population density, land use, distance from road network and open water bodies. Vulnerability was assessed at fine spatial resolution using a vulnerability index generated by combining all these factors.

Chapter 6 describes the critical gaps of medical practitioners across the country on knowledge and management practices of dengue. Participants were contacted via email addresses to access and respond to the survey questionnaire.

Chapter 7 discusses the key findings of the thesis. It also discusses the limitations of the studies, recommends future research ideas and provides a conclusion and recommendations arising from the thesis.

Literature review

2.1 Transmission, treatment and control of dengue

2.1.1 Dengue virus

DENV is a single-stranded ribonucleic acid (RNA) virus comprising four genetically and antigenically distinct serotypes (DENV1-4) (9). DENV is a member of genus *Flavivirus*, and the family *Flaviviridae*, which consists of approximately 70 viruses (10). The mature DENV is a spherical enveloped virus of approximately 50 nm in diameter. The virus has three structural proteins namely, E (Envelope), prM (precursor Membrane) / M (Membrane) and C (Capsid) (11). M and E proteins are anchored in the viral envelope via transmembrane domains. The nucleocapsid core of the virus is formed by an array of C protein subunits complexed with a positive sensed single-stranded RNA genome (11). In addition to structural proteins, the RNA genome encodes seven non-structural proteins, namely NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The structural protein forms the viral particle while non-structural proteins facilitate in the replication and invasion of the immune system (12). NS1 is the only protein that is continuously secreted by the infected host cells and is a marker of the severity of the disease (13).

Each of the DENV serotypes has distinct genotypes or lineages based on genetic variability. The current phylogenies based on complete DENV E gene confirms the presence of five genotypes in DENV-1 (genotype I, II, III, IV, and V), five genotypes in DENV-2 (Asian genotype, the cosmopolitan genotype, the American genotype, the Southeast Asian/American genotype, and the sylvatic genotype), four genotypes in DENV-3 (genotype I, II, III, and IV), and four genotypes in DENV-4 (genotype I, II, III, and IV) (14).

Hyperendemicity (co-circulation of multiple DENVs in a community) is a major risk factor for severe dengue (15, 16). It is proposed that a combination of an escalated movement of people between different countries/regions, changes in the genetic make-up of the circulating or newly introduced DENVs and the level of herd immunity to DENV serotypes, are responsible for the emergence of hyperendemicity (16). Infection with one DENV serotype confers long-term

immunity to that particular serotype (homotypic or homologous re-infection) and short-term cross-protective immunity (transient protection) against other viruses (17).

2.1.2 Dengue vector

Aedes (Ae) aegypti and *Ae. albopictus* mosquitoes are the two main vectors of DENV transmission (**Figure 2**). They breed in artificial and natural habitats such as tree holes, bamboo stumps, discarded tyres and flower bases (18, 19). However, *Ae. aegypti* predominantly rests on clothing and surfaces inside homes, where female mosquitoes frequently and almost exclusively bite human hosts (20). In addition, *Ae. aegypti* is an efficient epidemic vector and a highly anthropophilic, domesticated species (17). *Ae. aegypti* has spread to the Americas through the slave trade from Africa and then subsequently to the tropical and sub-tropical regions of the world (21). Due to climate change, some existing unfavourable areas such as inland Australia, the Arabian Peninsula, southern Iran and some parts of North America may become climatically favourable for *Aedes* mosquitoes (22).

The Asian tiger mosquito, *Ae. albopictus* is native to Oriental region, and has spread to Europe, America and Africa during the last 30–40 years (23). *Ae. albopictus* is considered to be a less effective transmitter of dengue, resulting partly from differences in host preferences (prefers vegetation and outdoor areas) and reduced vector competence (24).

While the flight range of *Ae. aegypti* and *Ae. albopictus* varies from 50–500 m (25), a century of rapid human population growth and international trade, with habitats such as tyres and potted plants, has enabled their spread (26). Transportation of containers containing mosquito eggs and larvae are the main drivers of such a wide geographic expansion. The desiccation-resistant eggs in the containers are hatched when they come into contact with rain or water, while the larvae are transported in vessels such as tyres, on ships. The population is established in a new area if the animal host is encountered and the climatic condition is suitable (25).

Both species feed during the daytime, from morning until dusk, although night-time biting is sometimes observed in *Ae. albopictus* (25). *Aedes* mosquitoes usually feed on multiple humans to complete their gonotrophic cycle (often as a result of interruption during feeding), resulting in multiple, clustered cases of dengue (27). Some people are particularly attractive or vulnerable to mosquito bites and can be fed on by more than one mosquito (28).

DENV gets into the mosquitoes from a viremic human host during infectious blood meals. DENV then invades the midgut, replicates and eventually disseminates throughout the

mosquito's body. Once the virus reaches the salivary gland, the mosquito becomes infectious. This extrinsic incubation period usually takes between 5–33 days at 25°C (average of 15 days) and 2–15 days at 30°C (average of 6.5 days) (29). Infected mosquitoes may pass the virus to next generation of mosquitoes through transovarian transmission but occur less frequently (30). Once infected, mosquitoes remain infectious for the rest of their lives (18).



Figure 2: *Aedes aegypti* *Aedes albopictus*

(Courtesy: Leonard E. Munstermann)

2.1.3 Clinical presentations

Once the DENV enters human host, it replicates in a wide range of cells including liver, spleen, lymph nodes, kidney and other organs prior to the development of viremia (12, 31). Most notably, the virus has been shown to infect monocytes, macrophages and dendritic cells (12). The clinical manifestation of DENV infection may be asymptomatic or lead to a wide range of clinical manifestations from classical dengue fever (with or without warning signs) to severe dengue (32). After an intrinsic incubation period of 4–10 days (average of 5.9 days), the illness begins abruptly and is followed by three phases: febrile, critical and recovery (29) (**Figure 3**).

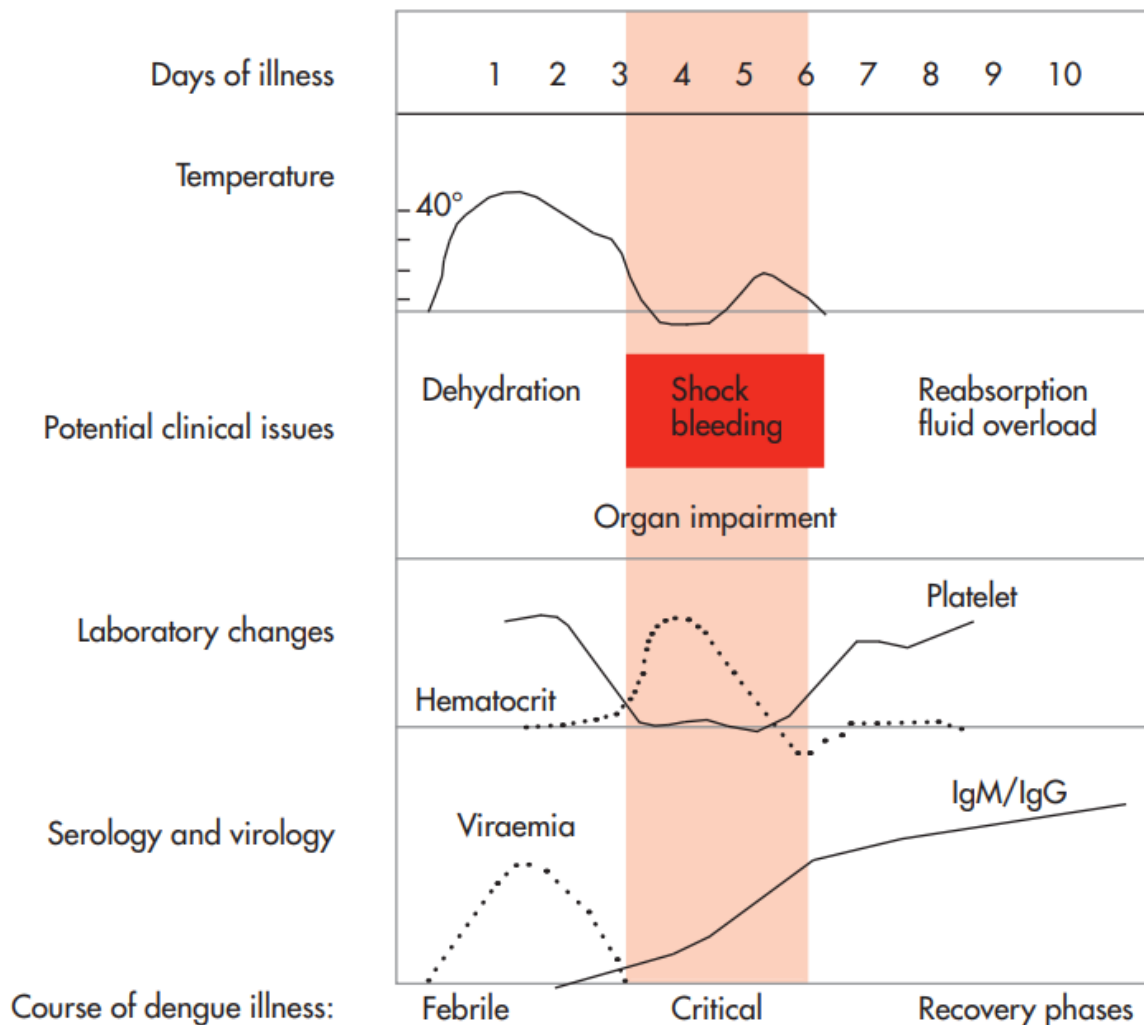


Figure 3: The course of dengue illness (33).

In the febrile phase, patients develop a high-grade fever that usually lasts for up to 2–7 days, and is often accompanied by facial flushing, skin erythema, frontal headache, retro-orbital pain, myalgia and arthralgia, gastrointestinal symptoms (anorexia, vomiting, nausea, diarrhoea) (32). A positive tourniquet test during this phase distinguish dengue from other febrile illness presenting with similar symptoms (34).

The majority of the patients make full recovery after the febrile period without entering the critical phase (34). However, around the time of defervescence, when the temperature drops, some patients may experience an increase in capillary permeability and a simultaneous relative increase in the haematocrit levels. This marks the beginning of the critical phase, which lasts for 24–48 hours. Depending on the degree of plasma leakage, patients may manifest ascites, pleural effusion, epistaxis, gingivorrhagias, metrorrhagia or hypermenorrhea (35). Warning

signs such as abdominal pain, vomiting, lethargy, increase in platelet count with concurrent decrease in platelet count are almost always present at this stage (33). Shock ensues when the critical volume of plasma is lost through such leakage. The hypoperfusion of organs due to prolonged shock results in progressive organ impairment, metabolic acidosis and disseminated intravascular coagulation (32).

If patients survive the 24–48 hours of the critical phase, they move to the recovery phase, during which there is gradual reabsorption of leaked fluid from the extravascular compartment such as the abdomen and subcutaneous space into intravascular compartment. This phase typically takes up to 48–72 hours. During this phase, patients restore their general well-being, improve their appetite, stabilise haemodynamics or blood count, recover from gastrointestinal illness and ensue diuresis (32).

2.1.4 Laboratory diagnosis

Viremia is detectable for 4–5 days after disease onset, which closely correlates to the duration of fever (36). During this early phase, viral nucleic acid and viral antigen (like NS1) can be detected in serum. In primary infection, IgM antibodies develop after 3–5 days of onset and reach a peak approximately 3 weeks later (1). This antibody persists for about 60 days (37, 38). During secondary infection, antibody titres rise quickly and IgM may be detected within 2–3 days and IgG within 4–5 days of disease onset (37).

2.1.4.1 Virus culture

The virus can be isolated during the viremic phase (within 5 days of illness onset) from serum, plasma or mononuclear cells in peripheral blood (39). The virus culture using *Ae. Albopictus* C6/36 mosquito cell line is widely practiced, although mosquito inoculation is the most sensitive method (36). The other cell line includes the use of either mosquito (AP-61, Tra-284, AP-64, CLA-1 cells) or mammalian cells (LLCMK2, Vero, and BHK-21 cells) (34). Due to anamnestic response associated with the production of cross-reactive antibodies in the early phase of the infection, this method is challenging for patients with secondary infection (38). More importantly, the isolation of the virus takes days to weeks and thus is not practised routinely (34).

2.1.4.2 Reverse-transcription polymerase chain reaction (RT-PCR)

The reverse-transcription polymerase chain reaction (RT-PCR) can be used for viral RNA detection. RT-PCR can detect RNA from the day of onset and is highly sensitive, and specific

(36). However, the performance of such tests requires specialised laboratory facilities and trained professionals, which is a major challenge in resource-limited settings (34).

2.1.4.3 Serology

Several serological tests are used for DENV diagnosis, including hemagglutination inhibition (HI), enzyme-linked immunosorbent assay (ELISA), complement fixation, western blotting, indirect immunofluorescent antibody test and plaque neutralization test. HI, and IgM and IgG-ELISA are the two most commonly used serological tests for routine testing. Diagnosis of DENV by serology is complicated in areas with multiple flavivirus infections such as Zika, yellow fever, and Japanese encephalitis, because of cross-reactivity of antibody response. In order to reduce the false positive result, IgM and IgG should be used in combination in NS1 antigen capture assay (34).

NS1-capture ELISA was developed in 2000 using rabbit polyclonal and monoclonal antibodies as capture and detection antibodies respectively (40). The development of the NS1rapid strip test revolutionised dengue diagnosis with its simple and low-technology assay – with high sensitivity and high specificity (34, 41, 42). This method of diagnosis has become standard for early diagnosis and management of dengue patients. However, in patients with secondary infection, a rapid anamnestic rise in NS1 cross-reacting antibodies is observed during the acute phase of the disease; the antibodies sequester NS1 in immune complexes that cannot be readily detected in the immune assay (34).

2.1.5 Treatment and control

Currently, there are no specific treatments for dengue, and the disease is managed through intravenous (IV) fluid replacement and supportive management (43, 44).

The first dengue vaccine, a live recombinant tetravalent dengue vaccine, is Dengvaxia (CYD-TDV). Developed by Sanofi Pasteur, it was first registered in Mexico in 2015, and is now being licensed in 20 countries, typically for use in persons with age 9–45 years. Two other tetravalent live recombinant vaccines, TV003/005 (Butantan Institute) and TDV (Takeda) are undergoing phase 3 clinical trials (45).

CYD-TDV is recommended for three-dose series on a 0/6/12 months basis. The overall pooled vaccine efficacy against virologically confirmed dengue cases was 65.6%, whilst that against severe dengue was 93.2%. The serotype specific vaccine efficacy was 54.7% (DENV-1), 43.0% (DENV-2), 71.8% (DENV-3) and 76.9% (DENV-4). When analysing for a subset of

the population, who were previously infected with dengue, vaccine efficacy was 81.9%, while it was only 52.5% in the seronegative individuals (17). Although it is difficult to achieve balanced tetravalent protection, the vaccine still has an important role to reduce the incidence of severe dengue and hospital admission, which is the main cause of burden due to dengue (17). WHO recently issued a conditional recommendation for the vaccine to be used only in highly endemic areas with seroprevalence of 70% or higher and not in those areas below 50% in order to maximise the public health benefit of vaccination. Further, WHO also recommends the use of vaccines among seropositive individuals to prevent the risk of getting severe dengue (46). In countries where the vaccination is based on out-of-pocket payment, it also depends on the affordability and purchasing power of the consumer, which is related to their income. Therefore, effective vector control strategies remain the cornerstone for reducing dengue-related morbidity and mortality.

Vector control efforts to remove breeding sites remain the mainstay of dengue control and prevention (1). However, due to poor community compliance and participation in source reduction (47), alternative methods such as insecticides (like organophosphate, organochloride and pyrethroids) (48, 49), mechanical methods (like ovitraps) (50), biological methods (like dragon fly – *Bacillus thuringiensis israelis*) (51) and genetic methods (like bacteria – *Wolbachia*, and fungus – *Beauveria bassiana*) had been adopted (52, 53). Since the single method of vector control does not yield an intended efficacy, an integrated approach with different control methods should be adopted. So, WHO recommended integrated vector management (IVM) of vector control, which integrates source reduction, chemical, biological and genetic methods as a sustainable approach of vector control (2). None of these approaches used in isolation is expected to control *Aedes* mosquitoes.

A systematic disease and entomologic surveillance are needed to measure the epidemiological effect of disease transmission, and facilitate the planning and allocation of resources (17). Every dengue-endemic country has a surveillance system, with reporting generally based on clinical diagnosis combined with laboratory tests, and epidemiological information (32). Effectively managed surveillance systems can be used to generate early warning signals for impending disease outbreaks, which is essential for the prevention and control of the disease (2). However, there are inconsistencies in the reporting standards between countries, and the WHO case definition of dengue is not uniformly adhered to while reporting the cases. Consequently, the surveillance system suffers from either underreporting or overreporting (2). Entomological surveillance runs parallel with disease surveillance to understand the spatial and

temporal distribution of vectors, prioritise areas and seasons for vector control, and monitor and evaluate control programs. The most common method of vector surveillance is estimating through larval indices (54), although adult mosquito surveys and oviposition traps have also been used in some settings (55). The selection of appropriate methods depends on a number of factors, such as surveillance objectives, the availability of resources, and vector abundance.

2.2 Global dengue situation

The first record of dengue-like illness can be traced back to the Chin Dynasty (265–425 AD) in China, where it was referred to as a “water poison” connected to flying insects associated with the water (56). Although epidemics of clinically recognizable dengue were documented in 1779–1780 in Africa, Asia and the Caribbean (56), DENV-1 was first isolated in Japan in 1943 and DENV-2 in 1945 in Hawaii (57). DENV-3 and DENV-4 serotypes were identified from cases of severe dengue in the Philippines and Thailand in 1954 (58).

An estimated 2.5–3.97 billion people living in the tropical and sub-tropical regions of the world are at risk of dengue (59, 60). With the current population growth and climatic conditions, WHO estimates 4.46 billion people will be at risk of dengue in 2050 (61). In 2010, it was estimated that there were 390 million dengue infections worldwide (95% credible interval [CI]: 284–528 million), of which 96 million (CI: 67–136 million) manifested clinically with varying degrees of severity (62). The number of deaths also increased from 12,300 in 2005 to 18,400 in 2015 (63).

Dengue imposes a high economic burden to the health sector and overall economy due to increased healthcare cost, school or work absenteeism and loss of productivity (64, 65). In the Americas, dengue illness costs US\$2.1 billion per year on average, excluding the cost of vector control (66). The cost of illness in the South-East Asian Region (SEAR) is \$950 million (67). Indirect costs such as patient productivity loss, substitute labour costs and care-givers cost accounts for the highest proportion of the patient expenditure as compared to the direct medical and non-medical costs (68).

In the SEAR, it is the leading cause of hospitalization and death among children (69). It is also the leading cause of fever among travellers returning from SEAR and the next leading cause of hospitalization after malaria for travellers returning from the tropics (70). While the hyperendemicity is classically seen in the SEAR, the 21st century also witnessed the endemic transmission of DENVs with all four DENV serotypes currently circulating throughout the Americas, and in some countries simultaneously (71, 72).

2.3 Situation and control strategies of dengue in Bhutan

Bhutan is situated in the Eastern Himalayas between People's Republic of China in the North and India in the South, West and East (**Figure 4**). Although Bhutan is a small country with a total area of 38,394 square kilometres, there is a wide variation in the distribution of the climatic zones. Bhutan is divided into six agro-ecological zones based on the elevations and climatic conditions. Himalayans foothills along the southern belt comprise of wet subtropical (100–600 masl), dry subtropical (1200–1800 masl) and humid subtropical (600–1200 masl) zones. This zone is characterised by high humidity and heavy rainfall with temperatures ranging from 15°C to 30°C all year round. The central valleys comprise of warm temperate (1800–2600 masl) and cool temperate (2600–3600 masl) zones. The temperature can range from -4°C to 15°C during winter and 15°C to 26°C during summers, with modest rainfall. The Alpine zone (3600–7500 masl) constitutes snow-capped peaks with very cold winters and cool summers (73). These heterogeneous climatic zones make some places vulnerable to dengue.

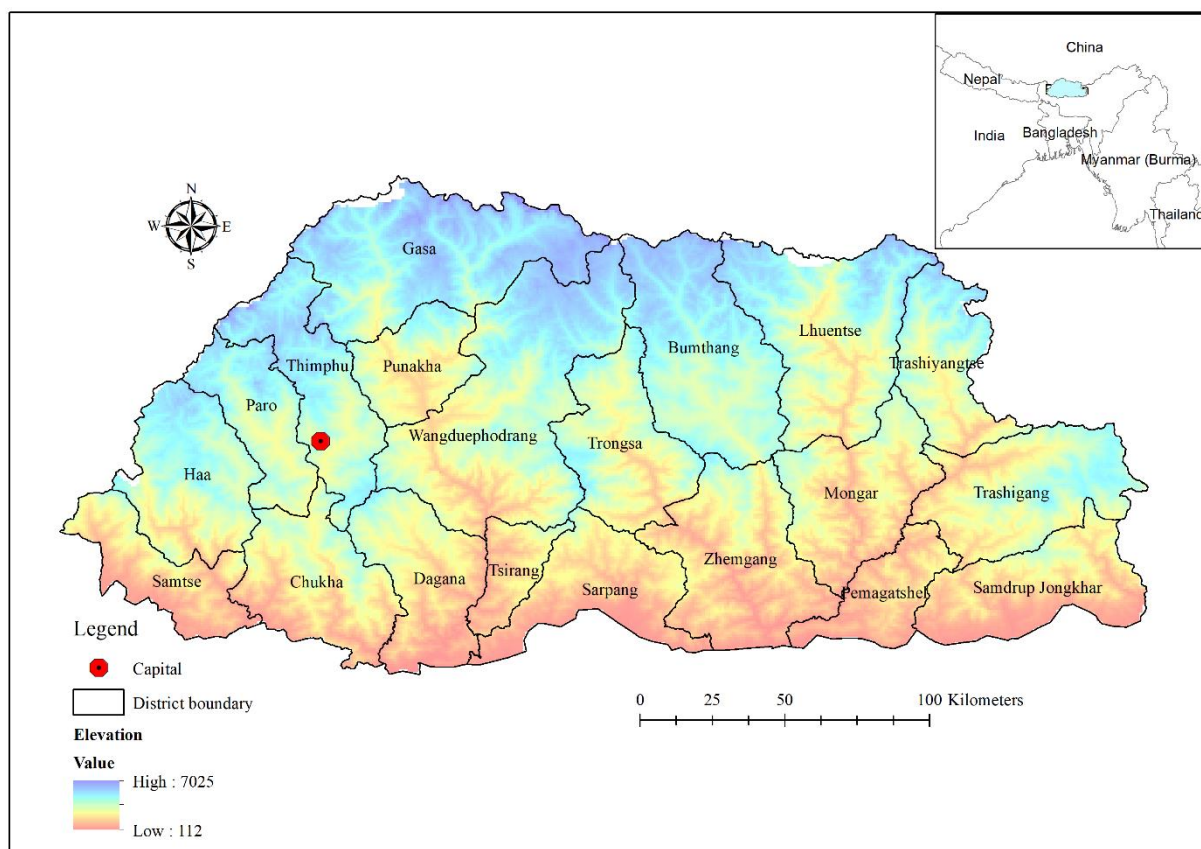


Figure 4. Map showing the location of Bhutan (top right) and 20 districts.

Dengue outbreak in Bhutan was first reported in 2004 from Phuntsholing municipality in Chukha District, with more than 2,500 cases (74). The detection of laboratory-confirmed primary dengue infections, as well as the occurrence of older patients (mean age of 31 years)

supports the recent emergence of dengue in Bhutan at that time. The burgeoning developmental activities, globalisation, increased trade, and travel potentially increased the risk of dengue in Bhutan. The outbreak was caused by DENV-2, possibly associated with the introduction of the Indian sub-continent strain. DENV-3 was identified in 2005, while both DENV-2 and DENV-3 were identified in 2006. DENV-1 was identified in 2013–2014, and again in 2016–2017 (75). For more than a decade, dengue was confined to Phuntsholing only; none of the other places ever reported dengue during this period. However, from 2013 onwards, the neighbouring areas sharing similar climate and environmental conditions also reported dengue. Between 2013 and 2018, seven more districts reported either sporadic cases or clusters of dengue. The worst ever dengue epidemic caused by DENV-3 was recorded in 2019 which resulted in 5,935 cases and six deaths. Cumulative cases of 11,159 were reported in the country from 2004–2019 (**Figure 5**).

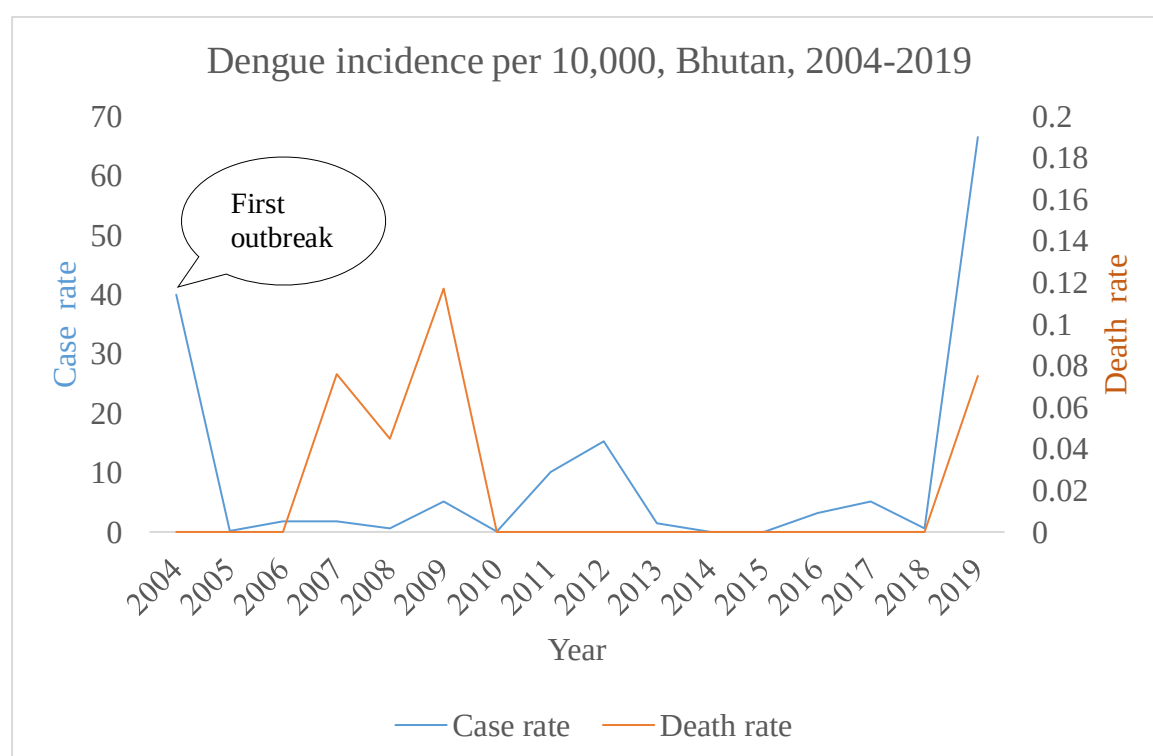


Figure 5. Progression of dengue in Bhutan, from the beginning of first outbreak detection until date (2019) (Source: Royal Centre for Disease Control, Annual Health Bulletin and the WHO).

The national strategy for dengue control is based on early case detection and management, reduction of mosquito breeding sites, insecticide spraying and surveillance. Dengue was first integrated with malaria and other vector-borne diseases in the Vector-borne Disease Control Program (VDCP) in 2003, having been known as the National Malaria Control Program

(NMCP) when launched in 1964. During the malaria eradication period from 1964–1971, VDCP centred its focus on vector control using indoor residual spraying (IRS) with dichlorodiphenyltrichloroethane (DDT). In 1995, DDT was replaced with the synthetic pyrethroid, deltamethrin, as a result of global concern over its environmental and safety issues, in addition to the development of insecticide resistance. In line with the Global Malaria Control Strategy, IRS was replaced with Insecticide Treated Bed Net (ITBN) in 1998. The use of ITBNs continued until it was replaced by Long-Lasting Insecticide Nets (LLINs) in 2006 along with IRS. The coverage and utilisation of LLIN and IRS in the country remained above 90% (76). These programmatic activities are all targeted towards malaria control which also substantially benefitted in the control of dengue.

VDCP supports, coordinates and evaluates vector control activities in district hospitals and basic health units (BHUs) or primary health care units (PHCs). Dedicated technicians / inspectors / insect collectors are placed in entomological units in all health centres in the southern districts or at sentinel sites to carry out regular inspection of breeding sites, vector surveys and control activities including health promotion and application of insecticides. The entomological unit also conducts periodic larval surveys and provides information to the Ministry of Health for necessary actions. However, these officials also carry out other activities besides dengue vector control, including control of other vector-borne diseases.

As the national surveillance and response system for priority diseases of public health concern, the National Early Warning Alert and Response Surveillance (NEWARS) was introduced in 2014 at Royal Centre for Disease Control (RCDC) (77). The system aims to detect cases early and provide efficient response leading to timely control of acute public health events and nationally important notifiable diseases. As per the NEWARS, mild dengue fever is a weekly notifiable disease, while severe dengue is an immediately notifiable disease. Acute public health events or outbreaks due to dengue are captured through an event-based platform in NEWARS. The reporting is done either through an online web-based system or a mobile-based short message service (SMS) to report cases from remote places with limited internet access. All health centres across the country are required to report the case/s and outbreaks to NEWARS, including zero cases reporting.

2.4 Knowledge gaps on the epidemiology of dengue in Bhutan

There has been limited research on the epidemiology of dengue in Bhutan. At the time of the commencement of this thesis, only two studies were published on dengue in Bhutan. Both of

these studies were on the molecular characterization of DENV, and aimed to study the circulating DENV serotypes and their phylogenies to establish the source of the infection (8, 74). A detailed understanding of the vulnerability and determinants of transmission of dengue is an invaluable asset to help design control strategies tailored to the country's need and limited public health resources.

Responding to dengue involves developing a comprehensive response plan – which should include both epidemiological (disease) and entomological surveillance – and implementing these plans. To trigger response action, surveillance needs to be sensitive enough to predict or detect outbreaks (78). The surveillance process starts with the notification, which then triggers a series of prevention and control measures (35). However, notification of cases is strongly influenced by the acceptability of the person in charge of reporting. Hence under-reporting is a huge problem in disease surveillance (79).

Entomological surveillance is essential for quantifying the magnitude and geographical distribution of mosquito populations to predict outbreaks and determine the appropriate control measures (32). Vector density should be maintained at a level that does not support the sustenance of DENV transmission. However, the use of vector indices for outbreak alerts varies across countries. For example, Breteau Index (BI) is commonly used in Sri Lanka, House Index (HI) in Peru, and HI and BI in Indonesia and Vietnam (80). Further, it is recommended to use adult mosquitoes instead of immature mosquitoes (larvae) to estimate the level of risk as the adults are directly involved in the transmission (81). Understanding the process of surveillance will be essential to improving the data quality and providing an efficient response. However, no reviews have been conducted so far in Bhutan to understand the effectiveness of the dengue surveillance, and whether its objectives were met or not.

Like other vector-borne diseases, dengue typically shows spatial and temporal variation driven by human, environmental and climatic factors (82). Dengue is more likely to cluster spatially in certain geographical locations due to the suitability of temperature, rainfall, humidity, vegetation type and human behaviours (83, 84). Quantifying the effect of individual climatic variables on the transmission of dengue and identifying spatial residual clusters will be essential for the development of a climate-based early warning system and the efficient allocation of limited resources (85). Focused interventions in specific areas would have a greater impact than the uniform allocation of resources (86).

Dengue incidence follows a specific seasonal pattern. For example, in Sri Lanka, the first peak of dengue occurs in June/July coinciding with the south-western monsoon that commences in late April. Then a second peak occurs at the end of the year, associated with the north-eastern monsoon that runs from October to December (87). In Timor-Leste, December to February are the wettest and hottest months of the year and are associated with high dengue incidence (88). Establishing a similar temporal trend in Bhutan will improve the accuracy of interventions with greater impact.

Bhutan experienced its worst dengue epidemic in 2019, with cases greater than the combined numbers reported in all the past years. This epidemic provides a unique opportunity to analyse the transmissibility and spatio-temporal patterns of dengue in real life situations where dengue control activities are implemented simultaneously during outbreaks. Through such analysis, we can understand the robustness of the preparedness plan and ascertain the effect of control activities. It is important to find the cause of the problem after the outbreak and record or document the lessons learnt to improve future dengue control strategies (89).

With the outburst of a nationwide dengue epidemic in 2019, there were a lot of uncertainties concerning the escalated risk. To respond to similar acute public health events and help direct the right interventions in the future, developing the vulnerability map to understand the risk of dengue at fine-scale resolution is of paramount importance. It is crucial to identify the priority regions and populations vulnerable to dengue so as to make informed policy decisions. Vulnerability assessment offers a novel way of conceptualising a complex web of factors and interactions that mediates the transmission of disease (90).

To develop a successful control strategy, understanding the knowledge on the management of dengue is crucial. Studies have recommended the need for training clinicians due to poor knowledge and skills associated with both clinical diagnosis and treatment of dengue patients (91). Having the right knowledge and ability to identify risk factors of severe dengue can significantly reduce the case fatalities (32). Hence, understanding the local characteristics of dengue management practices is essential to develop appropriate strategies to improve population health and save lives.

Epidemiology, severity, and surveillance of dengue in Bhutan

3.1 Introduction

Bhutan and other countries in the WHO-SEAR bear a high burden of dengue. The incidence of dengue has expanded geographically, and there are frequent outbreak events. Approaches for control need to be aligned with the current knowledge of the epidemiology of dengue and the challenges associated with dengue surveillance. In some countries, dengue is the main cause of morbidity and hospitalisation among children. Rapid identification of severe cases and appropriate clinical management is crucial to manage dengue effectively. Early detection of severe cases via identification of clinical warning signs including demographic characteristics and pre-existing co-morbidities will be useful to target at-risk groups for initiating prompt interventions. It has been shown that timely detection and correct management of severe dengue can significantly reduce the case fatality to less than 1%. This chapter is comprised of two studies.

In the first study, the epidemiological pattern on the distribution of dengue virus serotypes and genotypes in each country in the WHO-SEAR were described. The emergence or re-emergence of DENV serotypes or genotypes were more commonly associated with outbreaks or epidemics with high intensity of transmission. Efforts were made to provide the most recent updates on the incidence and case fatalities of dengue and described how it evolved over time. An argument was made to have a robust surveillance and monitoring system for rapid detection and characterization of the DENV in order to initiate timely and effective control measures. The implementation of immature mosquito (ovitrap, larval or pupal) sampling was highlighted as the major weakness of entomological surveillance as it does not directly relate to the risk of dengue transmission. A combination of methods (in particular using adult traps and larval indices together) should be routinely performed to better inform dengue control. The main challenge in dengue disease surveillance in Bhutan, and other countries in the region, is passive surveillance. Bhutan replaced paper-based reporting with a real-time web-based online platform in 2015, but under-reporting is a major impediment to the successful implementation of the surveillance system. Given that the huge number of cases were under-reported in the

passive surveillance, multi-faceted approaches were suggested including the engagement of communities using the available modern technologies such as mobile apps to report case/s and public health events of dengue. Such an approach will help detect cases in real-time and therefore, interventions can be implemented on time.

In the second study, a systematic review and meta-analysis was conducted using 143 eligible studies to identify demographic, pre-existing co-morbidities and clinical warning signs of severe dengue. The analysis was conducted using IVhet model to pool the effect sizes. Sub-group analysis was conducted according to WHO 1997 and 2009 dengue case classification. Sensitivity analysis was also performed to assess the robustness of the results. The main outcome of the study was that children, secondary infection and pre-existing conditions with diabetes and renal disease were associated with the development of severe dengue. Nearly all WHO-identified clinical warning signs were associated with severe dengue. The findings will be useful for clinicians to use those risk factors for management and timely interventions.

3.2 Article on epidemiology and disease surveillance

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Epidemiology and challenges of dengue surveillance in the WHO South-East Asia Region

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Dengue poses a significant health and economic burden in the WHO South-East Asia Region. Approaches for control need to be aligned with current knowledge on the epidemiology of dengue in the region. Such knowledge will ensure improved targeting of interventions to reduce dengue incidence and its socioeconomic impact. This review was undertaken to describe the contemporary epidemiology of dengue and critically analyse the existing surveillance strategies in the region. Over recent decades, dengue incidence has continued to increase with geographical expansion. The region has now become hyper-endemic for multiple dengue virus serotypes/genotypes. Every epidemic cycle was associated with a change of predominant serotype/genotype and this was often associated with severe disease with intense transmission. Classical larval indices are widely used in vector surveillance and adult mosquito samplings are not implemented as a part of routine surveillance. Further, there is a lack of integration of entomological and disease surveillance systems, often leading to inaction or delays in dengue prevention and control. Disease surveillance does not capture all cases, resulting in under-reporting, and has thus failed to adequately represent the true burden of disease in the region. Possible solutions include incorporating adult mosquito sampling into routine vector surveillance, the establishment of laboratory-based sentinel surveillance, integrated vector and dengue disease surveillance and climate-based early warning systems using available technologies like mobile apps.

Keywords: dengue emergence, dengue virus, early detection, epidemiology, surveillance, WHO-SEAR

Introduction

Dengue is an arthropod-borne disease caused by one or more of the four dengue viruses (DENV1–4), which belong to the genus *Flavivirus* and family *Flaviviridae*.¹ Infection with one DENV serotype confers long-term immunity to that particular serotype and short-term partial immunity to other serotypes.² Secondary infection with a different serotype (secondary heterotypic infection) can result in a severe form of the disease known as severe dengue (previously known as dengue haemorrhage fever [DHF] or dengue shock syndrome).³ In infants, even primary infection can lead to the severe form of the disease if the mother is immune to dengue.⁴ Dengue has repeatedly caused severe outbreaks, including in areas that were not previously affected, despite intensive surveillance and control.⁵ The disease constitutes a significant social and economic burden, with considerable disruption to health service delivery during epidemics.⁶

Aedes aegypti and *Aedes albopictus* are the two main mosquito vectors of dengue transmission. These vectors can

occur in both urban and rural areas, however, *Ae. aegypti* is predominantly found in urban areas while *Ae. albopictus* is found in more remote and forested areas.^{7,8} Both species breed in artificial and natural habitats such as tree holes, bamboo stumps, discarded tyres and flower pots. However, *Ae. aegypti* are commonly found around human dwellings, while *Ae. albopictus* prefer vegetation and outdoor areas.⁹ *Ae. aegypti* rest on clothing and surfaces inside homes where female mosquitoes frequently and almost exclusively bite human hosts.¹⁰ Both species feed during the daytime from morning until dusk, although nighttime biting is sometimes observed for *Ae. albopictus*.⁹ *Aedes* mosquitoes usually feed on multiple humans to complete their gonotrophic cycle (often as a result of interruption during feeding), resulting in multiple, clustered cases of dengue.¹¹ Once infected, mosquitoes remain infectious for the rest of their lives.¹² Besides mosquitoes, dengue infection is also transmitted vertically during pregnancy and breastfeeding,^{13,14} via blood transfusions^{15,16} and organ transplantation,¹⁷ as well as nosocomially through needle stick injuries.¹⁸

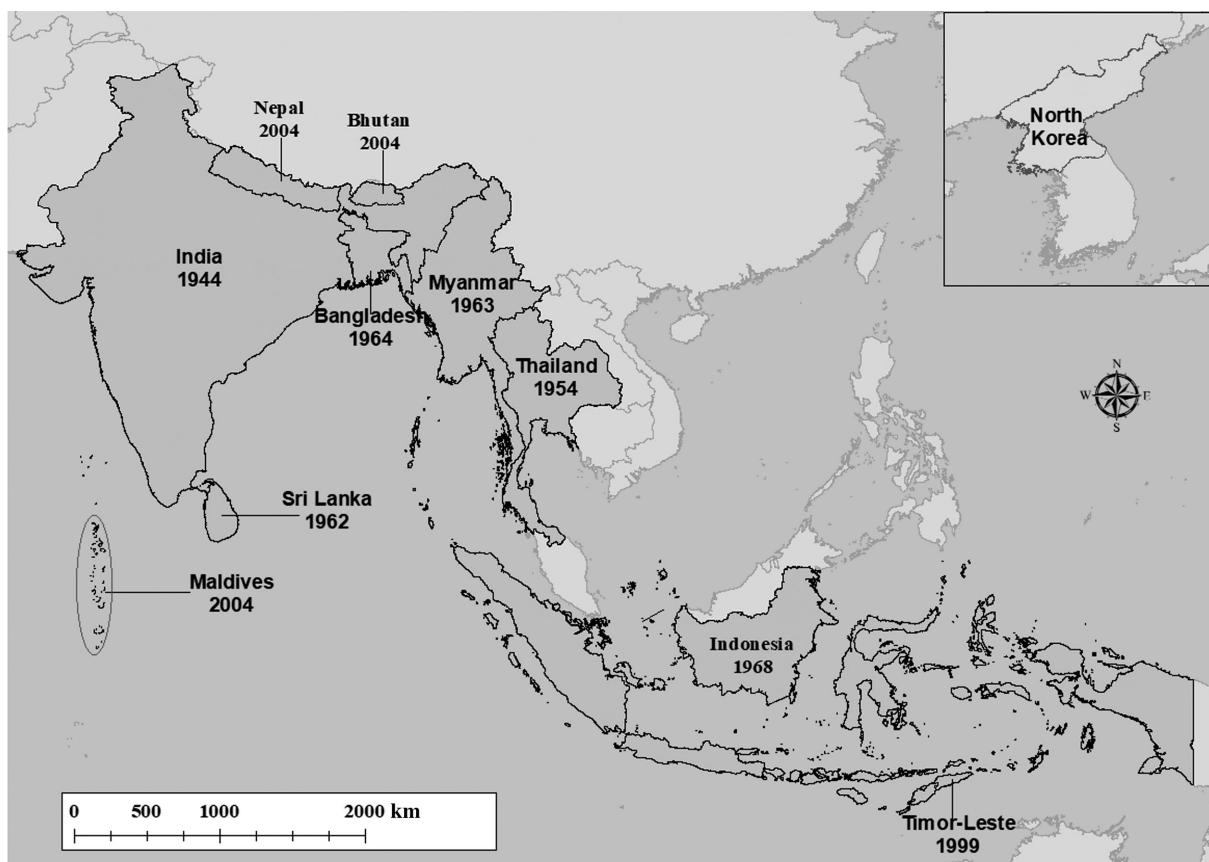


Figure 1. Member countries of the WHO South-East Asia Region. The shaded areas show endemicity of dengue and the year shows the first report of laboratory-confirmed dengue in each country.

Dengue fever may be asymptomatic or result in a wide range of clinical manifestations such as fever, headache, rash, myalgia, arthralgia/bone pain, retro-orbital pain and nausea/vomiting, with or without warning signs, after an incubation period of 4–10 d.¹⁹ Progression to severe dengue is marked by plasma leakage (that results in pleural effusion and ascites), respiratory distress, severe bleeding or organ impairment.^{2,19} Atypical clinical manifestations such as haemophagocytic syndrome, lymphadenopathy, splenomegaly and concomitant presentation with acute pancreatitis, acute cholecystitis and pancytopenia, have also been reported.^{20,21} Dengue in pregnant women is associated with miscarriage, preterm birth, low birthweight and maternal mortality.^{22,23}

The WHO South-East Asia Region (SEAR) has 10 dengue-endemic countries (Figure 1), where an estimated 1.3 billion at-risk people live, accounting for approximately 52% of the global population at risk of dengue.²⁴ Other than North Korea, countries in the region bear a high burden of dengue/severe dengue and experience frequent and cyclical epidemics.²⁵ It is evident that there has been an overall expansion of dengue in the region in recent decades,²⁶ favoured by tropical and subtropical climates in addition to human factors.²⁷ Frequent migration of people using modern transportation facilities has increased the potential risk of both dengue vector and virus dissemination to new locations.²⁸ Unplanned urbanisation and unprecedented movement of

people from rural areas have congested the cities²⁹ and a majority of these migrants live with poor water and sanitation provision and lack access to essential healthcare services.³⁰ Rubbish collecting in overcrowded cities provides breeding sites for dengue vectors. Rapid industrial and economic growth have led to deforestation and landscape fragmentation, which has increased exposure to mosquito bites and dengue transmission.³¹ Recently, dengue transmission has expanded from southern lowland areas to high elevation areas,^{32,33} largely as a result of a warming climate and high-altitude areas becoming more suitable for dengue vector habitation.³⁴

The first dengue vaccine, chimeric yellow fever virus–DENV tetravalent dengue vaccine (CYD-TDV) (Dengvaxia), developed by Sanofi Pasteur France, is licensed and currently only commercially available in Thailand and Indonesia within the SEAR.³⁵ Vaccine implementation is challenging, requiring thorough baseline information on burden and seroprevalence profiles of local dengue virus populations. Due to vaccine-induced severe dengue and deaths, the Philippines suspended the immunisation campaign within 2 y of commencement.³⁶ Therefore, dengue surveillance for early detection of cases and vector control remain the cornerstones for controlling dengue. It is thus essential to properly understand the epidemiology of dengue and existing surveillance strategies to develop effective dengue prevention and control programmes.³⁷ Improved prevention and control

could be achieved through the use of innovative entomological and epidemiological tools, which could enhance the use of available resources to provide early warnings of disease outbreaks in time and place. Vector surveillance in the region is routinely conducted using immature mosquitoes rather than adult mosquitoes.³⁸ However, there is little evidence of the quantifiable relationship between immature mosquitoes and dengue transmission.³⁹ Adult mosquito sampling has a better correlation with dengue risk, and should be considered for routine implementation along with vector indices to understand the transmission dynamics of the disease and to help select appropriate vector control strategies. Similarly, dengue disease surveillance is a critical component of disease prevention and control as it provides necessary information for risk assessment, epidemic preparedness and monitoring of the spread of dengue over space and time.¹⁹ However, there have been concerns about the effectiveness of surveillance due to massive under-reporting and a fragmented laboratory network in the region.^{40,41} A concerted effort involving electronic reporting using modern technologies such as the internet and mobile phones might help to address this gap.

This review was undertaken to describe the contemporary epidemiology of dengue and critically analyse the existing surveillance strategies for responding to dengue in the region. We aimed to describe the pattern of disease incidence and associated dengue viruses circulating in the region. We also examined the current efforts in both vector and disease surveillance and suggest possible solutions to enhance early detection and response to dengue outbreaks. The search strategy is described in Box 1.

Box 1. Review search strategy

The data source of this review comprised papers available in PubMed and Google Scholar until July 2020. We also reviewed grey literature to corroborate our findings. The key search terms used were 'dengue' or 'dengue virus' or 'severe dengue', 'epidemiology', 'dengue vector', 'early warning', 'surveillance' and 'Asia'. The search term 'Asia' was also replaced by specific names of the country in the region to capture all the relevant articles. For vector surveillance, we included papers that reported both mosquitoes and dengue incidence to compare the risk of dengue with different mosquito densities and to understand the most commonly used mosquito sampling method in the region. Each of the publications was screened for relevance using title and abstract. References from the articles were also searched depending on the relevance of the content. We excluded studies involving sero-survey, prediction, modelling, sociodemographic and ecological risk factors.

Epidemiology of dengue

Below, in describing the local epidemiology and surveillance approaches, we have focused on each endemic country in the chronological order that dengue virus was first detected.

India

Dengue virus in India was first isolated in 1944 in Calcutta.⁴² From 1962 to 2005, DENV-1 isolates predominantly belonged to genotype III.⁴³ A large outbreak with 12 000 cases and a case fatality rate (CFR) of 0.5% occurred in Tamil Nadu in 2012 due to the displacement of genotype III by genotype I.⁴⁴ This virus shared similar sequences with strains isolated in Myanmar in 2013 (that led to approximately 20 255 cases and 84 deaths)⁴⁵ and Sri Lanka in 2009 (that led to approximately 35 000 cases and 346 deaths).⁴⁶ The DENV-1 (genotype III) continued to predominate, even during 2014–2015.⁴⁷

The Indian DENV-2 viruses were initially of the American genotype but the Cosmopolitan genotype replaced it around 1974.⁴³ Although occasional cases of severe dengue were seen from 1988, circulation of DENV-2 (Cosmopolitan genotype) led to the largest outbreak of severe dengue in 1996 in Delhi, which resulted in 8900 cases and 374 deaths (giving a death rate of approximately 4.2%).^{48,49} The study indicated that the earlier American genotype had been replaced by the Cosmopolitan genotype due to frequent trade and travel between the Indian subcontinent and Africa, as well as other countries in the SEAR.⁴⁹ This same strain also caused an outbreak in Gwalior in 2001,⁵⁰ as well as outbreaks in Uttar Pradesh, Bihar and other locations during 2009–2012 and 2015–2016.^{51–53}

In Delhi, a shift in the DENV serotype occurred from DENV-2 to DENV-3 during 2003–2005^{54,55} and in 2016.⁵⁶ DENV-3 (genotype III) emerged in 2003 and was associated with a major outbreak of severe disease in northern India.^{54,55} Central India also observed a shift in dominant serotype from DENV-2 in 2013 to DENV-3 in 2016, which led to further expansion of dengue in rural areas.^{57,58} This virus continued to circulate in India by changing its genetic makeup and causing further geographical expansion.⁵⁹ This strain was considered to have spread to Sri Lanka, Latin America and East Africa, causing severe dengue outbreaks.^{60,61}

Without any report of DENV-4 for more than 3 decades,⁶⁰ this serotype re-emerged in Delhi in 2003.⁶² Andhra Pradesh in 2007,⁶³ Kerala in 2008⁶⁴ and Pune in 2009.⁶⁵ DENV-4 (genotype I) caused a severe dengue outbreak in Pune during 2009–2010.⁶⁵ All four DENV serotypes are cocirculating with changes in the dominant serotype leading to coinfection with multiple serotypes.^{66,67} Studies conducted in 2017 revealed DENV-2 and 3 as the predominant serotypes in the southern region and DENV-1 and 2 in the northern region.⁶⁸

Despite intensive dengue control programmes in India, dengue has gradually spread to the whole country, including rural areas.^{57,69} Although the CFR declined from 3.3% in 1996 to 0.2% in 2018, cases increased from 18 860 to 101 192 in 2018⁷⁰ and some areas still continue to report high numbers of fatalities.⁷¹

Thailand

In Thailand, severe dengue-like illness was first recognised in 1954 in a small outbreak among children.⁷² Four years later, a large outbreak of similar illness occurred that resulted in the hospitalisation of about 3000 children and 300 deaths associated with mixed infection of DENV-2 and Chikungunya virus.⁷² Dengue cases have been increasing annually from an average of 69 000 cases per year from 1985 to 1999⁷³ to 91 650

cases per year from 2009 to 2015.⁷⁴ However, mortality showed a decreasing trend.⁷⁵ The highest incidence occurred in 2013 with approximately 154 000 and 156 deaths (241.03 cases per 100 000 population and a CFR of 0.21). Thailand has the next highest economic burden from dengue in the region after Indonesia and India (Supplementary Table S1).^{76,77} Dengue is now reported from all parts of Thailand, including the Bangkok metropolitan area.⁷⁸

DENV-2 was the predominant strain during 1973–1986, DENV-1 and DENV-2 during 2000–2010 and DENV-3 during 2000–2002 and 2008–2010.^{79,80} The most common DENV-1 genotype was genotype I, including the recent strain isolated during 2015–2017.^{81,82} DENV-2 and DENV-4 were the main circulating serotypes during 2015–2017.^{81,83}

Phylogenetic analysis revealed two genotypes for DENV-2: Asian genotype I, a local strain, and a Cosmopolitan genotype, which was rarely found in Thailand in the past.⁸⁴ Asian genotype I was the most frequently isolated strain and has been circulating in Thailand since 1991.⁸⁵ DENV-3 (genotype III) has predominated since 2010 and continues to circulate today.^{81,83} DENV-3 (genotype I) was also detected for the first time during 2015–2016 in southern Thailand after its emergence in the country in 1998.⁸¹ The most recent DENV-4 virus belonged to genotypes I and II.^{81,83} Genotype I has been established in Thailand for decades and has also spread to other countries.⁸⁶ However, genotype II was also detected in southern Thailand during 2015–2016.⁸¹ The shifting of predominant serotypes in Thailand has been associated with cross-protective immunity and other immune alterations in the population,⁸⁷ and clade replacement has been postulated to result in improved viral fitness and increased transmissibility of the virus.⁸⁸

Sri Lanka

Sri Lanka confirmed its first dengue outbreak in 1962 and reported island-wide outbreaks of severe dengue from 1965 to 1968, caused by DENV-1 and 2.⁸⁹ The emergence of new clades in DENV-3 in 1989 and 2002–2004 coincided with an abrupt increase in cases and severe epidemics in Sri Lanka.⁹⁰ DENV-3 isolated before 1989 belonged to the Indian subcontinent genotype IIIA, while those after 1989 belonged to genotype IIIB.⁶¹ Subsequently, there was a serotype shift from DENV-3 to DENV-1 in 2009, which led to 35 008 cases and 346 deaths.⁴⁶ After 8 y of stable reporting, with 30 000–50 000 cases every year since the 2009 epidemic,⁹¹ the largest epidemic in the country, with over 185 688 clinical cases and 250 case fatalities, occurred in 2017. DENV-2 was the cause of the outbreak.^{92,93} Such high levels of morbidity and mortality may have been related to a new clade of the Cosmopolitan genotype of DENV-2, which had not previously been detected.⁹³

Dengue is now believed to be the number one killer among mosquito-borne diseases in Sri Lanka.^{28,94} Although the CFR decreased from 9.90 in 1989 to 0.24 in 2017, dengue incidence has increased dramatically during this period.⁹⁴ Today, dengue has become endemic to the whole island of Sri Lanka, with periodic outbreaks reported from all provinces and districts.^{95,96}

Myanmar

Dengue infection was first confirmed in 1965 in Myanmar by serological testing, although dengue-like illness was first reported in 1963 in Yangon city.⁹⁷ The first large outbreak was reported in Yangon in 1979, with 1654 hospitalisations and 81 deaths. Virological tests indicated both dengue and Chikungunya viruses as the causes of the outbreak.⁹⁸ Although dengue incidence showed an increasing trend, the CFR reduced from 5.5% in 1970 to 0.33% in 2015.⁹⁹ All states and regions have now reported dengue.¹⁰⁰

The highest peak of incidence was reported in 2015, which was predominantly associated with DENV-1 and DENV-2.¹⁰¹ DENV-1 was the dominant serotype from 1998 to 2013 in Myanmar.⁴⁵ In 2001, DENV-1 was associated with a large outbreak resulting in 15 361 severe cases and 192 deaths.¹⁰² The current DENV-1 virus circulating in the country belongs to genotype I, which was isolated in 2017 from cross-border travellers entering China from Myanmar.¹⁰³ Circulating DENV-2 belongs to the Asian I genotype,^{45,103} whereas the Cosmopolitan genotype has not been detected since 2013.⁴⁵ Both DENV-3 and DENV-4 were associated with severe dengue in 2006.¹⁰⁴ The current (2017) DENV-3 was classified as genotypes I and III, and DENV-4 as genotype II, which was implicated as contributing to the long-term epidemic (ongoing) in Myanmar and Thailand.¹⁰³

Bangladesh

In Bangladesh, DENV-3 was isolated in an outbreak of febrile illness, then known as ‘Dacca fever’, in 1964.¹⁰⁵ After this incident, infrequent sporadic cases were reported until a large outbreak of DENV-3 infection occurred during the monsoon season of 2000, resulting in 5275 hospitalised cases (25% with bleeding manifestations) and a CFR of 1.61%.¹⁰⁶ Phylogenetic analysis demonstrated that DENV-3 belonged to genotype II, which closely resembled the strain from Thailand and Myanmar.^{107,108} From 2013 to 2016, DENV-2 was the predominant strain, with coexistence of DENV-1. Neither DENV-3 nor DENV-4 were detected during this period.^{109,110}

From 2000 to 2009, 29 of the 64 Bangladeshi districts reported dengue, with Dhaka consistently reporting the largest number of cases.¹¹¹ Although dengue death rates have reduced from 2011 to 2017, the incidence had grown drastically during this time (Table 1). In 2019, 112 000 cases and 129 deaths were reported, making it the deadliest year since the 2000 epidemic (note, neither serotype nor genotype data were available).¹¹² The transmission of dengue across cities has been primarily associated with poor waste management and inadequate piped water supply, which encourages water storage in temporary containers.¹¹³

Indonesia

Dengue was first recognised in Indonesia in 1968 in an outbreak of severe dengue, which resulted in 58 cases and 24 deaths.¹¹⁴ The country bears the second highest economic burden of dengue in the region after India (Supplementary Table S1). Today, all 34 provinces report dengue, which occurs in >90% of districts.^{31,115} In addition to local endemic transmission, Bali is a location with significant transmission of dengue among international tourists.¹¹⁶ Dengue incidence appears to be cyclical,

Table 1. Country-specific incidence rate (per 100 000 population) of dengue cases and deaths in the WHO South-East Asia Region

Country	2011		2012		2013		2014		2015		2016		2017	
	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death	Case	Death
Nepal	0.289	0.000	0.662	0.000	2.601	0.000	1.278	0.000	0.471	0.003	5.269	0.003	7.204	0.014
Bangladesh	0.885	0.004	0.431	0.001	1.237	0.001	0.235	0.000	2.024	0.004	3.719	0.009	17.768	0.008
India	1.512	0.014	3.976	0.019	5.927	0.015	3.135	0.011	7.467	0.017	8.672	0.017	11.742	0.019
Myanmar	9.372	0.032	12.617	0.051	39.370	0.163	26.589	0.171	83.668	0.307	81.144	0.265	14.482	0.000
Indonesia	26.749	0.243	36.260	0.328	44.642	0.346	39.332	0.356	50.220	0.385	77.481	0.610	15.630	0.116
Timor-Leste	33.141	0.088	80.224	0.951	51.335	0.000	52.935	0.247	0.000	0.000	19.784	0.236	64.568	0.154
Bhutan	96.150	0.000	146.222	0.000	14.511	0.000	0.000	0.000	0.000	0.000	54.011	0.000	53.929	0.000
Thailand	103.361	0.093	117.319	0.128	227.129	0.200	60.048	0.070	211.123	0.205	92.837	0.093	77.045	0.091
Sri Lanka	140.157	0.911	217.679	1.077	155.759	0.879	227.457	0.467	142.941	0.267	262.793	0.457	865.921	0.000
The Maldives	775.462	3.199	280.422	0.259	171.365	0.252	180.773	0.490	451.479	1.434	451.426	0.701	228.726	0.000

Source: WHO-South-East Asian Regional Office.

peaking every 6–8 y. The CFR has decreased by half with each decade since 1980.¹¹⁷

The first well-characterised major outbreak associated with DENV-3 occurred during 1976 and 1977, resulting in 1260 cases and 32 deaths.^{118,119} Since then, several outbreaks occurred, with DENV-3 being the predominant serotype until the start of the 1990s.^{120–122} Subsequently, DENV-1 and DENV-2 were the predominant serotypes throughout the 1990s,^{123–125} however, DENV-3 re-emerged in 2004, resulting in 58 301 cases and 658 deaths.¹²⁶ DENV-4 appeared as the dominant serotype in 2006 in Yogyakarta¹²⁷ and also as the dominant serotype in travellers returning to Australia during 2007 and 2008.¹²⁸ DENV-1 became the predominant serotype from 2008 to 2012.^{129–133} From 2014 onwards, with occasional cases of DENV-1 in some areas,¹³⁴ DENV-2^{135,136} and DENV-3 predominated again.^{137,138}

Phylogenetic analysis demonstrated that genotypes I and IV are the most common strains of DENV-1 currently circulating in the country.^{137,139–141} DENV-2 belonged to the Cosmopolitan genotype, which has continued to circulate since its first detection in 1975,^{137,141} while the Asian II and American genotypes of DENV-2 have not been reported since 1975 and 1977, respectively.¹⁴² Similarly, genotype I of DENV-3 was the most commonly isolated, while genotype II of DENV-3 has not been reported since its first isolation in 1988.^{137,138,141} Genotype II of DENV-4 continues to be one of the most frequently isolated genotypes together with genotype III.^{131,139,141}

Timor-Leste

Although Timor-Leste reported its first outbreak in 2004,¹⁴³ dengue has been implicated as a cause of disease in travellers from Australia since 1999.¹⁴⁴ A major outbreak occurred in Timor-Leste in 2005, which resulted in 1070 cases and 39 deaths in the first 5 mo of the year. This outbreak was predominantly caused by DENV-3 (genotype I), along with DENV-2 (Cosmopolitan genotype) and DENV-1 (genotype IV).¹⁴⁵ Initially, when the country notified the WHO, the CFR of the outbreak was approximately 12%. With the support of the team from the Queen Sirikit National Institute of Child Health WHO collaborating centre, the CFR was

reduced to 3.6%.¹⁴⁶ Dengue remains a major public health problem with frequent outbreaks in the country.¹⁴⁷

The Maldives

Dengue has been reported from all atolls in the Maldives since 2004 and both *Ae. aegypti* and *Ae. albopictus* are present across the country. Annually, an estimated 1543 symptomatic dengue cases were reported in the country during 2011–2016.¹⁴⁸ In the region, the Maldives has the highest dengue incidence rate (Table 1).

Bhutan

Bhutan first reported dengue in 2004, during which there were 2579 cases. The DENV-2 Cosmopolitan genotype predominated in 2004 and DENV-3 (genotype III) predominated during 2005–2006.¹⁴⁹ A serotype shift with predominant DENV-1 (genotype V) was reported during 2013–2014 and 2016–2017.^{33,150} From 2004 to 2012, dengue was limited to one subdistrict in the western region of the country. However, it subsequently spread to at least eight new districts with no history of dengue transmission by the end of 2018, which further expanded to a total of 19 districts in 2019.¹⁵⁰

Nepal

In Nepal, the first case of dengue was reported from a Japanese traveller infected with DENV-2 in 2004.¹⁵¹ The first outbreak was reported in 2006 from nine districts in the lowland areas with an altitude of <300 m.¹⁵² All four DENV serotypes were detected during this outbreak with a predominance of DENV-3.

Subsequent outbreaks occurred in 2010, 2013 and 2016 with 917, 683 and 1527 cases in 12, 15 and 30 districts, respectively.¹⁵³ These outbreaks were mostly caused by DENV-1, DENV-2 and DENV-1, respectively.^{154–157} DENV-2 was the predominant serotype in 2017.¹⁵⁸ Today, dengue cases are reported even in the hilly regions at an altitude of 2500 m above sea level,¹⁵⁹ with 70% (20 million) of the total population of Nepal at risk of dengue.³²

Table 2. Dengue virus serotypes and corresponding genotypes (phylogenies) identified in the WHO South-East Asian Region

Country	DENV-1	DENV-2	DENV-3	DENV-4
Sri Lanka	I and IV	Asian I and Cosmopolitan	III	I
Indonesia	I, II, IV and V	Asian II, American and Cosmopolitan	I and II	I, II and III
Nepal	V	Asian II and Cosmopolitan	Not subtyped	Not subtyped
India	I, II, III and V	Asian I, Asian II, American, American/Asian and Cosmopolitan	I, II and III	I
Thailand	I, II and III	Asian I, Asian II, American, Cosmopolitan and Southeast Asian/American	I, II and III	I, II and III
Myanmar	I and V	Asian I and Cosmopolitan	II and III	I
Timor-Leste	IV	Cosmopolitan	I	NA
Bhutan	V	Cosmopolitan	III	NA
Bangladesh	V	Cosmopolitan	I and II	Not subtyped

NA, not applicable.

Note: The above classification of Dengue virus genotypes (phylogenies) are based on Weaver and Vasilakis.²¹¹

DENV-1: (a) genotype I: strains from Southeast Asia, China and East Africa; (b) genotype II: strains from Thailand collected in the 1950s and 1960s; (c) genotype III: sylvatic strains of Malaysia; (d) genotype IV: strains from West Pacific Islands and Australia; and (e) genotype V: all strains in the Americas, West Africa and a limited number from Asia.

DENV-2: (a) Asian 1: strains from Malaysia and Thailand; (b) Asian 2: strains from Vietnam, China, Taiwan, Sri Lanka and Philippines; (c) the Cosmopolitan genotype: strains from Australia, East and West Africa, the Pacific and Indian Ocean islands, the Indian subcontinent and the Middle East; and (d) Southeast Asian/American genotype: strains from Thailand, Vietnam and the Americas.

DENV-3: (a) genotype I: strains from Indonesia, Malaysia, the Philippines and South Pacific islands; (b) genotype II: strains from Thailand, Vietnam and Bangladesh; and (c) genotype III: strains from Sri Lanka, India, Africa and Samoa.

DENV-4: (a) genotype I: strains from Thailand, the Philippines, Sri Lanka and Japan; (b) genotype II: strains from Indonesia, Malaysia, Tahiti, the Caribbean and the Americas; and (c): genotype III: recent Thai strains different from other Thai isolates.

In summary, DENV strains remained closely related in the region with further geographical expansion of serotypical and genotypical diversity (Table 2). Frequent movement of people, ease of trade between countries and across the globe, and increased adaptation of *Aedes* mosquitoes, may be responsible for the movement of virus in the region.^{81,160} Phylogenetic studies have shown that DENV can move long distances between continents, between countries and between locations within countries.^{81,161} For example, the DENV-3 (genotype III) in East Africa was introduced to the Indian subcontinent sometime in 1984. This led to the establishment of stable DENV-3 (genotype III) transmission as the isolates from countries in East Africa had a distinct clade within subtype III.⁶¹ The Cosmopolitan genotype of DENV-2 isolated from a 2015–2016 dengue outbreak in southern Thailand was similar to strains circulating in Sri Lanka and northern Thailand.^{81,83} In Bangladesh, it has been suggested that the DENV-2 Cosmopolitan genotype has been imported more than once from India.¹⁶² These reports all suggest continuous introduction of the virus into the region and a requirement for continuous monitoring and surveillance of DENV. Additionally, epidemic cycles have been associated with shifts in predominant serotypes and genotypes and have often been associated with intense transmission, with higher dengue incidence and severe cases. Infection with any dengue virus serotype or genotype provides long-term immunity against the homologous strain and short-term immunity against other strains. People remain susceptible during subsequent outbreaks due to waning of this transient immunity and the low level of herd immunity.¹⁶³ Lowering of the herd immunity level is enhanced by implementation of rigorous vector control activities

during outbreaks, which protects people from the bite of *Aedes* mosquitoes and subsequently prevents people from acquiring natural immunity.¹⁶⁴ Notably, Bhutan and Timor-Leste have not yet reported DENV-4, while neither serotypical nor genotypical information was available from the Maldives (Table 2).

Surveillance strategies and challenges

Dengue control programmes should include entomological and epidemiological surveillance supported by well-established laboratories.¹⁹ Early detection and prediction of dengue outbreaks, enabling timely and targeted mobilisation of control resources, should be the ultimate goal of dengue surveillance systems.¹⁶⁵ This requires strong leadership and political will, and national partnerships with government bodies and the private sector as well as international collaborations.^{19,143}

Entomological surveillance

Entomological surveillance is important to understand the spatial and temporal distribution of vector populations, to identify the location of larval habitats for targeting interventions, to provide metrics for evaluating control programmes and to facilitate timely evidence-based interventions.¹⁴³ Here, we present entomological surveillance studies (as opposed to national surveillance findings) to understand the risk of dengue transmission using entomological parameters.

India

According to the operational guidelines published by the National Vector Borne Disease Control Programme (NVDCP) of India, an area is considered to be at a high risk of dengue if it has a house index (HI) > 10% and breteau index (BI) > 50.00.¹⁶⁶ However, high scores for surveillance indices have been recorded even in the absence of known epidemics. For example, larval surveillance carried out in 70 locations in the Kerala state reported values of HI (13.08%), container index (CI) (13.28%) and BI (16.57) that exceeded guideline cut-off values during the non-epidemic period.¹⁶⁷ Similarly, high scores for indices were also reported in Assam without any known dengue outbreak.¹⁶⁸ In all these conditions, the *Ae. albopictus* population was greater than the *Ae. aegypti* population. By contrast, high scores for indices in Puducherry (HI: 12.10%, CI: 14.80% and BI: 18.90)¹⁶⁹ and Assam (CI: 29.41–80.00%)¹⁷⁰ were reported during outbreaks (Table 3). These outbreaks were associated with the higher frequency of *Ae. aegypti* compared with *Ae. albopictus*.¹⁷⁰ This suggests that, in addition to larval indices, monitoring *Aedes* species is paramount to understand the risk profile of a community.

Basker and Kolandaswamy suggested thresholds for dengue outbreaks using HI < 2–3% and BI < 20, as these values were associated with a reduction in cases to non-epidemic levels.¹⁷¹ The authors observed that mean values of HI, CI and BI from 30 outbreaks in the Cuddalore district were reduced from 15.30%, 2.50% and 16.40 to 2.20%, 1.70% and 2.40, respectively, with intensive vector control.¹⁷¹ Similarly, in a quasi-experimental study in Delhi, HI, pupal index (PI), CI and BI were reduced by 63%, 62%, 64% and 99% in the study group compared with the control group, where increases of 59%, 102%, 73% and 71% were observed, respectively.¹⁷² The study group reported only one case, while the control group reported 38 dengue cases, and this was statistically significant. However, a study in Delhi reported an increasing trend of dengue incidence and deaths in spite of declining HI while continuous intervention actions were in place (Table 4).¹⁷³ This highlights the local differences in the transmission dynamics of dengue and the need to identify potential disease foci through robust methods.

Thailand

A study conducted in Ubon Ratchathani reported HI that ranged from 15.04% to 50.88% during a low transmission period (February–March 2007), while it increased from 25.16% to 56.36% during a high transmission period (June–July 2007). BI and CI followed the same trend.¹⁷⁴ The number of DHF cases also increased during the high transmission period compared with the low transmission period, hence suggesting a positive correlation with larval indices. Similarly, a nationwide analysis of DHF incidence among the entire Thai population found that incidence increased to approximately HI=30, but gradually declined above this HI value.¹⁷⁵ When age-stratified analysis was performed, a positive association was observed between DHF incidence and HI among the youngest age group of the population and a negative association with the oldest age group of the population.¹⁷⁵ This was observed because of the increased transmission intensity in the adult population due to antibodies against almost all

DENV serotypes from the previous infection, thereby conferring resistance to DHF.

Sri Lanka

In Gampaha district during 2014–2016, the *Aedes* vector density was assessed using multiple sampling methods.¹⁷⁶ Adult mosquito samplings were conducted using backpack aspirators and standard manual mouth aspirators with a 0.3 µm HEPA filter attached to stop insects entering the mouth of the aspirator.¹⁷⁶ Although a positive correlation was found between ovitrap index (OI) and dengue cases, it was not statistically significant. As per WHO guidelines, OI > 10% for *Aedes* species is an indication of a potential dengue outbreak once the first dengue case has been detected.¹⁷⁷ However, OI ranged from 56.9% to 94.7% during the 12-mo study period, despite the reduction of cases to non-epidemic levels. Even BI remained < 5% throughout the study period, hence prediction of dengue was not possible with these data.

In Sri Lanka, the BI is the main index used to guide vector control; a BI > 5 with reported dengue cases or a BI > 20 without any dengue cases will trigger vector control activities.³⁸ Udayanga et al. suggested that BI threshold values for determining control activities are problematic because they provide combined values for *Ae. aegypti* and *Ae. albopictus*.³⁸ They proposed threshold values for individual larval indices for *Ae. aegypti* and *Ae. albopictus* rather than combined indices (Table 3).³⁸

Nepal

Based on HI, the Pan American Health Organization defined three different risks of dengue: HI < 0.01% as low risk, 0.1–5% as medium risk and > 5% as high risk.¹⁷⁸ In the survey conducted during 2015 and 2016, HI values during the premonsoon season in a dengue-endemic district (Chitwan) were zero, while HI in non-dengue-endemic districts (Kathmandu and Lalitpur) varied from 0.1% to 5%.¹⁵³ This suggests that HI cannot predict the occurrence of dengue.

Indonesia

Multiple mosquito samplings were used to determine the risk of dengue using larvae, pupae and adult mosquito sampling.¹⁷⁹ Backpack aspirators were used to capture adult mosquitoes under resting and flying conditions. On the basis of risk characterisation using larval indices as per WHO recommendations,¹⁷⁷ villages categorised as medium risk (with endemic circulation) continued to have more dengue cases, but villages categorised as high risk (with sporadic circulation) did not report any dengue cases, and villages categorised as light to medium risk (being free from dengue) reported one dengue case during 2012–2013. By contrast, dengue-endemic and sporadic villages had significantly higher container pupae index and house pupae index scores than villages free from dengue, suggesting the prevalence of suitable conditions for mosquito survival in those villages. Notably, these indices were high in one sporadic village where there were no reports of any cases. Most adult mosquitoes captured in dengue-endemic villages were *Ae. aegypti*, whereas mosquito species that are not dengue vectors dominated in the high-risk villages,

Table 3. Studies showing vector density and associated dengue incidence in the WHO-South-East Asian Region

Country	Location/study period	HI	CI	BI	PI	+Larval density	OI (%)	Adult mosquitoes	Situation of dengue
India ¹⁶⁹	Puducherry	13.10	16.30	40.30	NR	NR	NR	NR	455 cases
India ¹⁷⁰	Assam	NR	29.41–80.00	NR	NR	NR	NR	NR	>4000 cases
India ¹⁶⁸	Assam	NR	NR	NR	NR	>30 15–30 <15	NR NR NR	NR NR NR	10 cases 2 cases 1 case
Thailand ¹⁷⁴	Low transmission	15.04–50.88	NR	NR	NR	NR	NR	NR	Only one area has >24 DHF cases
	High transmission	25.16–56.36	NR	NR	NR	NR	NR	NR	6 areas have >24 DHF cases
Thailand ¹⁷⁵	Nationwide	<30.00 >30.00	NR NR	NR NR	NR NR	NR NR	NR NR	NR NR	Positive correlation with DHF Negative correlation with DHF
Thailand ²¹²	Plaeng Yao District	NR	NR	NR	NR	NR	NR	PC=0.50	Significant positive correlation with dengue
*Sri Lanka ¹⁷⁶	Gampaha district	NR	NR	<5.00	NR	NR	56.90–94.70 (PC=0.56)	NR	Positive correlation but not significant
*Sri Lanka ³⁸	KMC and GK MOH	NR	PC: 0.29	PC: 0.79Agg, –0.38Alb	NR	NR	NR	NR	Significant positive correlation with Agg; negative correlation with Alb
	Akurana MOH	NR	PC: 0.45	PC: 0.76Agg, –0.25Alb	NR	NR	NR	NR	Significant positive correlation with Agg; negative correlation with Alb
	Gampola MOH	NR	PC: 0.35	PC: 0.68Agg, –0.22Alb	NR	NR	NR	NR	Significant positive correlation with Agg; negative correlation with Alb
±Nepal ¹⁵³	Kathmandu	0.59	4.00	0.00	NR	NR	NR	NR	High indices in non-endemic area
	Lalitpur	3.17	7.40	0.01	NR	NR	NR	NR	High indices in non-endemic area
	Chitwan	0.00	0.00	0.00	NR	NR	NR	NR	No cases in endemic area
#Indonesia ¹⁷⁹	Endemic area	18.00	4.00	18.00	4.00	NR	NR	12 [◇]	17 cases
	Endemic area	18.00	5.00	18.00	5.00	NR	NR	6 [◇]	22 cases
	Sporadic area	44.00	13.00	71.00	1.00	NR	NR	13 [◇]	0 cases
	Free area	16.00	3.00	19.00	1.00	NR	NR	0 [◇]	1 case

Agg, *Ae. aegypti*; Alb, *Ae. albopictus*; BI, breteau index; CI, container index (%); DHF, dengue haemorrhagic fever; HI, house index (%); KMC, Kandy Municipal Council; GK, Gangawata Korale; MOH, Medical Officer of Health; mon, monsoon; NR, not reported; PC, Pearson Correlation Coefficient; PI, pupal index (%).

*Analysis at 1-mo lag

+Number of larva per number of traps.

◇Number of *Aedes* mosquitoes.

#Rainy season.

±Premonsoon season.

Table 4. Studies showing variations in vector indices and dengue incidence, before and after intervention measures in India (Note: no studies were retrieved from other countries)

Authors (country)	Location/study group	Before intervention					After intervention				
		HI	CI	BI	PI	Status of dengue incidence	HI	CI	BI	PI	Status of dengue incidence
Basker et al. (India) ¹⁷¹	Cuddalore district	15.30	2.50	16.40	NR	1500	2.20	1.70	2.40	NR	Outbreak controlled
	Nagapattinam district	12.50	2.70	NR	NR	556	2.20	0.9	NR	NR	Outbreak controlled
	Tirunelveli district	13.20	11.20	13.20	NR	6 cases, 4 deaths	3.00	0.90	3.80	NR	Outbreak controlled
Nagpal et al. (India) ¹⁷²	Study group	7.00	3.60	8.04	11.54	NR	0.68	0.39	0.84	0.02	1 case
	Control group	6.80	4.60	4.19	11.91	NR	7.40	13.13	21.69	11.68	38 cases
*Katyal et al. (India) ¹⁷³	Delhi	5.30	NR	NR	NR	180 DHF, 2 deaths	4.2	NR	NR	NR	322 DHF, 3 deaths

BI, breteau index; CI, container index (%); DHF, dengue haemorrhagic fever; HI, house index (%); NR, not reported; PI, pupal index (%).
*Katyal et al. compares HI and DHF in the year 2000 (before intervention) and 2001 (after intervention) in Delhi. Health education and legislative actions resulted in a decrease in DHF incidence and HI from 1996 to 2000. However, an increase in both DHF cases and deaths was reported in 2001 despite continuous efforts of intervention in Delhi.

but with a comparable total number of *Ae. aegypti*.¹⁷⁹ The relationship between larval indices and adult dengue vector density is highly variable and depends on particular stressors like container size and type, degree of larval crowding and the availability of nutrients.¹⁸⁰

In summary, countries in the region frequently carry out *Aedes* surveys using larval indices to help monitor dengue risk and support the planning of vector control strategies. However, larval indices are not a direct measure of numbers of adult mosquitoes, which are directly involved in dengue transmission.¹⁸¹ The utility of traditional larval indices, particularly in their application to detecting dengue outbreaks, has to be established locally and evaluated periodically as it varies from place to place, as described above. As an alternative to larval surveys, pupal surveys can also be conducted. This approach involves calculating a pupal index as a proxy measure to estimate adult abundance.¹⁷⁶ Using this method, the surveyor strains the contents of water-filled containers through a sieve and resuspends the sieved contents into white enamel or plastic pans containing clean water. Pupae are then reared in the insectary to identify the species.¹⁸² However, this method is not routinely practised due to the requirement for large sample sizes, labour intensity and difficulties in obtaining pupae counts from large water containers.^{183,184}

The drawback of using an ovitrap is that it requires insectaries for counting eggs and rearing larvae or adult stages for species identification.¹⁷⁶ Oviposition might be affected by the availability of naturally occurring habitats, and the reliability of estimating adult populations is uncertain due to the skipping behaviour of female mosquitoes.¹⁸⁵ It is possible that an ovitrap with 500 eggs might have been visited by 5–500 female mosquitoes. It requires a considerable degree of skill to count eggs. Adult traps provide a faster and more direct estimation of the adult

population involved in dengue transmission, negating the need to rear mosquitoes. Adult mosquito monitoring is highly sensitive and rarely produces null indices compared with larval surveys.¹⁸⁵ However, adult vector sampling is not routinely practised as a part of surveillance and is currently mainly used for academic research.¹⁸¹ This is a major weakness of entomological surveillance in the region. The most important reason is that adult traps are designed for sampling specific stages of mosquitoes (e.g. BG-Sentinel is suitable for unfed mosquitoes and MosquiTRAP for gravid mosquitoes).¹⁸¹ Other reasons that may preclude their use in resource-poor countries include affordability, accessibility, reliance on a power supply (e.g. BG-Sentinel and the backpack aspirator operate with electric power) and labour intensity.¹⁸¹ Using a combination of methods (in particular using adult traps and larval indices together) is likely to provide the optimal level of information to inform dengue control. Adding information on mosquito infection status can provide an early warning about disease outbreaks and approaches for this are being developed.^{181,186}

Dengue disease surveillance

National notifiable disease surveillance data are used to provide epidemiological information on the spatial and temporal distribution of cases, to characterise infection risk, generate disease alerts and prioritise interventions.¹⁸⁷ Epidemiological and molecular techniques are routinely employed for the prevention and control of dengue outbreaks. As described in the previous section, virus genomic methods are powerful additions to traditional epidemiological and molecular methods. This analysis allows the monitoring of spatio-temporal patterns of the virus, changes in its virulence, connection with previous outbreaks,

vector competence and fitness of the virus.^{61,81} However, it is important to have quality samples for virus isolation and genomic sequencing along with the related metadata, such as date and location of sampling, onset of symptoms, suspected source and clinical outcomes of patients, to lead to an actionable measure.¹⁸⁸ Sentinel surveillance may be conducted to collect high quality data, as described in the section below. Relatively inexpensive, this approach selects representative health centres that monitor the situation more closely and provide timely and accurate information for initiating a public health response.

India

In India, dengue cases are notified to both the NVDCP and the Integrated Disease Surveillance Program (IDSP). The Government of India provides MAC ELISA tests free to laboratories at all levels of the health system. These test kits are developed by National Institute of Virology (NIV) in Pune, India and distributed annually based on the number of dengue cases recorded in the previous year. However, the reporting of dengue is not legally mandatory and thus reporting is inconsistent. Cases are reported daily during outbreaks, but during inter-epidemic periods reporting is influenced by the capacity of health facilities and the commitments of health workers. Further, private hospitals are the major source of dengue cases compared with public hospitals. Of the total hospitalised dengue cases observed in the Madurai district, Tamil Nadu, India, 75.77% of cases were from private hospitals, while public hospitals cared for only 24.23% of cases. Additionally, private hospitals are likely to test >90% of suspected dengue cases using serology, while only 70% of the cases are likely to be tested in public hospitals.⁴¹ However, attempts to include private hospitals in disease surveillance have failed, resulting in wide gaps in resources and quality of reporting.¹⁸⁹

The Government of India instituted sentinel surveillance during 2009–2010 at medical college, state and regional levels to strengthen laboratory capacity for timely identification of disease. The sentinel sites expanded from 20 in 2014 to 52 in 2017, covering 24 states. Patient details are collected and recorded in a web-based data entry system. This network has provided a unique opportunity to generate additional data on dengue virus serotypes along with the classification of dengue infection into primary and secondary infections. Such information can be critical for public health interventions, particularly for choosing sites for rolling out dengue vaccines.¹⁹⁰

There is an underestimation of the true burden of dengue in India, which is believed to be approximately 282 times the officially reported number.⁴¹ This was supported by a study conducted in Pune, where 47 000–59 000 dengue cases are expected per year; but only 6792 cases were reported from the entire Maharashtra state in 2016.¹⁹¹ Factors that impeded timely reporting were frequent changes in the reporting format and programme functioning. In addition, there was a lack of coordination, communication and collaboration between the two apex organisations (NVDCP and IDSP).¹⁹²

Thailand

Thailand has a National Communicable Disease Control Law, which mandates every public and private health sector member

to report dengue within 24 h of diagnosis.¹⁹³ However, surveillance has been found to suffer from under-reporting, particularly for non-hospitalised cases. In a cohort study involving children aged <15 y in the Kamphaeng Phet and Ratchaburi provinces of Thailand, the incidence of dengue leading to hospitalisation was 4.9 and 14.7 cases/1000 head of population, respectively, with average outpatient-inpatient ratios of 3.7:1 and 1:1. However, during the same period, the dengue surveillance system reported inpatient incidences of 2.6/1000 and 4.5/1000 cases, respectively.⁴⁰ The under-recognition of outpatient dengue cases during the period was 6.1-fold, while the under-recognition of inpatients was estimated at 2.6-fold.⁴⁰

Sri Lanka

Since 1996, dengue has been legally reportable in Sri Lanka based on clinical diagnosis and limited laboratory confirmation.⁴⁶ Sri Lanka has developed a laboratory-based, enhanced sentinel surveillance system in the Colombo district,¹⁹⁴ supported by DengueTools, a consortium formed in 2010 by scientists from 14 institutions in Asia, Europe and South America.¹⁹⁴ DengueTools supports the establishment of laboratory-based sentinel surveillance in outpatient clinics and government hospitals in the Colombo metropolitan area, providing detailed laboratory analysis of dengue viruses, including serotyping and molecular characterisation.¹⁹⁴

Myanmar

In Myanmar, dengue was made notifiable in 1966 after the dengue control programme was launched in the country.⁹⁹ Confirmed cases of dengue should be reported within 24 h of diagnosis. However, rapid diagnostics are in minimum supply in public hospitals, which potentially limits their use to critical care and undermines the treatment of large numbers of ambulatory patients.¹⁰⁰

Bangladesh

After 2010, syndromic surveillance in Bangladesh was replaced by laboratory-based surveillance, from which only serologically confirmed positives cases are reported. However, approaches to diagnosis are not uniform because diagnostic facilities in the public health sector are inadequate.^{195,196} The current national dengue surveillance system in Bangladesh captures only 2.8% of all the cases in Dhaka.¹¹³ Closer collaboration with the private health sector, including a mechanism to encourage its participation in dengue surveillance, is required.¹⁹⁶

Indonesia

Dengue is also largely under-reported in Indonesia, even although the reporting of dengue cases is compulsory by law.¹⁹⁷ One study found that only 20.3% of laboratory-diagnosed dengue cases were reported.¹⁹⁸ Reporting often takes days to months even although the regulations stipulate that dengue cases should be reported within 24 h after a confirmed diagnosis.¹⁹⁷ Different modes of reporting (like e-mail, online messaging or paper-based reporting) are used to submit the data from

the hospitals to the district health office, and at provincial and national levels. The varying modes of communication can delay the implementation of prevention and control measures.¹⁹⁹

Timor-Leste

Reporting of dengue is mandatory and reported cases are collated by the Department of Epidemiological Surveillance at the Ministry of Health. Rapid diagnostics are not widely used across the country although the current guidelines recommend their use.¹⁴⁷

Bhutan

Dengue control was first integrated with malaria and other vector-borne diseases in the Vector-borne Disease Control Program in 2003.²⁰⁰ In 2015, surveillance of dengue and other notifiable diseases in the country was shifted from paper-based reporting to a complete web-based system, whereby health centres collect data on cases and report in aggregate numbers. Although the real-time, web-based online system is simple to operate, dengue is largely under-reported.²⁰¹

The Maldives

The Maldives also has a population-based passive surveillance system supported by a web-based reporting platform similar to that of Bhutan. However, gross under-reporting was observed, with a reported surveillance sensitivity of 0.54.²⁰² The reporting rate was found to be extremely poor in medical wards (1.7%), where doctors were given the responsibility to both diagnose and report dengue. The acceptability of the local surveillance system by health professionals was found to be poor.^{150,202} Health professionals were not aware of surveillance operations and feedback provided by the Ministry of Health. Another reason for poor reporting was the conflicting priorities of clinicians (such as treating sick patients), which led to limited time for reporting.²⁰²

Nepal

Notification of dengue is mandatory and is a part of the early warning and reporting system.²⁰³ In Nepal, under-reporting was associated with limited access to health facilities, shortage of health workers, inadequate diagnostic facilities and political instability.²⁰⁴ For example, peripheral laboratories are only provided with diagnostic test kits during outbreaks, leading to under-diagnosis at other times.^{46,194,205}

Electronic surveillance approaches

Causes of untimely and poor reporting of surveillance data are multifactorial, including inadequate passive surveillance systems and the challenges associated with integrating private sector data. Clearly, a multifaceted approach is required, including the engagement of communities to ensure that they participate in the implementation of an effective surveillance and response system. Barde et al. suggested electronic reporting using innovative and available modern technologies such as the internet and mobile phones to strengthen the current surveillance

and response system for dengue.²⁰⁶ This could be enhanced by climate-based early warning systems such as MOSapp and DISapp in India²⁰⁷ and Mo-Buzz in Sri Lanka.²⁰⁸ These approaches use algorithms and computer simulations based on climate, dengue vector and human data to predict dengue outbreaks.

MOSapp allows field workers to report surveillance data, entomological indicators and key environmental variables by direct observation and via portable micro-climate stations. This application captures geotagged information on mosquito abundance, related environmental factors (like stagnant water, artificial containers) and micro-climate data (temperature, humidity, soil moisture, ambient light). The initial findings from the pilot study from 2015 to 2017 in Kerala demonstrated a positive correlation between the mosquito perception index (MPI; a measure of the number of mosquitoes perceived by an observer in their vicinity) and ovitrap counts.

The DISapp application provides ongoing risk estimates, education and recommendations based on the user's responses to a set of questions in the application. The collected data are then processed by a mathematical model for calibration of MPI for predicting mosquito abundance as well as the risk of a dengue outbreak. Mo-Buzz enables the general public to use their smartphones to inform health authorities about breeding sites, mosquito bites and symptoms using interactive forms on social media platforms like Twitter. The Global Positioning System-based sensor automatically captures the geographical coordinates of the user. This component provides additional real-time information about risk-related incidents occurring in the general population. The information received from communities is also reflected in hotspot maps. Using predictive and crowd-sourced information, the system disseminates health alerts and tailored messages to individuals and communities in their respective zones. Dengue early warning systems using such approaches have been widely accepted by public health officials and the general public,^{209,210} and could be replicated in other countries.

Conclusions

Dengue continues to be a disease of public health concern in WHO-SEAR, where disease incidence is increasing every year and there is an ongoing geographical expansion of transmission areas. All countries are hyper-endemic with cocirculation of multiple DENV serotypes and genotypes.

Better methods of dengue vector surveillance would be of the utmost importance for enhancing public health programmes and vector control. Adult mosquito data are not routinely collected, which limits the understanding of the correlation between vector density and dengue incidence. Inconsistent relationships between larval indices and dengue (that need further confirmation by quantitative approaches) indicates the necessity of including adult mosquito sampling in routine surveillance.

The current surveillance systems in the region do not provide adequate information for instituting informed dengue control activities, in particular due to massive under-reporting. Besides adopting dengue as a mandatory notifiable disease supported by legislation, additional efforts are needed to improve and maintain high-quality reporting. National surveillance systems in the region should fully adopt the 2011 WHO-SEAR Office guidelines, supported by national leadership and legislation,

focusing on both the public and private health sectors. Establishment of laboratory-based sentinel surveillance and efficient integration of entomological and disease surveillance can go a long way towards improving early diagnosis, prevention and control of dengue as well as facilitating the future roll-out of dengue vaccines. The use of information technologies like smartphones and the internet, and additional sources of information such as climate data, have great potential for early warning systems.

Supplementary data

Supplementary data are available at [Transactions](#) online.

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3.3 Article on predictors of severe dengue

Tsheten T, Clements ACA, Gray DJ, Adhikary RK, Furuya-Kanamori L, Wangdi K. Clinical predictors of severe dengue: a systematic review and meta-analysis. *Infect Dis Poverty*. 2021;10(1):123.

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SCOPING REVIEW

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Clinical predictors of severe dengue: a systematic review and meta-analysis

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Abstract

Background: Severe dengue is a life-threatening complication; rapid identification of these cases, followed by adequate management is crucial to improve the clinical prognosis. Therefore, this study aimed to identify risk factors and predictors of severe dengue.

Methods: A literature search for studies reporting risk factors of severe dengue among individuals with dengue virus infection was conducted in PubMed, Scopus and Web of Science database from inception to December 31, 2020. Pooled odds ratios (ORs) for patients' demographic characteristics, co-morbidities, and warning signs were estimated using an inverse variance heterogeneity model.

Results: We included 143 articles in the meta-analysis from a total of 13 090 articles retrieved from the literature search. The risk factors of severe dengue were: being a child [OR = 1.96; 95% confidence interval (CI): 1.22–3.13], secondary infection (OR = 3.23; 95% CI: 2.28–4.57), and patients with pre-existing diabetes (OR = 2.88; 95% CI: 1.72–4.81) and renal disease (OR = 4.54; 95% CI: 1.55–13.31). Warning signs strongly associated with severe disease were increased haematocrit with a concurrent decrease in platelet count (OR = 5.13; 95% CI: 1.61–16.34), abdominal pain (OR = 2.00; 95% CI: 1.49–2.68), lethargy (OR = 2.73; 95% CI: 1.05–7.10), vomiting (OR = 1.80; 95% CI: 1.43–2.26), hepatomegaly (OR = 5.92; 95% CI: 3.29–10.66), ascites (OR = 6.30; 95% CI: 3.75–10.60), pleural effusion (OR = 5.72; 95% CI: 3.24–10.10) and melena (OR = 4.05; 95% CI: 1.64–10.00).

Conclusions: Our meta-analysis identified children, secondary infection, diabetes and renal disease(s) as important predictors of severe dengue. Our finding also supports the predictive ability of the WHO warning signs to identify severe dengue. These findings are useful for clinicians to identify severe dengue for management and timely interventions.

Keywords: Severe dengue, Meta-analysis, Risk factor, Warning sign

Background

In 2010, it was estimated that there were 390 million dengue infections, of which 96 million manifested clinically [1] with severe dengue resulting in 21 000 deaths

worldwide [2]. Asia bears 70% of this global burden [1]. The incidence of dengue has surged dramatically with an eightfold increase over the last two decades, from 505 430 cases in 2000 to over 2.4 million in 2010, and 4.2 million in 2019 [3]. The increase in dengue incidence has been associated with explosive outbreaks and geographical expansion to new areas [3].

Dengue is an arboviral infection caused by a dengue virus (DENV) belonging to the Flaviviridae family. Four antigenically and genetically distinct DENV serotypes (DENV1–4) have been described to co-circulate around

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the world and cause human infections [4]. The infection leads to a wide spectrum of clinical manifestations from asymptomatic infection to life threatening severe dengue or dengue shock syndrome (DSS) [1]. In many Asian countries, severe dengue is the leading cause of hospitalization among children and the case fatality rate (CFR) is about 5% on average [5].

There is no specific treatment and the dengue vaccine [CYD-TDV (Dengvaxia®)] is licensed only in 20 countries [6]. The vaccine is not yet approved for younger children due to low efficacy and safety reasons [7]. In a randomized controlled, multicentre, phase III trial, the efficacy of CYD-TDV was reported at ~56% against virologically confirmed dengue among children in countries in the Asia-Pacific region [8]. Only adults aged 9–45 years living in an area of $\geq 70\%$ dengue prevalence, and whose serostatus is positive for past dengue infection are recommended for immunization [6]. Due to the challenges associated with the need to collect information on the burden and seroprevalence profiles of the local population, and the recent reports of vaccine-related severe dengue and deaths, the use of the dengue vaccines is not widespread [9]. Therefore, rapid identification of severe cases and appropriate clinical management remains the mainstay to avoid dengue-related case fatalities. This includes monitoring for plasma leakage and initiating intravenous fluid replacement to prevent shock and death [10]. A rational approach of case management through proper understanding of the determinants of severe dengue is key to improving clinical outcomes [11].

This systematic review and meta-analysis aimed to identify predictors of severe dengue. Such knowledge will be useful to clinicians for targeting at-risk groups of severe dengue for initiating prompt interventions to save lives.

Methods

Search strategy

The methods and results of the systematic review and meta-analysis are reported in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Additional file 1) [12]. No protocol was registered for this systematic review and meta-analysis.

Three databases, PubMed, Scopus and Web of Science, were searched from inception to December 31, 2020, for relevant articles. Key search terms were “dengue”, “dengue haemorrhagic fever”, “dengue shock syndrome” or “severe dengue”. The detailed search strategy is provided in Additional file 2. In addition, a backward citation search using the reference lists of relevant studies were reviewed for additional studies that may not have been captured using the search terms.

Eligibility criteria

This review was undertaken to identify predictors of severe dengue based on the patient’s demographic characteristics, comorbidities, and presentation of warning signs; therefore, the inclusion criteria were: (1) observational studies (cross-sectional, case control, or cohort study designs) conducted in humans; (2) which compared severe dengue and non-severe dengue cases; and (3) reported patients’ demographic characteristics (i.e., age, sex, ethnicity, socio-economic class, region/location, and primary or secondary dengue infection), comorbidities [i.e., asthma, chronic obstructive pulmonary disease (COPD), visual impairment, cardiovascular diseases (CVD), diabetes, obesity and overweight, hearing loss, cancer, oral health, alcohol use disorder, and haemoglobin disorders like thalassemia and sickle cell disease], and/or clinical warning signs [i.e., abdominal pain, vomiting, enlarged liver size, pleural effusion, ascites, gum bleeding, epistaxis, lethargy, melena, increase in haematocrit with concurrent decrease in platelet count, gastrointestinal (GI) bleeding, hematemesis and skin bleeding]. Exclusion criteria included: (1) case reports, case series, reviews, or letters; (2) in vitro and animal studies; (3) conference presentations; and (4) studies where patient outcomes were not separated into severe and non-severe dengue.

The classification of the severity of dengue of the selected studies was done either with the World Health Organization (WHO) 1997 or the revised WHO 2009 dengue case classification. The WHO 1997 dengue case classification categorized dengue into dengue fever (DF), dengue haemorrhagic fever (DHF) (i.e., grade I & II) and dengue shock syndrome (DSS) (i.e., grade III & IV) [5]. While the WHO 2009 dengue case classification categorized dengue into dengue without warning signs (DWoWS), dengue with warning signs (DWWS), and severe dengue (SD) [10]. In this review, we defined severe dengue as DSS according to the WHO 1997 dengue case classification and SD according to WHO 2009 dengue case classification. A detailed description of the WHO 1997 and 2009 dengue case classification along with the case definition of severe dengue used in this study are presented in Table 1.

Selection of studies and data extraction

All retrieved articles from the three databases (PubMed, Scopus and Web of Science) were imported into EndNote X7.7.1 (Clarivate Analytics, Philadelphia, PA, USA) and duplicates were removed. Then studies were screened by title and abstract in Rayyan (<http://rayyan.qcri.org/>). Using Rayyan, articles selected by title and abstract also underwent full text screening for the final selection. The

Table 1 Dengue severity stratification according to 1997 and 2009 World Health Organization (WHO) guidelines

1997 WHO classification		2009 WHO classification		
Dengue fever	DHF (Grade 1 and 2)	DSS (Grade 3 and 4)	DWoWS	SD
Acute febrile illness with ≥ 2 of the following symptoms: Headache Retro-orbital pain Myalgia Arthralgia Rash Haemorrhagic manifestations Leukopenia	Following must be all present: Fever or history of fever Haemorrhagic tendencies (as manifested by a positive tourniquet test, petechiae/purpura/ecchymoses, mucosal bleeding) Thrombocytopenia Plasma leakage (a rise in the haematocrit, pleural effusion, ascites)	All four criteria of DHF plus evidence of circulatory failure evidenced by: Rapid and weak pulse Narrow pulse pressure (< 20 mm Hg) Hypotension Cold clammy skin and restlessness	Fever and two of the following symptoms: Nausea or vomiting Rash Bodyaches Positive tourniquet test Leukopenia	Patients with any of the following features: Severe plasma leakage leading to Shock, fluid accumulation with respiratory distress Severe bleeding Severe organ impairment

DHF dengue haemorrhagic fever, DSS dengue shock syndrome, DWoWS dengue without warning signs, DWWS dengue with warning signs, SD severe dengue, HCT haematocrit

*Case definition of severe dengue used in the meta-analysis is shaded in grey

screening process was conducted by two independent reviewers (TT and RKA) and any discrepancies during the selection of studies were resolved through discussion and consensus following independent evaluation by another author (KW).

The same two reviewers (TT and RKA) extracted the data of the eligible articles. Differences in the extracted data were resolved by consensus between the reviewers. The following information was extracted: name of the first author, WHO dengue case classification type (guideline 1997 or 2009), country name, recruitment time, study design/size, study population (children, adults or mixed), median/mean age, infection type (primary or secondary), warning signs (i.e., abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy, liver enlargement, and increase in haematocrit with a concurrent decrease in platelets), co-morbidities (i.e., asthma, COPD, CVD, hypertension, diabetes, obesity, cancer, sickle cell disease), and the severity of disease (severe and non-severe dengue). When available, adjusted estimates were extracted, otherwise unadjusted estimates were calculated.

Quality assessment

The quality of the studies was assessed using the Methodological STAndards for Epidemiological Research (MASTER) scale [13]. This scale has 36 bias safeguards that were categorized into seven methodological standards or equivalence [13]. These standards reflect initial and ongoing equivalence in equal recruitment, equal retention, equal ascertainment, equal implementation, equal prognosis, sufficient analysis and temporal precedence. The studies were rated as ‘1’ or ‘0’ depending on the presence or absence of each of these safeguard items. Safeguards not relevant to the studies were rated ‘0’. Similar to the screening and data extraction process, two independent reviewers (TT & RKA) conducted the assessment and any discrepancies were resolved by the consensus and involvement of another author (KW).

Data analysis

The pooled odds ratios (ORs) with 95% confidence intervals (CI) comparing severe and non-severe dengue for each predictor was estimated using the inverse variance heterogeneity (IVhet) model [14]. Heterogeneity between studies was assessed using the Cochran Q and the I^2 test statistics. Levels of heterogeneity were categorized according to the I^2 index as low (<25%), low to moderate (25% to <50%), moderate to high (50% to <75%) or high ($\geq 75\%$). The same Cochran Q statistic was used to assess heterogeneity in the sub-group analysis.

Sub-group analyses were conducted to compare the differential effect based on (1) WHO dengue case

classification of disease severity (1997 vs 2009), and (2) children and adults to identify risk factors specific to an age group. We defined participants under the age of 20 years as children and as adults otherwise. This classification was based on the definition in the studies, with some studies reporting 19 years as children. Studies reporting only children or adults were excluded from the age predictor analysis. A minimum of four studies per strata was required for sub-group analysis.

For sensitivity analysis, a bias-adjusted (quality-effect model) meta-analysis was performed using the score generated from the MASTER Scale. The scores of all safeguards generated as described above were added and converted into a relative rank between 0 and 1 by dividing the cumulative score of each study by the highest score. We included these quality ranks into the model to estimate bias-adjusted pooled effect sizes as a sensitivity analysis [15].

The publication bias was assessed using the Doi plot and LFK index bias [16]. LFK values beyond ± 1 were considered to be indicative of asymmetry and suggest the presence of publication bias [16]. The analysis was performed in the statistical program Stata 16 (College Station, TX: StataCorp LLC) using *metan* and *lfk* modules.

Results

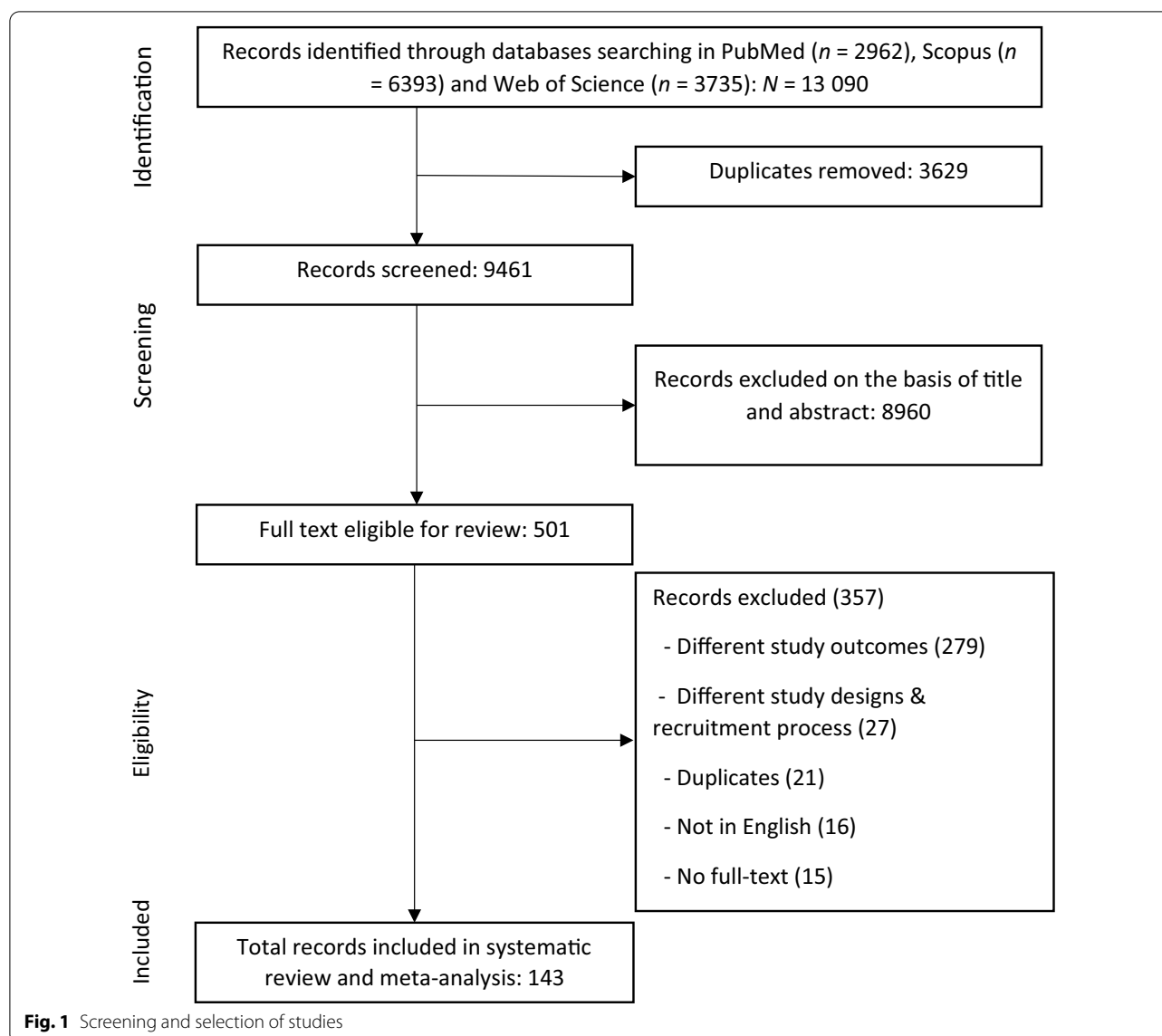
Literature search

A total of 13 090 records were retrieved from the initial search. After removing 3629 duplicates, 9461 records were screened by titles and abstracts. Subsequently, 501 articles were included for full-text review, of which 143 articles remained and were included in the systematic review and meta-analysis (Fig. 1). Studies included in this study are presented in Additional file 3.

Characteristics of the studies

Included studies were reported from the WHO regions as follows: South-East Asia ($n=74$, 51.8%), Western Pacific ($n=34$, 23.1%), Americas ($n=26$, 18.2%), Eastern Mediterranean ($n=7$, 4.9%), Europe ($n=2$, 1.4%) and Africa ($n=1$, 0.7%), respectively. Most of the studies were cross-sectional ($n=81$, 56.6%) followed by cohort ($n=36$, 25.2%) and case-control studies ($n=26$, 18.2%). In 59 studies, only children were included, while 36 studies reported only adults, both children and adults were reported in 47 studies, and one study did not provide information on the age of the participants. Dengue severity was classified using the WHO 2009 dengue case classification in 85 (59.4%) studies, while the rest used the WHO 1997 dengue case classification (Table 2).

Socio-demographic predictors including sex, age and primary/secondary infection variables were reported in 114, 87, and 29 studies, respectively. Diabetes was the



most reported co-morbidity in 10 studies, followed by hypertension in nine studies, obesity in five studies, and one each of CVD and renal disease in four studies. Other co-morbidities including asthma, pulmonary disease or sickle cell disease were not adequately reported to be analysed further. Finally, warning signs of severe dengue were reported as follows: abdominal pain ($n=55$ studies), vomiting ($n=53$ studies), enlarged liver size ($n=47$ studies), pleural effusion ($n=25$ studies), ascites ($n=22$ studies), gum bleeding ($n=12$ studies), epistaxis ($n=11$ studies), lethargy ($n=10$ studies), melena ($n=9$ studies), increase in haematocrit with concurrent decrease in platelet count ($n=7$ studies), gastrointestinal (GI) bleeding ($n=5$ studies), hematemesis ($n=5$ studies) and skin bleeding ($n=4$ studies).

Quality of the studies

The quality of the studies was assessed against each of the 36 safeguard items. Accordingly, the studies met all the pre-defined eligibility criteria and were from the same population and timeframe. Similarly, the attrition rate and missing values were either below 20% or non-existent in 143 studies. The procedures for data collection of covariates and outcomes were reliable and objective in 142 studies. Overall, the least deficient standards across studies were equal prognosis (88.6%), equal implementation (64.6%) and equal retention (59.4%). Temporal precedence was the most deficient standard across the studies (1.5%) (Additional file 4). This might be because most of the studies included in the review used cross-sectional designs where there is no temporal dimension.

Table 2 Characteristics of the studies included to assess the demographic characteristics, co-morbidities and clinical warning signs of severe dengue

Parameters	Frequency (n)	Percentage (%)
WHO Classification type		
1997	58	40.6
2009	85	59.4
WHO region		
South-East Asia	74	51.8
Western Pacific	33	23.1
American	26	18.2
Eastern Mediterranean	7	4.9
European	2	1.4
Africa	1	0.7
Study design		
Case control	26	18.2
Cohort	36	25.2
Cross-sectional	81	56.6
Study population		
Children	59	41.3
Adults	36	25.2
Mixed	47	32.9
No information	1	0.7
Recruitment year		
1990–2010	78	54.6
2011–2019	64	44.8
No information	1	0.7
Laboratory diagnosis		
ELISA	96	67.1
PCR	49	34.3
RDT	27	18.9
HI	10	7.0
Viral culture	4	2.8
Immunohistochemistry	2	1.4
Immunofluorescence assay	1	0.7
Neutralization test	1	0.7
Dotblot immuno assay	1	0.7
No information	13	9.1

ELISA enzyme linked immunosorbent assay, PCR polymerase chain reaction, RDT rapid diagnostic test, HI haemagglutination inhibition

Quantitative analysis

Demographic characteristics

Children were positively associated with the development of severe disease as compared to adults ($OR=1.96$, 95% CI : 1.22–3.13). Progression to severe dengue did not show a significant difference by sex ($OR=1.20$, 95% CI : 0.79–1.82). Secondary dengue infection was found to be significantly associated with the development of severe disease ($OR=3.23$, 95% CI : 2.28–4.57) (Table 3).

Table 3 Pooled estimates of odds ratio and corresponding 95% confidence intervals of patient demographic characteristics and severe dengue

Predictors	Number of studies	Pooled OR (95% CI)	Heterogeneity test I^2 (%)	P-value
Demography				
Children	22	1.96 (1.22–3.13)	90.00	<0.001
Female	114	1.20 (0.79–1.82)	80.3	<0.001
Secondary infection	29	3.23 (2.28–4.57)	33.20	0.044
Co-morbidities				
Diabetes	10	2.88 (1.72–4.81)	40.9	0.085
Cardiovascular disease	4	2.27 (0.38–13.71)	70.8	0.016
Obesity	5	0.76 (0.41–1.40)	32.9	0.202
Renal disease	4	4.54 (1.55–13.31)	45.1	0.162
Hypertension	9	1.82 (0.98–3.37)	63.1	0.006
Warning signs				
↑Hct & ↓Plt*	7	5.13 (1.61–16.34)	88.1	<0.001
Abdominal pain	55	2.00 (1.49–2.68)	70.9	<0.001
Vomiting	53	1.80 (1.43–2.26)	62.8	<0.001
Lethargy	10	2.73 (1.05–7.10)	85.1	<0.001
Hepatomegaly	47	5.92 (3.29–10.65)	89.3	<0.001
Ascitis	22	6.30 (3.75–10.60)	67.7	<0.001
Pleural effusion	25	5.72 (3.24–10.10)	76.3	<0.001
Gum bleeding	12	2.00 (0.86–4.66)	56.4	0.008
Epistaxis	11	1.85 (0.72–4.70)	64.4	0.002
Hemetemesis	5	12.35 (4.97–30.72)	52	0.080
Melena	9	4.05 (1.64–10.00)	78.1	<0.001
Skin bleeding	4	1.38 (0.47–4.06)	73.5	0.010
GI bleeding	5	9.49 (2.75–32.70)	78.4	<0.001

*Increase in haematocrit values with concurrent decrease in platelet count

OR odd ratio, CI confidential interval

Co-morbidities

Diabetes ($OR=2.88$, 95% CI : 1.72–4.81) and renal disease(s) ($OR=4.85$, 95% CI : 1.08–21.66) were associated with severe dengue. However, other co-morbidities including hypertension ($OR=1.82$, 95% CI : 0.98–3.37), CVD ($OR=2.27$, 95% CI : 0.38–13.71), and obesity ($OR=0.76$, 95% CI : 0.41–1.40) were not significantly associated with the severe disease (Table 3).

Warning signs

The definition of warning signs varied across the studies. Only one study defined abdominal pain as severe enough to warrant medical attention [17]. Persistent vomiting was defined in four ways: vomiting with signs of dehydration [18–20], ≥ 2 episodes of vomiting associated with fatigue or requiring intravenous fluid [17], at least six episodes of vomiting in 24 h [21] or vomiting during ≥ 2 consecutive days [22]. Similarly, liver

enlargement was defined as >2 cm in the midclavicular line in three studies [23–25]. No studies provided a definition of lethargy.

Progression to severe dengue was associated with a concurrent increase in haematocrit and decrease in platelet count compared to normal values ($OR = 5.13$, 95% CI : 1.61–16.34), abdominal pain ($OR = 2.00$, 95% CI : 1.49–2.68), lethargy ($OR = 2.73$, 95% CI : 1.05–7.09), vomiting ($OR = 1.80$, 95% CI : 1.43–2.26) and enlarged liver ($OR = 5.92$, 95% CI : 3.29–10.65) (Table 3).

Studies have used different definitions for mucosal bleeding and clinical fluid accumulation. Some studies used specific conditions like epistaxis [26, 27] or gum bleeding [28, 29] to refer to mucosal bleeding, while others have grouped them as mucosal bleeding [30, 31]. Similarly, clinical fluid accumulation was defined as ascites [24, 32] or pleural effusion or combined as clinical fluid accumulation [17, 33]. Here, we presented only specific conditions rather than the grouped variable. In terms of clinical fluid accumulation, both ascites ($OR = 6.94$, 95% CI : 3.75–10.60) and pleural effusion ($OR = 5.72$, 95% CI : 3.24–10.10) were significantly associated with severe dengue. In terms of mucosal bleeding, hematemesis ($OR = 12.35$, 95% CI : 4.97–30.72) was significantly associated, while gum bleeding ($OR = 2.00$, 95% CI : 0.86–4.66) and epistaxis ($OR = 1.85$, 95% CI : 0.72–4.70) were not significantly associated with severe dengue. In addition, GI bleeding ($OR = 9.49$, 95% CI : 2.75–32.70) and melena ($OR = 4.05$, 95% CI : 1.69–10.00) were also found to be positively associated with severe disease (Table 3). The forest plots are presented in additional file 5.

Subgroup analysis

All predictors that were significantly associated in the main analysis also showed similar results in the stratified analysis using the WHO 1997 and 2009 dengue case classifications. These included age groups, secondary infection, abdominal pain, vomiting, enlarged liver size, ascites, pleural effusion, hematemesis and melena. Similar to the main analysis, sex, epistaxis and gum bleeding were not significant in the stratified analysis (Additional file 6).

In the subgroup analysis by age, only adult females were significantly associated with severe dengue (relative to adult males, $OR = 2.12$, 95% CI : 1.13–3.97) (Additional file 7). Due to a limited number of studies, sub-group analysis could not be performed for all predictors in the co-morbidities category, GI bleeding, and increase in haematocrit values with a concurrent decrease in platelet count.

Sensitivity analysis

In the sensitivity analysis, when using the quality effects model, all pooled estimates were found to be consistent with the main analysis (Additional file 8).

Publication bias

The Doi plot and LFK index revealed major asymmetries for the estimates of age group ($LFK = -3.83$), CVD ($LFK = 2.92$), renal disease(s) ($LFK = -3.13$), hypertension ($LFK = 5.05$), vomiting ($LFK = 1.94$), lethargy ($LFK = 3.7$), gum bleeding ($LFK = 2.04$), melena ($LFK = 2.4$), skin ($LFK = 5.47$) and GI bleeding ($LFK = -2.05$). A moderate to high heterogeneity of the studies might have accounted for asymmetries in these estimates (Table 3 and Additional file 9).

Discussion

In this systematic review and meta-analysis, we found that the main predictors for severe dengue were being a child, secondary dengue infection, pre-existing co-morbidities [i.e., diabetes and renal disease(s)] and the presence of warning signs (i.e., increase in haematocrit with concurrent decrease in platelet count, abdominal pain, lethargy, vomiting, hepatomegaly, ascites, pleural effusion and melena). Most of these studies were reported from countries in the WHO-South-East Asia region.

Although there has been a shift in the incidence of DF towards older age groups [34], severe dengue continues to be an important cause of significant morbidity and mortality in children since it was first reported in the 1950s in South-East Asia [35]. Previous studies have demonstrated an increased risk of severe dengue or dengue shock syndrome in children and these conditions have been known to be common causes of hospitalization and mortality in tropical regions [36, 37]. The risk of severe dengue can be explained by greater vascular permeability in children [38]. Dengue shock results from a sudden generalized increase in microvascular permeability with less microvascular reserve to accommodate extraneous factors [38]. Therefore, clinicians should pay special attention to children in recognizing the severity of the disease and providing appropriate interventions on time. Such a strong positive association of severe disease with children also supports the delivery of future vaccines and therapeutics to pre-school and school-going children to achieve the greatest impact on disease burden.

Similar to the other reported studies [39], we found a strong association between secondary dengue infection and severe dengue. This pathogenesis might be related to antibody-dependent enhancement (ADE) in secondary infection with a different DENV serotype, where the pre-existing heterotypic antibodies bind to form immune

complexes with virions without neutralizing it [40]. These virus-immune complexes facilitate virus entry and enhanced virus replication in the FcγR (fragment crystallizable gamma receptors)-bearing cells, such as monocytes, dendritic cells and macrophages. The internalized DENV particles then initiate an immune cascade which results in the evasion of innate immunity, such as the inhibition of type-1 interferon, and subsequently leads to vascular leakage and severe disease [40, 41]. Further, cytokine levels are also assumed to be elevated in secondary dengue infection [42]. Cytokines like vascular cell adhesion molecule-1 (VCAM-1) facilitate chemotaxis by mediating the adhesion of lymphocytes and cells of the innate immune system to the vascular endothelium [43]. Other cytokines such as vascular endothelial growth factor-A (VEGF-A) enhance vascular permeability and activate the coagulation system by upregulating the production of tissue factors [44, 45]. Finally, biosynthesis of other pro-inflammatory cytokines such as interleukins (IL-6, IL-7, IL-8 and IL-10) facilitates an increased synthesis of DENV RNA (ribonucleic acid) and suppresses the host mediated and adaptive immune responses [41, 46]. However, it is important to note that the severity may be affected by certain DENV serotypes; the other meta-analysis study reported severe disease in secondary infection with DENV-2, 3 and 4 [39]. To provide accurate management of dengue, clinicians should rely on tests that detect both recent and past infections.

Our study also found a significantly higher risk of severe dengue due to pre-existing co-morbidities like diabetes and renal disease. This finding supports the need for hospitalization and monitoring of dengue patients with pre-existing co-morbidities [10]. Although no clear mechanism was postulated, in diabetes, patients with suboptimal glycaemic control ($HbA1c \geq 7\%$) were found to be strongly associated with severe dengue than were patients with adequate glycaemic control and without other co-morbidities [47]. In advanced diabetes, micro and macro-vascular functions are impaired, which might lead to increased plasma leakage and subsequently progress to severe dengue [48, 49]. In chronic kidney disease(s), pro-inflammatory cytokines are markedly elevated, which might cause vascular injury in dengue virus infection [50]. In addition, the uraemia associated with kidney disease induces endothelium dysfunction and contributes to greater vascular damage with dengue infection [51].

Patients with warning signs have to be admitted into the hospital for close monitoring and intravenous fluid therapy administration [10]. These interventions can reduce the frequency of patients progressing to severe dengue and deaths. However, none of the studies so far have comprehensively studied all warning signs identified

by the WHO. Some studies [52] used thrombocytopenia and elevated thrombocytopenia separately to assess the risk of developing severe disease. However, these parameters have to be interpreted with other concurrent laboratory results. For example, an increase in haematocrit with a concurrent decrease in platelet count is an important warning sign.

As expected, our study found all warning signs to be positively associated with severe dengue excepting cutaneous and mucosal bleeding (epistaxis and gum bleeding). Notably, gastrointestinal bleeding/melena was significantly associated with severe disease. A previous study on the clinical predictors of severe dengue also found similar findings [53]. Similar to a previously published study [52], fluid accumulation, vomiting and abdominal pain was found to be positively associated with the severe disease in this study. In addition, lethargy, abdominal pain, vomiting and hepatomegaly were strongly associated with an increase in haematocrit with a concurrent decrease in platelet count. None of the meta-analyses in the past have pooled this estimate, possibly due to a low number of studies.

The findings of this study should be interpreted in the context of some limitations. We were not able to consider the role of viremia, dengue virus serotypes, genetic, biomarker and other clinical parameters besides warning signs as predictors of severe dengue. Second, we were unable to analyse different co-morbidities such as sickle cell disease and bleeding disorders despite our broad search strategy. These disorders could affect the progression to severe disease and possible outcomes. Third, there was inconsistent reporting of heart diseases which made it difficult to assess these conditions individually as potential predictors, rather we combined different heart conditions into a single group. Fourth, many studies did not report adjusted effect sizes, and we based our pooled result on crude effect sizes. These might have overestimated the pooled effect sizes due to potential confounders. This is of particular concern with smaller studies and therefore our results need to be interpreted with caution. Fifth, limiting papers published in English might have influenced the precision of the pooled estimates. Importantly, most of the studies were from the South-East Asia region and the Pacific region, which bears more than 75% of the global burden of dengue. Sixth, we did not include biomarkers of severe dengue. Finally, we found both heterogeneity and publication bias in the included studies. These might be related to variations in study design, sample sizes, recruitment processes and exposure/outcome measurement across different studies. However, we conducted subgroup analysis and sensitivity analysis to account for these variations and tested the robustness of our results.

Conclusions

Our meta-analysis identified children, secondary infection, diabetes and renal disease(s) as important predictors of severe dengue. We also confirmed the predictive ability of all warning signs of severe dengue identified by the WHO. All warning signs were significantly associated with severe disease excepting mucosal and cutaneous bleeding. The knowledge generated from this study will help clinicians to identify early warning signals of severe dengue leading to timely interventions of dengue cases. Future studies using novel biomarkers and point of care methods including ultrasonography will be useful in predicting the onset of severe dengue.

Abbreviations

ADE: Antibody-dependent enhancement; CFR: Case fatality rate; COPD: Chronic obstructive pulmonary disease; CVD: Cardiovascular diseases; CYD-TDV: Chimeric yellow fever virus—DENV tetravalent dengue vaccine; DENV: Dengue virus; DF: Dengue fever; DHF: Dengue haemorrhagic fever; DWoWS: Dengue without warning signs; DWWS: Dengue with warning signs; DSS: Dengue shock syndrome; FcγR: Fragment crystallizable gamma receptors; GI: Gastrointestinal bleeding; HCT: Haematocrit; I²het model: Inverse variance heterogeneity model; OR: Odds ratio; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RNA: Ribonucleic acid; SD: Severe dengue; VCAM-1: Vascular cell adhesion molecule-1; WHO: World Health Organization.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40249-021-00908-2>.

- Additional file 1.** PRISMA Checklist for systematic reviews and meta-analysis
- Additional file 2.** Search strategy used in the review of literature
- Additional file 3.** Studies included in the review and meta-analysis
- Additional file 4.** Quality assessment of the studies using a MASTER Scale
- Additional file 5.** Forest plots with pooled OR of progression to severe dengue with potential predictors
- Additional file 6.** Subgroup analysis using WHO classification for demography, co-morbidities and clinical warning signs of dengue severity
- Additional file 7.** Subgroup analysis using age groups for demography and warning signs of severe dengue
- Additional file 8.** Sensitivity analysis
- Additional file 9.** Doi plots and LFK values used to assess publication bias

Authors' contributions

TT, LFK and KW conceived this study. TT, RKA and KW screened the studies and extracted the data from the eligible studies. TT undertook literature review and drafted the manuscript. LFK and TT analysed the result. KW helped in the drafting and revision of manuscript. DJG and ACAC were involved in the critical revision of manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are included this published article and the additional files, all of which are also available in the public domain.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Spatial and temporal patterns of dengue in Bhutan

4.1 Introduction

Dengue is a tropical disease and clustered in areas conducive for the breeding of *Aedes* mosquitoes. The transmission of the dengue infection is influenced by climatic and environmental drivers that facilitate the spread of *Aedes* mosquitoes. Understanding the spatio-temporal pattern of dengue including the identification of climatic and environmental drivers that are associated with the increase in transmission of the disease will be essential for planning preventive and control programs in the community. Dengue emerged as one of the top devastating diseases in 2019 in Bhutan which spread across 19 of the 20 districts resulting in six deaths. This is in sharp contrast to the previous years, when the disease was confined to a few districts in the southern region. This provided an opportunity to analyse the transmissibility and spatio-temporal patterns of the emergence of dengue in a newly affected region.

The first study in the chapter outlines the spatial and temporal pattern of dengue in the seven southern districts of Bhutan at the sub-district level. A Poisson regression model in a Bayesian Framework was constructed to analyse the epidemiological, demographic, environmental and meteorological data. The main outcome of the study was that dengue showed strong seasonality with peaks observed between July and September. The temperature was positively associated with the transmission, while the normalised difference vegetation index (NDVI) was negatively associated with the transmission of dengue. The model also revealed a significant residual spatial clustering, which was predominantly located in the eastern region.

The second study describes the spatial and temporal progression of the largest dengue epidemic to date in Bhutan and reported the estimated effective reproduction number. The pattern on how the epidemic progressed at different time periods and expanded to new sub-districts was illustrated using geographical information system. To estimate the effective reproduction number, four exponential growth rates were computed using the linear model to the log of the weekly number of cases. Accordingly, four temperature-dependent cohort reproduction numbers were computed at four phases of the epidemic. In addition, the reproduction number using incubation period and serial interval without the temperature variable was computed as

a supplementary analysis. The main finding of this study was that there was an increase in the number of affected areas during the epidemic with a very high reproduction number of 7.1. As in past epidemics, the Phuntsholing sub-district in the Chukha district was the worst affected area.

4.2 Article on drivers of dengue transmission

Tsheten T, Clements ACA, Gray DJ, Wangchuk S, Wangdi K. Spatial and temporal patterns of dengue incidence in Bhutan: a Bayesian analysis. *Emerg Microbes Infect.* 2020;9(1):1360-1371.

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Spatial and temporal patterns of dengue incidence in Bhutan: a Bayesian analysis

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ABSTRACT

Dengue is an important emerging vector-borne disease in Bhutan. This study aimed to quantify the spatial and temporal patterns of dengue and their relationship to environmental factors in dengue-affected areas at the sub-district level. A multivariate zero-inflated Poisson regression model was developed using a Bayesian framework with spatial and spatiotemporal random effects modelled using a conditional autoregressive prior structure. The posterior parameters were estimated using Bayesian Markov Chain Monte Carlo simulation with Gibbs sampling. A total of 708 dengue cases were notified through national surveillance between January 2016 and June 2019. Individuals aged ≤ 14 years were found to be 53% (95% CrI: 42%, 62%) less likely to have dengue infection than those aged >14 years. Dengue cases increased by 63% (95% CrI: 49%, 77%) for a 1°C increase in maximum temperature, and decreased by 48% (95% CrI: 25%, 64%) for a one-unit increase in normalized difference vegetation index (NDVI). There was significant residual spatial clustering after accounting for climate and environmental variables. The temporal trend was significantly higher than the national average in eastern sub-districts. The findings highlight the impact of climate and environmental variables on dengue transmission and suggests prioritizing high-risk areas for control strategies.

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KEYWORDS Dengue; temporal; spatial; Bayesian; Bhutan

Introduction

Dengue is a disease caused by arthropod-borne flaviviruses belonging to one or more of the four dengue virus serotypes (DENV1-4) [1]. An infection with one DENV serotype confers long-term immunity to that particular serotype, while a secondary infection with a different serotype (secondary heterotypic infection) can result in a severe form of the disease known as severe dengue or dengue haemorrhagic fever [2]. DENV-2 and DENV-3 are more commonly associated with hospitalization and greater mortality than the other two serotypes [3, 4].

Dengue transmission occurs through the bite of *Aedes* mosquitoes, namely, *Ae. aegypti* and *Ae. albopictus*. *Ae. aegypti* is most commonly found in tropical and sub-tropical regions, while *Ae. albopictus* has a larger geographical range due to its tolerance of colder temperatures [5]. *Ae. aegypti* typically breeds in man-made containers available in an urban environment, and rests and bites both indoors and outdoors [6]. *Ae. albopictus* is mostly an outdoor mosquito that breeds in natural containers such as banana trees, tree holes, coconut shells and cut bamboo [7]. Both

species feed during the day time from morning until dusk, although night-time biting is sometimes observed in *Ae. albopictus* [8].

The global burden of dengue has increased over the last few decades from over 8.3 million cases in 1990 to more than 58.4 million cases in 2013 [9]. Recent research estimates that there are 390 million dengue infections worldwide and 21,000 deaths due to severe dengue every year [10]. Generally, dengue viruses are associated with only mild illness during inter-epidemic periods (silent transmission), however, major epidemics occur every 3–5 years [11]. This interannual periodicity occurs because, after large epidemics, subsequent large epidemics do not reoccur for some time as a result of long-term protection against the infecting serotype and transient cross-protection to other serotypes [12]. The population remains susceptible to future outbreak/s due to waning of this transient immunity and the low level of herd immunity. Low herd immunity occurs due to vector control activities, which protects the people from the bite of *Aedes* mosquitoes, thereby preventing people from acquiring natural immunity [13].

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Climate plays a major role in the global and local spread of dengue infection [6, 14]. Epidemic transmission of dengue fever is strongly correlated with fluctuations in rainfall, temperature, relative humidity and vegetation types. Changes in these variables affect the population dynamics of mosquito vectors and the consequent risk of dengue transmission [14]. Increased precipitation during the monsoon season facilitates vector population growth by providing aquatic media for mosquitoes, although excessive rainfall can lead to flushing out of breeding sites and killing of mosquito larva [15, 16]. In some urban regions, stable larval habitats are provided by large domestic water tanks that are used to manage periodic water shortages [17]. Increased temperature due to global warming has been shown to enhance the transmission of dengue by favouring virus replication, proliferation and feeding behaviour of the vector [18]. Conversely, extreme hot weather may reduce transmission by increasing the mosquito mortality rate [19]. Increasing relative humidity is associated with increased oviposition, greater vector survival and larger numbers of infected mosquitoes [20]. Vegetation cover (as indicated by land surface greenness) influences the risk dengue transmission as vegetation provides resting or breeding sites for mosquitoes [21]. However, previous studies have reported inconsistent relationships between greenness indices and dengue incidence; some studies indicating a positive association [22], while others finding a negative association [23]. Choi et al. also showed that the relationship between dengue incidence and climatic variables varies by locality, suggesting that climate-based early warning should be established at the local scale [24]. Hence, understanding the local spatial and temporal dynamics of dengue, and recognizing the relationship between dengue incidence and climatic variables is essential to target surveillance and control efforts in a given area.

Various analytical approaches have been used to describe the spatio-temporal dynamics of dengue transmission using climate and environmental variables. Most of those studies have adopted a frequentist approach of analysis, where parameters are derived from the likelihood alone [20, 25]. Recently, Bayesian methods have been used to analyse the spatio-temporal distribution of dengue transmission. Bayesian methods have the flexibility of incorporating prior knowledge (including in relation to the spatial and temporal structure of the data), and to model spatial (and temporal) correlation simultaneously with the effect of covariates [26]. The Bayesian approach has the advantage of fully quantifying uncertainty in the estimates and the capability of dealing with small sample sizes [27]. Here, we used a Bayesian approach to analyse the spatio-temporal distribution of dengue in Bhutan to assist dengue control programmes to allocate limited resources to areas with high dengue transmission.

In Bhutan, dengue viruses have been continuously circulating for nearly two decades with the first report of dengue in 2004. The first documented outbreak of dengue was reported in the Phuentsholing sub-district in Chukha district, which shares a porous border with India [28]. By 2018, seven other neighbouring districts also reported dengue, either as an isolated case or as outbreaks. Past dengue outbreaks were associated with co-circulation of DENV-1–3, while DENV-4 is not yet detected in the country [28, 29].

Dengue is one of the important disease managed by the Vector-borne Disease Control Program (VDCP), of the Ministry of Health since 2003. The VDCP supports, coordinates and evaluates vector control activities in district hospitals and Basic Health Units (BHUs) [30]. All health centres in Bhutan follow the same guidelines and policies developed by the Ministry. Vector surveillance and control is the primary tool used to prevent and control dengue in Bhutan, through source reduction of mosquitoes, indoor residual spraying (IRS) of insecticide, thermal fogging, and health promotion activities. Separate to this, dengue disease surveillance is led by Royal Centre for Disease Control (RCDC), another division in the Ministry of Health. This system is designed to detect cases early and provide an efficient and timely response for control of the disease.

This study was undertaken to investigate the spatial and temporal patterns of dengue in Bhutan and quantify the role of local environmental factors in dengue fever transmission. This is the first study in the country to explore relationships between climate, and other environmental factors, and dengue incidence.

Materials and methods

Study setting

Bhutan is one of Asia's smallest nations, situated in the southern slopes of the eastern Himalayas bordering China in the North and India in the South, West and East. The country has a total area of 38,394 square kilometres and lies between 27° 30' N and 90° 30' E. Bhutan is administratively divided into 20 districts, which are further broken down into 205 sub-districts.

Seven districts where dengue transmission has been documented were included in the study. These districts include 82 sub-districts (Figure 1).

Data sources

Dengue cases

Weekly numbers of dengue cases were obtained from the National Early Warning, Alert, Response and Surveillance (NEWARS) database housed in the RCDC and aggregated at monthly intervals. NEWARS is an integrated online disease surveillance system that collates the notification of prioritized national notifiable

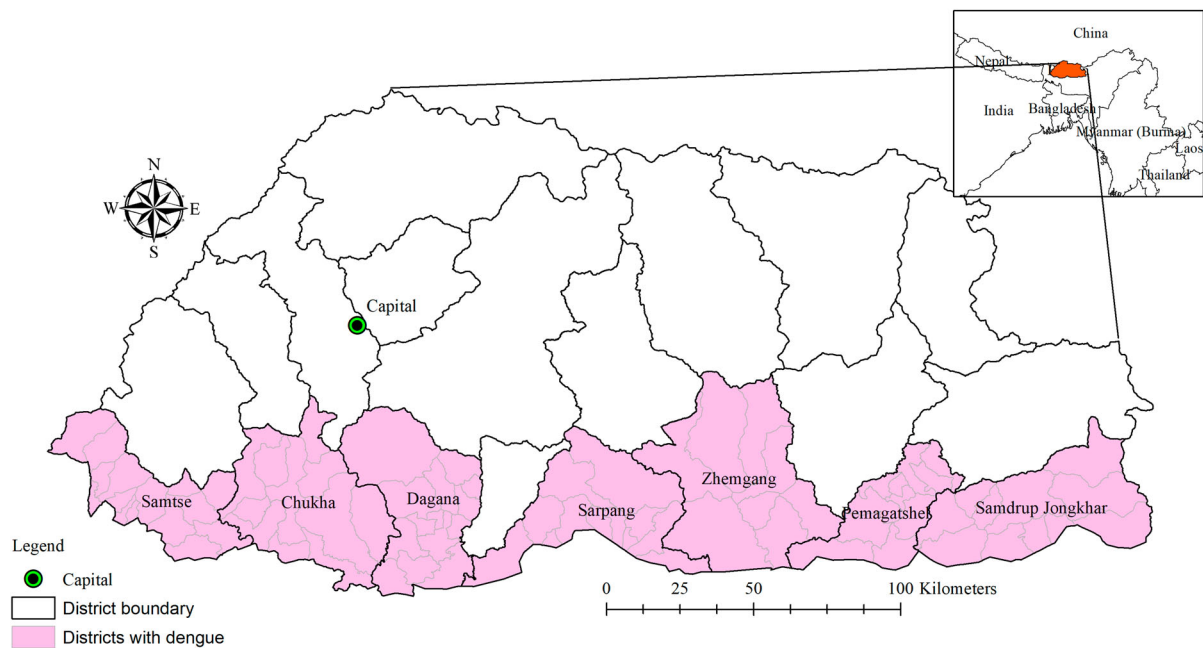


Figure 1. Administrative map of Bhutan and districts with documented dengue.

diseases and syndromes, including dengue, from health centres across the country. The reporting can be done either through an online web-based system or a mobile-based short message service (SMS) to report cases from remote places with limited internet access. All dengue fever cases are required to be reported into NEWARS from all health centres every week. Dengue cases are defined as any patients presenting fever with any two of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, positive tourniquet test and any warning signs of severe dengue (abdominal pain, persistent vomiting, mucosal bleeding, fluid accumulation, liver enlargement and increasing haematocrit value) [11]. Cases included in the study were all confirmed by rapid diagnostic tests and clinical-epidemiological criteria (those living in a dengue-endemic area or those with a recent history of travel to a dengue-endemic area and developing the symptoms described above). Clinicians and other health professionals are trained in the identification and reporting of dengue. When reporting to NEWARS, all cases are classified into following age groups: 1–29 days, 1–11 months, 1–4 years, 5–9 years, 10–14 years, 15–19 years, 20–24 years, 25–49 years, 50–64 years and ≥ 65 years. In our study, cases were categorized into two groups: ≤ 14 years and > 14 years.

Environmental and climate variables

Ten-day composites of 250-m spatial resolution normalized difference vegetation index (NDVI) data for the study period were obtained from the U.S. Geological Survey's (USGS) Earth Resources Observation and Science (EROS) Moderate Resolution Imaging Spectroradiometer (eMODIS) (<https://earthexplorer.usgs.gov/>) [31]. NDVI indicates vegetation greenness and is often

used as a proxy for mosquito-favoured habitats [22]. Prior to analysis, three 10-day composited raster images were used to obtain mean NDVI values by monthly windows for the study area. The spatial analyst tool (zonal statistics) in the geographical information system (GIS) software ArcGIS version 10.5 (ESRI, Redlands, CA) was used to extract the means for each sub-district [32].

Monthly data on climatic variables (rainfall, relative humidity, minimum and maximum temperature) were obtained from the National Centre for Hydrology and Meteorology of the Royal Government of Bhutan. The Centre collects data on these climatic variables from weather stations located in each district. Average monthly data of the district were calculated for each month of the study period from the daily records. District climatic variables were interpolated to the sub-districts within each district because sub-districts climatic records are unavailable. Population of the sub-districts was obtained from the National Statistical Bureau, Royal Government of Bhutan [33].

Crude standardized morbidity ratios

Raw standardized morbidity ratios (SMR) were calculated for each sub-district using the following formula:

$$Y_i = \frac{O_i}{E_i}$$

where Y_i is the SMR for the i th sub-district, O_i is the observed number of dengue cases in the i th sub-district, and E_i is the expected number of dengue cases in the i th sub-district. The expected number of dengue cases was calculated by multiplying the national incidence of dengue by the population of the sub-district during the study period.

Exploration of temporal trends of dengue

The monthly mean number of dengue cases was calculated for the full-time series (January 2016 to June 2019). The time series was then decomposed into three temporal components using locally-weighted regression or loess: seasonality, trend and residual variability [34]. The data, the trend component, the seasonal component and the residual component are denoted by Y_t , T_t , S_t and R_t , respectively, for $t=1$ to N . The formula for the loess model is:

$$Y_t = T_t + S_t + R_t$$

The function *stl* and parameter setting *periodic* were used to decompose the time series data and extract all the above parameters using R studio [35]. In the final model, a logarithmic transformation was used to assess the significance level of the trend.

Spatio-temporal models

An initial univariate Poisson regression analysis was performed to select covariates, with number of dengue cases as the dependent variable and NDVI and climatic variables with 0, 1, 2 and 3 months lag as independent variables. Lags for each variable with the lowest Akaike Information Criterion (AIC) [36] and a significant Incidence Rate Ratio (IRR) with a p -value <0.05 were selected. IRR was calculated by exponentiating the estimated regression coefficients from the Zero-inflated Poisson analysis output [37]. The collinearity of the selected covariates (temperature, humidity and NDVI) was tested using variance inflation factor (VIF) diagnostic tool [38]. Covariates with a VIF >4.0 were considered to be collinear and were removed from the final model. In this study, the final model contained rainfall and relative humidity lagged at 2 months, maximum temperature without lag, and NDVI lagged at 1 month (Supplementary Table).

The number of cells containing zero counts in our dataset was 6,775 (98%). So, the analysis was done to choose the best fitting model using AIC and Bayesian Information Criteria (BIC) [36]. Zero-inflated Poisson (ZIP) regression showed better fit over standard Poisson regression with lower AIC and BIC. A significant difference was observed between the two models as demonstrated by the Vuong test (Supplementary Table). ZIP regression models were constructed in a Bayesian framework. Model I contained independent variables (age, rainfall, maximum temperature, relative humidity and NDVI), unstructured and spatiotemporal random effects for the sub-districts (i.e. no spatial autocorrelation was assumed in the relative risk of dengue, but was assumed in the values of the district-level trends); in Model II, the unstructured random effects were replaced with spatially structured random effects but otherwise contained all of the same model

components (described below); and the final Model III included all of the components of Model I and Model II, that is: unstructured, structured and spatio-temporal random effects.

In the last model, dengue incidence in sub-district i ($1, \dots, 82$), month j was modelled using ZIP regression as follows:

$$P(Y_{ij} = y_{ij}) = \begin{cases} \omega + 1(1 - \omega)e^{-\mu_{ij}}, & y_{ij} = 0 \\ (1 - \omega)e^{-\mu_{ij}} \mu_{ij}^{y_{ij}} / y_{ij}!, & y_{ij} > 0; \end{cases}$$

$$Y_{ij} \sim \text{Poisson}(\mu_{ij})$$

$$\log(\mu_{ij}) = \log(E_i) + \theta_{ij}$$

$$\theta_{ij} = \alpha + \beta_1 \text{Age} + \beta_2 \text{Rainfall} + \beta_3 \text{Tmax} + \beta_4 \text{Humid} + \beta_5 \text{NDVI} + u_i + v_{ij} + w_{ij}$$

where y_{ij} is the number of dengue cases in $i=1, \dots, 82$ sub-districts and month j ; ω indicates the probability of being an excess zero, μ_{ij} is the mean number of cases, E_i is the expected number of cases (acting as an offset to control for population size); θ_{ij} is the log relative risk of dengue; α is an intercept; $\beta_1, \beta_2, \beta_3, \beta_4$, and β_5 , are the coefficients for age (over 15 years as a reference and assigned 0), rainfall lagged at 2 months, maximum temperature ($^{\circ}\text{C}$) without lag, relative humidity lagged at 2 months and NDVI lagged at 1 month respectively; u_i are unstructured random effects with a zero mean and variance σ_u^2 , v_i are spatially structured random effect with a zero mean and variance σ_v^2 and w_{ij} is the spatiotemporal random effect with a mean of zero and variance of σ_w^2 .

A conditional autoregressive (CAR) prior structure was used to model the spatially structured and spatiotemporal random effects. Spatial relationships between sub-districts were structured using an adjacency weight matrix, with a weight of “1” for contiguous and “0” for non-contiguous sub-districts. We specified a flat prior distribution for the intercept and a normal prior distribution for the random effects (mean of “0” and precision, inverse of variance, set at 0.0001). The priors for the precision of unstructured, structured and spatiotemporal random effects (inverses of variances shown above), were specified using non-informative gamma distributions with shape and scale parameters equal to 0.001.

An initial burn-in of 10,000 iterations was run, which was discarded. Model fitting was carried out using three separate chains starting at different initial values. Convergence was assessed by visualization of posterior density and history plots, and by computing the Gelman and Rubin diagnostic. After burn-in, subsequent blocks of 10,000 iterations were run and inspected for convergence. Thinning by a factor of 20 was specified after 40,000 iterations due to poor mixing of the chains and within-chain

autocorrelation. Convergence occurred at approximately 100,000 iterations for each model. The posterior distributions of each model parameter were stored following convergence and summarized for the analysis using posterior mean and 95% credible intervals (95% CrI). The model with the lowest deviance information criterion (DIC) was selected as the best-fit model.

In all the analyses, an α -level of 0.05 was adopted to indicate statistical significance (as indicated by 95% CrI for relative risks that excluded 1). Time series analysis with cross-correlations and univariate regression analysis was performed using Stata statistical software (version 15.1; Stata Corp, College Station, Texas, USA) [39]. The Bayesian models were developed using the WinBugs statistical software version 1.4.3 (Medical Research Council, Cambridge, UK) [40]. Choropleth maps of SMR and the random effects were produced using ArcGIS version 10.5 with shapefiles [32].

Ethical clearance for this study was approved by the Research Ethics Board of Health (REBH), Ministry of Health, Bhutan (REBH/Approval/2019/040) and Australian National University (ANU), Australia (2019/816).

Results

Descriptive statistics

A total of 708 dengue cases were notified to NEWARS from January 2016 to June 2019. The age-specific incidence rate of dengue was 155 per 100,000 inhabitants aged ≤ 14 years, 263 per 100,000 inhabitants aged > 14 years. Chukha district reported the highest number of dengue cases followed by Samdrup Jongkhar and Samtse, with means of 0.69 (range: 0–72), 0.43 (range: 0–76) and 0.18 (range: 0–40) cases per month respectively (Table 1). The distribution of dengue varied greatly between sub-districts, with incidence rate ranging from zero to as high as > 90 per 100,000 inhabitants each year during the study period (Figure 2).

The monthly averages of rainfall, maximum temperature, relative humidity and NDVI of the study districts were 7.95 mm, 25.08°C, 73.96% and 0.51 respectively. The mean maximum temperature was

highest in Chukha and Samtse districts (29.29°C and 28.47°C respectively), while these districts had the lowest NDVI values (0.46 and 0.41 respectively) (Table 1). There was wide spatial variation of SMR for dengue during the study period. The general pattern showed high SMR along the sub-districts located in the southern belt that shares a common boundary with India. Sub-districts with high SMR values (above 14.28) were Phuentsholing, Samtse and Dewathang (Figure 3).

Time series decomposition

The raw data exhibited a seasonal pattern with an apparent increase in 2016 and 2017. The seasonal decomposition plot demonstrated strong seasonality with a peak occurring between July and September each year. The inter-annual pattern showed a large peak in 2017 and an apparently increasing trend in 2019. The large grey bar in the third panel shows that the variation in the trend component is small as compared to variation in the data and seasonality component. The residuals in the fourth panel showed slightly elevated variability in the earlier years (2016–2017) and less apparent variability in the subsequent years (Figure 4).

Spatio-temporal model

We chose the mixed model containing the unstructured, structured and spatiotemporal random effects (Model III). Whilst this model didn't have the lowest DIC, it had a DIC value nearly as low as the unstructured model (Model I) and provided more information on the patterns of dengue risk. Individuals aged ≤ 14 years were found to be 53% (95% CrI: 42%, 62%) less likely to have dengue infection than those aged > 14 years. Dengue cases increased by 63% (95% CrI: 49%, 77%) for a 1°C increase in maximum temperature; and decreased by 48% (95% CrI: 25%, 64%) for a one unit increase in NDVI (Table 2). Mapping of the spatially structured random effect (v_i) showed higher mean dengue risk in Pemagatshel, Samdrup Jongkhar and some part of Zhemgang district (Figure 5(a)). The unstructured random effect (u_i) unsurprisingly

Table 1. Distribution of monthly means of dengue fever cases and climate and environmental variables in Bhutan, January 2016–June 2019.

Districts	No. of sub-districts	Mean (Minimum–Maximum)				
		Cases	Rainfall (mm)	Maximum temperature °C	Relative humidity (%)	NDVI
Chukha	11	0.69 (0.00–72.00)	11.52 (0.00–50.23)	29.29 (23.61–32.73)	71.30 (31.56–88.67)	0.46 (–0.01–0.98)
Dagana	14	0.01(0.00–3.00)	2.55 (0.00–15.37)	21.24 (11.32–26.50)	66.71 (44.43–92.80)	0.53 (0.01–0.95)
Pemagatshel	11	0.01(0.00–1.00)	3.52 (0.00–23.72)	21.81 (14.48–26.56)	82.60 (73.67–90.25)	0.61 (0.02–0.91)
Samdrup Jongkhar	11	0.43 (0.00–76.00)	7.79 (0.00–30.02)	25.41 (20.11–29.98)	74.38 (47.74–93.51)	0.47 (–0.01–0.88)
Samtse	15	0.18 (0.00–40.00)	12.62 (0.00–47.18)	28.47 (21.40–32.08)	71.00 (47.22–90.54)	0.41 (–0.06–0.98)
Sarpang	12	0.12 (0.00–13.00)	12.58 (0.00–79.73)	27.41 (21.60–33.60)	80.20 (60.25–93.03)	0.48 (–0.01–0.97)
Zhemgang	8	0.02 (0.00–2.00)	3.19 (0.00–19.01)	20.29 (12.51–26.84)	73.88 (54.74–89.64)	0.56 (0.02–0.92)
Overall	82	0.21(0.00–76.00)	7.95 (0–79.73)	25.08 (11.32–33.60)	73.96 (31.56–93.51)	0.51 (–0.06–0.98)

revealed a random distribution of posterior means (Figure 5(b)).

Dewathang sub-district located in the eastern part of the region showed >95% probability of a higher than national average trend. There was >50% probability of higher than national average trend in 26 sub-districts, that are mostly located along the eastern region, indicating a higher rate of increase in the incidence of dengue in this part of the country (Figure 6).

Discussion

The analysis demonstrated that the risk of dengue infection in those aged >14 years was significantly higher than in those aged ≤14 years. A similar

increased in the risk of older people was observed in other countries of the WHO-South-East Asia Region (SEAR) such as Indonesia [41], Thailand [42] and Nepal [43]. This may be because adult populations are engaged more in outdoor activities that render them vulnerable to dengue mosquito bites [44]. In addition, dengue infection in adults mostly results in symptomatic cases as compared to children, in whom up to 95% of infections had been reported to mild or sub-clinical [45]. Paediatric patients are less likely to manifest dengue-like illness including warning signs as compared to older patients. In a retrospective study conducted by Byrne et al. (2017), fever was absent in 25% of the children below 15 years of age, that were laboratory

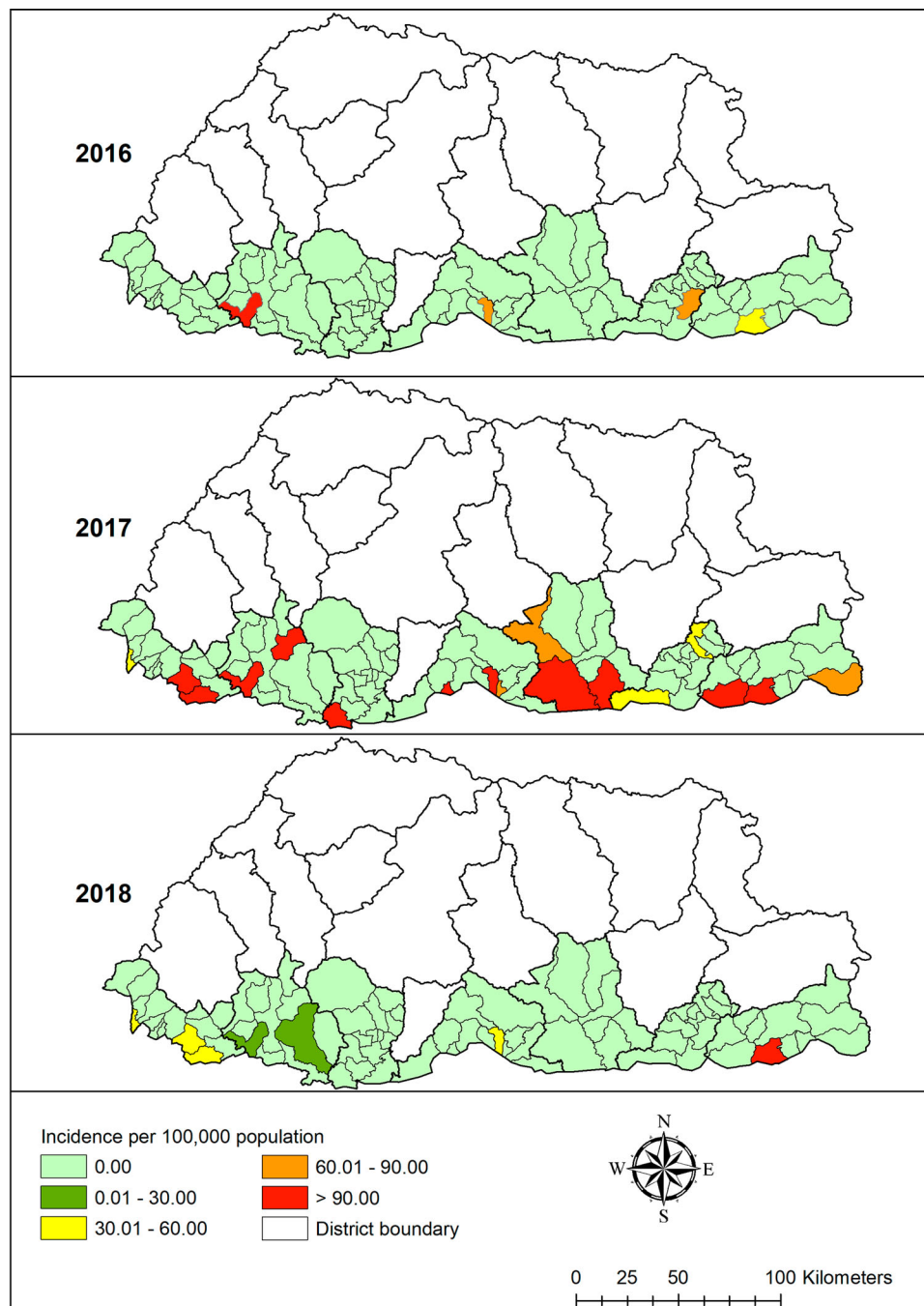


Figure 2. Dengue incidence rates by sub-districts, Bhutan, 2016–2018

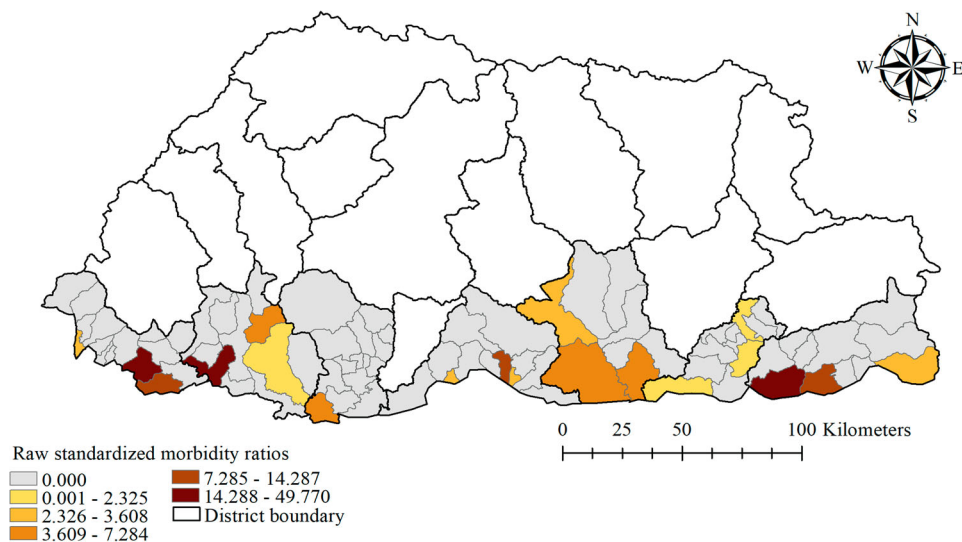


Figure 3. Raw standardized morbidity ratio of dengue by sub-districts in Bhutan, January 2016–June 2019

confirmed for dengue [46]. As a result, diagnosis of dengue among those children are likely to be overlooked and not reported through the surveillance system.

A clear seasonal pattern was observed during the study period. Time series analysis showed dengue as a highly seasonal disease with peaks observed in the

monsoon, which occurs from June to August each year (Supplementary figure). The monsoon is correlated with an extended period of rainy and wet days, and higher temperatures, which are favourable for mosquito breeding and population expansion [15, 16]. The finding is consistent with studies conducted in Indonesia [22], Timor-Leste [47] and Sri Lanka

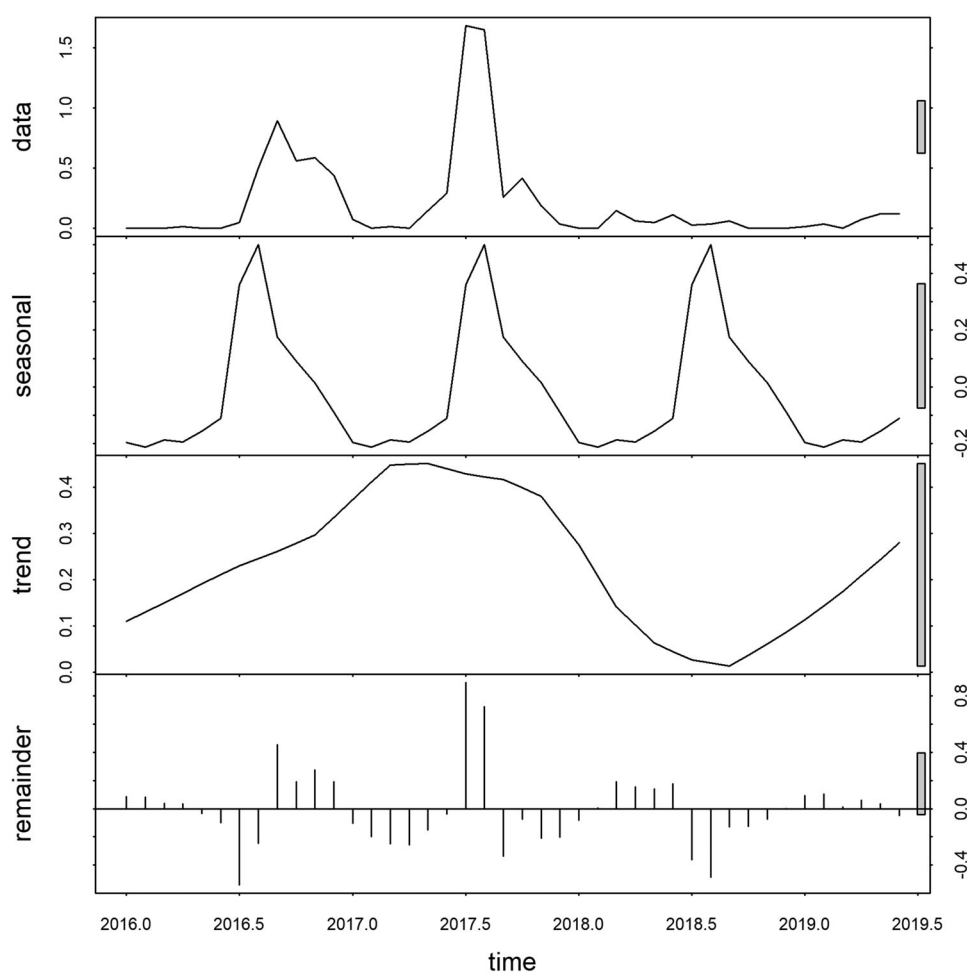


Figure 4. Temporal decomposition of numbers of dengue cases of Bhutan, January 2016–June 2019.

Table 2. Regression coefficients, relative risk and 95% credible interval from Bayesian spatial and non-spatial models of dengue cases in Bhutan, January 2016–June 2019.

Model/Variable	Coeff, posterior mean (95% CrI)	RR, posterior mean (95% CrI)
Model I (Unstructured)		
α (Intercept) ^a	−4.936 (−7.052, −3.481)	
Age (Above 14 years as base)	−0.752 (−0.969, −0.539)	0.471 (0.379, 0.582)
Mean monthly trend	1.023 (0.764, 1.325)	2.782 (2.146, 3.762)
Rainfall lagged 2 months (mm)	0.044 (−0.043, 0.132)	1.036 (0.965, 1.113)
Relative humidity lagged 2 months (%)	0.053 (−0.144, 0.252)	1.004 (0.988, 1.021)
Maximum temperature without lag (°C)	2.188 (1.824, 2.564)	1.619 (1.495, 1.759)
NDVI lagged 1 month	−0.666 (−1.036, −0.305)	0.514 (0.355, 0.737)
Probability of extra zero	0.702 (0.641, 0.758)	2.017 (1.898, 2.133)
Heterogeneity ^a		
Unstructured	0.112 (0.043, 0.221)	
Structured (trend)	23.88 (1.101, 152.600)	
DIC	1421.200	
Model II (Structured)		
α (Intercept) ^a	−4.699 (−6.413, −3.368)	
Age (Above 14 years as base)	−0.752 (−1.208, −0.319)	0.472 (0.298, 0.726)
Mean monthly trend	1.041 (0.541, 1.546)	2.832 (1.717, 4.693)
Rainfall lagged 2 months (mm)	0.046 (−0.134, 0.224)	1.038 (0.897, 1.199)
Relative humidity lagged 2 months (%)	0.045 (−0.351, 0.439)	1.004 (0.972, 1.036)
Maximum temperature without lag (°C)	2.254 (1.270, 3.345)	1.644 (1.323, 2.091)
NDVI lagged 1 month	−0.636 (−1.614, 0.283)	0.529 (0.199, 1.328)
Probability of extra zero	0.704 (0.629, 0.770)	2.022 (1.878, 2.159)
Heterogeneity ^a		
Structured (spatial)	0.044 (0.735, 39.700)	
Structured (trend)	7.048 (0.018, 0.086)	
DIC	1529.61	
Model III (Mixed)		
α (Intercept) ^a	−4.779 (−6.764, −3.468)	
Age (Above 14 years as base)	−0.753 (−0.972, −0.540)	0.471 (0.378, 0.582)
Mean monthly trend	1.023 (0.758, 1.327)	2.782 (2.133, 3.769)
Rainfall lagged 2 months (mm)	0.045 (−0.043, 0.133)	1.037 (0.966, 1.114)
Relative humidity lagged 2 months (%)	0.050 (−0.151, 0.249)	1.004 (0.988, 1.020)
Maximum temperature without lag (°C)	2.199 (1.818, 2.596)	1.625 (1.493, 1.772)
NDVI lagged 1 month	−0.663 (−1.040, −0.290)	0.515 (0.353, 0.748)
Probability of extra zero	0.703 (0.642, 0.759)	2.019 (1.900, 2.135)
Heterogeneity ^a		
Unstructured	0.126 (0.047, 0.259)	
Structured (spatial)	275.700 (0.593, 1843)	
Structured (trend)	38.740 (1.142, 349.500)	
DIC	1423.63	

Abbreviations: coeff: coefficients; CrI: credible interval; RR: relative risk; DIC: deviance information criterion.

^aCoefficient.

[48], which demonstrated an increasing number of cases during the monsoon and hottest season.

Time-series analysis also demonstrated large inter-annual variability with dramatic increase of dengue incidence in 2017. Such an increase can be explained by the geographical expansion of dengue to new geographical regions. From 2017 onwards, dengue cases were reported from Samtse, Samdrup Jongkhar, Pemagatshel, Sarpang, Dagana, Samtse and Zhemgang districts in addition to Chukha district. These districts are located along the international border with India. One plausible reason is importation of dengue from India across a highly porous border. Dengue cases in Bhutan increased following an upsurge of dengue cases in India along the international border [49]. The people living along the border areas in these districts freely cross to India during the day time and possibly got exposed to dengue. Other studies also found a higher incidence of dengue in districts bordering India [50].

From maps of the model posterior outputs, it is clear that cases in the newly affected areas are increasing

with the highest risk observed in a recently dengue infected sub-districts. In particular, Dewathang sub-district had the highest upward trend as compared to other sub-districts. Plausible reasons could include unplanned urbanization and development in the area in addition to favourable climate for dengue because of its lower altitude. Additionally, NK Darranga is one of the fastest-growing Indian towns along the Indo-Bhutan border and is located adjacent to Samdrup Jongkhar Town in Bhutan, with the towns being a walkable distance from each other. Individuals are allowed to move freely for cross-border shopping and employment opportunities. The developmental activities and the frequent migration of people across the border could have facilitated the transmission of dengue fever [43, 51]. All this evidence suggests that surveillance should be strengthened in the country with focus on point of entry in collaboration with Indian counterparts.

Maximum temperature was strongly associated with dengue incidence in our model. A temperature of more than 16°C is required for many tropical species of

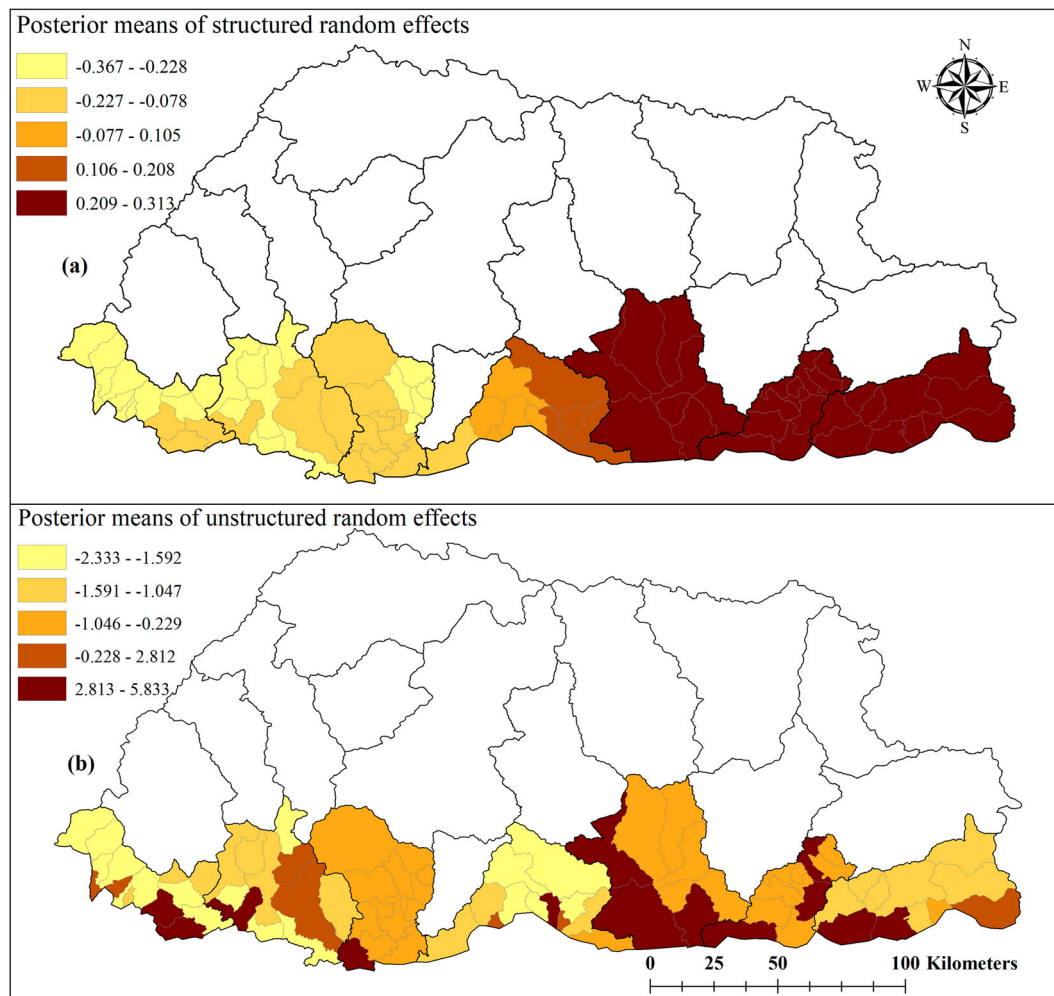


Figure 5. Spatial distribution of posterior means of structured (a) and unstructured random effects (b) in Bhutan, January 2016–June 2019 based on a Bayesian spatiotemporal model.

mosquitoes to complete their life cycles [52]. The threshold survival temperature for dengue virus is estimated at 11.9°C, while the optimum survival temperature for *Aedes* mosquitoes ranges from 22 to 26°C, and vector cease to feed when temperature falls below 17°C [20, 53]. Optimum temperatures prolong the life

expectancy of mosquitoes and facilitates their dispersal across geographical regions [54]. Higher temperature also reduces the extrinsic incubation period (EIP) of DENV in *Aedes* mosquitoes and promotes viral transmission [20, 47, 55]. The shortening of the EIP can increase the proportion of infectious *Aedes* mosquitoes

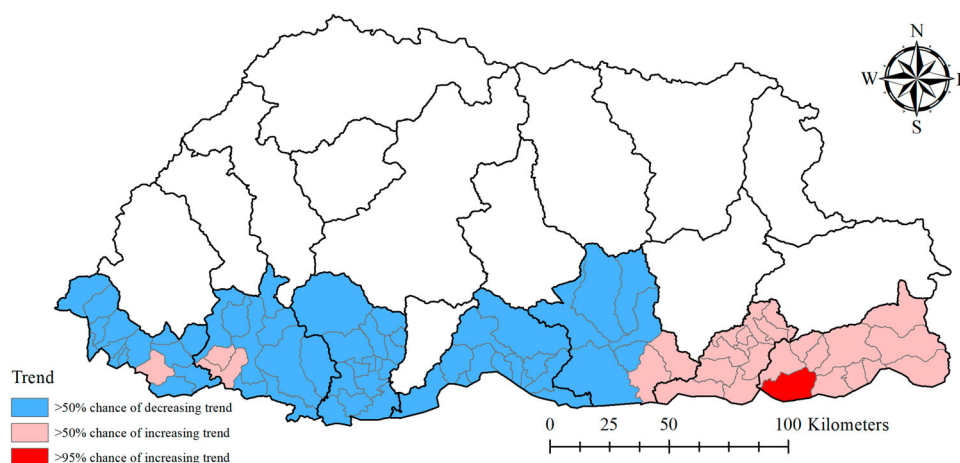


Figure 6. Trend analysis of dengue incidence in Bhutan, January 2016–June 2019, based on the spatiotemporal random effects of a Bayesian model.

before they die. Further, warmer temperature speeds up the gonotrophic cycle and makes *Aedes* mosquitoes more aggressive, which increases the biting rate and frequency of dengue virus transmission [56].

By contrast, NDVI was negatively associated with dengue incidence. The negative association between NDVI and dengue incidence was also reported in other studies [23, 57, 58, 59]. Lower NDVI is seen in areas with a higher population density (including urban and peri-urban areas), which tend to be areas that are favourable to *Aedes* mosquitoes [59]. Both *Ae. aegypti* and *Ae. albopictus* are detected in Chukha, Samtse and Samdrup Jongkhar districts, while other districts included in the study are known to have only *Ae. albopictus* [29].

This study is subject to a number of limitations. First, the time series data for this study was three years and six months in duration only, due to unavailability of earlier data. This short study duration might have affected the findings of our spatio-temporal model. Second, the passive surveillance data are subjected to under-reporting because sub-clinical dengue cases not seeking medical attention were unlikely to have been picked up by the surveillance system. Third, cases reported from each sub-districts or districts were not known of its source of infection, whether locally acquired within the sub-district or imported from other dengue endemic places. However, the number of cases presented here are based on the notification of data by health centres in each sub-districts or districts as per the existing surveillance system. Fourthly, district climatic factors were interpolated to the sub-district. This could have led to confounding of the results. But we believe that the climatic factors within a district to be generally homogenous due to small area of the districts. Finally, observed spatial and temporal variability might have been confounded by other factors such as socio-economic and ecological factors which are not included in the study. Future studies should include these variables to assess the true temporal relationship.

Overall, the number of notified dengue cases showed an increasing trend with strong seasonality observed during the monsoon season. The highest risk of dengue infections was observed in people aged >14 years. A significant association between dengue incidence and climatic factors indicates a crucial role for climatic conditions in driving the intensity of dengue virus transmission in the country. This finding could support the development of decision support tools that integrate climate data to establish climate-based early warning systems to provide informed decisions in implementing dengue control activities. High risk areas found in this study may be targeted for prioritizing allocation of resources to activities such as surveillance and vector control.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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4.3 Article on epidemiological analysis of dengue

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Article

Epidemiological Analysis of the 2019 Dengue Epidemic in Bhutan

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Abstract: Bhutan experienced its largest and first nation-wide dengue epidemic in 2019. The cases in 2019 were greater than the total number of cases in all the previous years. This study aimed to characterize the spatiotemporal patterns and effective reproduction number of this explosive epidemic. Weekly notified dengue cases were extracted from the National Early Warning, Alert, Response and Surveillance (NEWARS) database to describe the spatial and temporal patterns of the epidemic. The time-varying, temperature-adjusted cohort effective reproduction number was estimated over the course of the epidemic. The dengue epidemic occurred between 29 April and 8 December 2019 over 32 weeks, and included 5935 cases. During the epidemic, dengue expanded from six to 44 subdistricts. The effective reproduction number was <3 for most of the epidemic period, except for a ≈ 1 month period of explosive growth, coinciding with the monsoon season and school vacations, when the effective reproduction number peaked >30 and after which the effective reproduction number declined steadily. Interventions were only initiated 6 weeks after the end of the period of explosive growth. This finding highlights the need to reinforce the national preparedness plan for outbreak response, and to enable the early detection of cases and timely response.

Keywords: dengue; epidemic; Bhutan; dispersion; transmissibility



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1. Introduction

Dengue is an emerging vector-borne disease with a rapidly expanding global distribution and an increasing intensity of transmission in affected regions [1]. Globally, an estimated 390 million dengue infections occur each year and nearly 4 billion people are at risk of the disease [2,3]. A global dengue pandemic began in South-East Asia and the Pacific region during and after World War II [4]. The movement of troops during World War II, along with the destruction of the environment and human settlement has substantially contributed to the spread of dengue viruses and their vectors in these regions [5]. Climate change, economic booms (with uncontrolled urbanization and development), and unprecedented human travel have contributed to further spread of the dengue epidemic, which continues to encroach on new geographical locations [5–7]. Before the 1970s, only nine countries experienced severe dengue epidemics [8]. However, dengue is now endemic in over 140 countries in Africa, the Americas, the Eastern Mediterranean, and Asia [9]. In the South-East Asia Region, severe dengue is the leading cause of hospitalization and death among children [10]. For these reasons, the World Health Organization (WHO) has included dengue in the potential public health emergency of international concern (PHEIC) list under the current International Health Regulations (IHR) [11].

Dengue is caused by arthropod-borne flaviviruses belonging to one or more of the four known dengue virus serotypes (DENV1–4) [12]. The virus is transmitted through the

bites of an infective female *Aedes* mosquitoes, mainly *Aedes aegypti*, and to a lesser extent, *Aedes albopictus*. Both species feed during the daytime from morning until dusk, although night-time biting is also reported in the case of *Ae. albopictus* [13]. *Ae. aegypti* breeds both indoors and outdoors, while *Ae. albopictus* is well-adapted to the peridomestic environment and typically breeds outdoors [14].

Bhutan is a Himalayan country, bordering China in the north and India in all other directions. The climate near the border with China does not support the survival of mosquitoes and hence, there is no transmission of dengue. Transmission of dengue is observed mainly along the border with India, particularly along the southern foothills, where the climate is subtropical. Bhutan reported its first dengue outbreak in Phuntsholing (a subdistrict of Chukha) in 2004 with more than 2500 cases. From 2013, dengue cases began to be reported in other districts along the border with India, where both vector species of *Aedes* are found [15]. Except for DENV-4, all other DENV serotypes have been reported, with frequent changes in the dominant serotype [16]. The DENV-3 epidemic of 2019 was Bhutan's largest epidemic to date, with case numbers greater than the combined number reported in all previous years. At the peak of the epidemic, the government implemented a strategy of regular vector control, which included mass thermal fogging and public awareness messaging (on week 34), a mass cleaning campaign with a focus on larval source reduction (on weeks 34–37), travel advisory notifications to the general public (on week 35), and case-based indoor residual spraying and a postal health education campaign (on week 39).

Geographical information systems (GIS), combined with spatial analytical methods, have been increasingly utilized to collate, map, analyze, and interpret epidemiological information in relation to zoonotic and vector-borne diseases [17], and to facilitate better public health communication, collaboration, and decision making [18]. Spatiotemporal analysis utilizing GIS functionality can provide crucial information for control programs by allowing prompt assessment of patterns of the evolution of an outbreak. Additionally, the time-varying effective reproduction number (R_t) is used to quantify the transmissibility of infectious disease in a population over the course of an epidemic, and provides a measure of the strength of additional interventions required to halt the spread of a disease [19]. The transmission of vector borne diseases within a population depends on duration and degree of infectiousness, contact rate between vectors and hosts, and susceptibility of individuals. The combined effect of these variables are summarized by R_t , which is the average number of secondary cases produced by an index case over the course of its infectious period. In general, the number of cases will increase when $R_t > 1$ and decrease when $R_t < 1$ [20]. By maintaining $R_t < 1$, infectious diseases can be controlled and eradicated. Therefore, R_t can be used to estimate the intensity of control interventions necessary to contain an outbreak [21].

This epidemic provides an opportunity to analyze transmissibility and spatiotemporal patterns of emergence of dengue in real life situations where public health interventions are implemented simultaneously during outbreaks. The aim of this study was to quantify different spatial and temporal aspects of the dengue epidemic in Bhutan in 2019 to assist future epidemic management and planning. The objectives of the study were to explore the spatiotemporal characteristics of the epidemic and estimate the transmissibility of circulating dengue viruses.

2. Materials and Methods

2.1. Study Area

In Bhutan, elevation ranges from 160 m to more than 7000 m above sea level. The climate is humid and subtropical in the southern plains and foothills, temperate in the inner valleys of the central region, and cold in the northern Himalayas. There are four seasons in Bhutan. The dry spring season starts in early March and ends in late May. Summer weather commences in June, beginning with occasional showers, followed by heavy rain as it approaches the month of August. The rain continues in the Autumn season, which stretches from September to November. At higher elevations, this season is marked by

bright sunny days and some early snowfalls. From December, winter sets in with frost covering much of the country, and elevations above 3000 m above sea level are covered with snow [22].

Bhutan is administratively divided into 20 districts or *dzongkhags*. The population density in these districts ranges from as low as 1 person/km² in Gasa, to as high as 65 people/km² in Thimphu. In 2017, the mean population per district was 37,084 and ranged from 4086 to 146,466 people [23]. These districts are further broken down to a total of 205 subdistricts or *gewogs*.

2.2. Data Sources

We used both districts and subdistricts as the spatial units, and weeks as the temporal unit of analysis. The temporal unit is consistent with the existing reporting framework of the National Early Warning, Alert, Response and Surveillance (NEWARS). NEWARS is an integrated online disease surveillance system that collates data on all national notifiable diseases across Bhutan. All dengue cases are required to be reported into NEWARS from all health centers every week, while severe dengue or dengue hemorrhagic fever (DHF) has to be reported on the day of diagnosis. Dengue fever cases are defined as any patients with a history of travel or living in dengue-endemic areas presenting with fever and any two of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, positive tourniquet test, or any warning signs of severe dengue (abdominal pain, persistent vomiting, mucosal bleeding, fluid accumulation, liver enlargement, and increasing hematocrit). Severe dengue is characterized by one or more of the following: positive tourniquet test, petechiae/ecchymoses/purpura, bleeding (mucous, GI, or other), thrombocytopenia, or evidence of plasma leakage [11]. Serological confirmation of the cases was conducted using NS1 antigens and/or IgM/IgG antibodies in the respective health center using rapid test kits provided by the Ministry of Health. Both dengue fever and severe dengue cases were combined for this analysis as the numbers of severe dengue cases were small (Supplementary Table S1).

Population estimates used in the study were obtained from the recent 2017 Population and Housing Census of Bhutan [23]. The full epidemic was considered for the study in which the number of cases started to rise, peaked, and decreased to nonepidemic levels. This period ranged from week 18 (29 April) to week 49 (8 December).

2.3. Descriptive Analysis

The overall cumulative incidence was calculated for each district and subdistrict by dividing the total number of dengue cases reported by health centers located in that district and subdistrict by their respective population, multiplied by 10,000. The ages of the cases were categorized into four groups as follows: <9 years, 10–19 years, 20–49 years, and ≥50 years. These age categories were presented as a proportion of total cases in each district.

To visualize the changing spatial and temporal patterns of the dengue epidemic, separate maps were created at the subdistrict for four different time periods: weeks 18–25, 26–28, 29–34, and 35–49. The intervals between the time periods were created on the basis of the distribution of cases as per the phases of the epidemic curve explained in a supplementary analysis (Supplementary Material S1). The areas were classified as “newly affected”, “ever affected”, and “nonaffected” areas. “Newly affected” areas were defined as those subdistricts that have reported dengue for the first time during the time period, and these subdistricts were designated as “ever affected” in the subsequent time periods.

2.4. Growth Rate

The number of new cases of a disease often grows exponentially during the early stages of an epidemic and declines exponentially at the end of an epidemic. The exponential growth rate r —i.e., the per capita increase or decrease in the number of new cases per unit time—is a useful measure of the growth trajectory of an epidemic. Inspection of the number of new cases reported each week over the course of the epidemic revealed four

distinct phases in the epidemic. We estimated the value of the growth rate, r , for different stages of the epidemic by fitting a linear model to the log of the number of new cases reported each week during that phase, using the function *glm* from the *stats* R package [24].

2.5. Effective Reproduction Number

The effective reproduction number, R_t , for a vector borne disease is defined as the mean number of secondary cases arising from a typical primary case. Like the growth rate, the value of R_t can change over the course of an epidemic due to changes in environmental factors, human behaviors, healthcare responses, and the proportion of people with immunity to the disease.

When it is reasonable to assume that the serial interval, defined as the time between symptom onset in a primary case and a secondary case, does not change over the course of an epidemic, one can estimate the effective reproduction number as a simple function of the growth rate and the serial interval. We use such a method in a supplementary analysis (see Supplementary Material S1). However, as the length of the incubation period in mosquitoes is temperature-sensitive and therefore seasonally variable, for our primary analysis we estimated the time-varying, temperature-dependent cohort reproduction number using the method developed by Codeco et al. [25], which is a temperature-dependent extension of the method proposed by Wallinga and Teunis [26,27]. In brief, the method proposed by Wallinga and Teunis, which was first applied to SARS, used the distribution of the serial interval and the number of cases reported in each reporting period (e.g., each day or each week) to estimate for each case, the relative likelihood that they were infected by each case in the preceding reporting periods. The effective reproduction number for each reporting period was then estimated as the average number of cases thus attributed to each case in that reporting period. Codeco et al. extended this method to temperature-dependent vector-borne diseases by using observations of average daily temperature and the relationship between temperature and the extrinsic incubation period, to account for changes to the serial interval over time. They modeled the serial interval (measured in days) as the (truncated) sum of four independent periods: the intrinsic incubation period (gamma distributed with shape 16 and rate 2.7), the extrinsic incubation period (gamma distributed with shape 4.3 and rate $7.9 + 0.21 T$, where T is the average temperature in degrees Celsius), infectious period in humans and infection period in mosquitoes (exponentially distributed with mean 1). The serial interval distribution is truncated at 5 weeks, to account for the short lifespan of vectors.

We adapted this method and the published R code by Codeco et al. for our analysis. We calculated the time-dependent effective reproduction number for the whole country (i.e., using cases reported in all districts), and for Chukha only. Since cases were concentrated in Chukha district and the climatically similar southern districts, we used the (loess smoothed) weekly average daily temperature in Chukha to inform the temperature-dependent model of the extrinsic incubation period. To assess the effect of temperature-dependence on our estimates of the reproduction number, we also made temperature independent estimates of the effective reproduction number by adopting the counterfactual assumption that temperature was fixed at the average value across the epidemic period. As a sensitivity analysis exploring the effect of under-reporting in the early weeks of the epidemic, we calculated the temperature dependent time-varying reproduction number for Bhutan under the assumption that the proportion of dengue infections reported to NEWARS was up to 80% lower in the early weeks of the epidemic than the remainder of the epidemic (Supplementary Material S2).

3. Results

3.1. Descriptive Analysis

During the study period, 5935 cases of dengue were reported across the country. Of this, 26 (0.49%) cases were related to severe dengue. The epidemic curve showed a steep rise in cases from weeks 26 to 28 (Supplementary Figure S1). The overall cumulative

incidence for the entire country was 80.02 cases per 10,000 population. Chukha was the worst-affected district with a cumulative incidence of 607.55 cases per 10,000 population. Sarpang, Thimphu and Trashiyangtse followed next with ≈ 40 –60 per 10,000 population (Figure 1). At the subdistrict level, Phuntsholing, Bongo (in Chukha), Gelephu (in Sarpang), Lhamozingkha (in Dagana), and Khamdang (in Trashiyangtse) reported the highest incidences with 1204.1, 211.9, 137.3, 127.0, and 114.4 per 10,000 population, respectively (Figure 2). The maximum proportion was reported in the age group of 20–49 years in all districts followed by the 10–19 years age group (Figure 1). The overall proportion of age groups <9, 10–19, 20–49, and ≥ 50 years were 8.27%, 22.30%, 55.00%, and 14.43%, respectively (Table 1).

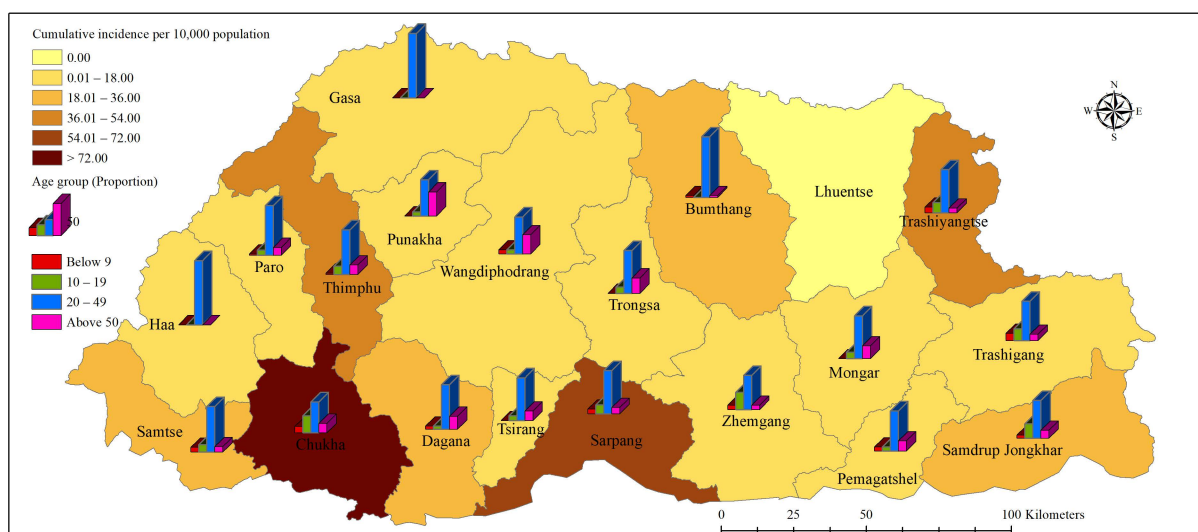


Figure 1. Distribution of cumulative incidence and proportion of cases by age groups as reported by health centers in each district during the dengue epidemic period in Bhutan, from weeks 18–49. The height of the bar corresponds to the proportionality of four age groups among dengue cases in each district.

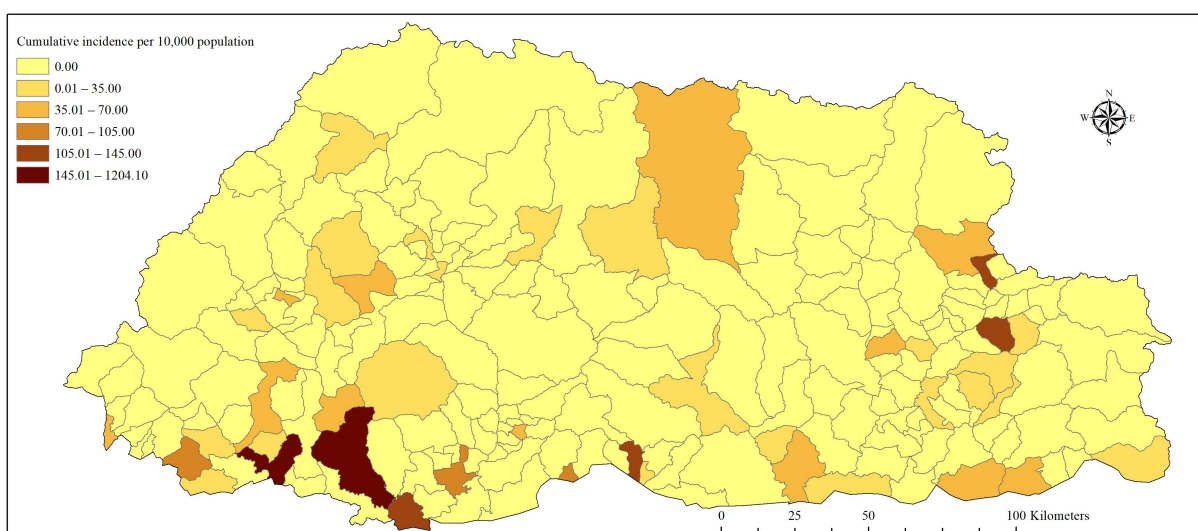


Figure 2. Distribution of cases by cumulative incidence by subdistrict during dengue epidemic in Bhutan, from weeks 18–49. Subdistricts with the darker color include Phuntsholing, Bongo, Gelephu, Lhamozingkha, and Khamdang subdistricts. Khamdang subdistrict in the top central-eastern region saw a cluster of cases (≈ 70 cases) for the first time in this epidemic.

Table 1. Proportion of affected dengue cases by age categories during the dengue epidemic in 2019, Bhutan (note: 688 records do not have information on age).

Age Categories (Years)	Number of Cases (<i>n</i>)	Proportion (%)
0–9	434	8.27
10–19	1170	22.30
20–49	2886	55.00
≥50	757	14.43

Only six subdistricts had ever reported cases before the 2019 epidemic. The cumulative number of newly affected subdistricts increased rapidly from zero in weeks 18–25 to eight in weeks 26–28, 22 in weeks 29–34 and eight in weeks 35–49. In total, 44 subdistricts were affected resulting in 5935 cases across a span of 32 weeks (Figure 3).

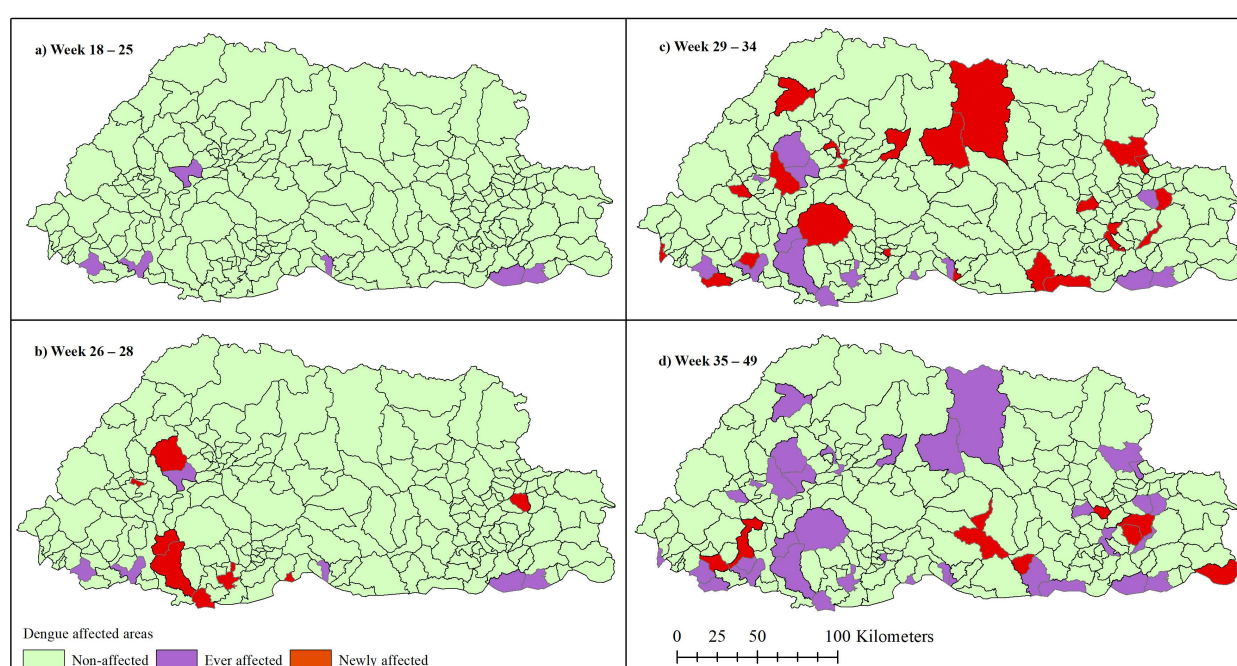


Figure 3. The changing spatiotemporal pattern of 2019 dengue epidemic in Bhutan at the subdistrict level. The epidemic initially started with six subdistricts as shown in the first panel in the top-left map (a). These subdistricts have also reported dengue in the past. The epidemic spread to 8, 22, and 8 new subdistricts in weeks 26–28 (b), 29–34 (c), and 35–49 (d), respectively, that summed to 44 subdistricts at the end.

3.2. Growth Rate

There were four apparent phases to the epidemic (Supplementary Figure S2, Supplementary Table S2). In the initial phase (weeks 18–25) the number of cases reported was <10 per week and the growth rate was slow ($r = 0.16$; 95%CI: -0.01 – 0.33). The second phase (weeks 25–28) was a short period of explosive growth ($r = 1.35$; 95%CI: 0.99 – 1.71), by the end of which there were >300 new cases reported each week. In the third phase (weeks 28–34) case numbers remained high, but growth slowed ($r = 0.12$; 95%CI: 0.07 – 0.17) to levels similar to phase 1. In the final phase (weeks 34–49), case numbers declined ($r = -0.29$; 95%CI: -0.32 – 0.27).

3.3. Effective Reproduction Number

The estimate of the effective reproduction number varied dramatically over the period (Figure 4). It was highest for cases that developed symptoms around epi-week 25, peaking at ≈ 30 ; however, the effective reproduction number declined sharply, reaching ≈ 1 in cases that developed symptoms around week 33 and continued to decline after this point

(Figure 4). The estimates of the effective reproduction number for Chukha-only and all of Bhutan exhibited the same temporal pattern; however, the reproduction was generally higher in the Chukha-only model and became <1 later in the Chukha-only model (week 34) than in the Bhutan model (week 32) (Figure 4). The weekly average temperature in Chukha ranged from 24 to 27 °C between weeks 18 and 39. The temperature-dependent and temperature-independent estimates of the effective reproduction number were largely the same; though in the Chukha-only models the peak effective reproduction number was somewhat higher (43 vs. 37) and earlier (week 24 vs. week 25) than in the temperature independent model (Figure 4). The alternate visualization of effective reproduction over the course of the epidemic is presented in Supplementary Figure S3. The peak value of R_t was sensitive to potential under-reporting. For instance, if we assumed that the reporting proportion (i.e., proportion of dengue infections reported to NEWARS) before week 27 was only 80%, 40%, or 20% of the proportion in week 27 and onward, the peak estimate of temperature-dependent R_t was reduced to 21 (95% CI: 19–24), 12 (95% CI: 10–13), or 6.2 (95% CI: 5.5–6.8), respectively, compared to 26 (95% CI: 23–29) when we assumed the reporting proportion was constant over the whole period (Supplementary Figures S4 and S5).

We were unable to use our methodology to measure the effectiveness of the wide-scale interventions initiated in week 34. However, we note that the wide-scale interventions began ≈ 6 weeks after the end of the period of rapid growth in case numbers and ≈ 10 weeks after the peak in the effective reproduction number (Figure 4). Similarly, we note that our estimates of the time-varying reproduction number are primarily descriptive in nature; we did not use our method to infer the underlying causes of variability. The relatively minor differences between temperature-dependent and temperature independent estimates do not imply that temperature had a minor or major role in dengue transmissibility during the epidemic.

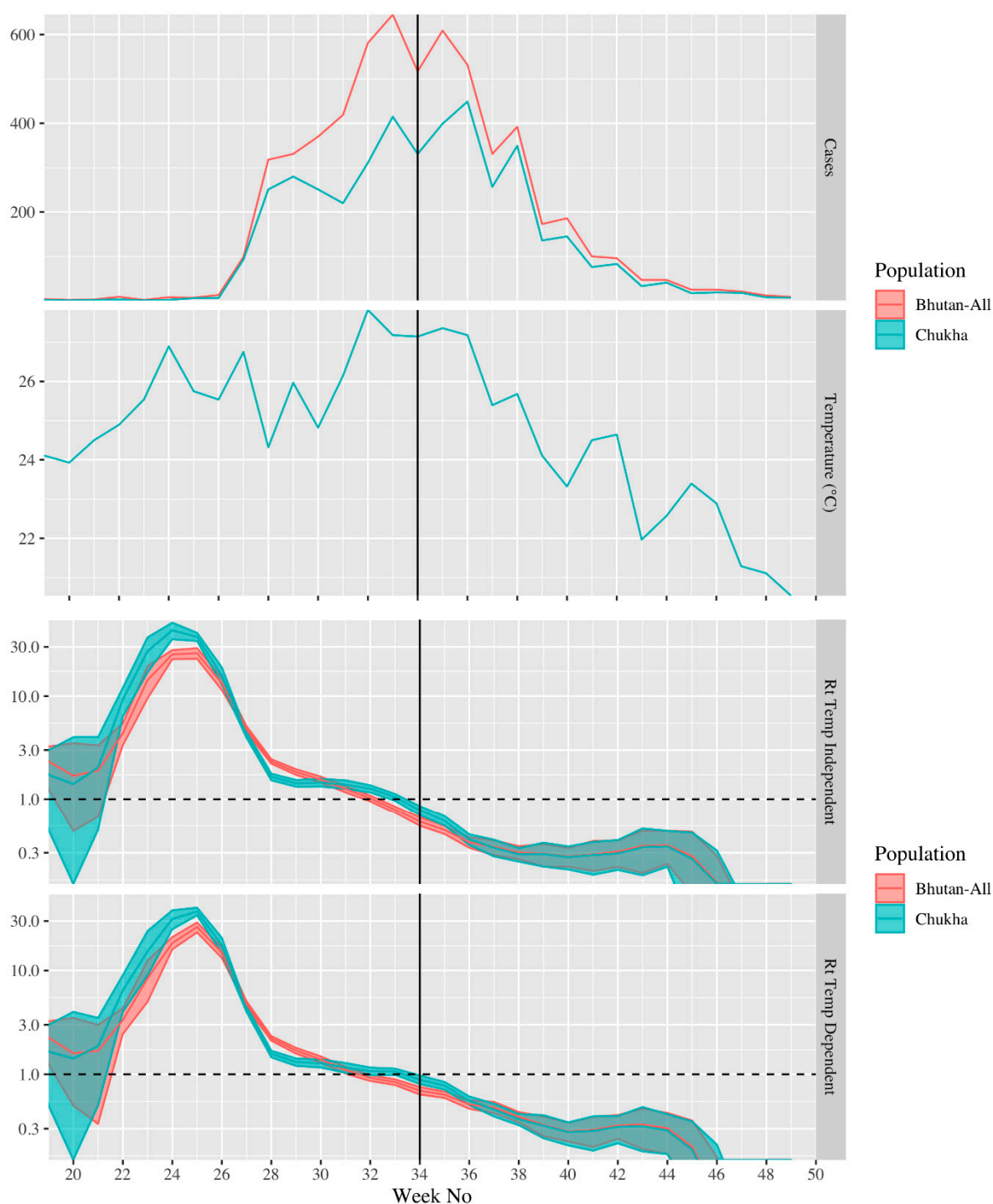


Figure 4. From top to bottom: the number of dengue cases reported in National Early Warning, Alert, Response and Surveillance (NEWARS) each week in all of Bhutan and Chukha only; the weekly average temperature (°C) in Chukha; the effective reproduction number for cases developing symptoms in each week, assuming constant temperature over the period; the effective reproduction number for cases developing symptoms in each week, accounting for changes in temperature over the epidemic. The solid vertical line indicates the start of interventions in week 34. The dashed horizontal line in the bottom two panels indicates the critical value of the effective reproduction number (1.0). In the bottom two panels, the shaded regions indicate 95% confidence intervals and the y-axis is on a log-scale for clarity.

4. Discussion

The 2019 epidemic was the first nation-wide dengue epidemic in Bhutan. The epidemic initially spread from low-lying areas in the southern foothills and diffused to other parts of the country, reaching 19 of the 20 districts at the end of the epidemic. There were 38 newly affected subdistricts during this epidemic in addition to the six previously-affected subdistricts. The estimate for the reproduction number was <3 for most of the epidemic period, except for a 1-month period of explosive growth at the start of the epidemic during which the reproduction number peaked >30 .

An explosive increase in cases, and very high reproduction number was observed between weeks 25 and 28, coinciding with the monsoon season which is characterized by increased rainfall and temperatures across the country (Supplementary Figure S6). Other countries have also observed increasing numbers of dengue cases during monsoon and warm seasons [28–30]. Rainfall increases the availability and productivity of breeding sites for female mosquitoes to lay eggs, and development of immature mosquitoes. Humidity maintains a conducive environment for the survival of adult mosquitoes. Humidity affects flight and host-seeking behavior, and lifespan of vectors [30]. The hot climate promotes dengue virus transmission by accelerating the biting rate, shortening the gonotrophic cycle and reducing the extrinsic incubation period [31].

An unprecedented geographical expansion of dengue was observed in the 2019 epidemic, expanding from six subdistricts with a history of sporadic cases of dengue outbreaks to a total of 44 (total of 205) subdistricts during this outbreak. The incidence among the affected subdistricts ranged from 1.8 to 1204.1 per 10,000 population. Human movement may have played an important role in the spread of dengue infection. Firstly, the outbreak season coincided with the school holidays which is generally associated with increased travel of students and parents from schools to their residential subdistricts and vice versa after the school holidays. Secondly, a majority of the cases were people aged 20–49 years. People in this age range are involved in economic activities and often travel from their subdistrict of residence to other subdistricts. For instance, people from other parts of the country travel to Phuentsholing Town located in Phuentsholing subdistrict to sell their farm produce and for other business purposes. Similarly, most of the goods and commodities entering Bhutan are ferried through this town. Historically and during this outbreak, Phuentsholing remained the epicenter of dengue epidemic. It is plausible for the people from other subdistricts to have got the infection from Phuentsholing during one of such visits and imported it to their subdistricts.

Local transmission in subdistricts is possible because *Ae. aegypti* had been reported in Chukha, Samtse, Samdrup Jongkhar, and Wangdiphodrang, while *Ae. albopictus* is present in 16 districts excepting Thimphu, Gasa, Bumthang, and Paro [15]. In addition, *Ae. aegypti* was detected in Khamdang subdistrict in Trashiyangtse district during this epidemic for the first time. Kraemer and colleagues have shown that even under current climatic conditions, continuous human movement will enable dengue vectors to spread and occupy suitable habitats, posing a risk to human health [32]. *Ae. aegypti* tend not to disperse far from households because human blood sources, mates, and places to lay their eggs are abundant around homes where they reside [33]. The limited flight range of mosquitoes means that rapid geographic spread of dengue during epidemics is more likely driven by the movement of viremic humans rather than the flight of infected mosquitoes [33].

Our results suggest that the dengue epidemic in 2019 consisted of four phases. After 3 weeks of explosive growth and 6 weeks of moderate transmission and consistently high case numbers, incidence peaked around week 34, coinciding with the beginning of interventions. However, decreasing rainfall and temperatures (Supplementary Figure S6), and naturally acquired immunity in the human population may have accounted for the reduction in transmission. Nevertheless, the size of the epidemic may have been smaller if these interventions were applied earlier, for instance around weeks 25–28 after the rapid growth of cases. However, late initiation of public health responses when the transmission is near its peak, or has started to decline, is a common issue in many countries [34].

We estimated that the effective reproduction number was approximately <3 for most of the epidemic prior to the onset of interventions, except for a 1-month period of explosive growth approximately one serial interval before the rapid rise in cases numbers, where the effective reproduction number was >10 . Our estimate of the effective reproduction number in the periods of slower growth is similar to estimates of the basic reproduction number in some countries, such as Singapore ($R_0 \approx 1.89$ to 2.23 , 2005) [34], Taiwan ($R_0 \approx 3.90$ to 4.60 , 2007) [35], and Mexico ($R_0 \approx 2.00$ – 3.09 , 2002) [21]. The effective reproduction number from the period of explosive growth in our study falls within the range basic reproduction number estimates of studies conducted in other countries such as Chile ($R_0 \approx 27.20$, 2002) [36], Brazil ($R_0 \approx 2.0$ – 103 , 1996–2003) [37], and Indonesia ($R_0 \approx 8.00$ – 39.45 , 2002–2007) [38].

Except DENV-4, the circulation of other three DENV serotypes varied over time in Bhutan. DENV-2 was the predominant serotype in 2004, while DENV-2 and 3 predominated in 2005 and 2006 [16]. A serotype shift with predominant DENV-1 was reported in 2013–2014, and DENV-1 and 2 in 2016–2017 [15,39]. After an absence of more than a decade, DENV-3 re-emerged and predominated in the current outbreak. As reported in other countries [40–42], the intense transmission and higher incidence in this outbreak might be related to a shift in the predominant serotype. Further, DENV-3 is often associated with intense force of transmission [43]. Previous outbreaks in Bhutan have been linked to the importation of dengue virus from neighboring countries in the South-East Asia region [16,39]. As the surveillance system did not capture information on the travel history of the dengue patients, the source that triggered this large epidemic could not be established. Phylogenetic analysis of the viral isolates might add this information by indicating the similarity of strains with other neighboring countries.

Estimates of basic and effective reproduction numbers can be sensitive to the type of methods used [21], complicating the comparison across studies that use different methods. Nevertheless, estimates of the effective reproduction number around 30 are remarkably high. Codeco et al. have shown that failing to account for changes in temperature can lead to the overestimation of effective reproduction numbers in the early stages of seasonally driven epidemics [25]. However, using their method, we found that the peak effective reproduction number remained high even after accounting for temperature changes. Moreover, in an alternative analysis in which we calculated the effective reproduction number from the growth rate in the four phases of the epidemic (see Supplementary Material S1), the peak estimated value of the reproduction number was also high (31; 95% CI 15–61).

The unprecedented size and geographic extent of the epidemic and the delay between the rapid rise in cases and implementation of interventions highlight the need to strengthen the existing surveillance and response mechanisms in Bhutan. Given the late initiation of dengue interventions in the 2019 outbreak, effort should be undertaken to develop a more rapid response, including the possible development and use of early warning systems, for future years.

Our study is subjected to several limitations. The data were obtained from the national surveillance database and it is likely that people with mild or asymptomatic illness did not seek care in public health facilities and were not captured by the surveillance system. Moreover, as dengue is a nonspecific febrile illness, diagnosis may be less likely when the clinical suspicion is low, such as in the early stages of epidemics and in regions with no history of dengue infection. Consequently, the completeness of cases in the database may have varied across the epidemic. In particular, if the rapid rise in reported case numbers observed in weeks 25–27 were in part due to increasing public awareness of healthcare-seeking behavior, then we will have overestimated the true reproduction number and growth rate in the early stages of the epidemic. Travel history of the cases was not captured by the surveillance system. As dengue has an intrinsic incubation period of 3–14 days [1], without this travel information, we were not able to rule out the acquisition of cases outside Bhutan or determine with certainty the region within Bhutan in which the infection was acquired. Moreover, the total number of cases in many districts was low and the center of the epidemic (Chukha) was a national travel

and commerce hub. We were therefore unable to estimate growth rates and reproduction numbers for every district. The reproduction number may have been much lower in districts with lower densities of dengue vectors and humans.

5. Conclusions

This study reports the spatiotemporal patterns and effective reproduction number of the first national dengue epidemic in Bhutan. Dengue was reported by 19 of the 20 districts in the country. A very high transmissibility of dengue virus was found, with a high reproduction number during a period of explosive growth. This finding highlights the need to reinforce the national preparedness plan for outbreak response, and to enable the early detection of cases and timely response.

Supplementary Materials: The following are available online at <https://www.mdpi.com/1660-4601/18/1/354/s1>. Supplementary Table S1: Dataset showing weekly and cumulative dengue cases of the 2019 dengue epidemic in Bhutan, Supplementary Table S2: Growth rate and reproduction number during phases of the dengue epidemic in Bhutan, 2019, Supplementary Material S1: Supplementary analysis, Supplementary Material S2: Sensitivity analysis, Supplementary Figure S1: Epidemic curve showing weekly number of new cases (left vertical axis) along with the weekly cumulative cases of dengue epidemic in Bhutan in 2019, Supplementary Figure S2: Epidemic curve showing the different growth rates across the four phases of the epidemic. The weekly reported cases numbers collected by NEWARS (National Early Warning, Alert, Response and Surveillance) are represented by dots, the fitted exponential growth curves and 95% confidence intervals are indicated by lines and shaded regions. The transition between phases one and two (week 25) coincided with the beginning of high rainfall and the start of school vacation. The transition between phases three and four (week 34) coincides with the beginning of national interventions, Supplementary Figure S3: The effective reproduction number over the course of the epidemic, assuming constant temperature and accounting the changes in temperature in all of Bhutan and Chukha only. Supplementary Figure S4: Sensitivity analysis to account the influence of under-reporting on the reproduction number by considering cut-offs in weeks 25, 26, 27, and 28, and reporting ratios of 0.2, 0.4, 0.6, 0.8, and 1.0. Supplementary Figure S5: The influence of under-reporting at the beginning phase of the epidemic on the estimated temperature dependent, time varying reproduction number for the whole country. Supplementary Figure S6: Total weekly rainfall and average temperature in Bhutan, 2019. Multiyear averages for these variables were calculated using data from 2008 to 2018. (Source: National Centre for Hydrology and Meteorology of the Royal Government of Bhutan).

Author Contributions: T.T. and K.W. conceived the study. T.T. undertook literature review, analyzed and drafted the manuscript, and prepared some figures. K.W. assisted in analysis, drafting and revision of the manuscript. A.M. estimated the growth rates and reproduction numbers and prepared some figures. D.J.G., A.C.A.C., A.M., S.W. and T.W. were involved in the critical revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declarations of Helsinki, and approved by the Research Ethics Board of Health, Bhutan (2019/082), and Human Research Ethics Committee, Australian National University (2019/923).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting the findings of the study is available in the supplementary material (Table S1).

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Conflicts of Interest: The authors declare no conflict of interest.

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Vulnerability assessment of dengue in Bhutan

5.1 Introduction

Dengue is the fastest growing vector-borne viral disease in the world. The emergence of dengue in new areas warrants further investigation into risk profiling to understand the emerging pattern of dengue. This chapter investigates the geographical vulnerability of dengue using a knowledge-driven model. MCDA model was used to generate a dengue vulnerability map for Bhutan. In this study, vulnerable areas were mapped at different seasons to show the variation of dengue risk at each season. Factors (or criteria) that demonstrated a significant relationship with dengue were used to compute vulnerability scores using WLC in the geographical information system software. Sensitivity analysis was conducted by computing the mean change in vulnerability score by changing the membership functions and weights of each factor. In addition, the predictive ability of the model was assessed using Receiver Operating Characteristic Curve in R software.

The study revealed heterogeneous geographical vulnerability to dengue. The most vulnerable areas were located in low-lying areas which were predominantly characterised by tropical and sub-tropical climates, high population density, agricultural and built-up landscapes, and relatively good road connectivity. The risk increased during the summer, even reaching up to the capital, Thimphu, and other places at higher elevations.

5.2 Article on vulnerability assessment

Tsheten T, Clements ACA, Gray DJ, Wangdi K. Dengue risk assessment using multicriteria decision analysis: A case study of Bhutan. *PLoS Negl Trop Dis*. 2021 10;15(2):e0009021.

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RESEARCH ARTICLE

Dengue risk assessment using multicriteria decision analysis: A case study of Bhutan

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Abstract

Background

Dengue is the most rapidly spreading vector-borne disease globally, with a 30-fold increase in global incidence over the last 50 years. In Bhutan, dengue incidence has been on the rise since 2004, with numerous outbreaks reported across the country. The aim of this study was to identify and map areas that are vulnerable to dengue in Bhutan.

Methodology/Principal findings

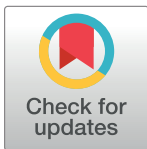
We conducted a multicriteria decision analysis (MCDA) using a weighted linear combination (WLC) to obtain a vulnerability map of dengue. Risk factors (criteria) were identified and assigned with membership values for vulnerability according to the available literature. Sensitivity analysis and validation of the model was conducted to improve the robustness and predictive ability of the map. Our study revealed marked differences in geographical vulnerability to dengue by location and season. Low-lying areas and those located along the southern border were consistently found to be at higher risk of dengue. The vulnerability extended to higher elevation areas including some areas in the Capital city Thimphu during the summer season. The higher risk was mostly associated with relatively high population density, agricultural and built-up landscapes and relatively good road connectivity.

Conclusions

Using MCDA, our study identified vulnerable areas in Bhutan during specific seasons when and where the transmission of dengue is most likely to occur. This study provides evidence for the National Vector-borne Disease Control programme to optimize the use of limited public health resources for surveillance and vector control, to mitigate the public health threat of dengue.

Author summary

Dengue is an important vector-borne viral disease affecting humans. In Bhutan, dengue incidence is on the rise with increased frequency of outbreaks and spread to new areas. Outbreaks were reported from places as high as above 900m above sea level in recent years. However,



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dengue control activities in Bhutan are usually initiated at the time of outbreaks. This often leads to a large number of cases and overburden the health system. To address these issues, we developed dengue risk maps at a fine spatial resolution by combining risk factors that mediate the transmission of dengue using a weighted linear combination. Vulnerability to dengue was spatially heterogeneous and varied by season. Dengue is highly vulnerable in low-lying areas throughout the season. However, the vulnerability extended to higher geographical elevations including the nation's capital during the summer season. The study provides a firm evidence-base to prioritize areas and seasons for dengue control strategies in Bhutan.

Introduction

The global incidence of dengue has increased over the last few decades from over 8.3 million reported infections in 1990 [1] to approximately 80 million dengue cases in 2015 (representing a ten-fold increase) [2]. The number of deaths also increased from 12,300 in 2005 to 18,400 in 2015 [3]. Before 1970, only nine countries experienced severe dengue epidemics. Today, more than 120 countries have endemic dengue virus (DENV) transmission, with severe dengue resulting in 21,000 deaths annually [4]. There are now estimated 390 million dengue infections worldwide every year (95% credible interval [CrI] 284–528 million), of which 96 million (95% CrI 67–136 million) manifest clinically with varying degree of severity [5]. Dengue is the leading cause of hospitalization and death among children in the South East Asia region [6].

Implementing effective interventions for dengue involves identification of priority areas and times of the year that are most vulnerable to the disease. However, this presents a challenge in developing countries without adequate data [7]. Misdiagnosis and asymptomatic infections impede the detection of cases, resulting in delayed responses [5]. Moreover, surveillance systems do not provide adequate information for instituting informed dengue control activities, in particular, due to massive under-reporting [8, 9]. Identifying areas at risk of dengue is of paramount importance to prioritize resources and provide effective responses to dengue outbreaks [10].

In the 2019 dengue epidemic, dengue cases were reported from 19 of the 20 districts in Bhutan, including many districts that had never previously had dengue. Unfortunately, the current national surveillance system does not capture information on the source of infection, i.e. whether it was acquired locally or imported from other districts or countries, and so it is not known whether all cases of dengue were acquired in the district where they were reported. This information is crucial to understand the epidemiology of dengue for planning control activities. Therefore, methods need to be applied to understand whether particular locations are likely to sustain local dengue transmission.

Recent advances in geographical information systems (GIS) and remote sensing technologies have been increasingly used to study the spatial relationships between vector-borne diseases and the factors that influence their distribution [11]. These technologies have been implemented in several ways, including as the basis for spatial decision support tools to improve resource allocation for disease surveillance and control activities [12] and predicting high risk locations for these diseases [13]. A GIS-based multi-criteria decision analysis (MCDA) is one such decision support tool, offering a straightforward approach to conceptualizing the complex web of factors and interactions that mediate the transmission of disease [14]. MCDA integrates multiple factors and weighs their importance by incorporating knowledge and data from diverse sources [15, 16]. This can be operationalised in different ways; here, we used a Weighted Linear Combination (WLC) algorithm for combining information to produce a dengue vulnerability map.

The aim of this study was to generate dengue vulnerability maps for Bhutan for different seasons. These maps can be used to visualise dengue vulnerability of different areas for prioritizing limited public health resources for controlling dengue in Bhutan.

Methods

Ethics statement

Ethical clearance for this study was approved by the Research Ethics Board of Health (REBH), Ministry of Health, Bhutan (REBH/Approval/2020/033) and the Australian National University (ANU), Australia (2020/297).

Study setting

Bhutan is one of Asia's smallest nations, situated in the southern slopes of the eastern Himalayas between the People's Republic of China in the North and India in the South, West and East. The country has a total area of 38,394 square kilometres and lies between 27° 30' N and 90° 30' E [17]. The country is administratively divided into 20 districts or *dzongkhags*, which are further divided into 205 sub-districts or *gewogs* (Fig 1).

Bhutan has a monsoonal climate with four seasons. The spring season starts in March and ends in May. The summer season, which commences in June and ends in August, is characterized by heavy rain and high temperatures. The rain continues into the autumn season, which stretches from September to November. From December, the winter sets in with frost covering much of the country, and it lasts until February [18].

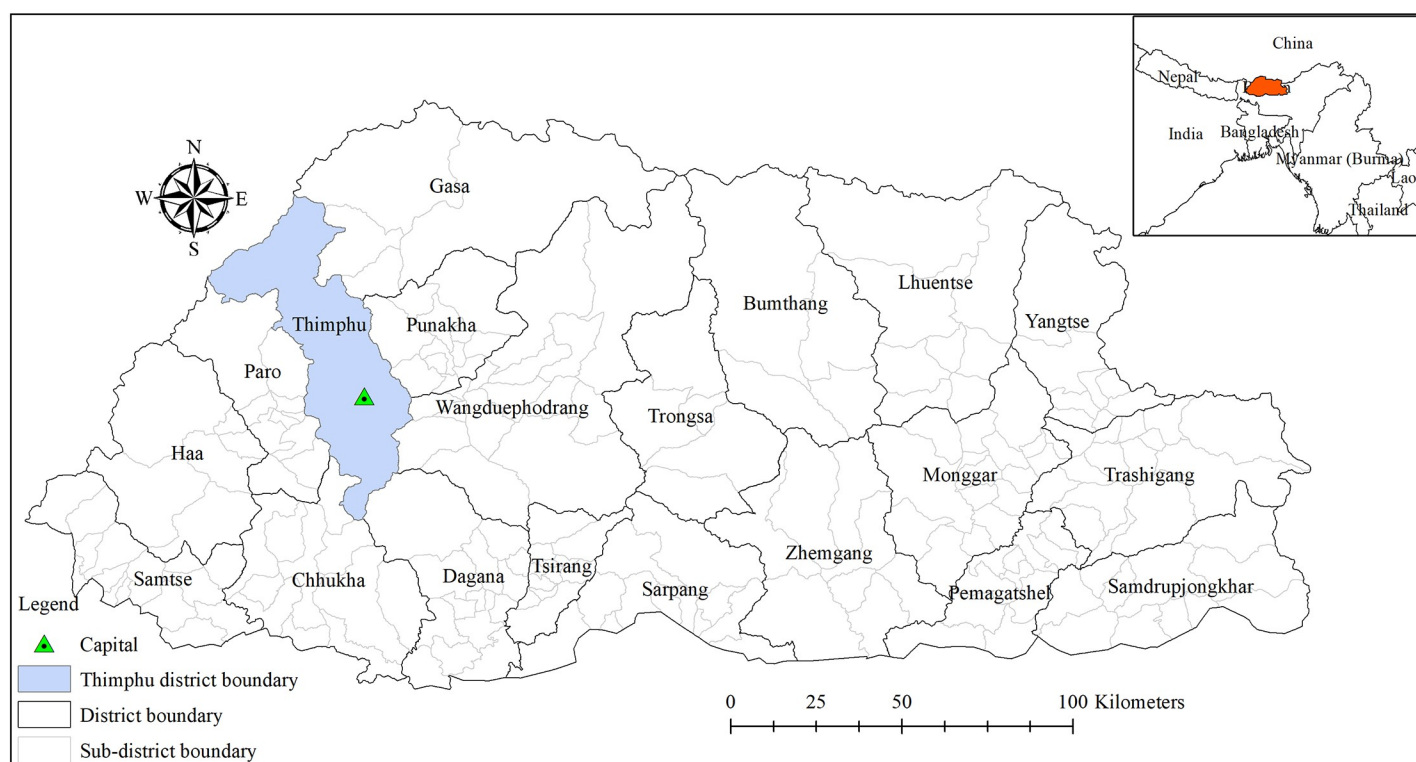


Fig 1. District (with name) and sub-district boundaries (in light grey colour) of Bhutan. The capital city (green triangle with dot inside) lies in Thimphu district, shown here in light blue.

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Study design

The MCDA process involves the following stages: 1) Defining the objective, 2) Identifying factors and constraints using different information sources (e.g. expert opinion, a literature search and analysis of historical data), 3) Defining the relationship between the factors and the vulnerability of a location for dengue, 4) Transforming or standardizing the values of the factors on a relative scale to allow comparison between each criterion, 5) Weighting the criteria on the basis of their relative importance to vulnerability, 6) Combining and aggregating all the layers/criteria to produce a final weighted estimate of vulnerability in each location and, 7) Conducting sensitivity analysis and validating the results.

Objective

The objective of MCDA was to identify and prioritize areas for dengue control. Dengue control activities such as vector control in Bhutan are initiated only during outbreaks and when the health system is already disrupted. During an outbreak, healthcare facilities are overcrowded with limited space to care for patients, and staffing is insufficient to meet the patient load. To avoid such delays and provide prompt intervention measures, identification of areas at risk of dengue is important. Areas that are vulnerable for dengue might then be sites for sentinel or other targeted surveillance approaches, and protocols and systems can be developed in advance for mobilization of resources when and where they are required.

Data sources

To validate the model, we used dengue cases between 2016–2019 from the National Early Warning, Alert, Response and Surveillance (NEWARS) database housed within the Ministry of Health of Bhutan. NEWARS is an integrated online disease surveillance system of all nationally notifiable diseases including dengue [19]. Dengue cases are defined as any patients presenting fever with any two of the following symptoms: headache, retro-orbital pain, rash, muscle/joint pain, positive tourniquet test and any warning signs of severe dengue, which include abdominal pain, persistent vomiting, mucosal bleeding, fluid accumulation, liver enlargement and elevated haematocrit value. These cases were further confirmed by rapid diagnostic test for dengue virus non-structural protein 1 (NS1), anti-dengue IgM and IgG antibodies. Only confirmed cases were reported into the NEWARS. Average annual dengue incidence in the districts varies between less than three cases to more than 21 cases per 10,000 inhabitants (S1 Fig). Dengue is seasonal with maximum dengue incidence reported during summer than other seasons (S1 Table).

Past studies have demonstrated multiple factors that influence the occurrence of dengue (S2 Table). We included temperature, rainfall, land use, population density, distance from the road network and the open water bodies/major rivers, upon which we base the vulnerability assessment.

Maximum temperature and rainfall variables were obtained from Worldclim (www.worldclim.org). These raster layers were developed at a spatial resolution of 30 seconds ($\sim 1 \text{ km}^2$) between 1970 and 2000 [20]. In this study, seasonal averages were generated for both rainfall and temperature for the following time periods: a) December, January and February for the winter season; b) March, April and May for the spring season; c) June, July and August for the summer season; and, d) September, October and November for the autumn season. As elevation has been already used as a covariate in Worldclim data production, it was not used in the model construction [21].

Population density was obtained from the Worldpop (www.worldpop.org) global high-resolution population denominators project, which provides gridded population counts at 100 m spatial resolution (3-arc seconds) [22].

Land use data were obtained as a vector layer from the Ministry of Agriculture and Forestry, Royal Government of Bhutan. This was the latest data available for land use in the country. This layer has 20 categories, including many specific land cover types. The layer was re-categorized into three categories based on relevance to the vulnerability of dengue transmission: built-up (areas occupied by humans), agricultural land and others (natural environment). This vector layer was converted into a raster layer using the *conversion tool* in ArcGIS.

Similarly, the road network data was obtained from the Ministry of Work and Human Settlement as a vector layer. Euclidean distance to the nearest road was computed throughout Bhutan using the *distance tool* in ArcGIS. Finally, we considered the distance to the open water bodies/major rivers, obtained from the National Land Commission, Bhutan. Euclidean distance to the nearest open water was computed following similar steps as the road network.

As data obtained from different sources were at diverse spatial scales, they had to be converted into uniform scales. All rasters were uniformly standardized at a pixel dimension of 0.0083 decimal degrees (which equals approximately 1km at the equator) covering the entire geographical extent of Bhutan. The land use and water bodies layers were reprojected from Geographic Coordinate System (GCS) Drukref_03 Transverse Mercator to the World Geodetic System (WGS) 1984 for spatial analysis. Raster maps were produced for all risk factors using this standardized spatial resolution and the same pixel size (Figs 2 and 3).

Standardization of criteria (Risk factors) using membership functions

There exist different ways of standardizing or normalizing criteria such as Boolean constraints, linear transformation, value/utility functions and membership functions (MF). Rescaling

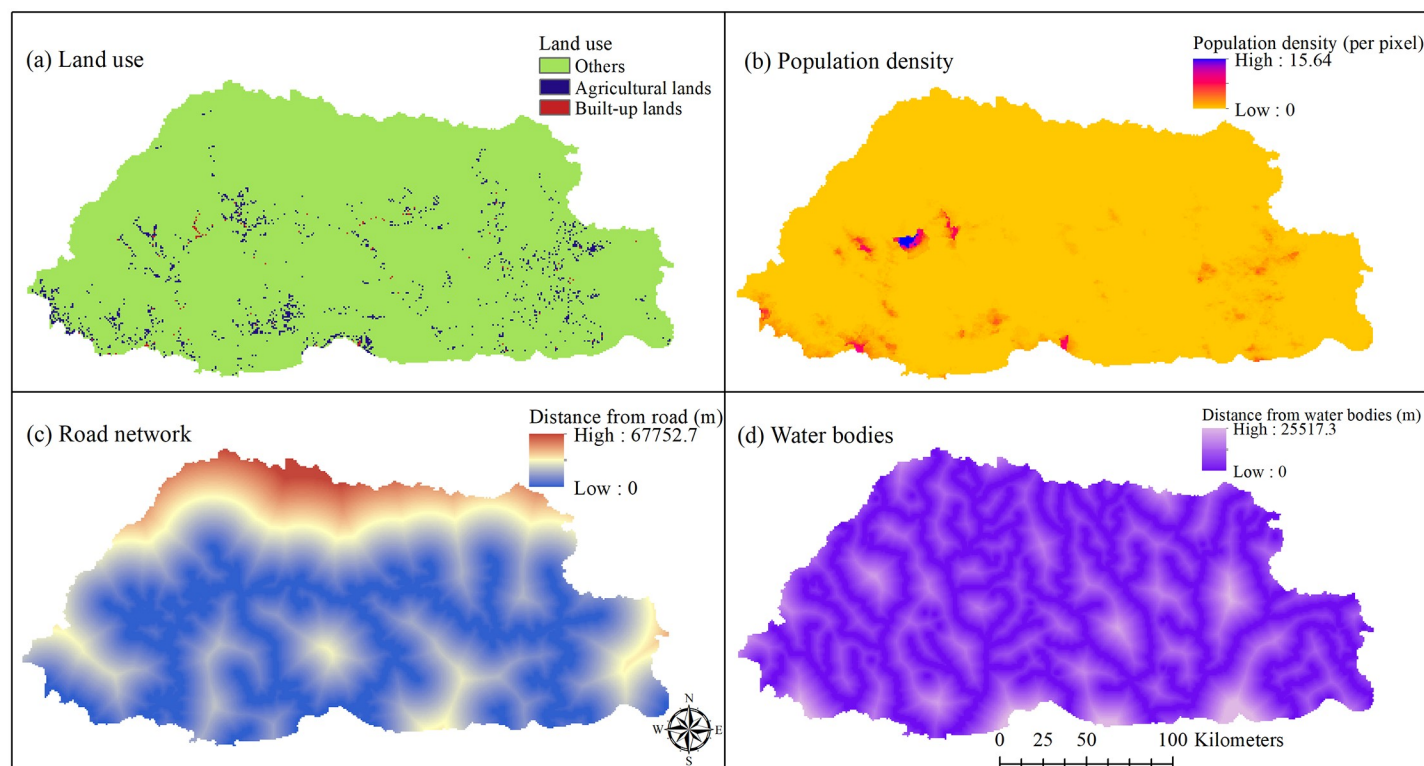


Fig 2. Patterns of land use types, distance from the road network (~1 decimal degrees = 111,000meters) and population density (number of people per pixel) in Bhutan.

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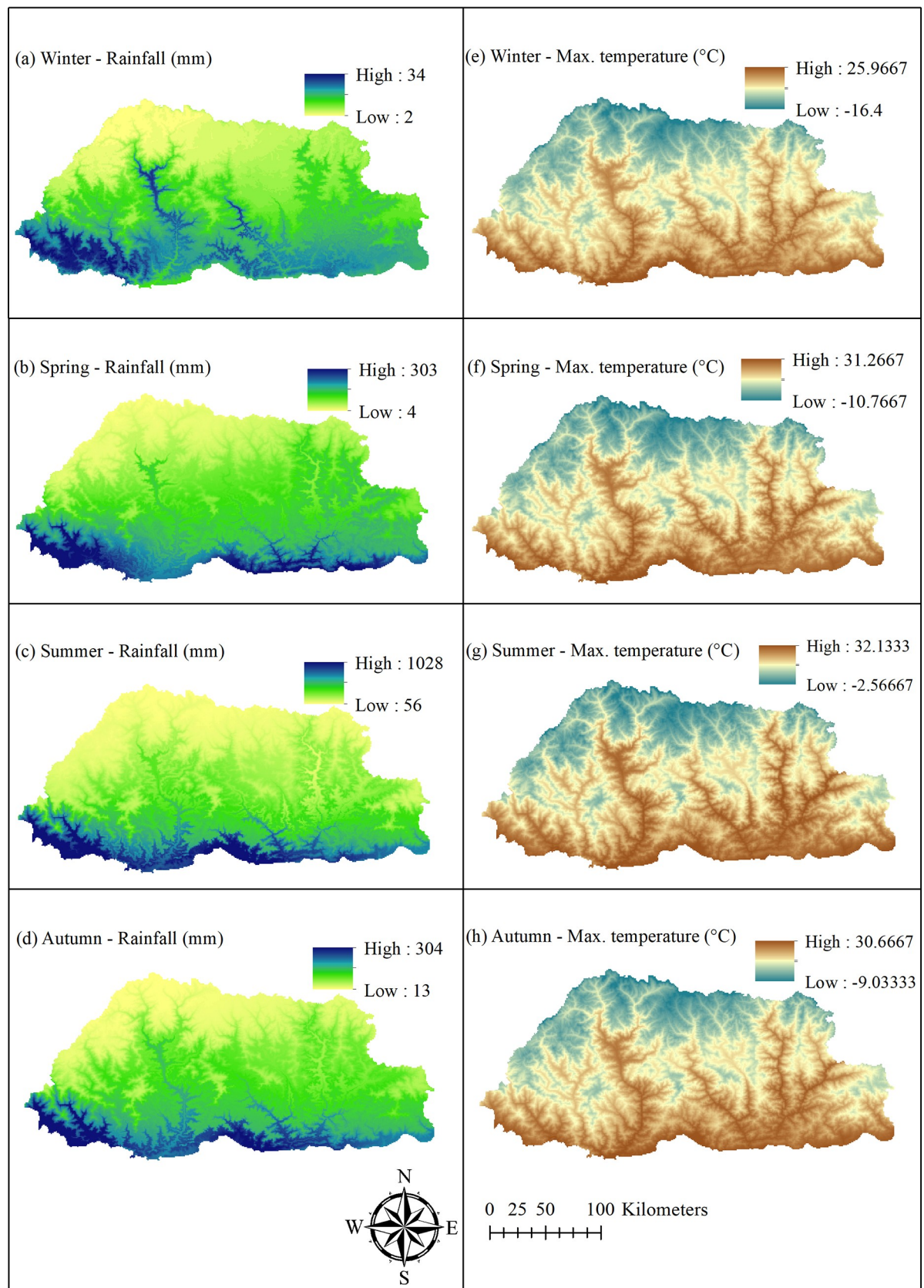


Fig 3. Patterns of seasonal average rainfall (left panel maps) and average maximum temperature (right panel maps) in Bhutan. Seasonal average data was computed by averaging three months for each season: Winter (December-February), Spring (March-May), Summer (June-August) and Autumn (September-November).

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factors into a standardized continuous scale allowed them to be compared and combined. In this study, fuzzy logic MF was used to determine the relationship between each factor and dengue vulnerability. Fuzzy set membership is the probability of an element belonging to a class (here, vulnerable/not vulnerable) in which boundaries between classes are not distinct. Using this approach can improve the accuracy of the output as the membership score represents a zone of gradual transition rather than a hard transition. The graded values ranged from 0 (i.e. complete non-membership or non-vulnerable in a fuzzy set) to 1 (i.e. complete membership or highly vulnerable in a fuzzy set).

Temperature

Climatic conditions, including temperature and rainfall, play a major role in the local spread of dengue infection by directly affecting the life cycle, feeding activity, biting rates and incubation period of the disease [23]. Vector populations increase through processes such as the laying of eggs, egg hatching and growth of larva and pupa, all of which are favoured by higher temperatures. Higher temperatures reduce the extrinsic incubation period of DENV in *Aedes* mosquitoes and promote viral transmission [24, 25]. Furthermore, higher temperatures increase the speed of blood meal digestion and thus boost the feeding habits of mosquitoes [26]. The longevity of *Aedes* mosquitoes increases with an increase in temperature, with the optimum survival temperature ranging from 27–30°C, while temperature below 17°C and above 34°C are associated with low mosquito activity and thus lower transmission [25, 27]. Thus we input a symmetric relationship between vulnerability and temperature, with vulnerability rising from 17°C (below which the environment is not vulnerable), peaking at 27–30°C, and falling to 34°C (above which the environment is also not vulnerable) (Table 1).

Rainfall

Increased rainfall facilitates vector population growth by providing water required for mosquito breeding [28]. The impact of rainfall on vector density and consequent dengue infection varies across different geographical regions. Cheong et. al had reported the highest dengue

Table 1. Parameters defining membership functions for dengue vulnerability in Bhutan.

Risk factors	Function shape	Not vulnerable	Maximum vulnerability	Rationale
Temperature (°C)	Symmetric	<17 & >34	27–30	Optimum surviving temperature for vector ranges from 27–30°C, while temperature <17°C & >34°C are associated with low mosquito activity & lower transmission
Monthly rainfall (mm)	Monotonic increasing (Linear)	<10 mm	>167 mm	Total monthly rainfall of 167mm is suitable for stable dengue transmission
Population density (per pixel)	Monotonic increasing (Linear)	< 1	>16	Populations >1 person per pixel are required for dengue transmission & vulnerability increases linearly to a maximum at 16 people per pixel
Distance from the road network (m)	Monotonic decreasing (Linear)	>5000m	<500m	Most dengue cases occur within 500m of a road, whilst dengue rarely occurs >5000m from the road network
Land use types	Categorical data	Natural environment	Built-up areas	Built-up areas are associated with highest dengue incidence followed by agricultural land. Natural environments such as forest, snow and barren land are not associated with dengue
Distance from the water bodies	Monotonic decreasing (Linear)	>20,000m	<500m	Most dengue cases occur within 500m of water bodies, whilst dengue rarely occurs >20,000m from water bodies

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incidence at a bi-weekly total rainfall between 215–302 mm in Malaysia [29]. In Taiwan, the relative risk of dengue was found to be significantly correlated with precipitation up to a maximum of 350mm/day at 70 days lag [30], while the peak dengue risk had been reported at a monthly cumulative rainfall of 720mm in China [31]. Countries in the World Health Organization South-East Asia Region (WHO-SEAR) including Bhutan are considered to have stable *Ae. aegypti* populations in geographical locations that receive an annual rainfall of greater than 2000mm [7]. Based on this relationship, dengue incidence varies seasonally (following a Sigmoidal function) with maximum incidence evident the month following a monthly cumulative rainfall of 167mm. Vulnerability in the current study was, therefore, assumed to increase monotonically with monthly rainfall above 10mm [32] (Table 1).

Population density

High population density and uncontrolled urbanization have created ideal conditions for increased transmission of dengue [33]. Dengue incidence is assumed to increase linearly with increasing population density. Areas with population density <1 person per square km were standardized to zero, while areas with the maximum population density (i.e., 16) were standardized to one. A simple linear increasing function was used to describe this relationship between vulnerability and population density (Table 1).

Land use

Ae. aegypti are commonly found around human dwellings, where female mosquitoes frequently and almost exclusively bite human hosts [26]. Therefore, this mosquito is predominantly found in high-density urban areas, and is rarely found in vegetated and forested land [34]. An abundance of *Ae. aegypti* is also associated with a high coverage of agricultural land use such as crops and paddy fields in some areas [35, 36]. Fragmentation of natural habitats can increase the local temperature and increase human-mosquito interactions, favouring the transmission of dengue [37]. On the continuous scale from 0 to 1, built-up areas were considered highly vulnerable and rated as “1”, agricultural lands were rated as “0.50”, while all others areas were considered not vulnerable to dengue and rated as “0”.

Distance from the road network

Using the road network as a measure of urbanization, studies have reported a positive relationship between the distribution of dengue epidemics and density of the road network. Well-developed road networks facilitate the movement of people and increase the probability of dengue transmission [38]. The density of the road network is also a proxy measure of the density of the human population. Cases have been found to concentrate within 500m of the road network and nearly all cases occur within a distance of 5000m [39]. Accordingly, maximum vulnerability for dengue was assumed to occur at a distance of up to 500m from the road network, then monotonically decrease to a maximum distance of 5000m, after which the location was deemed not to be vulnerable (Table 1).

Distance from open water bodies/major rivers

Distribution of mosquitoes and dengue incidence varies in response to the distribution of open water bodies and rivers. Studies in the past have shown greater dengue incidence [40, 41] as well as higher prevalence of dengue seropositive residents in areas adjacent open water bodies [42]. Standing water formed as a consequence of rivers can act as breeding sites for mosquitoes and enhance the transmission of dengue [43]. As for the road network, maximum

vulnerability was assumed to occur at a distance of up to 500m from open water, and then monotonically decrease to a maximum distance of 20,000m.

Applying decision rules

We used the analytical hierarchy process (AHP) developed by Saaty to generate weights for each factor using a pairwise comparison matrix [15]. With this method, the relative importance of each factor was determined through pair-wise comparison by creating a ratio-matrix using a numerical scale. Each comparison determines which factor is more important and to what extent on a nine-point scale ranging from extremely less important through equal importance to extremely more important [15].

Pairs of factors were compared based on the number of studies that reported significant associations with dengue incidence/outbreaks. To this end, a literature search was conducted to extract relevant publications in the PubMed database. We used the following combination of search terms: ("dengue virus" OR dengue OR "severe dengue") AND (temperature or precipitation or rainfall or "land use/land cover" road or water or river or "population density") AND (Asia). To retrieve recent articles, the search was restricted for the time period between 2010 and 2020. Factors that had been reported more frequently were considered to be more important and rated higher in a pairwise comparison. A detailed description can be found in S2 Table. The principal eigen vector obtained from the pairwise matrix was then used to calculate best-fit of weight for each factor [44]. To calculate weights, a normalized comparison matrix was computed from the pairwise comparison matrix by dividing all elements of each column by their respective column sum. Then the algebraic average of the six columns of risk factors was computed to generate an approximate weight-vector. The weights were normalized so that its additive value was equal to one.

To measure the inconsistency associated with the pairwise comparison matrix, a consistency index (*CI*) was calculated as follows:

$$CI = \frac{\lambda_{max} - p}{(p - 1)RI}$$

where, λ_{max} is the biggest eigenvalue that can be obtained once we have its associated eigenvector, p is the number of columns of the matrix (i.e., risk factors) and RI is a random index (its value depend on the size p). If $CI < 0.10$, then ratio indicates a reasonable or acceptable level of consistency, while $CI \geq 0.10$ indicates inconsistent judgements [45].

The Pearson correlation coefficient (r) was calculated for each pair of factors. If $r > 0.4$, weights of both factors were reduced by 10%, while other weights increased proportionally to result in the total weight of one [46].

Weights assigned to the factors were considered statistically acceptable with the CI of 0.013, which is below the threshold level of 0.10 (Table 2). These weights were further adjusted based on the results of the correlation analysis (S3 Table).

Combination of criteria using weighted linear combination

Weighted linear combination (WLC) was used to aggregate all standardized raster layers. A low vulnerability score in one factor is compensated by a high vulnerability score in another factor. WLC has the following expression [47]:

$$S_i = \sum_{j=1}^n w_j x_{ij}$$

Where, S_i represents the final score, w_j represents the weight of the criterion j , and x_{ij}

Table 2. Pairwise comparison of matrix of criteria (risk factors) and weights in analytical hierarchy process with respect to dengue vulnerability.

Risk factors	Temperature	Rainfall	Land use	Population Density	Road network	Water bodies	Adjusted weights
Temperature	1						0.290
Rainfall	1/2	1					0.269
Land use	1/3	1/2	1				0.136
Population density	1/4	1/3	1/2	1			0.133
Road network	1/6	1/5	1/3	1/3	1		0.071
Water bodies	1/6	1/5	1/3	1/3	1/2	1	0.101

RI = 1.24, λ_{max} = 6.084, CI = 0.013.

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represents the standardized score of the j^{th} criterion for the i^{th} pixel. Standardization of risk factors and WLC were operationalized in the TerrSet Geospatial Monitoring and Modelling software (in short TerrSet) (Clark Labs, Worcester, MA).

Sensitivity analysis

Sensitivity analysis was conducted by changing the MF (i.e., the structure or the shape) and weights of the risk factors. To determine the change in MF of the risk factors, following sensitivity analysis were conducted: a) All linear monotonic relationship were converted into sigmoidal relationship, b) Symmetric relationship of temperature was converted into a monotonic linear relationship and c) Rainfall was converted to a symmetric relationship from a monotonic relationship [32]. In order to assess the sensitivity of the weights, two new weights are applied to each factor by adding and subtracting 25% from the original weights [46]. We also used equal weights of 0.167 for all risk factors. Each of the newly constructed weights was individually incorporated into the model, while holding other factor weights constant. For each combination of weights obtained, a vulnerability map was created. Vulnerability estimates were extracted from both the original and new maps at the sub-district level using the *zonal statistics* tool in ArcGIS and the mean change in vulnerability was calculated. Average changes in vulnerability scores for each change in input values were assessed to determine the robustness of the model.

Map validation

The predictive ability of the MCDA was quantified using a Receiver Operating Characteristic Curve (ROC) constructed in the R software. Predicted values were obtained by averaging the dengue vulnerability scores of all four seasons at the sub-district level. National surveillance data from NEWARS were used to identify sub-districts that have reported dengue over the past five years. These sub-district-level observations were then compared with predicted vulnerability derived from the MCDA. The area under the curve (AUC) of the ROC was used to validate the predictive ability of the MCDA map. Note, using this approach, estimates of both sensitivity and specificity are dependent on all vulnerable areas of the country having experienced dengue cases during the past five years and all areas reporting dengue having had local transmission. Both are associated with considerable uncertainty.

Results

Vulnerability maps for dengue

Dengue vulnerability varied by different time periods, as displayed by four different vulnerability maps covering the country of Bhutan (Fig 4). The Winter season had the lowest vulnerability for dengue up to a maximum vulnerability score of 0.6. With the onset of spring, our model

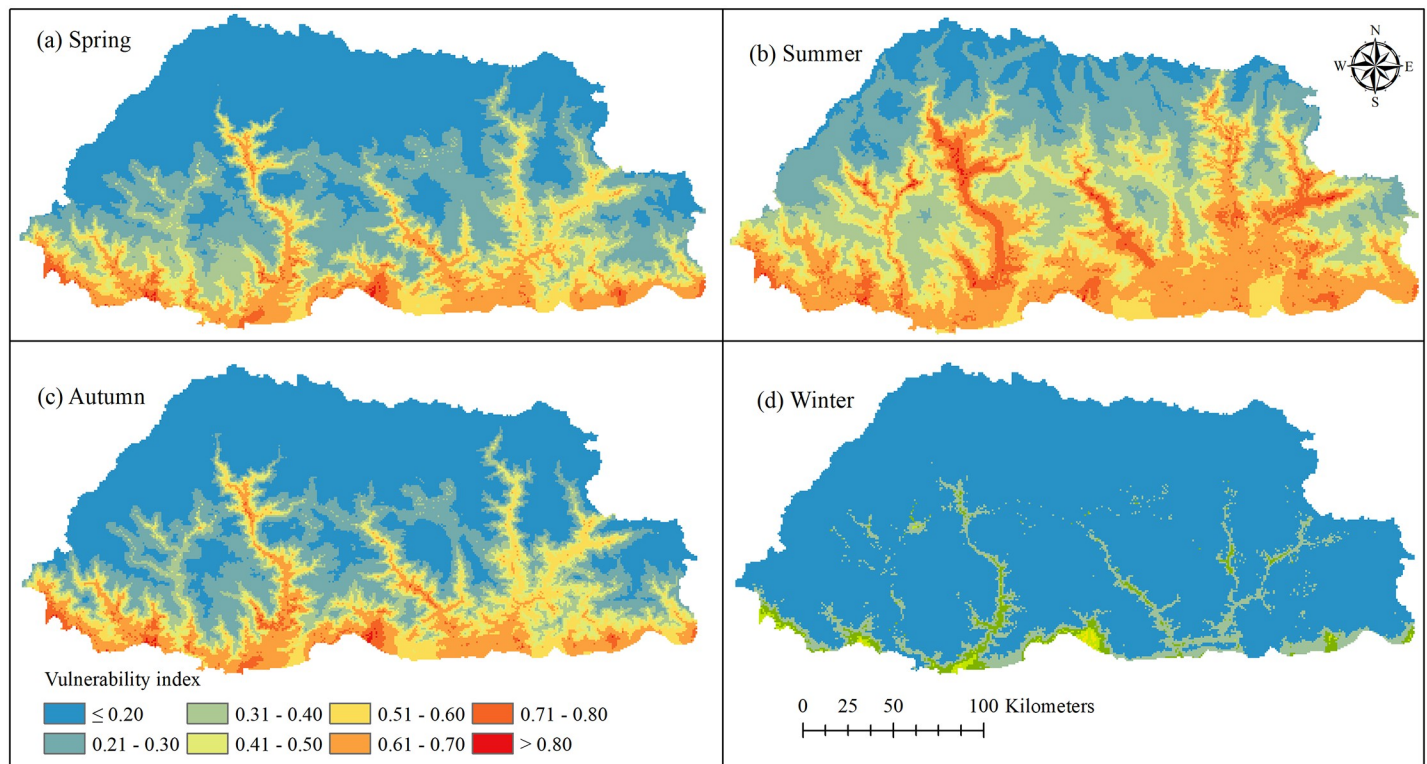


Fig 4. Dengue vulnerability map of Bhutan for winter, spring, summer and autumn seasons. Winter had vulnerability index upto 0.60, while all other seasons have >0.80. The geographical coverage of vulnerability index >0.80 was observed maximum in summer.

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projected an increase in vulnerability with extension of higher vulnerability to many geographical regions. Areas with high vulnerability (vulnerability score >0.80) were predominantly concentrated in low-lying elevations and included Chukha, Samtse, Sarpang, Dagana, Pema-gatshel and Samdrup Jongkhar districts.

Summer had the highest vulnerability for dengue, with further expansion of vulnerable areas. Areas of vulnerability even extended to the nation's capital, where the vulnerability score was >0.80 in three sub-districts, namely Kawang, Chang and Mewang (Fig 5). Other places with high vulnerability in summer included Wangdiphodrang, Punakha, Tsirang, Paro, Trongsa, Mongar, Trashiyangtse, Lhuentse, and Trashigang districts. In autumn, areas vulnerable for dengue started to decrease, mainly due to declining temperatures.

Sensitivity analysis

Assuming a sigmoidal relationship between risk factors and dengue vulnerability resulted in a mean change of vulnerability estimates of 0.0067, 0.0057, 0.0018 and 0.0054 for spring, summer, autumn and winter seasons respectively. Increasing or decreasing the weights of the factors by 25% whilst holding all other weights constant resulted in a mean change in vulnerability estimates from as low as 0.0006 to 0.0335. Changing the MF of rainfall from monotonic to symmetric structure resulted in the highest overall average change in vulnerability estimates of 0.0767 (Table 3).

Validation

The predictive performance (AUC) of the MCDA model at the sub-district level was 0.66 (95% CI: 0.57, 0.76), 0.64 (95%CI: 0.54, 0.73), 0.65 (95%CI: 0.55, 0.75), and 0.72 (95%CI: 0.64, 0.79)

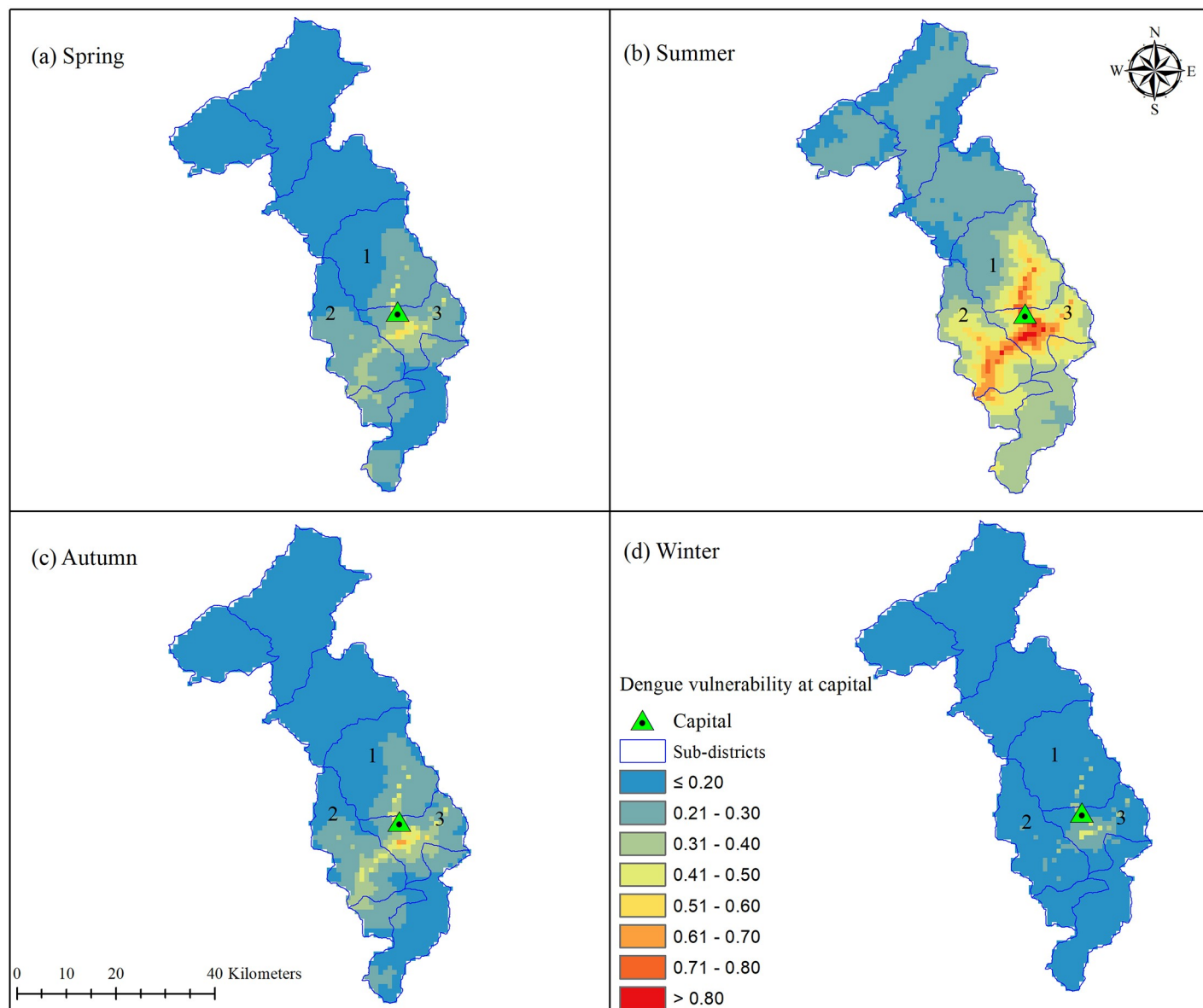


Fig 5. Dengue vulnerability at Thimphu sub-districts. Vulnerability index >0.80 were observed in Kawang (1), Mewang (2) and Chang (3) sub-districts during summer.

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for spring, summer autumn and winter seasons, respectively. The overall average performance of the model was AUC = 0.66 (95% CI: 0.57, 0.75).

Discussion

We implemented a knowledge-driven MCDA model using WLC to develop vulnerability maps for dengue, by season, in Bhutan. Areas with high vulnerability were predominantly located along the southern international boundary with India and in the river valleys projecting into the interior of the country. In agreement with the prediction map, dengue outbreaks in the past were predominantly reported from the southern border region. These areas are generally characterized by lower elevations, built-ups areas, high population densities and

Table 3. Average change in vulnerability scores generated by subtracting scores of the original map and the newly created maps.

Weights/Structural variation	Spring	Summer	Autumn	Winter	Overall change
Temperature (variation in weight)	↑ 25%	0.0021	0.0013	0.0040	0.0030
	↓ 25%	0.0059	0.0110	0.0045	0.0060
Rainfall	↑ 25%	0.0098	0.0205	0.0109	0.0124
	↓ 25%	0.0197	0.0335	0.0127	0.0181
Land use	↑ 25%	0.0157	0.0211	0.0117	0.0134
	↓ 25%	0.0088	0.0134	0.0125	0.0093
Pop. Density	↑ 25%	0.0164	0.0219	0.0125	0.0142
	↓ 25%	0.0092	0.0136	0.0129	0.0097
Road	↑ 25%	0.0049	0.0081	0.0009	0.0040
	↓ 25%	0.0028	0.0006	0.0010	0.0023
Water	↑ 25%	0.0056	0.0013	0.0097	0.0144
	↓ 25%	0.0135	0.0103	0.0098	0.0174
Equal weights		0.0344	0.0939	0.0320	0.0671
Linear to sigmoidal		0.0067	0.0057	0.0018	0.0054
Rainfall symmetric		0.0109	0.2312	0.0232	0.0767
Temperature monotonic		0.0496	0.0649	0.0529	0.0444

* Calculated by taking arithmetic mean of all four seasons.

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favourable climatic conditions for dengue transmission. People living in these areas can freely cross the international border in both directions, increasing their exposure to dengue virus infection. Other regions also reported an increasing incidence of dengue in places where there is free cross-border movement of people [48].

Vulnerability was entirely restricted to lower-elevation areas during the winter season, whereas many additional areas had enhanced risk in the other seasons, even including some higher-elevation areas. Areas found to be vulnerable during the summer season included the Thimphu (where capital city is located), Wangdiphodrang, Punakha, Tsirang, Paro, Trongsa, Mongar, Trashiyangtse, Lhuentse, and Trashigang districts. These are built-up areas, surrounded by agricultural land, with better road facilities and a high population density. High dengue incidence in built-up areas might be related to human activities such as using water tanks, plastic bottles, discarded tyres, ornamental ponds, metal drums, flower pots, buckets, trays and pots, which increases breeding habitats for immature mosquitoes [49]. Similarly, settlements with more gasoline stations and workshops, rice paddies and marshy or swampy land have been shown to have more dengue vectors [36]. As predicted by the model, a remote village in Trashiyangtse district reported its first-ever dengue outbreak in 2019. This area is located at an elevation of 915m above sea level, that few in the community expected to be vulnerable to dengue (Entomologist, Vector-borne Disease Control Programme, oral communication, 2nd March 2020). The method developed here could be extended to explore the effects of future global warming given that there are no actual data currently in existence that can enable an empirical analysis.

MCDA is still growing in public health applications, although it is widely practised in other disciplines [50, 51]. Using national surveillance data, validation of our model produced an average ROC-AUCs of 0.67. This shows that our model for generating vulnerability maps is reasonably reliable without the need for additional data to refine the parameters. Sensitivity analysis revealed higher mean differences in vulnerability estimates in all the vulnerability maps when varying the shape of the MF as compared to varying the weights. This indicates the

importance of defining the shape of the relationship between the risk factors and the vulnerability outcome [46]. Given that greater certainty exists around the shape of relationships than the values of the specific weightings, this increases our confidence in the model.

This study is subjected to several limitations. Factors such as literacy rate, poverty index, sanitation and hygiene could not be mapped as they were only available at coarse resolution (i.e. the district level) [52]. Up-to-date meteorological data were not available at higher resolution, and the classification of the road network into different types such as national highway or secondary road was not available. Dengue cases from the NEWARS were used to validate the model. However, those not captured by the surveillance systems who were asymptomatic or mild cases and did not seek care in public health facilities were not accounted for. Additionally, validating the vulnerability maps against data on locations where dengue had occurred doesn't account for areas that are vulnerable for dengue but where the disease has not yet reached, or for areas where dengue was reported but no local transmission had actually occurred (i.e., all cases were imported).

Overall, our study revealed peak season and areas of vulnerability to dengue at specific locations for prioritizing intervention strategies. The findings have implications for the development of surveillance and early warning systems for dengue, allowing identification of high-risk areas to mitigate public health effects of dengue in the country.

Supporting information

S1 Table. Seasonal dengue incidence per 10,000 inhabitants between 2016 and 2019, Bhutan (Data source: NEWARS).

(DOCX)

S2 Table. Total number of publications reporting association between dengue incidence and selected risk factors in the Asian countries.

(DOCX)

S3 Table. Pearson Correlation Coefficient (r) for pair of risk factors associated with occurrence of dengue transmission.

(DOCX)

S1 Fig. Average annual dengue incidence per 10,000 inhabitants between in 2016–2019, Bhutan. We calculated the incidence by dividing the average number of dengue cases reported to dengue surveillance (NEWARS) between 2016 and 2019 by the average district population. (Data source: NEWARS).

(TIFF)

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Clinical management and practice of dengue in Bhutan

6.1 Introduction

Reducing dengue incidence and case fatalities requires an organised process of early detection, treatment, and timely referral when indicated. Early detection and timely treatment of patients with dengue is critical to prevent patients from progressing to severe dengue and case fatalities. This study was conducted to investigate the knowledge and clinical management practices of dengue in Bhutan using an online survey. Qualtrics survey software was used to administer the survey questionnaire to the eligible participants. The questionnaire contained four domains with a list of related items, namely: transmission, clinical course and presentation, diagnosis and management, and surveillance and prevention of dengue. Participant's knowledge score was calculated as an aggregate of all the knowledge-based questions separately for each domain. The study revealed major gaps in the knowledge and clinical management practices of dengue in Bhutan. The level of knowledge significantly varied according to the type of profession, healthcare setting and past experience in managing dengue cases.

6.2 Article on knowledge of dengue management

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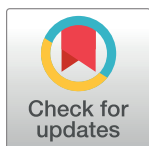
RESEARCH ARTICLE

Medical practitioner's knowledge on dengue management and clinical practices in Bhutan

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Abstract

Background

Dengue has emerged as a major public health problem in Bhutan, with increasing incidence and widening geographic spread over recent years. This study aimed to investigate the knowledge and clinical management of dengue among medical practitioners in Bhutan.

Methods

We administered a survey questionnaire to all practitioners currently registered under the Bhutan Medical and Health Council. The questionnaire contained items on four domains including transmission, clinical course and presentation, diagnosis and management, and surveillance and prevention of dengue. Participants were able to respond using an online Qualtrics survey, with the invitation and link distributed via email.

Results

A total of 97 respondents were included in the study (response rate: 12.7%), of which 61.86% were Health Assistants/Clinical Officers (HAs/COs) and 38.14% were medical doctors. The afternoon feeding behaviour of *Aedes* mosquito was correctly identified by only 24.7% of the respondents, and ~66.0% of them failed to identify lethargy as a warning sign for severe dengue. Knowledge on diagnosis using NS1 antigen and the clinical significance of elevated haematocrit for initial fluid replacement was strikingly low at 47.4% and 27.8% respectively. Despite dengue being a nationally notifiable disease, ~60% of respondents were not knowledgeable on the timing and type of cases to be reported. Respondent's median score was higher for the surveillance and reporting domain, followed by their knowledge on transmission of dengue. Statistically significant factors associated with higher knowledge included respondents being a medical doctor, working in a hospital and experience of having diagnosed dengue.

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Conclusion

The study revealed major gaps on knowledge and clinical management practices related to dengue in Bhutan. Physicians and health workers working in Basic Health Units need training and regular supervision to improve their knowledge on the care of dengue patients.

Introduction

Dengue, one of the most rapidly spreading vector-borne viral diseases, is found in tropical and sub-tropical regions around the world [1]. Before 1970, only nine countries experienced severe dengue epidemics [2]. However, dengue is now endemic in over 140 countries in Africa, the Americas, the Eastern Mediterranean and Asia [3]. The 2002 World Health Assembly Resolution WHA55.17 urged greater commitment to dengue control among member states, owing to its rapidly increasing public health importance. Subsequently, the IHR 1969 was revised in 2005 (WHA58.3) to include dengue among the public health emergencies of international concern (PHEIC) [4].

Reducing dengue morbidity and mortality requires an organized process of early detection, accurate classification, notification, treatment and referral when necessary [5]. Early detection and selecting the most appropriate treatment for dengue is of paramount importance to prevent the progression of the disease to severe dengue and subsequent case fatalities [6]. As a result of appropriate clinical management, case fatalities due to dengue have been significantly reduced from 10–20% to less than 1% in many countries over recent years [7]. Activities such as triaging and management decisions at the primary and secondary care levels are critical in reducing the number of unnecessary hospitalizations and deaths [5]. Timely notification of dengue allows rapid initiation of public health interventions, thereby reducing the transmission of dengue virus in the community [6].

Due to the non-specific clinical presentation, complex case definitions and a lack of routine laboratory diagnosis, dengue is often misdiagnosed, leading to poor clinical management and underreporting [8, 9]. In countries where dengue is imported by travellers, both diagnosis and treatment are delayed due to a lack of clinical suspicion of the disease [10]. Knowledge of dengue in such settings can be poor, with healthcare workers wrongly identifying *anopheles* mosquitoes as the vectors of dengue (15%) [11], use of paracetamol for preventing dengue [12], and prescribing antiviral and antimalarial drugs for treating dengue [13]. Whilst nearly all clinicians in a study in Texas demonstrated awareness that avoidance of mosquito bites is necessary to prevent dengue, only 33% knew that febrile dengue patients should take precautions to avoid mosquito bites to prevent dengue virus transmission to household members [14]. Similarly, in Singapore, only a small proportion of physicians (29.5%) reported performing an early diagnostic test for every suspected dengue cases [15]. A study in Taiwan had found poor knowledge of important clinical characteristics of dengue [6]. All these variations necessitate the need to understand the local characteristics of dengue management practices to develop appropriate strategies to improve population health.

Bhutan experienced its worst ever dengue epidemic in 2019. The Royal Centre for Disease Control under the Ministry of Health recorded 5,935 dengue cases during the 2019 epidemic, which is more than all the combined cases reported in previous years. Cases were reported from 19 of the 20 districts and six deaths were reported (Fig 1). The current study aims to characterise dengue-related knowledge and clinical management practices among medical practitioners (MP) in Bhutan in the year following the 2019 outbreak.

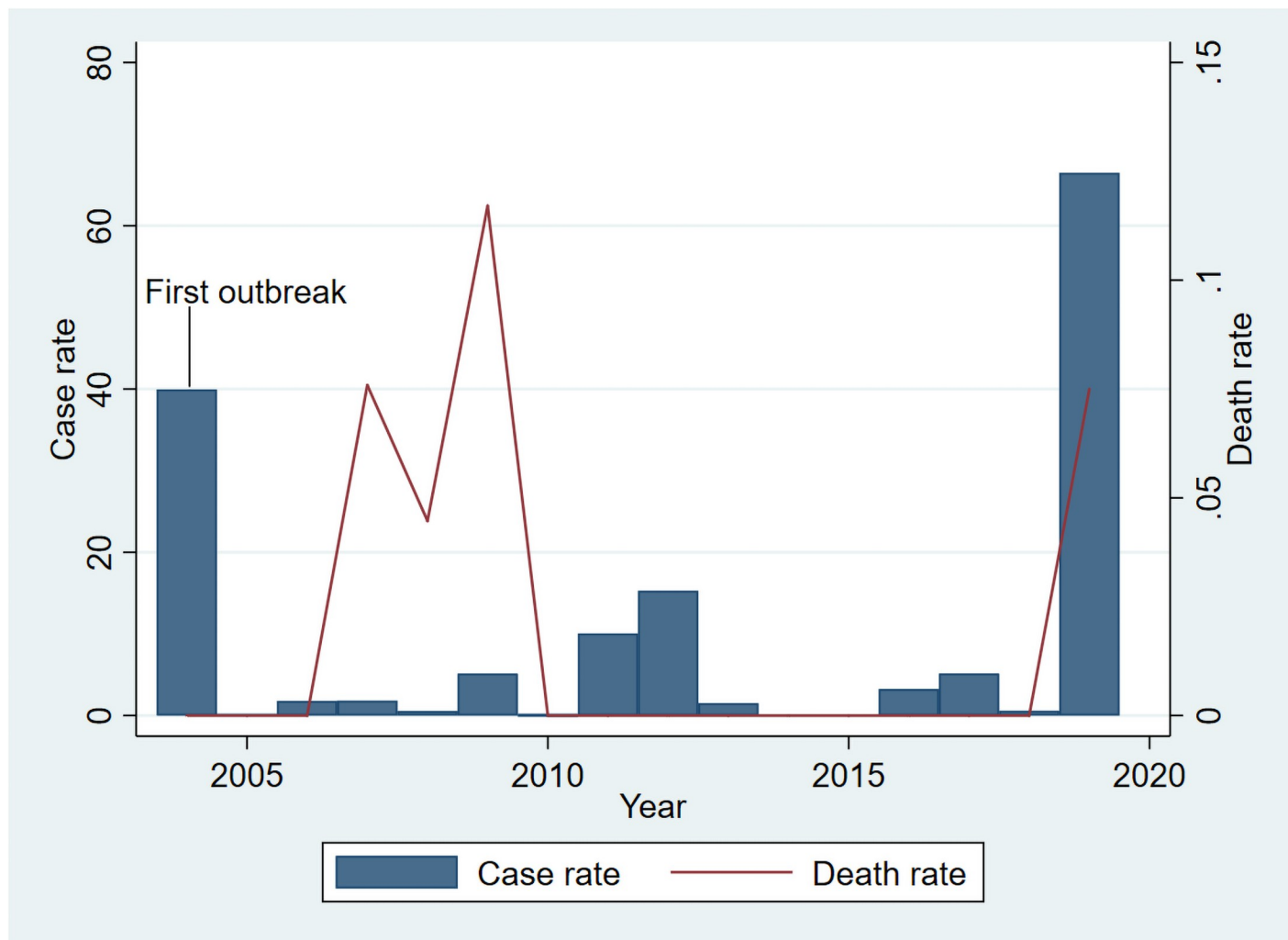


Fig 1. Dengue incidence and death rate (per 10,000) in Bhutan, 2004–2019 (source, Annual Health Bulletin, Royal Centre for Disease Control, and WHO-SEARO).

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Materials and methods

Study setting

Bhutan is a small landlocked country measuring 38,394 square kilometres, nestled in the Eastern Himalayas between India in the south, east and west, and China in the north. The right to free access to essential health services is mandated by the constitution, and currently, the expenditure for healthcare services including referrals to third countries is borne by the government of Bhutan. Healthcare services are delivered through 32 hospitals (one-national, two regional and the remainder district general hospitals) and 208 Primary Health Care (PHC) or Basic Health Units (BHUs—grade I and II) [16]. Primary care is delivered in each of these health centres, but medical technologies and healthcare services become more advanced and specialized from BHUs to district general hospitals, to regional hospitals and the national referral hospital. Over 903 MPs are currently registered by the Bhutan Medical and Health Council (BMHC). This includes 92 medical specialists (MD), 186 general duty medical officers (GDMO with a Bachelor of Medicine/Bachelor of Surgery), 21 clinical officers (COs) and 604

Health Assistants (HAs) [16]. HAs undergo two years in-country training for primary care based on a whole-of-society approach that includes health promotion, disease prevention, diagnosis, treatment, rehabilitation and palliative care. These HAs undertake an additional one-year diploma in clinical management to become COs. Although doctors and HA/COs work together in all health facilities, HA/COs predominantly work at BHUs.

Study design

We conducted a self-administered online survey among MPs in Bhutan from November to December 2019. This cross-sectional study sought to understand the MP's knowledge and management practices in relation to dengue.

We used Qualtrics (Qualtrics, Provo, UT) to administer the survey questionnaire. Each respondent was sent an invitation message in their email to complete the survey questionnaire, with a URL link embedded within the message. Once the respondents voluntarily agreed to participate in the study, they then navigated to the survey questionnaire. To increase our response rate, as explained by Cook et al. [17], we sent two reminders on days, 14 and 28 after sending out the initial email.

Respondent recruitment

Eligible respondents comprised of any practitioner practising in areas involved in diagnosis and management of dengue patients, including general medicine, paediatrics, emergency medicine, intensive care and laboratory medicine, at any level of the health system. Of the 903 MPs registered by the BMHC, email addresses were obtained for 777 of them. Five were excluded as they didn't work clinically in areas relevant to dengue (including health service managers, surgeons and anaesthesiologists), thus creating a potential respondent group of 772 MPs (Fig 2). Survey questionnaires were emailed to all respondents from December 2019 to January 2020. Email addresses were collected from the district health services. The district health managers contacted target respondents via telephone and email to encourage participation in the survey. Reminder telephone calls were also made by managers to enhance participation of the respondents. No incentives or compensations were offered to the respondents.

Survey questionnaire

Development of the study tool was informed by a review of the literature related to clinical practices for dengue care and past surveys on dengue management [11, 12, 14, 18, 19], and the tool was evaluated by three local experts on dengue. The tool was further modified after feedback from a pilot study which was conducted in Gomtu Hospital, Samtse district. Respondents from the pilot study were excluded in the final survey and analysis. Demographic questions included district, region, age, sex, highest qualification, number of years in service, whether they had diagnosed dengue and their specialty. The main questionnaire consisted of 25 multiple choice questions, which included the following domains: 1) Transmission; 2) Clinical course and presentation; 3) Diagnosis and management; 4) Surveillance and prevention of dengue (S1 Appendix). Since English is the official medium of pedagogy in both schools and medical institutes in the country, the questionnaire was designed in English and not translated into Dzongkha (the national language).

Data analysis

The response rate was calculated by dividing the total number of surveys returned by the total number of invitations (772). Characteristics of the respondents were described using

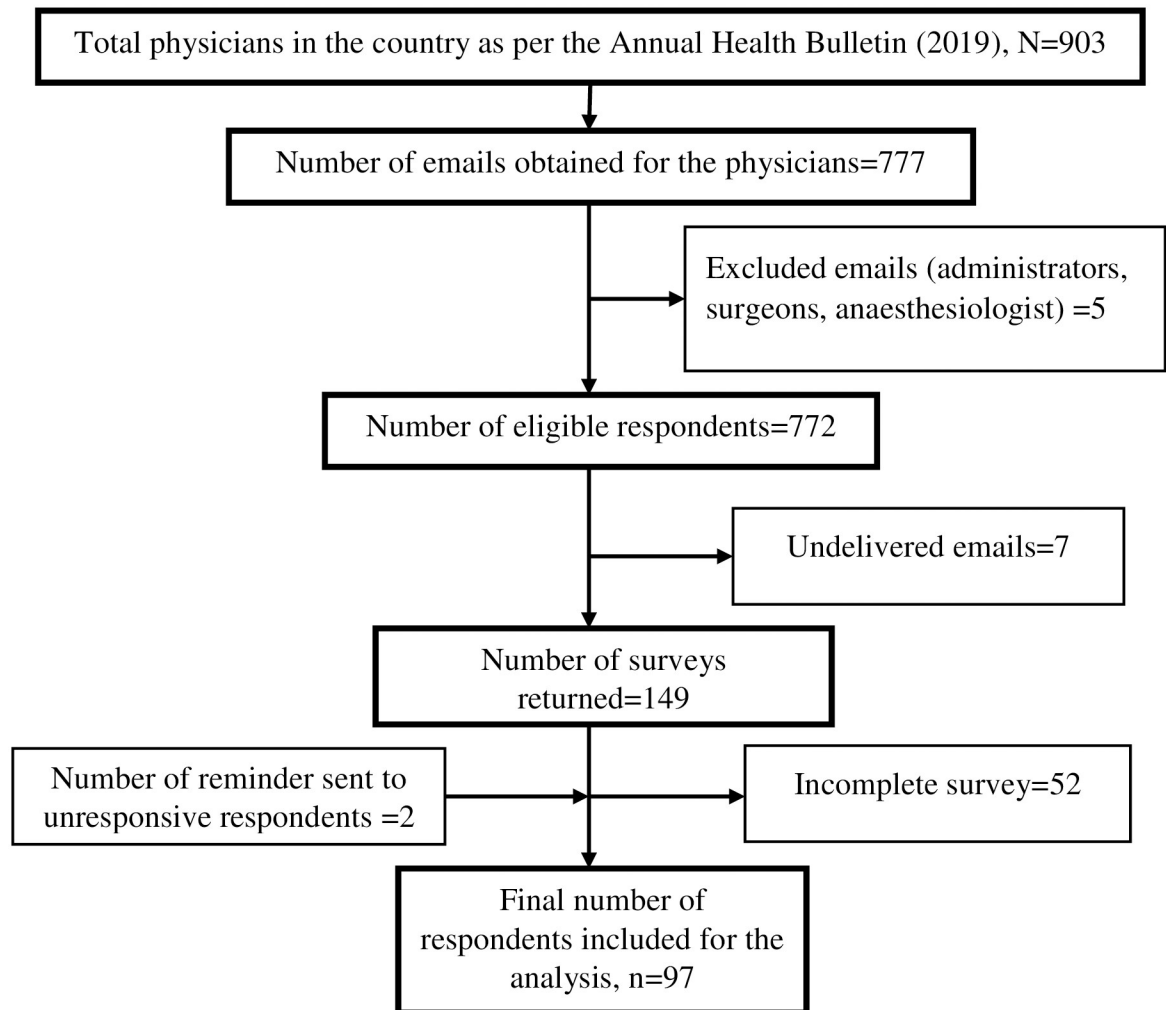


Fig 2. Study population recruitment for assessing knowledge on dengue management among physicians in Bhutan, 2019–2020.

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categorical variables and presented as frequencies and proportions. For simplicity, national, regional and district general hospitals were grouped as “hospital”, while BHU grade I and II were grouped as “BHU”. Correct responses to each question were summarized under separate domains, using frequencies and proportions. A choropleth map was developed in ArcGIS version 10.5 (ESRI, Redlands, CA) for visualizing the proportion of respondents (%) who participated in the survey [20].

To compare knowledge across respondents with different characteristics, a cumulative knowledge score was calculated as an aggregate of all the knowledge-based questions separately for each domain. A score of one was given for the right answer. For questions with multiple-choice questions, respondents received a score of one for every correct answer. Normalization was performed to standardize domain scores into values that ranged from 0 to 1 by dividing the respondent’s total score for each domain by the total domain score. Boxplots were created for each domain using the normalized scores. Since the distributions were non-normal as determined by a histogram, knowledge scores of all domains were compared across categories of respondent characteristics using non-parametric tests; i.e. the Man-Whitney U test and

the Kruskal-Wallis test. All data analyses were carried out using Stata statistical software (version 15.1; Stata Corp, College Station, Texas, USA) [21].

Ethical clearance

The study was reviewed and approved by the Research Ethics Board of Health (REBH), Ministry of Health, Bhutan (2019/081) and the Human Research Ethics Committee (HREC), Australian National University (ANU), Australia (2019/808).

Results

Response rate

Of the 772 respondents to whom the invitation was sent, we received notification that seven emails were not delivered. In addition, 52 respondents responded but did not complete the survey questionnaire, and were thus excluded from the response rate and subsequent analysis. Among those respondents who did not complete the survey, 14 were from the BHUs, two were from the national referral hospital and seven were from district hospitals. However, 27 of the respondents did not complete the health centre details. In terms of roles, 17 were HAs/COs, five were medical doctors and the rest did not provide any information. We got a response from a total of 97 respondents who had completed the questionnaire, yielding a total response rate of 12.7% (97/765) (Fig 2).

Characteristics of the respondents

Chukha district had the highest frequency of respondents ($n = 13$) (Fig 3). The age group with the largest number of respondents was <30 years (44.3% of the total) and most respondents were male (72.2%). There was a greater number of respondents who were HAs/COs (61.9%) than medical doctors (38.1%). Respondents from all health centres participated in the survey,

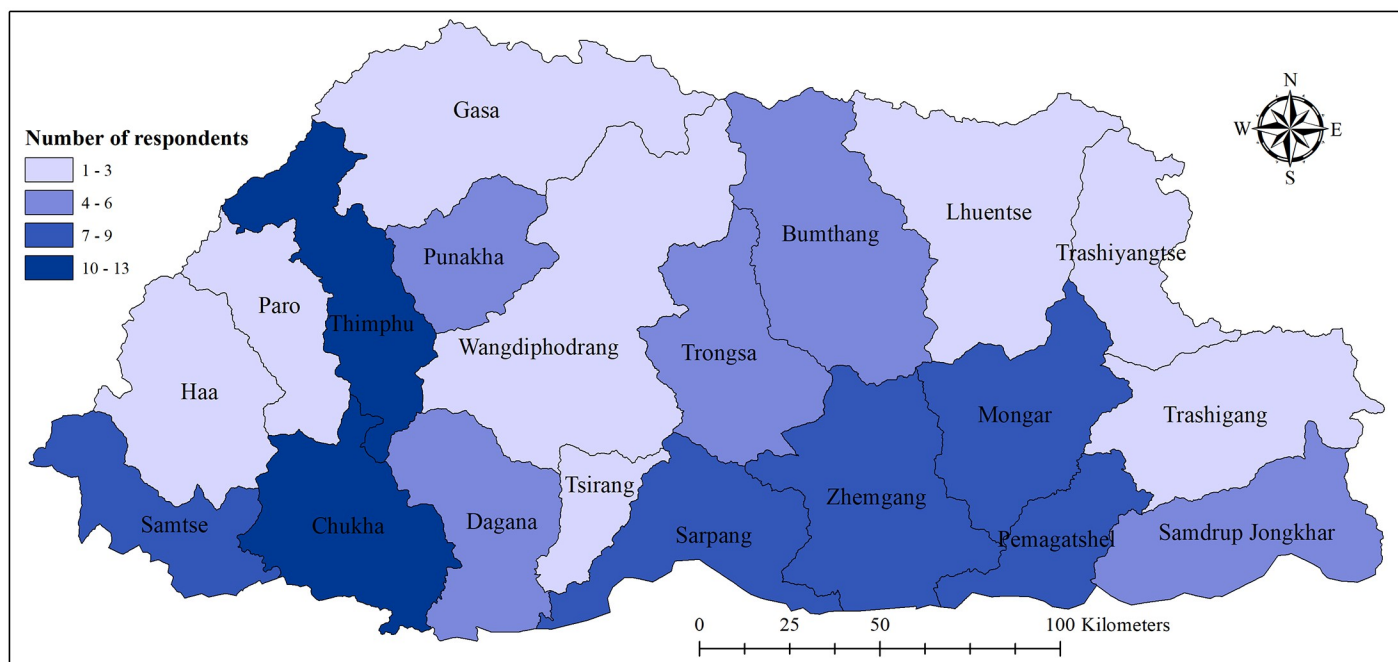


Fig 3. District-wise number of respondents included in the study, Bhutan, 2019–2020.

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Table 1. Characteristics of the study respondents.

Variables	Response selected	n	%
Age (years)	<= 29	43	44.3
	30–39	34	35.1
	>= 40	20	20.6
Sex	Male	70	72.2
	Female	27	27.8
Qualification	Certificate/Diploma	48	49.5
	BPH/M.Sc	5	5.2
	MBBS	36	37.1
	MD+PhD	8	8.3
Medical role	HAs/COs	60	61.9
	Medical doctors	37	38.1
Health centre level	Hospital	44	45.4
	BHU	52	53.6
	Private diagnostic	1	1.0
Medical speciality	General medicine	88	90.7
	Paediatric	2	2.1
	Emergency	3	3.1
	Obstetrics/gynaecology	2	2.1
	Laboratory/Radiology	2	2.1
Medical experience (years)	<= 5	50	51.6
	> 5	47	48.5
Ever diagnosed	Yes	54	55.7
	No	43	44.3

BPH: Bachelor of Public Health. HA: Health Assistant. CO: Clinical Officer. BHU: Basic Health Unit.

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including one from a private diagnostic centre. General medicine/practice was the most commonly reported specialty (90.7%) followed by emergency services (3.1%). More than half of the respondents had personally diagnosed dengue in a patient (55.7%) (Table 1).

Knowledge on the transmission of dengue (domain 1)

The majority of respondents (89.7%) correctly identified *Aedes* mosquitoes as the vector of dengue. Whilst more than half of the respondents reported mornings and evenings as times favourable for feeding behaviour of vector mosquitoes, only 24.7% correctly identified afternoons as a favourable feeding time. Only 16.7% reported that when a mosquito bites a dengue patient, it might become infected and then infect the next person it encounters during feeding time. About one-third of the respondents were aware of different dengue serotypes circulating in the country (Table 2).

Clinical course/presentation of dengue (domain 2)

Most of the study respondents (88.7%) were knowledgeable about the duration of the incubation period of infection with dengue virus. While more than half of the respondents correctly indicated options for identifying a patient with dengue at the time of presentation, only 45.4% would ask patients about vomiting, abdominal pain, lethargy and bleeding. Persistent vomiting, severe abdominal pain and bleeding were correctly identified as warning signs for severe dengue by 54.6%, 55.7% and 83.5% respectively. Lethargy was identified as a sign of severe

Table 2. Knowledge on the transmission of dengue (domain 1), Bhutan, 2019–2020.

Question	Response	n (%)
1. How is dengue transmitted?	1. By the bite of <i>Aedes</i> mosquitoes	87 (89.7)
2. When do <i>Aedes</i> mosquitoes bite a person?	2.1. Morning	60 (61.9)
	2.2. Afternoon	24 (24.7)
	2.3. Evening, until dark	73 (75.3)
3. When a mosquito bites a dengue patient, which of the following is true?	3.1. Mosquito might get infected and can infect next person during feeding time	16 (16.5)
	3.2. There is no infection of the mosquito if DENV is cleared from the human body	86 (88.7)
4. So far, which of the DENV serotypes are identified in Bhutan?	4.1. DENV-1	69 (71.1)
	4.2. DENV-2	61 (62.9)
	4.3. DENV-3	26 (26.8)

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dengue by only 34.0% of respondents. A notable lack of knowledge was observed on the classification of dengue based on the given clinical scenario; only 27.8% were able to correctly identify the right option of “dengue with warning signs” (Table 3).

Diagnosis and management of dengue (domain 3)

Respondents less frequently identified NS1 antigen for early diagnosis (47.4%) than anti-dengue IgM antibodies (62.9%). Chest X-ray with a lateral decubitus view for pleural effusion and haematocrit for diagnosing plasma leakage were reported as parts of the diagnostic approach for dengue by 27.8% and 56.7% respectively. Only 21.7% of the respondents correctly reported that patients with pre-existing co-morbidities need to be closely monitored before discharging them. Of the indications identified for administering IV crystalloids such as Normal Saline or Ringer’s Lactate to dengue patients, hypotension and tachycardia, or low urine output, were identified by 78.4% and 50.5% respectively. Surprisingly, only 27.8% correctly identified high haematocrit as an indication for initial fluid replacement. For a given scenario of a six-year-old boy with fever of a few days duration, distended painful abdomen and lethargy, 66.0% of the respondents reported that they would order a dengue test and admit the patient for 24 hours of observation (Table 4).

Surveillance and prevention of dengue (domain 4)

The majority of the respondents were knowledgeable about dengue being a nationally notifiable disease (80.4%). However, only 47.4% knew that suspected or confirmed cases have to be reported every week. More than 80.0% of respondents were able to identify both the variables (i.e., age and case type) included in surveillance reporting. About 95.0% of respondents were able to identify prevention messages such as using mosquito repellent and wearing long sleeve pants and shirts to prevent dengue infection (Table 5).

Comparisons of domains by respondent’s characteristics

The cumulative median score was 32 (IQR: 22–39) out of a possible score of 54 for all the four domains. Boxplots demonstrated higher median scores for domain 4 followed by domain 1.

Table 3. Knowledge of clinical course/presentation of dengue (domain 2), Bhutan, 2019–2020.

Question	Correct response/s	n (%)
1. How long does it take to develop symptoms of dengue?	1. Between 3–7 days after mosquito bite	86 (88.7)
2. How should a clinician identify patients with dengue at the time of presentation?	2.1. Take fever history	55 (56.7)
	2.2. Perform complete blood count	53 (54.6)
	2.3. Ask about headache, retro-orbital pain, body-aches and rash	75 (77.3)
	2.4. Ask about vomiting, abdominal pain, lethargy and bleeding	44 (45.4)
3. Identify the warning signs of dengue:	3.1. Persistent vomiting	53 (54.6)
	3.2. Lethargy	33 (34.0)
	3.3. Bleeding	81 (83.5)
	3.4. Severe abdominal pain	54 (55.7)
4. A patient presents to you with a history of fever, headache, joint pain, vomiting, mucosal bleeding, lethargy and liver enlargement. This patient is classified as:	4. Dengue with warning signs	27 (27.8)
5. Severe dengue is considered if the patient from an area of dengue risk presenting with 2–7 days of fever has any of the following clinical manifestations:	5.1. Evidence of plasma leakage	55 (56.7)
	5.2. Significant bleeding	63 (65.0)
	5.3. Lethargy or restlessness	46 (47.4)
	5.4. Persistent vomiting or acute abdominal pain	50 (51.6)
	5.5. Severe organ impairment	46 (47.4)
6. Which is the best early indicator of shock?	6. Tachycardia in the absence of fever or delayed capillary refill	43 (44.3)
7. When does clinically significant plasma leakage usually develop?	7. Between 3–7 days around the time of defervescence	69 (71.1)
8. Thrombocytopenia is expected to develop:	8. Following progressive leukopenia, around the time of defervescence	45 (46.4)

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Domain 2 and 3 had the same median score with slightly lower upper quartile scores for domain 2 (Fig 4). Being a medical doctor and having diagnosed dengue were significantly associated with a higher median score across all domains (p -value<0.05). Those working in the hospital had a significantly better score for the first three domains (p -value<0.05). Performance of the respondents did not vary by age group, sex or medical experience for any of the domains (S1 Table).

Discussion

MPs play a crucial role in early detection and timely management of dengue patients, and notification of dengue cases to national authorities. Having a high level of accurate knowledge on dengue management amongst these professionals can save the lives of patients and prevent other healthy people from becoming infected. The current study revealed major gaps in the knowledge of MPs on transmission, clinical presentation, diagnosis and treatment, and

Table 4. Knowledge on diagnosis and management of dengue (domain 3), Bhutan, 2019–2020.

Question	Correct response/s	n (%)
1. What is true about testing dengue virus?	1.1. Anti-dengue IgM antibodies are first detectable in most patients on days 3–5 after illness onset	61 (62.9)
	1.2. NS1 Ag appears as early as day 1 & can be used for early diagnosis	46 (47.4)
2. How is significant plasma leakage detected in a suspected dengue patient?	2.1. Increasing hematocrit above 20% of baseline	55 (56.7)
	2.2. Chest X-ray with lateral decubitus view to look for pleural effusion	27 (27.8)
3. The best course of action for a 6-year old boy with fever of a few days duration, distended painful abdomen and lethargy is to:	3. Order a dengue lab test and admit the patient for 24 hours of observation	64 (66.0)
4. Select the therapies that you would use for managing dengue fever	4.1. Paracetamol	80 (82.5)
	4.2. Oral rehydration	74 (76.3)
5. Which of the following are the correct criteria for sending a suspected dengue patient home?	5.1. Passing urine at least once every 6 hours	36 (37.1)
	5.2. Does not have major co-morbidities	21 (21.7)
	5.3. Does not have any warning signs	82 (84.5)
	5.4. No hemoconcentration	39 (40.2)
6. Under what circumstances do you tell a patient with suspected dengue to return to the clinic?	6.1. Persistent vomiting	69 (71.1)
	6.2. Drowsiness or lethargy	64 (66.0)
	6.3. Hematemesis	65 (67.0)
7. When should IV crystalloids (like Ringer's Lactate or Normal Saline) be given to suspected dengue patients?	7.1. Hypotension (as initial fluid replacement)	76 (78.4)
	7.2. High hematocrit (as initial fluid replacement)	27 (27.8)
	7.3. Tachycardia, delayed capillary refill, or low urine output (as initial IV fluid therapy)	49 (50.5)
8. When should a blood transfusion be given to patients with suspected dengue?	8.1 Significant clinical bleeding	54 (55.7)
	8.2. Low hematocrit and persistent shock after a trial of IV crystalloids and/or colloids	41 (42.3)

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prevention of dengue in Bhutan. Knowledge varied according to the type of profession, health-care setting and past experience in managing dengue cases. To our knowledge, this is the first study of health practitioner's knowledge and practices with regards to dengue in the WHO-South East Asia Region (WHO-SEAR).

In this study, poor knowledge on transmission of dengue was evident from the respondent's inability to recognize the feeding time of dengue mosquitoes. Similar findings have been documented by Huang et al. in Taiwan, where, only 14.4% of respondents correctly identified the feeding behaviour of dengue mosquitoes [22]. Having accurate knowledge on the behaviour of dengue mosquitoes will help MPs to impart appropriate health education [22], which remains one of the cornerstones of preventing dengue.

Table 5. Surveillance reporting and prevention of dengue (domain 4), Bhutan, 2019–2020.

Question	Correct response/s	n (%)
1. Which of the following are true about the reporting of dengue in Bhutan?	1.1. Dengue is a notifiable disease	78 (80.4)
	1.2 All suspected or confirmed dengue cases should be reported every week	46 (47.4)
	1.3. Severe dengue should be reported immediately after detection	57 (58.8)
2. Variables required to report dengue cases as per the national surveillance are:	2.1. Age	78 (80.4)
	2.2. Case type (whether death occurred or not)	82 (84.5)
3. Dengue vaccination is recommended for people aged ≥ 9 years in areas with:	3. $\geq 70\%$ prevalence of dengue	57 (58.8)
4. What prevention message should be given to patient to prevent dengue infection?	4.1. Use of mosquito repellent, and wearing long sleeve shirts and pants	92 (94.8)
	4.2. Taking precautions to avoid the bite of mosquitoes if any member of the household is infected with dengue	68 (70.1)

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Respondents had low knowledge of the signs that lead to dengue shock and thrombocytopenia. Such findings were also reported in Puerto Rico, where only 29.0% of the respondents correctly identified early signs of shock, and 48.0% identified severe abdominal pain and persistent vomiting as warning signs of severe dengue [23]. This could be due to a lack of training on the recognition of warning signs and case classification of dengue as per the updated WHO guidelines. Identification of warning signs of dengue and indications that lead to shock is critical for managing dengue [1]. Yusuf and Ibrahim reported that 56.0% of respondents lacked adequate training to manage dengue patients, including identifying warning signs, and recommended to close this gap [24].

In the diagnosis and management domain, respondents failed to recognize the importance of NS1 antigen as an early marker of dengue infection. This might indicate a lack of training, experience of managing dengue patients or a lack of diagnostic test kits for detecting NS1 antigen in health facilities as has been reported in other countries [24]. IgM would be negative during the febrile phase (it may be positive at or after defervescence), while NS1 antigen becomes detectable as early as day one of onset of the disease [1]. Ruberto et al. [25] reported that less than half of the healthcare providers were choosing the right diagnostic tools in the U.S. Respondents also performed poorly on knowledge regarding the detection of plasma leakage using Chest X-rays. This could be in part due to unavailability of X-ray machines in many facilities like BHUs, where they depend on simple blood test parameters, noting that 47.7% of the respondents from hospitals reported using Chest X-ray, while only 11.5% from BHUs reported the use of Chest X-ray for detecting plasma leakage. The feasibility of cheaper, portable and a point-of-care ultrasound machines may be considered to detect plasma leakage and other lifesaving diagnoses in remote areas without X-ray facilities [26].

Respondents also overlooked the importance of recognizing co-morbidities that require admission for patients suspected of having dengue. A similar pattern was also observed in Ecuador, where only 22.0% of healthcare providers were reported to be monitoring dengue patients having co-morbidities for hospital admission [12]. Patients with significant co-morbidities should be closely monitored before they can be sent back home regardless of the severity of the disease [14]. Respondents also had a poor understanding of elevated haematocrit as an indication for fluid replacement during the course of dengue illness. A similar finding was

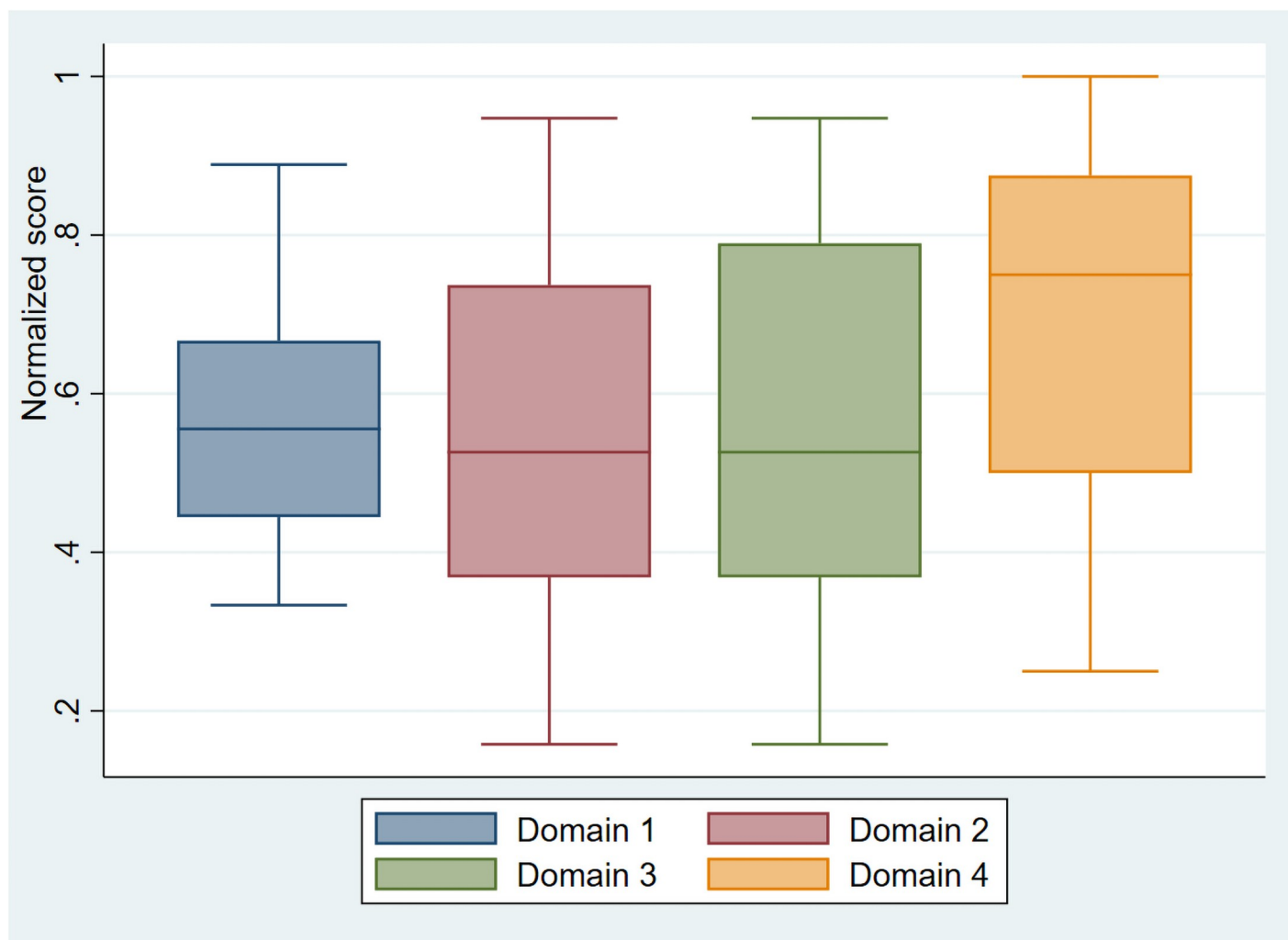


Fig 4. Boxplot showing respondent scores for four domains on knowledge of dengue management in Bhutan, 2019–2020. Domain 1: Transmission; domain 2: Clinical course; domain 3: Diagnosis and treatment; domain 4: Surveillance and prevention.

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reported in Puerto Rico, where 30.0% of respondents correctly identified elevated haematocrit after an initial trial of intravenous crystalloids [23].

Only 47.4% of participants knew that aggregate cases, whether suspected or confirmed (based on the WHO case definition) have to be reported weekly. This suggests the need to evaluate and reinforce training of physicians on surveillance and reporting. Training on national disease surveillance and reporting is conducted every year by the Ministry of Health to sensitize healthcare workers, including physicians. There is a need to review the existing strategy of training healthcare workers on the surveillance system in the country to improve reporting.

The high proportion of respondents who had poor knowledge of transmission and clinical course might be due to the lack of experience in dengue case management by the respondents. This is evident with 44% of the respondents having never seen dengue in their professional career. In Bhutan, dengue transmission only occurred in Chukha district (Fig 3) from 2004 to 2012 [27]. Eventually, it spread to eight new districts by the end of 2018, and a further 10 districts in 2019 [28]. Prior to 2019 dengue epidemic, training on dengue management was mostly conducted among clinicians and healthcare workers in the dengue-endemic districts.

Since 2019, dengue is reported from 19 districts in Bhutan. Therefore, it is an opportune time to train and provide refresher courses to all clinicians and health workers of Bhutan.

Overall, knowledge on transmission, clinical course, diagnosis and management, and surveillance and prevention of dengue was higher for medical doctors, physicians working in hospitals and healthcare workers who had experience in managing dengue cases in their clinical practice. In hospitals, most of the physicians are medical doctors, which might have led to increased performance as compared to those working in BHUs, which are mostly staffed by HAs/COs. Variation in performance between the type of profession may be related to their educational qualifications and responsibilities. A study in Ethiopia has shown that physicians were 39 times more likely to have a high level of knowledge on dengue prevention than nurses [24]. The same study also demonstrated that healthcare workers who worked in referral hospital settings were 75 times more likely to have a high level of knowledge on dengue prevention when compared to those who worked in health centres [24]. Additionally, physicians with past dengue clinical experience had better knowledge than dengue-inexperienced physicians [14], and findings of significant differences in knowledge of dengue among healthcare workers in different clinical settings have been reported in other regions [6, 15]. This emphasizes the need to prioritize specific health centres and novice health professionals in dengue case management training and continuing medical education to reduce morbidity and mortality due to dengue.

This study has some limitations worth mentioning. The response rate was low despite our endeavours to increase it by extending the duration of the survey for collecting the data by more than two months. Firstly, respondents located in remote or hard-to-reach areas with limited internet connectivity may not have accessed the survey despite a follow-up reminder to participate in the survey. Secondly, due to limited health workforce, respondents might have other competing priorities like patient care and health promotion activities at the time of the survey and had to forego their participation. Thirdly, as the number of respondents were higher in the southern region, we speculate that respondents working in the dengue-endemic districts including Chukha, Samtse, Sarpang, Zhemgang, Pemagatshel and Samdrup Jongkhar were more likely to take part in a survey as compared to those working in non-endemic areas due to the familiarity with the disease. High response rates in Thimphu can be attributed to the national referral hospital, where severe dengue patients are referred from other districts. However, this survey does include respondents from all 20 districts, thus covering the whole country.

Conclusion

The study reveals a critical gap and an urgent need to strengthen the knowledge of dengue management and clinical practices among physicians in Bhutan. Emphasis has to be given to educating physicians on transmission, clinical manifestations, early diagnosis and appropriate management of dengue patients. In addition, strategies should be put in place to enhance the physician's awareness of surveillance and reporting requirements. Exploratory studies such as focus group discussions with healthcare workers in both hospitals and BHUs or PHCs is suggested to understand critical gaps in dengue management.

Supporting information

S1 Table. Comparison of responses across four domains by the characteristics of the study respondents, Bhutan, 2019–2020.

(DOCX)

S1 Appendix. Research instrument.
(DOCX)

S2 Appendix. Dataset.
(XLSX)

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Discussion

7.1 Introduction

The work in this thesis addresses the knowledge gaps from previous studies and extends our understanding of the epidemiology of dengue in Bhutan.

Dengue is the most rapidly spreading vector-borne disease, occurring with explosive outbreaks and continuing to expand in previously unaffected areas with high intensity of transmission. WHO identified dengue as being among the top 10 threats to global health in 2019 (92). In Bhutan, dengue was first identified in 2004 and has become endemic in low-lying areas in the southern foothills with outbreaks occurring every 3–5 years. The disease continues to pose a serious public health risk and substantial economic loss to society. Despite an increase in the burden of the disease, there is limited research on the epidemiology of dengue in Bhutan. Therefore, this thesis was structured by incorporating important studies to better understand the epidemiology of dengue in Bhutan.

A narrative review was conducted in this thesis to understand the contemporary epidemiology including the surveillance system in the WHO-SEAR countries (Chapter 3). In addition, Chapter 3 also includes a study that used systematic review and meta-analysis to identify clinical predictors of severe dengue. This was followed by a detailed study of dengue in Bhutan using both primary and secondary data. Hotspots and drivers of dengue were assessed through a Bayesian analysis (Chapter 4). Using the 2019 surveillance data from the largest dengue outbreak in Bhutan, an in-depth study was undertaken to understand the outbreak by quantifying the effective reproductive number. Further, the spatial vulnerability of dengue using MCDA was conducted to identify risk profiles of places across the nation using demographic and environmental variables (Chapter 5). Finally, a study was conducted on the knowledge of dengue management and clinical practices in Bhutan (Chapter 6).

This chapter summarises the key findings of this thesis and enables a better understanding of the epidemiological characteristics and management of dengue in Bhutan. This chapter also highlights weaknesses and strengths of the research and suggests areas for future research.

7.2 Emergence of dengue and surveillance strategies

The first dengue outbreak in Bhutan was in 2004 in Phuntsholing Municipality in Chukha District, which shares a porous border with the Indian state of West Bengal. More than 2,500 cases were reported and the outbreak was associated with the importation of DENV-2 from India. Dengue transmission was limited to Phuntsholing Municipality until it later spread to other districts of Bhutan in 2013. Genetic studies have confirmed the presence of DENV-1 (genotype I), DENV-2 (Cosmopolitan genotype) and DENV-3 (genotype III) (75) while other genotypes (of DENV1-3) and DENV-4 have not been detected in the country.

Globally, asymptomatic dengue infections accounts for >70% of all infection (93). However, 0.5%–5% of the infection progresses to severe dengue resulting in severe bleeding, organ impairment and plasma leakage (94). Twenty percent of severe dengue can be fatal and lead to death. Therefore, WHO recommends that clinicians should have experience in the management of dengue and strive toward decreasing mortality from 20% to <1% (95). Understanding the predictors of severe dengue can help clinicians in providing the right treatment to the dengue patients and save lives. Meta-analysis of risk factors of severe dengue identified the demographic and clinical predictors of severe dengue. Children continues to be at-risk for severe dengue; the plausible reasons are the fragile capillaries and greater permeability with less microvascular reserve that responds to extraneous factors in children (96).

Secondary infection with a DENV serotype different from the primary infecting serotype also increased the risk of severe dengue as previously reported (97). This indicates that severe dengue is mediated by an immunological mechanism, the most common being antibody dependent enhancement (ADE). According to this mechanism, non-neutralizing antibodies form immune complexes with the virus and facilitate the entry and enhanced replication of virus in Fc γ receptor-bearing cells (dendritic cells, monocytes and macrophages) (98). Anamnestic response of T-lymphocyte also increases the release of pro-inflammatory cytokines, such as VCAM-1, VEGF-A and interleukins (99, 100). These cytokines increases the risk of vascular permeability and subsequently results in shock (101).

Dengue infection tends to be severe in patients with pre-existing co-morbidities such as diabetes and renal disease, as found in this study. Patients with poor glycaemic control were most likely to experience dengue infections as severe dengue, compared to those with normal glycaemic control (102). This can be due to the damaged vascular functions in advanced stage of diabetes leading to plasma leakage and severe dengue (103). Similarly, in renal disease, pro-

inflammatory cytokines are elevated, which might lead to vascular damage (104). In contrast to mild dengue, an elevated serum creatinine level is observed in severe dengue. Serum creatinine values above 4 mg/dl has been known to increase the risk of severe dengue (105, 106). This finding further supports the WHO recommendation of close observation of dengue patients with comorbidities irrespective of the severity of the disease (32).

WHO proposed seven warning signs of severe dengue and recommended any patients with those signs to be hospitalised for observation and management (32). The warning signs are abdominal pain, persistent vomiting, lethargy, hepatomegaly (>2 cm), mucosal bleeding, clinical fluid accumulation, and increase in haematocrit with concurrent decrease in platelet count. Several studies in the past assessed the utility of those signs as a guide to hospitalise and predict the disease progression to severe dengue (107). However, these studies failed to report the combined effects of those warning signs.

Severe dengue was also associated with abdominal pain, lethargy, vomiting, hepatomegaly, and increased haematocrit with concurrent decrease in platelet count. Ascites and pleural effusion were the most common type of clinical fluid accumulation that predicted the severity of dengue, while hematemesis, GI bleeding, and melena were the two common types of mucosal bleeding associated with severe dengue. However, epistaxis, skin bleeding, and gum bleeding were not associated with severe dengue. As there was an inconsistent association across different sites of mucosal bleeding, the findings imply that the site of mucosal bleeding is playing a critical role in determining the severity of dengue. This finding is in agreement with the previous meta-analysis study where gum bleeding and epistaxis were not associated with the development of severe dengue (108).

In order to improve an early case detection, response, and effective management including the treatment of dengue, surveillance system should be established in dengue-endemic settings. Dengue surveillance encompasses both epidemiological and entomological surveillance. Dengue is a notifiable disease in Bhutan and has been integrated with other notifiable diseases in the NEWARS, led by RCDC, Ministry of Health. NEWARS, a web-based reporting, replaced paper-based reporting in 2015, which substantially improved the timeliness of reporting and this has been used for timely control of the disease. However, there is still under-reporting despite providing annual training and sensitization to healthcare professionals on NEWARS. This might be related to poor acceptability and conflict of priorities between

clinical and public health activities (109). So, there is a need to undertake an evaluation study on NEWARS to improve reporting and strengthen the surveillance.

Entomological surveillance of dengue is managed by the VDCP since 2003. The VDCP supports, coordinates and evaluates vector control activities in district hospitals and BHUs/PHUs (76). Larval or pupal indices are the main sampling strategies used for dengue risk stratification to support vector control activities. However, adult mosquito sampling is not performed routinely and this is the major drawback of the current entomological surveillance. Larval or pupal indices are not an accurate indicator of transmission because only adult mosquitoes can transmit dengue infection (110). In addition, these immature mosquitoes have to be reared in insectaries to identify the species (111), which results in delays for subsequent control activities. The reason for not including adult mosquitoes in the routine surveillance might be related to affordability and accessibility of the traps, and associated high labour intensity (110). Incorporating adult mosquito in a routine surveillance will provide the necessary information for dengue control, particularly in understanding the risk and forecasting disease outbreaks.

7.3 Temporal and spatial distributions of dengue

Dengue transmission is seasonal and correlates with the onset of monsoon in the Himalayas (112). Monsoon seasons provides opportunities for immature mosquitoes to breed in a number of natural habitats such as tree holes, leaves, and other receptacles on accumulated water (55, 113). In Bhutan, dengue cases start to increase from spring (April/May) and peak in summer, coinciding with monsoon between July and September. During this period, there is abundant rainfall averaging 300 mm with conducive temperatures (minimum 17°C and maximum 28°C) for mosquito growth.

In the Bayesian spatio-temporal analysis, a positive association was found between maximum temperature and dengue, and a negative association between NDVI and dengue. Temperature is the most important factor that supports the transmission of dengue. An increase of temperature shortens the replication of DENV by reducing the extrinsic incubation period and increases the feeding frequency of *Aedes* mosquitoes (114). Higher temperature also accelerates the gonotrophic cycle in mosquitoes and makes them more aggressive. Consequently, the frequency of mosquito bites increases resulting in the enhanced transmission of dengue (85).

In contrast, NDVI was negatively associated with the transmission of dengue in Bhutan. Areas with the lower NDVI are indicative of less vegetation, such as urban and peri-urban areas, which are accompanied with poor infrastructure, water supply and sewerage treatment, especially in developing countries (115). Such settlements facilitate the breeding of mosquitoes and hence increase the transmission of dengue (116). However, some studies have reported a positive association between dengue and NDVI (117). Such heterogeneous association might be related to different socio-demographic characteristics in different settings, such as vector control strategies, behaviour of the people, sanitation and hygiene – implying the need to assess the role of location-specific risk factors in the transmission of dengue. The findings suggest a need to undertake regular dengue surveillance, including vector surveillance at high risk areas, including the capital city Thimphu.

Dengue was confined to Phuntsholing Municipality for around a decade. However, since 2013 cases have been reported from neighbouring districts with similar environmental and climatic conditions. In 2019, Bhutan experienced its first and largest nation-wide dengue outbreak which affected 19 of the 20 districts with more than 5000 cases and six deaths. The number of cases during this epidemic was greater than all cases combined in all the previous years.

Both *Ae. albopictus* and *Ae. aegypti* are prevalent in Bhutan. Even though *Ae. albopictus* is the dominant vector in 16 districts, *Ae. aegypti* has been rapidly emerging in these districts in recent years (8). For example, *Ae. aegypti* was reported for the first time in 2019 in Khamdang sub-district under Trashiyangtse district. This finding suggests that the endemic circulation of dengue vector in previously identified places and its emergence in newer places supported by conducive environment might have led to the outbreak in 2019. Dengue vectors are usually introduced and dispersed into new places due to increased trade, human movement and transportation (118). The fact that vectors are transported by humans are supported by the limited flight range of *Aedes* mosquitoes. For example, *Ae. aegypti* has a flight range of 512 metres (119), while *Ae. albopictus* tend to not disperse beyond 1 kilometre of their breeding site (120).

In addition to the geographical expanding of the dengue vector, the constellation of other factors, including the presence of a large number of susceptible populations in these areas, might have led to greater transmission (121, 122). The rate at which a susceptible population becomes infected is a measure of the intensity of transmission. However, no studies were conducted to assess the herd immunity or sero-prevalence of dengue in Bhutan. Having such

information would have led to a better understanding of the susceptibility of the population to dengue in Bhutan. As dengue has a high prevalence of asymptomatic infections, quantifying the transmission based on clinical cases will result in inaccurate estimates (123). Another limitation of the study was related to the unavailability of the data on the travel history of the reported cases as the surveillance system captures cases as aggregates. Without this information, it was not able to determine whether infection was acquired from their place of residence or from the neighbouring dengue-endemic places. This highlights the need to review the existing surveillance system to capture critical information from the individual cases.

7.4 Spatial vulnerability to dengue

Without any evidence on the spatial vulnerability of dengue, there are speculations that dengue is endemic to some specific areas or associated with travel history to those areas (124). In order to assess the vulnerability and improve the surveillance and control strategies for dengue, vulnerability assessment was carried out using a knowledge-based MCDA model that utilised all relevant factors associated with dengue transmission.

The MCDA model demonstrated spatially heterogeneous vulnerability to dengue which varied with season. Places located at lower elevations were vulnerable throughout the year. However, the vulnerability in higher geographical elevations, including the nation's capital, Thimphu, was observed during summer. The vulnerability was mostly associated with places characterised by high population density, agricultural land, better road connectivity and built-up areas. These inhabited areas are marked by increased human activities that support the habitats for mosquito through inadequate solid waste management and indoor water storage containers (125, 126).

The model showed areas as high as 900 metres above sea level to be highly vulnerable to dengue as opposed to a low index of public suspicion. As predicted by the model, high levels of transmission occurred in these areas during the 2019 outbreak. The MCDA map can be used to prioritise vector control strategies and develop robust surveillance and response plan. Rather than uniformly distributing resources across the country, allocating resources according to the geographical need as identified in this map may be more efficient and effective in a resource-constrained setting like Bhutan.

7.5 Knowledge and clinical management practices of dengue

To better understand the knowledge on the clinical management and identification of warning signs of severe dengue, a cross-sectional study was conducted involving clinicians (medical practitioners) in Bhutan. This was the first study that explored a detailed understanding of the dengue management practices in the WHO-SEAR.

Clinicians had poor knowledge on the peak hours of feeding activity and infectiousness of the dengue vector; only 24.7% of the respondents correctly identified the timing of the peak activity of the dengue vector, and 16.5% reported that infected mosquitoes are responsible for transmitting the DENV during the feeding time. Poor knowledge on the transmission of dengue has also been documented in other countries (127). The plausible reason might be related to lack of training, particularly among clinicians working in non-dengue endemic areas. Clinicians' knowledge on transmission is important to empower and bring about appropriate behaviour changes in patients and their families through providing correct information (128).

The study also found that a very low proportion of clinicians were knowledgeable on the use of tools to detect plasma leakage, the pathognomonic sign of severe dengue. For instance, only 27.8% correctly reported that plasma leakage (clinically manifested as pleural effusion or ascitis) can be detected using an X-ray machine. This finding can be due to the lack of such equipment in BHUs.

Knowledge on warning signs of severe dengue was also found to be poor. Lethargy, persistent vomiting and abdominal pain were correctly reported in 34%, 54.5% and 55.7% of the clinicians respectively. This might perhaps be related to a lack of awareness and training on the identification of warning signs of severe dengue as reported in other studies (129). As evident in the meta-analysis, these signs are significant predictors of severe dengue and have to be monitored closely (32). Another finding was related to a low frequency of respondents (21.7%) who reported the use of co-morbidities as a risk factor for progression to severe dengue. As depicted by the meta-analysis and in previous studies (130), co-morbidities such as diabetes and renal disease increase the risk of progression to severe dengue.

Dengue is fast spreading in every corner of the country. Hence, the Ministry of Health should be well prepared to respond to outbreaks by strengthening the capacity of every health centre, particularly by improving the knowledge and skills of clinicians on managing dengue. Sensitization and refresher training should be systematically initiated, and no one should be left behind untrained.

7.6 Limitations

This thesis has some limitations which are worth mentioning in conjunction with the findings. Although specific limitations were included in each study, it is worthwhile to elaborating here.

1. The narrative review on the epidemiology and control challenges of dengue surveillance might have resulted in both information bias (due to personal/expert opinion) and selection bias (due to inclusion of studies). However, selection bias might have minimal influence as standard search strategies and inclusion criteria were adopted.
2. The Bayesian and epidemic analysis was computed using secondary data from the surveillance system. Under-reporting is a major weakness of any surveillance system as the mild or sub-clinical cases would not have been captured, and reporting is likely influenced by the motivation of the staff working in the field. The addition of other variables, such as human behaviour, is worth considering in the model and exploring their impact on the transmission of dengue. Furthermore, the study might have potentially generated additional insights had the models been run separately according to the different DENV serotypes.
3. Due to unavailability of data, water bodies used to generate vulnerability maps for dengue were not categorised into flowing and stagnant water. As the dengue vectors commonly breeds in stagnant water, including all types of water bodies might have possibly influenced the vulnerability index. Studies in the future might include separate models for both flowing and stagnant water to compare the vulnerability in distribution maps of dengue.
4. There was strong heterogeneity and publication bias among studies included in the meta-analysis. However, subgroup and sensitivity analyses were conducted to account for this limitation.
5. The response rate for the knowledge and clinical management of dengue was low, despite two rounds of reminders sent to take part in the study. However, respondents were included from all 20 districts, thus covering the whole country.

7.7 Recommendation for future research

This thesis provides an evidence base to understand the epidemiology of dengue in Bhutan. However, there are certain gaps which needs to be addressed to gain better understanding of dengue epidemiology in Bhutan to develop robust control strategies.

1. Dengue disease surveillance and entomological surveillance were never evaluated, raising a serious question on its efficiency and effectiveness. As highlighted in the narrative review, a detailed understanding of the surveillance needs to be undertaken to identify gaps and improve it further.
2. Linking dengue disease and entomological surveillance data would be an important milestone to develop early warning system for dengue control. Studies involving both dengue disease and entomological components are limited globally.
3. The Bayesian analysis incorporated limited covariates due to lack of data on other risk factors such as poverty level, sanitation and hygiene (including access to safe drinking water), and the proportion of urban/rural population. Including these factors might improve the predictive ability of the model and yield better study outcomes.
4. Due to insufficient data collected over a short period (<10 years), prediction of the dengue outbreaks could not be conducted. Such studies will be essential for the program to plan ahead to implement control strategies and prevent such transmission events from occurring.
5. To understand the prevalence of asymptomatic dengue and force of infection during outbreaks as in 2019, the future research should focus on a nation-wide sero-prevalence study. This study will also be crucial to support the future plans for vaccine rollout campaigns.
6. The last study on the disease economic burden dates back to 2013 (67), when the dengue infection was confined to few places. Hence, there is a need to conduct a study on economic burden of the disease with the latest data.

7.8 Conclusion

This thesis employed a combination of empirical approaches to better understand the epidemiology of dengue with implications for dengue control and prevention in Bhutan.

Bhutan has now become hyper-endemic for multiple DENV serotypes/genotypes, with outbreaks flaring up after every three to five years. Outbreaks are marked by increasing geographical expansion to previously dengue-naïve areas. A meta-analysis involving a pool of large studies corroborated the WHO recommendation to closely monitor the pre-existing morbidities and warning signs of severe dengue. However, the current surveillance system does not provide adequate information, including reporting, for early case detection and managing

dengue effectively. Vector surveillance is primarily supported by immature mosquito sampling, which limits the understanding of the true risk of dengue. The surveillance may be strengthened by incorporating innovations and modern technologies such as mobile apps to improve reporting from the communities, and include adult mosquito sampling in routine vector surveillance to enhance vector control.

Fluctuations in the temperature and vegetation significantly influenced the transmission of dengue. The 2019 dengue epidemic was the worst of all the records affecting 19 of the 20 districts. Intensive transmission with maximum cases corresponded to the summer season, which was marked by a monsoon, higher temperatures, and increased mobility of people to dengue-endemic areas. This increased their exposure to dengue vectors. Hotspots or areas found to be vulnerable to dengue can be used to target surveillance and early warning systems for dengue. A survey conducted among clinicians revealed major gaps in the knowledge and clinical management practices of dengue. We recommend acting on the need to systematically institutionalise sensitisation and refresher training for managing dengue in the country of Bhutan.

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APPENDICES

Appendix 1

Statement of author's contribution to articles

[1.1] Tsheten T, Gray DJ, Clements ACA, Wangdi K. **Epidemiology and challenges of dengue surveillance in the WHO South-East Asia Region**. Trans R Soc Trop Med Hyg. 2021;115(6):583-599.

Contributions

TT and KW conceived this study. TT undertook literature review and drafted the manuscript. KW helped in the drafting and revision of manuscript. DJG and ACAC were involved in the conception and critical revision of manuscript. All authors read and approved the final manuscript.

Name	Contribution (%)
Tsheten Tsheten (TT)	75
Darren J Gray (DJG)	5
Archie CA Clements (ACAC)	7
Kinley Wangdi (KW)	13

[1.2] Tsheten T, Clements ACA, Gray DJ, Adhikary RK, Furuya-Kanamori L, Wangdi K. **Clinical predictors of severe dengue: a systematic review and meta-analysis**. Infect Dis Poverty. 2021 Oct 9;10(1):123.

Contributions

TT, LFK and KW conceived this study. TT, RKA and KW screened the studies and extracted the data from the eligible studies. TT undertook literature review and drafted the manuscript. LFK and TT analysed the result. KW helped in the drafting and revision of manuscript. DJG and ACAC were involved in the critical revision of manuscript. All authors read and approved the final manuscript.

Name	Contribution (%)
Tsheten Tsheten (TT)	71
Archie CA Clements (ACAC)	6
Darren J Gray (DJG)	5
Ripon K Adhikary (RKA)	2
Luis Furuya-Kanamori (LFK)	8
Kinley Wangdi (KW)	8

[1.3] Tsheten T, Clements ACA, Gray DJ, Wangchuk S, Wangdi K. **Spatial and temporal patterns of dengue incidence in Bhutan: a Bayesian analysis**. Emerg Microbes Infect. 2020;9(1):1360-1371.

Contributions

TT and KW conceived this study. TT undertook literature review, analysed and drafted the manuscript. KW helped in the analysis, drafting and revision of manuscript. AC, DG and SW were involved in the critical revision of manuscript. All authors read and approved the final manuscript.

Name	Contribution (%)
Tsheten Tsheten (TT)	76
Archie CA Clements (ACAC)	7
Darren J Gray (DJG)	5
Sonam Wangchuk (SW)	2
Kinley Wangdi (KW)	10

[1.4] Tsheten T, Mclure A, Clements ACA, Gray DJ, Wangdi T, Wangchuk S, Wangdi K. **Epidemiological Analysis of the 2019 Dengue Epidemic in Bhutan**. Int J Environ Res Public Health. 2021;18(1):354.

Contributions

TT and KW conceived the study. TT undertook literature review, analysed and drafted the manuscript, and prepared some figures. KW assisted in analysis, drafting and revision of the

manuscript. AM estimated the growth rates and reproduction numbers and prepared some figures. DJG, AC, AM, SW, and TW were involved in the critical revision of the manuscript. All authors read and approved the final manuscript.

Name	Contribution (%)
Tsheten Tsheten (TT)	72
Angus Mclure (AM)	8
Archie CA Clements (ACAC)	7
Darren J Gray (DJG)	5
Tenzin Wangdi (TW)	2
Kinley Wangdi (KW)	8

[1.5] Tsheten T, Clements ACA, Gray DJ, Wangdi K. **Dengue risk assessment using multicriteria decision analysis: A case study of Bhutan**. PLoS Negl Trop Dis. 2021;15(2):e0009021.

Contributions

TT and KW conceived the study. TT undertook a literature review, analysed and drafted the manuscript. KW assisted in analysis, drafting and revision of the manuscript. DJG and ACAC were involved in the critical revision of the manuscript. All authors read and approved the final manuscript.

Name	Contribution (%)
Tsheten Tsheten (TT)	75
Archie CA Clements (ACAC)	10
Darren J Gray (DJG)	5
Kinley Wangdi (KW)	10

[1.6] Tsheten T, Clements ACA, Gray DJ, Gyeltshen K, Wangdi K. **Medical practitioner's knowledge on dengue management and clinical practices in Bhutan**. PLoS One. 2021;16(7):e0254369

Contributions

TT and KW conceived this study. TT undertook the literature review, developed questionnaire, piloted it, and administered them, data analysis and drafted the manuscript. KW helped in the analysis, drafting and revision of the manuscript. DG, AC and KG were involved in the critical revision of the manuscript. All authors read and approved the final manuscript.

Name	Contribution (%)
Tsheten Tsheten (TT)	80
Archie CA Clements (ACAC)	6
Darren J Gray (DJG)	5
Kinley Wangdi (KW)	9

Appendix 2

Supplementary information from the publications

[2.1] Clinical predictors of severe dengue: a systematic review and meta-analysis (Chapter 3)

Additional file 1. PRISMA Checklist for systematic reviews and meta-analysis

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 4-5
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 5
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Page 5-6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 5
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Additional file 2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 6-7
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 6-7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were	Page 5-6

Section and Topic	Item #	Checklist item	Location where item is reported
		sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Page 5-6
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 7
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Page 8
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 8
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Page 8
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Page 8 and additional file 3
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 8
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Page 8
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Page 8
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Page 8
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Page 8
RESULTS			

Section and Topic	Item #	Checklist item	Location where item is reported
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 9
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Page 9 and figure 1
Study characteristics	17	Cite each included study and present its characteristics.	Page 9 and additional file 3
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Page 10 and additional file 4
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Page 10-11
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Page 10 and additional file 4
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Page 10-11
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Page 12 and additional file 8)
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Page 12 and additional file 7)
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Page 12
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Page 10-12
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page 12-15

Section and Topic	Item #	Checklist item	Location where item is reported
	23b	Discuss any limitations of the evidence included in the review.	Page 15-16
	23c	Discuss any limitations of the review processes used.	Page 15-16
	23d	Discuss implications of the results for practice, policy, and future research.	Page 16
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Page 5 (Not registered)
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 5 (Not registered)
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Page 5 (Not registered)
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 17
Competing interests	26	Declare any competing interests of review authors.	Page 16
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Page 16 and additional files

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. doi: 10.1136/bmj.n71

Additional file 2. Search strategy used in the review of literature

((("Severe Dengue" OR "dengue hemorrhagic fever" OR "dengue haemorrhagic fever" OR "dengue shock syndrome" OR "dengue complication*" OR "dengue death*" OR "dengue mortalit*" OR "dengue fatalit*" OR "dengue intensive care") AND ("undifferentiated dengue fever" OR "non-severe dengue" OR "dengue fever" OR "dengue without warning signs" OR "dengue with warning signs" OR dengue)) AND (comorbidit* OR "chronic disease*" OR "Nutritional and Metabolic Disease*" OR "Central Nervous System Disease*" OR "Respiratory Tract Disease*" OR "Digestive System Disease*" OR "Urologic Disease*" OR "Metabolic Disease*" OR "Endocrine System Disease*" OR diabetes OR hypertension OR "cardiac disorder" OR "cardiovascular disease*" OR "coronary heart disease*" OR "cerebrovascular disease*" OR "arterial disease*" OR deafness OR "hearing loss*" OR obesity OR cancer OR alcohol OR arthritis OR osteoporosis OR "sickle-cell disease" OR "sickle cell disease" OR "hemoglobin disorder*" OR "haemoglobin disorder*" OR oral OR dental OR malnutrition OR overweight OR underweight OR asthma OR allerg* OR stroke OR "heart disease*" OR embolism OR thrombosis OR anaemia OR anemia OR Demography OR "Age Factors" OR Sex OR "Ethnic Groups" OR "Social Class" OR demograph* OR gender OR age OR sex OR ethnic OR location OR region* OR background OR residen* OR nation* OR class OR income OR econom* OR "socio-economic"))

Additional file 3. Studies included in the review and meta-analysis

Author	Country	Study population	Classification	Reference
Tan VPK et al	Malaysia	Adults	2009	[1]
Burattini MN et al	Brazil	Mixed	1997	[2]
Kuo HJ et al	Taiwan	Adults	2009	[3]
Junior JJD et al	Brazil	Mixed	2009	[4]
Richards AL et al	Indonesia	Mixed	1997	[5]
Sahu AK et al	India	Adults	2009	[6]
Ditsuwan T et al	Thailand	Mixed	1997	[7]
Patra G et al	India	Mixed	2009	[8]
Mayetti M et al	Indonesia	Children	1997	[9]
Mangione JNA et al	Vietnam	Children	1997	[10]
Singh J et al	India	Mixed	1997	[11]
Yeung W et al	Singapore	Adults	2009	[12]
Yacoub S et al	Vietnam	Adults	2009	[13]
Giraldo D et al	Brazil	Children	2009	[14]
Hair GM et al	Brazil	Mixed	2009	[15]
Sabeena S et al	India	Mixed	1997/2009	[16]
Lin YP et al	China	Mixed	2009	[17]
Rathakrishnan A et al	Malaysia	Adults	2009	[18]
Shah I et al	India	Children	1997	[19]
Wakimato MD et al	Brazil	Children	2009	[20]
Kumar A et al	Barbados	Children	2009	[21]
Hanafusa S et al	Thailand	Mixed	1997	[22]
Trung DT et al	Vietnam	Mixed	1997	[23]
Agrawal VK et al	India	Mixed	2009	[24]
Adam AS et al	Indonesia	Children	2009	[25]
Sahana KS et al	India	Children	2009	[26]
Bisoyi SK et al	India	Adults	2009	[27]
Mishra S et al	India	Children	2009	[28]
Tun MMN et al	Vietnam	Children	2009	[29]
Mittal H et al	India	Children	1997	[30]
Vaddadi K et al	India	Children	2009	[31]

Suwarto S et al	Indonesia	Adults	2009	[32]
Gupta S et al	India	Children	2009	[33]
Kerdpanich P et al	Thailand	Mixed	1997	[34]
Dussart P et al	Cambodia	Children	1997/2009	[35]
Tsai CY et al	Taiwan	Mixed	2009	[36]
Noecker CA et al	Mexico	Adults	2009	[37]
Rathakrishnan A et al	Malaysia	Adults	2009	[38]
Khongphatthanayothin A et al	Thailand	Children	1997	[39]
Pongsumpun P et al	Thailand	Mixed	1997	[40]
Karyanti MR et al	Indonesia	Children	1997	[41]
Rafi A et al	Bangladesh	Adults	2009	[42]
Narayanan M et al	India	Children	1997	[43]
Boillat-Blanco N et al	Tanzania	Adults	2009	[44]
Kabra SK et al	India	Children	1997	[45]
Alvis-Guzman N et al	Colombia	Mixed	2009	[46]
Elenga N et al	France	Children	2009	[47]
Anita C et al	India	Adults	1997	[48]
Nunes PCG et al	Brazil	Mixed	2009	[49]
Rocha BAM et al	Brazil	Mixed	2009	[50]
ABUALAMAH WA et al	Saudi Arabia	Mixed	2009	[51]
Srinivasan P et al	India	Children	2009	[52]
Lee Ik et al	Taiwan	Adults	2009	[53]
Pongpan S	Thailand	Children	1997	[54]
Barros TAC et al	Brazil	Adults	2009	[55]
Naranjo-Gómez JS et al	Colombia	Adults	2009	[56]
Kaur N et al	Malaysia	Mixed	2009	[57]
Koraka P et al	Indonesia	Children	1997	[58]
MD San SS et al	Malaysia	Adults	2009	[59]
Pozo-Aguilar JO et al	Mexico	Mixed	2009	[60]
Vejchapipat p	Thailand	Children	1997	[61]
Nguyen MT et al	Vietnam	Children	2009	[62]
Aung KLL et al	Thailand	Adults	2009	[63]
Pereira MS et al	India	Adults	2009	[64]

Khan MIH et al	Pakistan	Mixed	1997	[65]
Devignot S et al	Cambodia	Children	1997	[66]
Low GKK et al	Malaysia	Mixed	2009	[67]
Mercado ES et al	Philippines	Children	1997	[68]
Niriella MA et al	Sri Lanka	Mixed	2009	[69]
Singla M et al	India	Children	2009	[70]
Maneerattanasak S et al	Thailand	Children	1997	[71]
Heringer M et al	Brazil	Mixed	2009	[72]
Shabrish S et al	India	Mixed	2009	[73]
Juffrie M et al	Indonesia	Children	1997	[74]
Vicente CR et al	Brazil	Mixed	2009	[75]
Yolanda N et al	Indonesia	Children	2009	[76]
Arayasongsak U et al	Thailand	Children	1997	[77]
Shaheedha SM et al	India	Children	2009	[78]
van de Weg CA et al	Indonesia	Children	1997	[79]
Biswas HH et al	Nicaragua	Children	2009	[80]
van de Weg CA	Brazil	Mixed	2009	[81]
Temprasertudee S et al	Thailand	Adults	2009	[82]
Humayoun MA et al	Pakistan	Mixed	1997	[83]
Vasey B et al	Venezuela	Mixed	2009	[84]
Buntubatu S et al	Indonesia	Children	1997	[85]
Zhang H et al	China	Adults	2009	[86]
Davidson TD et al	Jamaica	Children	2009	[87]
Phuong NTN et al	Vietnam	Mixed	2009	[88]
Phakhounthong K et al	Cambodia	Children	2009	[89]
Potts JA et al	Thailand	Children	1997	[90]
Low GKK et al	Malaysia	Adults	2009	[91]
Carrasco LR et al	Singapore	Adults	2009	[92]
Jain S et al	India	Adults	1997	[93]
Shams N et al	Pakistan	Adults	1997	[94]
Torrentes-Carvalho A et al	Brazil	Not reported	2009	[95]
Dang TN et al	Thailand	Children	1997	[96]
Vazhayil PP et al	India	Children	2009	[97]

Hoffmeister B et al	Germany	Mixed	2009	[98]
Wichmann O et al	Thailand	Mixed	1997	[99]
Hegazi MA et al	Saudi Arabia	Mixed	2009	[100]
Perdomo-Celis F et al	Colombia	Children	2009	[101]
Chaiyaratana W et al	Thailand	Children	1997	[102]
Soundravally R et al	India	Mixed	2009	[103]
Liao B et al	China	Adults	2009	[104]
Trairatvorakul P et al	Thailand	Children	1997	[105]
Balmaseda A et al	Nicaragua	Mixed	1997	[106]
Soundravally R et al	India	Adults	1997	[107]
Falconar AKI et al	Colombia	Mixed	2009	[108]
Zhao L et al	China	Adults	2009	[109]
Wangdi K et al	Timor-Leste	Mixed	1997	[110]
Rathi M et al	India	Children	2009	[111]
Cherupanakkal C et al	India	Mixed	2009	[112]
Bandyopadhyay D et al	India	Adults	1997	[113]
Popper SJ et al	Nicaragua	Children	1997	[114]
Chaudhary R et al	India	Mixed	1997	[115]
Duangmala T et al	Thailand	Children	1997	[116]
Park S et al	Thailand	Children	1997	[117]
Srivastava G et al	India	Children	2009	[118]
Saqib MA	Pakistan	Mixed	1997	[119]
Mohamed NA et al	Yemen	Mixed	1997	[120]
Ahmed FU et al	Bangladesh	Children	1997	[121]
Lam pk et al	Vietnam	Children	1997	[122]
Wong jg et al	Singapore	Adults	1997	[123]
Hoang lt et al	Vietnam	Mixed	1997	[124]
Andries AC et al	Cambodia	Children	2009	[125]
Furuta T et al	Vietnam	Children	1997	[126]
Gopal SSS	India	Adults	2009	[127]
Mairuhi ATA et al	Indonesia	Children	1997	[128]
Vuong NL et al	Vietnam	Mixed	2009	[129]
Pothapregada S et al	India	Children	1997	[130]

Thanachartwet V et al	Thailand	Adults	1997	[131]
May WL et al	Myanmar	Children	2009	[132]
Machain-Williams C et al	Mexico	Adults	2009	[133]
de Kruif MD et al	Indonesia	Children	1997	[134]
Zhao H et al	China	Mixed	2009	[135]
Pillai AB et al	India	Mixed	2009	[136]
Marin-Palma D et al	Sri Lanka	Adults	2009	[137]
Cherupanakkal C et al	India	Mixed	2009	[138]
Utama IMGD et al	Indonesia	Children	1997	[139]
Gibson G et al	Brazil	Children	1997	[140]
Prasad D et al	India	Children	2009	[141]
Uddin MN et al	Bangladesh	Adults	1997	[142]
Thanachartwet V et al	Thailand	Adults	2009	[143]

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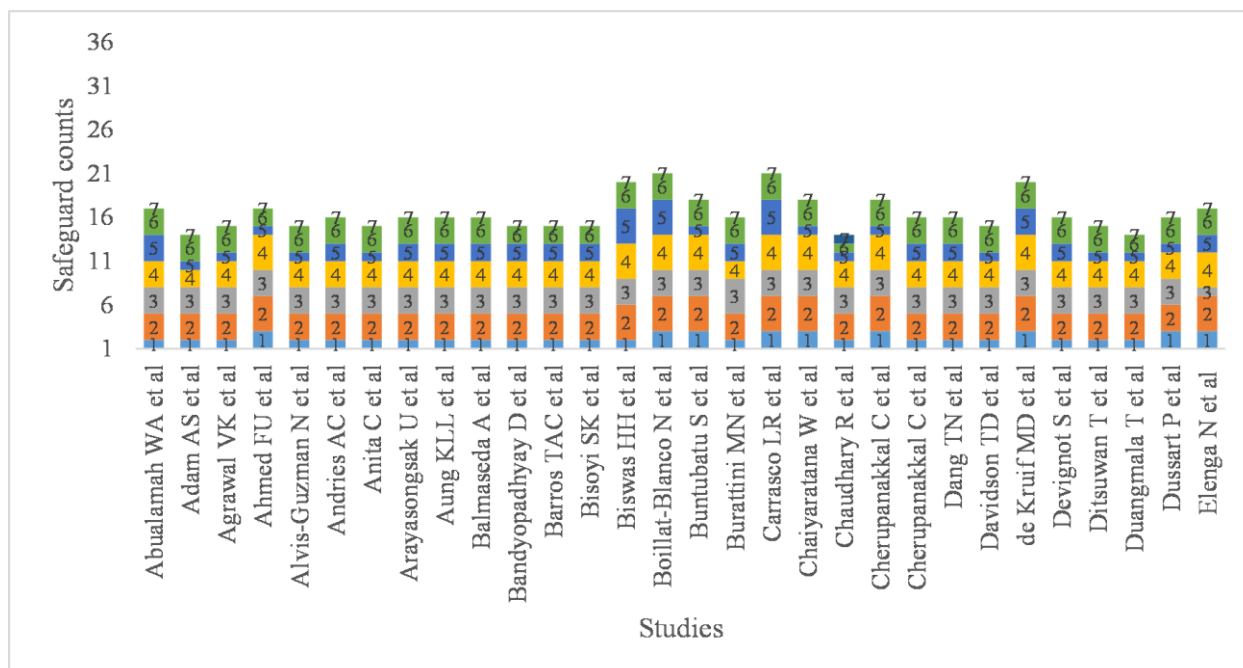
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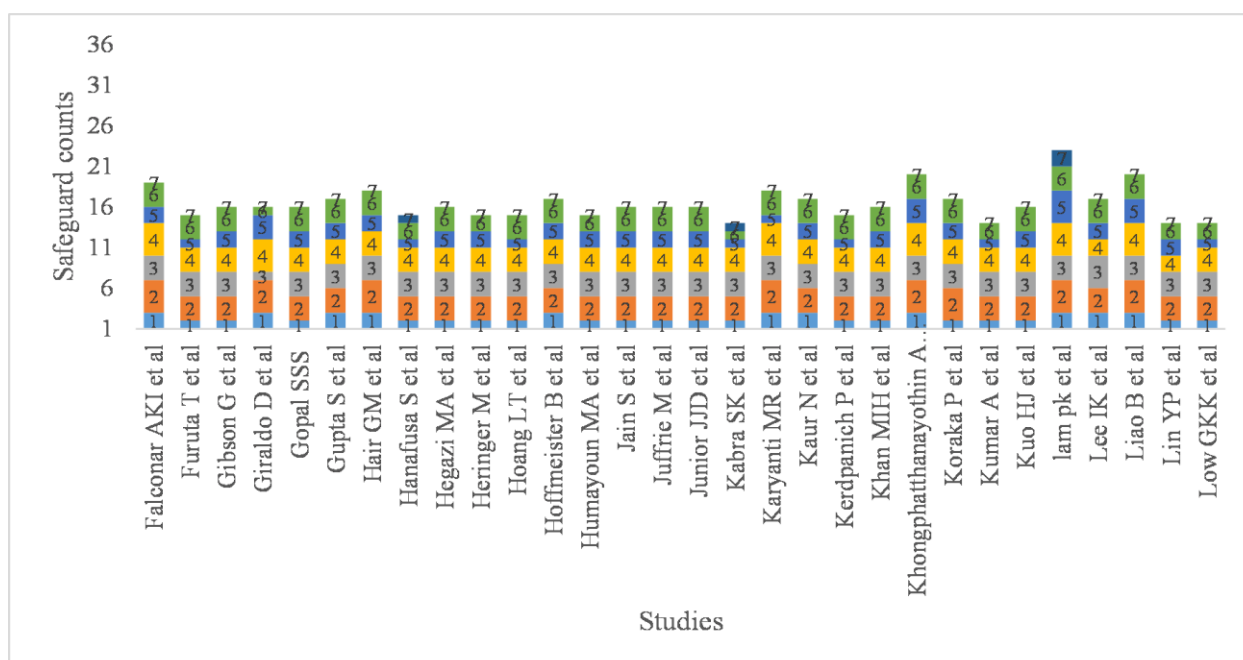
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Additional file 4. Quality assessment of the studies using a MASTER Scale.

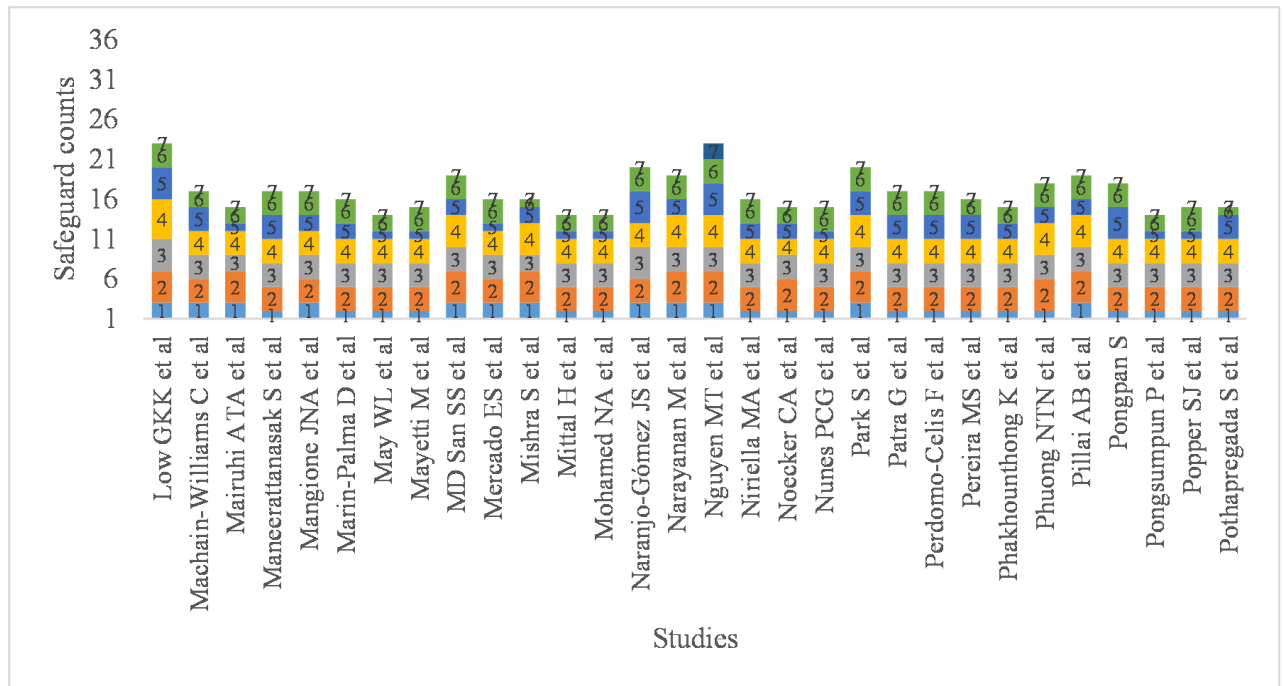
Additional file 4-A



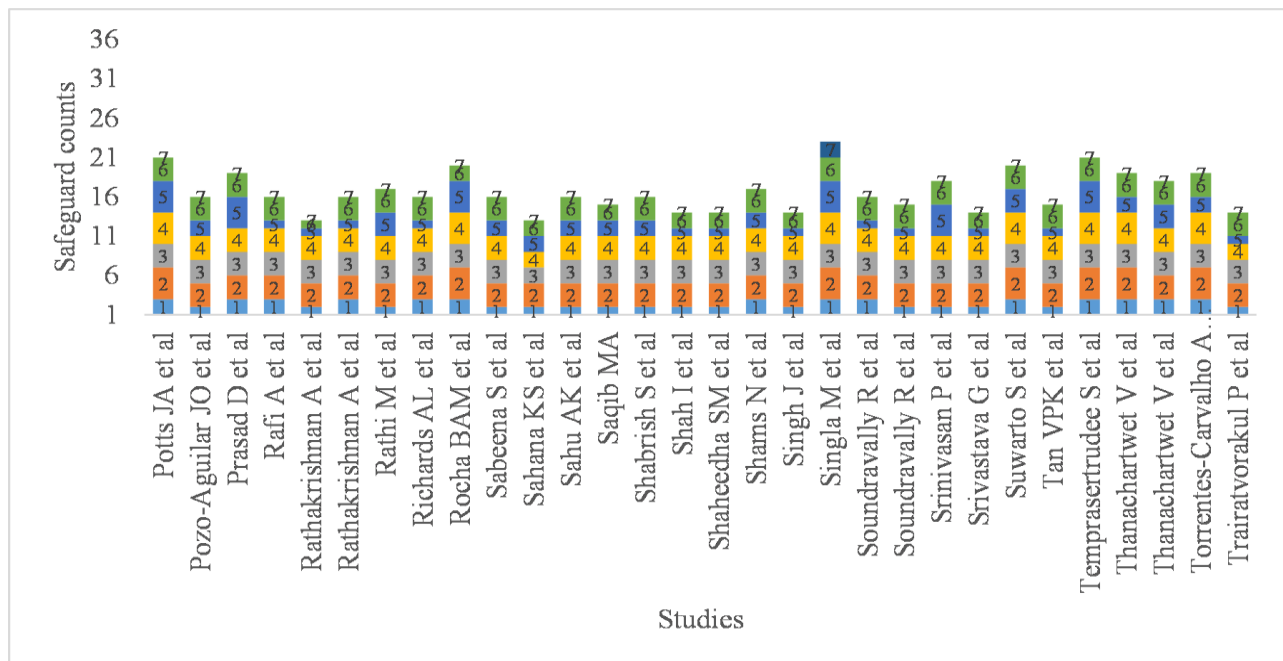
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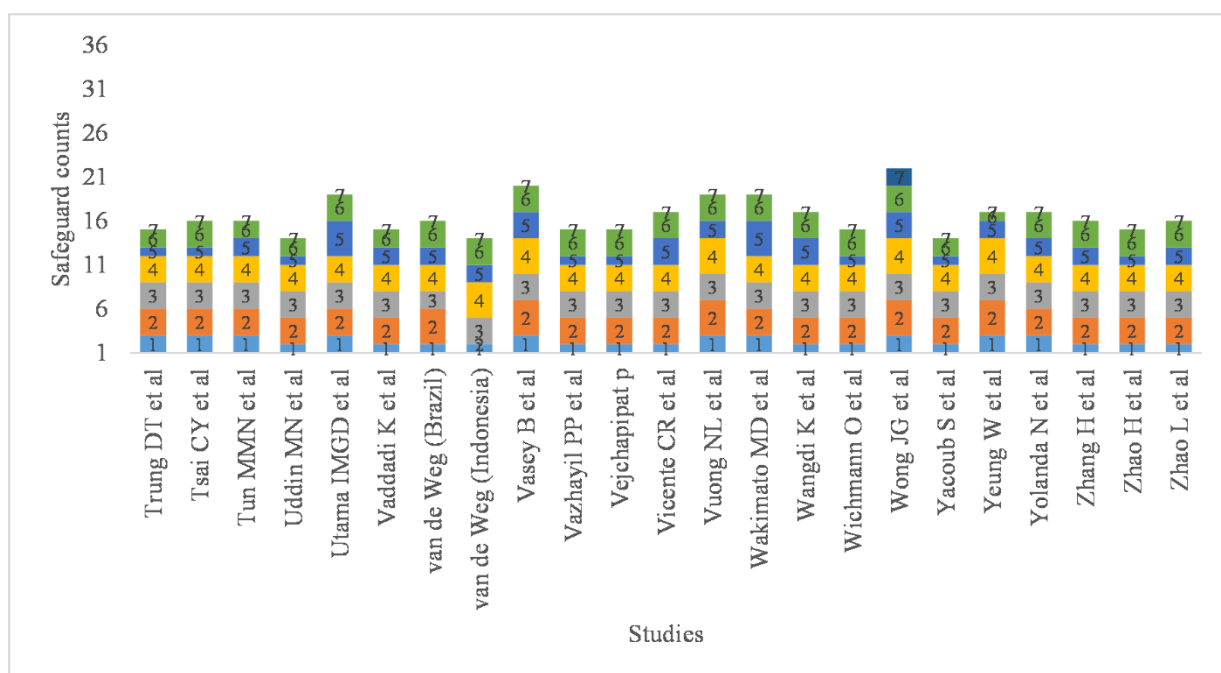
Additional file 4-C



Additional file 4-D

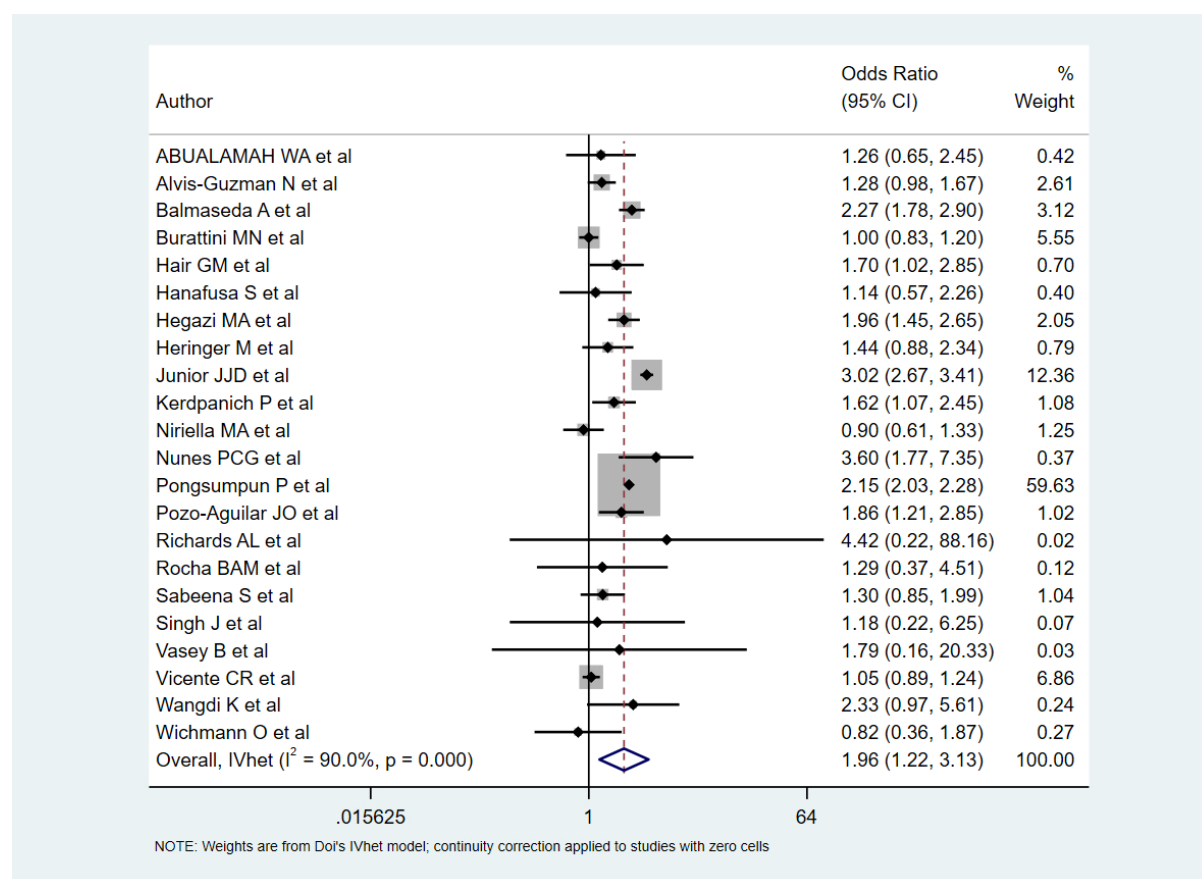


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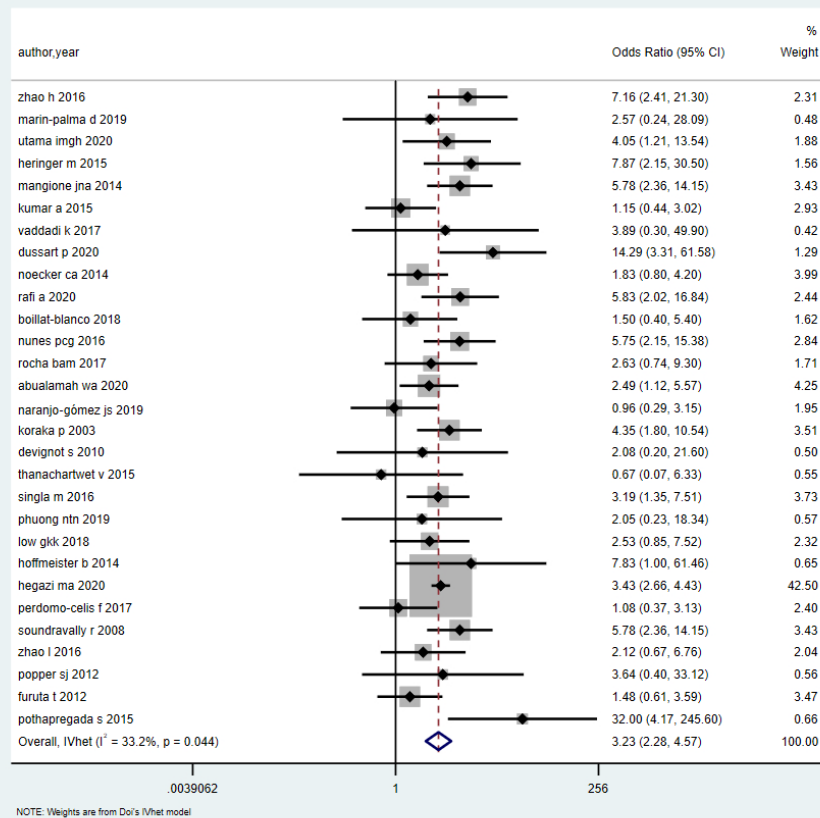


Additional file 5. Forest plots with pooled OR of progression to severe dengue with potential predictors.

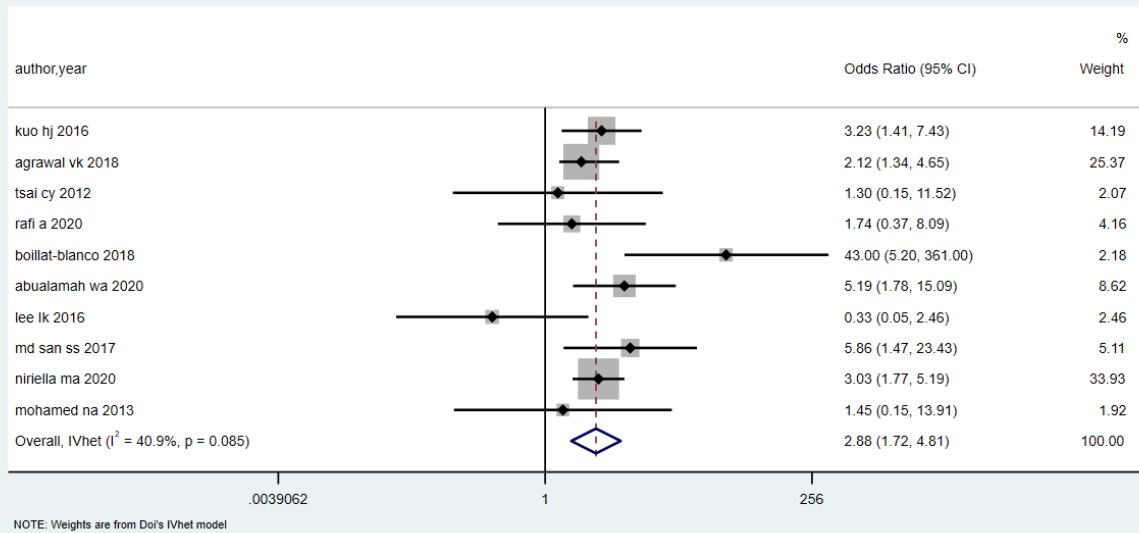
Additional file 5 - Children



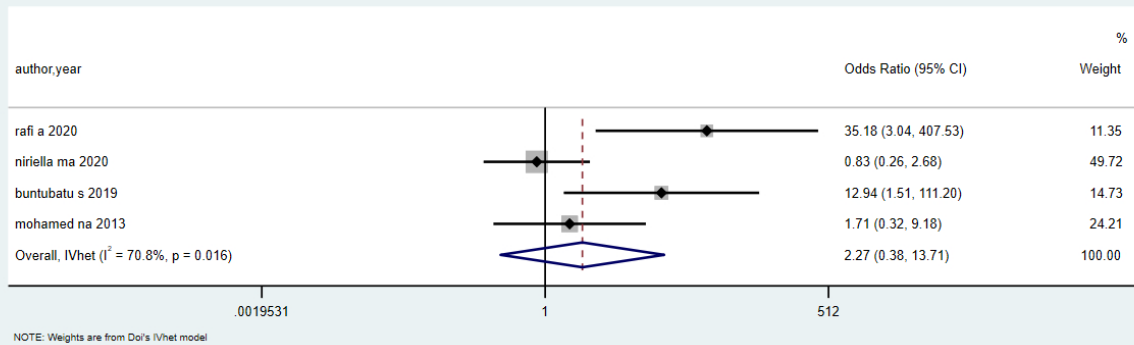
Additional file 5 - Infection



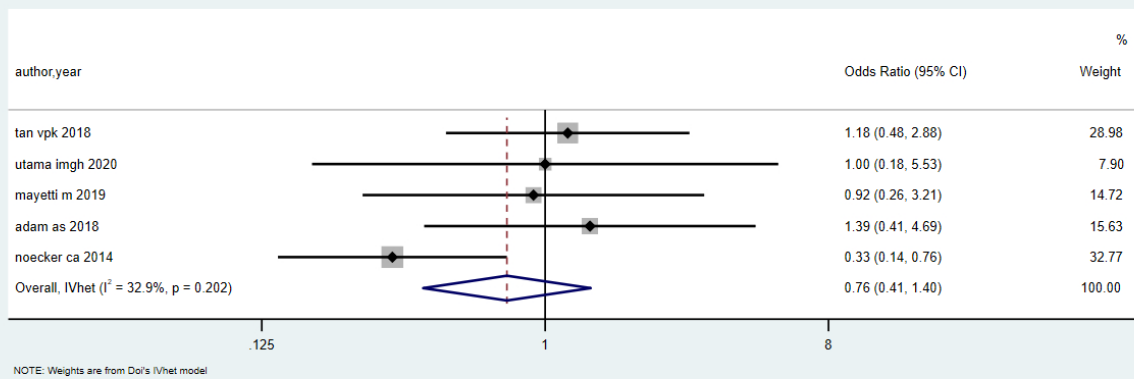
Additional file 5 - Diabetes



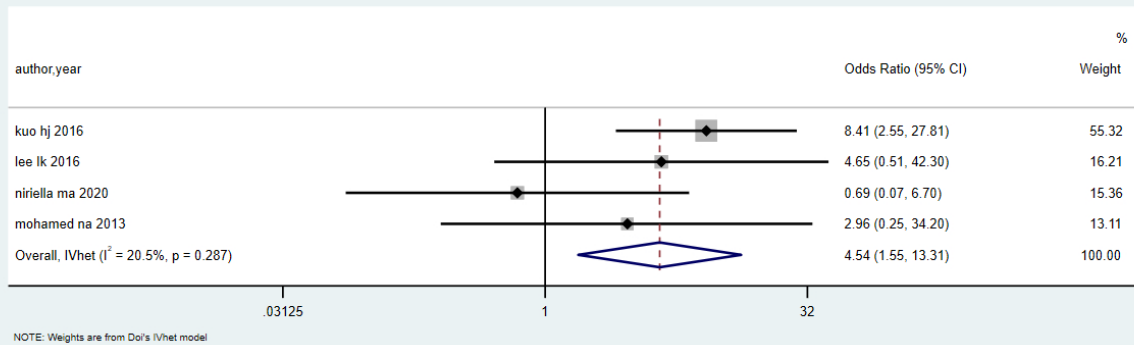
Additional file 5 - Cardiovascular disease



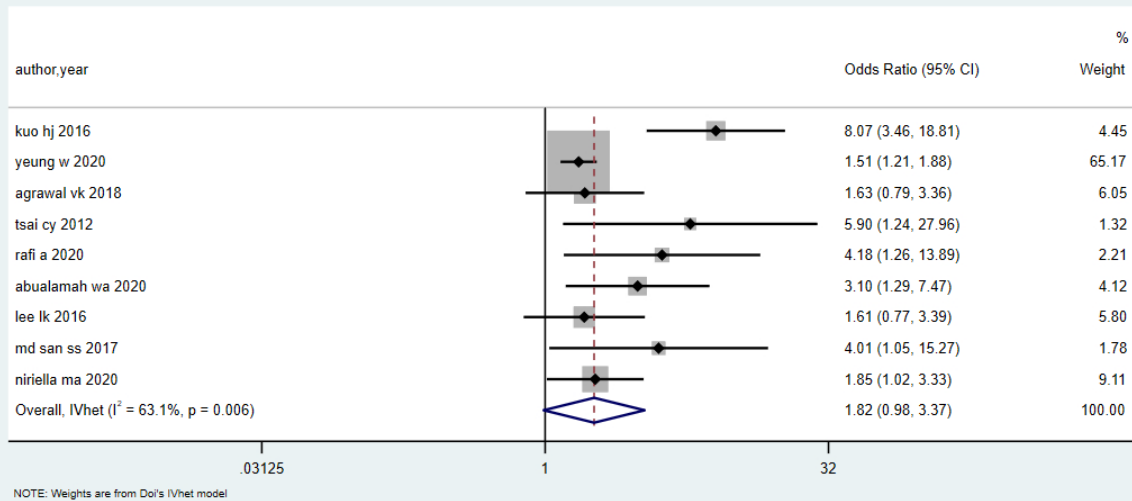
Additional file 5 - Obesity



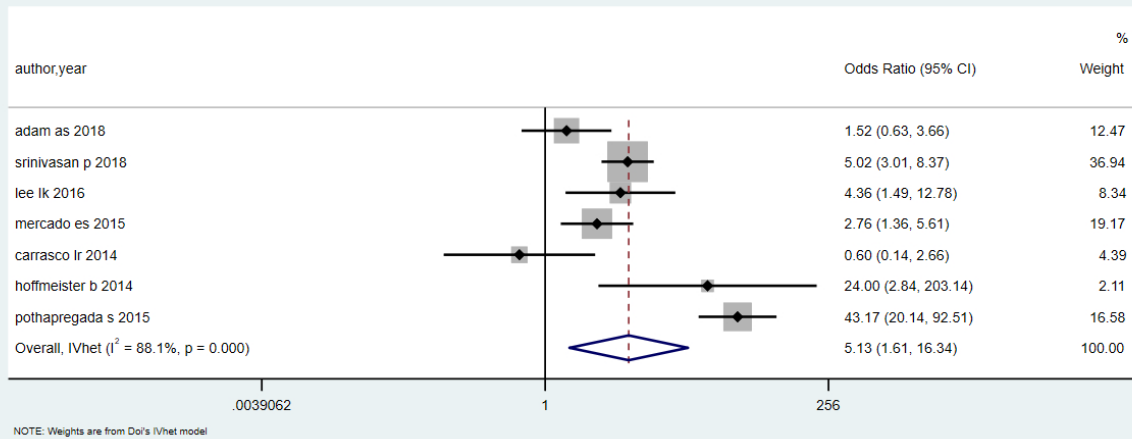
Additional file 5 - Renal disease



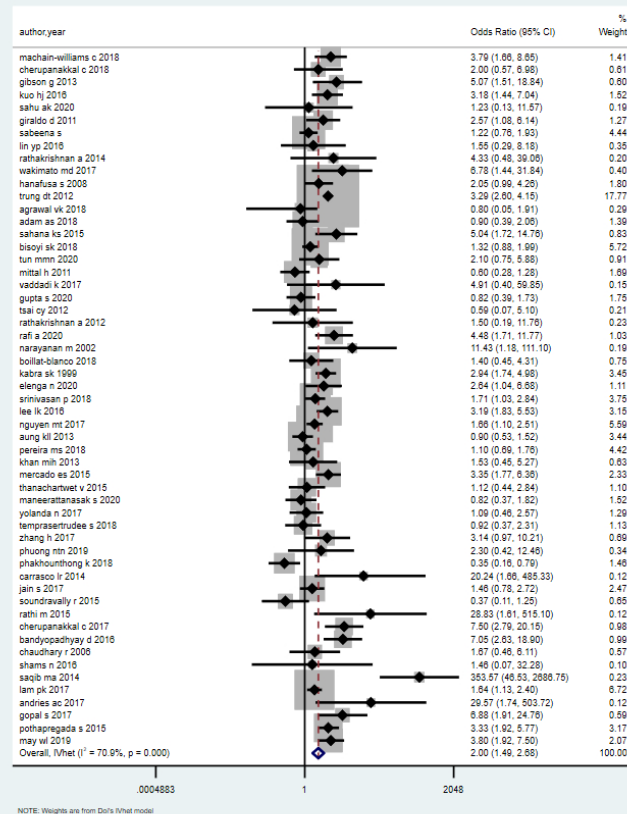
Additional file 5 - Hypertension



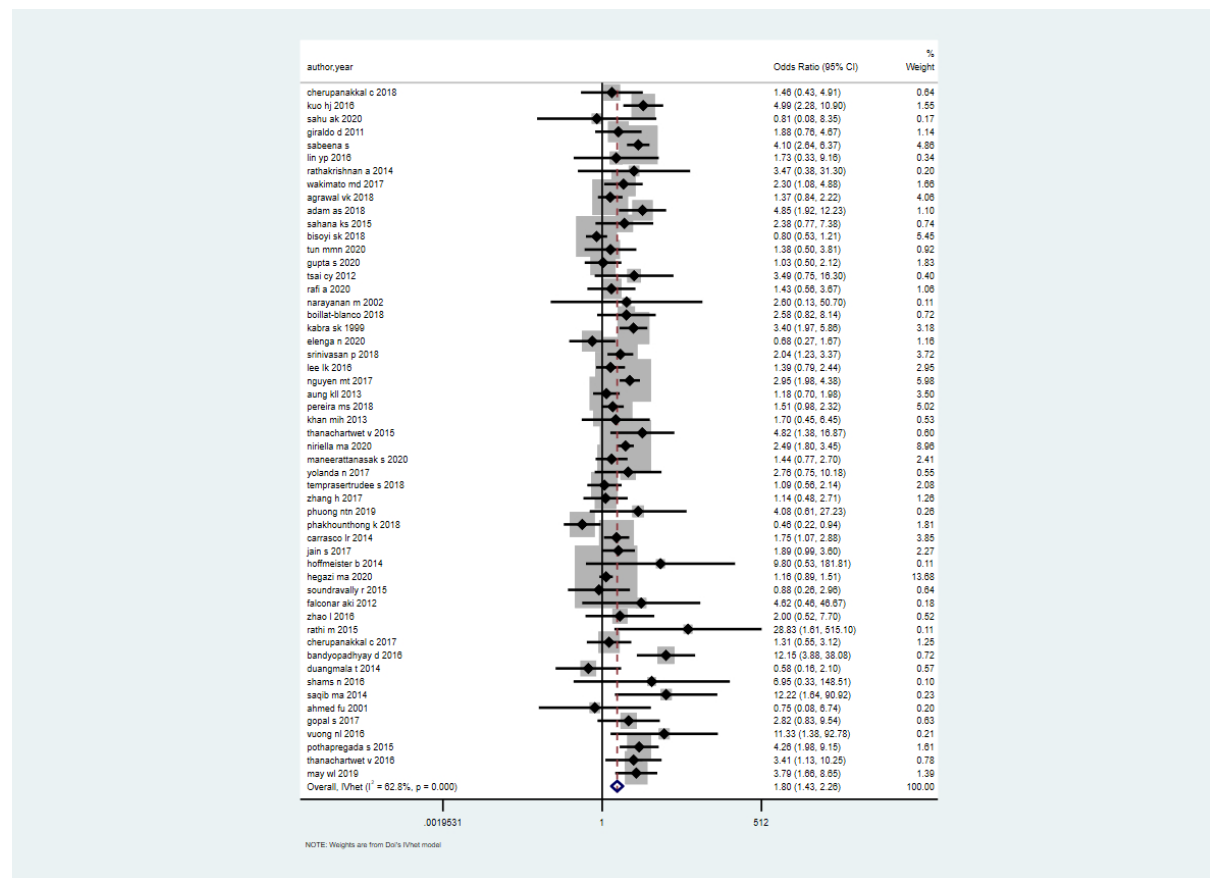
Additional file 5 – Increase in haematocrit with concurrent decrease in platelet count



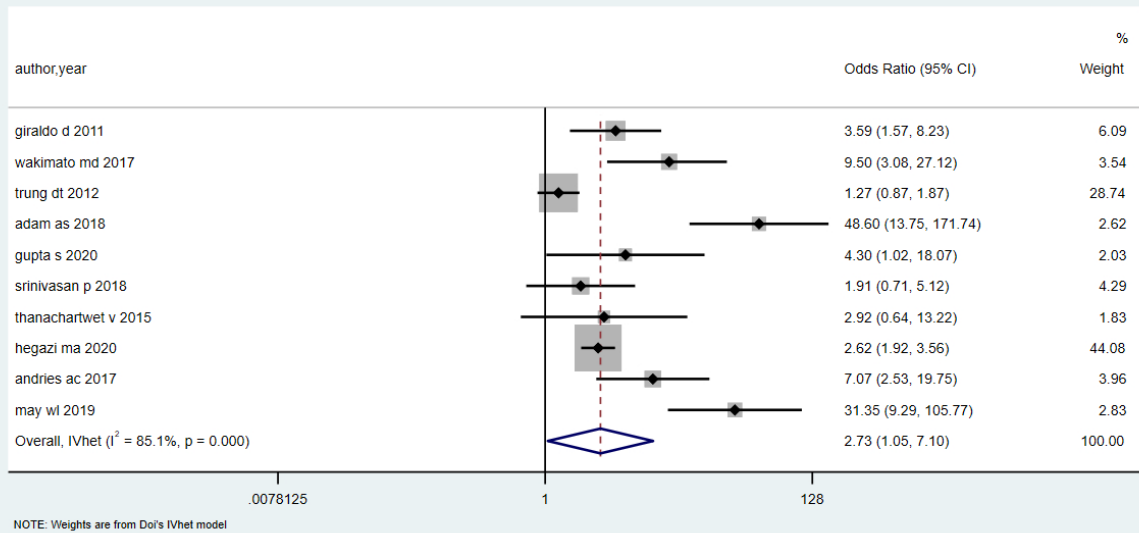
Additional file 5 - Abdominal pain



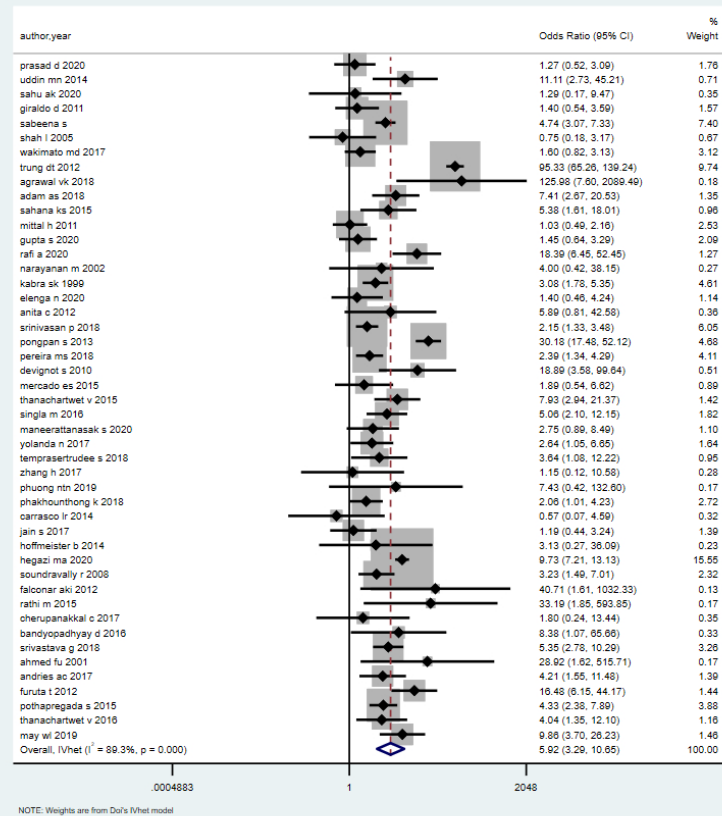
Additional file 5 - Vomiting



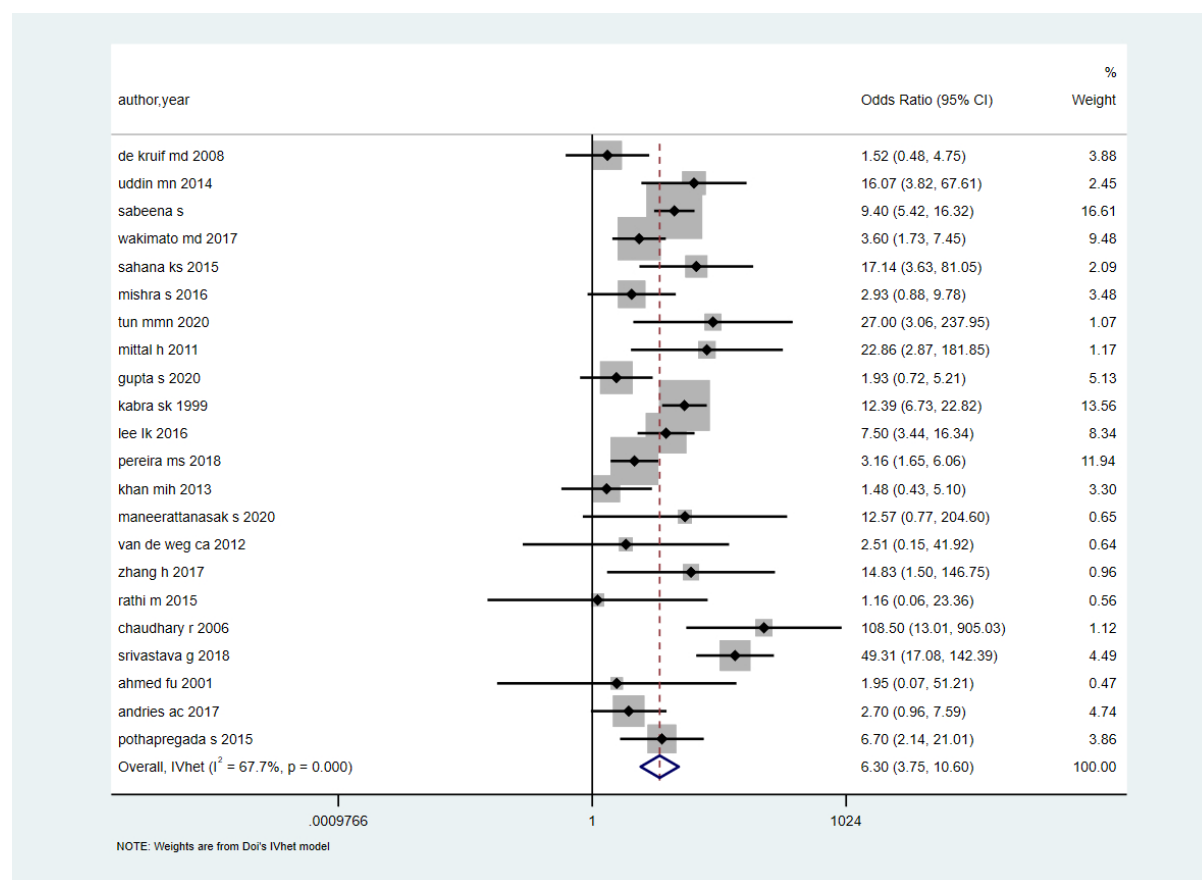
Additional file 5 - Lethargy



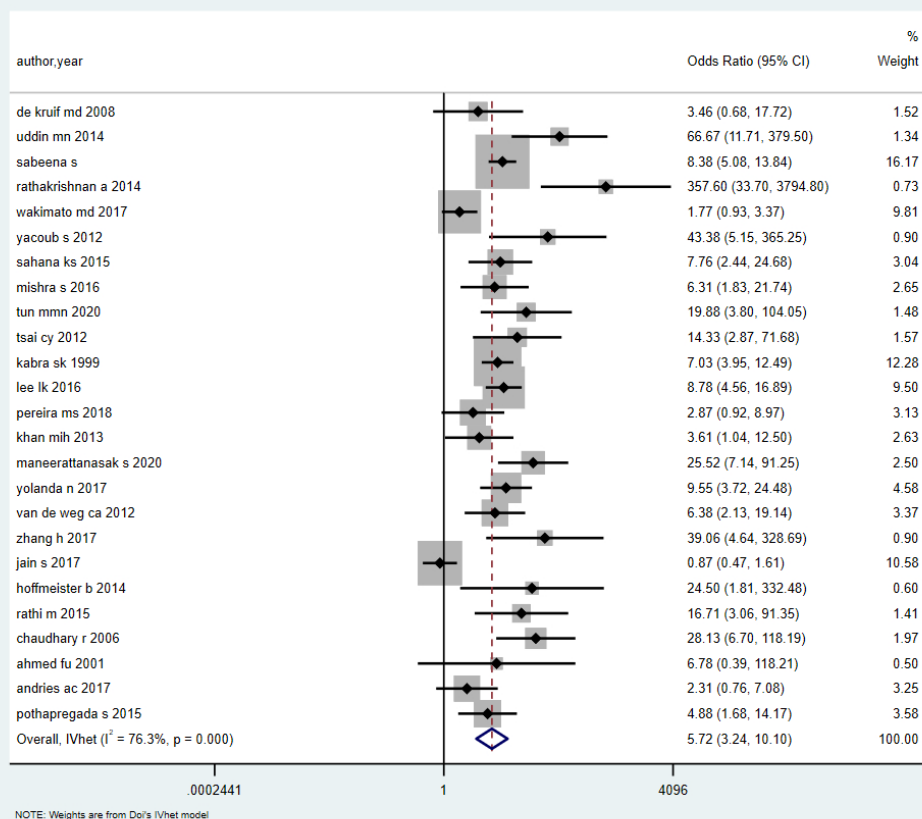
Additional file 5 - Hepatomegaly



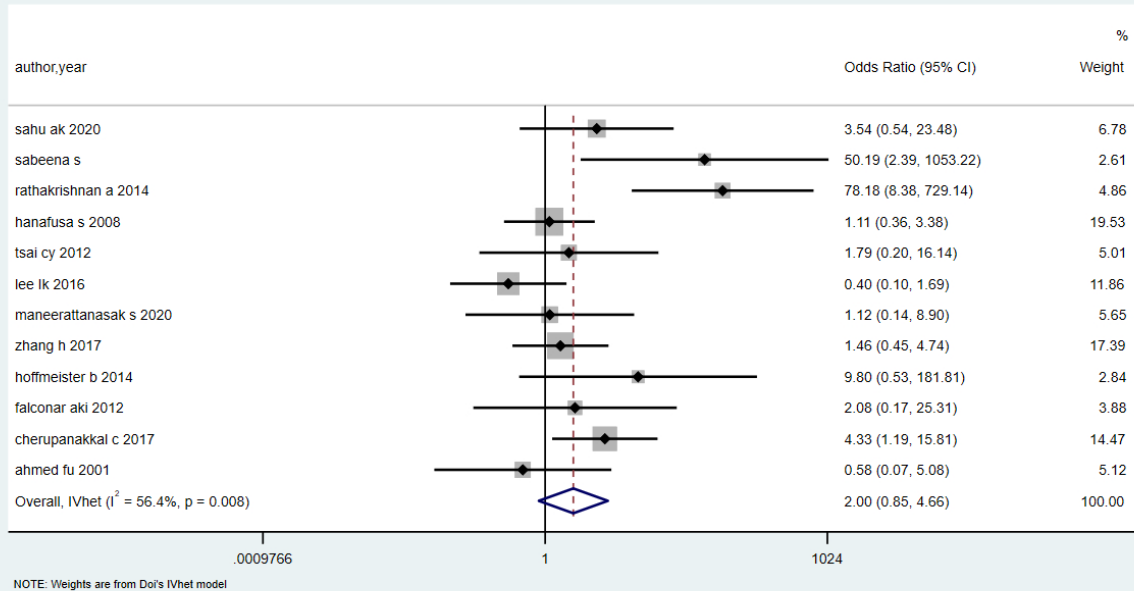
Additional file 5 - Ascitis



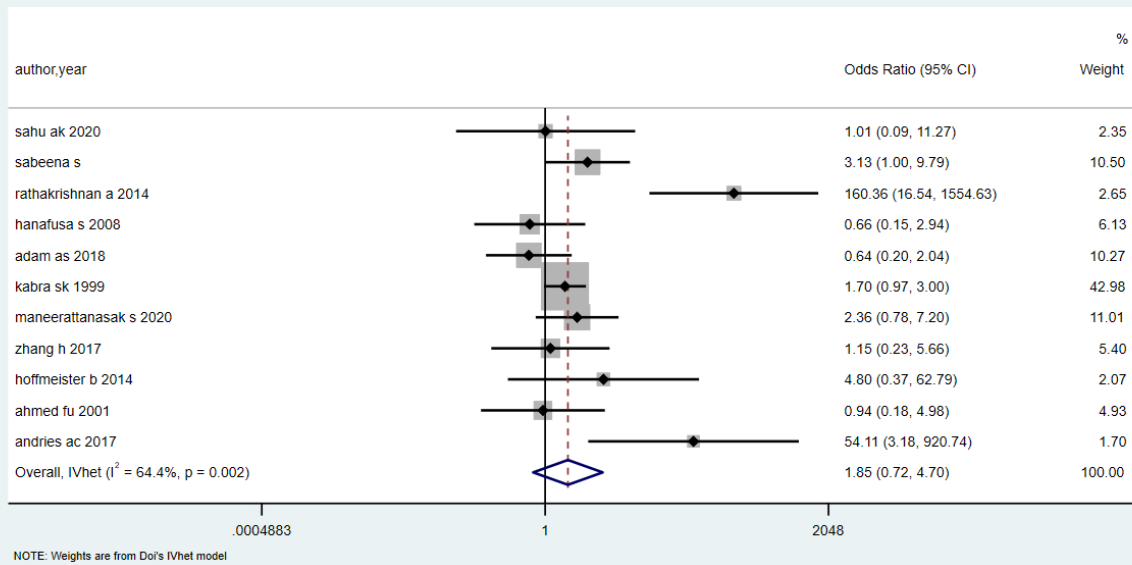
Additional file 5 - Pleural effusion



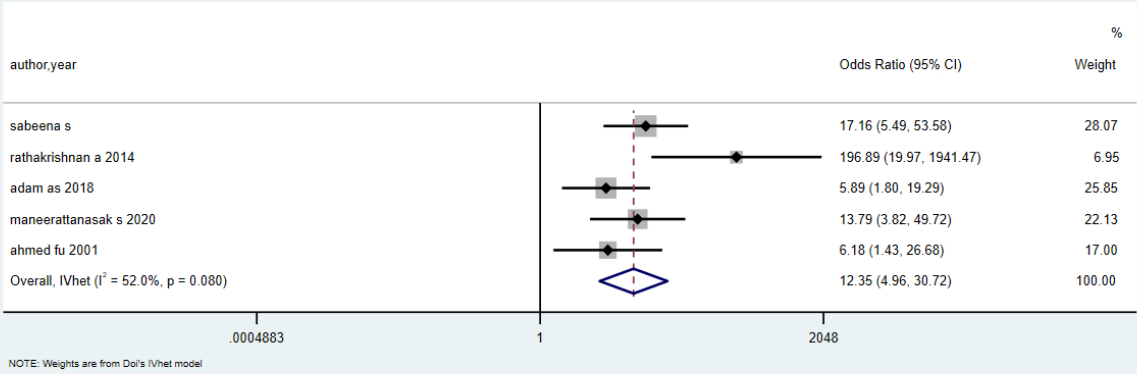
Additional file 5 - Gum bleeding



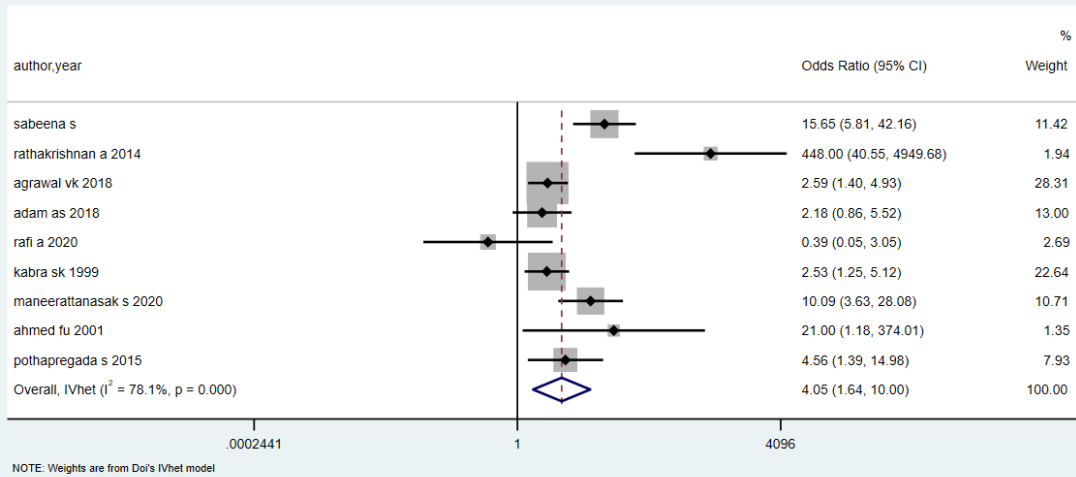
Additional file 5 - Epistaxis



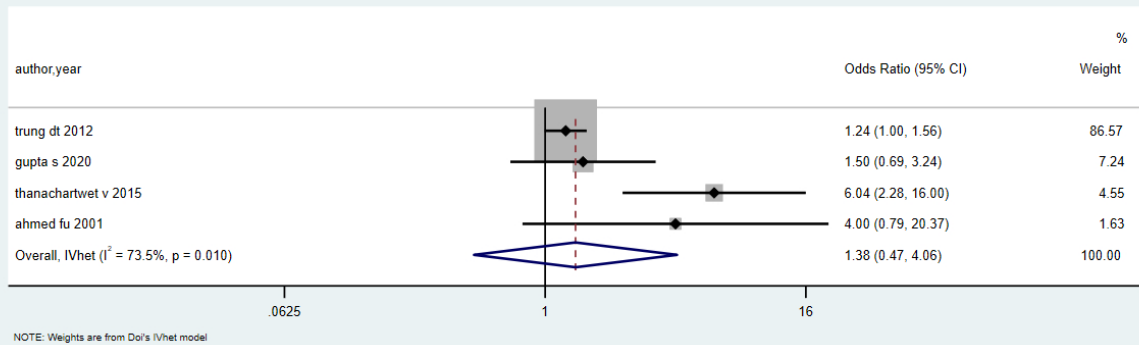
Additional file 5 - Hemetemesi



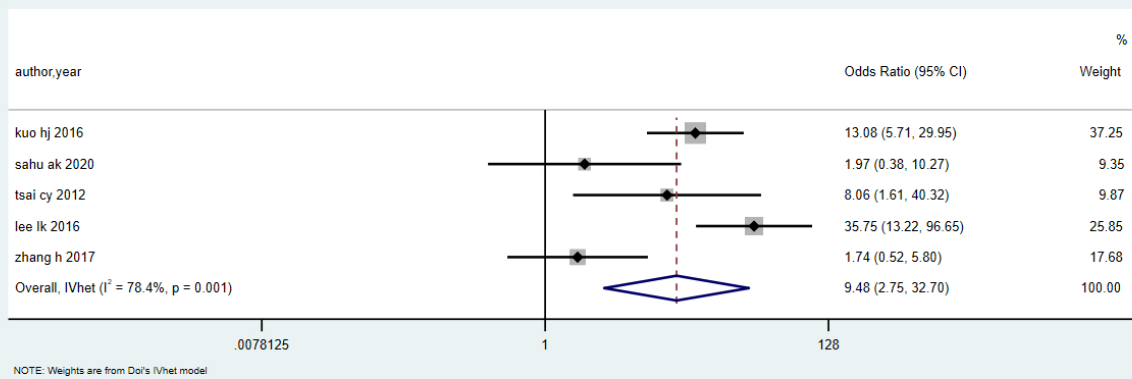
Additional file 5 - Melena



Additional file 5 - Skin bleeding



Additional file 5 - Gum bleeding



Additional file 6. Subgroup analysis using WHO classification for demography, co-morbidities and clinical warning signs of dengue severity.

Risk factors	WHO classification	Number of studies	Pooled OR (95% CI)	<i>P-value</i>
Children	1997	9	2.01 (1.03–3.92)	0.042
	2009	13	1.84 (1.10–3.07)	0.019
Female	1997	45	1.16 (0.57–2.37)	0.679
	2009	69	1.27 (0.92–1.75)	0.147
Secondary				
Infection	1997	7	4.09 (2.22–7.55)	< 0.001
	2009	22	3.09 (2.12–4.49)	< 0.001
Abdominal pain	1997	16	2.54 (1.44–4.47)	0.001
	2009	39	1.66 (1.25–2.19)	< 0.001
Vomiting	1997	12	2.59 (1.57–4.27)	< 0.001
	2009	41	1.70 (1.33–2.18)	< 0.001
Hepatomegaly	1997	18	10.62 (3.19–35.31)	< 0.001
	2009	29	4.21 (2.63–6.74)	< 0.001
Ascitis	1997	10	7.50 (2.67–21.07)	< 0.001
	2009	12	5.83 (3.12–10.89)	< 0.001
Pleural effusion	1997	10	4.54 (1.56–13.17)	0.005
	2009	15	6.70 (3.49–12.85)	< 0.001
Gum bleeding	1997	3	1.00 (0.41–2.43)	0.992
	2009	9	2.70 (0.88–8.26)	0.081
Epistaxis	1997	4	1.58 (1.00–2.49)	0.052
	2009	7	2.48 (0.53–11.68)	0.25
Melena	1997	4	4.28 (1.80–10.17)	0.001
	2009	5	3.89 (0.72–20.95)	0.114

Additional file 7. Subgroup analysis using age groups for demography and warning signs of severe dengue.

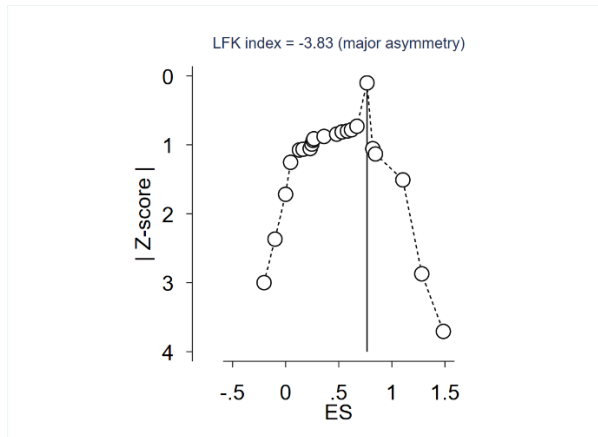
Predictors		Pooled OR		
		Studies	(95% CI)	P-value
Demography				
Female	Children	50	1.00 (0.88– 1.13)	0.978
	Adult	29	2.12 (1.13– 3.97)	0.019
Warning signs				
Secondary Infection	Children	12	3.02 (1.79– 5.12)	< 0.001
	Adult	9	2.50 (1.57– 3.97)	< 0.001
Abdominal pain	Children	23	1.85 (1.29– 2.64)	0.001
	Adult	19	1.75 (1.21– 2.54)	0.003
Vomiting	Children	19	2.05 (1.42– 2.98)	< 0.001
	Adult	19	1.57 (1.14– 2.16)	0.006
Hepamegaly	Children	26	3.58 (2.26– 5.66)	< 0.001
	Adult	13	3.56 (2.03– 6.25)	< 0.001
Ascitis	Children	15	6.14 (3.03– 12.44)	< 0.001
	Adult	4	5.40 (2.40– 12.13)	< 0.001
Pleural effusion	Children	13	5.48 (3.28– 9.15)	< 0.001
	Adult	7	4.22 (0.62– 28.78)	0.141

Additional file 8. Sensitivity analysis.

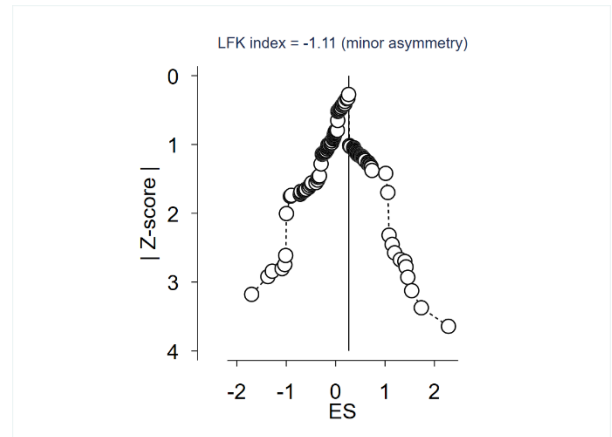
Predictors	Number of Studies	Pooled OR (95% CI)
Demography		
Children	22	1.85 (1.30, 2.64)
Female	114	1.17 (0.90, 1.53)
Secondary Infection	29	3.18 (2.37, 4.26)
Co-morbidities		
Diabetes	10	2.96 (1.83, 4.82)
CVD	4	2.61 (0.45, 15.14)
Obesity	5	0.78 (0.42, 1.43)
Renal disease	4	4.46 (1.53, 13.00)
Hypertension	9	1.83 (0.98, 3.39)
Warning signs		
↑Hct & ↓Plt	7	4.86 (1.61, 14.67)
Abdominal pain	55	2.11 (1.64, 2.71)
Vomiting	53	1.94 (1.58, 2.39)
Lethargy	10	3.06 (1.30, 7.17)
Hepatomegaly	47	5.41 (3.32, 8.82)
Ascitis	22	6.00 (3.69, 9.76)
Pleural effusion	25	6.18 (3.65, 10.45)
Gum bleeding	12	2.06 (0.90, 4.71)
Epistaxis	11	1.95 (0.83, 4.60)
Hemetemesis	5	12.40 (4.99, 30.84)
Melena	9	4.38 (1.84, 10.42)
Skin bleeding	4	1.55 (0.59, 4.04)
GI bleeding	5	9.54 (2.79, 32.60)

Additional file 9. Doi plots and LFK values used to assess publication bias.

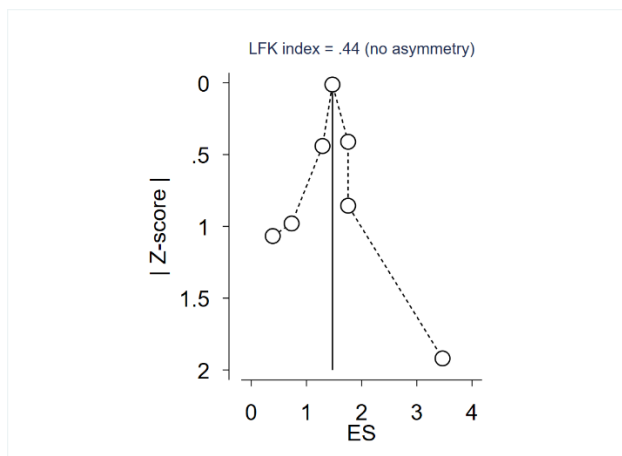
Additional file 9 - Age group



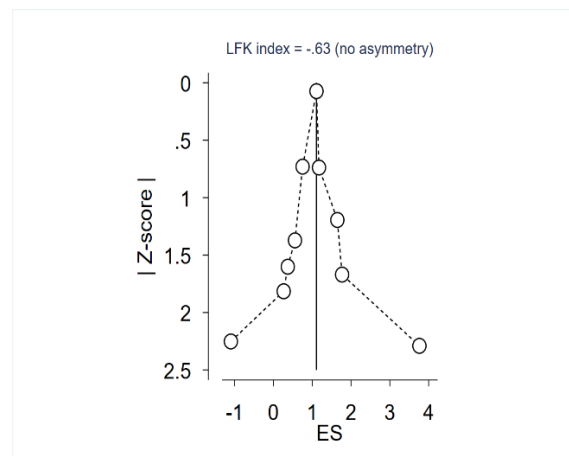
Additional file 9 - Sex



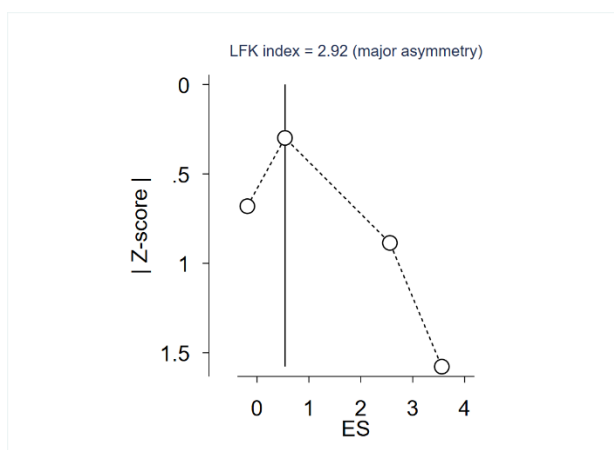
Additional file 9 - Secondary infection



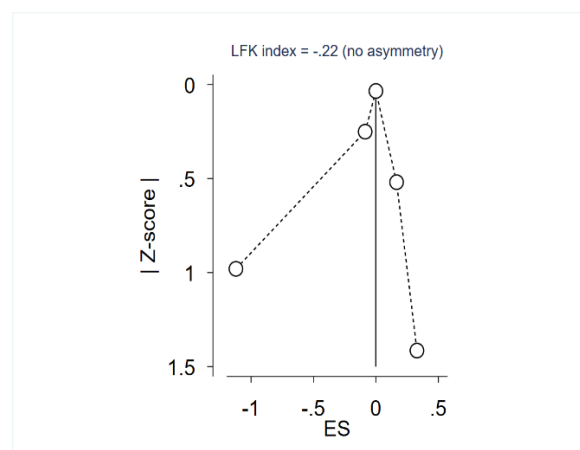
Additional file 9 - Diabetes



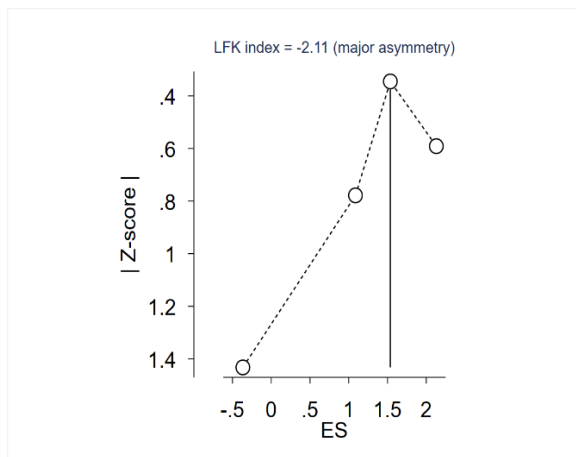
Additional file 9 – Cardiovascular disease



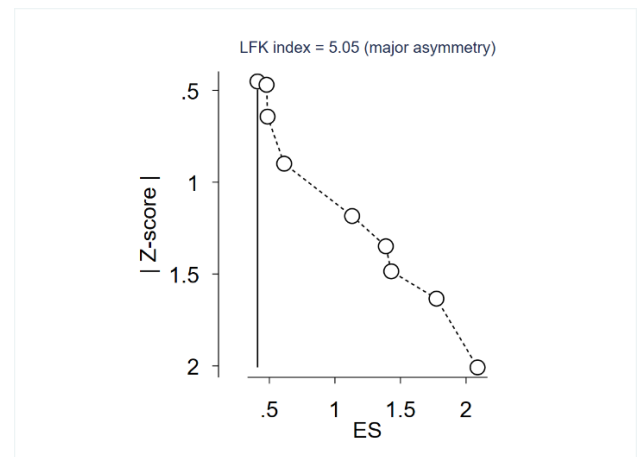
Additional file 9 - Obesity



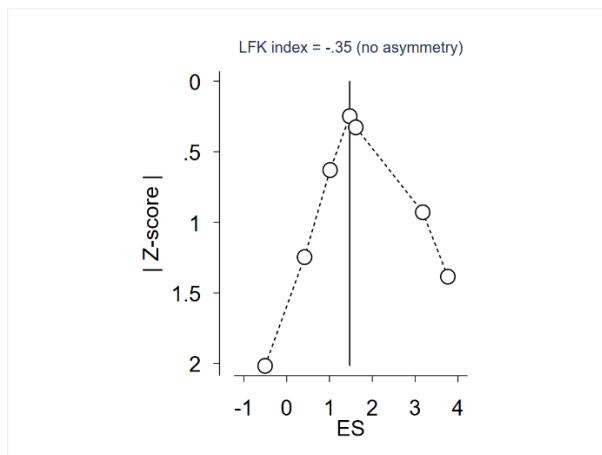
Additional file 9 - Renal disease



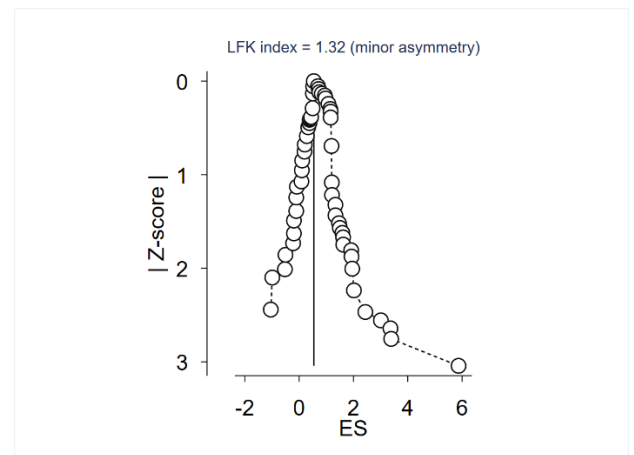
Additional file 9 - Hypertension



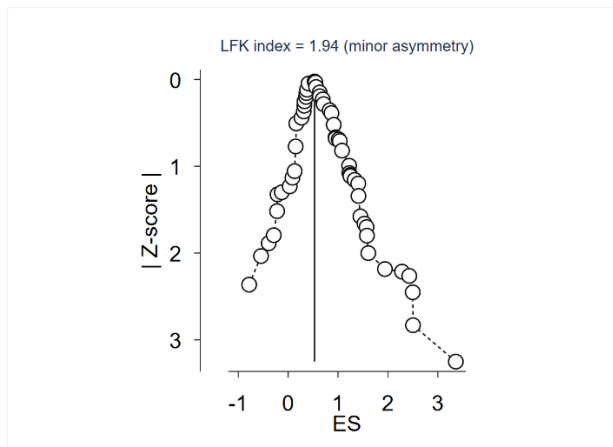
Additional file 9 - ↑Hct & ↓Plt



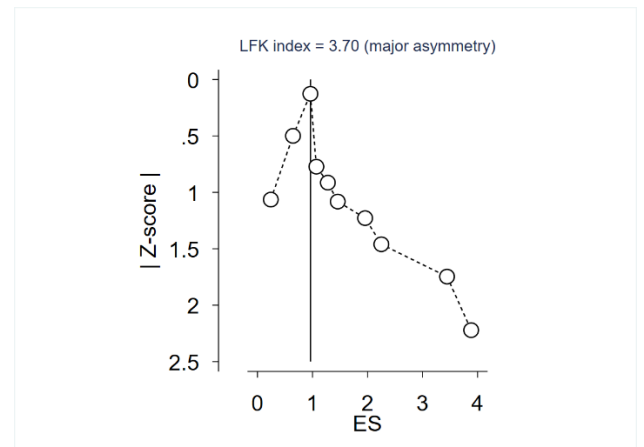
Additional file 9 - Abdominal pain



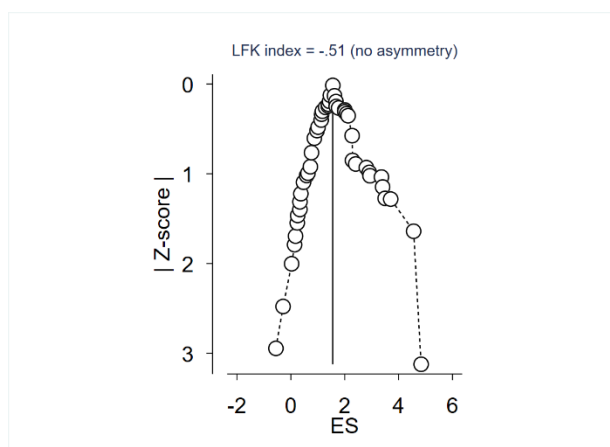
Additional file 9 - Vomiting



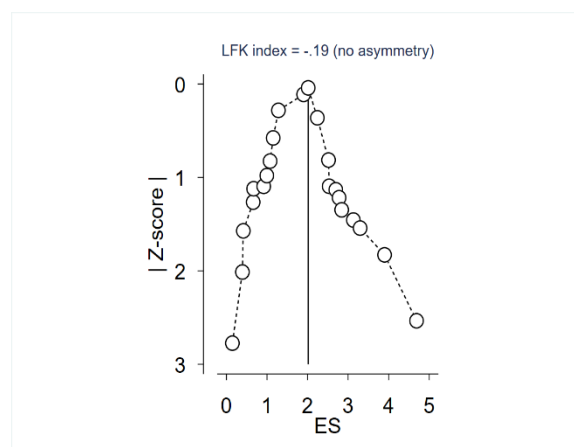
Additional file 9 - Lethargy



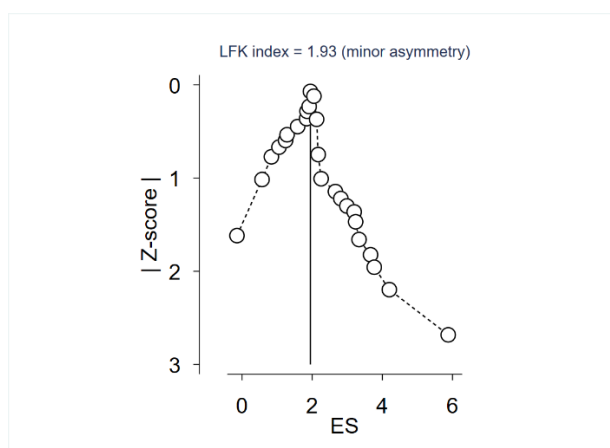
Additional file 9 - Enlarged liver size



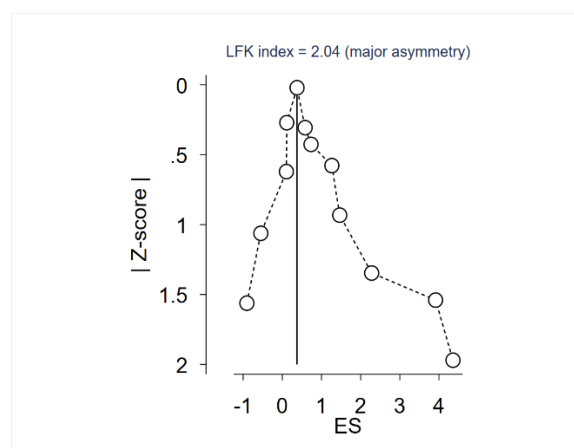
Additional file 9 - Ascitis



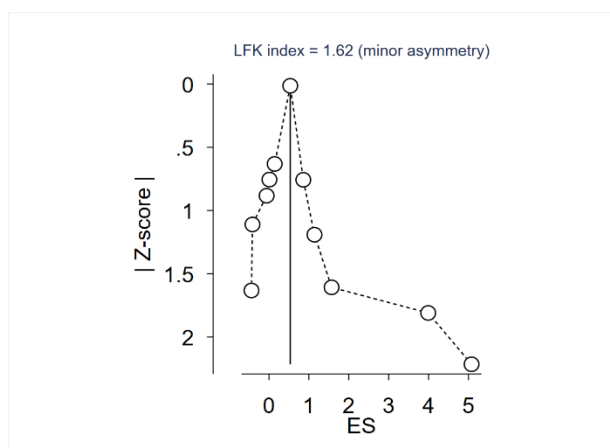
Additional file 9 - Pleural effusion



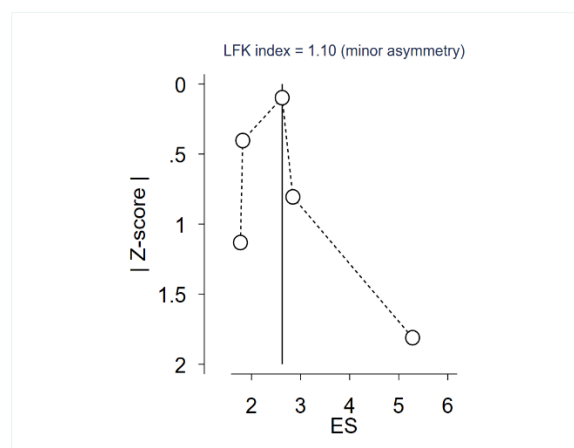
Additional file 9 - Gum bleeding



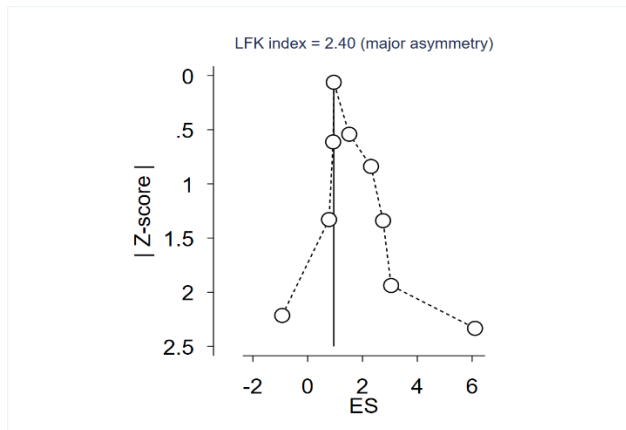
Additional file 9 - Epistaxis



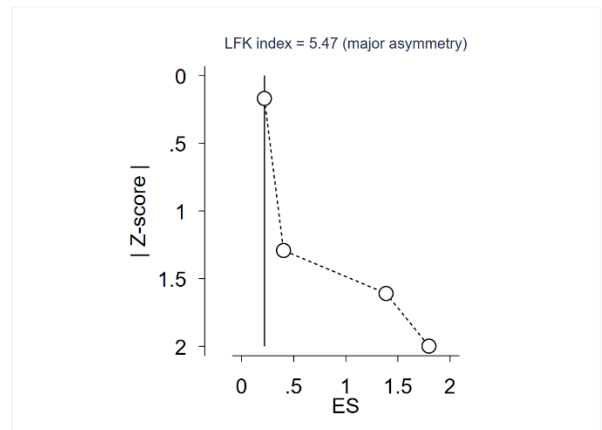
Additional file 9 - Hemetemesi



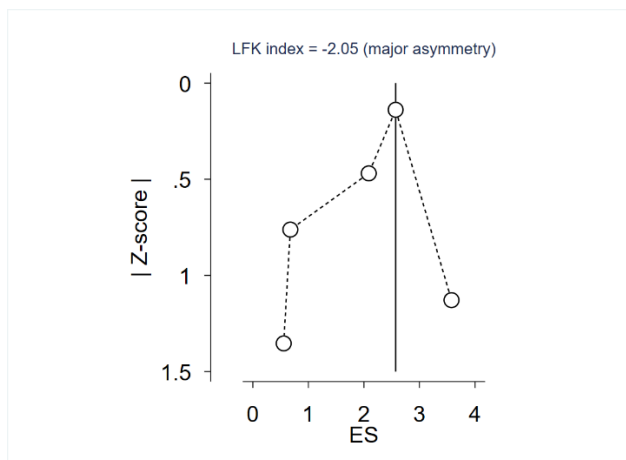
Additional file 9 - Melena



Additional file 9 - Skin bleeding



Additional file 9 - GI bleeding



[2.2] Spatio-temporal drivers of dengue transmission (Chapter 4)

Supplementary Table 1. Univariate preliminary analysis of variables using Poisson regression

Variables	IRR	95% CI	p value	AIC	BIC
Without lag					
Rainfall	1.001	1.0011 - 1.0014	<0.0001	6477.950	6490.239
Max Temp	1.550	1.5000 - 1.6036	<0.0001	5764.781	5777.070
Min Temp	1.342	1.3110 - 1.3735	<0.0001	5891.090	5903.379
Relative humidity	1.042	1.0349 - 1.0494	<0.0001	6711.124	6723.413
NDVI	0.133	0.101 - 0.175	<0.0001	6639.113	6651.402
One month lag					
Rainfall	1.001	1.0011 - 1.0014	<0.0001	6512.729	6525.018
Max Temp	1.516	1.468 - 1.565	<0.0001	5825.582	5837.871
Min Temp	1.352	1.321 - 1.384	<0.0001	5843.605	5855.894
Relative humidity	1.048	1.041 - 1.056	<0.0001	6671.967	6684.255
NDVI	0.070	0.052 - 0.093	<0.0001	6485.699	6497.988
Two months lag					
Rainfall	1.001	1.0011 - 1.0013	<0.0001	6453.907	6466.196
Max Temp	1.418	1.377 - 1.460	<0.0001	6022.012	6034.301
Min Temp	1.285	1.259 - 1.312	<0.0001	6045.974	6058.263
Relative humidity	1.050	1.043 - 1.058	<0.0001	6655.148	6667.437
NDVI	0.095	0.059 - 0.103	<0.0001	6502.977	6515.266
Three months lag					
Rainfall	1.001	1.0006 - 1.0009	<0.0001	6750.636	6762.925
Max Temp	1.330	1.2956 - 1.3645	<0.0001	6195.055	6209.344
Min Temp	1.200	1.1788 - 1.2216	<0.0001	6333.666	6345.954
Relative humidity	1.021	1.0149 - 1.0277	<0.0001	6817.475	6829.763
NDVI	0.182	0.140 - .235	<0.0001	6692.141	6704.430

Supplementary Table 2. Variance inflation factor (VIF) for collinearity of the selected variables.

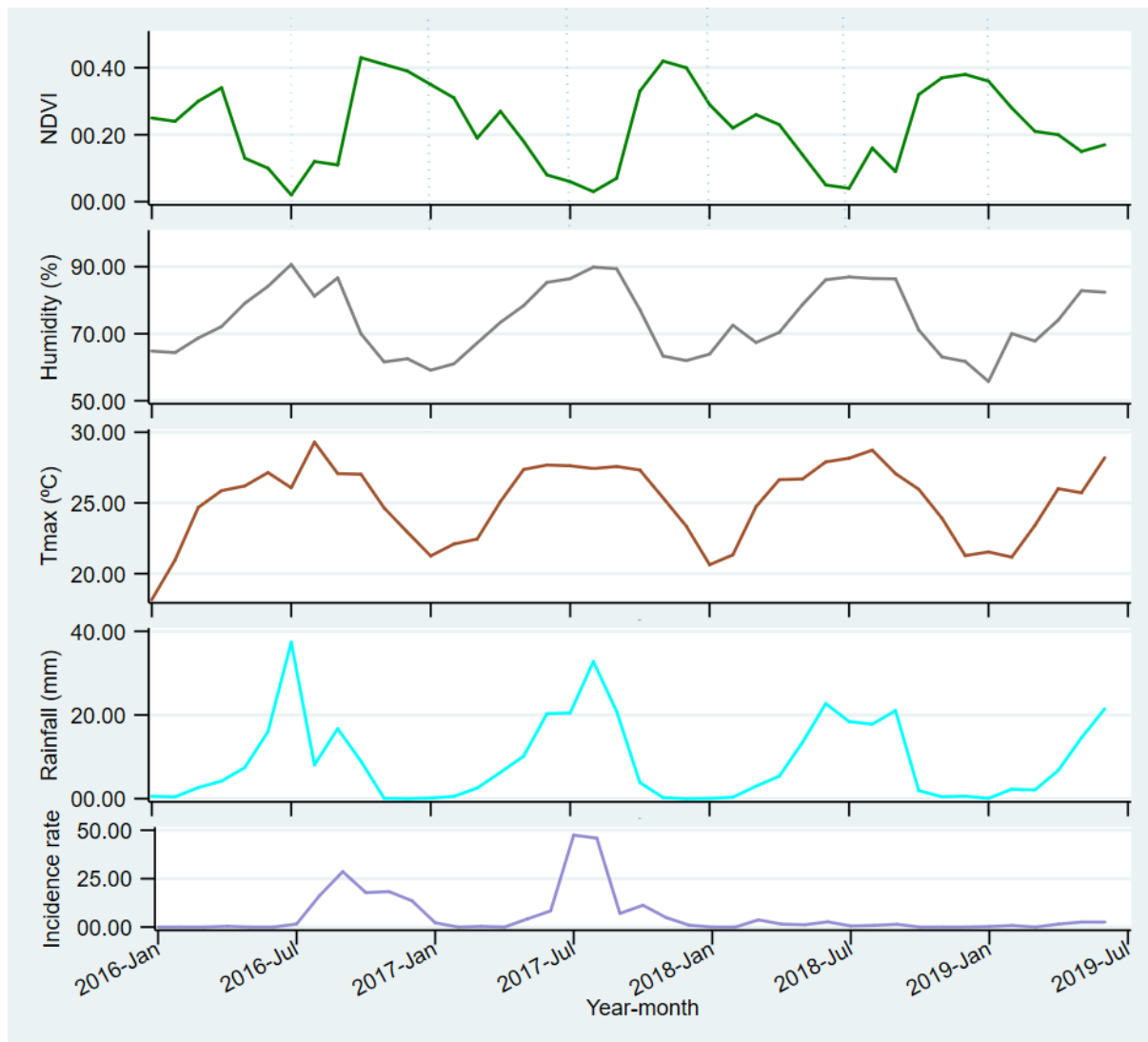
Variable	VIF
----------	-----

Rainfall 2 months lag	1.79
NDVI one month lag	2
Relative humidity 2 months lag	1.53
Max Temperature without lag	1.52
Mean VIF	1.59

Supplementary Table 3. Model comparison using Akaike's Information Criterion (AIC), Bayesian Information Criterion (BIC) and Vuong test.

Model	Observation	AIC	BIC	Vuong test p-value
Poisson	3444	5684.59	5715.31	< 0.001
ZIP	3444	2302.99	2339.85	

Supplementary Figure 1. The monthly average NDVI, relative humidity, maximum temperature, rainfall and dengue incidence rate (per 100,000) for districts reporting dengue in Bhutan.



[2.3] Epidemiological analysis of dengue epidemic in Bhutan (Chapter 4)

Supplementary Table S1: Dataset showing weekly and cumulative dengue cases of the 2019 dengue epidemic in Bhutan.

Week No	New cases	Cumulative cases
18	2	2
19	4	6
20	2	8
21	3	11
22	9	20
23	2	22
24	8	30
25	7	37
26	13	50
27	99	149
28	318	467
29	331	798
30	370	1168
31	419	1587
32	581	2168
33	645	2813
34	517	3330
35	609	3939
36	532	4471
37	331	4802
38	392	5194
39	173	5367
40	186	5553
41	100	5653
42	96	5749
43	47	5796
44	47	5843
45	25	5868
46	25	5893
47	21	5914
48	12	5926
49	9	5935

Supplementary Material S2. Supplementary analysis

As a supplementary analysis we estimated the reproduction number from the growth using the formula:

$$Rt = (1 + r \tau_H)(1 + r \tau_M)(1 + r \tau_e)(1 + r \tau_i).$$

where τ_e is the mean extrinsic incubation period, τ_i is the mean intrinsic incubation period, τ_H is the mean duration of viremia in humans, and τ_M is the mean duration of viremia in mosquitoes, which is assumed to be the expected additional life of the vector. Other authors have used the formulae $Rt = e^{r(\tau_e + \tau_i)}$ or $Rt = (1 + r \tau_H)(1 + r \tau_M)e^{r(\tau_e + \tau_i)}$ [1]. However, the first formula fails to account for the duration of viremia, while both formulae implicitly assume that the incubation period is exactly τ_i for all human cases and that the incubation period is exactly τ_e for all mosquitos; an assumption that tends to over-estimate the reproduction number [2].

Inspection of the number of new cases reported each week over the course of the epidemic revealed four distinct phases in the epidemic. We estimated the value of the growth rate, r , for different stages of the epidemic by fitting a linear model to the log of the number of new cases reported each week during that phase, using the function *glm* from the *stats* R package [3]. For the purposes of these estimates, the four phases were defined with three breakpoint weeks which were considered the last week of the preceding phase and the first week of the following phase. The breakpoint between phases one and two (week 25) was chosen to correspond to the start of summer school vacation and a period of high rainfall (i.e. the monsoon season). The breakpoint between phases three and four (week 34) was chosen to correspond to the beginning of large-scale interventions. The breakpoint between stages two and three (week 28) was chosen by inspection. We confirmed our choice of breakpoints by using the function *segmented*, from the R package, *segmented* [4], to fit a piecewise log-linear model and estimate the choice of the breakpoints. The breakpoint estimates using this procedure were week 25.7 (95% CI 25.2–26.3), week 27.6 (95% CI 26.9–28.2) and week 35.2 (95% CI 33.9–36.6). Moreover, the best fit model with four phases had a lower Akaike information criterion (26.2) than the best fit with three phases (53.4), while models with five or more phases failed to converge, confirming the suitability of a four-phase model. Breakpoints at 25, 28, and 34 were preferred as these aligned with the weekly reporting structure of the NEWARS data, provided a more robust estimate of the growth rate in phase two by including two additional data points, and allowed for the comparison of growth rates before and after interventions.

The estimates for the growth rate (r) in each phase was combined with estimates of τ_H , τ_M , τ_e , and τ_i drawn from the literature [Table 1 in [5]], to estimate the reproduction number for each period. We used Monte Carlo sampling from each parameter's uncertainty distribution to calculate confidence intervals for the reproduction number. We modelled the uncertainty in the growth rate, r , using a normal distribution with mean and standard deviation determined by the maximum likelihood estimate and standard error. Following Enduri and Jolad [5] we used gamma distributions to model the uncertainty in the remaining parameters: lifespan of vector (mean 8.88 days; s.d. 1.33 days) [6], duration of viremia (mean 5 days; s.d. 1 day) [6], extrinsic incubation period (mean 10 days; s.d. 1 day) [7], and intrinsic incubation period (mean 5.38 days, s.d. 0.73 days) [7]. By drawing 10^7 sets of parameters from these distributions and calculating R_t for each set, we calculated the median and 95% highest density intervals using the package *coda* in R [8].

Using this method, the estimated reproduction number was approximately the same in phases one and three with $R_t = 1.8$ (95% CI 0.95–3.26) and $R_t = 1.5$ (95% CI 1.30–1.92) respectively. However, the reproduction number was very high in phase two ($R_t = 31.0$, 95% CI 15.2–60.7) and less than one in phase four ($R_t = 0.22$, 95% CI 0.16–0.29) (Supplementary Table S6).

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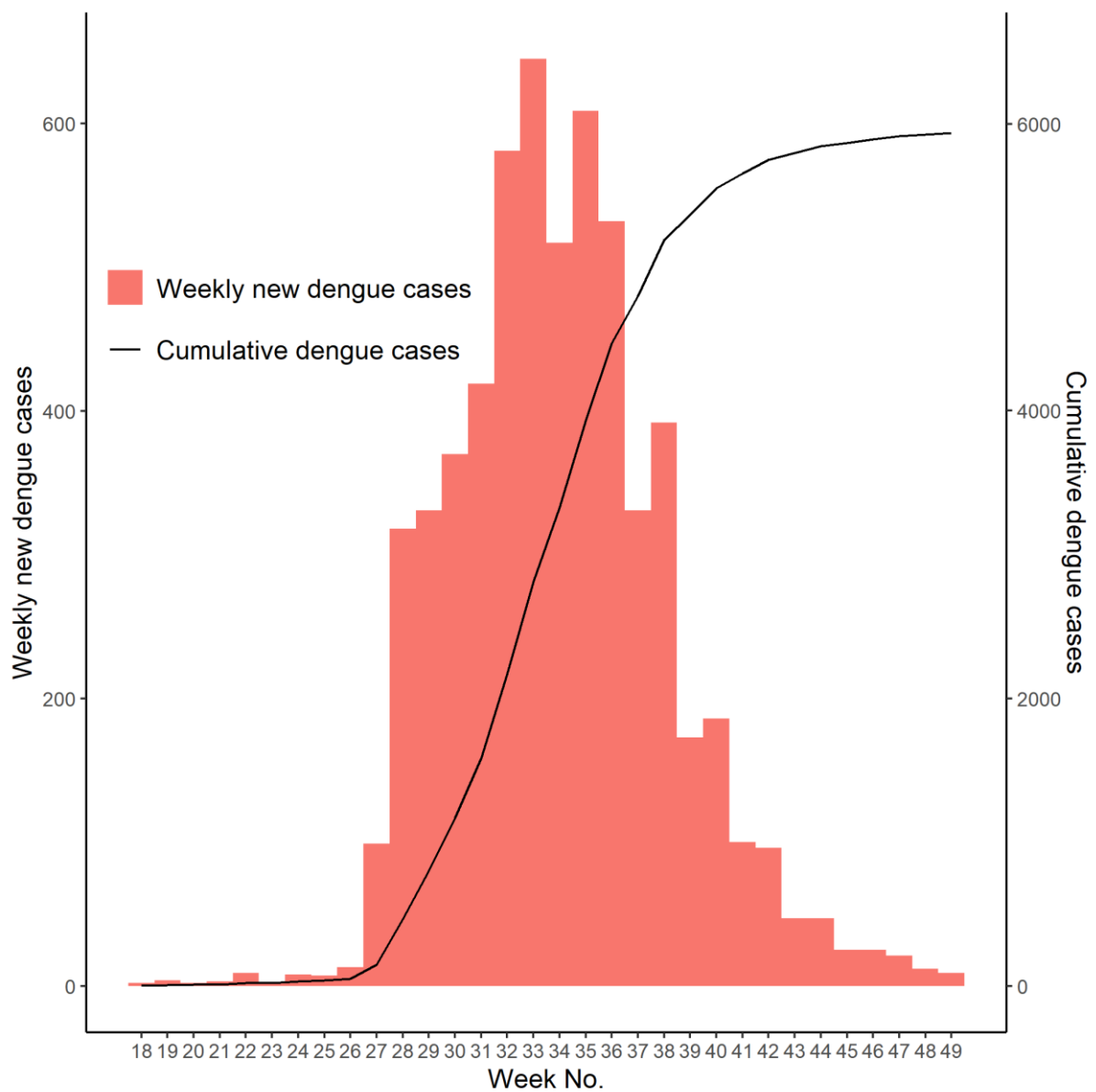
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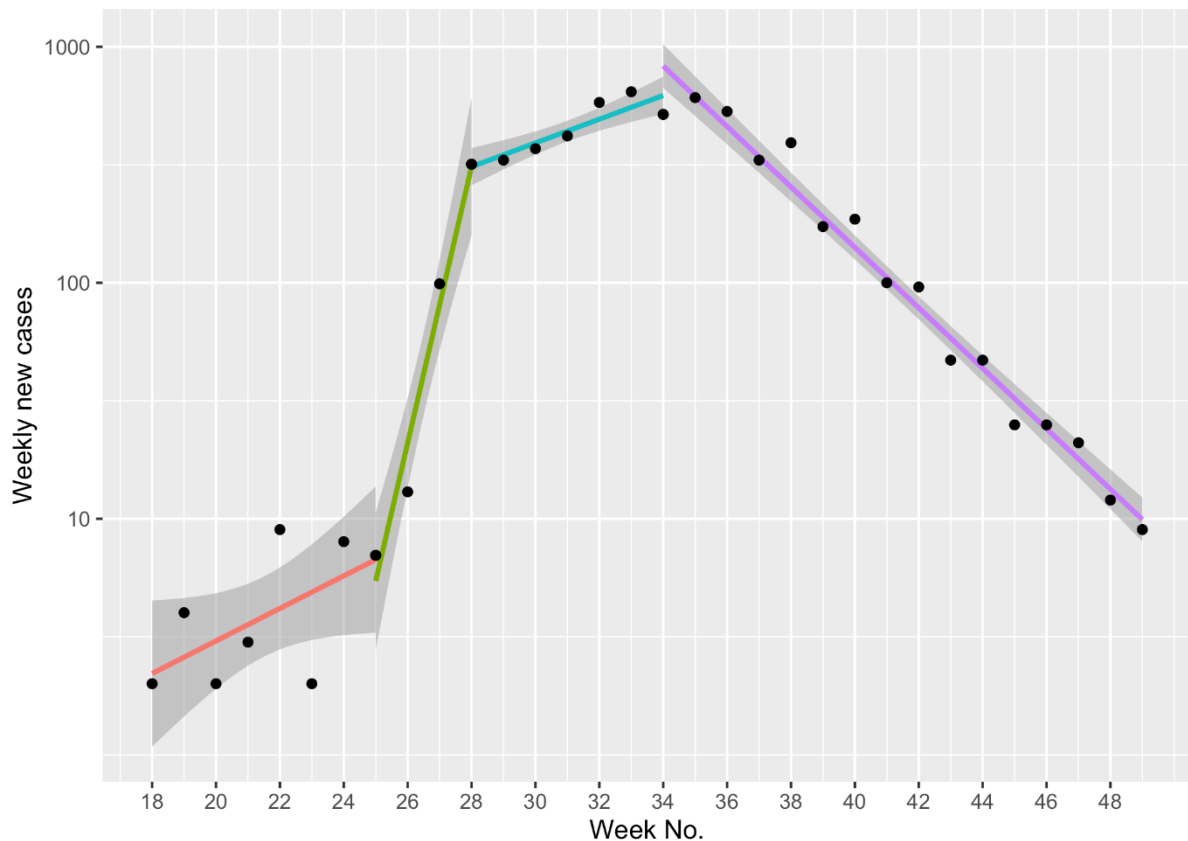
Supplementary Material S3. Sensitivity analysis

Public awareness, treatment seeking behaviour and clinical suspicion of dengue may have varied over the period. This may have led to different reporting rates over the study period. We considered a sensitivity analysis in which the proportion of cases reported in the early part of the epidemic was lower than for the remainder of the period. We considered cut-offs in weeks 25, 26, 27 and 28, and reporting ratios of 0.2, 0.4, 0.6, 0.8 and 1.0. For each combination of cut-off week and reporting ratio, we multiplied the number of cases reported in all weeks prior to the cut-off by the inverse of the reporting ratio (Supplementary Figure S8) and estimated the temperature dependant, time varying reproduction number for the whole country (Supplementary Figure S9) using these inflated case numbers. Assuming lower reporting ratios in the early weeks of the epidemic reduced the estimates of the reproduction number five weeks either side of the cut-off, and reduced the estimated peak value of the reproduction number. For a given reporting ratio, a cut-off at week 27 led to the lowest estimates of time-varying reproduction numbers.

Supplementary Figure S4. Epidemic curve showing weekly number of new cases (left vertical axis) along with the weekly cumulative cases of dengue epidemic in Bhutan in 2019



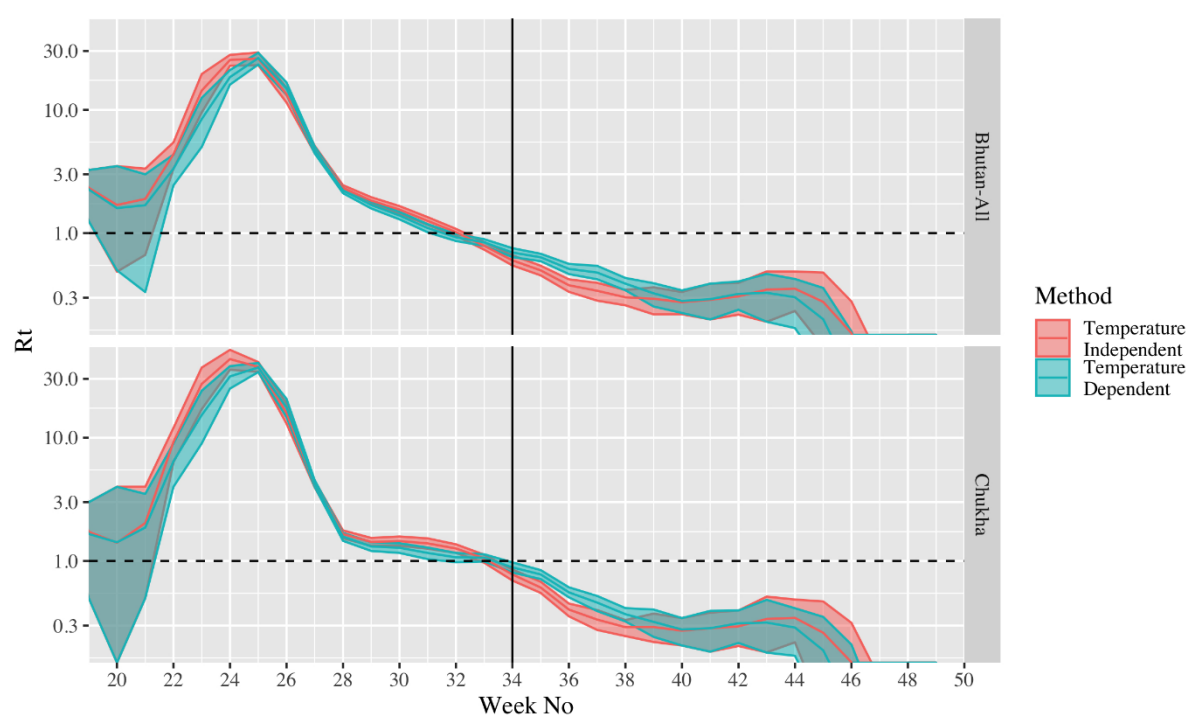
Supplementary Figure S5. Epidemic curve showing the different growth rates across the four phases of the epidemic. The weekly reported cases numbers collected by NEWARS (National Early Warning, Alert, Response and Surveillance) are represented by dots, the fitted exponential growth curves and 95% confidence intervals are indicated by lines and shaded regions. The transition between phases one and two (week 25) coincided with the beginning of high rainfall and the start of school vacation. The transition between phases three and four (week 34) coincides with the beginning of national interventions



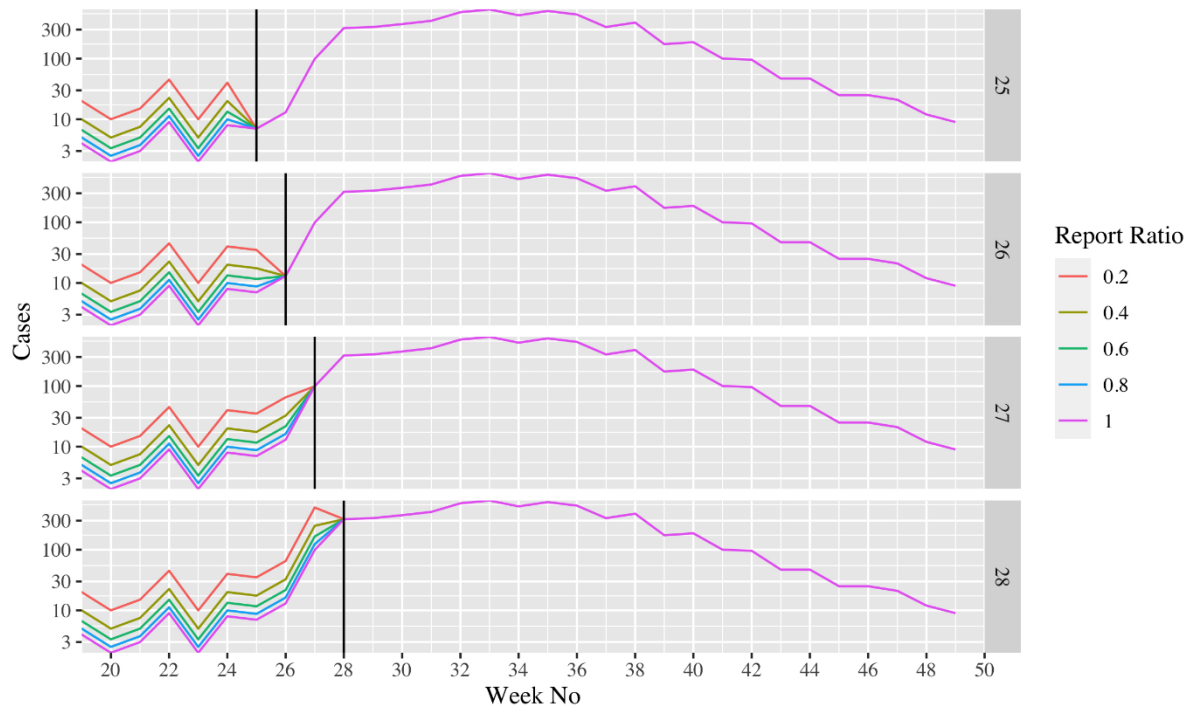
Supplementary Table S6. Growth rate and reproduction number during phases of the dengue epidemic in Bhutan, 2019

Week number	Growth rate, r (95% CI)	Reproduction number, R (95% CI)
18 – 25	0.16 (-0.01, 0.33)	1.8 (0.95, 3.26)
25 – 28	1.35 (0.99, 1.71)	31 (15.20, 60.70)
28 – 34	0.12 (0.07, 0.17)	1.5 (1.30, 1.92)
34 – 49	-0.29 (-0.32, -0.27)	0.22 (0.16, 0.29)

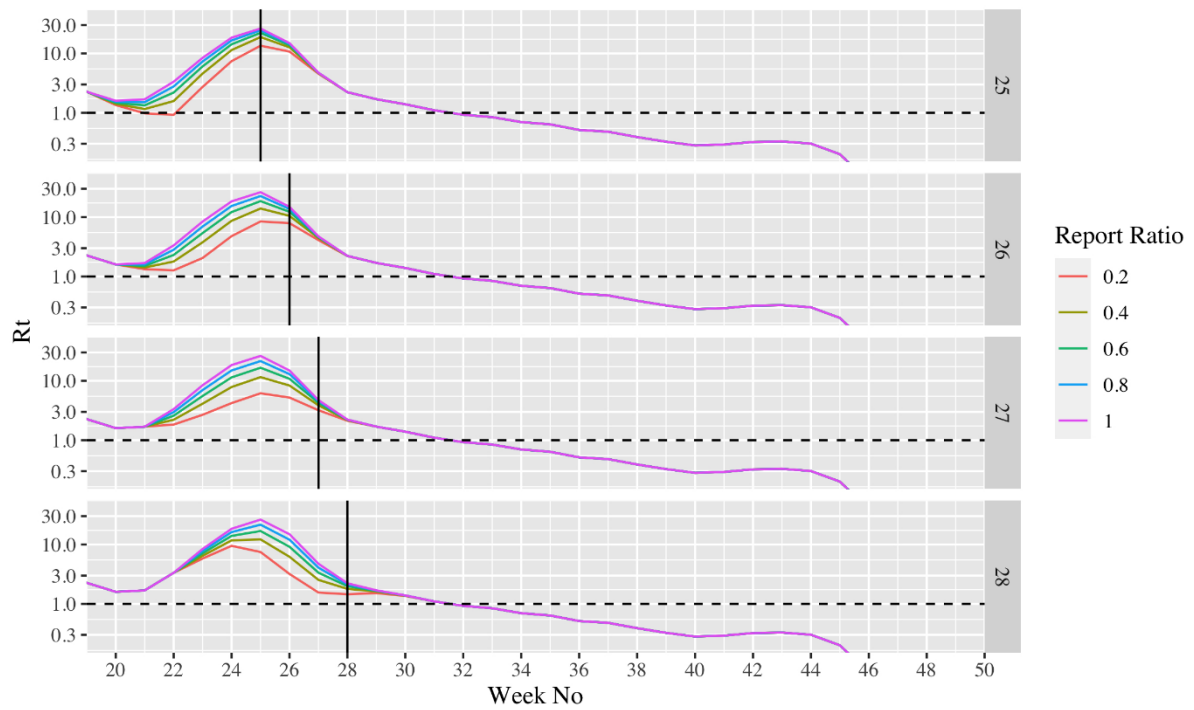
Supplementary Figure S7. The effective reproduction number over the course of the epidemic, assuming constant temperature and accounting the changes in temperature in all of Bhutan and Chukha only.



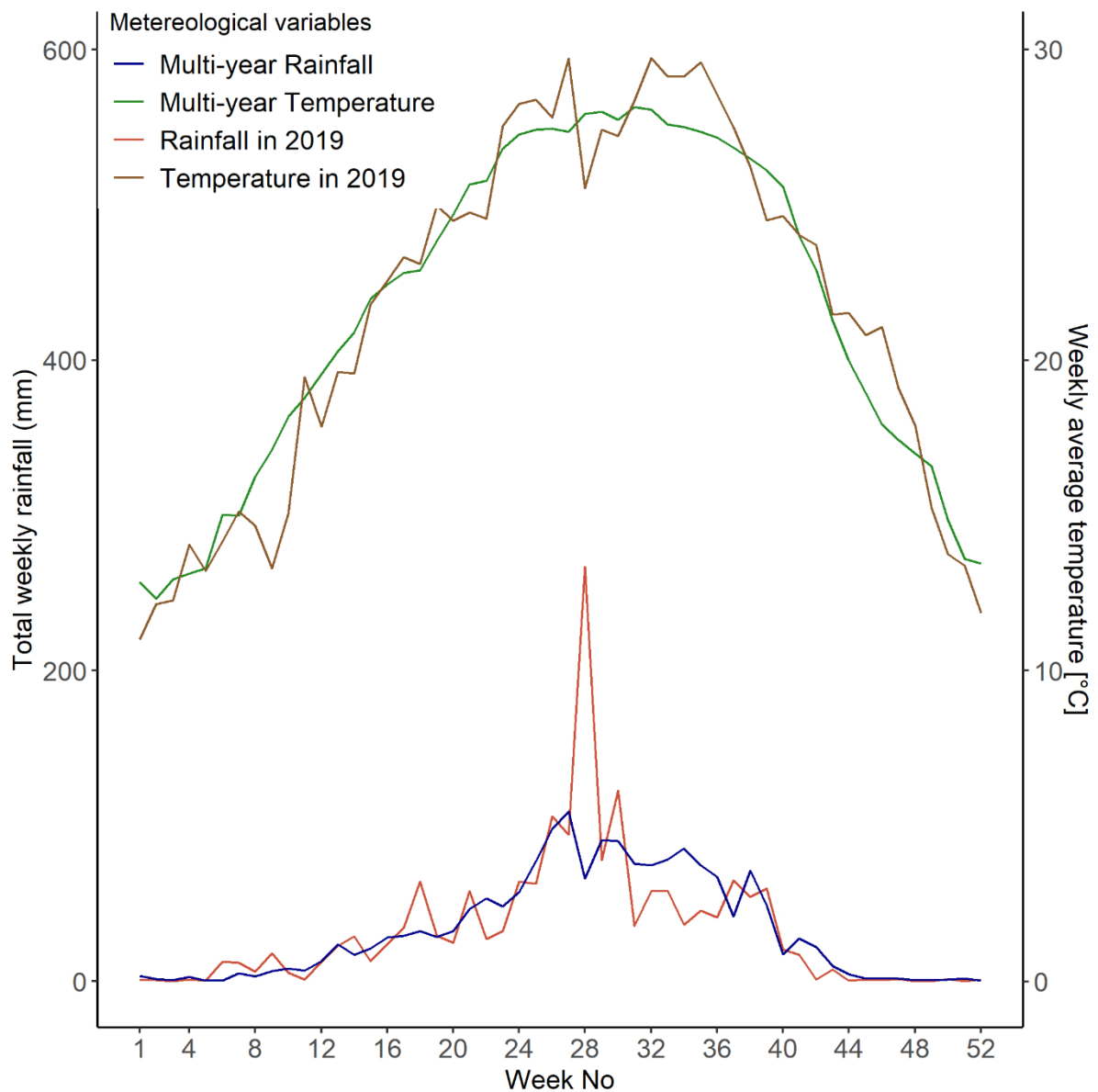
Supplementary Figure S8. Sensitivity analysis to account the influence of under-reporting on the reproduction number by considering cut-offs in weeks 25, 26, 27, and 28, and reporting ratios of 0.2, 0.4, 0.6, 0.8, and 1.0.



Supplementary Figure S9. The influence of under-reporting at the beginning phase of the epidemic on the estimated temperature dependent, time varying reproduction number for the whole country.



Supplementary Figure S10. Total weekly rainfall and average temperature in Bhutan, 2019. Multiyear averages for these variables were calculated using data from 2008 to 2018. (Source: National Centre for Hydrology and Meteorology of the Royal Government of Bhutan).



[2.4] Dengue risk assessment using multicriteria decision analysis: A case study of Bhutan (Chapter 5)

S1 Table. Seasonal dengue incidence per 10,000 inhabitants between 2016 and 2019, Bhutan (Data source: NEWARS)

Season	2016	2017	2018	2019
Winter (Dec - Feb)	0.50	0.12	0.00	0.28
Spring (Mar - May)	0.01	0.18	0.35	0.65
Summer (Jun - Aug)	0.63	4.13	0.20	52.99
Autumn (Sep - Nov)	2.33	1.09	0.08	17.80

S2 Table. Total number of publications reporting association between dengue incidence and selected risk factors in the Asian countries.

Studies in the past have reported a statistical association between dengue incidence with temperature, rainfall, elevation, population density, land use, road network and water bodies. We used combination of search terms as described below to retrieve articles that showed association between dengue and its independent variables to calculate weights in AHP. Due to large volumes of literature available online in peer-reviewed journals, the search was confined only to Asian countries which is logically chosen to represent Bhutan. We used following combination of search terms: (((("dengue virus" OR dengue OR "severe dengue"Title OR "dengue fever" OR "dengue hemorrhagic fever"[MeSH Terms]) AND (temperature or precipitation or rainfall or "land use/land cover" road or water or river or "population density")) AND (("2010"[Date - Create]: "2020"[Date - Create]))) AND (asia[MeSH Terms]).

Variables	Country	Total publications	Reference
Temperature	Cambodia	1	(1)
	China	12	(2-13)
	India	1	(14)
	Malaysia	1	(15)
	Pakistan	2	(16, 17)
	Philippines	1	(18)
	Saudi Arabia	1	(19)
	Taiwan	4	(20-23)

	Thailand	1	(24)
	Vietnam	3	(25-27)
	Indonesia	1	(28)
	Timor-Leste	1	(29)
Rainfall	Cambodia	1	(1)
	China	4	(6, 7, 9, 10)
	Pakistan	2	(16, 17)
	Philippines	2	(18, 30)
	Taiwan	3	(20, 21, 23)
	Vietnam	2	(25, 26)
	Timor-Leste	1	(29)
Land use	China	5	(2, 3, 9, 13, 31)
	Pakistan	1	(16)
	India	1	(14)
	Taiwan	1	(32)
	Thailand	1	(33)
	Vietnam	1	(25)
Population density	China	4	(2, 3, 9, 13)
	Pakistan	2	(16, 34)
Road network	China	4	(3, 31, 35, 36)
Water bodies	Bangladesh	1	(37)
	Taiwan	1	(38)

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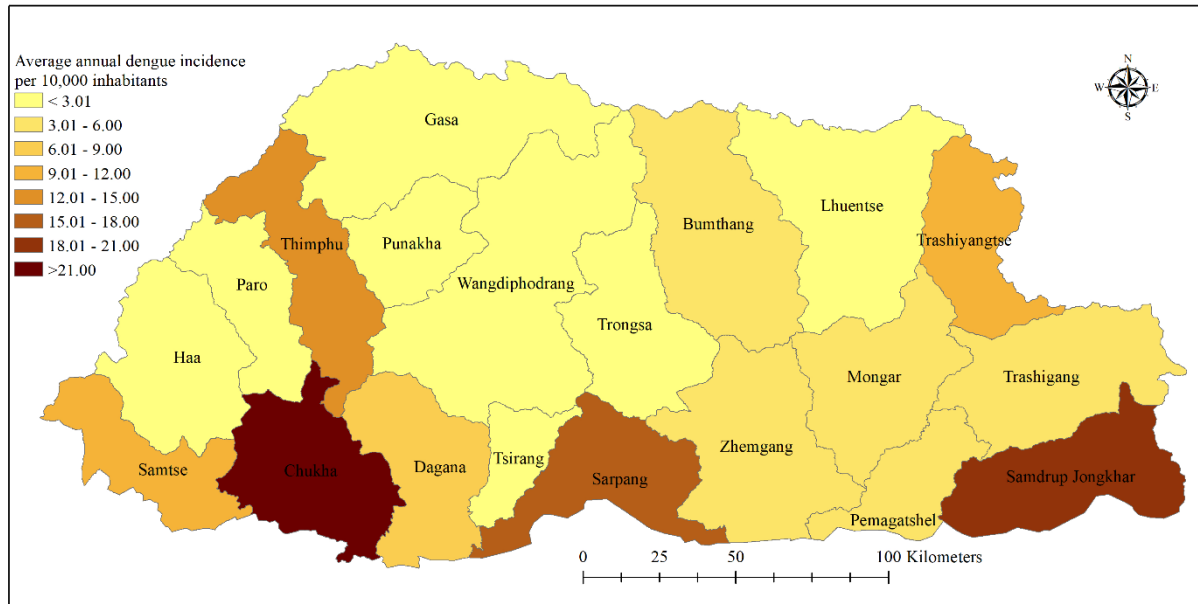
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S3 Table. Pearson Correlation Coefficient (r) for pair of risk factors associated with occurrence of dengue transmission.

Risk factors	Temp	Rain	Land use	Pop. density	Road network	Water bodies
Temp	1					
Rain	0.751	1.000				
Land use	0.427	0.336	1.000			
Pop. density	0.268	0.189	0.631	1.000		
Road network	-0.496	-0.205	-0.441	-0.429	1.000	
Water bodies	0.018	0.063	-0.133	-0.121	0.039	1.000

Bolded numbers relate to multicollinearity issues with $r > 0.4000$)

S1 Figure. Average annual dengue incidence per 10,000 inhabitants between in 2016-2019, Bhutan. We calculated the incidence by dividing average of dengue cases reported to dengue surveillance (NEWARS) between 2016 and 2019 by the average district population. (Data source: NEWARS)



S1 Table. Comparison of responses across four domains by the characteristics of the study respondents, Bhutan, 2019-2020.

Variables	Response selected	Domain 1 (n=9)		Domain 2 (19)		Domain 3 (n=19)		Domain 4 (n=8)	
		Median (IQR)	p-value	Median (IQR)	p-value	Median (IQR)	p-value	Median (IQR)	p-value
Age groups (years)	<= 29	5 (4 - 6)		9 (6 - 14)		9 (7 - 15)		6 (5 - 7)	
	30-39	5 (4 - 6)	0.778*	10.5 (7 - 15)	0.607*	11.5 (6 - 16)	0.879*	7 (4 - 7)	0.234*
	>=40	5 (4 - 6.5)		9 (7 - 11)		10.5 (8 - 13)		5.5 (4 - 6)	
Sex	Male	5 (4 - 6)	0.817#	10 (7 - 15)	0.558#	11.5 (7 - 15)	0.594#	6 (4 - 7)	0.522#
	Female	5 (4 - 6)		9 (5 - 14)		9 (7 - 13)		6 (5 - 7)	
Medical role	HA/COs	5 (4 - 6)	0.024#	8.5 (5 - 10)	< 0.001#	8 (6 - 10.5)	< 0.001#	6 (4 - 7)	0.002#
	Doctor	6 (4 - 7)		14 (10 - 16)		15 (13 - 17)		7 (6 - 7)	
Medical experience (years)	<=5	5 (4 - 6)	0.319#	10 (5 - 15)	0.902#	12 (7 - 15)	0.447#	6 (4 - 7)	0.556#
	> 5	5 (4 - 7)		10 (7 - 13)		9 (7 - 13)		6 (5 - 7)	
Facility type	Hospital	6 (5 - 7)	<0.001#	13 (9.5 - 15)	< 0.001#	15 (12 - 16)	< 0.001#	6 (5 - 7)	0.120#
	BHU	4.5 (4 - 5)		8 (5 - 10)		8 (5.5 - 10.5)		6 (4 - 7)	
Ever diagnosed dengue	Yes	5.5 (4 - 7)	0.012#	12.5 (9 - 15)	< 0.001#	14 (12 - 16)	< 0.001#	7 (5 - 7)	0.001#
	No	5 (4 - 5)		7 (5 - 10)		7 (5 - 9)		5 (4 - 6)	

*Kruskal-Wallis test; #Man-Whitney U test

S2 Table. Research instrument

As a part of our efforts to understand the knowledge and clinical practices among medical practitioners on dengue, we are conducting this survey. The survey has 40 questions and it might take 15-20 minutes to complete. Your time is highly valuable and thank you for the participation

Part I: General Information

Date of survey: _____

1. May I know the Dzongkhag under which your health center is located?	1. Bumthang 2. Chukha 3. Dagana 4. Gasa 5. Haa 6. Lhuentse 7. Mongar 8. Paro 9. Pemagatshel 10. Punakha 11. S/Jongkhar 12. Samtse 13. Sarpang 14. Thimphu 15. Trashigang 16. Trongsa 17. Tsirang 18. Wangdiphodran 19. T/Yangtse 20. Zhemgang	
2. Which level of healthcare do you practice your medicine?	1. National 2. Regional 3. General hospital 4. Basic Health Unit (BHU)	
3. What is your age (in completed year)		

4. What is your gender?	1. Male 2. Female 3. Others	
5. What is the title/position of your profession?	1. Health Assistant 2. Clinical Officer 3. Doctor	
6. Which of the following best describes your area of speciality, or what is your current working place of speciality?	1. Community Health 2. General medicine 3. Paediatric 4. Emergency 5. Intensive 6. Laboratory 7. Others, specify	
7. How many years have you been in this profession?(from the start of career till date):		

Part II: Knowledge and clinical practices

a. Dengue transmission

8. How is dengue transmitted?

1. Bite of *Anopheles* mosquitoes
2. Bite of *Aedes* mosquitoes
3. Bite of *Culex* mosquitoes
4. Bite of ticks/mites

9. At what time of the day is people more likely to get infected by dengue? (Multiple choice question)

1. Morning
2. Afternoon
3. Evening
4. Night

10. When mosquito feeds dengue case, which of the following is true?

1. No infection of the mosquito, if the dengue virus concentration in the blood is too low

2. The mosquito becomes infected and can infect others when it feeds next
 3. The mosquito will live for another 2-3 months
 4. Female mosquitoes will spread the dengue virus to other mosquitoes when they mate
11. So far, which of the following dengue virus (DENV) serotypes are found in Bhutan?

(Multiple choice question)

1. DENV-1
2. DENV-2
3. DENV-3
4. DENV-4

b. Clinical presentation and course

12. How many cases of dengue have you personally diagnosed during your career?

1. 0
2. 5 or less
3. 6–10
4. 11–20
5. >21 cases

13. After being bitten by dengue infected mosquitoes, how long does it take to develop dengue illness?

1. Less than 3 days after exposure
2. Between 3-10 days after exposure
3. As long as 3 weeks after exposure
4. Anytime in the month after exposure

14. How should a clinician identify a patient with dengue at the time of presentation? (Multiple choice question)

1. Take a fever history to identify any reported fever within the past week
2. Only consider dengue if the patient is febrile on presentation
3. Perform a complete blood count to look for hemoconcentration, leukopenia, or thrombocytopenia
4. Ask about headache, retro-orbital pain, joint pain, rash, and muscle aches
5. Ask about vomiting, abdominal pain, bleeding, and lethargy
6. Only consider dengue if thrombocytopenia is present

15. Identify warning signs of dengue fever or severe dengue from the following list (multiple choice question)

- | | | |
|-----------------------|------------------------|--------------------|
| 1. Fever | 2. Persistent vomiting | 3. Muscle pain |
| 4. Dyspnoea | 5. Giddiness | 6. Constipation |
| 7. Retro-orbital pain | 8. Lethargy | 9. Diarrhoea |
| 10. Persistent cough | 11. Bleeding | 12. Abdominal pain |

16. A 10-year-old female patient presents to you with 2 days history of fever, headache, joint pain, vomiting, mucosal bleeding, lethargic and liver enlargement (>2cm). Based on the latest WHO guideline, this patient is classified as:

1. Dengue fever
2. Dengue haemorrhagic fever
3. Dengue with warning signs
4. Severe dengue

17. A severe dengue should be considered if the patient from an area of dengue risk presenting with fever of 2-7 days plus any of the following features (Multiple choice question)

1. An evidence of plasma leakage
2. A significant bleeding
3. Altered level of consciousness (lethargy or restlessness, coma)
4. A severe gastrointestinal involvement (persistent vomiting, increasing or intense abdominal pain, jaundice)
5. Guillain–Barre syndrome
6. Severe organ impairment (acute renal/liver failure, encephalitis, cardiomyopathy)

18. Which of the following is the best early indicator of shock?

1. Hypotension
2. Bradycardia
3. Mental status changes
4. Tachycardia in the absence of fever or delayed capillary refill

19. When do you expect a patient to develop clinically significant plasma leakage?

1. During the first 3 days of illness while the patients is febrile

2. Usually on days 3–7 of illness, around the time of defervescence
3. Following a rapid increase in platelet count and progressive leucocytosis
4. During the recovery phase

20. When do you expect a patient with dengue to develop thrombocytopenia?

1. In the first 3 days of the illness, while the patient is febrile
2. During the recovery phase
3. Thrombocytopenia is not seen in dengue
4. Following progressive leukopenia, around the time of defervescence

c. Diagnosis

21. What is true about testing for dengue virus? (Multiple choice question)

1. Absence of anti-dengue IgM antibodies rules out acute infection
2. Anti-dengue IgM antibodies are first detectable in most patients on days 3–5 after illness onset
3. No test exists to diagnose acute dengue virus infection before antibody production
4. Anti-dengue IgG antibodies detected in days 1– 3 after onset can be used to diagnose an acute dengue virus infection

22. How can clinically significant plasma leakage be detected in a suspected dengue patient? (Multiple choice question)

1. Decreasing haematocrit
2. Increasing hematocrit above 20% of baseline
3. Decreasing platelet count
4. Chest X-ray with lateral decubitus view to look for pleural effusion

d. Clinical management

23. A 6-year-old boy patient presents to your office with a few days of fever and a distended, painful abdomen. Her mother states that she has been less active over the past three days. It is currently April and you have seen six patients in the past three weeks with dengue infections. The best course of action in managing this patient is to:

1. Order dengue lab tests and ask the patient to return to your office in 24 hours
2. Order dengue lab tests and admit the patient to the hospital for 24 hours of observation

3. Order dengue lab tests and admit the patient to the Intensive Care Unit for close monitoring and access to emergency care
24. Select an appropriate treatment you could use to treat dengue fever (Multiple choice question)
- | | | |
|-------------------|------------------------|----------------------|
| 1. Aspirin | 2. Opioids | 3. Paracetamol |
| 4. Oral hydration | 5. Antiviral treatment | 6. Blood transfusion |
25. Which of the following are the correct criteria for sending a suspected dengue patient home? (Multiple choice question)
1. Passing urine at least once every 6 hours
 2. Has abdominal pain but no persistent vomiting
 3. Does not have coexisting conditions such as diabetes
 4. Has a pulse pressure less than 20
 5. Does not have any warning signs
 6. No hemoconcentration
26. When and under what circumstances should you tell a patient with suspected dengue to return to the clinic or present to local emergency department? (Multiple choice question)
1. Persistent vomiting
 2. Drowsiness or lethargy
 3. Hematemesis
 4. High fever ($> 38^{\circ}\text{C}$)
27. When should intravenous (IV) crystalloids (i.e., Ringer's lactate, normal saline) be given to suspected dengue patients? (Multiple choice question)
1. All cases regardless of clinical evidence of shock or hemoconcentration
 2. Hypotension, as initial fluid replacement therapy
 3. Significant clinical bleeding, as initial fluid replacement therapy
 4. Low hematocrit and persistent shock after initial IV fluids given
 5. High hematocrit, as initial fluid replacement therapy
 6. Tachycardia, delayed capillary refill, or low urine output as initial IV fluid therapy
28. When should blood transfusions (whole blood or packed red blood cells) be given for a patient suspected of having dengue? (Multiple choice question)

1. Hypotension as a presenting symptom in a suspected dengue patient
2. Low platelet count without bleeding, shock, or hypotension
3. Significant clinical bleeding
4. Low hematocrit and persistent shock after a trial of IV crystalloids and/or colloids
5. Blood transfusions should never be given

e. Surveillance and prevention of dengue

29. Which of the following statements is correct regarding the reporting of dengue in Bhutan?
(Multiple choice question)

1. Dengue is not a notifiable disease
2. Dengue reporting should occur with laboratory confirmed cases only
3. All suspected and confirmed cases should be reported within 1 week
4. Severe dengue should be reported immediately after it is detected in the healthcenter

30. Which variables are required to report dengue in the surveillance system (NEWARS)?
(Multiple choice question)

1. Age
2. Sex
3. Type of case (whether case or death)
4. Signs and symptoms
5. Travel history

f. Prevention messages

31. Dengue vaccine is recommended for those aged 9 years and above in an area with

- | | |
|---------------------------|---------------------------|
| 1. $\leq 49\%$ prevalence | 2. 50-59% prevalence |
| 3. 60-69 %prevalence | 4. $\geq 70\%$ prevalence |

32. What prevention messages should be given to patients to avoid dengue virus infection?
(Multiple choice question)

1. Travelers to endemic areas should be offered vaccination
2. Use mosquito repellent and wear long sleeves and pants for protection
3. Avoid mosquitos during the peak feeding time at night
4. If someone in the household is sick with dengue, take precautions so that a mosquito does not bite the infected person and then bite others in the household.

THANK YOU FOR THE PARTICIPATION!

Appendix 3

Conference presentations

[3.1] Tsheten T, Gray DJ, Clements ACA, Wangdi K. **Epidemiology and challenges of dengue surveillance in the WHO South-East Asia Region.** *National Centre for Epidemiology and Population Health (NCEPH) student conference, College of Health and Medicine, Australian National University, Canberra, Australia, September 11, 2019.*

[3.2] Tsheten T, Clements ACA, Gray DJ, Wangchuk S, Wangdi K. **Spatial and temporal patterns of dengue incidence in Bhutan: a Bayesian analysis.** *National Centre for Epidemiology and Population Health (NCEPH) student conference, College of Health and Medicine, Australian National University, Canberra, Australia, October 12, 2021.*

[3.3] Tsheten T, Penjor K, Tshering C, Clements ACA, Gray DJ, Wangdi K. **Identifying socio-ecological drivers of common cold in Bhutan: a national surveillance data analysis.** *5th Annual Southern Cross Travel Medicine Conference and the Inaugural Southern Cross Tropical Medicine Conference, University of Queensland, Brisbane, Australia, September 16-18, 2022.*

[3.4] Tsheten T, Clements ACA, Gray DJ, Wangchuk S, Wangdi K. **Spatial and temporal patterns of dengue incidence in Bhutan: a Bayesian analysis.** *20th International Congress for Tropical Medicine and Malaria (ICTMM2020), Bangkok, Thailand, October 24-28, 2022.*