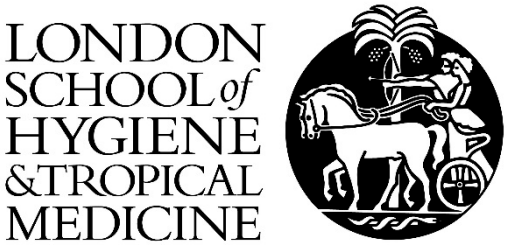


Undocumented Burden of Dengue in Africa

Jacqueline Kyungah Lim

Thesis submitted in accordance with the requirements for the degree of

Doctor of Philosophy
of the
University of London

October 2019

Department of Infectious Disease Epidemiology

Faculty of Epidemiology and Population Health

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

The thesis was based on studies supported by a grant to Dengue Vaccine Initiative awarded by the Bill and Melinda Gates Foundation (OPP 1053432)

Declaration by candidate

I, Jacqueline Kyungah Lim, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Date: October 24, 2019

Abstract

In Africa, information on dengue burden in Africa is limited. Dengue diagnostics is also a key challenge in defining the true burden. Among the various diagnostic options, rapid diagnostic test (RDT) is a convenient and prompt tool for dengue diagnosis, especially in resource-limited environments. To assess current knowledge on the use of RDTs for dengue with respect to their economic impact, a systematic review was conducted of published data. Overall, data were limited to demonstrate an economic impact of dengue RDTs and the available two studies reached different conclusions: one concluded that one particular RDT would be a cost-effective tool in endemic setting, and the other, based on a modeling, showed that a dengue RDT would not be advantageous in terms of cost and effectiveness compared to current practice of antibiotics prescription for undifferentiated fever.

This thesis presents patterns of dengue epidemiology and outbreak based on passive fever surveillance studies in Mombasa, Kenya, and Ouagadougou, Burkina Faso. To estimate the proportion and understand clinical patterns of dengue-positive cases among non-malarial febrile patients, we conducted passive health facility-based fever surveillance studies in Ouagadougou, Burkina Faso and Mombasa, Kenya. In Mombasa, of 482 non-malarial febrile patients, 223 (46%) were identified as dengue–confirmed and 92 (19%) as dengue-probable. The surveillance covered the beginning of a dengue outbreak in April-May 2017, during which 67% of enrolled patients were dengue-confirmed. In Ouagadougou, of 2929 non-malarial febrile patients, 540 (18%) were identified as dengue–confirmed and 571 (19%) as dengue-probable. During the study period, a dengue outbreak occurred in September-November 2016, during which 46% of enrolled patients were dengue-confirmed.

To understand DENV transmission in the community, 4 repeated serosurveys were conducted among the same individuals at 6 month intervals in Ouagadougou. Seroprevalence at enrollment was 66%. The binomial regression based on IgG positivity by age, assuming constant force of infection (FoI) over calendar time, resulted in the FOI of 6% per year.

In summary, in both Burkina Faso and Kenya, there is considerable transmission of DENV, in terms of proportion of DENV confirmed infections among

non-malarial febrile patients in the healthcare facilities as well as seroprevalence and FoI in the community. These burden estimates can facilitate evidence-based decision making on interventions for dengue prevention and control, including a dengue vaccine. However, given the currently available information on dengue burden in Africa and the status of dengue vaccine development, including the only licensed vaccine with restrictions in public health use, consideration of dengue vaccine introduction may be premature for Africa and more data would be necessary to build evidence base on dengue in African settings.

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Appendices

Table of abbreviations

AFI	Acute febrile illness
AGIR	Action-Gouvernance-Integration-Renforcement
BMGF	Bill & Melinda Gates Foundation
°C	Celsius degrees
CI	Confidence Interval
COI	Cost of illness
CPGH	Coast Province General Hospital
CRCHUM	Centre du Recherche du Centre Hospitalier de l'Université de Montréal
CHUYO	*Centre Hospitalier Universtaire Yalgado Ouédraogo*
CRF	Case Report Form
CFR	Case Fatality Rate
CSPS	Centre de Santé et de Promotion Sociale (Health and Social Promotion Center)
DALY	Disability-adjusted life year
DENV	Dengue virus
DCF	Data Collection Form
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DMU	Data Management Unit
DSS	Dengue shock syndrome
DVI	Dengue Vaccine Initiative
ELISA	Enzyme-linked immunosorbent assay
FoI	Force of infection
FRNT	Focus Reduction Neutralization Test
GAVI	Global Alliance for Vaccines and Immunization
GCP	Good Clinical Practice
GIS	Geographic Information System
GDH	Ganjoni District Hospital
IRSS	Institute de Recherche en Sciences de la Santé de Burkina Faso
ICF	Informed Consent Form

ICH-GCP	International Conference on Harmonization-Good Clinical Practice
IgM/IgG	Immunoglobulin type M and type G
IPD	Patients admitted in the health institution (Patients hospitalized)
IRB	Institutional Review Board
IRD	Institute de Recherche pour le Développement
IVI	International Vaccine Institute
KEMRI	Kenya Medical Research Institute
LRTI	Lower Respiratory Tract Illness;
MAC-ELISA	IgM Antibody Capture Enzyme-Linked Immunosorbent Assay
MoH	Ministry of Health
NPV	Negative Predictive Value
OPD	Patients treated in the clinic (External consultation)
PI	Principal Investigator
PPV	Positive Predictive Value
PRNT	Plaque Reduction Neutralization Test
RDT	Rapid Diagnostic Test
RT-PCR	Reverse Transcriptase -Polymerase Chain Reaction
SD	Standard Diagnostics
SOP	Standard Operating Procedure
TDH	Tudor District Hospital
UI	Uncertainty Interval
URI	Upper Respiratory Illness
WHO	World Health Organization
WHO SAGE	WHO Scientific Advisory Group of Experts
YF	Yellow fever

Acknowledgments

First, I would like to thank my PhD supervisors, Prof. Neal Alexander and Tansy Edwards for their guidance, support, and encouragement throughout my PhD. My supervisors were there throughout the 5 years of my study and, without them, I would never have reached the finish line. I also would like to thank Prof. Peter Smith and Dr. Gian Luca di Tanna for their advice and support during my PhD.

I would like to thank previous directors of Dengue Vaccine Initiative (DVI), Dr. Luiz da Silva, Dr. Georges Thiry, and Dr. In-Kyu Yoon for encouraging me to start my PhD and for their continued support throughout the study. I would like to thank the Bill and Melinda Gates Foundation for funding the DVI field studies, based on which I was able to conduct my research study toward the PhD.

I would like to thank IVI and the DVI team in IVI (Jung-Seok, Kang Sung, Ian, Sl-Ki, Soo, Eunju, and Kyung Jin) for their patience and support during the field projects as well as my time away to fulfill my PhD study requirements. Also, there are many people to thank for the field work used toward my PhD. I would like thank Sammy M. Njenga, Sultani Hadley Matendechero, Esther Andia, Noah Oyembo, Henry Kanyi, and other team members in Mombasa for the study in Kenya. I would like to thank Yaro Seydou, Mabel Carabali, Ahmed Barro, Desire Dahourou, Teguewende Nikiema, Therese Kagone, Losseni Kaba, and Valéry Ridde as well as the other team members in Ouagadougou for the study in Burkina Faso. I have learnt so much while working with them on these field studies. Without these collaborators, it would not have been possible to conduct the research studies and see them to completion.

Finally, I would like to express my gratitude to my family: my mother (Ms. Dong-Ryun Jang), my father (Mr. Woong-So Lim), my sister Ranah, my brother Jung Min, my sister-in-law Sara, my mother- and father-in-law (Ms. Jong-Ok Anh and Mr. Dong-Jun Lee), and most importantly, my husband, Dr. Jung-Seok Lee. Without their patience, encouragement, and support, I could not have completed this 5–year PhD journey. I must confess that it was not easy. At times, it was definitely turbulent, but looking back, I did enjoy every step of the way.

Table of Contents

Chapter 1. Introduction

1 Introduction

Dengue fever is a mosquito-borne flavivirus infection caused by four related but antigenically distinct dengue viruses (DENVs, serotypes 1–4), and is a major and rapidly increasing global public health problem (1). The clinical illness ranges from self-limited mild febrile illness to classic dengue fever (DF) to the more severe form of illness, dengue hemorrhagic fever (DHF). Recent studies have estimated an annual incidence of 50–100 million symptomatic infections globally, with 50,000 DHF cases requiring hospitalization and approximately 20,000 deaths (2-6). The case fatality is known to be about 2.5%, but reported to be as high as 10% (7, 8). Dengue is now endemic in more than 120 countries with over half the world's population at risk of infection (9). Dengue disproportionately affects countries in the tropics and subtropics, many of which have limited health care resources (10).

Dengue virus is transmitted by *Aedes* mosquitoes. Despite mosquito control efforts, over the last few decades, diseases transmitted by *Aedes* mosquitoes have spread rapidly in tropical and subtropical parts of the world, including in Africa (11, 12). Some of the factors that have driven epidemics of *Aedes*-transmitted viruses are: population growth, climate change, urbanization, globalization and geographic expansion of mosquitoes (13).

The rapid spread of DENV transmission worldwide and its associated morbidities underscore the need for effective control and prevention measures. It is expected that dengue vaccines will significantly reduce burden of dengue, and there are continued efforts to develop ones that are safe, efficacious, and cost effective. There were some recent key developments in the dengue vaccine field, such as: licensure of Dengvaxia® by Sanofi Pasteur in many dengue endemic countries, but its use is limited by the complexities in the performance and safety of Dengvaxia®; progress made by other dengue vaccine manufacturers, including TDV by Takeda and TV003 by Butantan in Phase III clinical trials. With these ongoing dengue preventive and control efforts, in 2013 there was the analysis of GAVI's vaccine investment strategy (VIS), in which dengue, one of the vaccine-preventable diseases under consideration, was not selected after review of the available data. The GAVI VIS acknowledged that the incidence has grown dramatically worldwide without effective treatments and vector control. However, due to unknown vaccine efficacy and the uncertainties of the disease burden, especially in Africa, dengue was not

selected in the vaccine policy. In the follow-up GAVI VIS in 2018, dengue was not considered.

1.1 Global Distribution of Dengue

There have been attempts to assess the global burden of dengue. Dengue transmission is well documented and the risk is known to be high in the Americas and Asia (2). Based on existing records on dengue occurrence globally, global distribution of dengue risk was assessed using modeling and cartographic approaches (2). The estimated number of dengue infections per year was 390 million (95% credible interval: 284–528), with 96 million (67–136) being apparent with any level of disease severity (2). This number of total infections is more than three times higher than the estimates of the World Health Organization (WHO) (2). Among the apparent cases, the majority were from Asia [67 (95% credible interval: 47–94) million infections] and the Americas regions [13 (95% credible interval: 9–18) million infections] (2). The number of apparent infections from Africa was noteworthy [16 (95% credible interval: 11–23) million infections], being similar to that of the Americas, indicating a significantly larger burden than previously documented (2). In terms of inapparent infections, the pattern is similar. Of 294 (95% credible interval: 217–392) million infections, the highest burden is found in Asia [204 (95% credible interval: 151–273) million infections] (2). It is followed by Africa [48 (95% credible interval: 34–65) million infections], which showed to be, again, similar to what was found for the Americas region [41 (95% credible interval: 31–53) million infections] (2).

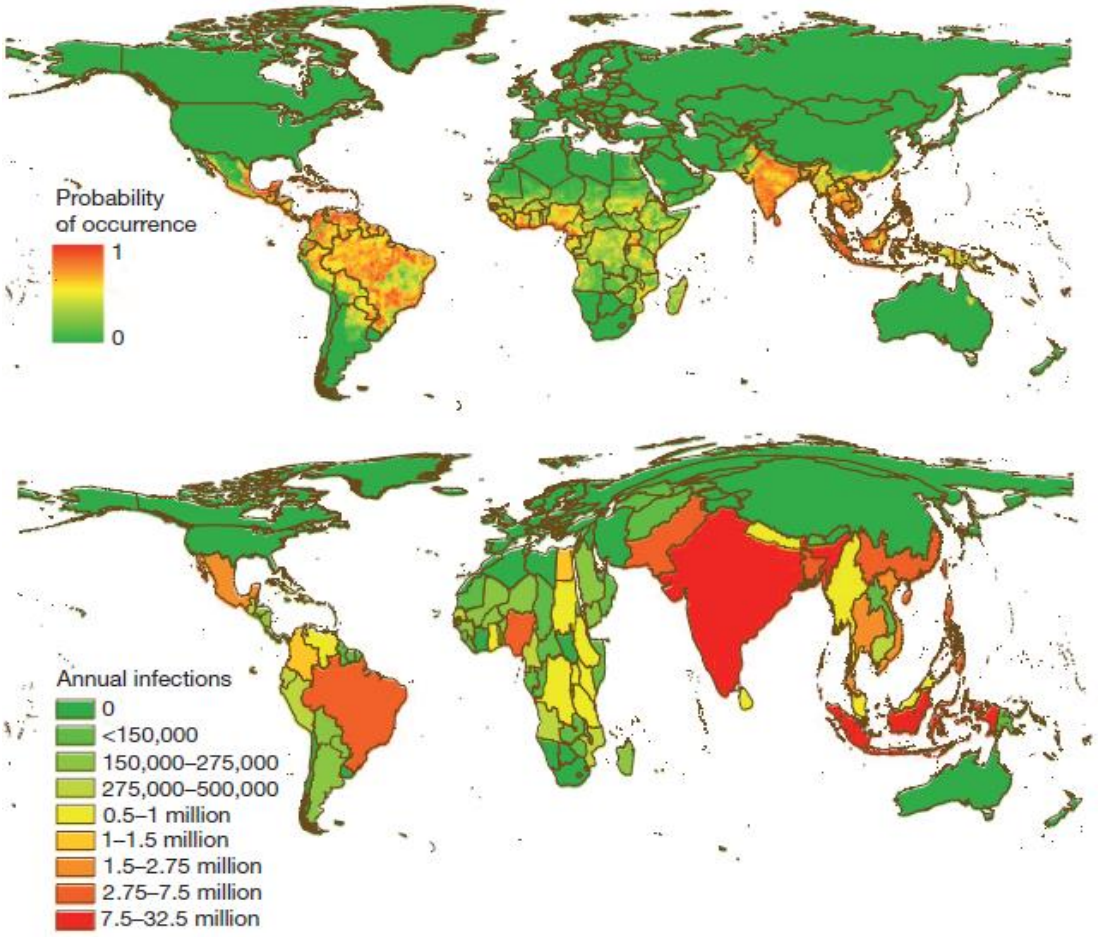


Figure 1. Global map of countries showing predicted risk of dengue and reports of dengue cases.
Source: Bhatt S. Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature. 2013; 496:504–507. (2)
Accessed: 14 May 2019.

1.2 Dengue in Africa – Literature review

In light of the progress and updates made in the field, the gap in terms of reliable data from Africa became more evident, with the dengue burden there being largely unknown (2). It is now timely to have population-based data generated from selected sites in Africa to facilitate better understanding of the disease and its significant impact in these lower-income, possibly GAVI-eligible, countries and evidence-based decision-making for control and preventive interventions.

With an objective to explore what is available in the literature on dengue in Africa, a search of published data was performed using "((Dengue[Title]) AND (Africa[MeSH Terms]))" as a search term in PubMed (www.pubmed.org). In this

search, literature published up to May 2019 was covered. The search generated 224 articles and title screening was performed on all 224 articles. Breakdown of articles based on the main topic based on title screening is provided in Table 1.

Table 1. Articles on dengue in Africa, reviewed for title-screening

Main topic of the articles in title screening (n=224)	No. of articles
Vector, vector interventions	32
Cost of illness	1
Editorial	23
Systematic or literature review	15
Data generation	82
Outbreak investigation	4
Case reports	6
Military, travellers or imported/exported cases	33
Virus strains, phylogenetic analysis	14
Immune response	5
Laboratory assays and development of diagnostics	5
Risk mapping using vector or environmental parameters	2
Study protocol	1
Undetermined language	1

From 224 articles, there were 15 articles based on systematic and literature reviews. Abstract review of these articles was done and they were reviews: on vectors (n=2); dengue in general, such as epidemiology and pathogenesis (n=4); dengue in the context of other co-circulating pathogens, such as yellow fever (n=2) and chikungunya viruses (n=1); and dengue mortality, as editorial (n=1). Five articles covered outcomes of systematic reviews or included findings from literature. One was by Amarasinghe et al. on overview of evidence on presence of dengue in Africa (14), described in greater details in section 1.2.1 below; and another with similar description of dengue in Africa was by Were (15). Another was based on data from Tanzania, reporting the estimated seroprevalance of past dengue infection to be up to 51% in health facility-based survey and 11% in a community-based study (16). The third one was a systematic review with a focus on Middle East and North Africa

region and exploring the potential for outbreaks of multiple *Aedes*-transmitted diseases (17). The fourth was a report from expert conference on Dengue in Africa in 2013 summarizing the existing evidence on literature and key action points to advance knowledge of the epidemiology of dengue in Africa (18). This is further described in detail in section 1.3. These review papers reached a common conclusion of insufficient data supporting *Aedes* or DENV presence in countries in Africa and highlighted the need for better DENV surveillance for control measures.

Also, abstract review was done on 86 articles, which reported on outbreak (n=4) and data generation (n=82). All 86 articles were selected for abstract review. Abstract review resulted in exclusion of 20 articles that reported on virus and phylogenetic analysis (n=9); review (n=2); case report (n=1); travellers and exported cases (n=2); immune response (n=1); and outbreak alerts only (n=2). In addition, abstract review also excluded 3 articles with study results without denominator (population) and/or cases confirmed by lab results. If the studies were conducted on special groups (i.e. measles positive patients, blood donors, and pregnant women, etc.), these were not excluded.

There were 66 articles, which reported study results with dengue case confirmation using laboratory tests. Breakdown of articles based on the country of data reporting and year of publication is provided in Table 2.

Table 2. Articles on results of studies conducted in Africa, categorized by country and year of publication

Region	**Country**	**No. of articles**	**Year of publication [no. published in the year, if more than 1]**
East Africa (n= 36)	Sudan	12	2018(19), 2015 [3](20-22), 2014 [2](23, 24), 2012[2](25, 26), 2011(27), 2010(28), 2006(29), 1986(30)
	Kenya	9	2018(31)*, 2017[3](32, 33), 2016 [2](34, 35), 2015[2](36, 37)*, 2011(38), 1982 (39)
	Tanzania	8	2018(40), 2016[3](41-43)*, 2014 [2](44, 45), 2012[2](46, 47)
	Ethiopia	2	2018 [2](48, 49)
	Republic of	2	2016 (50)*, 1996 (51)*

	Djibouti		
	Reunion Is.	1	2011 (52)
	Somalia	1	1989 (53)
	Zambia	1	2014(54)
West Africa (n= 14)	Burkina Faso	4	2018(55)*, 2017(56), 2016(57), 1985(58)*
	Nigeria	3	2017(59), 2016(60) , 1977 (61)
	Senegal	2	2014(62)*, 1986 (63)
	Sierra Leone	2	2017(64), 2016 (65)
	Côte d'Ivoire,	1	2015(66)
	Ghana	1	2015(67)
	Mali	1	2011(68)
Central Africa (n= 9)	Gabon	5	2016(69), 2013(70), 2012(71), 2011(72), 2009 (73)*
	Cameroon	3	2018[2](74, 75), 2014(76)
	Democratic Republic of the Congo	1	2018(77)
Southern Africa (n= 7)	Mozambique	4	2018[2](78, 79)*, 2017(80) , 2016(81)*
	Angola	2	2015(82)*, 2013(83)*
	South Africa	1	1987(84)

*based on outbreak investigation or cases identified during a dengue outbreak

These 66 articles all contained some defined population with laboratory-based detection of dengue cases. In the abstract review, it was found that 14 articles were based on cases identified in the outbreak or routine outbreak investigation.

Of these 66 articles, 57 were published in the recent decade, in 2009 or after. More than half of the articles were published based on studies conducted in East Africa, in particularly there were 29 publications that were based on studies conducted in Sudan, Kenya and Tanzania. In West Africa, the majority of the articles were published based on studies conducted in Burkina Faso and Nigeria, but most of the articles were published after 2016. In Central Africa, the majority of the articles

were published based on studies conducted in Gabon and all of the studies were published after 2009. More detailed reviews by country will be provided below.

The review showed scarcity of data on dengue in Africa. They were mostly focused in some selected countries, such as Sudan, Kenya, Tanzania, etc. Also, with more heightened awareness and repeated outbreaks, it was in the more recent years that these studies became available as published articles. In terms of the currently available information based on this literature review, there was a consensus, in the literature, a considerable occurrence of dengue infections to occur in Africa, but with limited data to support it, especially due to the lack of reports with robust diagnostic confirmation. Available evidence underscores the need for improved surveillance and accurate assessment of epidemiology of dengue in Africa to document the largely hidden burden of dengue in Africa.

1.2.1 Dengue and *Aedes* mosquito presence

Two mosquito species, *Aedes aegypti* and *Aedes albopictus* are the vectors for some of the common arboviruses, including DENV. Both are widely distributed in the African continent (85-87). Presence of *Aedes* has been documented as early as 1823 in Africa (86, 88). Transmission of different *Aedes*-transmitted diseases continues between known epidemics. However, except for well-known and historically important pathogens like the yellow fever virus, reports of cases of the other *Aedes*-transmitted viral diseases are limited to a few countries in Africa. It is mainly based on a few sporadic outbreaks and individual case reports, often among travelers (14, 89, 90).

Previously, dengue was not recognized as an important etiology of non-malarial febrile episodes in Africa, but this is being revised in the light of recent repeated outbreaks (91-93). However, most of the data are from studies are often not representative or population-based, being limited by their retrospective design using existing samples or outbreak investigations (14, 92, 94).

The first isolation of dengue virus in Africa was in Nigeria in the 1960's (86, 95). In 2011, Amarasinghe et al. conducted a review of published literature, country reports, and WHO library database (14) covering 1960–2010. With this, two review papers described dengue cases to have been reported in 34 African countries with some documentation of reported dengue cases and *Aedes aegypti* presence (14, 15)

(Table 3). Of the 34 countries, 22 have reported local disease transmission with the majority of (n=20) cases reported with laboratory-confirmation and the remaining two based on only clinically-confirmed cases (14). The remaining 12 were included based not on endemicity, but on cases among travelers returning from Africa (14). Also, there have been 20 outbreaks in 15 countries, mostly in East Africa, reported between 1960 and 2010 (14). Some of the countries with frequent reports of epidemics were Zanzibar (1823, 1870), Burkina Faso (1925), Egypt (1887, 1927), South Africa (1926–1927), and Senegal (1927–1928) (14). However, the authors pointed out that not all have been lab-confirmed. In addition to those 34, there were 13 countries with evidence on only the presence of *Aedes* mosquitoes, and 5 with no evidence of dengue cases or *Aedes*.

Table 3. Countries in Africa showing level of transmission of DENV* (14)

Level of transmission	**Countries**
Locally acquired (n=7)	Cape Verde, Egypt, Eritrea, Mauritius, Réunion Seychelles, Sudan
Locally and travel acquired (n=15)	Angola, Burkina Faso, Cameroon, Comoros, Republic of Djibouti, Côte d'Ivoire, Ghana, Kenya, Madagascar, Mozambique, Nigeria, Senegal, Somalia, South Africa, Zanzibar
Travel/expatriate acquired (n=12)	Benin, The Democratic Republic of Congo, Ethiopia, Equatorial Guinea, Gabon, Mali, Namibia, Rwanda, Tanzania, Togo, Uganda, Zambia
Dengue has not been reported but that have *Ae. aegypti* mosquitoes (n=13)	Mauritania, The Gambia, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Niger, Chad, Central African Republic, Republic of the Congo, Malawi, Zimbabwe, and Botswana
Data not available for dengue and *Aedes* mosquitoes (n=5)	Western Sahara, Morocco, Algeria, Tunisia, and Libya

* table modified by author based on data in the specified source

1.2.2 Available data on dengue in Africa

There have been some reports of cases and epidemics of dengue in Africa. However, data on incidence and seroprevalence are rare and limited to some countries. A study in Nigeria estimated the prevalence of flavivirus infections among 1,816 children and adults in urban and rural areas during the early 1970s using virus-specific hemagglutination inhibition and neutralization testing and the prevalence was 38% for DENV-1 infection and 45% for DENV-2 infection (61). Also, other previous studies in Nigeria reported dengue IgM antibody prevalence of 30% in febrile children in Ilorin, 17.2% in Ogbomoso, and 23.4% in 2014 in Ibadan (60, 96, 97). By NS1 antigen, 35% prevalence was found in Ibadan (96).

In Burkina Faso, 683 samples from pregnant women and blood donors were tested using IgG ELISA, and the authors estimated the prevalence to be 26.3% in rural settings and 36.5% in urban settings (98). While these estimates are much lower when compared against those from Asia and the Americas, it should be noted that there is a small number of studies and often the studies were not population-based, with limited generalizability.

In terms of serotypes, all four have been isolated in Africa, with DENV2 being the most prevalent serotype in epidemics (14). Table 4 lists the countries in Africa by serotype and the year of reported epidemic (14). Burkina Faso reported all four serotypes of DENV at different time points, Senegal 3 serotypes, and several other countries reported 2.

Table 4 Dengue epidemics in Africa by the circulating serotype*(14)

Serotype	Countries	Year
DENV1	Burkina Faso, Comoros, Ivory Coast, Kenya, Madagascar, Nigeria, South Africa, Sudan.	1927, 1964, 1968, 1979, 1984, 1984, 1992, 1993, 1998, 2006, 2007, 2013
DENV2	Burkina Faso, Comoros, Republic of Djibouti, Gabon, Ghana, Ivory Coast, Kenya, Mali, Reunion (France), Senegal, Seychelles, Somalia, Sudan.	1977, 1978, 1979, 1982, 1983, 1984, 1985, 1986, 1987, 1990, 1991, 1992, 1993, 1999, 2002, 2005, 2008
DENV3	Burkina Faso, Cameroon, Cape Verde, Ivory Coast, Mozambique, Senegal.	1984, 1985, 1992, 1993, 2006, 2007, 2008, 2009, 2013
DENV4	Burkina Faso, Senegal.	1980, 1985, 2013

* generated by author using data from the specified source

1.2.3 Modelling of dengue transmission in Africa

In 2014, Messina et al. generated a series of global maps to show the extent of global spread of lab-confirmed dengue cases from 1943 to 2013 for each DENV serotype, and the expansion of dengue hyperendemicity (86). The authors used data sources to identify “occurrence” defined as the sub-national distribution of reported confirmed human infections with each DENV type (86). The authors identified 1000 to 1956 occurrence points of geographical location of the cases, variable by serotype, excluding those with lack of evidence on testing (86). These maps showing the reporting history of each DENV type and the cumulative number of DENV types for the periods 1943–1959, 1960–1969, 1970–1979, 1980–1989, 1990–1999, and 2000–2013, suggested a rapid growth in the reported DENV types (86). While this is more evident for the Asia-Pacific, the Americas, and the Indian subcontinent regions, more reported cases are becoming available recently for the Africa region, showing countries in West and East Africa with three or more DENV types reported (86). With such geographical expansion in the presence of one or more DENV types, it should be also noted that there is also potential increase in co-circulation of all four viruses, indicating possible hyperendemicity of DENV transmission (86).

As part of the Global Burden of Disease study in 2013, Stanaway et al. modelled mortality using the Cause of Death Ensemble Modelling tool and incidence from officially reported cases, with adjustment for under-reporting using published estimates of expansion factors (99). Using 1780 country-years of mortality data from 130 countries, 1636 country-years of dengue case reports from 76 countries, and expansion factor estimates for 14 countries, various estimates indicating the burden due to dengue were generated (99). Also, using expansion factors estimated for 14 countries to account for under-reporting, the authors reported exponentially increasing number of apparent cases globally, from 8.3 million in 1990 to 58.4 million in 2013 (99). The authors reported incidence using mixed-effects negative binomial models, and found the highest age-standardized incidence rates in southeast Asia, with an annual average of 34·3 [95% uncertainty interval (UI): 12·7–75·0] cases per 1000 people in the region (99). It was 18.2 (95% UI: 7.7-37.4) for Caribbean and 9.7 (95% UI: 4.2-19.6) cases per 1000 people for Tropical Latin America regions in 2013 (99). What is noteworthy was that it was 10.7 (95% UI: 4.6-21.6) cases per 1000 people for Western sub-Saharan, 5.0 (95% UI: 1.9-11.0) for Central sub-Saharan, and 3.0 (95% UI: 1.0-7.2) for Eastern sub-Saharan Africa.

In addition, the authors modeled dengue mortality using the Global Burden of Disease Cause of Death database, which contains data for 240 causes of death, and reported that, again, the highest age-standardized mortality rates were found in southeast Asia, with an annual average of 8.5 (95% UI: 3.9–10.7) cases per million people in the region (99). Similar to patterns found for incidence, mortality rate for Tropical Latin America regions at 1.4 (95% UI: 0.2-1.7) and this was comparable to Western sub-Saharan at 0.8 (95% UI: 0.6-1.1) and Central sub-Saharan regions at 0.6 (95% UI: 0.4-0.9) (99). Number of deaths also increased from 1990 to 2013, from 8657 to 9110, but the difference was not as dramatic as number of cases of apparent dengue. Overall, this study documented increased transmission of DENV in 2013, in all regions, compared to 1990, and these estimates by region, again, show that DENV transmission in Africa is at a similar level, in terms of equal numbers of infections (both apparent and inapparent) as in Latin America (2), where hyperendemicity of DENV is well-documented (99).

1.3 Challenges leading to under-recognition of dengue in Africa

The presence of *Aedes* mosquitoes and human DENV infection have been confirmed in Africa (14, 86). Also, there are modelling estimates predicting the intensity of transmission and level of disease burden. However, there is still limited data generated on dengue in Africa, on magnitude of DENV transmission, extent of its spread, serotype-specific information, with lack of adequate surveillance systems and research efforts (14, 86).

There are several challenges that resulted in such data scarcity. African countries commonly have many competing public health problems. The frequently non-specific clinical presentation of dengue makes it difficult to distinguish from other causes of febrile illness (2, 15). Also, possible issues of under-reporting and treatment-seeking behavior different from other regions may also contribute to the challenges faced in Africa (2).

This is further complicated by complexities of diagnostics with cross-reactivity across flaviviruses and not widely available diagnostic assays and laboratory capacity (18). Common tests for dengue diagnosis include serologic, virus isolation, molecular, and virus antigen detection as well as combination of these methods, variable in terms of test accuracy (i.e. sensitivity and/or specificity), extent to which technical expertise and infrastructure are required, etc. (100-102). There are rapid diagnostic tests (RDTs), with advantages of quick turnaround time and user-friendliness, but the sensitivity and specificity may be compromised. Nonetheless, in Africa where laboratory diagnostic resources are limited, tests that are require technical resources, including equipment and set-up, and costly would not be adoptable and RDTs can be useful tools for dengue diagnosis (103).

1.3.1 Laboratory diagnosis of dengue

Limited diagnostic capacity for dengue contributes to the problem of a largely unknown dengue burden in Africa. In addition to the problem of various causes of acute febrile illness (AFI) with similar symptoms limiting accurate assessment of dengue burden, there are challenges due to the extent of availability of tests and inherent reliability of existing tests. Dengue diagnostics is complex with multiple considerations interplaying. The main concern is cross-reactivity across flaviviruses and that there are often multiple co-circulating flaviviruses in areas of dengue

endemicity (100, 101). Results of dengue serologic tests are hampered by cross-reactivity as these serologic tests detect antibodies of other flaviviruses, such as Japanese encephalitis, St. Louis encephalitis, West Nile, yellow fever, and Zika (100, 101). However, often dengue-endemic areas also have other flaviviruses circulating. In order to accurately determine the cause of infection, it may be necessary to perform additional analyses, but there is no known test that can perfectly distinguish specific flaviviruses (100).

There are various testing options for dengue diagnosis: serology, molecular methods, and virus antigen detection as well as combination of these methods (100-102). Among serologic tests, there is IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (MAC-ELISA), which captures dengue virus-specific IgM antibodies, and IgM levels remain positive for 3-5 days after symptom onset and may remain up to 12 weeks following infection (101, 104). Often, a second sample should be obtained after day 7 of symptoms for interpretation of results to distinguish between recent, presumptive infection from current, infection (104). As with IgM, there is IgG ELISA test, which captures dengue virus-specific IgG antibodies. The difference is that IgG remains detectable after several months, and possibly life-long (101). Especially due to secondary dengue infection (previous infection with another serotype of a dengue virus, or infection or vaccination against another flavivirus), antibody titres may remain detectable and react against many flaviviruses (101). Then, even in the acute phase, IgG level can be detected and, with this feature of IgG, IgG and IgM levels, calculated as IgM/IgG antibody ratios, can distinguish primary and secondary dengue infections (101). Benefit of IgM and IgG ELISA is that these are available as laboratory-developed tests and commercial diagnostic kits (104).

Due to cross-reaction with other flaviviruses, serologic test results may require further analyses by plaque reduction neutralization testing (PRNT) for confirmation (104). PRNT is a more precise test that can determine cause of infection by detecting specific neutralizing antibodies (104). Hence, it can distinguish specific flaviviruses and DENV serotypes. However, it is known to be labor intensive and expensive, thus not widely available (104). And, a single PRNT is limited to determine the timing of infection (104).

Molecular methods detect dengue virus RNA in the first 1-7 days in the course of illness (105). Commonly used is RT-PCR assay. This can distinguish the four

dengue virus serotypes and is available, in forms of multiplex or trioplex (dengue with chikungunya and Zika viruses) (105). A positive result from PCR indicates confirmed dengue infection, but a negative PCR result does not mean non-dengue (105). As a method to detect DENV antigen, there are NS1 tests that detect the non-structural protein NS1 of dengue virus commonly in serum (106). NS1 can be detected in the acute phase of illness up to 7 days since onset of symptoms (106). A positive NS1 test is indicative of a dengue infection but it does not provide serotype information (106). Also, a negative NS1 test result does not mean non-dengue (106). Conveniently, dengue NS1 tests are available as commercial diagnostic kits.

Among this wide range of diagnostic options, different tests are used at different times, depending on the illness progression (time of sample collection), purpose of diagnosis (point-of-care vs. surveillance), and availability of resources (laboratory facilities and expertise available, i.e. at national and regional reference laboratory-levels vs. lower-level clinical setting) (101). There are more reliable methods, such as molecular (PCR) assays, RNA detection, and PRNTs, but they are more labour-intensive and costly, requiring some infrastructure including technical expertise (101). In this context, RDTs can be useful tools for point-of-care diagnosis, especially in resource-limited settings (103).

Given limited diagnostic capacity and laboratory facilities in the region, one way to address this challenge might be using dengue RDTs. In the expert conference held in Accra, Ghana, in February 2013 on Dengue in Africa, key questions regarding the expansion of dengue in Africa were addressed (18). Consistent with the points addressed in the introduction, key areas were identified to be in need for further advancement of our understanding of the epidemiology of dengue in Africa. In light of the need for representative data to be collected across Africa to understand the true burden of dengue, better collaboration among established networks was encouraged along with dengue diagnostic tools to be made more widely available in the healthcare setting in Africa to produce such data (18). It is so that policy recommendations could be developed based on such data generated for necessary actions to provide dengue vector control and health services (18).

1.3.1.1 Rapid diagnostic tests for dengue

According to the ASSURED criteria defined by WHO for evaluation of POC devices for resource-limited settings, diagnostic tests would be ideal if they are: (1) Affordable; (2) Sensitive; (3) Specific; (4) User-friendly (simple to use with minimal training); (5) Rapid (for prompt treatment) and Robust (no refrigerated storage); (6) Equipment-free; (7) Delivered to those who need it (107). There are several rapid tests available with reasonable sensitivity and specificity. While these are not considered the standard reference and their usefulness is not yet proven in clinical settings, they are known to be convenient and prompt option to support clinical diagnosis (108, 109). The conference on dengue in Africa held in Ghana concluded that it is necessary to make such tests available at sentinel sites and health facilities to support accurate diagnosis (18).

There is a number of different commercially available rapid tests, based on the detection of dengue virus non-structural protein 1 (NS1) antigen, IgM, IgG, and IgA antibodies (101, 103). While the RDTs have advantages, such as more rapid turnaround time and user-friendliness in field settings for point-of-care diagnosis, these tests have variable sensitivity and specificity (101). Not all commercial rapid tests are validated by reference laboratories and rapid test results for diagnosis should be interpreted with caution (101). Also, to improve accuracy of RDTs, it has been suggested that RDTs could be used in combination with others, for example the combined test with NS1 antigen and IgM antibody (110, 111). Nonetheless, in resource-limited countries where there might be limited infrastructure and expertise, major benefits of using RDTs would be that they are available as user-friendly point-of-care kits, with no other equipment or training needed. Also, these countries with no surveillance system established to monitor incidence of dengue, use of the dengue RDTs could be helpful for case detection to predict outbreak, and to allow individual dengue cases to be identified in the early phase of illness, hence facilitating better case management of dengue illness and possibly reducing the duration of illness, leading to lower cost-of-illness due to dengue (112, 113).

Given the overall convenience and benefit of using RDTs, a systematic review was performed on published data to explore the economic impact of using RDTs, the cost-aspect of the benefit and impact when using dengue RDTs for dengue case detection (chapter 2). The hypothesis behind this review was that there may be

economic impact due to prompt detection of dengue in the early phase of illness using RDTs and economic impact is defined to be broad: both from the point of view of cost-effectiveness and from the perspective of financial impact of RDT in patients, i.e. early diagnosis possibly leading to cost-saving in patients. Evidence in the current literature on this was explored in more detail in chapter 2 of the thesis.

1.3.1.2 Secondary versus primary infections

Different testing options need to be applied considering the progress of illness. For example, molecular tests, such as PCR, are used within 7 days after onset of symptoms as well as NS1 antigen detection (100). However, serologic tests can be used after 7 days since onset of symptoms (100). Other considerations for the choice of diagnostic method include the set-up of laboratory facilities and technical expertise available, costs, and the time of sample collection.

Test results also depend on whether it is primary versus secondary infection. It is important to differentiate the two in order to apply proper case management, given that secondary dengue infection, a known risk factor for dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), can cause more severe disease than the primary infection (7, 114). In general, once a person is infected with DENV, the virus is found in serum or plasma for about 2-7 days and this coincides with the duration of fever (101, 115). And then, anti-dengue antibodies will appear days later and stay detectable with different patterns of immune response for primary and secondary infections. For primary, dengue as well as flavivirus-naïve, infections, there will be detectable IgM levels for 80% by day 5 of illness and this will stay detectable for 2 weeks after onset of symptoms, but will not stay detectable after 3 months or so (101, 115). In case of primary infections, anti-dengue IgG appears afterwards.

In secondary infection, antibody titres rise rapidly and IgG level can be detected at high levels from the initial phase of illness (115). It has been documented that it can last about 10 months to as long as life time (115). Also, in secondary infections, IgG shows cross reaction across different flaviviruses (115). What is different from primary infection is the lower IgM level in secondary infections. Due to these differences, to distinguish primary and secondary dengue infections, one common way to identify is to use IgM and IgG indices for calculation of IgG/IgM ratio

(114-116). Different cut-off values have been studied and a study reported that the IgG/IgM ratio of ≥ 1.10 had a sensitivity of 100%, specificity of 97.4%, and accuracy of 67.5% in differentiating secondary from primary dengue (114). Also, use of the ratio to discriminate primary from secondary dengue was shown to perform better later in the course of illness (116)

1.3.2 Surveillance systems

Moreover, many dengue endemic countries in Asia and Latin America have mandatory reporting of dengue cases to public health authorities or national surveillance systems to monitor incidence patterns (117). However, coupled with limitation in diagnostic resources, most African countries lack such mechanisms, and only sporadic outbreaks and individual case reports have been documented (117). Burkina Faso is one of the few countries with an established national routine surveillance of potential epidemic diseases, which has included dengue in the notification system since 2016. This is in addition to investigations during outbreak periods, conducted by the Ministry of Health of Burkina Faso, based on few health centers (118). Especially during the outbreak in 2017 in Burkina Faso, a laboratory-based arbovirus sentinel surveillance was implemented during fall 2017, which was built on existing routine surveillance with enhancement of sample testing, improvements in case reporting as well as data management (119).

There may be surveillance studies launched in response to these epidemics, but such activities would not remain in place during the non-epidemic seasons. Also, several disease burden studies assessing the causes of febrile illnesses were conducted in the past years in Africa. Yet, most studies solely identified the causes without comprehensive evaluation of clinical and epidemiologic patterns of infections with lab-confirmation of respective pathogens. Actual results from the field, whether from research study or local surveillance systems, provide better inputs for more accurate modeling outcomes on transmission and dynamics of DENV in the region.

1.4 Gaps in knowledge

A considerable level of dengue transmission may be occurring in Africa and, in selected few countries, there are more data becoming available on this recurring problem. However, with limited diagnostic capacity and surveillance systems, the

lack of data underscores the need for robust evidence based on population-based studies equipped with laboratory confirmation to support the extent of transmission and burden of dengue in the region. Due to the uncertainties of the disease burden in Africa and complexities associated with dengue vaccines (introduction of the only licensed one as well as development of the other candidates), it may be premature to consider vaccine introduction as an intervention in Africa. However, population-based data generated from local studies will serve as important factors to be considered later, once a safe and cost-effective vaccine becomes available, for evidence-based policy decisions for control and prevention strategies, including introduction of vaccines.

1.5 Study background and objectives

Given the gap in the literature and to address the knowledge gap on the magnitude of the dengue problem and generate improved data on dengue epidemiology, passive facility-based fever surveillance studies were conducted among residents of Ouagadougou in Burkina Faso, and Mombasa in Kenya (120), funded by a 4-year grant to Dengue Vaccine Initiative (DVI) from the Bill and Melinda Gates Foundation in 2013. As part of the field operation of DVI, I was in charge of the studies in Burkina Faso and Kenya, the PhD program covers the work for which I led the grant writing to study design, project execution, and closing (more on the role of the candidate in the current project in section # 1.10).

In both sites, partial or full outbreaks were captured during the study period and this allowed our data generated to: 1) assess the epidemiology of DF; and 2) compare dengue-positive cases to non-dengue cases and assess differences in clinical features of dengue during the outbreak and non-outbreak periods. Outpatient dengue accounts for the greatest burden of disease, both epidemiologically and economically; however, there continues to be a lack of data on dengue among non-hospitalized cases (121, 122). The surveillance studies cover both hospitalized and outpatient department. Also, due to difference in the clinical and epidemiological patterns of dengue between adults and children, the age range for the surveillance studies covered 1 to 55 years of age (123, 124).

In addition to the surveillance studies looking at symptomatic dengue fever episodes, to assess baseline population-based seroprevalence and calculate

subsequent rates of dengue infection by IgG seroconversion, repeated serosurveys were conducted among residents of Ouagadougou, Burkina Faso. In the same catchment area as the facility-based surveillance, 4 serosurveys, at intervals of about 6 months, were conducted in 3000 randomly selected residents between 1 and 55 years of age. The last interval covered the 2016 outbreak and sero-conversion rates were compared across demographic and clinical characteristics, and in the interval covering the outbreak versus those with no outbreak reported.

To date, there had not been data generated from Africa, assessing the burden due to dengue fever episodes that sought healthcare and also estimating the ongoing rate of infection, measured by sero-conversions, in the community residents that do not seek care, from the same catchment area population. The data generated will not only provide the most updated estimates of the disease burden currently available but also will be used to make informed policy to facilitate decisions on adoption of various measures for dengue prevention and control.

1.6 Study sites

Study sites were selected, in part, based on their likelihood of supporting DENV transmission. To select sites, we considered dengue outbreaks and case reports in the literature, available seroprevalence studies, as well as country-specific dengue risk maps of the probability of DENV transmission and the level of evidence of dengue presence, reporting the consensus estimates based on modeling of probability of dengue presence in Africa (9, 86).

In addition to referring to limited, but existing, data available from surveillance and research studies, site selection in 2013 was supported by modeling outcomes. One in particular, the published data on dengue incidence as well as vectors, and other supplementary data sources were applied to build country-specific models to assess the level of evidence consensus indicative of the probability of dengue presence and a range of evidence was reported (9, 86, 125). The authors generated country-specific dengue risk maps and burden estimates for all GAVI-eligible countries based on determined consensus on presence or absence of dengue and mapping of the country-specific probability of dengue occurrence (9, 86). According to the authors, Burkina Faso was reported to have evidence consensus of 88% in prediction of dengue presence (9, 86, 125). This high level of consensus of evidence

for dengue presence in Burkina Faso could be supported by the reported outbreak in 2006 in Ouagadougou and 36.5% sero-positivity found in 2004 in the urban part of the country (9, 98). Kenya reported evidence consensus of 83% in prediction of dengue presence (9, 86, 125). This high level of consensus of evidence for dengue presence in Kenya could also be supported by the reported outbreaks reported in 1982 and 2011 with 14% sero-positivity in adults in 2004 (9, 126, 127). The consensus level estimates for Burkina Faso and Kenya are indicative of "complete consensus" (if higher than 79%)(125).

Another factor for consideration in site selection is adequate research infrastructure to implement the studies was taken into account. Finally, inclusion of different regions of Africa was also a factor in site selection. Thus, Ouagadougou, Burkina Faso, and Mombasa, Kenya were selected to measure the burden of dengue in selected sites from West and East Africa.

1.6.1 Dengue in Burkina Faso

PubMed (www.pubmed.org) was used to search for existing evidence on dengue in Burkina Faso. No other database was considered, but outbreak reports on WHO website or local MoH websites were reviewed. The search term combination used was; "(Dengue[MeSH Terms]) AND (Burkina Faso[MeSH Terms])". This search generated 13 articles. All but 1 were published 2006 or later. Abstract review was done on all the articles and basic details of the obtained articles are summarized in Table 5.

Table 5. Articles on dengue in Burkina Faso

Topic (No. of articles)	Authors (ref.)	Year	Aim	Methods
Vector control (1)	Ouedraogo et al.(128)	2018	To evaluate the effectiveness of a community-based intervention for dengue vector control in Ouagadougou.	Effectiveness study on a vector control intervention
Cost of illness (1)	Lee et al.(12)	2019	To capture the entire cost incurred during the period of dengue illness in Burkina Faso, Kenya, and Cambodia and to understand how the economic burden of dengue is distributed between private and non-private payers.	Cost-of-illness survey on dengue RDT-positive patients within a fever surveillance
Review/ editorial (2)	Sanou et al.(119)	2018	To describe the successful implementation of laboratory-based arbovirus sentinel surveillance during a dengue outbreak during fall 2017, as an effort to build capacity to better understand the burden of disease caused by arboviruses in Burkina Faso.	Description of the implementation, surveillance methods, and associated costs of enhanced surveillance as an outbreak response
	Ridde et al.(118)	2014	To describe the need for rapid deployment of research and interventions on dengue fever in Burkina Faso, given the conventional focus being on malaria.	Review on the need for more research and public health interventions for dengue
Data generation (4)	Diallo et al. (56)	2017	To study epidemiology, diagnostic and outcomes of dengue patients hospitalized for fever and painful syndrome with a positive test to the dengue non-structural antigen 1, based on a retrospective study covering a period from January 2013 to December 2014 in a private clinic in Ouagadougou.	A retrospective study using data from patients who sought care at a private clinic
	Fournet et al. (129)	2016	To evaluate flavivirus presence in Ouagadougou and the link between anti-flavivirus antibody seroprevalence and urbanization modes.	A population-based cross-sectional survey conducted and among children
	Ridde et al. (57)	2016	To describe epidemiology and vector ecology based on an exploratory cross-sectional survey of febrile	A prospective cross-sectional

			patients performed from December 2013 to January 2014 at six primary healthcare centers in Ouagadougou, as well as collection of data on potential *Aedes* breeding sites and larvae.	survey based on patients identified at primary healthcare centers
	Collenberg et al. (98)	2006	To assess seroprevalence of six different human pathogenic viruses: human immunodeficiency virus (HIV); hepatitis B virus (HBV); hepatitis C virus (HCV); human T-cell leukemia virus (HTLV); human herpesvirus type 8 (HHV-8);and dengue virus, based on a seroprevalence study among pregnant women and blood donors from rural (Nouna) and urban (Ouagadougou) parts of Burkina Faso.	A seroprevalence study among pregnant women and blood donors
Outbreak investigation (1)	Tarnagda et. al. (55)	2018	To report on 1,327 probable cases of dengue in Burkina Faso in 2016.	Outbreak investigation conducted among suspected dengue cases using a RDT
Case reports, including	Mamoudou et al. (130)	2016	To report three cases of hemorrhagic dengue observed at the Infectious Diseases Department CHU Yalgado Ouédraogo, Ouagadougou, and describe its epidemiological and clinical characteristics.	A case report of 3 DHF patients
travellers' or imported cases (3)	Hashimoto et al. (131)	2017	To present a case of dengue fever imported from Burkina Faso to Japan, with results from phylogenetic analysis.	A case report of an imported DF patient to Japan
	Eldin et al. (132)	2016	To report on two cases of dengue fever in travellers returning from Burkina Faso to France.	A case report of two French travellers
Virus strains (1)	Gonzalez (133)	1985	To present viral identification and isolation of strains virus isolations, describe the observed syndrome and discuss the epidemiological patterns of the outbreak in the rainy season of 1982 in Ouagadougou.	A prospective study of dengue-like patients

Of the 13 articles, 5 presented results from field studies and detailed results of outbreak investigation (55-57, 98, 129). Full text review was done on articles on outbreak investigation and on locally generated data. And the main results extracted from these studies are summarized below.

The first outbreak of dengue in Burkina Faso occurred in 1925 (14). In 1982, DENV2 was reported in humans and mosquitoes after an outbreak in Ouagadougou

(134, 135). There were declared outbreaks in 2013, 2016, and 2017 (118, 136-138). In 2016, between August and November, there were 1061 dengue RDT positive cases identified from all districts of Ouagadougou and 15 deaths reported (55, 137). The outbreak in September 2017 proved to be even larger, with 9029 suspected dengue cases, 5773 dengue RDT-positive cases, and 18 deaths throughout the country (138).

In terms of proportion of acute dengue, Ridde et al. conducted a cross-sectional survey among non-malarial febrile patients at 6 primary healthcare centers from December 2013 to January 2014 in Ouagadougou (57). Of the 379 subjects, 8.7 % (33/379) had positive RDTs for dengue and almost 40% were either probable- or confirmed- dengue (57). In terms of seroprevalence, Collenberg et al. reported, based on a study based on testing 683 samples from pregnant women and blood donors using IgG ELISA, that the estimated prevalence was 26.3% in rural settings and 36.5% in urban settings (98). This was similar to what Fournet et al. reported based on a seroprevalence study (129). The authors reported the prevalence of past flavivirus infections among the enrolled children (n = 685) to be 22.7%, indicating active transmission of flaviviruses without distinguish across flaviviruses (129)

These repeated epidemics and seroprevalence estimates suggest a considerable dengue burden in Burkina Faso. Nonetheless, there are not much data on comprehensive evaluation to understand patterns of dengue epidemiology (57). Also, Burkina Faso, like other countries in Africa, has other competing public health problems, and several of them have similar presenting symptoms, and the availability dengue diagnostic assays is limited (15, 118).

Most African countries lack national surveillance systems to monitor dengue incidence. However, Burkina Faso is one of the few countries with an established national routine surveillance of potential epidemic diseases, which has included dengue in the notification system since 2016. In outbreak periods, the Ministry of Health of Burkina Faso conducts investigations, based on few health centers (118).

1.6.2 Dengue in Kenya

Similarly, PubMed was used to search for existing evidence on dengue in Kenya. The search term combination used was; “(Dengue[MeSH Terms]) AND (Kenya[MeSH Terms])”. The search generated 23 articles. Compared to Burkina Faso, there were more articles on Kenya from 1980’s and there were more articles

based on data generated from local studies. There were 2 letters published in Lancet by Johnson et al. in 1982 (139) and 1990 (140). Excluding these letters, abstract review was done on the remaining 21 articles and basic details of the obtained articles are summarized in Table 6.

Table 6. Articles on dengue in Kenya

Topic (No. of articles)	Authors (ref.)	Year	Aim	Methods
Vector – related (8)	Chepkorir et al.(141)	2018	To reports on the *Aedes* mosquito species occurrence, diversity, and blood feeding patterns, as means of measuring the risk of transmission of YF and DEN viruses in Kacheliba sub-county, West Pokot County	Entomological surveillance
	Nyasembe et al (142)	2018	To assess selectivity in plant feeding using a DNA-based approach targeting trnH-psbA and matK genes and identify host plants of field-collected Afro-tropical mosquito vectors of dengue, Rift Valley fever and malaria	biochemical and molecular analyses of mosquito samples
	Agha et al. (143)	2017	To survey water-holding containers for mosquito immature (larvae/pupae) indoors and outdoors from selected houses during the long rains, short rains and dry seasons (100 houses/season) in each County from October 2014-June 2016, and we compared DEN and YF risk in Kilifi County (DEN-outbreak-prone), and Kisumu and Nairobi Counties (no documented DEN outbreaks)	Household-based entomological survey
	Ngugi et al. (144)	2017	To characterize breeding habitats and establish container productivity profiles of Ae. aegypti for a period of 24 months (June 2014 to May 2016)in rural and urban sites in western and coastal Kenya	Household-based entomological survey
	Lutomiah et al. (145)	2016	To conduct an entomologic investigation and establish the mosquito species, and densities, causing the outbreak	Entomologic surveillance

	Owino et al. (146)	2015	To describe the development and use of synthetic human odor baits for improved sampling of adult Ae. aegypti, in two dengue and chikungunya fevers endemic areas in Kenya; Kilifi and Busia counties	biochemical and molecular analyses of human volunteers and mosquito samples
	Attaway et al. (147)	2014	To identify highest and lowest areas of dengue risk within Kenya using a geospatial analysis, using the bioclimatic variables and elevation and mosquito habitat in environmental susceptibility analysis and geographical information systems	Dengue risk mapping based on bioclimatic and entomological variables
	Midega et al. (148)	2006	to identify the types of domestic container that are most productive for *Aedes aegypti* (L.) pupae	Assessment of pupal/demographic-survey methodology
Cost of illness (1)	Lee et al.(12)	2019	to capture the entire cost incurred during the period of dengue illness in Burkina Faso, Kenya, and Cambodia and to understand how the economic burden of dengue is distributed between private and non-private payers	Cost-of-illness survey
Data generation (8)	Konongoi et al. (149)	2018	To report on entomologic investigations and laboratory confirmed chikungunya cases in northeastern Kenya using the patient blood samples received at the Kenya Medical Research Institute viral hemorrhagic fever laboratory and the immunoglobulin M enzyme linked immunosorbent assay (IgM ELISA) was used to test for the presence of IgM antibodies against chikungunya and dengue	entomologic investigations and laboratory testing of existing patients samples
	Ngoi et al. (34)	2016	To tested plasma samples obtained in a cross-sectional study from febrile adult patients aged 18-35 years seeking at seven health facilities in coastal Kenya in 2014-2015and evaluated for AHI and malaria as well as dengue and chikungunya	A cross-sectional study nested within a facility-based acute HIV

		viruses	infection study conducted among febrile adult
Vu et al. (32)	2017	To study a cluster of dengue virus infections in children in Kenya during July 2014-June 2015	A prospective cohort of children
Vu et al. (33)	2017	To measure neutralizing antibody against DENV and, to evaluate assay specificity, WNV in serum samples that tested positive for serum anti-DENV IgG by enzyme-linked immunosorbent assay	A prospective seroprevalence survey
Kononogoi et al.(35)	2016	To detect the presence of IgM antibodies against dengue, yellow fever, West Nile and Zika using 868 samples from febrile patients were received from hospitals in Nairobi, northern and coastal Kenya from September 2011 to December 2014	Hospital-based fever surveillance
Ochieng et al. (36)	2015	to test for the presence of IgG antibodies to dengue virus (DENV), chikungunya virus (CHIKV) and Rift Valley fever virus (RVFV) using 1,091 HIV-negative blood specimens from the 2007 Kenya AIDS Indicator Survey	Retrospective testing of using HIV-negative blood specimens from a national population-based survey on AIDS
Ellis et al. (37)	2015	To investigate several individuals with dengue-like illnesses and negative malaria blood smears were identified in Mombasa, Kenya in February 2013, and to estimate the magnitude of local transmission including a serologic survey to determine incident dengue virus (DENV) infections	Household-based serosurvey
Blayloc k et al. (38)	2011	To describes the seroincidence and seroprevalence of dengue infection in western Kenya based on testing for antibodies to dengue virus using an IgG indirect ELISA using banked	Dengue seroprevalence survey

			sera obtained from 354 healthy, afebrile children ages 12-47 months from Kisumu District, Kenya	
Virus isolation (1)	Johnson et al. (150)	1982	To describe the first virologically confirmed DENV 2 in Kenya using 7 strains of virus identified in 1982 from outpatients attending hospital on the Northern coast of Kenya	serological study
Outbreak investigation (1)	Obonyo et al (31)	2018	To investigate a suspected dengue outbreak in Mandera town from September to October 2011	Outbreak investigation
Case reports, including Imported cases (1)	Martyn-Simmons et al. (151)	2007	To report a case of widespread skin eruption in a UK women returning from a visit to Kenya	Case report of 1 UK traveler
Diagnostic-related (1)	Wasonga et al. (152)	2015	to develop and evaluate an in-house IgM-capture enzyme linked immunosorbent assay (ELISA) for the detection of chikungunya virus infections and test performance among clinically suspected dengue patient samples from Eastern Kenya, collected in 2013	Validation of an in-house ELISA

Of the 21 articles, 9 presented results from field studies and detailed results of outbreak investigation. Full text review was done on articles on outbreak investigation and on locally generated data. And the main results extracted from these studies are summarized below.

In Kenya, compared to other African countries, there is more evidence available for the presence of dengue, with several documented outbreaks in different locations. The most recent outbreak reported was in Mombasa in May 2017 (153). In 2011, an outbreak was confirmed in Mandera, North Eastern region, and, in 2013, another in Mombasa continuing into 2014 (31, 37). In addition to outbreak investigations, a study based on 868 samples from febrile patients in multiple locations in Kenya from 2011 to 2014 reported 40% (345/868) of the samples to be dengue-positive by either IgM ELISA or RT-PCR (35).

A cohort study among 1258 children 1-17 years of age conducted in 2014-2015 reported that among 1104 samples tested, 7.4% were positive for DENV RNA and all 4 serotypes were found (32). Also, a cross-sectional study among febrile adult patients aged 18-35 years tested for acute HIV infection (AHI) and malaria were used to evaluate presence of dengue and chikungunya virus infections (34). Authors found that 8.8% were positive for DENV infection, indicating a substantial level of DENV infections in coastal Kenya(34).

In terms of seroprevalence, dengue was found to be the most common viral pathogen in retrospectively tested blood specimens from HIV-negative survey samples from the 2007 Kenya AIDS Indicator Survey, with 12.5% having dengue IgG (36). Similarly, a household survey in Mombasa reported that 13% of individuals had serological evidence of either past or current DENV infection, by IgM anti-DENV ELISA (37). When 830 anti-DENV IgG positive samples from children ≤ 10 years of age were tested with neutralization assay, 23% had neutralizing antibody to DENV, indicating DENV transmission in the region in the past decade (33). In 1982, when DENV2 was first isolated, prevalence of DENV2 antibody was measured in Malindi and it was found to be as high as 52% among outpatients (38). However, additional studies found this to have dropped to 1% seroprevalence of DENV 2 antibody among asymptomatic individuals, suggesting that DENV was not endemic (38). Also, a study testing antibodies to dengue virus using an IgG indirect ELISA among 354 afebrile children ages 12-47 months in Kisumu also reported a seroprevalence of 1.1% and sero-incidence of 8.5 seroconversions per 10000 persons per year (38).

While such information suggests notable dengue transmission in Kenya, what has been reported is variable, without much data on comprehensive evaluation on dengue epidemiology (36, 37). Often, published studies were based on retrospective testing of collected samples that are not well representative of the general population. Additionally, Kenya, like other African countries, has many competing public health problems with similar symptoms, and suffer from limited diagnostic capacity (15).

Table 7. Characteristics of the sites chosen for dengue burden studies in Africa

	East	West
	Kenya	Burkina Faso
GDP per capita (2017 Est.)(154)	1,594.8 (current USD)	642 (current USD)
Population(155)	48,466,927	19,173,322
% Urban pop. (156)	25%	29%
% pop. below poverty line (World Bank)	45.9% (2005)	46.7% (2009)
National surveillance system for dengue	No	No*
Outbreak (14)	1982, 2011, 2013	1984, 2013, 2016, 2017
Annual incidence of DENV infection	8.5/1000(38, 157)	-
Reported seroprevalence	1.1-52% (38)	26.3 - 36.5% (98)

*not available at the time of site selection, but system was established during the outbreak in 2016, during the study period

1.7 Aim, objectives and structure

1.7.1 Aim

The principal aim of the thesis is to measure the burden of dengue among non-malarial febrile patients seeking care at selected health facilities in Mombasa, Kenya, and Ouagadougou, Burkina Faso. Also, the thesis aims to assess seroprevalence and age-specific rates of infection measured by seroconversion of dengue IgG among randomly selected residents of Ouagadougou. Such data generated could facilitate evidence-based decision making on dengue prevention and control interventions in the region.

1.7.2 Objectives

In order to measure the burden of dengue fever and determine seroprevalence and rates of infection of DENV, several approaches were pursued. The specific objectives of the thesis include:

1) Description of different dengue diagnostic tests and benefits of using dengue RDTs, based on review of currently available literature, in the context of cost-effectiveness of dengue RDTs
2) Estimation of the proportion of dengue among non-malarial febrile patients in Mombasa, Kenya
    a. Assessment of performance of clinical diagnosis of suspected dengue
    b. Description of epidemiologic characteristics of DF patients vs. non-DF patients during and before the outbreak period
3) Estimation of the proportion of DF among non-malarial febrile patients in Ouagadougou, Burkina Faso
    a. Description of epidemiologic characteristics of DF patients vs. non-DF patients during and outside the 2016 outbreak
4) Estimation of the seroprevalence of dengue and age-specific force of infection, measured by IgG ELISA, in repeated serosurveys conducted in Ouagadougou, Burkina Faso; and measure the effects, in terms of seroconversion rate ratios, of potential demographic and clinical risk factors.

To address the gaps in knowledge on dengue in Africa, the surveillance studies and serosurveys conducted in Burkina Faso and Kenya provide information on epidemiologic patterns and clinical characteristics of dengue-positive cases among non-malarial febrile episodes, and, in Burkina Faso, prevalence as well as age-specific rate of infection of DENV measured by seroconversion. As a major global public health problem, efforts have been made to develop effective tools to prevent and control against dengue, such as vector control and vaccines. In absence of population-based reliable data on dengue in Africa, an accurate, up to date description of the burden of dengue, evaluated through health facility-based fever surveillance studies and community-based serosurveys, will facilitate informed decision-making on implementation of control and preventive measures for dengue.

1.7.3 Structure and contribution of research papers

Continuing on from this introduction (chapter 1) where the background of the study is laid out, there is a brief description of dengue diagnostic options, focusing on dengue RDTs, as rapid tests might be a feasible diagnostic option in resource-limited

settings. In the context of benefits of using dengue RDTs, there is a literature review paper assessing existing evidence on cost aspect of the benefit of using dengue RDTs (chapter 2, paper 1) assessing economic impact of dengue RDTs where tests may lead to avoiding unnecessary treatments if found to be dengue positive on RDT. Then, the overall study design and methods are described in a study protocol paper (chapter 3, paper 2). It is followed by two papers, one examining the characteristics and epidemiology of Dengue in Mombasa (chapter 4, paper 3) and another in Ouagadougou (chapter 5, paper 4), in the health facility settings. These two papers report the proportions of dengue among non-malarial febrile patients in two sites and describe epidemiologic and clinical characteristics of dengue versus non-dengue patients during and before the outbreak period (in early 2017 in Mombasa and late 2016 in Ouagadougou). The fifth paper of the thesis investigates the burden and characteristics of dengue by measuring seroprevalence and force of infection, as well as ratios of rate of dengue IgG seroconversions, in Ouagadougou in greater detail (chapter 6, paper 5). Discussion and conclusions are found in paper 6 (chapter 7).

1.7.3.1 Paper 1 (Chapter 2)

Paper 1 in chapter 2, entitled “A systematic review of the economic impact of rapid diagnostic tests for dengue”, was published in *BMC Health Services Research* in 2017 (158). With description of different testing options for dengue diagnosis and RDTs as a useful diagnostic option in resource-limited environments as in Africa, benefits of use of RDTs are described by this work based on the systematic review of published data on the use of RDTs for dengue with respect to their economic impact.

1.7.3.2 Paper 2 (Chapter 3)

Chapter 3 is a protocol paper (Paper 2) which was published as “Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative” in *BMJ Open* in 2018 (120). This paper described the design and methods of the studies in Africa, composed of the passive fever surveillance and serosurveys in 3 sites of the Dengue Vaccine Initiative, including Mombasa, Kenya, and Ouagadougou, Burkina Faso. While Gabon, as a 3rd

site of the DVI study was included in this paper, the thesis focuses only on studies conducted in Burkina Faso and Kenya.

.

1.7.3.3 Paper 3 (Chapter 4)

Paper 3 in Chapter 4 was submitted and under review as “Clinical and epidemiologic characteristics associated with dengue fever before and during the 2017 outbreak in Mombasa, Kenya” in the *PLoS Neglected Tropical Diseases* in 2019. This work describes the epidemiologic and clinical characteristics of dengue before and during the outbreak in Mombasa, which started in April 2017, and compares performance of clinical diagnosis of suspected dengue during and before the outbreak.

1.7.3.4 Paper 4 (Chapter 5)

Paper 4 in Chapter 5 was submitted and under review as “Clinical and epidemiologic characteristics associated with dengue identified in the health facility-based surveillance before and during outbreak in 2016 in Ouagadougou, Burkina Faso” in the *Journal of Infectious Diseases* in 2019. This work is the first to describe the epidemiologic and clinical characteristics of dengue during and outside the large outbreak in Ouagadougou, which took place between September and November 2016

.

1.7.3.5 Paper 5 (Chapter 6)

Paper 5 has been written in preparation for publication and is titled “Dengue virus seroprevalence and force of infection in Ouagadougou, Burkina Faso”. This work details results from the repeated serosurveys conducted in Ouagadougou and describes seroprevalence by age and other characteristics. In addition, seroconversion rates are measured between the repeat surveys, including those periods containing the outbreak or not, and rate ratios for demographic and clinical characteristics are estimated.

1.8 Ethical approval

Approvals for the studies covered in this PhD were given by the Research Ethics Committee of the London School of Hygiene & Tropical Medicine (Reference

number: 17096, dated 1 May 2019, for the study in Burkina Faso; Reference number: 10457, dated 18 February 2016, for the study in Kenya), as well as by the local IRBs and ethical committees. Details are provided in each chapter.

1.9 Funding

The thesis was based on part-time PhD program, supported by self-funding. The studies described in this thesis were supported by a grant to Dengue Vaccine Initiative awarded by the Bill and Melinda Gates Foundation (OPP 1053432).

1.10 Role of the candidate in the current project and prior work

1.10.1 Work prior to PhD

I joined the International Vaccine Institute (IVI) in September 2009 as an epidemiologist in the dengue program in IVI, known as the Pediatric Dengue Vaccine Initiative (PDVI). PDVI was terminated in 2010 and Dengue Vaccine Initiative (DVI) was formed in a consortium format in 2011, funded by the Bill and Melinda Gates Foundation (OPP 1016669) for 4 years from 2011-2015. This grant supported activities of DVI program, including field studies, until 2015. In DVI, I was responsible for the field studies, including the passive health facility-based fever surveillance, cost-of-illness survey, repeated community-based serological survey, and healthcare utilization survey. With the aim of generating solid, high-quality data on comprehensive burden of the dengue disease among children and adults in a defined geographical area, in 2011, I designed and oversaw execution of the same package of studies, initially, in potential early adopter countries of dengue vaccines: Thailand, Colombia, and Vietnam.

In late 2012 and early 2013, based on the continued interaction with the donor, DVI program decided to have the same package of field studies expanded to countries of low economic setting, likely GAVI-eligible countries. When this plan was in discussion, it was prior to GAVI VIS and the aim was to generate data to convince that dengue should be included in the GAVI VIS. In addition to being responsible for the field operation of DVI, by 2013, I was the acting program leader for dengue within IVI, and led the grant writing process for the upcoming phase of the DVI consortium, known as DVI II, including the expansion of field studies.

With the expansion, the proposal included to conduct the study package in West, Central, and East Africa (Burkina Faso, Gabon, and Kenya, respectively), with Cambodia as an additional country in SE Asia. The grant was confirmed in mid-2013 (OPP 1053432) and study preparation, including designing of the studies and protocol writing, began immediately after the award confirmation. The studies in additional four sites were launched in 2014.

1.10.2 Current project in the PhD thesis

Being in charge of the field work of DVI, I was responsible for the overall execution of epidemiological studies during the project period, from protocol drafting, obtaining IRB approvals, data review/analysis, and presentations of the data. In all the sites, the standardized study design and methods were applied to conduct field studies. All the studies were based on minimal modification, to fit local context, based on the original DVI protocol I developed for the field site in Thailand in 2011. Using the work for which I led the grant writing and fundraising processes to support my own research initiatives, I decided to start the PhD study in LSHTM as a part-time student (enrollment in November 2014).

Therefore, for the studies included in this thesis, in addition to obtaining funds to support them, I was responsible for finding the right partners, finalizing of the study scope, study design, protocol preparation, obtaining IRB approvals, launch, monitoring of data collection, project execution, data analysis, and write-up of results. I was not the main on-site staff handling day-to-day study operation, but there were weekly monitoring of data transfer and fortnightly calls for study update with the study teams for each site. I travelled to the field sites regularly for data collection monitoring and control of data quality, in close collaboration with a designated data manager and the site PI. After discussion with the local collaborators, I requested to use the data from Kenya and Burkina Faso to analyse and draft manuscripts toward the PhD. These resulted in drafts of manuscripts of study findings included in the thesis (chapters 3, 4, 5, and 6).

While I have not performed any laboratory work, I developed laboratory testing plans, including selection of samples to undergo further laboratory analyses, and oversaw sample inventory (i.e. to maximize the use of the existing sample volumes). Data collection in Burkina Faso ended in 1Q of 2017 and lab testing in both Burkina

Faso and in IVI, after samples were brought to Korea, continued until mid-2018. Data collection in Kenya ended in 2Q of 2016 and lab testing in both Burkina Faso and in IVI, after samples were brought to Korea, continued until mid-2017.

The PhD study enabled me to maximize the epidemiological insights from the data, in particular with respect to my specific interests which include characterizing the clinical profile of dengue to understand patterns of dengue among healthcare seeking individuals, and estimating force of infection to understand community-based DENV transmission.

1.11 Contributions by candidate and others

Unless otherwise noted, I, Jacqueline K. Lim, have performed all analyses and written all manuscripts included in this thesis. I shared responsibilities in setting up some of the included studies, as described below. I have independently performed all literature reviews and have written the introduction and discussion sections of the thesis. I have not performed any laboratory work. Specific contributions to each chapter follow.

Paper 1: *A systematic review of the economic impact of rapid diagnostic tests for dengue*

I am first author, and worked with Gian Luca di Tanna on conceiving the design of the review. I conducted the review and generated the draft of the manuscript including all tables and figures. I, as the first author, led the process of manuscript preparation, revision, and submission. Gian Luca di Tanna co-designed the study and provided oversight of the review. Neal Alexander contributed to the design of the review and to the writing of the manuscript.

Paper 2: *Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative*

I am first author. I developed the grant proposal that was successful in obtaining funds for the studies described in this protocol paper. The protocols of the studies in Africa were based on minimal modification, to fit local context, based on the original DVI protocol I developed for the field site in Thailand in 2011. I wrote the complete first draft of this protocol manuscript, drawing on the study protocol. I, as the first

author, led the process of manuscript preparation, revision, and submission. The study PIs are authors (Sammy M Njenga, Selidji Todagbe Agnandji, Seydou Yaro). Those that were involved in protocol development and participated in study set-up and data collection on site are also authors (Mabel Carabali, Jung-Seok Lee, Kang-Sung Lee, Suk Namkung, Sl-Ki Lim, Valery Ridde, Jose Fernandes, Bertrand Lell, Sultani Hadley Matendechero, Meral Esen, Esther Andia, Noah Oyembo, Ahmed Barro, and Emmanuel Bonnet). Neal Alexander, as my PhD supervisor, and In-Kyu Yoon, the Director of DVI, provided oversight and guidance on the overall study design, project execution, and specific methodologies, e.g. epidemiology, statistical, and virology and other laboratory methods.

Paper 3: *Clinical and epidemiologic characteristics associated with dengue fever before and during the 2017 outbreak in Mombasa, Kenya*

I am first author. I developed the grant proposal that was successful in obtaining funds for the study described in this paper. I led the study design and wrote the protocol. I oversaw the ethical approval process, supported study set-up, monitored data collection, performed literature review, performed data cleaning, and conducted statistical analysis including generating SAS code for analysis and all the figures. I wrote the first draft of the manuscript. I then, as the first author, led the process of manuscript revision, and submission. Sultani Hadley Matendechero and Sammy M. Njenga, as the study PI and co-investigator, were responsible for study set-up and execution at the facilities in Mombasa. Neal Alexander, In-Kyu Yoon, and Sammy M. Njenga provided oversight and guidance on the overall study design, project execution, and specific methodologies, e.g. epidemiology, statistical, and virology and other laboratory methods. Jung-Seok Lee, Kang Sung Lee, Suk Namkung, Sl-Ki Lim, Esther Andia, and Noah Oyembo provided support in setting up the study at the sites, in sample and data collection, and data management and analysis. Henry Kanyi, So Hee Bae, and Jae Seung Yang performed laboratory work. Neal Alexander and Tansy Edwards oversaw statistical analysis, and contributed to manuscript preparation.

Paper 4: *Clinical and epidemiologic characteristics associated with dengue identified in the passive health facility-based surveillance before and during outbreak in 2016 in Ouagadougou, Burkina Faso*

I am first author. I led the study design and co-developed the protocol with Dr. Mabel Carabali. Some site-specific details were added in collaboration with the key investigators (Y Seydou and V Ridde, as the PI and co-investigator, and M Carabali). I oversaw the ethical approval process, supported study set-up, monitored data collection, performed literature review, performed data cleaning, and conducted statistical analysis including generating SAS code for analysis and all the figures, except for Fig. 1 (by Emmanuel Bonnet, one of co-authors). I wrote the first draft of the manuscript. I, as the first author, led the process of manuscript preparation, revision, and submission. M. Carabali also supported data and sample collection. Yaro Seydou and Valéry Ridde, as the PI and co-investigator, were responsible for study set-up and execution in Ouagadougou. Ahmed Barro, Desire Dahourou, Kang Sung Lee, Teguewende Nikiema, Suk Namkung, Jung Seok Lee, Losseni Kaba, and Paul-André Somé provided support in setting up the study at the sites, in sample and data collection, and data management and analysis. Mee Young Shin, Therese Kagone, and Jae Seung Yang performed laboratory work. Emmanuel Bonnet provided support in data cleaning. Neal Alexander and In-Kyu Yoon provided oversight and guidance on the overall study design, project execution, and specific methodologies, e.g. epidemiology, statistical, and virology and other laboratory methods. Neal Alexander and Tansy Edwards oversaw statistical analysis, and contributed to manuscript preparation.

Paper 5: *Dengue virus seroprevalence and force of infection in Ouagadougou, Burkina Faso*

I am first author. I led the study design and co-developed the protocol with Dr. Mabel Carabali. Some site-specific details were added in collaboration with the key investigators (Y Seydou and V Ridde, as the PI and co-investigator, and M Carabali). I oversaw the ethical approval process, supported study set-up, monitored data collection, performed literature review, performed data cleaning, and conducted statistical analysis including generating SAS code for analysis and all the figures, except for Fig. 1 (by Emmanuel Bonnet, one of co-authors). I wrote the first draft of the manuscript. I, as the first author, led the process of manuscript preparation, revision, and submission. M. Carabali also supported data and sample collection. Yaro Seydou and Valéry Ridde, as the PI and co-investigator, were responsible for study set-up and execution in Ouagadougou. Ahmed Barro, Desire Dahourou, Kang

Sung Lee, Teguewende Nikiema, Suk Namkung, Jung Seok Lee, Losseni Kaba, and Paul-André Somé provided support in setting up the study at the sites, in sample and data collection, and data management and analysis. Mee Young Shin, Therese Kagone, and Jae Seung Yang performed laboratory work. Emmanuel Bonnet provided support in data cleaning. Neal Alexander and In-Kyu Yoon provided oversight and guidance on the overall study design, project execution, and specific methodologies, e.g. epidemiology, statistical, and virology and other laboratory methods. Neal Alexander and Tansy Edwards oversaw statistical analysis, and contributed to manuscript preparation.

References

1. Boisier P, Morvan JM, Laventure S, Charrier N, Martin E, Ouledi A, et al. [Dengue 1 epidemic in the Grand Comoro Island (Federal Islamic Republic of the Comores). March-May 1993]. Ann Soc Belg Med Trop. 1994 Sep;74(3):217-29.
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013 Apr 25;496(7446):504-7.
3. Halstead S. Pathogenesis of dengue: challenges to molecular biology. Science. 1988 1998 Jan 29;239(4839):476-81.
4. Gubler DJ MM. Impact of dengue/dengue hemorrhagic fever on the developing world. Adv Virus Res. 1999;53:35-70.
5. Singhi SK, Bansal A. Dengue and dengue hemorrhagic fever: management issues in an intensive care unit. J Pediatr (Rio J). 2007 2007;83(2 Suppl):S22-35.
6. World Health Organization. Dengue and dengue haemorrhagic fever. Fact sheet No 117. 2009 [cited 2019 May 12]; Available from: https://www.who.int/neglected_diseases/integrated_media/integrated_media_dengue/en/
7. Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. Archives of Virology. 2013;158(7):1445-59.
8. World Health Organization. Dengue and severe dengue. Fact sheets 2019 2 February 2018 [cited 2019 12 May]; Available from: http://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
9. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6(8):e1760.
10. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol. 2013;5:299-309.
11. Tedjou A, Kamgang B, Yougang A, Njiokou F, Wondji C. Update on the geographical distribution and prevalence of Aedes aegypti and Aedes albopictus (Diptera: Culicidae), two major arbovirus vectors in Cameroon. PLoS Negl Trop Dis 2018 2019 Mar 18;13(3):e0007137.
12. Lee J, Mogasale V, Lim JK, Ly S, Lee K, Sorn S, et al. A multi-country study of the economic burden of dengue fever based on patient-specific field surveys in Burkina Faso, Kenya, and Cambodia. PLoS Negl Trop Dis 2019 2019 Feb 28;13(2):e0007164.
13. Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR. Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. PLoS Negl Trop Dis. 2019 March 28, 2019;13(3):e0007213.
14. Amarasinghe A, Kuritsky J, Letson G, Margolis H. Dengue virus infection in Africa. Emerging Infectious Diseases. 2011;17(8):1349-54.
15. Were F. The dengue situation in Africa. Paediatr Int Child Health. 2012;1:18-21.
16. Ward T, Samuel M, Maoz D, Runge-Ranzinger S, Boyce R, Toledo J, et al. Dengue data and surveillance in Tanzania: a systematic literature review. Trop Med Int Health. 2017;22(8):960-70.
17. Humphrey J, Cleton N, Reusken C, Glesby M, Koopmans M, Abu-Raddad L. Dengue in the Middle East and North Africa: A systematic review. PLoS Negl Trop Dis. 2016;10(12).
18. Jaenisch T, Junghanss T, Wills B, Brady OJ, Eckerle I, Farlow A, et al. Dengue expansion in

Africa-not recognized or not happening? Emerg Infect Dis. 2014 2014 Oct;20(10):e140487.
19. Adam A, Schuttoff T, Reiche S, Jassoy C. High seroprevalence of dengue virus indicates that dengue virus infections are frequent in central and eastern Sudan. Trop Med Int Health. 2018;23(9):960-7.
20. Himatt S, Osman K, Okoued S, Seidahmed O, Beatty M, Soghaier M, et al. Sero-prevalence of dengue infections in the Kassala state in the eastern part of the Sudan in 2011. J Infect Public Health. 2015;8(5):487-92.
21. Abdalla T, Karsany M, Ali AA. Correlation of measles and dengue infection in Kassala, Eastern Sudan. J Med Virol. 2015;87(1):76-8.
22. Soghaier M, Himatt S, Osman K, Okoued S, Seidahmed O, Beatty M, et al. Cross-sectional community-based study of the socio-demographic factors associated with the prevalence of dengue in the eastern part of Sudan in 2011. BMC Public Health. 2015;15(558):015-1913.
23. Elduma A, Osman W. Dengue and hepatitis E virus infection in pregnant women in Eastern Sudan, a challenge for diagnosis in an endemic area. Pan Afr Med J. 2014;19(391).
24. Soghaier MA, Mahmood SF, Pasha O, Azam SI, Karsani MM, Elmangory MM, et al. Factors associated with dengue fever IgG sero-prevalence in South Kordofan State, Sudan, in 2012: Reporting prevalence ratios. J Infect Public Health. 2014 Feb;7(1):54-61.
25. Seidahmed O, Hassan S, Soghaier MA, Siam HAM, Ahmed FTA, Elkarsany MM, et al. Spatial and temporal patterns of dengue transmission along a Red Sea coastline: a longitudinal entomological and serological survey in Port Sudan city. PLoS Negl Trop Dis. 2012;6(9):27.
26. Abdallah T, Ali A, Karsany MS, Adam I. Epidemiology of dengue infections in Kassala, Eastern Sudan. J Med Virol. 2012;84(3):500-3.
27. Malik A, Earhart K, Mohareb E, Saad M, Saeed M, Ageep A, et al. Dengue hemorrhagic fever outbreak in children in Port Sudan. J Infect Public Health. 2011;4(1):1-6.
28. Adam I, Jumaa A, Elbashir HM, Karsany MS. Maternal and perinatal outcomes of dengue in PortSudan, Eastern Sudan. Virol J. 2010;7(153):7-153.
29. Ageep A, Malik A, Elkarsani MS. Clinical presentations and laboratory findings in suspected cases of dengue virus. Saudi Med J. 2006;27(11):1711-3.
30. Hyams K, Oldfield EC, Scott RM, Bourgeois AL, Gardiner H, Pazzaglia G, et al. Evaluation of febrile patients in Port Sudan, Sudan: isolation of dengue virus. Am J Trop Med Hyg. 1986;35(4):860-5.
31. Obonyo M, Fidhow A, Ofula V. Investigation of laboratory confirmed dengue outbreak in North-eastern Kenya, 2011. PLoS One 2018 2018 Jun 7;13(6):e0198556.
32. Vu DM, Mutai N, Heath CJ, Vulule JM, Mutuku FM, Ndenga B, et al. Unrecognized Dengue Virus Infections in Children, Western Kenya, 2014-2015 Emerg Infect Dis. 2017 November 2017 23(11).
33. Vu D, Banda T, Teng C, Heimbaugh C, Muchiri E, Mungai P, et al. Dengue and west nile Virus transmission in children and adults in coastal Kenya. Am J Trop Med Hyg. 2017;96(1):141-3.
34. Ngoi CN, Price MA, Fields B, Bonventure J, Ochieng C, Mwashigadi G, et al. Dengue and chikungunya virus infections among young febrile adults evaluated for acute HIV-1 infection in coastal Kenya. PLoS One. 2016;11(12):e0167508.
35. Konongoi L, Ofula V, Nyunja A, Owaka S, Koka H, Makio A, et al. Detection of dengue virus serotypes 1, 2 and 3 in selected regions of Kenya: 2011-2014. Virology Journal. 2016 4 November

2016;13(182).
36. Ochieng C, Ahenda P, Vittor A, Nyoka R, Gikunju S, Wachira C, et al. Seroprevalence of infections with dengue, rift valley fever and chikungunya viruses in Kenya, 2007. PLoS One. 2015;10(7).
37. Ellis EM, Neatherlin JC, Delorey M, Ochieng M, Mohamed AH, Mogeni DO, et al. A household serosurvey to estimate the magnitude of a dengue outbreak in Mombasa, Kenya, 2013. PLoS Negl Trop Dis. 2015 Apr;9(4):e0003733.
38. Blaylock JM, Maranich A, Bauer K, Nyakoe N, Waitumbi J, Martinez LJ, et al. The seroprevalence and seroincidence of dengue virus infection in western Kenya. Travel Medicine and Infectious Disease. 2011.
39. Johnson B, Ocheng D, Gichogo A, Okiro M, Libondo D, Kinyanjui P, et al. Epidemic dengue fever caused by dengue type 2 virus in Kenya: preliminary results of human virological and serological studies. East African medical journal. 1982;59(12):781.
40. Boillat-Blanco N, Klaassen B, Mbarack Z, Samaka J, Mlaganile T, Masimba J, et al. Dengue fever in Dar es Salaam, Tanzania: clinical features and outcome in populations of black and non-black racial category. BMC Infect Dis. 2018;18(1):018-3549.
41. Kajeguka D, Kaaya R, Mwakalinga S, Ndossi R, Ndaro A, Chilongola J, et al. Prevalence of dengue and chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. BMC Infect Dis. 2016;16(183):016-1511.
42. Mboera L, Mweya C, Rumisha S, Tungu P, Stanley G, Makange M, et al. The risk of dengue virus transmission in Dar es Salaam, Tanzania during an epidemic period of 2014. PLoS Negl Trop Dis. 2016;10(1).
43. Vairo F, Mboera LEG, De Nardo P, Oriyo NM, Meschi S, Rumisha SF, et al. Clinical, virologic, and epidemiologic characteristics of dengue outbreak, Dar es Salaam, Tanzania, 2014. Emerg Infect Dis. 2016;22(5):895-9.
44. Chipwaza B, Mugasa J, Selemani M, Amuri M, Mosha F, Ngatunga S, et al. Dengue and chikungunya fever among viral diseases in outpatient febrile children in Kilosa district hospital, Tanzania. PLoS Negl Trop Dis. 2014;8(11).
45. Vairo F, Nicastri E, Yussuf SM, Cannas A, Meschi S, Mahmoud MA, et al. IgG against dengue virus in healthy blood donors, Zanzibar, Tanzania. Emerg Infect Dis. 2014 Mar;20(3):465-8.
46. Hertz JT, Munishi OM, Ooi EE, Howe S, Lim WY, Chow A, et al. Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. The American journal of tropical medicine and hygiene. 2012;86(1):171-7.
47. Vairo F, Nicastri E, Meschi S, Schepisi MS, Paglia MG, Bevilacqua N, et al. Seroprevalence of dengue infection: a cross-sectional survey in mainland Tanzania and on Pemba Island, Zanzibar. International Journal of Infectious Diseases. 2012;16(1):e44-6.
48. Ferede G, Tiruneh M, Abate E, Wondimeneh Y, Gadisa E, Howe R, et al. A study of clinical, hematological, and biochemical profiles of patients with dengue viral infections in Northwest Ethiopia: implications for patient management. BMC Infect Dis. 2018;18(1):018-3557.
49. Ferede G, Tiruneh M, Abate E, Wondimeneh Y, Damtie D, Gadisa E, et al. A serologic study of dengue in northwest Ethiopia: Suggesting preventive and control measures. PLoS Negl Trop Dis. 2018;12(5).
50. Le Gonidec E, Maquart M, Duron S, Savini H, Cazajous G, Vidal P, et al. Clinical survey of

dengue virus circulation in the Republic of Djibouti between 2011 and 2014 identifies serotype 3 epidemic and recommends clinical diagnosis guidelines for resource limited settings. PLoS Negl Trop Dis. 2016;10(6).
51. Rodier GR, Gubler DJ, Cope SE, Cropp CB, Soliman AK, Polycarpe D, et al. Epidemic dengue 2 in the city of Djibouti 1991-1992. Trans R Soc Trop Med Hyg. 1996 May-Jun;90(3):237-40.
52. D' Ortenzio E, Balleydier E, Baville M, Filleul L, Renault P. [Dengue fever in the Reunion Island and in South Western islands of the Indian Ocean]. Med Mal Infect. 2011;41(9):475-9.
53. Botros B, Watts D, Soliman AK, Salib AW, Moussa MI, Mursal H, et al. Serological evidence of dengue fever among refugees, Hargeysa, Somalia. J Med Virol. 1989;29(2):79-81.
54. Mazaba-Liwewe M, Siziya S, Monze M, Mweene-Ndumba I, Masaninga F, Songolo P, et al. First sero-prevalence of dengue fever specific immunoglobulin G antibodies in Western and North-Western provinces of Zambia: a population based cross sectional study. Virol J. 2014;11(135):11-135.
55. Tarnagda Z, Cissé A, Bicaba B, Diagbouga S, Sagna T, Ilboudo A, et al. Dengue fever in Burkina Faso, 2016. Emerg Infect Dis 2018 2018 Jan;24(1):170-2.
56. Diallo I, Sondo K, Tieno H, Tamelokpo E, Zoungrana J, Sagna Y, et al. [About 98 cases of dengue hospitalized in a private clinic of Ouagadougou: epidemiology, diagnostic and evolution] [Article in French]. Bull Soc Pathol Exot 2017 2017 Dec;110(5):291-6.
57. Ridde V, Agier I, Bonnet E, Carabali M, Dabiré K, Fournet F, et al. Presence of three dengue serotypes in Ouagadougou (Burkina Faso): research and public health implications. Infect Dis Poverty. 2016 2016 Apr;5(5):23.
58. Gonzalez JP, Du Saussay C, Gautun JC, McCormick JB, Mouchet J. [Dengue in Burkina Faso (ex-Upper Volta): seasonal epidemics in the urban area of Ouagadougou]. Bull Soc Pathol Exot Filiales. 1985;78(1):7-14.
59. Kolawole O, Seriki A, Irekeola A, Bello K, Adeyemi O. Dengue virus and malaria concurrent infection among febrile subjects within Ilorin metropolis, Nigeria. J Med Virol. 2017;89(8):1347-53.
60. Onoja AB, Adeniji JA, Olaleye OD. High rate of unrecognized dengue virus infection in parts of the rainforest region of Nigeria. Acta Tropica. 2016 August 2016;160:39-43.
61. Fagbami A, Monath T, Fabiyi A. Dengue virus infections in Nigeria: a survey for antibodies in monkeys and humans. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1977;71(1):60-5.
62. Faye O, Ba Y, Faye O, Talla C, Diallo D, Chen R, et al. Urban epidemic of dengue virus serotype 3 infection, Senegal, 2009. Emerg Infect Dis. 2014 Mar;20(3):456-9.
63. Saluzzo J, Cornet M, Adam C, Eyraud M, Digoutte JP. [Dengue 2 in eastern Senegal: serologic survey in simian and human populations. 1974-85]. Bull Soc Pathol Exot Filiales. 1986;79(3):313-22.
64. Dariano D, Taitt C, Jacobsen K, Bangura U, Bockarie A, Bockarie M, et al. Surveillance of vector-borne infections (chikungunya, dengue, and malaria) in Bo, Sierra Leone, 2012-2013. Am J Trop Med Hyg. 2017;97(4):1151-4.
65. de Araujo LJ, Mores C, Bausch D, Christofferson R. Short report: Serological evidence of under-reported dengue circulation in Sierra Leone. PLoS Negl Trop Dis. 2016;10(4).
66. L' Azou M, Succo T, Kamagate M, Ouattara A, Gilbernair E, Adjogoua E, et al. Dengue: etiology of acute febrile illness in Abidjan, Cote d'Ivoire, in 2011-2012. Trans R Soc Trop Med Hyg. 2015;109(11):717-22.
67. Stoler J, Delimini R, Bonney J, Oduro A, Owusu-Agyei S, Fobil J, et al. Evidence of recent

dengue exposure among malaria parasite-positive children in three urban centers in Ghana. Am J Trop Med Hyg. 2015;92(3):497-500.
68. Phoutrides EK, Coulibaly MB, George CM, Sacko A, Traore S, Bessoff K, et al. Dengue Virus Seroprevalence Among Febrile Patients in Bamako, Mali: Results of a 2006 Surveillance Study. Vector-Borne and Zoonotic Diseases. 2011;11(11):1479-85.
69. Gabor J, Schwarz N, Esen M, Kremsner P, Grobusch M. Dengue and chikungunya seroprevalence in Gabonese infants prior to major outbreaks in 2007 and 2010: A sero-epidemiological study. Travel Med Infect Dis. 2016;14(1):26-31.
70. Caron M, Grard G, Paupy C, Mombo IM, Bikie Bi Nso B, Kassa Kassa FR, et al. First evidence of simultaneous circulation of three different dengue virus serotypes in Africa. PLoS One. 2013;8(10):e78030.
71. Caron M, Paupy C, Grard G, Becquart P, Mombo I, Nso BB, et al. Recent introduction and rapid dissemination of Chikungunya virus and Dengue virus serotype 2 associated with human and mosquito coinfections in Gabon, central Africa. Clin Infect Dis. 2012 Sep;55(6):e45-53.
72. Pourrut X, Nkoghe D, Gonzalez J-P, Leroy E. No evidence of dengue virus circulation in rural Gabon. Emerg Infect Dis. 2011;17(8):1568-9.
73. Leroy EM ND, Ollomo B, Nze-Nkogue C, Becquart P, Grard G, Pourrut X, Charrel R, Moureau G, Ndjoyi-Mbiguino A, De-Lamballerie X. Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007. Emerg Infect Dis 2009;15(4):591-3.
74. Tchuandom S, Tchouangueu T, Antonio-Nkondjio C, Lissom A, Djang J, Atabonkeng E, et al. Seroprevalence of dengue virus among children presenting with febrile illness in some public health facilities in Cameroon. Pan Afr Med J. 2018;31(177).
75. Yousseu F, Nemg F, Ngouanet S, Mekanda F, Demanou M. Detection and serotyping of dengue viruses in febrile patients consulting at the New-Bell District Hospital in Douala, Cameroon. PLoS One. 2018;13(10).
76. Demanou M, Pouillot R, Grandadam M, Boisier P, Kamgang B, Herve J, et al. Evidence of dengue virus transmission and factors associated with the presence of anti-dengue virus antibodies in humans in three major towns in Cameroon. PLoS Negl Trop Dis. 2014;8(7).
77. Makiala-Mandanda S, Ahuka-Mundeke S, Abbate J, Pukuta-Simbu E, Nsio-Mbeta J, Berthet N, et al. Identification of dengue and chikungunya cases among suspected cases of yellow fever in the Democratic Republic of the Congo. Vector Borne Zoonotic Dis. 2018;18(7):364-70.
78. Muianga A, Pinto G, Massangaie M, Ali S, Oludele J, Tivane A, et al. Antibodies against chikungunya in Northern Mozambique during dengue outbreak, 2014. Vector Borne Zoonotic Dis. 2018;18(8):445-9.
79. Mugabe V, Ali S, Chelene I, Monteiro V, Guiliche O, Muianga A, et al. Evidence for chikungunya and dengue transmission in Quelimane, Mozambique: Results from an investigation of a potential outbreak of chikungunya virus. PLoS One. 2018;13(2).
80. Oludele J, Lesko B, Mahumane GI, de Bruycker-Nogueira F, Muianga A, Ali S, et al. Dengue virus serotype 2 established in Northern Mozambique (2015-2016). Am J Trop Med Hyg. 2017;97(5):1418-22.
81. Massangaie M, Pinto G, Padama F, Chambe G, da Silva M, Mate I, et al. Clinical and epidemiological characterization of the first recognized outbreak of dengue virus-type 2 in Mozambique, 2014. Am J Trop Med Hyg. 2016;94(2):413-6.

82. Sharp T, Moreira R, Soares MJ, Miguel da Costa L, Mann J, DeLorey M, et al. Underrecognition of dengue during 2013 epidemic in Luanda, Angola. Emerg Infect Dis. 2015;21(8):1311-6.
83. Centers for Disease Control and Prevention. Ongoing dengue epidemic - Angola, June 2013. MMWR Morb Mortal Wkly Rep. 2013 Jun 21;62(24):504-7.
84. Blackburn N, Meenehan G, Aldridge N. The status of dengue fever virus in South Africa—serological studies and diagnosis of a case of dengue fever. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1987;81(4):690-2.
85. Surtees G. The distribution, density and seasonal prevalence of Aedes aegypti in West Africa. Bull World Health Organ. 1967;36(4):539-40.
86. Messina J, Brady O, Scott T, Zou C, Pigott D, Duda K, et al. Global spread of dengue virus types: mapping the 70 year history. Trends Microbiol. 2014;22(3):138-46.
87. Kamgang B, Ngoagouni C, Manirakiza A, Nakouné E, Paupy C, Mirdad K. Temporal patterns of abundance of *Aedes aegypti* and *Aedes albopictus* (*Diptera: Culicidae*) and mitochondrial DNA analysis of *Ae. albopictus* in the Central African Republic. PLoS Negl Trop Dis 2013 2013 Dec 12;7(12):e2590.
88. Paupy C, Ollomo B, Kamgang B, Moutailler S, Rousset D, Demanou M, et al. Comparative role of Aedes albopictus and Aedes aegypti in the emergence of dengue and chikungunya in central Africa. Vector Borne Zoonotic Dis. 2010;10(3):259-66.
89. Zeller H, Van Bortel W, Sudre B. Chikungunya: Its History in Africa and Asia and Its Spread to New Regions in 2013-2014. The Journal of infectious diseases. 2016 Dec 15;214(suppl 5):S436-s40.
90. Monath TP, Vasconcelos PF. Yellow fever. Journal of clinical virology. 2015;64:160-73.
91. Kiemde F, Spijker R, Mens P, Tinto H, Boele M, Schallig H. Aetiologies of non-malaria febrile episodes in children under 5 years in sub-Saharan Africa. Trop Med Int Health. 2016 Aug. 2016;21(8):943-55.
92. Baba M, Villinger J, Masiga DK. Repetitive dengue outbreaks in East Africa: A proposed phased mitigation approach may reduce its impact Reviews in Medical Virology. 2016 29 February 2016;26(3):183-96.
93. Amoako N, Duodu S, Dennis FE, Bonney JHK, Asante KP, Ameh J, et al. Detection of dengue virus among children with suspected malaria, Accra, Ghana. Emerg Infect Dis 2018 2018 Aug;24(8):1544–7.
94. Kraemer MU, Sinka ME, Duda K, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLife. 2015 Jun 30, 2015 4:e08347.
95. Gubler D, Clark G. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerg Infect Dis 1995 1995 Apr-Jun;1(2):55-7.
96. Fagbami AH, Onoja AB. Dengue haemorrhagic fever: An emerging disease in Nigeria, West Africa. Journal of Infection and Public Health. 2018 November-December 2018;11(6):757-62.
97. Oladipo EK, Awoyelu EH, J.K. O. Yellow fever, dengue fever, and West Nile viruses co-circulation in Ogbomoso. International Journal of Medicine in Developing Countries. 2018 Feb. 15, 2018;2(2):50-4.
98. Collenberg E, Ouedraogo T, Ganame J, Fickenscher H, Kynast-Wolf G, Becher H, et al. Seroprevalence of six different viruses among pregnant women and blood donors in rural and urban

Burkina Faso: A comparative analysis. J Med Virol. 2006 May;78(5):683-92.
99. Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. Lancet Infect Dis. 2016;16:712-23.
100. Centers for Disease Control and Prevention. Testing guidance. Dengue-Testing 2019 May 3, 2019 [cited 2019 May 26]; Available from: https://www.cdc.gov/dengue/healthcare-providers/testing/testing-guidance.html
101. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention, and control. Geneva: World Health Organization; 2009.
102. Muller DA, Depelsenaire ACI, Young PR. Clinical and Laboratory Diagnosis of Dengue Virus Infection The Journal of Infectious Diseases. 2017 10 April 2017 215(suppl_2):S89–S95.
103. Blacksell SD. Commercial dengue rapid diagnostic tests for point-of-care application: Recent evaluations and future needs? Journal of Biomedicine and Biotechnology. 2012 11 February 2012;2012(Article ID 151967).
104. Centers for Disease Control and Prevention. Serologic tests for dengue virus. Dengue-Testing 2019 May 3, 2019 [cited 2019 May 26]; Available from: https://www.cdc.gov/dengue/healthcare-providers/testing/serologic-tests.html
105. Centers for Disease Control and Prevention. Molecular tests for dengue virus. Dengue-Testing 2019 May 3, 2019 [cited 2019 May 26]; Available from: https://www.cdc.gov/dengue/healthcare-providers/testing/molecular-tests/index.html
106. Centers for Disease Control and Prevention. Dengue virus antigen detection. Dengue-Testing 2019 May 3, 2019 [cited 2019 May 26]; Available from: https://www.cdc.gov/dengue/healthcare-providers/testing/antigen-detection.html
107. Wu G, Zaman MH. Low-cost tools for diagnosing and monitoring HIV infection in low-resource settings. Bulletin of the World Health Organization 2012 19 October 2012;2012(90):914-20.
108. Teles FR, Prazeres DM, Lima-Filho JL. Trends in dengue diagnosis. Reviews in medical virology. 2005 Sep-Oct;15(5):287-302.
109. Blacksell SD, Bell D, Kelley J, Mammen MP, Jr., Gibbons RV, Jarman RG, et al. Prospective study to determine accuracy of rapid serological assays for diagnosis of acute dengue virus infection in Laos. Clin Vaccine Immunol. 2007 Nov;14(11):1458-64.
110. Blacksell SD, Jarman RG, Bailey MS, Tanganuchitcharnchai A, Jenjaroen K, Gibbons RV, et al. Evaluation of six commercial point-of-care tests for diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. Clin Vaccine Immunol. 2011 Dec;18(12):2095-101.
111. Osorio L, Ramirez M, Bonelo A, Villar LA, Parra B. Comparison of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection. Virol J. 2010;7:361.
112. Guzman MG JT, Gaczkowski R, Ty Hang VT, Sekaran SD, Kroeger A, Vazquez S, Ruiz D, Martinez E, Mercado JC, Balmaseda A, Harris E, Dimano E, Leano PS, Yoksan S, Villegas E, Benduzu H, Villalobos I, Farrar J, Simmons CP. Multi-country evaluation of the sensitivity and specificity of two commercially-available NS1 ELISA assays for dengue diagnosis. PLoS Negl Trop Dis 2010 2010 Aug 31;4(8):e811.
113. Gan V, Tan L, Lye D, Pok K, Mok S, Chua R, et al. Diagnosing dengue at the point-of-care: utility of a rapid combined diagnostic kit in Singapore. PLoS One. 2014 2014 Mar 19;9(3):e90037.

114. Changal KH, Raina AH, Raina A, Raina M, Bashir R, Latief M, et al. Differentiating secondary from primary dengue using IgG to IgM ratio in early dengue: an observational hospital based clinico-serological study from North India. BMC Infectious Diseases. 2016;16(715).
115. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. Geneva: World Health Organization; 1997.
116. Nguyen THT, Clapham HE, Phung KL, Nguyen TK, DInh TT, Nguyen THQ, et al. Methods to discriminate primary from secondary dengue during acute symptomatic infection. BMC Infectious Diseases. 2018;18(375).
117. Beatty M, Stone A, Fitzsimons D, Hanna J, Lam S, Vong S, et al. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas Dengue Prevention Boards. PLoS Negl Trop Dis 2010 2010 Nov 16;4(11):e890.
118. Ridde V, Carabali M, Ly A, Druetz T, Kouanda S, Bonnet E, et al. The need for more research and public health interventions on dengue fever in Burkina Faso. PLoS Neglected Tropical Diseases. 2014;8(6):e2859.
119. Sanou A, Dirlikov E, Sondo K, Kagone T, Yameogo I, Sow H, et al. Building laboratory-based arbovirus sentinel surveillance capacity during an ongoing dengue outbreak, Burkina Faso, 2017. Health Secur. 2018;16(S1):S103-S10.
120. Lim J, Carabali M, Lee J-S, et al. Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative. BMJ Open. 2018;2018(8):e017673.
121. Anderson K, Chunsuttiwat S, Nisalak A, Mammen M, Libraty D, Rothman A, et al. Burden of symptomatic dengue infection in children at primary school in Thailand: a prospective study. Lancet. 2007;369(9571):1452--9.
122. Okanurak K, Sornmani S, Mas-ngammueng R, Sitaputra P, Krachangsang S, Limsomboon J. Treatment seeking behavior of DHF patients in Thailand. . Southeast Asian J Trop Med Public Health 1997;28:351-8.
123. Porter K, Beckett C, Kosasih H, Tan R, Alisjahbana B, Rudiman P, et al. Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. . Am J Trop Med Hyg. 2005;72(1):60--6.
124. Kittigul L, Pitakarnjanakul P, Sujirarat D, Siripanichgon K. The differences of clinical manifestations and laboratory findings in children and adults with dengue virus infection. J Clin Virol. 2007;39:76-81.
125. Messina JP, Brady OJ, Hay SI. Dengue risk and burden estimates in GAVI countries. Spatial Ecology and Epidemiology Group University of Oxford; 2014.
126. Mease LE CR, Musila LA, Prosser T, Ogolla F, Ofula VO, Schoepp RJ, Rossi CA, Adungo N. Seroprevalence and distribution of arboviral infections among rural Kenyan adults: a cross-sectional study. Virol J. 2011;8(371).
127. Biggar RJ JB, Oster C, Sarin PS, Ocheng D, Tukei P, Nsanze H, Alexander S, Bodner AJ, Siongok TA, et al. Regional variation in prevalence of antibody against human T-lymphotropic virus types I and III in Kenya, East Africa. Int J Cancer. 1985;35(6):763-7.
128. Ouedraogo S, Benmarhnia T, Bonnet E, Some P-A, Barro AS, Kafando Y, et al. Evaluation of effectiveness of a community-based intervention for control of dengue virus vector, Ouagadougou, Burkina Faso. Emerg Infect Dis. 2018;24(10):1859-67.

129. Fournet F, Rican S, Vaillant Z, Roudot A, Meunier-Nikiema A, Kassie D, et al. The influence of urbanization modes on the spatial circulation of flaviviruses within Ouagadougou (Burkina Faso). Int J Environ Res Public Health. 2016;13(12).
130. Mamoudou S, Boushab B. [Hemorrhagic form of dengue fever observed at the Infectious Diseases Department CHU Yalgado Ouedraogo, Burkina Faso]. Pan Afr Med J. 2016;23(168).
131. Hashimoto T, Kutsuna S, Maeki T, Tajima S, Takaya S, Katanami Y, et al. A case of dengue fever imported from Burkina Faso to Japan in October 2016. Jpn J Infect Dis. 2017;70(6):675-7.
132. Eldin C, Gautret P, Nougairede A, Sentis M, Ninove L, Saidani N, et al. Identification of dengue type 2 virus in febrile travellers returning from Burkina Faso to France, related to an ongoing outbreak, October to November 2016. Euro Surveill. 2016;21(50):1560-7917.
133. Gonzalez J, Du Saussay C, Gautun JC, McCormick JB, Mouchet J. [Dengue in Burkina Faso (ex-Upper Volta): seasonal epidemics in the urban area of Ouagadougou]. Bull Soc Pathol Exot Filiales. 1985;78(1):7-14.
134. Gonzalez J, Du Saussay C, Gautun J, McCormick J, Mouchet J. Dengue in Burkina Faso (ex-upper Volta): seasonal epidemics in the urban area of Ouagadougou. Bulletin de la Societe de Pathologie Exotique. 1985;78(1):7-14.
135. Robert V, Lhuillier M, Meunier D, Sarthou J, Monteny N, Digoutte J-P, et al. Yellow fever virus, dengue 2 and other arboviruses isolated from mosquitos, in Burkina Faso, from 1983 to 1986. Entomological and epidemiological considerations. Bulletin de la Societe de Pathologie Exotique. 1993;86(2):90-100.
136. Ministère de la Santé. Rapport d'étape de l'investigation de cas suspects de Dengue dans la région sanitaire du Centre. Ouagadougou, Burkina Faso: Direction de la lutte contre la maladie; 2013.
137. World Health Organization. Dengue fever - Burkina Faso. Disease outbreak news 2016 2016 November 18 [cited 2018 August 18]; Available from: http://www.who.int/csr/don/18-november-2016-dengue-burkina-faso/en/
138. World Health Organization. Dengue fever - Burkina Faso. Disease outbreak news 2017 2017 November 6 [cited 2018 August 18]; Available from: http://www.who.int/csr/don/6-november-2017-dengue-burkina-faso/en/
139. Johnson B, Musoke S, Ocheng D, Gichogo A, Rees PH. Dengue-2 virus in Kenya. Lancet. 1982;2(8291):208-9.
140. Johnson B, Okoth F, Tukei PM, Mugambi M, Woody JN, Morrill JC, et al. Dengue-2 virus in Kenya. Lancet. 1990;336(8722):0140-6736.
141. Chepkorir E, Venter M, Lutomiah J, Mulwa F, Arum S, Tchouassi D, et al. The occurrence, diversity and blood feeding patterns of potential vectors of dengue and yellow fever in Kacheliba, West Pokot County, Kenya. Acta Trop. 2018;186:50-7.
142. Nyasembe V, Tchouassi D, Pirk C, Sole C, Torto B. Host plant forensics and olfactory-based detection in Afro-tropical mosquito disease vectors. PLoS Negl Trop Dis 2018 2018 Feb 20;12(2):e0006185.
143. Agha S, Tchouassi D, Bastos A, Sang R. Assessment of risk of dengue and yellow fever virus transmission in three major Kenyan cities based on Stegomyia indices. PLoS Negl Trop Dis. 2017;11(8).
144. Ngugi H, Mutuku F, Ndenga B, Musunzaji P, Mbakaya J, Aswani P, et al. Characterization and productivity profiles of Aedes aegypti (L.) breeding habitats across rural and urban landscapes in

western and coastal Kenya. Parasit Vectors. 2017;10(1):017-2271.
145. Lutomiah J, Barrera R, Makio A, Mutisya J, Koka H, Owaka S, et al. Dengue outbreak in Mombasa city, Kenya, 2013-2014: Entomologic investigations. PLoS Negl Trop Dis. 2016;10(10).
146. Owino E, Sang R, Sole C, Pirk C, Mbogo C, Torto B. An improved odor bait for monitoring populations of Aedes aegypti-vectors of dengue and chikungunya viruses in Kenya. Parasit Vectors. 2015;8(253):015-0866.
147. Attaway DF, Jacobsen KH, Falconer A, Manca G, Bennett LR, Waters NM. Mosquito habitat and dengue risk potential in Kenya: alternative methods to traditional risk mapping techniques. Geospatial health. 2014;9(1):119-30.
148. Midega J, Nzovu J, Kahindi S, Sang RC, Mbogo C. Application of the pupal/demographic-survey methodology to identify the key container habitats of Aedes aegypti (L.) in Malindi district, Kenya. Ann Trop Med Parasitol. 2006;100(1):S61-S72.
149. Konongoi S, Orcid X, Nyunja A, Ofula V, Owaka S, Koka H, et al. Human and entomologic investigations of chikungunya outbreak in Mandera, Northeastern Kenya, 2016. PLoS One. 2018;13(10).
150. Johnson B, Ocheng D, Gichogo A, Okiro M, Libondo D, Kinyanjui P, et al. Epidemic dengue fever caused by dengue type 2 virus in Kenya: preliminary results of human virological and serological studies. East Afr Med J. 1982;59(12):781-4.
151. Martyn-Simmons C, Powell S, Sudhanva M, Selim AGA, Creamer D, Pearson IC. A florid skin rash in a returning traveller. Clin Exp Dermatol. 2007;32(6):779-81.
152. Wasonga C, Inoue S, Kimotho J, Morita K, Ongus J, Sang R, et al. Development and evaluation of an in-House IgM-Capture ELISA for the detection of chikungunya and its application to a dengue outbreak situation in Kenya in 2013. Jpn J Infect Dis. 2015;68(5):410-4.
153. World Health Organization. Weekly bulletin on outbreaks and other emergencies. Regional Office for Africa, Health Emergencies Programme. 2017 June 2017;Week 24(10 - 16 June 2017).
154. The World Bank. GDP per capita (current US$). Data 2019 [cited 2019 May 22]; World Bank national accounts data, and OECD National Accounts data files]. Available from: https://data.worldbank.org/indicator/NY.GDP.PCAP.CD
155. PopulationPyramid.net. PopulationPyramid. 2017 [cited 2019 May 22]; Population Pyramids of the World from 1950 to 2100]. Available from: http://populationpyramid.net/
156. United Nations Population Division. Urban population (% of total). World Urbanization Prospects: 2018 Revision 2019 [cited 2019 June 11]; Available from: https://data.worldbank.org/indicator/sp.urb.totl.in.zs
157. Centers for Disease Control and Prevention. Dengue map. 2013 Outbreak 2013 [cited 2013 July 1]; Available from:
158. Lim JK, Alexander N, Di Tanna GL. A systematic review of the economic impact of rapid diagnostic tests for dengue. BMC Health Services Research. 2017;17(850).

2 Literature review

A systematic review of the economic impact of rapid diagnostic tests for dengue

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	LSH1405874	**Title**	Ms.
First Name(s)	Jacqueline Kyungah		
Surname/Family Name	LIM		
Thesis Title	Undocumented burden of dengue in Africa		
Primary Supervisor	Prof. Neal Alexander		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMC Health Services Research		
When was the work published?	December 2017		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am first author, and worked with Gian Luca di Tanna on conceiving the design of the review. I conducted the review and generated the draft of the manuscript including all tables and figures. I, as the first author, led the process of manuscript preparation, revision, and submission. Gian Luca di Tanna co-designed the study and provided oversight of the review. Neal Alexander contributed to the design of the review and to the writing of the manuscript.

SECTION E

Student Signature	[redacted]
Date	23 June 2019

Supervisor Signature	[redacted]
Date	17 June '19

Lim et al. BMC Health Services Research (2017) 17:850
DOI 10.1186/s12913-017-2789-8

BMC Health Services Research

RESEARCH ARTICLE Open Access

A systematic review of the economic impact of rapid diagnostic tests for dengue

Jacqueline Kyungah Lim[1,2*], Neal Alexander[2] and Gian Luca Di Tanna[3]

Abstract

Background: Dengue fever is rapidly expanding geographically, with about half of the world's population now at risk. Among the various diagnostic options, rapid diagnostic tests (RDTs) are convenient and prompt, but limited in terms of accuracy and availability.

Methods: A systematic review was conducted of published data on the use of RDTs for dengue with respect to their economic impact. The search was conducted with combinations of key search terms, including "((Dengue[Title]) AND cost/economic)" and "rapid diagnostic test/assay (or point-of-care)". Articles with insufficient report on cost/economic aspect of dengue RDTs, usually on comparison of different RDTs or assessment of novel rapid diagnostic tools, were excluded. This review has been registered in the PROSPERO International prospective register of systematic reviews (registry #: CRD42015017775).

Results: Eleven articles were found through advanced search on Pubmed. From Embase and Web of Science, two and 14 articles were obtained, respectively. After removal of duplicate items, title screening was done on 21 published works and 12 titles, including 2 meeting abstracts, were selected for abstract review. For full-text review, by two independent reviewers, 5 articles and 1 meeting abstract were selected. Among these, the abstract was referring to the same study results as one of the articles. After full text review, two studies (two articles and one abstract) were found to report on cost-wise or economic benefits of dengue RDTs and were selected for data extraction. One study found satisfactory performance of IgM-based Panbio RDT, concluding that it would be cost-effective in endemic settings. The second study was a modeling analysis and showed that a dengue RDT would not be advantageous in terms of cost and effectiveness compared to current practice of antibiotics prescription for acute febrile illness.

Conclusions: Despite growing use of RDTs in research and clinical settings, there were limited data to demonstrate an economic impact. The available two studies reached different conclusions on the cost-effectiveness of dengue RDTs, although only one of the two studies reported outcomes from cost-effectiveness analysis of dengue and the other was considering febrile illness more generally. Evidence of such an impact would require further quantitative economic studies.

Keywords: Dengue, Dengue fever, Diagnostic, rapid diagnostic test (RDT), Cost-effectiveness

Background

Dengue fever, a mosquito-borne flavivirus infection caused by four related but antigenically distinct dengue viruses (DENVs, serotypes 1–4), is a major and rapidly increasing public health problem. Its geographic range now includes about half of the world's population and continues to expand, with epidemics that disrupt health care systems [1–4]. Current WHO estimates are of about 50–100 million annual infections globally, while Bhatt et al. recently estimated 390 million infections annually with 96 million disease episodes [5–7].

However, there are no other suitable disease prevention methods: mosquito vector control is often ineffective [8, 9]. There is a vaccine, Sanofi Pasteur's live attenuated Dengvaxia®, recently registered in multiple countries in Southeast Asia and Latin America and it shows to have

* Correspondence: kajlim@gmail.com
[1]Global Dengue and Aedes-transmitted Diseases Consortium (GDAC), International Vaccine Institute (IVI), SNU Research Park, Gwanak-ro 1, Seoul, Gwanak-gu 151-191, South Korea
[2]Epidemiology and Public Health Department, London School of Hygiene and Tropical Medicine, London, UK
Full list of author information is available at the end of the article

variable efficacy [10–13]. At present, there are no drugs for specific treatment and there is a need for accurate and cheap dengue diagnostic tests to be widely used in clinical settings [14–16]. Thus, many dengue endemic countries in the tropics are still experiencing a rise in cases and in deaths due to dengue [17–20].

Recently the WHO Strategic Advisory Group of Experts (SAGE) on Immunization emphasized the need for estimation of the true burden of dengue disease, including cost of illness [6]. Data are available, but mostly focused in countries in Asia and Latin America, with well-documented hyper-endemicity and a long history of dengue transmission, such as Thailand [21, 22], the Philippines [23], Brazil [24, 25], Mexico [26], and Colombia [27]. Most of the available burden data are from studies of the epidemiology and evidence based on economic studies is limited [28, 29].

Among the key limitations of economic studies of dengue are the challenges in its diagnosis. Often, cost-related studies for dengue are based on clinical, rather than laboratory, confirmation [30]. Available methods include virus isolation, serology, and molecular methods [31]. One test routinely used by research laboratories for virus identification is Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay [32]. While this is a definite proof of infection and confirms the serotype, commercial kits that include serotyping are often expensive and would require serum samples collected in early phase during the illness [33]. Another commonly used method is immunoglobulin type M (IgM) antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) [31]. With IgM staying elevated for 2 to 3 months, interpretation could be challenging given that elevated IgM could be due either to recent past infection or to cross-reactivity with other flaviviruses [33]. Any clinical management decision reached on the basis of a single blood sample collected in the acute phase is not conclusive. Levels of immunoglobulin type G (IgG) stay elevated for months to years, so a positive result on one of the available assays could indicate a past infection, thus has limited implications for clinical management [31]. Moreover, it may cross-react across the Flavivirus group (dengue virus, Japanese encephalitis virus, West Nile virus, yellow fever virus, Zika virus, etc.) [34]. There are other assays such as Plaque Reduction Neutralization Test (PRNT) which detect serotype-specific antibodies [35]. Compared to others mentioned, these are time-consuming and labour-intensive, thus expensive [36, 37].

Amongst different diagnostic tools, rapid diagnostic tests (RDTs) are a convenient (easy to use) and prompt option, despite their limitations in terms of accuracy [38]. While their availability could be limited, especially in resource-limited settings, RDTs are commonly used for dengue detection in many endemic countries [38]. There could be a number of different commercially available tests and they could be based on the detection of dengue virus non-structural protein 1 (NS1) antigen, IgM, IgG, and IgA antibodies [39]. Often, these tests have high specificity (usually around 90%), but lower levels of sensitivity, ranging from 10 to 99%, in detection of dengue and could be cross-reactive with other flaviviruses [39–42]. However, the speed of RDTs provides early diagnosis of dengue possibly leading to timely case management. Given their limited accuracy, these RDTs are not considered the standard reference and their usefulness is not yet proven in clinical settings [15, 43]. However, some literature supports the use of such tests in combination with others, for example the combined test with NS1 antigen and IgM antibody [42, 44].

Especially in terms of economic studies, one major benefit of using RDTs would be that they allow dengue detection in the early phase of illness (at presentation), hence facilitating capture of the entire spectrum of costs incurred throughout illness. Previous studies reported that early detection is effective in reducing the duration of illness, possibly leading to lower cost-of-illness due to dengue [45, 46]. In recognition of the need to balance speed, accuracy, and availability to maximize utility when using RDTs for dengue detection for the patients in clinical settings, a systematic review was performed to explore the economic impact of using RDTs for dengue. The hypothesis behind this review was that there may be economic impact due to prompt detection of dengue in the early phase of illness using RDTs and economic impact is defined to be broad: both from the point of view of cost-effectiveness and from the perspective of financial impact of RDT in patients, i.e. early diagnosis possibly leading to cost-saving in patients.

Methods

In this review, literature published in English up to September 2017 was covered. Scientific databases used for the search were: Embase, IBSS, Medline (including PubMed), and Web of Science. In order to take more caution and not miss articles that may imply on economic benefit of RDTs, the literature search was conducted in a comprehensive approach. In Pubmed, advance search was performed with search terms "((Dengue[Title]) AND cost)" OR "((Dengue[Title]) AND economic)" AND:

1. "rapid diagnostic test[MeSH Terms]"
2. "RDT[MeSH Terms]"
3. "rapid test[MeSH Terms]"
4. "rapid assay[MeSH Terms]"
5. "rapid diagnostic assay[MeSH Terms]"
6. "point-of-care [MeSH Terms]"
7. "POC[MeSH Terms]"
8. "point-of-care test[MeSH Terms]"

MeSH terms are assigned by indexers of the National Library of Medicine [47]. While the search on Pubmed

was performed with above search terms with "rapid diagnostic test" and "point-of-care test" were used as MeSH terms, additional articles were identified through IBSS, EMBASE, and Web of Science via general search using keywords:

1. "dengue and rapid diagnostic test (or RDT) and cost"
2. "dengue and rapid diagnostic test (or RDT) and economic"
3. "dengue and point-of-care (or POC) and cost"
4. "dengue and point-of-care (or POC) and economic".

From Embase, Web of Science, and WHOLIS, outcomes of general search included meeting abstracts in addition to full articles. Preliminary screening needed to be done for search outcomes through Embase and Web of Science, as their general search led to journals, not articles, where each key word may appear in different articles.

After such preliminary screening was done for search outcomes through Embase and Web of Science, title screening, abstract review, and full-text review were done. The development of this literature review is shown in the flow chart (Fig. 1). Rationales for excluding articles obtained through this multiple searches using different sources were described in Fig. 1. Exclusion criteria were not relevant articles that:

- mainly report on cost associated with a new diagnostic technology
- report on different technologies or performance of the tests without addressing cost or economic aspect of RDT use
- report on RDT-confirmed dengue case numbers in a study with insufficient information on economic impact

Also included for full-text review were those describing, in addition to those with direct reporting of quantitative costs, some qualitative economic benefit, i.e. mention of cost-effectiveness of RDTs without any quantitative valuation of it. This was done to prevent loss of any articles containing cost-related implication, even if not quantitatively specified in the article. Data were then extracted from the full texts of the selected articles. The data extraction table was developed following the Consolidated Health Economic Evaluation Reporting Standards (CHEERS) statement and the reporting Checklist [48]. Also, the reporting Checklist for Cost-effectiveness Analyses from Second Panel on Cost-Effectiveness in Health and Medicine (Additional file 1: Table S1) [48, 49]. In addition, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were followed [48, 50]. This review is registered in the PROSPERO international prospective register of systematic reviews, under the title "Systematic review of health economic assessments of dengue rapid diagnostic tests" (registry number: CRD42015017775). Full text review was performed independently by two individuals.

Results

As shown in Fig. 1, a more focused advanced search on Pubmed resulted in 11 articles and, after preliminary screening of the outcomes of general search on EMBASE and Web of Science, there were 15 published works (2 articles from EMBASE and 14 titles from Web of Science with one in overlap). After removal of duplicative articles, 21 titles from all three sources underwent screening. For abstract review, 10 articles and 2 meeting abstracts were considered relevant and among these 5 articles were selected for full-text review and 1 meeting abstract was retained and, as an abstract, skipped the full-text review step.

On review of the full text of the articles, it was found that only two studies reported quantitative or qualitative economic impact of RDT use for dengue: one by Lubell et al. and another by Mitra et al. The second study was reported in an abstract and an article, the former being published first [51, 52]. As the abstract was referring to the same study results as the articles, they were merged in the data extraction stage and were presented as one combined piece of work. Some others found with "cost" or "economic" as one of the key search terms and reached the full-text review stage were proven to contain information on the cost aspects of dengue RDTs. However, some were found to report on the actual price of the test or cost of production of a new assay as they assess performance of it. The extracted data and findings from these two studies are included in Additional file 1: Tables S1, 2a, and 2b [51–53].

The article (2016) and meeting abstract (2014) by Mitra et al. report that Panbio RDT alone is highly sensitive and cost effective for diagnosis of dengue infection in their comparative evaluation of performance and cost-effectiveness of commercially available immunochromatography-based RDT kits [51, 52]. This study is not, in fact, a cost-effectiveness analysis and reports a high sensitivity (97%) for Panbio RDT and a cost of 13.6 USD (reported in the abstract in 2014, 6.90 USD in the article in 2016) which they refer to as being cost-effective without clearly defining the basis of measurements, i.e. denominator [51, 52]. They compared four commercially available RDTs [Panbio Dengue Duo cassette, Standard Diagnostics (SD) Bioline Dengue Duo, J. Mitra Dengue Day-1 test and Reckon Dengue IgG/IgM] against composite reference criteria (CRC), and compared the cost of the tests. The authors conducted this study among stored blood samples from 281 patients who sought care for acute febrile illness at Christian Medical College (CMC) hospital in Vellore,

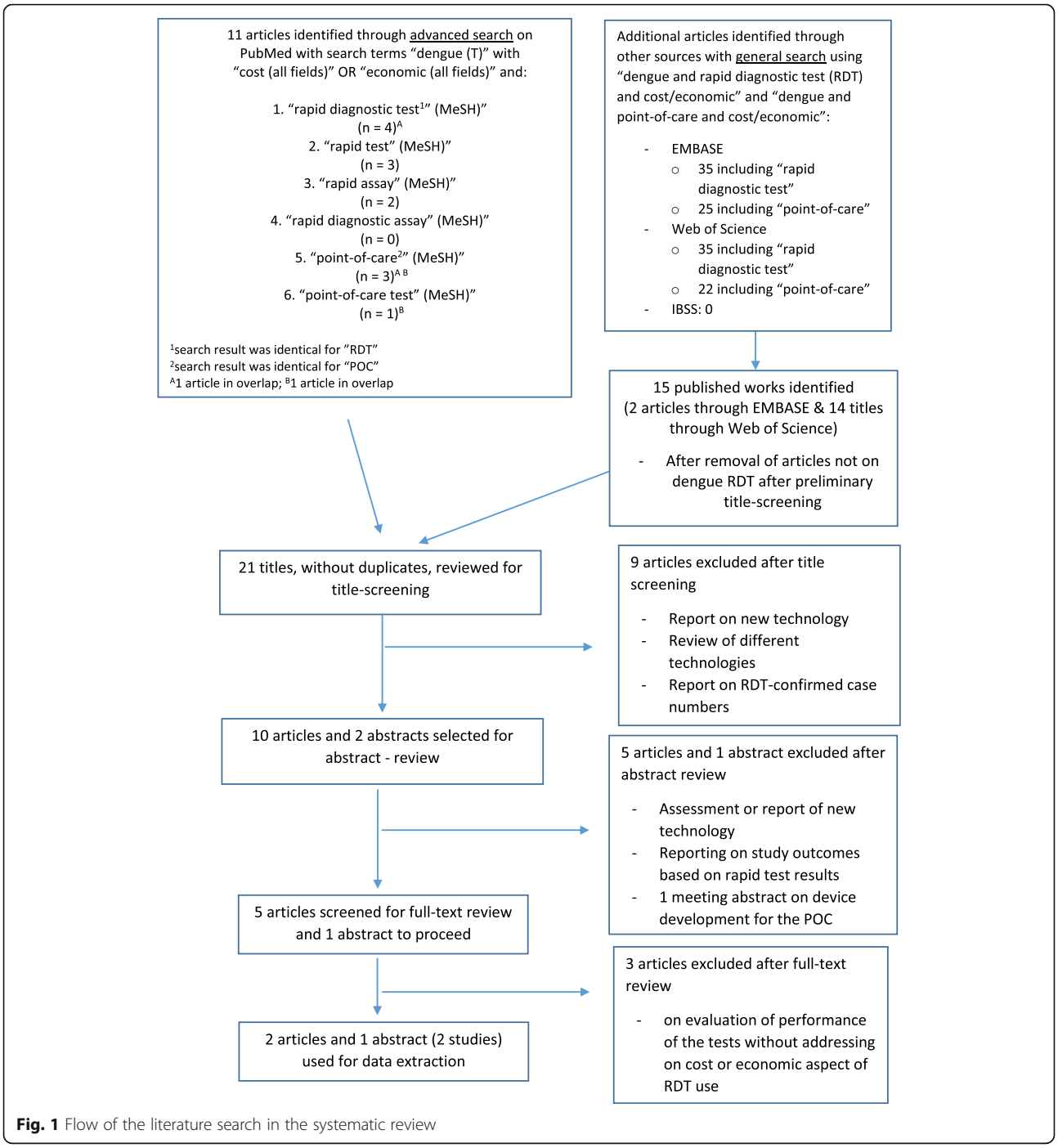


Fig. 1 Flow of the literature search in the systematic review

India [52]. The CRC was locally developed by infectious diseases expert, virologist, epidemiologist, reflecting WHO guidelines and this was used to identify dengue cases while lab-confirmed etiology of other cases of fever was needed to identify non-dengue controls. The authors measured sensitivity, specificity, and predictive values of these commercial RDTs in dengue cases and non-dengue controls. Based on IgM capture positivity of the four selected RDT kits, Panbio test was found to have the highest sensitivity, followed by SD Duo (97.7 and 64.3% respectively) [52]. However, specificity was higher for the Reckon RDT and SD Duo at 99.3 and 96.6%, respectively, compared to Panbio at 87.8%. Based on NS1 antigen capture assay, none were found to show satisfactory results in terms of sensitivity, while specificity was high, around 90% [52]. Therefore, even though the cost of Panbio test was the highest at 6.90 USD (in 2016) compared to the rest three ranging

between 3.29 to 4.27 USD, it was concluded that IgM assay by Panbio would be the test of choice and a cost-effective option for diagnosis of acute dengue infection in endemic settings.

An economic evaluation based on cost-effectiveness modeling by Lubell et al. (2016) reported that use of a dengue RDT is found to be not advantageous, more costly and less effective, when compared to the common practice of presumptive treatment with antibiotics prescription [53]. The authors developed a model to measure the impact and cost-effectiveness of testing for elevated C-reactive protein (CRP), compared with RDTs for dengue and scrub typhus in the management of undifferentiated fever. They used data from 1083 outpatients between 5 and 49 years of age from three provincial hospitals in rural Laos [53]. A decision tree model was developed to determine cost effectiveness of different testing approaches for undifferentiated fever and measure the ability of dengue and scrub typhus rapid tests, compared with testing for elevated CRP, to inform antibiotic treatment as currently practiced in clinical settings. The authors assumed sensitivity and specificity of a dengue RDT to be 95% and conducted economic evaluation to calculate the median incremental cost, the number of disability adjusted life years (DALYs) averted, and incremental cost-effectiveness ratios (ICER) for each strategy compared to the current practice of antibiotics prescription. For this, the model adopted assumptions in sensitivity and specificity of tests, costs of tests, the cost of a course of antibiotic, duration of all self-limiting viral infections and treated bacterial infection, as well as duration of bacterial infections that do not receive an appropriate treatment, mortality rate, a mean loss of life-years for a case of death, etc. Another important parameter in the model was incidence. The authors used incidence estimates of different pathogens to calculate proportion of patients who were given antibiotics for bacterial infections and proportion of those given antibiotics for viral infections. Furthermore, variable level of incidence between half to double of what was found in the fever study was applied in the model to test robustness of model outcomes. The model output reported that a dengue RDT is dominated by current practice, with a higher cost (median incremental cost = $1.5, Crl: 0.5; 3.2) and fewer numbers of DALYs averted (−0.006 DALYs, CrI: −0.301; 0.089) on average.

Discussion

The hypothesis behind this review was that prompt detection of dengue in the early phase of illness using RDTs may lead to economic benefit in terms of patients' cost of illness. The review was from both the point of view of cost effectiveness of RDT and the perspective of financial impact of RDT. We found two studies with different conclusions [51–53]. Two studies were heterogenous in terms of design — cost-effectiveness modelling or comparative evaluation of performance of RDTs. They both took place in dengue-endemic locations, in India and in Laos, over different time periods between 2008 and 2013 [51–53]. In both studies, the authors acknowledged limited generalizability to other populations of febrile patients, possibly due to specific epidemiological characteristics of each study area [51–53]. Epidemiological profiles, such as a varying level of sero-prevalence and likely high proportion of secondary infections [42], and particular serotype profiles [54, 55] could affect performance of RDTs for detection of dengue.

In the comparative evaluation of performance of RDTs, the authors concluded that Panbio RDT is cost-effective. Performance of IgM assay by Panbio was the most satisfactory in the diagnosis of acute dengue infection and the cost of the test was acceptable. This was although the cost based on the manufacturer's quoted price in India for Panbio was the highest at 6.90 USD compared to the rest three: SD, Reckon and J. Mitra at US$ 4.27, 3.29 and 3.61, respectively. The authors also explored different combinations. When NS1 antigen capture positivity alone was considered, all three tests (Panbio is IgM assay only) showed sensitivity below 30% while specificity was satisfactory, higher than 90% for all three tests. Thus, the authors concluded the NS1-based test to be unreliable. Also, the authors explored changes in performance when combined tests were used. Paired with Panbio RDT, other three RDTs only marginally increased the sensitivity while combination of Reckon with any of the three RDTs was found to increase specificity to higher than 99%. However, such combined testing would double the cost. Thus, the authors concluded that Panbio IgM-based RDT alone would be a cost-effective and sensitive option especially during the times of outbreak in dengue-endemic settings [51].

The main limitation of the study is that the RDT performance was not compared with other standard tests, such as NS1 or IgM capture based ELISA or RT-PCR. There are standard ways of laboratory-based confirmation of dengue infection using various assays that are available. While the authors indicate that using CRC as case definition is commonly done, such an assessment of RDT performance may not be most accurate. Also, as acknowledged by the authors, the study results could have been affected by cross-reactivity with other flaviviruses circulating in the study area [52]. Also, dengue RDTs are commonly used especially in the areas of high incidence of dengue [56]. However, when the study measured prevalence of dengue, the authors found 15.9 to 49.3% of IgG positivity among the samples in the study and it was comparatively lower than prevalence of IgG positivity previously measured by other studies. If prevalence of dengue or other flaviviruses is lower than what was previously estimated, then performance of the RDTs

would have been different in cases of low-level transmission of dengue or other flaviviruses.

Based on an economic evaluation using cost-effectiveness modeling, Lubell et al. showed that the a dengue RDT would provide little or no advantage in terms of health outcomes among patients with AFI while resulting in higher costs than current practice of antibiotics prescription [53]. As well as a dengue RDT, they had also modeled cost-effectiveness of a scrub typhus RDT and CRP test. For these two, the model showed that there are advantages over current practice of antibiotics prescription while cost would increase. There may be limited generalizability of the model outcomes, due to some of specific assumptions used in the model for this particular study sample obtained from Laos. For example, the years of life lost per death was assumed to be 45 years, based on the median age of outpatients and life expectancy in Laos. For the costs of tests, a gamma distribution was applied with a mean of $1.5, which may be lower than the current price of commonly used RDTs. The study was conducted in an outpatient-sample where dengue was confirmed in about slightly higher than 10% of the patients. While the study explored how the model outcomes would change if the incidence of dengue were to be variable between 50 and 200% of what was found in the fever study in Laos and found out that still CRP test would outperform both RDTs for dengue and scrub typhus, the study does not report how higher incidence of dengue will impact the median incremental cost and median DALYs averted by using dengue RDTs.

Also, the authors acknowledged limitations due to diagnostic uncertainty where multiple pathogens are detected for some patients whereas some others had no identifiable pathogen as the cause of illness. Depending on misclassification due to diagnostic limitations, there may be changes in economic benefit of dengue RDTs. Another limitation of the study was that the model does not consider societal impact of such viral infections where use of dengue RDTs may not be immediately cost-effective, but diagnosis based on dengue RDTs may provide benefit by raising awareness for signs of severe manifestation of illness or alerting health authorities of outbreaks for preventive and control measures, etc.

The authors, qualitatively, report that there would be improvements to current practice of antibiotics prescription whereby a dengue RDT would be used to prevent antibiotics prescribed to patients with viral infections [53]. Although not measured, there are long-term benefits of vigilant antibiotics prescription where dengue RDTs could be used for non-dengue confirmation to prompt antibiotics prescription, leading to a higher probability of bacterial infections receiving appropriate treatment. If these societal impact and long-term indirect benefits were considered in economic evaluation, dengue RDTs may be associated with higher cost-effectiveness than what was predicted in the current model.

The main assumption behind the topic of this review was the prompt detection of dengue in the early phase of illness using RDTs leading to economic impact, with both perspectives of cost-effectiveness and financial benefit. There are RDTs that detect IgA, IgM or IgG antibodies, as well as NS1 antigen [39]. Depending on the detection methods, the utility of these RDTs may be quite different and there can be variable performance characteristics. Only the study by Mitra et al. used commercially available RDTs for comparison, and Lubell et al. conducted a modeling analysis using a hypothetical RDT for dengue with 95% sensitivity and specificity in the model assumption. With limited evidence, such comparison among different test methods (or kits) could not be made in this review [42, 57]. Also, RDT performance could vary depending on factors such as the type of infection (primary vs. secondary infection), the time since onset of illness, and the serotype. It was assumed that the decision to use RDTs and refer to the test result for diagnosis and to guide clinical management would be at discretion of clinicians. Although there were no data reporting such findings, different RDTs' variable range of performance and accuracy could lead to misclassification in terms of dengue diagnosis. And this could affect the test performance and lead to bias by under or over-estimating the economic impact of early detection of dengue. Limited by data availability and lack of assurance on the direction of bias, these factors influencing performance were not considered in this literature review.

With 2.5 billion people at risk, efforts to develop vaccine and other preventive tools continue, but dengue remains a substantial burden to the healthcare system and society in the endemic countries. [7, 58]. The total annual global cost of dengue illness was estimated at US$8·9 billion and in a large country like Brazil, it is reported that the estimated cost for dengue for the epidemic season in the societal perspective would reach as high as US$ 1212 million after adjusting for under-reporting [28, 58, 59]. In a study reviewing medical costs associated with case management for dengue fever patients in Mexico, real costs for patients, reported to the Secretariat of Health, were US$33 for outpatients, and US$491 for inpatients [60]. How burdensome dengue treatment costs would be to households was shown in a study conducted in Cambodia where survey results were compared in households with dengue positive and the ones with dengue-negative children [61]. On average, the total cost of lab-confirmed dengue was 31.5 USD and the total cost per hospitalized dengue case was 40.1 USD [61]. To finance the cost of a febrile illness, 67% of households incurred an average debt of 23.5 USD [61]. Compared to an average one-week expenditure on food in Cambodia, about 9.5 US dollars per household, the

costs of treatment for dengue, whether outpatient or hospitalized, put enormous financial strain on the household [61].

Given this burden and financial strain placed by dengue on the health system, as well as individuals and households, many of the articles reviewed acknowledge the need for accurate and simple diagnostic assays for infection in resource-limited settings in regions of high dengue endemicity. However, we have found only two studies with different conclusions reached: one concluded that Panbio RDT at 6.90 USD was cost-effective; the other concluded that a dengue RDT is associated with negative DALYs averted while resulting in higher costs than current practice of antibiotics prescription. The two studies differ in design and findings cannot be directly compared. With no additional studies that explicitly estimated the cost-effectiveness of RDTs for dengue other than these two studies, such assessments must await future studies for more conclusive evidence. Likewise, any economic impact of RDT use in clinical settings, for patients, to health systems, and for particular situations such as outbreaks, remains to be assessed. Such work would guide appropriate interventions to improve patient management in resource-limited settings to reduce the burden of dengue.

Conclusions

Existing studies of dengue RDTs are largely epidemiological and we found two studies which reported quantitative and qualitative economic impact of their use. However, these two studies reported different conclusions and there is a need for new studies to specifically measure economic impact of dengue RDTs. Such studies would yield greater understanding of the benefit of RDTs for dengue and hence could help reduce the costs incurred due to dengue illness.

Additional file

Additional file 1: Table S1. Basic information and introduction of the articles. **Table S2A.** Data extracted from the articles (study methods). **Table S2B.** Data extracted from the articles (results and discussion). (DOCX 25 kb)

Abbreviations

AFI: Acute febrile illness; CHEERS: Consolidated Health Economic Evaluation Reporting Standards; CRP: C-reactive protein; DALYs: Disability adjusted life years; DENV: Dengue virus; ELISA: Enzyme-linked immunosorbent assay; ICER: Incremental cost-effectiveness ratios; IgM/IgG: Immunoglobulin type M and type G; NS1: Nonstructural protein 1; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PRNT: Plaque Reduction Neutralization Test; RDTs: rapid diagnostic tests; RT-PCR: Reverse Transcriptase-Polymerase Chain Reaction

Acknowledgements

We would like to thank the DVI team at the International Vaccine Institute and the team at Faculty of Epidemiology and Public Health at London School of Hygiene and Tropical Medicine for their helpful comments during the preparation of this manuscript and support during the study.

Funding

This literature review project did not require any funding. NA receives support from the United Kingdom Medical Research Council (MRC) and Department for International Development (DFID) (MR/K012126/1).

Availability of data and materials

Data sharing is not applicable to this article as no datasets were analyzed during the current study.

Authors' contributions

JKL designed the study, conducted the review, and was a major contributor in writing the manuscript. GLDT co-designed the study and conducted the review. NA was a contributor in designing of the study and supported in writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study did not involve human participants or human data. Therefore, the study did not involve consenting of participants and did obtain ethical approvals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details

[1]Global Dengue and Aedes-transmitted Diseases Consortium (GDAC), International Vaccine Institute (IVI), SNU Research Park, Gwankak-ro 1, Seoul, Gwanak-gu 151-191, South Korea. [2]Epidemiology and Public Health Department, London School of Hygiene and Tropical Medicine, London, UK. [3]Centre for Primary Care and Public health, Queen Mary University of London, London, UK.

Received: 9 May 2017 Accepted: 11 December 2017
Published online: 29 December 2017

References

1. Alphey N, Alphey L, Bonsall MB. A model framework to estimate impact and cost of genetics-based sterile insect methods for dengue vector control. PLoS One. 2011;6(10):e25384.
2. Clark DV, Mammen MP Jr, Nisalak A, Puthimethee V, Endy TP. Economic impact of dengue fever/dengue hemorrhagic fever in Thailand at the family and population levels. Am J Trop Med Hyg. 2005;72(6):786–91.
3. Endy TP, Yoon IK, Mammen MP. Prospective cohort studies of dengue viral transmission and severity of disease. Curr Top Microbiol Immunol. 2010;338:1–13.
4. Stephenson JR. The problem with dengue. Trans R Soc Trop Med Hyg. 2005;99(9):643–6.
5. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–7.
6. WHO: Global Strategy for Dengue Prevention and Control: 2012–2020 WHO report In. 2012.
7. Brady OJ, Gething PW, Bhatt S, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6(8):e1760.
8. Chang M, Christophel E, Gopinath D. Abdur R, on behalf of malaria OVaPD, World Health Organization regional Office for the Western Pacific: challenges and future perspective for dengue vector control in the western Pacific region, regional analysis. Western Pacific Surveill Response J. 2011;2(2):9–16.
9. Eisen L, Beaty B, Morrison A, Scott T. ProactiveVector control strategies and improved monitoring and evaluation practices for dengue prevention. J Med Entomol. 2009;46(6):1245–55.
10. Villar L, Dayan G, Arredondo-García J, Rivera D, Cunha R, Deseda C, Reynales H, Costa M, Morales-Ramírez J, Carrasquilla G, et al. Efficacy of a tetravalent

dengue vaccine in children in Latin America. N Engl J Med. 2015;372(2): 113–23.
11. Capeding M, Tran N, Hadinegoro S, Ismail H, Chotpitayasunondh T, Chua M, Luong C, Rusmil K, Wirawan D, Nallusamy R, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet. 2014; 384(9951):1358–65.
12. Larano C. Sanofi's Dengue Vaccine Made Widely Available for First Time - Philippines plans to immunize schoolchildren starting in April World 2016; http://www.wsj.com/articles/dengue-vaccine-made-widely-available-for-first-time-1456247746. Accessed May 31, 2016.
13. Sanofi Pasteur. First Dengue Vaccine Approved in More than 10 Countries. Focus on Dengue - Press releases 2016; http://www.sanofipasteur.com/en/articles/first_dengue_vaccine_approved_in_more_than_10_countries.aspx. Accessed Oct. 31, 2017.
14. Osorio L, Uribe M, Ardila G, Orejuela Y, Velasco M, Bonelo A, Parra B. The use of rapid dengue diagnostic tests in a routine clinical setting in a dengue-endemic area of Colombia. Mem Inst Oswaldo Cruz. 2015;110(4):510–6.
15. Blacksell SD, Bell D, Kelley J, Mammen MP Jr, Gibbons RV, Jarman RG, Vaughn DW, Jenjaroen K, Nisalak A, Thongpaseuth S, et al. Prospective study to determine accuracy of rapid serological assays for diagnosis of acute dengue virus infection in Laos. Clin Vaccine Immunol. 2007;14(11):1458–64.
16. Peeling RW, Artsob H, Pelegrino JL, et al. Evaluation of diagnostic tests: dengue. Nature Reviews Microbiology. 2010;8:S30.
17. Lam SK. Two decades of dengue in Malaysia. Nagasaki Univ Bull Paper Dept Tropical Med. 1994;35(4):195–200.
18. Chen CD, Seleena B, Nazni WA, Lee HL, Masri SM, Chiang YF, Sofian-Azirun M. Dengue vectors surveillance in endemic areas in Kuala Lumpur city Centre and Selangor state, Malaysia. Dengue Bulletin. 2006;30:197–203.
19. da Silva LGA, Gurgel AM, Costa AM, et al. Aedes aegypti control in Brazil. The Lancet. 2016;387(10023):1052–1053.
20. Undurraga E, Betancourt-Cravioto M, Ramos-Castañeda J, Martínez-Vega R, Méndez-Galván J, Gubler D. Economic and Disease Burden of Dengue in Mexico. . PLoS Negl Trop Dis 2015;9(3):e0003547.
21. Anderson KB, Chunsuttiwat S, Nisalak A, Mammen MP, Libraty DH, Rothman AL, Green S, Vaughn DW, Ennis FA, Endy TP. Burden of symptomatic dengue infection in children at primary school in Thailand: a prospective study. Lancet. 2007;369(9571):1452–9.
22. Okanurak K, Sornmani S, Indaratna K. The cost of dengue hemorrhagic fever in Thailand. Southeast Asian J Trop Med Public Health. 1997;28(4):711–7.
23. Shepard DS, Undurraga EA, Halasa YA. Economic and disease burden of dengue in Southeast Asia. PLoS Negl Trop Dis. 2013;7(2):e2055.
24. Taliberti H. Zucchi P: [direct costs of the dengue fever control and prevention program in 2005 in the City of Sao Paulo]. Rev Panam Salud Publica. 2010;27(3):175–80.
25. de Araujo JM, Schatzmayr HG, de Filippis AM, Dos Santos FB, Cardoso MA, Britto C, Coelho JM, Nogueira RM. A retrospective survey of dengue virus infection in fatal cases from an epidemic in Brazil. J Virol Methods. 2009; 155(1):34–8.
26. Castaneda-Orjuela C, Diaz H, Alvis-Guzman N, et al. Burden of Disease and Economic Impact of Dengue and Severe Dengue in Colombia, 2011. Value in Health Regional Issues. 1 (2) (pp 123-128), 2012. Date of Publication: December 2012.; 2012.
27. Shepard D, Undurraga E, Halasa Y, Stanaway J. The global economic burden of dengue: a systematic analysis. Lancet Infect Dis. . 2016;S1473(3099(16)): 146-148.
28. Martelli C, Siqueira JJ, Parente M, et al. Economic Impact of Dengue: Multicenter Study across Four Brazilian Regions. PLoS Negl Trop Dis. 2015; 9(9):e0004042.
29. Constenla D, Garcia C, Lefcourt N. Assessing the Economics of Dengue: Results from a Systematic Review of the Literature and Expert Survey. Pharmacoeconomics. 2015;33(11):1107-1135.
30. US CDC. Laboratory Guidance and Diagnostic Testing. Dengue>Clinical/Laboratory Guidance 2010. Accessed April 6, 2017.
31. Sa-ngasang A, Wibulwattanakij S, Chanama S, O-rapinpatipat A, A-nuegoonpipat A, Anantapreecha S, Sawanpanyalert P, Kurane I. Evaluation of RT-PCR as a tool for diagnosis of secondary dengue virus infection. Jpn J Infect Dis. 2003;56:205–9.
32. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martinez E, et al. Dengue: a continuing global threat. Nat Rev Microbiol. 2010;8(Suppl 12):S7–16.
33. Buchy P, Yoksan S, Peeling RW, Hunsperger E. Laboratory Tests For The Diagnosis Of Dengue Virus Infection. In: in WHOobotSPfRaT, Tropical Diseases, eds. Scientific Working Group, Report on Dengue, 1-5 October 2006, Geneva, Switzerland 2007.
34. Thomas SJ, Nisalak A, Anderson KB, Libraty DH, Kalayanarooj S, Vaughn DW, Putnak R, Gibbons RV, Jarman R, Endy TP. Dengue plaque reduction neutralization test (PRNT) in primary and secondary dengue virus infections: how alterations in assay conditions impact performance. Am J Trop Med Hyg. 2009;81(5):825–33.
35. Putnak J, de la Barrera R, Burgess T, Pardo J, Dessy F, Gheysen D, Lobet Y, Green S, Endy T, Thomas S, et al. Comparative evaluation of three assays for measurement of dengue virus neutralizing antibodies. Am J Trop Med Hyg. 2008;79(1):115–22.
36. Liu L, Wen K, Li J, Hu D, Huang Y, Qiu L, Cai J, Che X. Comparison of plaque- and enzyme-linked immunospot-based assays to measure the neutralizing activities of monoclonal antibodies specific to domain III of dengue virus envelope protein. Clin Vaccine Immunol. 2012;19(1):73–8.
37. Kao CL, King CC, Chao DY, Wu HL, Chang GJ. Laboratory diagnosis of dengue virus infection: current and future perspectives in clinical diagnosis and public health. J Microbiol Immunol Infect. 2005;38(1):5–16.
38. Blacksell SD. Commercial Dengue Rapid Diagnostic Tests for Point-of-Care Application: Recent Evaluations and Future Needs? Journal of Biomedicine and Biotechnology. 2012;2012(Article ID 151967):12 pages.
39. Krishnananthasivam S, Fernando A, Tippalagama R, Tennekoon R, De Man J, Seneviratne D, Premawansa S, Premawansa G, De Silva A. Evaluation of a commercial rapid test kit for detection of acute dengue infection. Southeast Asian J Trop Med Public Health. 2015;46(4):602–10.
40. Hunsperger E, Sharp T, Lalita P, Tikomaidraubuta K, Cardoso Y, Naivalu T, Khan A, Marfel M, Hancock W, Tomashek K, et al. Use of a rapid test for diagnosis of dengue during suspected dengue outbreaks in resource-limited regions. J Clin Microbiol. 2016;54(8):2090–5.
41. Blacksell SD, Jarman RG, Bailey MS, Tanganuchitcharnchai A, Jenjaroen K, Gibbons RV, Paris DH, Premaratna R, de Silva HJ, Lalloo DG, et al. Evaluation of six commercial point-of-care tests for diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. Clin Vaccine Immunol. 2011;18(12):2095–101.
42. Teles FR, Prazeres DM, Lima-Filho JL. Trends in dengue diagnosis. Rev Med Virol. 2005;15(5):287–302.
43. Osorio L, Ramirez M, Bonelo A, Villar LA, Parra B. Comparison of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection. Virol J. 2010;7:361.
44. Guzman MGJT, Gaczkowski R, Ty Hang VT, Sekaran SD, Kroeger A, Vazquez S, Ruiz D, Martinez E, Mercado JC, Balmaseda A, Harris E, Dimano E, Leano PS, Yoksan S, Villegas E, Benduzu H, Villalobos I, Farrar J, Simmons CP. Multi-country evaluation of the sensitivity and specificity of two commercially-available NS1 ELISA assays for dengue diagnosis. PLoS Negl Trop Dis. 2010;4(8):e811.
45. Gan VCTL, Lye DC, Pok KY, Mok SQ, Chua RC, Leo YS, Ng LC. Diagnosing dengue at the point-of-care: utility of a rapid combined diagnostic kit in Singapore. PLoS One. 2014;9(3):e90037.
46. U.S. National Library of Medicine. Indexing with MeSH Vocabulary. PubMed Online Training 2001; https://www.nlm.nih.gov/bsd/disted/pubmedtutorial/015_030.html. Accessed 21 March, 2017..
47. Husereau D, Drummond M, Petrou S, Carswell C, Moher D, Greenberg D, Augustovski F, Briggs AH, Mauskopf J, Loder E, et al. Consolidated health economic evaluation reporting standards (CHEERS)–explanation and elaboration: a report of the ISPOR health economic evaluation publication guidelines good reporting practices task force. Value Health. 2013;16(2):231–50.
48. Sanders GD, Neumann PJ, Basu A, Brock DW, Feeny D, Krahn M, Kuntz KM, Meltzer DO, Owens DK, Prosser LA, et al. Recommendations for Conduct,Methodological practices, and reporting of cost-effectiveness analyses - second panel on cost-effectiveness in health and medicine. JAMA. 2016;316(10):1093–103.
49. Moher D, Liberati A, Tetzlaff J, Altman D, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Medicine. 2009;6(7):e1000097.
50. Mitra S, Choudhari R, Nori H, et al. Performance and cost-effectiveness of immunochromatography based rapid diagnostic test (RDT) kits in diagnosis of dengue infection in resource limited set up. Int J Infect Dis. 2014; 21(Suppl 1 Meeting Abstract: 64.018):450.

51. Mitra S, Choudhari R, Nori H, et al. Comparative evaluation of validity and cost-benefit analysis of rapid diagnostic test (RDT) kits in diagnosis of dengue infection using composite reference criteria: a cross-sectional study from south India. J Vector Borne Dis. 2016;53(1):30–6.
52. Lubell Y, Althaus T, Blacksell SD, et al. Modelling the impact and cost-effectiveness of biomarker tests as compared with pathogen-specific diagnostics in the Management of Undifferentiated Fever in remote tropical settings. PLoS One. 2016;11(3):e0152420.
53. Ngwe Tun M, Kyaw A, Makki N, Muthugala R, Nabeshima T, Inoue S, Hayasaka D, Moi M, Buerano C, Thwe S, et al. Characterization of the 2013 dengue epidemic in Myanmar with dengue virus 1 as the dominant serotype. Infect Genet Evol. 2016;43:31–7.
54. Kotaki T, Yamanaka A, Mulyatno K, Churrotin S, Sucipto T, Labiqah A, Ahwanah N, Soegijanto S, Kameoka M, Konishi E. Divergence of the dengue virus type 2 cosmopolitan genotype associated with two predominant serotype shifts between 1 and 2 in Surabaya, Indonesia, 2008-2014. Infect Genet Evol. 2016;37:88–93.
55. Acestor N, Cooksey R, Newton P, Menard D, Guerin P, Nakagawa J, et al. Mapping the aetiology of non-malarial febrile illness in Southeast Asia through a systematic review - terra incognita impairing treatment policies. PLoS One. 2012;7(9):e44269.
56. Hang VT, Nguyet NM, Trung DT, Tricou V, Yoksan S, Dung NM, Van Ngoc T, Hien TT, Farrar J, Wills B, et al. Diagnostic accuracy of NS1 ELISA and lateral flow rapid tests for dengue sensitivity, specificity and relationship to viraemia and antibody responses. PLoS Negl Trop Dis. 2009;3(1):e360.
57. Simmons CP, Farrar JJ. Chau NvV, Wills B: Dengue. N Engl J Med. 2012;
58. Shepard D, Undurraga E, Halasa Y, Stanaway J. The global economic burden of dengue: a systematic analysis. Lancet Infect Dis. 2016;16(8):935–41.
59. Lee JS, Mogasale V, Lim JKC, Mabel, et al. A Multi-country Study of the Household Willingness-to-Pay for Dengue Vaccines: Household Surveys in Vietnam, Thailand, and Colombia. PLoS Negl Trop Dis 2015;9(6).
60. Zubieta-Zavala A, Salinas-Escudero G, Ramírez-Chávez A, et al. Calculation of the Average Cost per Case of Dengue Fever in Mexico Using a Micro-Costing Approach. PLoS Negl Trop Dis. 2016;10(8):e0004897.
61. Huy R, Wichmann O, Beatty M, Ngan C, Duong S, Margolis H, Vong S. Cost of dengue and other febrile illnesses to households in rural Cambodia: a prospective community-based case-control study. BMC Public Health. 2009;9:155.

3 Methods

Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	LSH1405874	**Title**	Ms.
First Name(s)	Jacqueline Kyungah		
Surname/Family Name	LIM		
Thesis Title	Undocumented burden of dengue in Africa		
Primary Supervisor	Prof. Neal Alexander		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMJ Open		
When was the work published?	January 2018		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am first author. I developed the grant proposal that was successful in obtaining funds for the studies described in this protocol paper. I wrote the generic protocol of the DVI field studies and further developed it for the protocol of the studies in Africa. I wrote the complete first draft of this protocol manuscript, drawing on the study protocol. I, as the first author, led the process of manuscript preparation, revision, and submission. The study PIs are authors (Sammy M Njenga, Selidji Todagbe Agnandji, Seydou Yaro). Those that were involved in protocol development and participated in study set-up and data collection on site are also authors (Mabel Carabali, Jung-Seok Lee, Kang-Sung Lee, Suk Namkung, Sl-Ki Lim, Valery Ridde, Jose Fernandes, Bertrand Lell, Sultani Hadley Matendechero, Meral Esen, Esther Andia, Noah Oyembo, Ahmed Barro, and Emmanuel Bonnet). Neal Alexander, as my PhD supervisor, and In-Kyu Yoon, the Director of DVI, provided oversight and guidance on the overall study design, project execution, and specific methodologies, e.g. epidemiology, statistical, and virology and other laboratory methods.

SECTION E

Student Signature	[redacted]
Date	23 June 2019

Supervisor Signature	[redacted]
Date	17 June 2019

BMJ Open

Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative

Jacqueline Kyungah Lim,[1,2] Mabel Carabali,[1,3] Jung-Seok Lee,[4] Kang-Sung Lee,[4] Suk Namkung,[1] Sl-Ki Lim,[1] Valéry Ridde,[5] Jose Fernandes,[6] Bertrand Lell,[6] Sultani Hadley Matendechero,[7] Meral Esen,[8] Esther Andia,[9] Noah Oyembo,[9] Ahmed Barro,[10] Emmanuel Bonnet,[11] Sammy M Njenga,[9] Selidji Todagbe Agnandji,[6] Seydou Yaro,[12] Neal Alexander,[2] In-Kyu Yoon[1]

To cite: Lim JK, Carabali M, Lee J-S, *et al*. Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative. *BMJ Open* 2018;**8**:e017673. doi:10.1136/bmjopen-2017-017673

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2017-017673).

Received 10 May 2017
Revised 25 September 2017
Accepted 18 October 2017

For numbered affiliations see end of article.

Correspondence to
Jacqueline Kyungah Lim;
kalim@ivi.int

ABSTRACT

Introduction Dengue is an important and well-documented public health problem in the Asia-Pacific and Latin American regions. However, in Africa, information on disease burden is limited to case reports and reports of sporadic outbreaks, thus hindering the implementation of public health actions for disease control. To gather evidence on the undocumented burden of dengue in Africa, epidemiological studies with standardised methods were launched in three locations in Africa.

Methods and analysis In 2014–2017, the Dengue Vaccine Initiative initiated field studies at three sites in Ouagadougou, Burkina Faso; Lambaréné, Gabon and Mombasa, Kenya to obtain comparable incidence data on dengue and assess its burden through standardised hospital-based surveillance and community-based serological methods. Multidisciplinary measurements of the burden of dengue were obtained through field studies that included passive facility-based fever surveillance, cost-of-illness surveys, serological surveys and healthcare utilisation surveys. All three sites conducted case detection using standardised procedures with uniform laboratory assays to diagnose dengue. Healthcare utilisation surveys were conducted to adjust population denominators in incidence calculations for differing healthcare seeking patterns. The fever surveillance data will allow calculation of age-specific incidence rates and comparison of symptomatic presentation between patients with dengue and non-dengue using multivariable logistic regression. Serological surveys assessed changes in immune status of cohorts of approximately 3000 randomly selected residents at each site at 6-month intervals. The age-stratified serosurvey data will allow calculation of seroprevalence and force of infection of dengue. Cost-of-illness evaluations were conducted among patients with acute dengue by Rapid Diagnostic Test.

Ethics and dissemination By standardising methods to evaluate dengue burden across several sites in Africa, these studies will generate evidence for dengue burden in Africa and data will be disseminated as publication in peer-review journals in 2018.

Strengths and limitations of this study

- ► There have not been population-based studies conducted with a multidisciplinary approach (ie, surveillance, healthcare utilisation and serosurvey in one catchment area population). Data from the passive surveillance will be used to calculate annual incidences of dengue and data from the serosurvey will estimate the force of infection and prevalence.
- ► The studies were conducted in three locations in Africa, based on standardised methods and laboratory algorithm. Thus, comparison by site would be possible.
- ► This is not a cohort study. The passive facility-based surveillance may lead to underestimation of the burden of dengue fever by measuring incidence based on only those that sought care at our study facilities.
- ► There may be limited generalisability of our study results to other dengue-endemic parts of Africa.

BACKGROUND

Dengue fever, a mosquito-borne flavivirus infection caused by four related but antigenically distinct dengue viruses (DENVs, serotypes 1–4), is a major and rapidly increasing global public health problem. Recent studies have estimated an annual incidence of 50–100 million symptomatic infections globally.[1] Dengue is a high burden disease that disproportionately affects countries in the tropics and subtropics, many of which have limited healthcare resources.[2] Although one dengue vaccine has been recently licensed in several endemic countries, the vaccine has restricted age and epidemiological indications. Other prevention and control measures such as vector control are suboptimal as

stand-alone interventions,[3 4] and no drugs for treatment are currently available.

Like in Asia and the Americas, epidemics of dengue were reported from Africa in the late 19th and early 20th centuries.[5 6] Specifically for Africa, there are records of multiple dengue case reports between 1964 and 1968 with DENV 2 in Nigeria.[7] Data from several studies conducted in the 1960–1970s in Nigeria supported a substantially high level of immunity in adults as well as children.[8 9] In 2011, Amarasinghe *et al* conducted a comprehensive review of literature on dengue in Africa and described that dengue cases have been reported in 34 countries in Africa, with most of these countries also having *Aedes* mosquitoes.[6] However, prior studies which suggested the presence of dengue in Africa were limited by their retrospective design or sample collection (blood donors or sample collected from surveys of other diseases), and often from travellers, with a small number of reported autochthonous cases, to demonstrate the true, population-based, burden of dengue. Also, while many dengue endemic countries in Asia and Latin America have mandatory reporting of dengue cases to public health authorities and national surveillance systems in place to monitor incidence patterns,[10] most African countries lack such established reporting mechanisms and only sporadic outbreaks and individual case reports have been documented. In addition, the frequently non-specific clinical presentation of dengue may be difficult to distinguish from the myriad other infectious diseases present in Africa, since dengue diagnostic assays are not widely available. Thus, the burden of dengue remains largely unknown in Africa.[6 11] Without such dengue burden data, informed decision-making about prevention and control measures, including dengue vaccine introduction, in Africa are not possible.

Limited by surveillance capacity hindering continuous reporting in the region, there had not been frequent and systematic reporting of dengue in Africa. African ancestry is known to be protective against severe dengue and the candidate genes were recently identified in a Cuban patient.[12 13] Bhatt *et al*'s modelling of the global dengue burden suggests high burden in Africa in terms of equal numbers of infections (both apparent and inapparent) as in Latin America.[1] There are new findings about dengue in Africa, but there is still much unknown about the magnitude of the dengue problem in the continent. To improve estimates of population-based dengue disease burden in Africa and validate whether the undocumented burden of dengue is as high in Africa as in the Americas with empirical data, the Dengue Vaccine Initiative (DVI) initiated field studies at three sites in West (Ouagadougou, Burkina Faso), West-Central (Lambaréné, Gabon) and East Africa (Mombasa, Kenya). In each of the three sites, a standardised package of study components, including passive facility-based fever surveillance, healthcare utilisation surveys, cost-of-illness surveys and serological surveys (figure 1), was initiated between December 2014 and March 2016.

METHODS

Site selection

Study sites were selected, in part, based on their likelihood of supporting DENV transmission. To select sites, we considered dengue outbreaks and cases reports in the literature, available seroprevalence studies as well as country-specific dengue risk maps of the probability of DENV transmission and the level of evidence of dengue presence, reporting the uncertainty of the consensus estimates of dengue in Africa.[7 14] In addition, adequate research infrastructure to implement the studies was taken into account. Finally, inclusion of different regions of Africa was also a factor in site selection. Thus, Ouagadougou, Burkina Faso; Lambaréné, Gabon and Mombasa, Kenya were selected, respectively, to measure the burden of dengue in selected sites from West, (West-) Central and East Africa.

In Burkina Faso, the first reported dengue outbreak occurred in Ouagadougou in 1982 due to DENV-2.[6] Serological prevalence of dengue antibodies among pregnant women and blood donors was found to be 26.3% in a rural setting (Nouna village) and 36.5% in an urban setting (Ouagadougou) in 2006.[15] More recently, an observational study conducted by Ridde *et al* among febrile patients consulting at selected study facilities in 2013–2014 showed 8.7% (33/379) to be positive by dengue rapid diagnostic test (RDT) and 15 of 60 samples tested by RT-PCR to be dengue-positive.[16] With evidence for the presence of dengue, along with a strong health and demographic surveillance system (Ouaga-HDSS) which could be used to describe the demographic characteristics of the catchment area, a field study was initiated in Ouagadougou, Burkina Faso in December 2014.

In Gabon, cases of dengue haemorrhagic fever (DHF) caused by up to three different DENV serotypes have been reported, and dengue seroprevalence has been found to be between 5% and 20%.[17–19] Results of a recently published study demonstrated seroprevalence of 12.3% among toddlers approximately 30 months of age in semirural Lambaréné between 2007 and 2010.[20] However, a different study in 2005–2008 suggested minimal DENV transmission in rural areas of Gabon.[21] This latter study examined antibodies against dengue in individuals from randomly selected villages representing about 10% of all Gabonese villages. Blood samples were tested by anti-DENV IgG and IgM capture ELISA and found to have only minimal IgG (0.5%) and IgM (0.5%) seroprevalence. Based on these low prevalences, the authors concluded that there was no active circulation of DENV in rural Gabon. However, the low seroprevalence may have been affected by low sensitivities of the tests used, leading to a high rate of false negative results and/or selection bias in the blood sample pool among the selected villagers.[22] Seroprevalence estimates in the 2007/2010 study may have also been impacted by the possibility of false-positive results due to IgG cross-reactivity among flaviviruses.[21] Nevertheless, given the

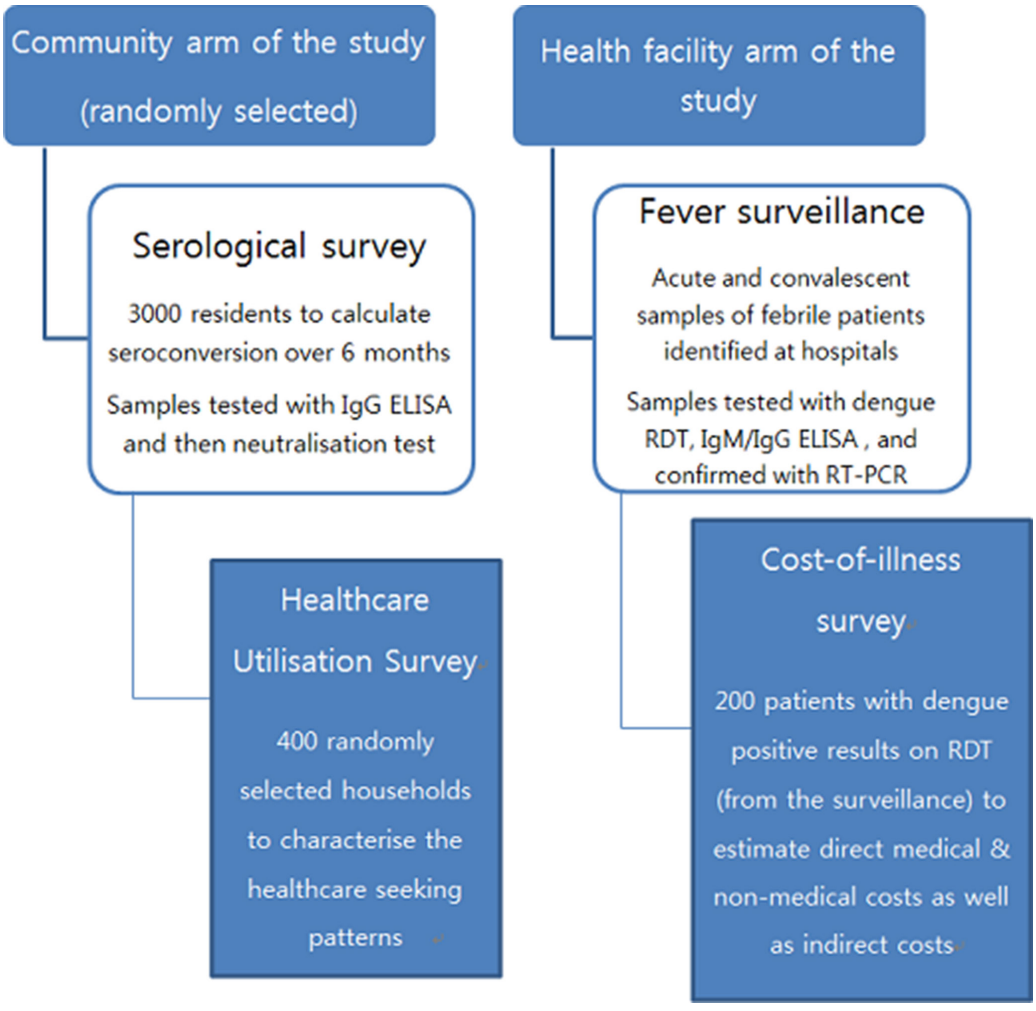


Figure 1 Description of the study components, including passive facility-based fever surveillance, healthcare utilisation surveys, cost-of-illness surveys and serological surveys. There are two arms in the study package, composed of four parts. In the health facility-based arm of the study package, there are passive facility-based fever surveillance and cost-of-illness survey embedded within the surveillance. In the community arm of the study, there are serological survey and healthcare utilisation survey.

possibility of DENV circulation in Gabon, a field study was initiated in Lambaréné in March 2015 in a community with a catchment population of about 77 000 residents, using the clinical research infrastructure of the Centre de Recherches Medicales de Lambaréné (CERMEL), benefiting from experienced research staff who conducted a large Phase 3 malaria vaccine trial.[23 24]

In Kenya, more evidence is available for the presence of dengue based on local data. Dengue was the most common viral pathogen in retrospectively tested blood specimens from HIV-negative survey samples from the 2007 Kenya AIDS Indicator Survey. Antibody testing for dengue as well as chikungunya and Rift Valley fever was performed by IgG ELISA using either commercial kits or CDC assays; 12.5% were found to be dengue-positive.[25] Similarly, a household survey found 13% of individuals from 701 households in Mombasa had serological evidence of either past or current DENV infection.[26] These data suggest that there is more dengue in Kenya than indicated by public health reporting, possibly due to misdiagnosis.[25 26] A field study was initiated in Mombasa, Kenya in March 2016.

Study participants

For the passive facility-based fever surveillance, individuals who met the following criteria were eligible for study enrolment:

1. Age 1–55 years old.
2. Resident of the catchment area covered by healthcare facilities participating in the study, without plans to move out of the catchment area within 12 months.
3. Signed informed consent and assent for those aged between 7 (13 for Kenya) and 17 years.
4. Patients presenting with current fever (axillary temperature ≥37.5°C) or history of fever for ≤7 days duration without localising signs (fever caused by a localised infection as well as fever with a known and confirmed aetiology other than dengue, such as malaria

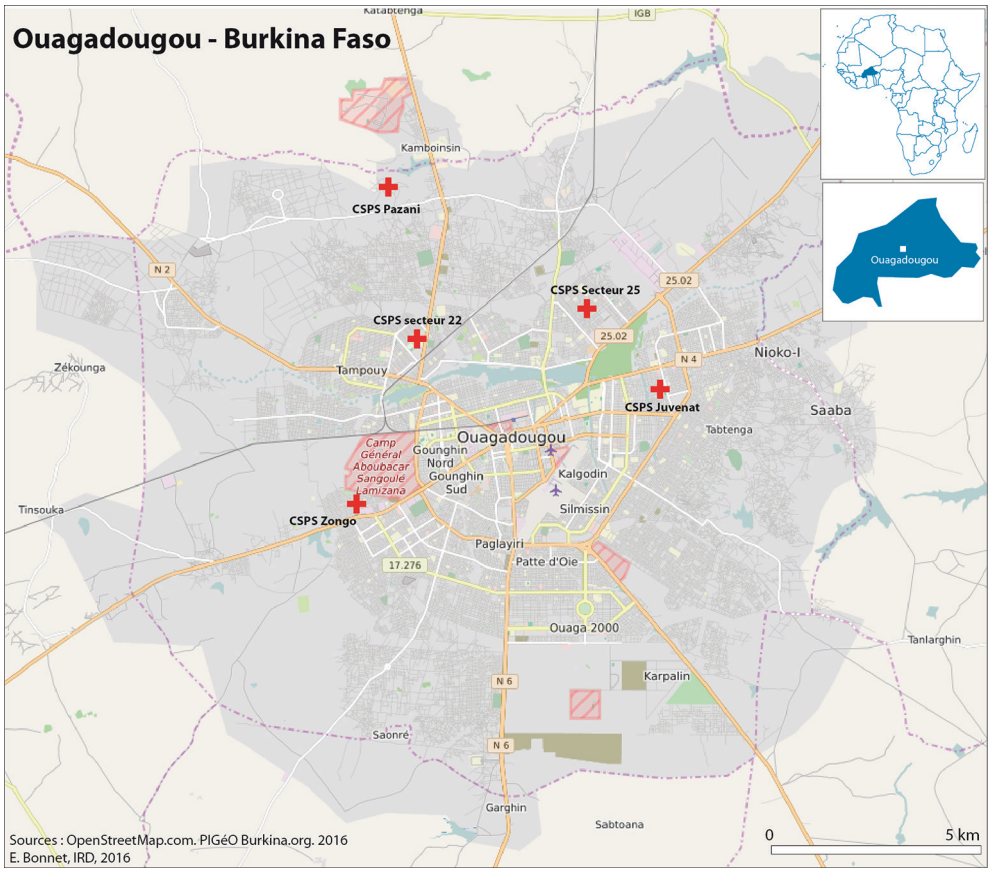


Figure 2 Map of the study area in Ouagadougou, Burkina Faso.

confirmed by malaria RDT, as listed in the patient identification standard operating procedure [SOP]).

For the serological survey, criteria 1–3 were applied. For the healthcare utilisation survey, household interviews were conducted among the heads or representatives of the household invited from each family participating in the serosurvey.

Study area and population

Burkina Faso, located in West Africa, has a population of 14 017 462. The country is mainly rural with about 29% of the population reported to be living in urban areas in 2014. However, Burkina Faso is urbanising rapidly and is positioned as the country with the fourth fastest urbanisation in the last 25 years.[27 28] The capital, Ouagadougou, has a population of 2 741 128. The majority of the population live in urban settings. About 45% of the population are under 15 years of age.[29] The city is divided into 12 districts and 52 sectors. Ouagadougou is the country's largest city and the cultural and economic centre. The city is part of the Soudano-Sahelian area, with a rainfall of about 800 mm per year. The rainy season is from May to October, with a mean temperature of 28°C (82°F). The cold season runs from December to January, with a minimum average temperature of 16°C (61°F). During the hot season, which runs from March to May, the temperature can reach as high as 43°C (109°F).

The HDSS is in place in Ouagadougou. Ouaga-HDSS monitors a population of 81 717 residents; according to this surveillance system, the city population is very stable with a rate of migration of 4.1% and more than 80% of the inhabitants with ownership of their houses [20]. A map of the city and the study area is shown in figure 2.

Gabon, located on the west coast of Central Africa, has an area of nearly 270 000 square kilometres (100 000 sq. mi) with a population estimated at 1.5 million. Its capital and largest city is Libreville. In 2014, it is reported that 87% of the Gabonese population lived in urban areas.[28] The sixth largest city, Lambaréné, the capital of Moyen-Ogooué province, is located 75 km south of the equator, with a population of 25 257 in 2009. The majority of Lambaréné residents live in semirural areas. About 42% of the Gabonese population is under 15 years of age.[29] Similarly, Lambaréné's population is relatively young with about 50% under 20 years of age.

The health services of Gabon are mostly public, but there are some private institutions as well. With one of the best medical infrastructure in the region, almost 90% of the population have access to healthcare services. Albert Schweitzer Hospital (ASH) is a private institution which served as a study site for the passive fever surveillance study.[30 31] The study area in Lambaréné is shown in figure 3.

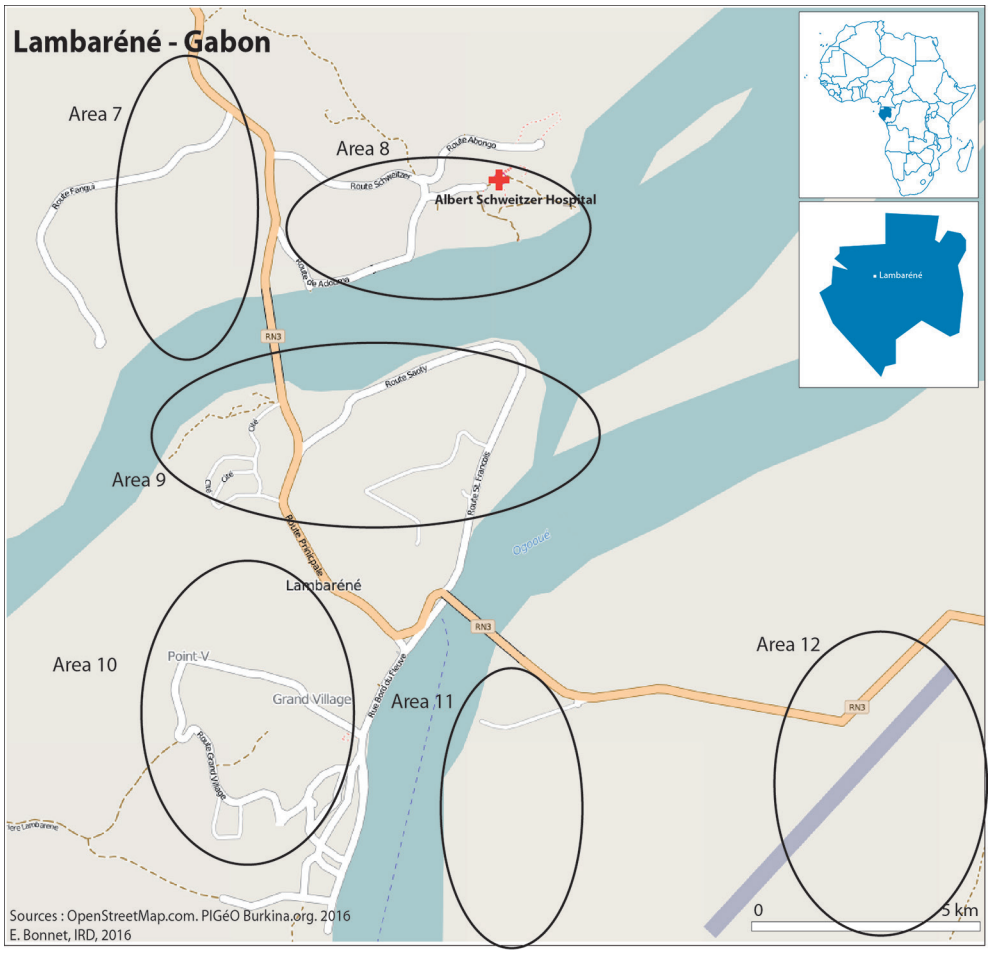


Figure 3 Map of the study area in Lambaréné, Gabon.

Kenya, located in East Africa, lies on the equator, covering 581 309 km^2 (224 445 sq. mi), with a population of approximately 45 million people in 2014.[32] Kenya generally has a warm and humid tropical climate but is diverse, ranging from the cooler climate around the capital city, Nairobi, to a hot and dry climate inland as well as a desert-like climate in the north-eastern regions along the border with Somalia and Ethiopia.[32] The capital, Nairobi, is a regional commercial hub. The main industries include agriculture, exporting tea and coffee as well as the service industry.

Kenya is divided into 47 semiautonomous counties. Mombasa is the country's second largest city after Nairobi and is located on the east coast of the country.[32] Administratively, Mombasa is the capital of Mombasa County, which was formerly called Coast Province. This overall Coast region covers over 80 000 km^2 in the south-eastern part of Kenya, constituting about 15% of the country's land area, with a population of 3 325 307 residents.

The main economic driver of Mombasa is tourism and trading industry. Mombasa itself has a population of about 1.3 million with almost 50% of the population under 15 years of age.[29] Increasingly, the population of the province lives in urban areas; at present about 45% live in Mombasa and other urban centres. The 'long rains' period begins around April and the 'short rains' period begins in October.[32] Mean annual temperature ranges from 24°C to 27°C, but maximum temperature averages over 30°C between January and April.

Figure 4 shows the area of Mvita subcounty of Mombasa, which was the catchment area for the study in Kenya, with a catchment population of 74 735 residents. The map indicates the three facilities involved in the study.

Sample size

Given the paucity of available age-specific dengue incidence data in the study countries or nearby countries, it was difficult to obtain population-based incidence to make assumptions when calculating sample sizes. The required catchment population for the passive facility-based fever surveillance was roughly estimated based on the limited data available in the literature. Annual incidence estimates were calculated based on available prevalence estimates with the assumption that the outcome of interest has zero prevalence at age zero, and that force of infection is constant. It was assumed that prevalence estimates found for one particular age group would be adjusted as the annual incidence and used across all ages.

Wichmann *et al* calculated an expansion factor for children by comparing data from three cohort studies to national surveillance data in Southeast Asia.[33] For children in Thailand, the age-specific expansion factors

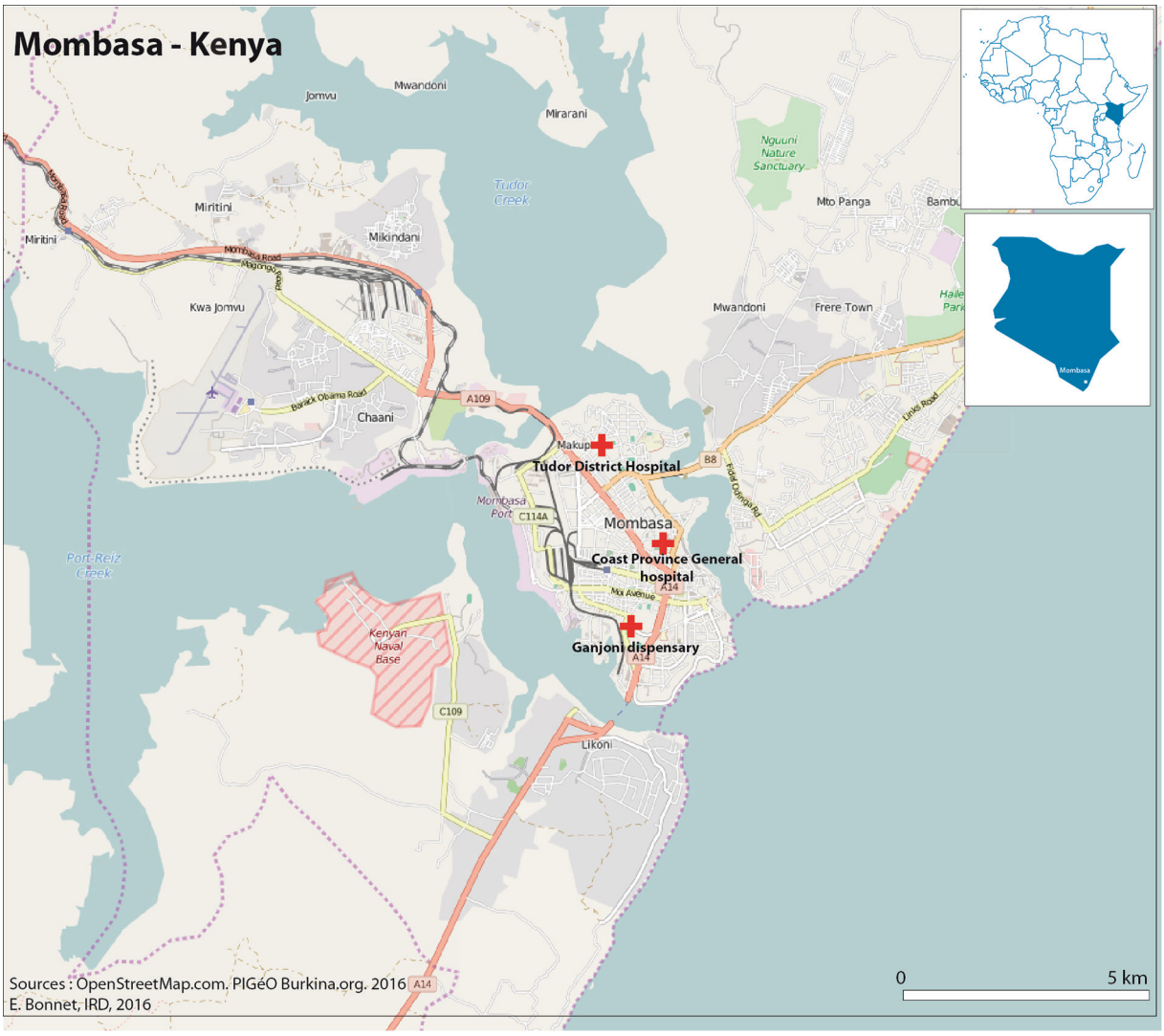


Figure 4 Map of the study area in Mombasa, Kenya.

calculated were 11.85 for <5 years, 8.76 for 5–9 years and 7.81 for 10–14 years.[33] The results show that, even for Asia where better reporting and surveillance systems are available, there is a considerable degree of under-reporting. For Africa, there may be more dengue cases under-ascertained (not seeking care) and under-reported (not reported even if a patient with dengue seeks care, given that dengue is not one of the routinely notifiable diseases in Africa), but such information on the extent of underestimation of dengue was not available.[34 35] Also, the incidence estimates used in our sample size calculations were not from population-based studies. While it would have been ideal to adjust the incidence further for likely underestimation, the annual incidence used in sample size calculations could not be adjusted for possible under-reporting due to the lack of data. The sample sizes were calculated with 95% confidence levels and a margin of error at a fixed significance level within 25% of the true proportion of incidence. This gives relative precision of 75%, considering the gap in evidence for dengue incidence in the study areas. The final sample sizes were calculated by assuming 10%–20% (variable by site) non-response rate or loss to follow-up. The required catchment population size for the fever surveillance study in Burkina Faso was estimated to be 100 000, Gabon to be 77 000 and Kenya to be 70 000. In these catchment populations, the number of enrolled subjects depends on the number of eligible patients who seek care at the study facilities. How many eligible febrile episodes would actually present at our study facilities was difficult to predict; but after assessment of the volume of febrile patients at the facilities, a realistic upper limit for enrolment for a study period of approximately 1.5 years was set at 3000 subjects to offer enrolment to all consenting eligible patients.

For the serological survey, the sample size was calculated similarly using the prevalence proportion based on published literature. Seroprevalence of 0.304 for Burkina Faso,[15] 0.123 for Gabon,[21] and 0.144 for Kenya[36] were used. With the same confidence levels and allowed margin of error and assuming 10%–30% (variable by site) non-response rate, the sample size was calculated to be 3000 participants at each site. Again, with the scarcity of data from the selected countries, there were no other

prevalence estimates reported or estimates from different age groups. As prevalence is expected to increase with age and higher prevalence would give a smaller sample size, our calculations are likely to be conservative.

Study components

Fever surveillance—design and methods

To determine burden due to symptomatic dengue in each of the three sites in Burkina Faso, Gabon and Kenya, passive facility-based fever surveillance was implemented in a well-defined catchment area population. In Burkina Faso, the surveillance study was initiated in December 2014 in five selected primary healthcare centres, locally called 'Centre de Santé et de Promotion Sociale', in the municipality of Ouagadougou, with a catchment population of 105 000 residents. This project was implemented in collaboration with Centre Muraz in Bobo-Dioulasso, EQUITE sante programme (a collaborative programme between University of Montreal and Action-Gouvernance-Integration-Reinforcement, AGIR, based in Ouagadougou, funded by the Canadian Institute of Health Research) and DVI. In Gabon, the surveillance study was initiated in the ASH serving a catchment population of 130 000 residents in the Moyen-Ogooué and surroundings within Lambaréné, in collaboration with CERMEL and Institute of Tropical Medicine in Tubingen, Germany. In Kenya, the surveillance study was implemented at Ganjoni dispensary, Tudor subcounty Hospital and Coast Provincial General Hospital, serving a catchment population of 70 000 residents in Mombasa, in collaboration with Kenya Medical Research Institute and Ministry of Health of Kenya.

As described in figure 5, both outpatients and inpatients at the designated study facilities, who meet inclusion criteria as mentioned earlier were tested for dengue, first with SD Dengue Duo RDT. Dengue confirmation was done by detection of dengue virus in serum samples using PCR as well as antidengue IgM and IgG antibodies in acute and convalescent serum by ELISA (SD Dengue IgM & IgG capture ELISA tests, Standard Diagnostics, Yongin-Si, Korea).[10 37] Every consecutive patient meeting inclusion criteria was eligible for enrolment during the study period. Infants<1 year old were not included due to operational limitations, such as difficulty of infantile bleeding.

In Ouagadougou, Burkina Faso, the fever surveillance was initiated in December 2014 and continued until February 2017 (approximately 2 years). In Lambaréné, Gabon, the fever surveillance was initiated in April 2015 and continued until January 2017 (approximately 1.5 years). In Mombasa, Kenya, the fever surveillance was initiated in March 2016 and continued until May 2017 (15 months).

Among subjects enrolled in the fever surveillance, those who were positive by dengue rapid diagnostic test were offered further enrolment in the cost-of-illness survey, consisting of interviews on the day of acute illness visit, day 10–14 from the first visit and day 28, if illness continues. The cost-of-illness survey questionnaire was designed to estimate the direct medical, direct non-medical and indirect costs associated with dengue-positive patients identified at study facilities. This survey also estimates the cost of treating dengue at the facility level. Data were gathered by linking patients' medical records concerning outpatient visits, inpatient visits and service consumption (eg, diagnostic tests, medication and other services provided to patients). The cost-of-illness portion of the study will be described separately.

Fever surveillance—laboratory testing

As shown in figure 6, in all three sites, acute samples were tested using a commercial RDT for dengue NS1 and IgM/IgG (Dengue Duo, Standard Diagnostics, Yongin-Si, Korea). Dengue Duo RDT was used on the day of acute illness visit at the site of patient presentation (day 1). The acute and convalescent samples were subsequently tested at a local laboratory using dengue IgM/IgG ELISA (SD Dengue IgM & IgG Capture ELISA, Standard Diagnostics, Yongin-Si, Korea). The serum was separated and stored in 4 aliquots of about 500 µL for various laboratory tests, as indicated in consent documents.

After ELISA testing, samples were shipped to the International Vaccine Institute (IVI) in Korea. Samples with positive results by RDT or ELISA, as well as a small number of samples with negative results, undergo further testing by RT-PCR at the Clinical Immunology Laboratory of IVI. Four DENV serotype-specific real-time RT-PCR assays are used for laboratory confirmation of dengue and serotyping.[38] The DENV 1–4 RT-PCR assays are carried out in 25 µL reaction mixtures containing 5 µL template RNA, TagMan Fast Virus 1-step mastermix (Applied Biosystems), 0.9 µM of each primer and 0.2 µM probe.[38] Amplification and detection are performed in a StepOne Plus real-time PCR system, and the baseline and threshold are determined using the auto-baseline and threshold feature in StepOne Software V.2.2.2 (Applied Biosystems). Thermocycling parameters are as follows: reverse transcription at 50°C for 5 min, inactivation at 95°C for 20 s, followed by 45 cycles of fluorescence detection at 95°C for 3 s and annealing at 60°C for 30 s.[38] A specimen is considered positive if target amplification is recorded within 40 cycles.

Serological survey—design and methods

While the facility-based fever surveillance studies provide estimates of the burden of medically attended dengue disease, evaluation of all DENV infections in a population—including subclinical and mildly symptomatic infections, which impact immune status—is needed to capture the overall impact of dengue. As part of the study package, population-based serological surveys were conducted in the same catchment population used for the fever surveillance. At each of the three sites in Africa, the serosurvey was conducted on a cohort of approximately 3000 randomly selected residents of urban and semiurban parts of Ouagadougou, Lambaréné and

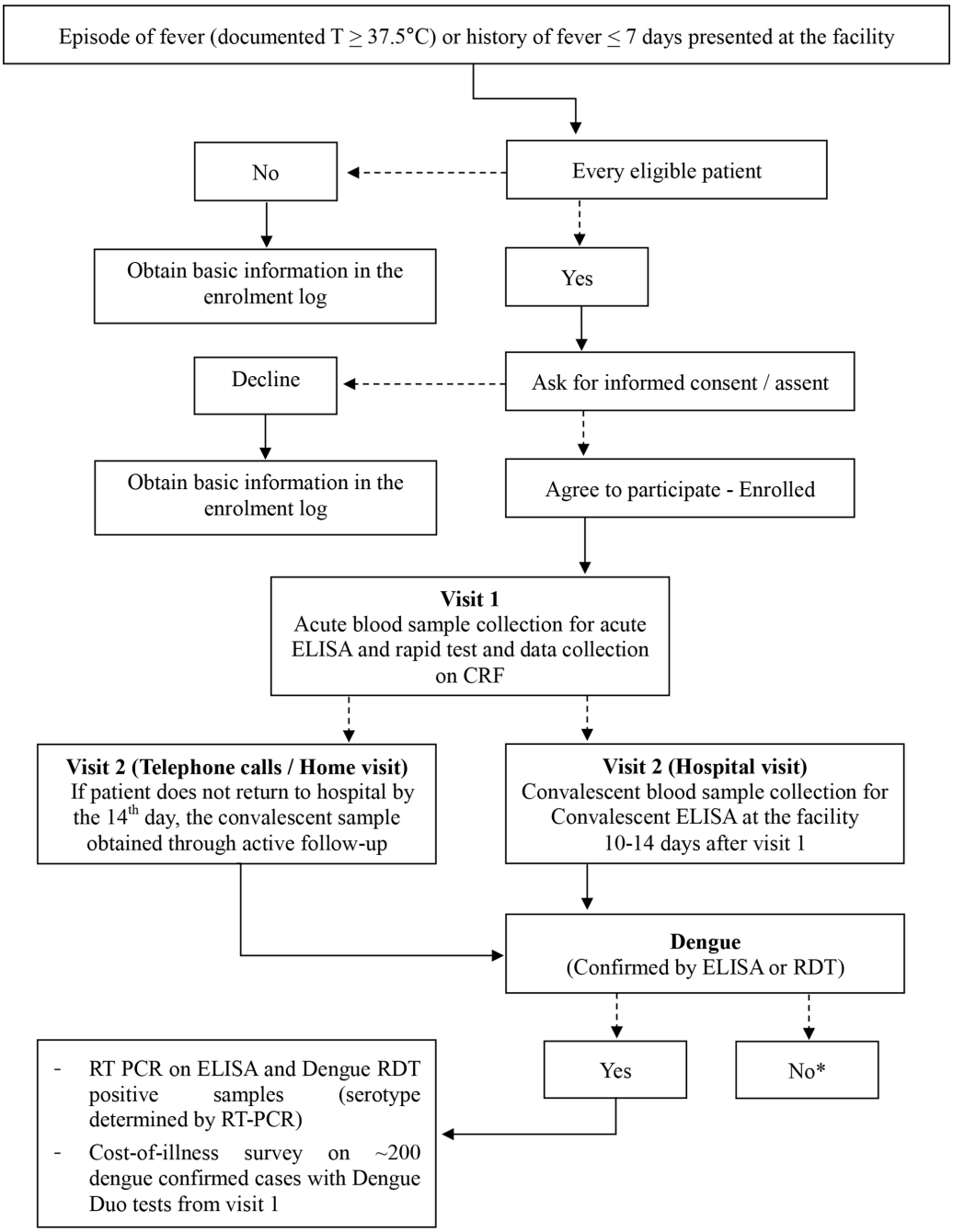


Figure 5 Patient flow in the fever surveillance. Eligible febrile patients identified and enrolled as study subjects followed these steps to complete participation in the passive fever surveillance. * A small number of those samples that are negative on ELISA or NS1 are tested with PCR to exclude false negative results of the ELISA. CRF, case report form.

Mombasa. Without individual-level census information on all residents of Lambaréné and Mombasa, with help of community/village health workers, randomisation was done based on neighbourhoods (or defined areas for which the health workers/volunteers are responsible) as cluster units. As the community/village health workers are familiar with the villages and their residents, they are good entry points into the communities. With these health workers, the field team screened houses in the selected villages by knocking on doors of every 5–7 houses, depending on the household density per neighbourhood. Also, demographic information collected in previous research projects conducted in the same area was used as a guide, if available. In the case of the site in Ouagadougou, HDSS data were available and the EQUITE SANTE, a CIHR funded research programme of the University of Montreal, had set up a geographic information system database of houses in the study area. Using these data, households of potential enrolees of the serosurvey were preselected randomly and household visits were made in Ouagadougou. In the three sites, about 45% of the serosurvey samples were targeted to be collected from children 1 to 14 years of age, and 55% were targeted to be collected from adults between 15 and 55 years of

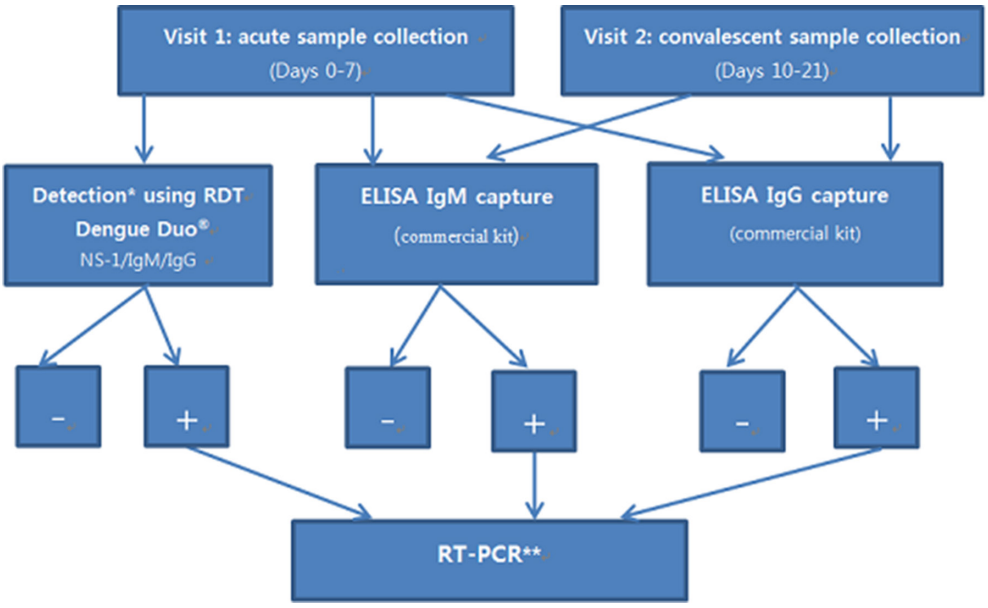


Figure 6 Laboratory testing algorithm for dengue. Samples from subjects of the passive fever surveillance would follow these steps of the testing algorithm for confirmation of dengue. *Dengue Duo®test is performed on enrolled febrile patients to identify dengue cases for immediate follow-up of dengue-confirmed cases in the cost-of-illness survey. **Selected samples, including those that were found positive by IgM and NS1 on Dengue Duo®,as well as those positive by IgM and IgG capture ELISA, will be tested with RT-PCR.

age to reflect the age distribution of the general population of the area. Household-based enrolment was offered to the head of the household until the specific cap for the age-group was reached in Lambaréné and Mombasa.

Randomly selected subjects 1–55 years of age underwent phlebotomy (5 mL for children and 7 mL for adults) twice—before the rainy season and after the rainy season, at approximately 6-month intervals. The sera were evaluated using IgG indirect ELISA at baseline and after 6 months. The presence of dengue IgG antibodies at 6-month intervals will be used to estimate the level of occurrence of inapparent DENV infection and to calculate the rate of infection in the catchment population. Flow cytometry-based DENV neutralisation assays will be applied to a subset of samples to assess for presence of dengue neutralising antibodies and seroconversion over the 6-month interval. In addition to overall seroconversion, age-specific seroconversion estimates in the catchment population as well as the proportion of inapparent infections will be determined.

Serological survey—laboratory testing

From the samples collected in the serosurvey, about 200 µL of serum were used and tested at a local laboratory using dengue IgG ELISA (Panbio Dengue IgG Indirect ELISA, Alere North America, Florida, USA). After ELISA testing for dengue IgG at the local laboratories, samples were shipped to IVI. Given potential serological cross-reactivity among flaviviruses,[39] flow cytometry-based neutralisation assays will be performed against selected flaviviruses to include yellow fever virus, West Nile virus, Zika virus and Japanese Encephalitis virus, in addition to DENV 1-4, at the Clinical Immunology Lab of IVI.[40 41] About 50 samples per bleed for four bleeds in Burkina Faso and two bleeds in Gabon and Kenya will be tested.

About 1000 µL of serum is allotted for this procedure. The flow cytometry-based neutralisation assays are performed in duplicate in 96-well cell culture plates with flat-bottom wells, each containing DC-SIGN-expressing U937 cells.[40] The amount of virus used in the assay infects between 7% and 15% of the cells. Human immune sera are serially diluted and the virus is preincubated with the sera for 1 hour at 37°C.[40] The cells are washed, the virus and serum mixture is added to the cells for 1 hour at 37°C and the cells are further incubated for 24–48 hours at 37°C in 5% CO_2. The cells are fixed, permeabilised and stained with fluoresce-conjugated monoclonal antibody 4G2, which recognises the flavivirus E protein.[42] FACScan flow cytometer (Becton Dickinson, San Diego, California, USA) is used to analyse the cells.[40] The serum dilution that neutralises 50% of the viruses is calculated by nonlinear, dose-response regression analysis with Prism 4.0 software (GraphPad Software, San Diego, California, USA).

In addition, a Luminex-based multiplex immunoassay will be performed on a randomly selected subsample to assess for IgG to different flaviviruses.[43] About 200 samples per bleed for four bleeds in Burkina Faso and two bleeds in Gabon will be tested. Detection of IgG against ZIKV and each of the four DENV serotypes will be performed on patient serum samples using an in-house microsphere-based multiplex immunoassay (arbo-MIA) at the Clinical Immunology Lab of IVI.[44 45] The arbo-MIA is based on a mixture of microspheres covalently coupled with either DENV-1, DENV-2, DENV-3, DENV-4 or ZIKV

recombinant antigens (E protein domain III) produced in Drosophila S2 expression system. Briefly, microsphere mixtures were sequentially incubated in the dark under constant shaking with a 1:400 dilution of patient serum samples, with 2 µg/mL antihuman IgG biotin-conjugated antibody (Jackson Immunoresearch, West Grove, Pennsylvania, USA) and with 2 µg/mL streptavidin-R-phycoerythrin conjugate (Life technologies). After the final incubation, the median fluorescence intensity (MFI) of each microsphere set is quantified using a BioPlex 200 instrument (Bio-Rad Laboratories, Hercules, California, USA). Samples are considered seropositive if the ratio of MFI values obtained for the viral antigen to the control antigen is superior to the defined cut-off. The cut-off of the MIA is determined for each viral antigen by receiving operating characteristic (ROC) curve analysis using well-characterised sera.

In Lambaréné, the enrolment bleed took place in November–December 2015, while the second blood collection occurred in May 2016. In Ouagadougou, the enrolment bleed took place in May–June 2015 with follow-up blood collections in December 2015, June 2016 and January 2017. In Mombasa, the enrolment bleed took place in May 2016 with the second blood collection in November 2016–February 2017.

Healthcare utilisation survey

As the passive fever surveillance was conducted at study facilities, patients with potential dengue could be missed if they seek care elsewhere. To identify the proportion of fever and dengue cases potentially missed by the passive surveillance system due to patients living in the study area but seeking care outside of study facilities, a population-based healthcare utilisation survey was conducted in 400 randomly selected households from the study catchment area to characterise the healthcare utilisation patterns of the households when they have (self-reported) febrile episodes among the family members. In addition to assessing health-seeking behaviours of the residents, preferences in terms of health-seeking behaviour and respective reasons for their preferences were investigated. The questionnaire was administered to 400 heads of households. Among 3000 residents who participated in the serosurvey, there were about 600 households. From these households, 400 heads of households were randomly selected and offered enrolment in the health utilisation survey. Heads of households or a senior representative within the household were asked questions on health seeking patterns of their family members.

Study questionnaires

For the fever surveillance study, questionnaires were administered at the acute illness visit and the convalescent visit. The convalescent visit may take place at the healthcare facility (10–14 days later) or at the patient's home (15–21 days after the acute visit), according to patient preference and availability. The questionnaires were completed by medical staff of the study facilities, including demographic and clinical information (eg, signs, symptoms, past medical history, treatments prescribed and diagnoses). The same staff also completed the follow-up questionnaire at the convalescent visit within 21 days from the acute visit. Study nurses completed surveillance enrolment log. Lab technicians completed the lab section (mostly dengue-related diagnostics) and the forms were compiled by the study coordinator on site.

For the serosurvey component, questionnaires were administered at the household by trained field team staff at each serosurvey visit. Study nurses completed the questionnaire after a brief physical and medical examination. At the follow-up visit(s) in about 6 months, the same staff made the household visits to complete the follow-up questionnaire. Enrolment log was maintained by the study coordinator on site.

Variables of the surveillance questionnaires

The variables collected are listed in table 1.

Planned statistical analysis

From the fever surveillance data, incidence of symptomatic dengue among patients that seek healthcare at the study facilities will be calculated. Age-specific incidence rates in all the children and adults will be determined by referring to the size and distribution of the general population of the study area at the time of surveillance as the denominator in calculation of the incidence of symptomatic dengue cases. Each person residing in the study area is assumed to contribute 12 months of person time to the denominator. Although the study areas all report a low migration rate, the in-migration is assumed to balance the out-migration of the population during the study period. Age-specific incidence of symptomatic dengue will be calculated by using age-specific denominators and the number of symptomatic dengue cases in eligible individuals as the numerator.

Using the data collected in the Healthcare Utilisation Survey, the proportion of febrile cases missed by the passive surveillance system will be determined. Then using the proportion, the numerator will be further adjusted in recognition of those missed fever cases from the study area, which could have been dengue. Also, comparison will be made between those that agreed to participate and those that declined participation among the eligible potential enrolees. The enrolment log, which records basic information obtained during the screening process of potential enrolees, will be reviewed. In addition to checking that our sample of febrile cases is representative of febrile patients of the general population in the catchment area, refusal rates will be determined based on information in the log. Then, the refusal rates will be used to adjust the numerator.

SPSS software will be used for analysis of the fever surveillance data. Multivariable logistic regression will be used to compare confirmed patients with dengue versus patients with non-dengue febrile in terms of symptomatic presentation, based on signs and symptoms collected from all

Lim JK, *et al*. *BMJ Open* 2018;**8**:e017673. doi:10.1136/bmjopen-2017-017673

Table 1 List of variables collected in the passive fever surveillance data collection form

Topic	Description	Items
Basic information	Demographic and basic information about the patient and the treatment received	Type of treatment, where patient is enrolled (IPD vs OPD) Date of fever onset, duration of fever Current temperature Tourniquet test results Patient's address (district and village-level) Date of visit, date of birth, age and sex Weight and height
General health condition	Current condition of the patient (self-report) and underlying diseases of the patient	How well the patient could handle daily activities Pre-existing conditions
Signs and symptoms during this illness	A set of signs and symptoms that may be related to fever and dengue (dengue fever and dengue haemorrhagic fever) at both visits 1 and 2	Rash, fatigue, headache, retro-orbital pain, neck/ear pain, sore throat, breathing difficulty, cough, expectoration, gastrointestinal signs (nausea/vomiting, diarrhoea, abdominal pain and so on), haemorrhagic signs (nose/gum bleeding, ecchymosis, petechiae and so on), signs of shock (cyanosis, capillary refill), arthralgia, myalgia, loss of appetite, jaundice and so on
Medical history	Previous dengue-related or other flavivirus infection as well as vaccination history (self-report)	Previous dengue infection and related hospitalisation Previous infection to other commonly circulating arboviral infection in the area (ie, Yellow fever vaccination history)
Laboratory findings	Records from the routine laboratory tests widely used in clinical fever/dengue patient management, as part of the hospital care procedure	Platelet count, haematocrit, haemoglobin, leucocytes, neutrophils, protein level, AST, ALT, urine test results and so on
Clinical diagnosis	Clinician's diagnosis with or without referring to the RDT	Diagnosis given by the physician based on clinical presentation after physical examination of the patient
Dengue testing results	Results from the dengue tests, mainly RDTs for dengue as well as other commonly circulating arbovirus in the area	Dates of blood draw Test results of the RDT IgM/IgG capture ELISA results PCR results (if available)
Treatment	Medicine(s) prescribed and the starting and end dates	Antibiotics, paracetamol, ibuprofen, aspirin and others that may be site-specifically prescribed
Outcome	Outcome of this particular visit	Hospitalised, returned home or referral
Hospitalisation	Information collected only among hospitalised patients in the surveillance to record other severe signs and progression of illness	Admission and discharge diagnoses Presence of haemorrhagic signs or shock syndrome
Hospital charges	Expenses and hospital charges incurred by patient on the visit 1	Amount of the out of pocket payment by the patient or the family/or guardian Breakdown of the hospital charges (laboratory, medication, admission-related charges)
Final outcome	Outcome of the patient's illness at the second visit	Final diagnosis given for the patient, outcome of illness Completion of study participation (early termination and the reason and so on)

ALT, Alanine AminoTransferase; AST, Aspartate aminotransferase; IPD, Inpatient department; OPD, Outpatient department; RDT, Rapid Diagnostic Test.

patients with laboratory-confirmed dengue by serology and RT-PCR, adjusting for possible confounders, such as age, days since onset of fever, primary versus secondary infection, inpatient versus outpatient and so on. Differences in symptomatic complex of dengue fever (DF) (and DHF, if data allows) by age and serotype will be also determined using multivariable logistic regression.

As outpatient disease accounts for the greater part of dengue disease burden, clinical profile of individuals with DENV infection will be characterised by the type of treatment (hospitalised vs outpatients) as well as by severity of the disease (severe vs non-severe by the 2009 WHO criteria).[46] Classification is determined after the course of illness is completed (typically during the convalescent visit). Symptomatic dengue is classified as outpatient or hospitalised. Progression of dengue is recorded as DF, DHF I, DHF II, DHF III or DHF IV, and clinical patterns will be compared by the severity grade.[46 47] These will be compared with results obtained from other DVI studies in Latin America (Colombia) and Asia (Thailand, Vietnam and Cambodia). Overall, comparisons will be made across Burkina Faso, Gabon and Kenya.

With the age-stratified sera that reflect the age distribution of the general population of the country, the serological survey sampling strategy ensures sufficient subjects to obtain precise age-specific estimates of seropositivity and seroconversion of the catchment area population. The seroconversion rate and change in the immune status will be determined by age group during the study period. The age-stratified serosurvey data will also allow calculation of the force of infection of dengue in the study population. After enrolment, there are subjects who drop out in the follow-up bleeds about 6 months later. Basic demographic information will be compared between those that completed participation and those with incomplete participation to check whether study subjects represent the catchment area population. Comparisons will be made among Burkina Faso, Gabon and Kenya.

Ethical considerations

To minimise inconvenience of the study to patients, clinicians and nurses were sensitised and trained regarding the study requirements and procedures in order for data collection to be integrated into routine patient care. The clinicians and nurses selected for the study receive coordinated support from study field staff throughout the study process. Written informed consent and assent for participants 7 (13 for Kenya)–17 years of age were obtained from patients by study staff. Study staff go through consent and assent documents for short summary of the disease, detailed description of study procedures and information on reimbursement. Patient data are documented in the study designated office; only the study staff have access to the data that are de-identified. Data are exclusively handled in the study office and stored safely in a protected database in the study office as well as on the DVI main server.

DISCUSSION

Dengue cases have been detected since the 1960s in Africa, and there has been continued presence of *Aedes* vectors in the continent.[5 7] However, very few dengue studies have been conducted in Africa, and little evidence is based on population-based studies.[6] Compared with the volume of evidence from SE Asia and the Americas, there is critical data scarcity on dengue in Africa. Suspicion of substantial dengue burden in Africa is based on limited reports of outbreaks and a handful of seroprevalence studies testing different viruses among samples that likely do not represent the general population. In the three countries selected for our field studies, somewhat more data are available, but are still very limited. In Burkina Faso, a recent observational study conducted in 2013 reported that 8.7% of the febrile patients showed positive results on dengue RDT.[16] In Gabon, one study suggested minimal DENV circulation in rural areas,[21] while another study reported 12.3% seroprevalence, by IgG antibodies against dengue, among toddlers 30 months of age in semirural parts of Lambaréné.[20] In Kenya, about 13% of the individuals in Mombasa have been reported to have evidence of past or current DENV infection by RT-PCR and IgM antidengue ELISA after the 2013 outbreak.[26] Despite the limited scope and generalisability of these studies, they suggest that there may be more dengue than previously appreciated due to underestimation and misdiagnosis.[25 26]

These studies suggest the presence of dengue and some level of underlying seroprevalence in the countries of our field studies. However, often these studies are limited by their retrospective design or sample collection (blood donors or sample collected from surveys of other diseases) to demonstrate the true, population-based, burden of dengue. We proposed to address this gap by population-based dengue surveillance and seroprevalence studies in West, (West-) Central and East Africa.

The present studies at three sites in Africa will provide important information on undocumented DENV circulation in Africa. Such data will help to strengthen the evidence base for dengue burden in Africa. Better defined disease burden data based on our studies could be used to assess the relative need for dengue prevention and control measures, such as whether a dengue vaccine would be a cost-effective public health intervention for countries in Africa. Clinical findings from our studies could also be used as a guide for dengue case detection and case management.

The studies have some important limitations. We recognise variability of dengue epidemiology over time and by region. Due to resource constraints, our studies are limited in terms of time frames and geographical extent. These constraints may limit the generalisability of our study results.

One potential source of bias in estimating the incidence of symptomatic dengue is under-ascertainment due to the community residents with relevant symptoms seeking care from other healthcare providers and facilities than the study facilities. As the study design remains passive surveillance, cases are ascertained only at our study facilities. By estimating the proportion of febrile patients seeking care elsewhere as well as refusal rates among the potential enrolees that were screened for eligibility criteria, the degree of febrile patients missed by the study can be determined. Inverse probability weighting will be used to account for these potential subjects missed by the surveillance as adjustments in incidence calculation. Also, depending on the transmission volume of dengue or other cocirculating diseases with onset of fever, there may be patients that are diagnosed with other diseases and ruled out for dengue. Furthermore, with respect to dengue diagnostics for our serological surveys, there are other circulating flaviviruses in Africa leading to challenges in identifying antibodies to past dengue infections. While our testing plan assesses for some flaviviruses, others known to circulate in Africa, such as Banzi and Usutu viruses, are not part of the testing plan.[48–50] Due to resource limitations, serological testing will be limited to yellow fever virus, West Nile virus, Zika virus and Japanese

Encephalitis virus as well as DENV 1–4. Therefore, in some cases, it may be difficult to determine prior exposure to DENV versus other flaviviruses based on serological data. This cross-reactivity may lead to overestimation of dengue force of infection.

In addition, the serosurvey and healthcare utilisation survey are conducted on a randomised subsample of the catchment area population and there may be limited generalisability of the data collected from these surveys. With unknown differences among those that agree to participate and those that do not agree, the data may not be representative of the general population of the study countries.

CONCLUSION

The data collected from our studies will contribute to the assessment of the unknown dengue disease burden in Burkina Faso, Gabon and Kenya. These data can fill a gap in undocumented burden of dengue in the region and, collectively, may be used to infer dengue burden in other areas of Western, Central and Eastern Africa. Countries in Africa may not consider introduction of a dengue vaccine as a priority in the near future due to many other competing public health problems and limited resources. For cost-effective implementation of public health interventions, accurate data on dengue burden from epidemiological studies would be needed for policy makers to make evidence-based decisions on control and prevention of dengue. Our studies will provide some much needed information based on population-based research to assess dengue burden in Africa.

Author affiliations
[1]Global Dengue and Aedes-transmitted Diseases Consortium, International Vaccine Institute, Gwanak-gu, The Republic of Korea
[2]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK
[3]Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, QC, Canada
[4]Development and Delivery, International Vaccine Institute, Gwanak-gu, The Republic of Korea
[5]School of Public Health, University of Montreal, Montreal, Quebec, Canada
[6]Centre de Recherches Médicales de Lambaréné, Fondation Internationale de l'Hôpital Albert Schweitzer, Lambaréné, Gabon
[7]Department of Communicable Disease Prevention and Control, Ministry of Health, Nairobi, Kenya
[8]Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany
[9]Eastern and Southern Africa Centre of International Parasite Control (ESACIPAC), Kenya Medical Research Institute, Nairobi, Kenya
[10]Program Equité, Action-Gouvernance-Integration-Reinforcement, Ouagadougou, Burkina Faso
[11]UMI Résiliences, Institut de recherche pour le developpement (IRD), Paris, France
[12]Centre Muraz, Bobo Dioulasso, Hauts Bassins, Burkina Faso

Acknowledgements We thank the doctors and laboratory staff of CSPS of Ouagadougou, ASH, Ganjoni dispensary, Tudor subcounty Hospital and Coast Provincial General Hospital as well as at Centre Muraz, CERMEL and KEMRI. We thank collaborators at AGIR, IRD France and University of Montreal, and University of Tubingen. Last, we would like to thank the DVI team as well as statisticians, laboratory, and administrative staff at the International Vaccine Institute for their helpful comments during the preparation of this manuscript and support during the studies.

Contributors JKL designed the study, is overseeing data collection and was a major contributor in writing the manuscript. MC codesigned the study, oversaw some parts of data collection and supported in writing of the manuscript. JSL was a contributor in designing of the study and oversight of parts of data collection. KSL was a contributor in oversight of data collection. SN, SKL, EA, NO and AB supported in data collection. VR supported in designing of the study and was a major contributor in finalisation of the manuscript. JF was a contributor in data collection. BL was a contributor in designing of the study and data collection. SHM was a contributor in designing of the study and site establishment. ME was a contributor in designing of the study. EB supported in data generation. SMN was a contributor in designing of the study and site establishment. STA and SY were contributors in designing of the study and site establishment. NA was a major contributor in providing oversight of the data collection and finalisation of the manuscript. IKY was a major contributor in designing of the study and finalisation of the manuscript. All authors read and approved the final manuscript.

Funding The current study was supported by funds from the Bill and Melinda Gates Foundation (OPP 1053432). NA receives support from the United Kingdom Medical Research Council (MRC) and Department for International Development (DFID)(MR/K012126/1). VR holds a CIHR-funded Research Chair in Applied Public Health (CPP-137901).

Disclaimer The funding body had no role in the design of the study and collection, analysis and interpretation of data and in writing the manuscript.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The protocol for each study obtained ethical approvals from the Institutional Review Boards (IRBs) of the International Vaccine Institute, the London School of Hygiene and Tropical Medicine and the Ethics Committee of host country institutions, including KEMRI Scientific and Ethical Review Unit in Kenya, Gabon National Ethics Committee and Institutional Ethics Committee, Scientific Review Board of CERMEL in Gabon and the IRB of Centre Hospitalier de l'Universitéde Montréal (CRCHUM) at University of Montreal and the National Health Ethical Committee of Burkina Faso.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. Bhatt S, Gething PW, Brady OJ, *et al*. The global distribution and burden of dengue. *Nature* 2013;496:504–7.
2. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. *Clin Epidemiol* 2013;5:299–309.
3. Chang MS, Christophel EM, Gopinath D, *et al*. Challenges and future perspective for dengue vector control in the Western Pacific Region. *Western Pac Surveill Response J* 2011;2:e1–16.
4. Eisen L, Beaty BJ, Morrison AC, *et al*. ProactiveVector control strategies and improved monitoring and evaluation practices for dengue prevention. *J Med Entomol* 2009;46:1245–55.
5. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerg Infect Dis* 1995;1:55–7.
6. Amarasinghe A, Kuritsky JN, Letson GW, *et al*. Dengue virus infection in Africa. *Emerg Infect Dis* 2011;17:1349–54.
7. Messina JP, Brady OJ, Scott TW, *et al*. Global spread of dengue virus types: mapping the 70 year history. *Trends Microbiol* 2014;22:138–46.
8. Fagbami AH, Monath TP, Fabiyi A. Dengue virus infections in Nigeria: a survey for antibodies in monkeys and humans. *Trans R Soc Trop Med Hyg* 1977;71:60–5.
9. Carey DE, Causey OR, Reddy S, *et al*. Dengue viruses from febrile patients in Nigeria, 1964-68. *Lancet* 1971;1:105–6.
10. Beatty ME, Stone A, Fitzsimons DW, *et al*. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas Dengue Prevention Boards. *PLoS Negl Trop Dis* 2010;4:e890.
11. Were F. The dengue situation in Africa. *Paediatr Int Child Health* 2012;32(Suppl 1):18–21.

12. Sierra B, Triska P, Soares P, *et al*. OSBPL10, RXRA and lipid metabolism confer African-ancestry protection against dengue haemorrhagic fever in admixed Cubans. *PLoS Pathog* 2017;13:e1006220.
13. Chacón-Duque JC, Adhikari K, Avendaño E, *et al*. African genetic ancestry is associated with a protective effect on Dengue severity in colombian populations. *Infect Genet Evol* 2014;27:89–95.
14. Brady OJ, Gething PW, Bhatt S, *et al*. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Negl Trop Dis* 2012;6:e1760.
15. Collenberg E, Ouedraogo T, Ganamé J, *et al*. Seroprevalence of six different viruses among pregnant women and blood donors in rural and urban Burkina Faso: a comparative analysis. *J Med Virol* 2006;78:683–92.
16. Ridde V, Agier I, Bonnet E, *et al*. Presence of three dengue serotypes in Ouagadougou (Burkina Faso): research and public health implications. *Infect Dis Poverty* 2016;5:23.
17. Caron M, Grard G, Paupy C, *et al*. First evidence of simultaneous circulation of three different dengue virus serotypes in Africa. *PLoS One* 2013;8:e78030.
18. Leroy EM, Nkoghe D, Ollomo B, *et al*. Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007. *Emerg Infect Dis* 2009;15:591–3.
19. Caron M, Paupy C, Grard G, *et al*. Recent introduction and rapid dissemination of Chikungunya virus and Dengue virus serotype 2 associated with human and mosquito coinfections in Gabon, central Africa. *Clin Infect Dis* 2012;55:e45–e53.
20. Gabor JJ, Schwarz NG, Esen M, *et al*. Dengue and chikungunya seroprevalence in Gabonese infants prior to major outbreaks in 2007 and 2010: A sero-epidemiological study. *Travel Med Infect Dis* 2016;14:26–31.
21. Pourrut X, Nkoghé D, Gonzalez JP, *et al*. No evidence of dengue virus circulation in rural Gabon. *Emerg Infect Dis* 2011;17:1568–9.
22. Andries AC, Duong V, Ngan C, *et al*. Field evaluation and impact on clinical management of a rapid diagnostic kit that detects dengue NS1, IgM and IgG. *PLoS Negl Trop Dis* 2012;6:e1993.
23. Lell B, Agnandji ST, von Glasenapp I, *et al*. A randomized trial assessing the safety and immunogenicity of AS01 and AS02 adjuvanted RTS,S malaria vaccine candidates in children in Gabon. *PLoS One* 2009;4:e7611.
24. Agnandji ST, Lell B, Soulanoudjingar SS, *et al*. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med* 2011;365:1863–75.
25. Ochieng C, Ahenda P, Vittor AY, *et al*. Seroprevalence of infections with Dengue, rift valley fever and Chikungunya viruses in Kenya, 2007. *PLoS One* 2015;10:e0132645.
26. Ellis EM, Neatherlin JC, Delorey M, *et al*. A household serosurvey to estimate the magnitude of a dengue outbreak in Mombasa, Kenya, 2013. *PLoS Negl Trop Dis* 2015;9:e0003733.
27. United Nations Development Programme. Population, urban (% of population). *Reports HD*: UNDP, 2013.
28. United Nations. *World urbanization prospects: The 2014 revision, highlights*. United Nations: Department of Economic and Social Affairs PD, 2014.
29. Central Intelligence Agency. Age structure. In: *The world factbook*: Central Intelligence Agency, 2016.
30. Ramharter M, Adegnika AA, Agnandji ST, *et al*. History and perspectives of medical research at the Albert Schweitzer Hospital in Lambaréné, Gabon. *Wien Klin Wochenschr* 2007;119(Suppl 3):8–12.
31. Schwarz NG, Gysels M, Pell C, *et al*. Reasons for non-adherence to vaccination at mother and child care clinics (MCCs) in Lambaréné, Gabon. *Vaccine* 2009;2009:5371–5.
32. Wikipedia. Kenya. https://en.wikipedia.org/w/index.php?title=Kenya&oldid=737329303
33. Wichmann O, Yoon I, Vong S, *et al*. Dengue in Thailand and Cambodia: An assessment of the degree of underestimated disease burden based on reported cases. *PLoS NTD* 2010.
34. Gibbons CL, Mangen MJ, Plass D, *et al*. Measuring underreporting and under-ascertainment in infectious disease datasets: a comparison of methods. *BMC Public Health* 2014;14:147.
35. European Centre for Disease Prevention and Control (ECDC). *Data quality monitoring and surveillance system evaluation - A handbook of methods and applications*. Stockholm: European Centre for Disease Prevention and Control (ECDC), 2014.
36. Mease LE, Coldren RL, Musila LA, *et al*. Seroprevalence and distribution of arboviral infections among rural Kenyan adults: a cross-sectional study. *Virol J* 2011;8:371.
37. World Health Organization. *Guidelines for the clinical evaluation of dengue vaccines in endemic areas*. Geneva: World Health Organization, 2008.
38. Alm E, Lindegren G, Falk KI, *et al*. One-step real-time RT-PCR assays for serotyping dengue virus in clinical samples. *BMC Infect Dis* 2015;15:493.
39. Peeling RW, Artsob H, Pelegrino JL, *et al*. Evaluation of diagnostic tests: dengue. *Nat Rev Microbiol* 2010;8:S30–S37.
40. Kraus AA, Messer W, Haymore LB, *et al*. Comparison of plaque- and flow cytometry-based methods for measuring dengue virus neutralization. *J Clin Microbiol* 2007;45:3777–80.
41. de Alwis R, de Silva AM. Measuring antibody neutralization of dengue virus (DENV) using a flow cytometry-based technique. *Methods Mol Biol* 2014;1138:27–39.
42. Lambeth CR, White LJ, Johnston RE, *et al*. Flow cytometry-based assay for titrating dengue virus. *J Clin Microbiol* 2005;43:3267–72.
43. Cabral-Castro MJ, Peralta RHS, Cavalcanti MG, *et al*. A Luminex-based single DNA fragment amplification assay as a practical tool for detecting and serotyping dengue virus. *J Virol Methods* 2016;236:18–24.
44. Balasuriya UB, Shi PY, Wong SJ, *et al*. Detection of antibodies to West Nile virus in equine sera using microsphere immunoassay. *J Vet Diagn Invest* 2006;18:392–5.
45. Beck C, Desprès P, Paulous S, *et al*. A high-performance multiplex immunoassay for serodiagnosis of flavivirus-associated neurological diseases in horses. *Biomed Res Int* 2015;2015:1–13.
46. Hadinegoro SR. The revised WHO dengue case classification: does the system need to be modified? *Paediatr Int Child Health* 2012;32(Suppl 1):33–8.
47. Guilarde AO, Turchi MD, Siqueira JB, *et al*. Dengue and dengue hemorrhagic fever among adults: clinical outcomes related to viremia, serotypes, and antibody response. *J Infect Dis* 2008;197:817–24.
48. Mossel EC, Crabtree MB, Mutebi JP, *et al*. Arboviruses isolated from mosquitoes collected in Uganda, 2008-2012. *J Med Entomol* 2017;54:1403–9.
49. Shope R. *The flaviviruses: Detection, diagnosis and vaccine development*: Academic Press, 2003.
50. Nikolay B, Diallo M, Boye CS, *et al*. Usutu virus in Africa. *Vector Borne Zoonotic Dis* 2011;11:1417–23.

BMJ Open

Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative

Jacqueline Kyungah Lim, Mabel Carabali, Jung-Seok Lee, Kang-Sung Lee, Suk Namkung, Sl-Ki Lim, Valéry Ridde, Jose Fernandes, Bertrand Lell, Sultani Hadley Matendechero, Meral Esen, Esther Andia, Noah Oyembo, Ahmed Barro, Emmanuel Bonnet, Sammy M Njenga, Selidji Todagbe Agnandji, Seydou Yaro, Neal Alexander and In-Kyu Yoon

*BMJ Open*2018 8:
doi: 10.1136/bmjopen-2017-017673

Chapter 4. Clinical and epidemiologic characteristics associated with dengue fever before and during the 2017 outbreak in Mombasa, Kenya

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	LSH1405874	**Title**	Ms.
First Name(s)	Jacqueline Kyungah		
Surname/Family Name	LIM		
Thesis Title	Undocumented burden of dengue in Africa		
Primary Supervisor	Prof. Neal Alexander		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Plos Neglected Tropical Diseases
Please list the paper's authors in the intended authorship order:	Jacqueline K. Lim, Sultani Hadley Matendechero, Neal Alexander, Jung-Seok Lee, Kang Sung Lee, Suk Namkung, Esther Andia, Noah Oyembo, Henry Kanyi, So Hee Bae, Jae Seung Yang, Tansy Edwards, In-Kyu Yoon, Sammy M. Njenga
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am first author. I developed the grant proposal that was successful in obtaining funds for the study described in this paper. I led the study design and wrote the protocol. I oversaw the ethical approval process, supported study set-up, monitored data collection, performed literature review, performed data cleaning, and conducted statistical analysis including generating SAS code for analysis and all the figures. I wrote the first draft of the manuscript. I then, as the first author, led the process of manuscript revision, and submission. Sultani Hadley Matendechero and Sammy M. Njenga, as the study PI and co-investigator, were responsible for study set-up and execution at the facilities in Mombasa. Neal Alexander, In-Kyu Yoon, and Sammy M. Njenga provided oversight and guidance on the overall study design, project execution, and specific methodologies, e.g. epidemiology, statistical, and virology and other laboratory methods. Jung-Seok Lee, Kang Sung Lee, Suk Namkung, Sl-Ki Lim, Esther Andia, and Noah Oyembo provided support in setting up the study at the sites, in sample and data collection, and data management and analysis. Henry Kanyi, So Hee Bae, and Jae Seung Yang performed laboratory work. Neal Alexander and Tansy Edwards oversaw statistical analysis, and contributed to manuscript preparation.

SECTION E

Student Signature	[redacted]
Date	23 June 2019

Supervisor Signature	[redacted]
Date	17 June 2019

Target journal: PLoS NTD

Title: Clinical and epidemiologic characteristics associated with dengue fever before and during the 2017 outbreak in Mombasa, Kenya

Short title: Epidemiology of dengue fever before and during the recent outbreak in Mombasa, Kenya

Jacqueline K. Lim[1 2], Sultani Hadley Matendechero[3], Neal Alexander[2], Jung-Seok Lee[4], Kang Sung Lee[4], Suk Namkung[1], Esther Andia[5], Noah Oyembo[5], Sl-Ki Lim[1], Henry Kanyi[5], So Hee Bae[6], Jae Seung Yang[6], Mary A. Ochola[7], Tansy Edwards[2], In-Kyu Yoon[1], Sammy M. Njenga[5]

[1]International Vaccine Institute, Global Dengue and *Aedes*-transmitted Diseases Consortium, Seoul, Korea, Republic of; [2]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine; [3]Communicable Disease Prevention and Control, Ministry of Health, Nairobi, Kenya; [4]International Vaccine Institute, Development and Delivery, Seoul, Korea, Republic of; [5]Eastern and Southern Africa Centre of International Parasite Control (ESACIPAC), Kenya Medical Research Institute (KEMRI), Nairobi, Kenya; [6]International Vaccine Institute, Clinical Research Lab, Seoul, Korea, Republic of; [7]Coast Provincial General Hospital, Mombasa County, Kenya

*Corresponding author
Jacqueline K. Lim[1*], MPH
Research Scientist
Global Dengue and *Aedes*-transmitted Diseases Consortium (GDAC)
International Vaccine Institute (IVI)
SNU Research Park, Gwankak-ro 1
Gwanak-gu, Seoul, Korea 151-191
T: +82-2-881-1340 (Dir) / +82-2-872-2801 (ext. 340)
Email: kalim@ivi.int

38 pages

Word count of the text: 5079 (not counting the abstract and tables)

Word count of the abstract: 307

Figures and tables: 3 figures and 4 tables

Abbreviations: CI, confidence interval; CPGH, Coast Province General Hospital; °C, Celsius degrees; CRF, Case Report Form; DENV, Dengue virus; DF, Dengue fever; DHF, Dengue hemorrhagic fever; DSS, Dengue shock syndrome; DVI, Dengue Vaccine Initiative; ELISA, Enzyme-linked immunosorbent assay; ICF, Informed Consent Form; IgM/IgG, Immunoglobulin type M and type G; IRB, Institutional Review Board; KEMRI, Kenya Medical Research Institute; KEPH, Kenya Essential Package for Health; LRTI, lower respiratory tract illness; NPV, negative predictive value; PPV, positive predictive value; RDT, Rapid Diagnostic Test; RT-PCR, Reverse Transcriptase-Polymerase Chain Reaction; URI, upper respiratory illness; WBC, white blood cell

Keywords: Dengue, Kenya, Africa, surveillance, children, outbreak

Contributor's statement page

All persons designated as authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content. And all the authors contributed in some or all areas of acquisition of funding, conception of the study, collection of data, analysis and interpretation of data, drafting the article, article revision, scientific support, and final approval of the version to be published. The authors meet the criteria for authorship and qualify for authorship of this manuscript.

Abstract

Information on dengue burden in Africa is limited. To estimate the proportion of dengue-positive cases among febrile patients, we conducted passive health facility-based fever surveillance in Mombasa, Kenya. The occurrence of an outbreak during the study enabled us to compare clinical indicators of dengue before and during the outbreak.

Non-malarial febrile patients between 1 and 55 years of age, from Mvita sub-county, were enrolled at three health facilities. Acute and convalescent blood samples were collected within an interval of 10-21 days of fever onset. Acute samples were tested with Dengue Duo® RDT and a selected subset with RT-PCR, and acute/convalescent samples with IgM/IgG ELISA.

Among 482 non-malarial febrile patients enrolled, 295 (61.2%) were identified as dengue-positive based on laboratory results. The passive surveillance covered the beginning of a dengue outbreak in April-May 2017, during which 73.9% (122/165) of enrolled patients were dengue-positive. During non-outbreak period, 54.6% (173/317) were dengue-positive. When clinical diagnosis was compared against dengue positivity, the sensitivity (82.8%) was higher during the outbreak than the non-outbreak period (49.1%), but the specificity was lower (86.1% vs. 91.7%). Both positive predictive and negative predictive rates were higher during the outbreak than non-outbreak (PPV=94.4 vs. 87.6% and NPV=63.8 vs 60.0%, respectively). In adjusted analyses, nausea/vomiting, during the outbreak, and arthralgia, headache, and loss of appetite, during non-outbreak period, were found to be associated with dengue-positivity.

In our study, about half of the dengue cases were identified during the outbreak that occurred in the last two months of the 15-month surveillance period. Nonetheless, there was a substantial occurrence of dengue cases even in the non-outbreak period. Clinical features of dengue patients differed between the outbreak and non-outbreak period, and clinical diagnosis was less accurate in the latter. More data from additional prospective and longitudinal studies would help to further define patterns of dengue in Kenya for improved case detection.

Author summary: There are fewer studies on dengue in Africa, relative to the Asia-Pacific and Latin American regions. To estimate the proportion of dengue among patients with fever, and to identify clinical features of dengue during outbreak and non-outbreak periods, we studied 482 patients with non-malarial fever, aged 1-55 years, who attended three health facilities in Mombasa, Kenya. Cases were tested with a rapid test for dengue, and further tests were carried out on paired blood samples taken 10-21 days apart. Even before the dramatic increase during the outbreak, there was a substantial number of dengue patients. Clinical diagnosis was more accurate, relative to dengue-positive cases, during the outbreak period. On average, dengue symptoms varied between the outbreak, and the non-outbreak periods. For example, nausea/vomiting were associated with dengue in the outbreak period, and joint pain, loss of appetite, and headache in the non-outbreak period. More data from additional studies would more clearly identify characteristics of dengue in Mombasa.

Introduction

Dengue fever (DF) is a mosquito-borne flavivirus infection caused by four related but antigenically distinct dengue viruses (DENVs, serotypes 1–4), and is a major and rapidly increasing global public health problem (1). Recent studies have estimated an annual incidence of 50–100 million symptomatic infections globally, with 50,000 dengue hemorrhagic fever (DHF) cases requiring hospitalization and approximately 20,000 deaths annually (2-6). Dengue disproportionately affects countries in the tropics and subtropics, many of which have limited health care resources (7).

Aedes mosquitoes and dengue cases were documented as early as 1823 in 34 countries in Africa, and *Ae. aegypti* and *Ae. albopictus* are widely distributed in the continent (8-11). The first isolation of dengue virus in Africa was in Nigeria in the 1960's (10, 12). However, most reports have come from a small number of countries, and few prospective and population-based dengue studies have been conducted (11, 13, 14). Moreover, the ability of many previous studies to demonstrate the true, population-based, burden of dengue was limited by their retrospective design or sample collection among special groups, such as blood donors. Also, while many dengue endemic countries in Asia and Latin America have mandatory reporting of dengue cases (15), most African countries lack such mechanisms, and only sporadic outbreaks and individual case reports have been documented.

In Kenya, compared to other African countries, there is more evidence available for the presence of dengue, with several documented epidemics and outbreaks in different locations. The most recent outbreak reported was from Mombasa in May 2017 (16). In 2011, an outbreak was confirmed in Mandera, North Eastern region, and, in 2013, another in Mombasa continuing up to 2014 (17, 18). In addition to outbreak investigations, a study based on 868 samples from febrile patients identified from September 2011 to December 2014 in multiple locations in Kenya reported 40% (345/868) of the samples to be dengue-positive for dengue by either IgM Enzyme-linked immunosorbent assay (ELISA) or by RT-PCR (19). In terms of sero-prevalence, dengue was found to be the most common viral pathogen in retrospectively tested blood specimens from HIV-negative survey samples from the 2007 Kenya AIDS Indicator

Survey, with 12.5% having dengue IgG (20). Similarly, a household survey in Mombasa reported that 13% of individuals had serological evidence of either past or current DENV infection, by IgM anti-DENV ELISA. While such information suggests a notable dengue transmission in Kenya, its magnitude remains largely unknown (17, 20). Often, published studies were based on retrospective testing of collected samples that are not well representative of the general population. Additionally, Kenya, like other African countries, has many competing public health problems. The frequently non-specific clinical presentation of dengue makes it difficult to distinguish from other causes of febrile illness, especially since dengue diagnostic assays are not widely available (21).

The Dengue Vaccine Initiative (DVI), in collaboration with the Kenya Medical Research Institute (KEMRI) and the Ministry of Health of Kenya, conducted passive facility-based fever surveillance in Mombasa, Kenya. The study served three objectives. First, to estimate the proportion of dengue cases and compare their clinical patterns to other non-malarial febrile patients. The occurrence of an outbreak during the study period allowed us to include the following two further objectives: to evaluate the performance of clinical diagnosis, in terms of sensitivity and specificity relative to status of dengue positivity, before and during the outbreak, and, finally, to identify signs and symptoms associated with dengue patients, before and during the outbreak.

Methods

Site selection

Site selection was based on published literature on dengue transmission, and reports of outbreaks and cases, seroprevalence studies, dengue burden modelling and research infrastructure (10, 22, 23). Ganjoni health centre, Tudor sub-county Hospital, and Coast Provincial General Hospital (CPGH) in Mombasa, Kenya were selected in consultation with collaborators in Kenya Medical Research Institute (KEMRI) and Ministry of Health of Kenya, serving catchment population of 74,735 residents in Mombasa (Fig. 1) (22, 24, 25).

In the 6-level system of healthcare service delivery in Kenya, Ganjoni health

centre is a Kenya Essential Package for Health (KEPH) level 2 health service provider, focusing on primary care and health promotion for the community. Tudor sub-county Hospital is KEPH level 4, district-level health centre with outpatient and observation care; and CPGH is KEPH level 5, the largest tertiary referral center in the entire coast region.

Study area and population

Coastal Kenya, in eastern Africa, has a warm and humid tropical climate (25). Mombasa, the country's second largest city after Nairobi, has a population of about 1.3 million, of whom almost 50% are under 15 years of age (6, 24). The "long rains" period begins around April and the "short rains" around October (25). This study took place between March 2016 and May 2017 (15 months) (Figure 1).

Study design

Passive fever surveillance

Investigational methods used in this study have previously been described (22). To determine the burden of symptomatic dengue in Mombasa, among non-malaria febrile patients, passive facility-based surveillance was implemented in the three facilities, enrolling both outpatients and inpatients (22). First, patients who were febrile, or with a history of fever in the past 7 days, were tested for malaria using RDT (either CareStart™ Malaria or SD BIOLINE Malaria kit) as part of routine practice. Those eligible and agreeing to participate were referred to a study physician (Figure 2). Malaria RDT-negative patients were enrolled in the study and tested with dengue RDTs. An acute sample of blood was taken at first presentation with current or history of fever. Phlebotomists performed a blood draw of 7-10 ml with aseptic measures using disposable needles and syringes. The whole blood was used for the dengue RDT. After collection of blood samples, a study physician conducted interviews and physical

examinations and the surveillance case report forms were completed to capture symptom history, medical history, treatment and laboratory results (22).

The patient was asked to return to the facility for the convalescent sample collection 10-14 days after the first visit. After the 14th day, if the patient had not returned, a house visit was made and the second blood sample was collected within 21 days of the first visit.

Confirmation of positive RDT was done by detection of IgM/IgG antibodies against dengue virus using ELISA (on all the samples) and dengue virus using PCR (on a selected subset) in serum samples as described below (15, 26).

Study participants

For the passive facility-based fever surveillance, individuals who met the following criteria were eligible for study enrollment:

1. Age 1- 55 years;
2. Resident of the catchment area covered by healthcare facilities participating in the study, without plans to move out within 12 months;
3. Signed informed consent, and assent for those aged between 13 and 17 years; and
4. Patients presenting with current fever (body temperature ≥ 37.5º C) or history of fever for ≤ 7 days duration without localizing signs (fever caused by a localized infection as well as fever with a known and confirmed etiology other than dengue, such as malaria confirmed by malaria RDT).

Laboratory Testing Algorithm

Acute samples were tested using a commercial RDT for dengue NS1 and IgM/IgG (Dengue Duo®, Standard Diagnostics, Yongin-Si, Korea) on the day of the first visit at the site of patient presentation (day 1). The acute and convalescent samples were subsequently tested in the KEMRI laboratory using dengue IgM/IgG ELISA (SD

Dengue IgM & IgG Capture ELISA®, Standard Diagnostics, Yongin-Si, Korea). The results from IgM/IgG ELISA and RDT were used for selecting samples that would go on to further testing. Those samples that met the following criteria underwent molecular analysis with RT-PCR (27): (i) NS1- or IgM-positive on the rapid test in acute phase; and/or (ii) sero-converted between acute and convalescence phase on IgM and IgG capture ELISA. RT-PCR was also performed on a limited number of randomly selected samples that were sero-positive at both acute and convalescent time points by IgM and IgG capture ELISA as well as those negative by RDT and IgM/IgG ELISA at all time points. In addition, convalescent samples were tested using chikungunya IgM ELISA kit (SD Chikungunya IgM ELISA®, Standard Diagnostics, Yongin-Si, Korea). The detailed laboratory testing procedure has been previously described (22).

Laboratory confirmation for dengue infection was performed according to WHO diagnostic criteria (28). Sero-conversion of anti-dengue IgM and IgG between the acute and convalescent phases and/or virus detection (RT-PCR) in the acute serum specimen was considered to be confirmed dengue. A positive IgM serology in single serum and/or positive on NS1 or IgM of RDT in single acute serum were criteria of probable dengue infection (28). Confirmed and probable dengue infections were grouped to be dengue-positive in this analysis. Samples with negative results on RT-PCR and sero-negative results on paired IgM and IgG ELISA results were classified as non-dengue. Also, a positive IgG serology in single serum, with negative results from all other tests, was classified as non-dengue.

Statistical analysis

There were three components in the analysis.

1. *Clinical characteristics of dengue-positive versus non-dengue cases*

A descriptive summary of characteristics is presented for dengue-positive versus non-dengue cases. Age was initially broken down to 8-level categorical variable for descriptive purposes. A body temperature ≥38.0°C, the 75th percentile of the body

temperature measured at the time of enrollment, was used to create a dichotomous variable indicating a higher body temperature at the patient's first presentation. Clinical diagnosis at admission, prior to lab-confirmation, was grouped with suspected dengue, undifferentiated fever, and non-dengue.

In the penultimate month of surveillance (April 2017) as we observed a steep rise in the dengue caseload, there was a public health alert issued by Mombasa County health officials over a dengue outbreak and it was declared an outbreak in May (29-32). The last two months of surveillance were analyzed as an outbreak period and the previous months as non-outbreak. Yellow fever vaccination history was dichotomized between those who reported having been vaccinated versus those who did not remember or reported no vaccination. Dichotomous variables were also created for various signs and symptoms (presence vs. absence). For nausea and vomiting, patients were asked whether they had nausea and/or vomiting during their illness. Categorical pair-wise comparisons were made across dengue status using χ^2 or Fisher's exact tests. Continuous variables were compared using Student's *t*-test or ANOVA.

2. *Accuracy of the clinical diagnosis of suspected dengue*

Clinical diagnosis as categorized as suspected dengue based on clinician judgement or other than suspected dengue. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), relative to dengue-positive status, were reported with corresponding 95% CIs.

3. *Differences in dengue cases between outbreak and non-outbreak periods*

Finally, clinical indicators associated with dengue-positive cases were identified using multivariable logistic regression. The analysis was to assess how the clinical profile of dengue cases differed between those presenting during the outbreak versus non-outbreak periods.

A final model was built for the outcome of dengue positivity (dengue-positive vs. non-dengue) for the outbreak and non-outbreak periods with the same *a priori* confounders of age, gender, treatment center, and fever duration. Age is a known

confounder for dengue and presentation as a primary or subsequent infection. It was categorized into three and then two levels due to data sparsity. Gender may be related to *Aedes* exposure and may mediate clinical presentation (33). Treatment center may be a proxy for otherwise any unexplained variation. Fever duration prior to the visit, reflecting disease progression, may affect level of viremia and clinical presentation (34, 35).

A backward stepwise process was used to select variables to be entered in the final multivariable model for the outbreak and non-outbreak periods, with a significance level of 0.2 for entry and 0.1 for retention. Other variables investigated were variables on clinical presentation, such as high body temperature at enrollment, various signs and symptoms. Clinical diagnosis of suspected dengue was considered too closely related to dengue positivity as well as some of the signs and symptoms, and was not included in the model. Due to data sparsity, some signs and symptoms from the descriptive and univariable analyses were not included in the model building. All analyses were performed using SAS® version 9.4 (SAS Institute, Cary, North Carolina).

Ethical considerations

The study protocol obtained ethical approvals from the Institutional Review Boards (IRBs) of the International Vaccine Institute, the London School of Hygiene and Tropical Medicine, and the KEMRI Scientific and Ethical Review Unit.

All adult subjects provided informed consent, and a parent or guardian of any child participant provided informed consent on the child's behalf with assent from the child aged between 13 and 17 years. For all subjects, written consent was obtained.

Results

General and clinical characteristics of dengue cases

Of 513 enrolled individuals, 31 had incomplete visit 1 (acute) lab data (i.e. RDT results available but no sample for ELISA or PCR, Figure 2). These patients were found to be non-differential to those that were in the analysis sample, in terms of age, gender,

status of being kept under observation, and days into illness at the time of enrollment. However, they were more likely (25/31) to be enrolled at CPGH, where the majority of our analysis sample was also enrolled. Of the remaining 482 patients, 223 (46%) had confirmed dengue infections based on paired ELISA and/or PCR, 72 (15%) were classified as dengue-probable, and 187 (39%) as non-dengue cases. Table 1 describes the demographic and clinical characteristics between dengue-positive and non-dengue cases and the breakdown by 3-level dengue-status (dengue-confirmed, probable, and non-dengue) is presented in supplementary tables. Of these dengue- positive cases, 69% (205/295) were based on PCR confirmation (Fig. 2). Also, 29% (48 of 166 paired samples tested) and 24% (39 of 160 paired samples tested) were lab-confirmed with dengue infection by seroconversion between acute and convalescent samples using IgM and IgG capture ELISA, respectively. There were 32 patients confirmed by both PCR and ELISA (of either IgM or IgG seroconversion) and 18 patients by seroconversion on ELISA alone.

Only two of the 482 patients were required to be kept under observation <3 days and both were dengue-positive patients. Close to 80% of the dengue-positive patients were between 15 and 34 years-of-age (Table 1). Of the 482 patients, the average time between fever onset and presentation was 3.0 days, with no evidence that this differed between dengue-positive and non-dengue cases. However, the average entire duration of fever illness was significantly longer for dengue cases than non-dengue cases — 6.9 versus 4.9 days — among 309 patients with data on endpoint of their fever illness duration.

Table 1. Demographic and clinical characteristics between dengue-positive and non-dengue cases among febrile enrollees of the health facility-based fever surveillance in Mombasa, Kenya in 2016-2017

Characteristics	N (%)			p-value
	Dengue-positive (n=295)	Non-dengue (n=187)	Total (n=482)	
Place of enrollment				0.645

CPGH	139 (47.12)	94 (50.27)	233 (48.34)	
Tudor	123 (41.69)	70 (37.43)	193 (40.04)	
Ganjoni	33 (11.19)	23 (12.30)	56 (11.62)	
Mean age (SD)	23.35 (9.23)	23.14 (13.46)	23.27 (11.05)	0.839
Age group (years)				**<.001**
1-4	8 (2.71)	31 (16.58)	39 (8.09)	
5-9	10 (3.39)	6 (3.21)	16 (3.32)	
10-14	13 (4.41)	6 (3.21)	19 (3.94)	
15-19	45 (15.25)	21 (11.23)	66 (13.69)	
20-24	124 (42.03)	39 (20.86)	163 (33.82)	
25-34	61 (20.68)	44 (23.53)	105 (21.78)	
35-44	24 (8.14)	28 (14.97)	52 (10.79)	
45-55	10 (3.39)	12 (6.42)	22 (4.56)	
Female	117 (39.66)	90 (48.13)	207 (42.95)	0.067
IPD/OPD	2 (0.68)/293 (99.32)	0/187 (100.0)	2 (0.41)/480 (99.59)	0.259
Fever duration prior to visit (days, SD)	2.96 (1.92)	2.84 (1.79)	2.91 (1.87)	0.513
Fever duration, entire illness (days, SD)*	6.88 (3.75)	4.91 (2.76)	6.17 (3.55)	**<.001**
Temperature at presentation (SD)	37.85 (0.66)	37.71 (0.73)	37.80 (0.69)	**0.024**
Temperature at presentation				**0.014**
Below 38.0°c	179 (60.68)	134 (71.66)	313 (64.94)	
≥ 38.0°c	116 (39.32)	53 (28.34)	169 (35.06)	
Prev. dengue infection**	3 (1.02)	3 (1.60)	6 (1.24)	0.323
YF vaccination**	146 (49.49)	77 (41.18)	223 (46.27)	0.074
Clinical diagnosis				
Suspected dengue	186 (63.05)	18 (9.63)	204 (42.32)	**<.001**
Undifferentiated fever	76 (25.76)	121 (64.71)	197 (40.87)	
Non-dengue	33 (11.19)	48 (25.67)	81 (16.80)	
URI	18 (54.55)	27 (56.25)	45 (55.56)	
Malaria	1 (3.03)	3 (6.25)	4 (4.94)	

UTI	2 (6.06)	2 (4.17)	4 (4.94)	
Diarrheal illness	1 (3.03)	1 (2.08)	2 (2.47)	
Others	11 (33.33)	15 (31.25)	26 (32.10)	
Signs and symptoms** (presence)				
Rash	34 (11.53)	10 (5.35)	44 (9.13)	**0.022**
Fatigue/weakness	269 (91.19)	156 (83.42)	425 (88.17)	**0.010**
Headache	282 (95.59)	155 (82.89)	437 (90.66)	**<.001**
Retro-orbital pain	166 (56.27)	69 (36.90)	235 (48.76)	**<.001**
Neck pain	90 (30.51)	43 (22.99)	133 (27.59)	0.072
Ear pain	23 (7.80)	10 (5.35)	33 (6.85)	0.300
Breathing difficulty	1 (0.34)	5 (2.67)	6 (1.24)	**0.035**
Nasal congestion	15 (5.08)	26 (13.90)	41 (8.51)	**0.001**
Rhinorrhea	27 (9.15)	37 (19.79)	64 (13.28)	**0.001**
Sore Throat	17 (5.76)	22 (11.76)	39 (8.09)	**0.019**
Cough	46 (15.59)	48 (25.67)	94 (19.50)	**0.007**
Sputum production	9 (3.05)	15 (8.02)	24 (4.98)	**0.015**
Nausea & vomiting	151 (51.19)	75 (40.11)	226 (46.89)	**0.018**
Diarrhea	31 (10.51)	25 (13.37)	56 (11.62)	0.340
Constipation	13 (4.41)	9 (4.81)	22 (4.56)	0.835
Abdominal pain	101 (34.24)	55 (29.41)	156 (32.37)	0.270
Nose bleeding	8 (2.71)	0	8 (1.66)	**0.026**
Gum bleeding	10 (3.39)	0	10 (2.07)	**0.008**
Flushed face	6 (2.03)	5 (2.67)	11 (2.28)	0.647
Loss of appetite	195 (66.10)	93 (49.73)	288 (59.75)	**<.001**
Myalgia	221 (74.92)	114 (60.96)	335 (69.50)	**0.001**
Arthralgia	222 (75.25)	104 (55.61)	326 (67.63)	**<.001**

*only among those that reported the end of fever illness (n=309; 199 dengue and 110 non-dengue patients)

**based on self-report

Of the 482 RDT results, 189 patients (39.2%) were positive for NS1 and/or IgM. In terms of clinical diagnosis, about 42% (204/482) of enrolled febrile patients had

clinically suspected dengue (Table 1). Among dengue-positive patients, 63% (186/295) were clinically suspected dengue, prior to lab-confirmation of dengue. Of the non-dengue patients, the majority, 64.7 and 25.7% were diagnosed with undifferentiated fever and non-dengue, respectively.

There were peaks of dengue incidence in April-June 2016 and April-May 2017 (Figure 3), coinciding with the “long rains” season. Of 295 dengue cases, 173 were identified before (173/317), and 122 during the outbreak (122/165). DENV-2 was the predominant serotype during the 1 year period before the outbreak, with a lower level of DENV 1 throughout the study period (Figure 3). The outbreak was also largely DENV 2.

In terms of symptoms, rash, fatigue, headache, retro-orbital pain, nausea/vomiting, nose bleeding, gum bleeding, loss of appetite, myalgia, and arthralgia were found more commonly among dengue-positive cases, compared to non-dengue. Breathing difficulty, nasal congestion, rhinorrhea, sore throat, cough, and sputum production were found more commonly among non-dengue cases, compared to dengue-positive cases.

Accuracy of dengue case detection by clinical diagnosis with suspected dengue

Among 482 patients, clinical diagnosis (suspected dengue vs. other than suspected dengue) was compared against dengue positivity (dengue-positive vs. non-dengue cases). While clinical diagnosis of dengue and RDT positive result were found to be closely related in our data, clinical diagnosis was often made in the absence of knowledge of the dengue RDT result. Even though the rapid test results become available in 15-20 minutes, due to the patient flow and the volume of febrile patients as well as the level of physician availability, the results may or may not have been available at the time of diagnosis.

Clinical diagnosis of dengue had quite high specificity (90%) while sensitivity was 63% (Table 2). During the outbreak, the sensitivity of clinical diagnosis was significantly higher (83% vs. 49%) than prior to it, but specificity was slightly lower (86% vs. 92%). Both PPV and NPV were higher during the outbreak.

Table 2. Sensitivity and positive predictive value of clinical diagnosis vs. lab confirmation of dengue among febrile patients in Kenya

		Overall			Outbreak			Non-outbreak		
		Clinical diagnosis (by the physician*)		Total	Clinical diagnosis (by the physician*)		Total	Clinical diagnosis (by the physician*)		Total
		Suspected dengue	Other than dengue		Suspected dengue	Other than dengue		Suspected dengue	Other than dengue	
Dengue positivity (defined in this study)	Dengue-positive (dengue confirmed & probable cases)	186	109	295	101	21	122	85	88	173
	Non-dengue	18	169	187	6	37	43	12	132	144
Total		204	278	482	107	58	165	97	220	317
		Sensitivity = 63.05% (57.26 to 68.57%) Specificity = 90.37 %% (85.21 to 94.19%) PPV = 91.18% (86.85 to 94.18%) NPV = 60.79 % (57.01 to 64.45%)			Sensitivity = 82.79% (74.90 to 89.02%) Specificity = 86.05% (72.07 to 94.70%) PPV = 94.39% (88.86 to 97.26%) NPV = 63.79% (53.97 to 72.59%)			Sensitivity = 49.13% (41.47 to 56.83%) Specificity = 91.67% (85.90 to 95.62%) PPV = 87.63% (80.14 to 92.56%) NPV = 60.00% (56.24 to 63.64%)		

PPV, positive predictive value; NPV, negative predictive value

* referring to the RDT test results was at the discretion of the attending physician and in some cases, RDT test was performed at the lab while the patient is being examined by the physician

Differences in dengue cases between outbreak and non-outbreak periods

From univariable analyses during the outbreak (Table 3A), the variables found to be associated with increased odds of dengue positivity, compared to non-dengue, were fatigue/weakness and nausea/vomiting. During the non-outbreak period (Table 3B), gender, age, retro-orbital pain, myalgia, arthralgia, loss of appetite, and headache, were associated with increased odds of dengue compared to non-dengue. Rhinorrhea and nasal congestion were associated with decreased odds.

Table 3A. Univariable analysis showing associations with dengue positivity during the period of outbreak in the health facility-based fever surveillance

Characteristics	During outbreak (n=165)					
	Total N	N (%) Dengue-positive (n=122)	N (%) Non-dengue (n=43)	Univariable analysis Dengue-positive vs. no dengue		
				OR	95% CI	p-Value
Gender						0.659
Male	93	70 (75.27)	23 (24.73)	Ref	-	
Female	72	52 (72.22)	20 (27.78)	0.85	0.43-1.72	
Age (years)						0.123
1-19	38	28 (73.68)	10 (26.32)	Ref	-	
20-24	77	62 (80.52)	15 (19.48)	1.48	0.59-3.69	
25-55	50	32 (64.00)	18 (36.00)	0.64	0.25-1.60	
Treatment center						0.929
CPGH	56	41 (73.21)	15 (26.79)	Ref	-	
Tudor	87	64 (73.56)	23 (26.44)	1.02	0.47-2.18	
Ganjoni	22	17 (77.27)	5 (22.73)	1.24	0.39-3.97	
Fever duration prior to visit						0.449
1-2 days	71	54 (76.06)	17 (23.94)	Ref	-	
3 days	49	33 (67.35)	16 (32.65)	0.65	0.29-1.46	
4-7 days	45	35 (77.78)	10 (22.22)	1.10	0.45-2.68	
Temperature at presentation						0.130
Below 38.0°c	70	56 (80.0)	14 (20.0)	Ref	-	

≥ 38.0°c	95	66 (69.47)	29 (30.53)	1.76	0.85-3.65	
Presence of signs and symptoms (ref. absence)						
Retro-orbital pain	109	84 (77.06)	25 (22.94)	1.59	0.78-3.26	0.204
Myalgia	127	97 (76.38)	30 (23.62)	1.68	0.77-3.69	0.195
Arthralgia	125	96 (76.80)	29 (23.20)	1.78	0.83-3.86	0.142
Fatigue/weakness*	148	113 (76.35)	35 (23.65)	**2.87**	**1.03-8.00**	**0.044**
Loss of appetite	126	97 (76.98)	29 (23.02)	1.87	0.86-4.07	0.112
Headache	162	120 (74.07)	42 (25.93)	1.43	0.13-16.16	0.773
Neck pain	50	40 (80.0)	10 (20.0)	1.61	0.72-3.59	0.245
Ear pain	11	9 (81.82)	2 (18.18)	1.63	0.34-7.87	0.541
Rhinorrhea	9	7 (77.78)	2 (22.22)	1.25	0.25-6.25	0.788
Nasal congestion	4	2 (50.0)	2 (50.0)	0.34	0.05-2.50	0.291
Cough	20	12 (60.0)	8 (40.0)	0.48	0.18-1.26	0.136
Nausea & vomiting*	86	71 (82.56)	15 (17.44)	**2.56**	**1.26-5.36**	**0.001**
Diarrhea	18	14 (77.78)	4 (22.22)	1.26	0.39-4.07	0.695
Abdominal pain	67	49 (73.13)	18 (26.87)	0.93	0.46-1.89	0.846
Sore Throat	10	6 (60.0)	4 (40.0)	0.50	0.14-1.88	0.308

Statistical significance of the frequencies: *p-value<0.05 **p-value<.001

Table 3B. Univariable analysis showing associations with dengue positivity during the period of non-outbreak (n=317) in the health facility-based fever surveillance

Characteristics	During non-outbreak (n=317)					
	Total N	N (%) Dengue-positive (n=173)	N (%) Non-dengue (n=144)	Univariable analysis Dengue-positive vs. no dengue		
				OR	95% CI	p-Value
Gender*						**0.048**
Male	182	108 (59.34)	74 (40.66)	Ref	-	
Female	135	65 (48.15)	70 (51.85)	0.64	0.41-1.00	
Age (years)**						**<.001**
1-19	102	48 (47.06)	54 (52.94)	Ref	-	
20-24	86	62 (72.09)	24 (27.91)	2.91	1.58-5.35	

25-55	129	63 (48.84)	66 (51.16)	1.07	0.64-1.81	
Treatment center						0.649
CPGH	177	98 (55.37)	79 (44.63)	Ref	-	
Tudor	106	59 (55.66)	47 (44.34)	1.01	0.62-1.64	
Ganjoni	34	16 (47.06)	18 (52.94)	0.72	0.34-1.50	
Fever duration prior to visit						0.258
1-2 days	176	98 (55.68)	78 (44.32)	Ref	-	
3 days	69	32 (46.38)	37 (53.62)	0.69	0.39-1.20	
4-7 days	72	43 (59.72)	29 (40.28)	1.18	0.68-2.06	
Temperature at presentation						0.147
Below 38.0°c	218	113 (51.83)	105 (48.17)	Ref	-	
≥ 38.0°c	99	60 (60.61)	39 (39.39)	1.43	0.88-2.32	
Presence of signs and symptoms (ref. absence)						
Retro-orbital pain*	126	82 (65.08)	44 (34.92)	**2.05**	**1.29-3.26**	**0.002**
Myalgia*	208	124 (59.62)	84 (40.38)	**1.81**	**1.13-2.89**	**0.013**
Arthralgia**	201	126 (62.69)	75 (37.31)	**2.47**	**1.54-3.94**	**<.001**
Fatigue/weakness	277	156 (56.32)	121 (43.68)	1.74	0.89-3.41	0.104
Loss of appetite*	162	98 (60.49)	64 (39.51)	**1.63**	**1.05-2.55**	**0.031**
Headache**	275	162 (58.91)	113 (41.09)	**4.04**	**1.95-8.37**	**<.001**
Neck pain	83	50 (60.24)	33 (39.76)	1.37	0.82-2.28	0.228
Ear pain	22	14 (63.64)	8 (36.36)	1.50	0.61-3.68	0.379
Rhinorrhea*	55	20 (36.36)	35 (63.64)	**0.41**	**0.22-0.74**	**0.003**
Nasal congestion*	37	13 (35.14)	24 (64.86)	**0.41**	**0.20-0.83**	**0.014**
Cough	74	34 (45.95)	40 (54.05)	0.64	0.38-1.07	0.090
Nausea & vomiting	140	80 (57.14)	60 (42.86)	1.20	0.77-1.88	0.414
Diarrhea	38	17 (44.74)	21 (55.26)	0.64	0.32-1.26	0.197
Abdominal pain	89	52 (58.43)	37 (41.57)	1.24	0.76-2.04	0.390
Sore Throat	29	11 (37.93)	18 (62.07)	0.48	0.22-1.04	0.063

Statistical significance of the frequencies: *p-value<0.05 **p-value<.001

During the outbreak, the multivariable model building process selected nausea/vomiting. During the non-outbreak period, the process selected age, high body temperature, diarrhea, arthralgia, nasal congestion, loss of appetite, and headache. With age, gender, treatment center, and fever duration prior to visit as a prior adjustments and high body temperature identified to be significantly associated in the variable screening/selection process, the final model was run for the outbreak and non-outbreak periods, with significant signs and symptoms (nausea/vomiting, diarrhea, arthralgia, nasal congestion, loss of appetite, and headache). Table 4 shows that, during the outbreak period, dengue cases were associated with 2.6 times greater odds of presenting with nausea/vomiting, compared to non-dengue cases. Table 4 (on the right) shows that, during the non-outbreak period, dengue cases were 2.0 times more likely to present with arthralgia, 3.1 times more likely to present with headache, and 1.9 times more likely to present with loss of appetite, compared to non-dengue cases.

Table 4. Multivariable associations with dengue positivity by outbreak status

Characteristics	Multivariate analysis					
	During the outbreak* (n=165) Dengue-positive (n=122) *ref.* no dengue (n=43)			During non-outbreak (n=317) Dengue-positive (n=173) *ref.* no dengue (n=144)		
	aOR	95% CI	p-Value	aOR	95% CI	p-Value
Gender			0.378			0.221
Male				Ref	-	
Female	0.70	0.32-1.55		0.73	0.44 – 1.21	
Age (years)			0.319			**0.009**
1-19				Ref	-	
20-24	1.47	0.55-3.94		**2.36**	**1.16-4.83**	
25-55	0.72	0.26-1.95		0.90	0.47-1.74	
Treatment center			0.978			0.161
CPGH				Ref	-	
Tudor	1.09	0.46-2.55		1.28	0.68-2.40	
Ganjoni	1.13	0.30-4.24		0.53	0.22-1.26	
Temperature at presentation			0.212			0.066
Below 38.0°c				Ref	-	
≥ 38.0°c	1.71	0.74-3.96		1.72	0.97-3.08	
Fever duration prior to visit			0.451			0.088
1-2 days				Ref	-	
3 days	0.57	0.23-1.43		0.52	0.27-0.98	
4-7 days	0.94	0.35-2.50		1.05	0.56-1.98	
Presence of signs and symptoms (*ref.* absence)						
Nausea/vomiting	**2.55**	**1.11-5.87**	**0.028**	1.21	0.71-2.07	0.474
Nasal congestion	0.22	0.03-1.82	0.160	0.52	0.23-1.18	0.117
Arthralgia	1.71	0.65-4.55	0.280	**2.01**	**1.15-3.50**	**0.014**
Headache	1.37	0.08-23.96	0.831	**3.06**	**1.26-7.42**	**0.013**
Loss of appetite	1.19	0.47-3.02	0.722	**1.92**	**1.10-3.37**	**0.023**
Diarrhea	1.28	0.35-4.69	0.710	0.47	0.22-1.02	0.057

*in April-May of 2017; aOR = adjusted odds ratio

Discussion

Overall, evidence on dengue in Africa is limited (11). For Kenya, most of the evidence on dengue from outbreak investigations and retrospective testing of existing serum banks from other studies (17, 18). Our data showed that dengue infection is an important cause of non-malarial febrile illness in patients seeking care at public health facilities in Mombasa. Approximately half the dengue cases of the 15-month study were identified in the last two months, which coincided with an outbreak. This allowed us to demonstrate differences in clinical and symptomatic patterns between the outbreak and non-outbreak periods.

General and clinical characteristics of dengue cases

A key finding of our study was that a substantial number of dengue-positive cases was identified, including a notable outbreak in Apr/May 2017, in Mombasa. Dengue cases were 2.4 times more common during the outbreak, than in non-outbreak period (O.R=2.36, 95% C.I=1.56-3.57). Of 482 non-malarial febrile patients, 295 (61.2%) were identified to be dengue-positive. Of those patients enrolled before the outbreak, about half (173/317, 54.6%), were identified to be dengue-positive, compared to more than two-thirds of those enrolled during the outbreak (122/165, 73.9%). This shows the magnitude of the dengue outbreak captured in the first two months, compared to the baseline caseload in non-outbreak period.

The high proportion of dengue-positive cases should be interpreted bearing in mind that malaria cases were excluded from the denominator. Also, it is expected that the proportion of cases would be increased during a dengue outbreak. In the current study, although it was only the first two months of the outbreak, the study happened to capture the start of a dengue outbreak in April 2017 (Fig. 3) (29, 31, 32). This could still have caused the proportion of dengue cases, over the duration of the whole study, to be higher than in non-epidemic years. Officially reported to WHO, this outbreak in Mombasa had more than half of the individuals (540/945) lab-confirmed with dengue in May and June 2017 (16).

Among comparable previous studies, few reported levels of dengue as high as in the current study (18). In particular, a surveillance study conducted in seven Mombasa hospitals in 2013 found that, among 267 cases with suspected dengue, 58% were lab-

confirmed with a current infection (17). In our data, of 204 dengue-suspected patients, 156 (76%) were dengue-confirmed by either PCR and/or ELISA (suppl. Table 1). There may be differences in study settings, but these still indicate similarly high proportions of dengue cases.

However, most other studies report different findings from ours. A study tested 500 samples from febrile patients identified in CPGH from January 2014 to March 2015 using in-house indirect ELISA and Focus Reduction Neutralization tests (FRNT) (36). This study found DENV to account for 15% of all the fevers presenting, even without screening out malaria RDT-positive cases (36). A study conducted in Kilifi, about 70km along the coast from Mombasa, reported that about 10% of febrile adults that were not acute HIV infection and also RDT-negative for malaria were PCR-confirmed with dengue in 2014-2015 (37). Among the corresponding subgroup in our study, there were 173 (50.4%) dengue cases confirmed by either PCR and/or ELISA among 343 febrile patients between 18-35 years-of-age. Even if we consider the differences in the study setting (location, outbreak in the study period) and methods (different diagnostic tests, i.e. dengue-confirmation using only PCR among HIV and malaria negative febrile patients in Kilifi and ELISA/FRNT among all febrile patients in Mombasa), the estimate of proportion of dengue was higher in our data.

Those cases who were RDT-negative at baseline were less likely than RDT-positive cases to provide a convalescent sample, despite reminders and home visits. Thus, our analysis dataset included those without a convalescent sample in order to minimize likely upward bias it could have resulted in estimation of the proportion of dengue-positive cases.

During the study period, there was a programmatic challenge to patient recruitment when there was a strike among medical officers. This lasted 71 days between September and November of 2016. Health facilities remained operational and there were clinical officers and other health facility staff on duty. The official absence of medical officers at health facilities could have influenced health-seeking behavior and our surveillance might otherwise have enrolled a larger volume of febrile patients. If so, our denominator of non-malarial fever cases could have been bigger and the proportion of dengue, before the outbreak, could have been lower.

There are limited data from Kenya and Africa on how dengue affects different age groups. Among those attending health facilities in the current study, dengue cases were more concentrated between 15 and 34 years-of-age, higher on average than in the 2011 outbreak in Mandera in which 30% of dengue cases were below 10 years of age with

another 20% between 10 and 19 years (18). However, our findings may have been impacted by age-specific health-seeking behavior, with 70% of all enrollees being teenagers or young adults.

In the context of other co-circulating pathogens, chikungunya was also suspected to be a possible co-circulating virus in the area. Retrospectively, we performed chikungunya testing to see if there could have been co-infections with dengue. On all the convalescent samples, we performed Chikungunya IgM ELISA tests and none was found positive. Furthermore, our study did not enroll malaria RDT positive patients in the patient screening process, even though some of them could have had co-infection with dengue. A study on the 2011 dengue outbreak in Mandera town, Kenya, reported 4 out of 30 lab-confirmed dengue cases to have malaria co-infection (18). However, overall, such concurrent infection is reported to be uncommon (38, 39).

Accuracy of dengue case detection by clinical diagnosis with suspected dengue

Overall, of 295 dengue-positive cases, 186 (63.1%) were clinically diagnosed with suspected dengue. Their clinical diagnosis of suspect dengue was made prior to lab-confirmation of dengue and, often, in the absence of knowledge of the dengue RDT results. The sensitivity of clinical diagnosis, relative to dengue-positivity, was significantly higher during the outbreak compared to non-outbreak (83% vs. 49%), while specificity was similar (86% vs. 92%). In terms of PPV and NPV, clinical diagnosis performed better during the outbreak (PPV=94% and NPV=64%), compared to non-outbreak (PPV=88% and NPV=60%).

Guidelines for case detection and management remained unchanged throughout the study period and, being part of the routine practice, was not influenced by the study procedure. Despite possibly limited applicability, for clinical judgement, the 1997 WHO dengue case classification criteria were used in the study facilities for diagnosis and management for suspected dengue (40). In addition to referring to the criteria, clinicians suspected dengue if the patient tested negative for malaria (by RDT or microscopy) or did not respond to antimalarial treatment. With increased dengue transmission and caseload during the outbreak, clinicians may be more alert to identify dengue cases during outbreak. One potential factor influencing performance of clinical diagnosis could have been a difference in duration of illness before presentation, although, in fact, this was similar in the

outbreak and non-outbreak periods (34, 35).

Differences in dengue cases between outbreak and non-outbreak periods

The main objective of the third component of this analysis was to assess how the clinical presentation of dengue-positive cases might differ between outbreak and non-outbreak periods. The final model, with a priori confounders and controlled for high body temperature, showed that, during the outbreak period, dengue cases were more likely to present with nausea/vomiting. During the non-outbreak period, dengue-positive cases were more likely to present with arthralgia, headache, and loss of appetite, compared to non-dengue cases.

These differences in symptomatic presentation of dengue between the outbreak and non-outbreak periods were reported in other studies. A previous surveillance study of the 2011 dengue outbreak in Mandera, Kenya, also reported high frequencies of vomiting, arthralgia, and headache among dengue cases, albeit with a small sample size of 30 dengue cases (18). In various other study settings, these variables have been reported as being positively associated with dengue, some specifically in outbreak settings (41-43). Specifically for gastrointestinal symptoms, there had been previous studies, which indicated higher odds of nausea and vomiting among dengue cases compared against non-dengue cases (44-46).

The 1997 case definition differentiates probable and confirmed DF, DHF, and dengue shock syndrome (DSS) (40). As in the 1997 case definition, nausea/vomiting, arthralgia, and headache were signs associated with dengue in the current study (40). The revised 2009 WHO case definitions classified the illness into dengue with and without warning signs, and severe dengue (40, 47). According to this revised scheme, warning signs, possibly leading to severe dengue, include abdominal pain, persistent vomiting and/or diarrhea, lethargy, clinical fluid accumulation, and mucosal bleeding (40, 47). Of these warning signs, we found only nausea/vomiting to be associated with dengue positivity. Also, there are some data suggesting association of gastrointestinal signs, such as nausea/vomiting, to hospitalized and more severe dengue (44, 48). Also, a meta-analysis on symptoms of severe dengue disease reported vomiting/nausea to be significant predictor for progression into severe dengue whereas headache showed protective effect against progression into severe dengue (49).

In the current study, most of the dengue-positive cases were mild. In the whole study, only two cases required hospitalization and both were dengue-positive. Both were clinically diagnosed with dengue, not DHF, and no complications were recorded. Of these two, one had a complete record of admission: no complication was reported, and they spent two days in hospital before discharge. While our study did not collect data on other indicators of dengue severity, nausea/vomiting being associated with dengue cases in the outbreak, but not before, may indicate likely severity of dengue illness during the outbreak (44, 48, 49).

For the non-outbreak period, arthralgia, loss of appetite, and headache, were found to be associated with dengue-positivity, consistent with previous reports in epidemic and non-epidemic settings (42, 50, 51). This is also consistent with previous findings that these symptoms were more strongly associated with classical DF than with severe forms (45, 52), with the case profile of the current study being closer to the former.

In our study, DENV-2 was the predominant serotype during both before and during the outbreak (Figure 3). A study of febrile patients in CPGH from January 2014 to March 2015 also reported that DENV 2 was the predominant serotype, followed by DENV 3 and DENV 1(36). Outbreaks may coincide with a shift in dengue serotypes (53-55), but this was not observed in the current study. In Mombasa, between 2011 and 2014, the most frequent serotype was DENV 1 followed by DENV 3 (19). The outbreak recorded in Mombasa in 2013 was also documented to be DENV 1 (56). Although in different regions, in a cohort of children in Western Kenya, in Kisumu and Chulaimbo, the serotypes identified among dengue cases in March and April 2016 was also DENV 1 (57). DENV2 may have partially replaced DENV1 prior to our study. However, lacking more detailed information on virus strain, it is difficult to determine whether there were virological differences between outbreak and non-outbreak periods.

Limitations and strengths

Dengue transmission can vary substantially over time and space. In endemic areas, dengue epidemics occur at between 3 and 5-year intervals (53). Hence, the generalizability of the current study is limited by its duration of 15 months and geographical restriction to one area of Mvita sub-county. Furthermore, one source of bias could be due to the study design, where cases were enrolled only at our study facilities and we missed those

community residents with relevant symptoms seeking care from other healthcare providers than the study facilities, including private clinics. This may further restrict the generalizability of the findings.

Nonetheless, our study held several strengths not found in previous studies. By implementing the surveillance at three different KEPH levels of public health facilities, we were able to capture the wide spectrum of clinical manifestations of dengue. Unlike previous dengue studies in Kenya focusing mostly on outbreaks, this study captured the time before the outbreak as well as the first two months of the outbreak, with a large sample size and high dengue caseload, enabling an exploration of the differences between dengue and non-dengue cases (Fig. 3) (29, 31, 32).

Conclusion

Our data provide evidence for a high level of transmission of dengue in Mombasa and demonstrate the magnitude of the 2017 outbreak, compared to baseline during the non-outbreak period. The study findings also provide some insight into differences in clinical and epidemiological patterns of dengue cases between the outbreak and non-outbreak periods. Dengue detection by clinical diagnosis was more accurate during the outbreak than before it. More data from additional prospective and longitudinal studies would further define patterns of dengue in Kenya for improved case detection and monitoring of dengue outbreaks.

Acknowledgments.

The authors thank the doctors and staff of Ganjoni Health Center, Tudor sub-county Hospital, and Coast Provincial General Hospital. Also, we thank the laboratory staff of Tudor sub-county Hospital and Coast Provincial General Hospital. The authors would also like to thank the health authorities of Mombasa and Kenya, and particularly the Ministry of Health, for their support in the study execution. Lastly, we would like to thank the DVI team and statisticians and administrative staff at the International Vaccine Institute for their helpful comments during the preparation of this manuscript and support during the studies.

Financial disclosure: This study was supported by funding from the Bill and Melinda Gates Foundation (grant #: OPP 1053432), as well as from the governments of Sweden, India, and the Republic of Korea.
NA and TE were supported by award MR/R010161/1, which is jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 programme supported by the European Union.

Conflict of interest statement: I certify that the authors do not have any relevant financial relationships or potential conflicts of interest to disclose regarding the material discussed in this manuscript.

Author contributions

JKL took the lead on field surveys, monitored the data collection, conducted formal analysis, created all figures, and wrote the manuscript. SHM, SN, EA, NO, SKL, and MAO were involved in the data and sample collection. JSL, KSL, TE provided statistical support. HK, SHB, and JSY provided laboratory support. NA, IKY, and SMN provided scientific support and overall supervision.

References

1. Boisier P, Morvan JM, Laventure S, Charrier N, Martin E, Ouledi A, et al. [Dengue 1 epidemic in the Grand Comoro Island (Federal Islamic Republic of the Comores). March-May 1993]. Ann Soc Belg Med Trop. 1994 Sep;74(3):217-29.
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013 Apr 25;496(7446):504-7.
3. World Health Organization W. Dengue and dengue haemorrhagic fever.Fact sheet N°117. 2009 [cited 2011; Available from:
4. Halstead S. Pathogenesis of dengue: challenges to molecular biology. Science. 1988 1998 Jan 29;239(4839):476-81.
5. Gubler DJ MM. Impact of dengue/dengue hemorrhagic fever on the developing world. Adv Virus Res. 1999;53:35-70.
6. Singhi SK, Bansal A. Dengue and dengue hemorrhagic fever: management issues in an intensive care unit. J Pediatr (Rio J). 2007 2007;83(2 Suppl):S22-35.
7. Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol. 2013;5:299-309.
8. Surtees G. The distribution, density and seasonal prevalence of Aedes aegypti in West Africa. Bull World Health Organ. 1967;36(4):539-40.
9. Kamgang B., Ngoagouni C., Manirakiza A., Nakouné Emmanuel., Paupy C, Mirdad K. Temporal Patterns of Abundance of Aedes aegypti and Aedes albopictus(Diptera: Culicidae) and Mitochondrial DNA Analysis of Ae. albopictus in the Central African Republic. PLoS Negl Trop Dis. 2013;12:e2590.
10. Messina J, Brady O, Scott T, Zou C, Pigott D, Duda K, et al. Global spread of dengue virus types: mapping the 70 year history. Trends Microbiol. 2014;22(3):138-46.
11. Amarasinghe A, Kuritsky J, Letson G, Margolis H. Dengue virus infection in Africa. Emerging Infectious Diseases. 2011;17(8):1349-54.
12. Gubler D, Clark G. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerg Infect Dis 1995 1995 Apr-Jun;1(2):55-7.
13. Kraemer MU, Sinka ME, Duda K, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLife. 2015 Jun 30, 2015 4:e08347.
14. Baba M, Villinger J, Masiga DK. Repetitive dengue outbreaks in East Africa: A proposed phased mitigation approach may reduce its impact Reviews in Medical Virology. 2016 29 February 2016;26(3):183-96.
15. Beatty M, Stone A, Fitzsimons D, Hanna J, Lam S, Vong S, et al. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas Dengue Prevention Boards. PLoS Negl Trop Dis 2010 2010 Nov 16;4(11):e890.

16. World Health Organization. Weekly bulletin on outbreaks and other emergencies. Regional Office for Africa, Health Emergencies Programme. 2017 June 2017;Week 24(10 - 16 June 2017).
17. Ellis EM, Neatherlin JC, Delorey M, Ochieng M, Mohamed AH, Mogeni DO, et al. A household serosurvey to estimate the magnitude of a dengue outbreak in Mombasa, Kenya, 2013. PLoS Negl Trop Dis. 2015 Apr;9(4):e0003733.
18. Obonyo M, Fidhow A, Ofula V. Investigation of laboratory confirmed dengue outbreak in North-eastern Kenya, 2011. PLoS One 2018 2018 Jun 7;13(6):e0198556.
19. Konongoi L, Ofula V, Nyunja A, Owaka S, Koka H, Makio A, et al. Detection of dengue virus serotypes 1, 2 and 3 in selected regions of Kenya: 2011-2014. Virology Journal. 2016 4 November 2016;13(182).
20. Ochieng C, Ahenda P, Vittor A, Nyoka R, Gikunju S, Wachira C, et al. Seroprevalence of infections with dengue, rift valley fever and chikungunya viruses in Kenya, 2007. PLoS One. 2015;10(7).
21. Were F. The dengue situation in Africa. Paediatr Int Child Health. 2012 May;32 Suppl 1:18-21.
22. Lim J, Carabali M, Lee J-S, et al. Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative. BMJ Open. 2018;2018(8):e017673.
23. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6(8):e1760.
24. Central Intelligence Agency. Age structure: Central Intelligence Agency.
25. Wikipedia. Kenya. [cited 2016 September 2]; Available from: https://en.wikipedia.org/w/index.php?title=Kenya&oldid=737329303
26. World Health Organization. Guidelines for the clinical evaluation of dengue vaccines in endemic areas. Geneva: World Health Organization; 2008. Report No.: WHO/IVB/08.12.
27. Alm E, Lindegren G, Falk KI, Lagerqvist N. One-step real-time RT-PCR assays for serotyping dengue virus in clinical samples. BMC Infectious Diseases. 2015 2 November 2015;15(493).
28. World Health Organization. Handbook for clinical management of dengue. Geneva, Switzerland: World Health Organization,; 2012.
29. Impouma B. Weekly Bulletin on outbreaks and other emergencies: World Health Organization Regional Office for Africa; 2017.
30. Githeko A. How Kenya can manage its increasing dengue fever cases. Health & Medicine 2017 [cited 2018 April 21]; Available from: https://theconversation.com/how-kenya-can-manage-its-increasing-dengue-fever-cases-77329
31. Sanga B. Mombasa issues alert over Dengue Fever outbreak after 150 cases diagnosed.

Health 2017 May 7th 2017 [cited 2018 April 21]; Available from: https://www.standardmedia.co.ke/health/article/2001238909/mombasa-issues-alert-over-dengue-fever-outbreak

32. Onsarigo C. 119 infected after Mombasa dengue fever outbreak. The Star 2017 [cited 2018 April 21]; Available from: Available from: https://www.the-star.co.ke/news/2017/05/07/119-infected-after-mombasa-dengue-fever-outbreak_c1556136

33. Ankera M, Arimab Y. Male-female differences in the number of reported incident dengue fever cases in six Asian countries. Emerging Diseases Surveillance and Response, Division of Health Security and Emergencies, World Health Organization Regional Office for the Western Pacific. 2011;2(2):17-23.

34. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue in the early febrile phase: viremia and antibody responses. The Journal of Infectious Diseases. 1997;176 ((August)):322-30.

35. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. The Journal of Infectious Diseases. 2000 1 January 2000;181(1):2–9.

36. Munyuga K, Ng'ang'a J, Inoue S, Syengo C, Ndege Co, Kwallah A, et al. Co-circulation evidence of dengue virus serotypes at the Kenyan coast in 2014, 2015. Journal of Pharmacy and Biological Sciences. 2016 December 2016;11(6):83-7.

37. Ngoi CN, Price MA, Fields B, Bonventure J, Ochieng C, Mwashigadi G, et al. Dengue and chikungunya virus infections among young febrile adults evaluated for acute HIV-1 infection in coastal Kenya. PLoS One. 2016;11(12):e0167508.

38. Wiwanitkit V. Concurrent malaria and dengue infection: a brief summary and comment. Asian Pac J Trop Biomed 2011 2011 Aug;1(4):326–7.

39. Epelboin L, Hanf M, Dussart P, Ouar-Epelboin S, Djossou F, Nacher M, et al. Is dengue and malaria co-infection more severe than single infections? A retrospective matched-pair study in French Guiana. Malar J. 2012;11(142).

40. Hadinegoro SRS. The revised WHO dengue case classification: does the system need to be modified? Paediatr Int Child Health 2012 2012 May;32(S1):33-8.

41. Guo C, Zhou Z, Wen Z, Liu Y, Zeng C, Xiao D, et al. Global epidemiology of dengue outbreaks in 1990–2015: A systematic review and meta-analysis. Front Cell Infect Microbiol 2017;7(317).

42. Humayoun MA, Waseem T, Jawa AA, Hashmi MS, Akram J. Multiple dengue serotypes and high frequency of dengue hemorrhagic fever at two tertiary care hospitals in Lahore during the 2008 dengue virus outbreak in Punjab, Pakistan. International Journal of Infectious Diseases. 2010 September 2010;14(Supplement 3):e54–e9

43. Hotchandani A. Loss of appetite and strength in the geriatric population: diagnostic

symptoms for dengue. Trop Doct 2014 2014 Jul;44(3):182-5.
44. Ramos-De La Medina, Remes-Troche JM, González-Medina MF, Anitúa-Valdovinos Mdel M, Cerón T ZC, A. D-V. [Abdominal and gastrointestinal symptoms of Dengue fever. Analysis of a cohort of 8559 patients]. Gastroenterol Hepatol 2011 2011 Apr;34(**4**):243-7.
45. Low JG, Ooi EE, Tolfvenstam T, Leo YS, Hibberd ML, Ng LC, et al. Early Dengue infection and outcome study (EDEN) - study design and preliminary findings. Ann Acad Med Singapore. 2006 2006 Nov;35(11):783-9.
46. Ahmed S, Arif F, Yahya Y, Rehman A, Abbas K, Ashraf S, et al. Dengue fever outbreak in Karachi 2006 - A study of profile and outcome of children under 15 years of age. J Pak Med Assoc. 2008 January 2008;58(1):4-8.
47. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention, and control. Geneva: World Health Organization; 2009.
48. Pignataro SB, Barcia T, Argento R. Frequency of gastrointestinal symptoms of dengue infection in an adult population. Analysis of a cohort of 1463 patients in a South American country. Gastroenterology. 2017 April 2017;152(5):S444
49. Zhang H, Zhou YP, Peng HJ, Zhang XH, Zhou FY, Liu ZH, et al. Predictive Symptoms and Signs of Severe Dengue Disease for Patients with Dengue Fever: A Meta-Analysis. Biomed Res Int 2014;2014:359308. .
50. World Health Organization. Dengue and severe dengue. Fact sheets 2019 2 February 2018 [cited 2019 12 May]; Available from: http://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
51. Gurugama P, Garg P, Perera J, Wijewickrama A, Seneviratne S. Dengue viral infections. Indian J Dermatol 2010 2010 Jan-Mar;55(1):68-78.
52. Domingues R, Kuster G, Onuki de Castro F, Souza V, Levi J, Pannuti C. Headache features in patients with dengue virus infection. Cephalalgia 2006 2006 Jul;26(7):879-82.
53. Bennett SN, Drummond AJ, Kapan DD, Suchard MA, Muñoz-Jordán JL, Pybus OG, et al. Epidemic dynamics revealed in dengue evolution. Mol Biol Evol. 2010 2010 Apr;27(4):811–8. .
54. Gubler DJ. Dengue and Dengue Hemorrhagic Fever. Clin Microbiol Rev 1998 1998 Jul;11(3):480–96.
55. Saha K, Ghosh M, Firdaus R, Biswas A, Seth B, Bhattacharya D, et al. Changing pattern of dengue virus serotypes circulating during 2008-2012 and reappearance of dengue serotype 3 may cause outbreak in Kolkata, India. Journal of Medical Virology. 2016 18 March 2016;88(10):1697-702.
56. Koech BJ. Seroprevalence of dengue fever virus in the adult kenyay population in Nairobi, Eldoret and Kisumu regions: Univeristy of Nairobi; 2015.
57. Vu DM, Mutai N, Heath CJ, Vulule JM, Mutuku FM, Ndenga B, et al. Unrecognized Dengue Virus Infections in Children, Western Kenya, 2014-2015 Emerg Infect Dis. 2017 November 2017 23(11).

Supporting Information

S1 STROBE Checklist

S2 Table S1: Data by 3-level dengue-confirmation status (confirmed-, probable dengue, and non-dengue)

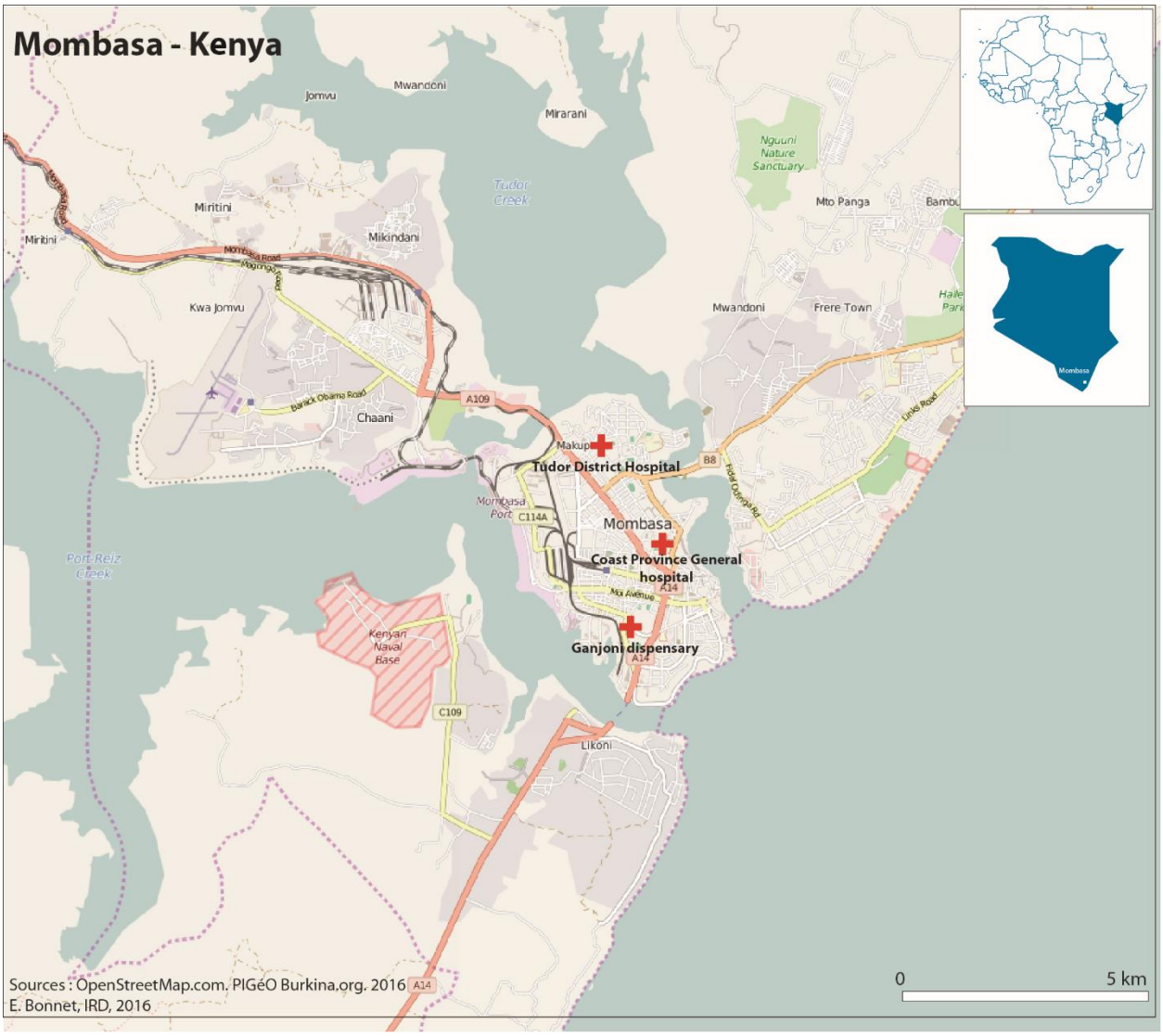


Figure 1. A map of the area of catchment population and study facilities.

Notes: The map shows the approximate location of the three selected study facilities (Ganjoni health centre, Tudor sub-county Hospital, and Coast Provincial General Hospital), covering the catchment area population of residents of Mvita sub-county, Mombasa, Kenya. Source: Lim et. al (22).

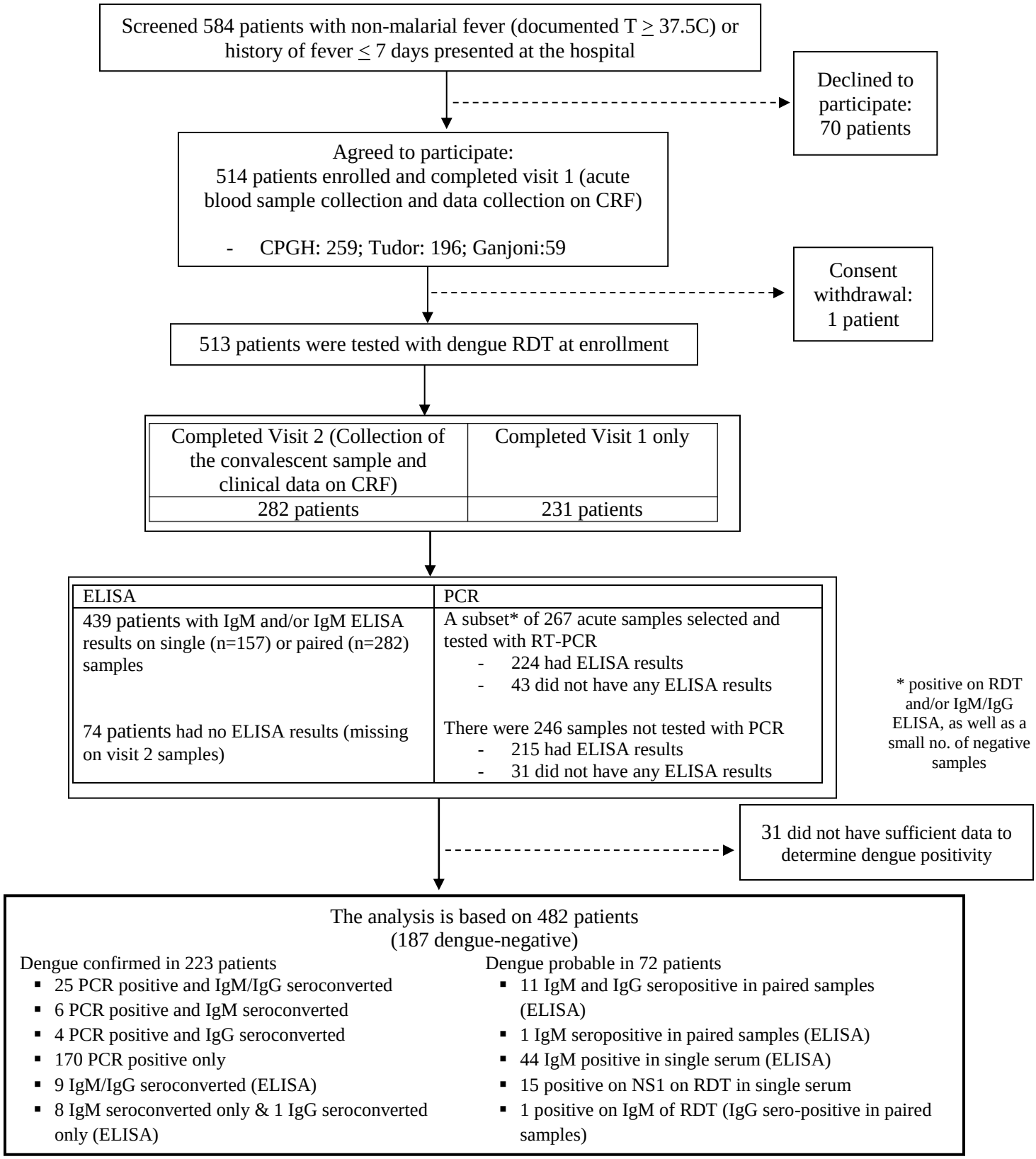


Figure 2 A chart of patient flow in the passive fever surveillance at the study facilities

Notes: The chart shows the flow of patients from screening, enrollment to study participation, with determination of laboratory-based status of dengue infection, as well as how the analysis sample was reached.

Figure 3. Monthly distribution of the enrolled febrile patients and patients by dengue status as well as serotype distribution.

Multytipic infections
June 2016 - 1 DENV II and III
Oct. 2016 - 1 DENV I and II
Nov. 2016 - 4 DENV I and II
- 1 DENV I and III
- 1 DENV II and III
Dec. 2016 - 1 DENV I and II
Jan. 2017 - 1 DENV I, II, and III
Mar. 2017 - 3 DENV II and IV
Apr. 2017 - 1 DENV I and II
- 1DENV II and III
- 7 DENV II and IV
May 2017 – 10 DENV II and IV

Figure 3 Monthly distribution of the enrolled febrile patients by dengue-positivity as well as serotype distribution

Notes: The figure has two parts: the upper part shows monthly distribution of dengue-positive and non-dengue cases among the enrolled patients; and the lower part shows distribution of serotypes identified (numbers shown in the bars) by month.

Chapter 5. Clinical and epidemiologic characteristics associated with dengue during and outside the 2016 outbreak identified in health facility-based surveillance in Ouagadougou, Burkina Faso

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	LSH1405874	**Title**	Ms.
First Name(s)	Jacqueline Kyungah		
Surname/Family Name	LIM		
Thesis Title	Undocumented burden of dengue in Africa		
Primary Supervisor	Prof. Neal Alexander		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	The Journal of Infectious Diseases
Please list the paper's authors in the intended authorship order:	Jacqueline K. Lim, Yaro Seydou, Mabel Carabali, Ahmed Barro, Desire Dahourou, Kang Sung Lee, Teguewende Nikiema, Suk Namkung, Jung Seok Lee, Mee Young Shin, Emmanuel Bonnet, Therese Kagone, Losseni Kaba, Tansy Edwards, Paul-André Somé, Jae Seung Yang, Neal Alexander, In-Kyu Yoon, Valéry Ridde

Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am first author. I led the study design and co-developed the protocol with Dr. Mabel Carabali. Some site-specific details were added in collaboration with the key investigators (Y Seydou and V Ridde, as the PI and co-investigator, and M Carabali). I oversaw the ethical approval process, supported study set-up, monitored data collection, performed literature review, performed data cleaning, and conducted statistical analysis including generating SAS code for analysis and all the figures, except for Fig. 1 (by Emmanuel Bonnet, one of co-authors). I wrote the first draft of the manuscript. I, as the first author, led the process of manuscript preparation, revision, and submission. M. Carabali also supported data and sample collection. Yaro Seydou and Valéry Ridde, as the PI and co-investigator, were responsible for study set-up and execution in Ouagadougou. Ahmed Barro, Desire Dahourou, Kang Sung Lee, Teguewende Nikiema, Suk Namkung, Jung Seok Lee, Losseni Kaba, and Paul-André Somé provided support in setting up the study at the sites, in sample and data collection, and data management and analysis. Mee Young Shin, Therese Kagone, and Jae Seung Yang performed laboratory work. Emmanuel Bonnet provided support in data cleaning. Neal Alexander and In-Kyu Yoon provided oversight and guidance on the overall study design, project execution, and specific methodologies, e.g. epidemiology, statistical, and virology and other laboratory methods. Neal Alexander and Tansy Edwards oversaw statistical analysis, and contributed to manuscript preparation.

SECTION E

Student Signature	[redacted]
Date	23 June 2019

Supervisor Signature	[redacted]
Date	17 June 2019

Target journal: JID

Title: Clinical and epidemiologic characteristics associated with dengue during and outside the 2016 outbreak identified in health facility-based surveillance in Ouagadougou, Burkina Faso

Short title: Dengue outbreak in 2016 in Burkina Faso

Jacqueline K. Lim[1 2*], Yaro Seydou[3,], Mabel Carabali[1 4], Ahmed Barro[5], Desire Dahourou[3 6], Kang Sung Lee[1], Teguewende Nikiema[3], Suk Namkung[1], Jung-Seok Lee[1], Mee Young Shin[1], Emmanuel Bonnet[7], Therese Kagone[3], Losseni Kaba[8], Tansy Edwards[2], Paul-André Somé[5], Jae Seung Yang[1], Neal Alexander[2], In-Kyu Yoon[1], Valéry Ridde[7 9]

Author affiliation

[1]International Vaccine Institute, Seoul, Korea, Republic of; [2]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine; [3]Centre MURAZ, Bobo-Dioulasso, Burkina Faso; [4]McGill University, Montreal, Quebec Canada; [5]Action-Gouvernance-Integration-Renforcement (AGIR), Program Equité, Ouagadougou, Burkina Faso; [6]Institut de Recherché en Sciences de la Santé, Ouagadougou, Burkina Faso; [7]Institute for Research on Sustainable Development (IRD), CEPED, Université de Paris, ERL INSERM SAGESUD; [8]Centre National de Transfusion Sanguine, Ouagadougou, Burkina Faso; [9]University of Montreal Public Health Research Institute (IRSPUM), Montreal, Canada

*Corresponding author

Jacqueline K. Lim[1 2*]

Research Scientist

Global Dengue and *Aedes*-transmitted Diseases Consortium (GDAC)

International Vaccine Institute (IVI)

SNU Research Park, Gwankak-ro 1

Gwanak-gu, Seoul, Korea 151-191

T: +82-2-881-1340 (Dir) / +82-2-872-2801 (ext. 340)

Email: kalim@ivi.int

28 pages (not counting figures)

Word count of the text: 3652 (not counting the Abstract and tables)
Word count of the abstract: 161
Figures and tables: 5 figures and 4 tables

Abbreviations: AGIR, Action-Gouvernance-Integration-Renforcement; CI, Confidence Interval; CSPS, Centre de Santé et de Promotion Sociale (Health and Social Promotion Center); °C, Celsius degrees; CRF, Case Report Form; DENV, Dengue Virus; DF, Dengue Fever; DHF, Dengue Hemorrhagic Fever; DSS, Dengue Shock Syndrome; DVI, Dengue Vaccine Initiative; ELISA, Enzyme-Linked Immunosorbent Assay; ICF, Informed Consent Form; IgM/IgG, Immunoglobulin type M and type G; IRB, Institutional Review Board; IRD, Institute for Research on Sustainable Development; IVI, International Vaccine Institute; LRTI, lower Respiratory Tract Illness; MoH, Ministry of Health; NPV, Negative Predictive Value; PPV, Positive Predictive Value; RDT, Rapid Diagnostic Test; RT-PCR, Reverse Transcriptase-Polymerase Chain Reaction; URI, Upper Respiratory Illness; WBC, White Blood Cell; YF, Yellow fever

Conflict of interest statement: I certify that the authors do not have any relevant financial relationships or potential conflicts of interest to disclose regarding the material discussed in this manuscript.

Financial disclosure: This study was supported by funding from the Bill and Melinda Gates Foundation (grant #: OPP 1053432), as well as from the governments of Sweden, India, and the Republic of Korea. This research project was part of the “Community research studies and interventions for health equity in Burkina Faso” program funded by the Canadian Institutes of Health Research (ROH-115213).
NA and TE were supported by award MR/R010161/1, which is jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 programme supported by the European Union.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Contributor’s statement: All persons designated as authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content. And all the authors contributed in some or all areas of acquisition of funding, conception of the study, collection of data, analysis and interpretation of data, drafting the article, article revision, scientific support, and final approval of the version to be published. The authors meet the criteria for authorship and qualify for authorship of this manuscript.

Abstract

Background: In Africa, the magnitude of dengue transmission is largely unknown.

Methods: To better understand dengue epidemiology and clinical characteristics in Burkina Faso, a fever surveillance study was conducted among patients aged 1-55 years, who presented with non-malarial febrile illness at five primary healthcare facilities in Ouagadougou, Burkina Faso from December 2014 to February 2017, encompassing a 3-month dengue outbreak in 2016.

Results: Among 2929 patients tested with dengue RDT, RT-PCR, and IgM/IgG ELISA, 740 (25%) were dengue–positive; 55% and 14% were dengue-positive during outbreak and non-outbreak periods, respectively. DENV2 predominated during the outbreak, whereas DENV3 predominated before the outbreak. Only 25% of dengue-positive cases were clinically diagnosed with suspected dengue. Dengue-positive cases were 11 times more likely than non-dengue cases to require observation for ≤3 days (versus routine outpatient care).

Conclusion: Dengue is an important pathogen in Burkina Faso, accounting for a substantial proportion of non-malarial fevers both during and outside outbreak, but is only infrequently suspected by clinicians.

Author summary:

There is not much evidence on dengue in Africa, relative to the Asia-Pacific and Latin American regions. To estimate the proportion of dengue among patients with fever, and to identify clinical features of dengue during outbreak and non-outbreak periods, we studied 2929 patients with non-malarial fever, aged 1-55 years, who attended five primary healthcare centers in Ouagadougou, Burkina Faso. Patients were tested with a rapid test for dengue, and further tests were carried out on paired blood samples taken 10-21 days apart. Overall, a quarter of non-malarial febrile episodes identified between December 2014 and February 2017 were dengue-positive. Dengue-positive cases were 11 times more likely than non-dengue cases to require observation for ≤3 days. During the study period in 2016, there was a dengue outbreak where more than half of non-malarial febrile patients were identified to be dengue-positive. DENV2 was the main serotype in circulation during the outbreak, whereas DENV3 was the main serotype before the outbreak. There was a low level of clinical suspicion of dengue even during the 2016 outbreak, broader use of RDTs and more epidemiologic data would help to improve dengue case detection and surveillance in Burkina Faso.

Keywords: Dengue, surveillance, outbreak, Burkina Faso, Africa

Introduction

Dengue Fever (DF) is a mosquito-borne disease caused by four related but antigenically distinct dengue viruses (DENVs, serotypes 1–4). Approximately 50 to 100 million cases of DF and 500,000 severe dengue cases requiring hospitalization reportedly occur annually worldwide (1-3).

The *Aedes* mosquito vectors of DENV are widely distributed in Africa, and dengue cases have been reported in 34 African countries (4-6). However, data are limited to retrospective testing of existing samples or outbreak investigations from a few countries (5, 7-9). Several studies have identified DENV as a common cause of febrile illness in Africa, but there is a continued challenge to distinguish dengue from other causes of febrile illness given limited diagnostic capabilities (10-12).

In Burkina Faso, several outbreaks have been reported since 1925 (5, 13, 14), including an outbreak declared in November 2013 by the Burkina Faso Ministry of Health (MoH) (11, 15). Between 5 August and 12 November 2016, the Burkina Faso MoH conducted an outbreak investigation as part of emergency response in collaboration with WHO and 1266 suspected dengue cases were identified by the MoH, with 1061 cases positive by dengue RDT, and 15 deaths from all 12 districts of Ouagadougou (16, 17). Most recently, an even larger outbreak occurred in September 2017, with 9029 suspected dengue cases, 5773 dengue RDT-positive cases, and 18 deaths throughout the country (18). These repeated outbreaks suggest a considerable dengue burden in Burkina Faso.

Most African countries lack mandatory reporting or national surveillance systems for dengue (19). Burkina Faso added dengue to its routine national surveillance system for potential epidemic diseases in 2016. Also, the MoH conducts outbreak investigations at several sentinel health centers (11).

To better understand the dengue problem in Burkina Faso (11), a passive facility-based fever surveillance study was conducted in Ouagadougou, from 2014-2017. During the study period, the 2016 dengue outbreak occurred, allowing for: 1) characterization of dengue epidemiology and; 2) comparison of clinical features during and outside the outbreak.

Methods

Study area and population

The study area was selected based on the existence of previous outbreaks and

case reports, past seroprevalence and modelling studies, as well as the availability of research infrastructure (4, 20, 21). Ouagadougou is the capital city of Burkina Faso in West Africa with most of its population residing in urban settings (22). The hot season occurs in March-May with temperatures reaching 43 °C, followed by the rainy season in May-September. Health services in Ouagadougou are provided by three university hospitals, five district hospitals, and 60 primary healthcare centers (CSPS, Centres de Santé et de Promotion Sociale), as well as private clinics (23).

The current study was implemented in five CSPSs (Pazani, CSPS22, CSPS25, Juvenat Fille, Zongo), serving a catchment population of 110,000 residents (Fig. 1). The population in Ouagadougou is stable with an annual transmigration rate of 4.1% and >80% with home ownership (24).

Study design

The passive facility-based fever surveillance study enrolled outpatients and observation patients (for ≤3 days), as previously described (20), between December 2014 and February 2017 (27 months). Patients presenting with fever (body temperature ≥ 37.5º C) or history of (self-reported) fever for ≤7 days were tested for malaria using RDT (SD BIOLINE Malaria kit, Standard Diagnostics, Yongin-Si, Korea) as part of routine practice. Patients were eligible for study enrolment if they were malaria RDT-negative without localizing signs (i.e., no localized infection or known/confirmed non-dengue etiology), aged 1-55 years, resident of the catchment area covered by the study CSPSs, and provided informed consent, plus assent for individuals aged 8-17 years.

Malaria RDT-negative patients were tested using dengue RDTs. During the enrollment visit, an acute blood sample (7-10 ml) was collected (Fig. 2). Then, a study physician/nurse conducted interviews and physical exams, and a surveillance case report form was completed capturing symptom history, medical history, treatment and laboratory results (20). A convalescent blood sample was collected at the facility between 10-14 days after the initial visit, or if not possible within this timeframe, the patient was followed up at home within 21 days.

Laboratory Testing Algorithm

As described previously (20), acute samples were tested at enrollment at the CSPS using a commercial RDT for dengue NS1 and IgM/IgG (Dengue Duo®, Standard

Diagnostics, Yongin-Si, Korea). The acute and convalescent sera were subsequently tested at the Centre Muraz laboratory using dengue IgM/IgG ELISA (SD Dengue IgM & IgG Capture ELISA®, Standard Diagnostics, Yongin-Si, Korea). Furthermore, RT-PCR was performed at IVI on acute sera from patients who had (25): (i) NS1 or IgM positive by RDT in the acute sample; and/or (ii) sero-conversion between acute and convalescent samples by IgM and IgG capture ELISA. RT-PCR was also performed on a limited number of randomly selected acute sera that were: (iii) sero-positive in both acute and convalescent samples by IgM and IgG capture ELISA; or (iv) IgG positive by RDT in the acute sample; or (v) negative by RDT and ELISA on all samples.

Dengue infection status was categorized based on interpretation of laboratory results, following WHO diagnostic criteria (26). Sero-conversion by dengue IgM and/or IgG between acute and convalescent samples and/or virus detection by RT-PCR in the acute sample were considered to be laboratory-confirmed dengue. Positive IgM by ELISA in a single acute sample or paired acute/convalescent samples, or NS1 and/or IgM positive by RDT were considered as probable dengue (26). Confirmed and probable dengue cases were combined into a dengue-positive group for this analysis. Patients with negative RT-PCR and negative paired acute/convalescent IgM ELISA were classified as non-dengue.

Statistical analysis

There were 2 components in the analysis. First, a descriptive summary of clinical and laboratory characteristics is presented for dengue-positive and non-dengue cases. Elevated body temperature, as a dichotomous variable, was defined as body temperature ≥38.5°C, the 75th percentile of the body temperature measured at enrollment. Clinical diagnosis (i.e., made by clinician prior to laboratory confirmation) was grouped as suspected dengue, undifferentiated fever, and other illness. Our surveillance covered the entire outbreak from September to November 2016. Cases were also designated as outbreak or non-outbreak depending on date of occurrence, with outbreak cases considered as those occurring between September and November 2016, defined to be consistent with the outbreak period declared by Burkina Faso MoH/WHO (16, 17). Yellow fever (YF) vaccination history was dichotomized between those who reported having been vaccinated versus those who did not remember or reported no vaccination. Categorical pair-wise comparisons were made across dengue infection status using $\chi 2$ or Fisher's exact tests with significance level of 0.05. Continuous variables were compared using Student's t-

test or ANOVA.

Secondly, based on our a priori hypothesis that clinical presentation associated with dengue-positivity would be different between the outbreak and non-outbreak periods, logistic regression was used to build a multivariable model of clinical indicators associated with dengue-positive vs. non-dengue cases, to separately fit the outbreak and non-outbreak periods. The models contained age and gender as a priori confounders, possibly associated with exposure to *Aedes* vectors, and with some clinical features (27). A backward stepwise process was used to select a final multivariable model for each outbreak status, with a significance level of 0.2 for entry and 0.1 for retention. Further variables investigated included: demographic and clinical variables such as YF vaccination history, requirement for observation, fever duration prior to enrollment, temperature at presentation, and clinical signs/symptoms. Some signs and symptoms were used only in the descriptive and univariate analyses, due to data sparsity. Clinical diagnosis of suspected dengue was considered to be closely related to dengue-positivity and was not included.

Finally, a single set of variables was obtained as the union of the sets of variables from regression modelling in the outbreak and non-outbreak periods. Variables found to be significant in only one period were applied to both periods, producing a single list of variables. These variables were fitted to both outbreak and non-outbreak periods to give comparable results between them.

As part of sensitivity analysis, a descriptive summary of clinical and laboratory characteristics using three categories for dengue infection status — confirmed, probable, and non-dengue — is presented in supplementary S2 table. Between dengue-confirmed and non-dengue groups, univariate logistic analyses were conducted for during and outside the outbreak (S3 and S4 tables). All analyses were performed using SAS® version 9.4 (SAS Institute, Cary, North Carolina).

Ethical considerations

The study protocol received ethical approvals from the Institutional Review Boards (IRBs) of IVI, the London School of Hygiene and Tropical Medicine, the National Ethical Committee for Health Research of Burkina Faso, and the Ethics Committee of the Centre Hospitalier de l'Université de Montréal (CRCHUM) at University of Montreal.

Results

Analysis was performed on 2929 out of 3012 enrolled patients with complete clinical and laboratory data; 83 withdrew consent or had incomplete laboratory data to determine dengue infection status (Fig 3). Although similar in terms of age, gender, requirement for observation, and days of illness before enrollment, these 83 patients were significantly different from the analysis sample in terms of residential neighborhood — the majority from Zongo (40%) and Pazani (28%) — and being mostly from non-outbreak periods (87%). In terms of missing data, only the patients requiring observation had information on the complete blood count (CBC) test and the results from CBC were not included in the analysis.

Clinical characteristics between dengue-positive and non-dengue cases

Table 1 describes demographic and clinical characteristics of dengue-positive vs. non-dengue cases. Of 2929 analyzed patients, 2189 (74.7%) were non-dengue and 740 (25.3%) were dengue-positive. Of the 740 dengue-positive patients, 540 were laboratory-confirmed and 200 were probable dengue. Of the dengue-positive cases, 42% (n=317) were confirmed by PCR and the remainder by paired ELISA (Fig. 3). A small peak in dengue-positive cases was observed in October-December 2015. A much larger peak occurred in August-December 2016 (Fig. 4). Both peaks occurred at the end or after the May-September rainy season. Of 777 fever cases from the outbreak, 55.1% (n=428) were dengue-positive, with DENV2 predominating. Of 2152 non-outbreak fever cases, 14.5% (n=312) were dengue-positive, mostly with DENV3 and a few DENV1.

Table 1. Demographic and clinical characteristics of dengue-positive and non-dengue cases in the facility-based fever surveillance established in Ouagadougou, Burkina Faso, between December 2014 and February 2017

Characteristics	Dengue-positive (n=740)	Non-dengue (n=2189)	Total (n=2929)	p-value
Age group (years)				**<.001**
1-4	37 (5.0)	275 (12.6)	312 (10.7)	
5-9	43 (5.8)	149 (6.8)	192 (6.6)	
10-14	45 (6.1)	129 (5.9)	174 (5.9)	

15-19	85 (11.5)	231 (10.6)	316 (10.8)	
20-24	110 (14.9)	366 (16.7)	476 (16.3)	
25-29	134 (18.1)	375 (17.1)	509 (17.4)	
30-34	94 (12.7)	269 (12.3)	363 (12.4)	
35-39	71 (9.6)	155 (7.1)	226 (7.7)	
40-44	57 (7.7)	111 (5.1)	168 (5.7)	
45-49	33 (4.5)	67 (3.1)	100 (3.4)	
50-55	31 (4.2)	62 (2.8)	93 (3.2)	
Female	465 (62.8)	1563 (71.4)	2028 (69.2)	**<.001**
CSPS				**<.001**
Pazani	113 (15.3)	400 (18.3)	513 (17.5)	
Zongo	91 (12.3)	592 (27.0)	683 (23.3)	
CSPS22	65 (8.8)	240 (11.0)	305 (10.4)	
CSPS25	266 (36.0)	502 (22.9)	768 (26.2)	
Juvenat Fille	205 (27.7)	446 (20.4)	651 (22.2)	
Under observation ≤3 days/OPD	135 (18.2)/605 (81.8)	45 (2.1)/2144 (97.9)	180 (6.2)/2749 (93.9)	**<.001**
Mean days, fever duration prior to visit (SD)	2.92 (1.21)	2.61 (1.22)	2.69 (1.23)	**<.001**
Fever duration prior to visit				**<.001**
1-2 days	301 (40.7)	1153 (52.7)	1454 (49.6)	
3 days	238 (32.2)	634 (29.0)	872 (29.8)	
4-7 days	201 (27.2)	400 (18.4)	603 (20.6)	
Mean temperature at enrollment (SD)	38.29 (0.77)	38.03 (0.78)	38.09 (0.78)	**<.001**
Temperature at enrollment				**<.001**
Below 38.5°c	478 (64.6)	1681 (76.8)	2159 (73.7)	
≥ 38.5°c	262 (35.4)	508 (23.2)	770 (26.3)	
Mean days, fever duration, entire illness (SD)	4.72 (2.52)	4.04 (2.46)	4.21 (2.49)	**<.001**

Prev. dengue infection	14 (1.9)	2 (0.1)	16 (0.6)	**<.001**
YF vaccination				**<.001**
Received	122 (16.5)	824 (37.6)	946 (32.3)	
Not received	618 (83.5)	1365 (62.4)	1983 (67.7)	
Clinical diagnosis				
Suspected dengue	187 (25.3)	12 (0.6)	199 (6.8)	**<.001**
Undifferentiated fever	529 (71.5)	1987 (90.8)	2516 (85.9)	
Other illness	24 (3.2)	190 (8.7)	214 (7.3)	
URI (% of other illness)	5 (20.8)	27 (14.2)	32 (15.0)	
Bronchitis	4 (16.7)	30 (15.8)	34 (15.9)	
Pneumonia	6 (25.0)	21 (11.1)	27 (12.6)	
Viral syndrome	3 (12.5)	11 (5.8)	14 (6.5)	
Diarrheal illness	2 (8.3)	28 (14.7)	30 (14.0)	
Influenza	1 (4.2)	4 (2.1)	5 (2.3)	
Others	3 (12.5)	69 (36.3)	72 (33.6)	
Signs and symptoms (presence)				
Rash	95 (12.8)	163 (7.5)	258 (8.8)	**<.001**
Fatigue	603 (81.5)	1526 (69.7)	2129 (72.7)	**<.001**
Headache	708 (95.7)	1899 (86.8)	2607 (89.0)	**<.001**
Retro-orbital pain	131 (17.7)	107 (4.9)	238 (8.1)	**<.001**
Neck pain	13 (1.8)	47 (2.2)	60 (2.1)	0.517
Ear pain	2 (0.3)	10 (0.5)	12 (0.4)	0.741
Nasal congestion	20 (2.7)	105 (4.8)	125 (4.3)	**0.015**
Rhinorrhea	30 (4.1)	132 (6.0)	162 (5.5)	**0.042**
Sore Throat	11 (1.5)	64 (2.9)	75 (2.6)	**0.032**
Cough	91 (12.3)	354 (16.2)	445 (15.2)	**0.011**
Sputum production	4 (0.5)	30 (1.4)	34 (1.2)	0.075
Nausea & vomiting	270 (36.5)	635 (29.0)	905 (30.9)	**<.001**
Diarrhea	23 (3.1)	128 (5.9)	151 (5.2)	**0.004**
Constipation	12 (1.6)	85 (3.9)	97 (3.3)	**0.003**
Abdominal pain	271 (36.6)	639 (29.2)	910 (31.1)	**<.001**

Nose bleeding	7 (1.0)	10 (0.5)	17 (0.6)	0.130
Gum bleeding	5 (0.7)	2 (0.1)	7 (0.2)	0.013
Loss of appetite	331 (44.7)	739 (33.8)	1070 (36.5)	**<.001**
Capillary refill >2 sec	8 (1.1)	19 (0.9)	27 (0.9)	0.600
Myalgia	319 (43.1)	560 (25.6)	879 (30.0)	**<.001**
Arthralgia	426 (57.6)	953 (43.5)	1379 (47.1)	**<.001**

Overall, dengue-positive cases were older than non-dengue cases (Table 1). Among dengue-positive cases, those after the 2016 outbreak were younger than those before or during the outbreak (about 75% <30 years old, compared to before and during the outbreak with about 50% <30 years) (Fig. 5); the age difference before, during and after the outbreak was statistically significant (ANOVA, p-value<.001).

There were 180 patients requiring observation at the CSPS. Patients later determined to be dengue-positive were more likely, on presentation, to require observation: 18% of dengue-positive cases versus 2% of non-dengue cases (Table 1). A small but significant difference was observed in average time between fever onset and enrollment for dengue-positive versus non-dengue cases (2.9 days vs. 2.6 days, p <.001). Likewise, the entire duration of fever illness on average was significantly longer for dengue-positive cases (mean 4.7 versus 4.0 days, among the 2926 patients with such data, p <.001). Dengue-positive cases were half as likely to self-report that they had been vaccinated for YF (17%, versus 38% for non-dengue cases, p <.001).

Of 2929 available RDT results, 11% (316/2929) and 4% (129/2929) were positive for NS1 and IgM, on the RDT kit, respectively (Fig. 1). There were 38 patients with positive results for both NS1 and IgM on the RDT. During the outbreak period, 86% (271/316) were NS-1 positive and 40% (52/129) were IgM positive (28 showing positive on both NS1 and IgM).

Only 25% of dengue-positive cases were clinically diagnosed with suspected dengue, prior to lab-confirmation, and more than 90% of non-dengue cases were clinically diagnosed with undifferentiated fever. During the outbreak, 31.3% (131/428) of dengue-positive cases were diagnosed with suspected dengue, while 17.0% (53/312) were diagnosed with suspected dengue during non-outbreak periods.

Clinical features associated with dengue during and outside the 2016 outbreak

Demographic and clinical associations with dengue-positivity are shown in Table 2 for the outbreak and in Table 3 for non-outbreak periods. During the outbreak, independently associated symptoms were: rash, retro-orbital pain, cough, headache, nausea/vomiting, and loss of appetite. During non-outbreak periods, retro-orbital pain, headache, nausea/vomiting, and constipation were independently associated. In addition to the symptoms, the multivariable model selected requirement for observation and lack of YF vaccination to be associated with dengue-positivity in both outbreak and non-outbreak periods. Age in non-outbreak periods and, gender, elevated temperature at enrollment, and fever duration prior to enrollment in the outbreak period were also selected. Age and gender were a priori confounders and were significantly associated with dengue. Enrolled CSPS may be a proxy for otherwise any unexplained variation across centers, but was not selected for either of the outbreak or non-outbreak periods. In the absence of observation of variation with respect to dengue-positivity, it was not entered in the models.

Table 2. Univariate logistic analyses showing significant indicators and their odds ratios of dengue-positivity during the outbreak period, from the facility-based fever surveillance established in Ouagadougou, Burkina Faso, between December 2014 and February 2017

Characteristics	During outbreak (n= 777)					
	Total N	N (%) dengue-positive (n=428)	N (%) Non-dengue (n=349)	Univariate analysis Dengue-positive vs. non-dengue		
				OR	95% CI	p-Value
Age group (years)						0.195
1-14	129	63 (48.8)	66 (51.2)	Ref	-	
15-24	213	121 (56.8)	92 (43.2)	1.38	0.89-2.14	
25-34	242	128 (52.9)	114 (47.1)	1.18	0.77-1.80	
35-55	193	116 (60.1)	77 (39.9)	**1.58**	**1.01-2.47**	
Gender*						**0.004**
Male	293	181 (61.8)	112 (38.2)	Ref	-	
Female	484	247 (51.0)	237 (49.0)	**0.65**	**0.48-0.87**	
Under observation** (*ref.*	128	110 (85.9)	18 (14.1)	**6.36**	**3.77-10.71**	**<.001**

OPD)						
Fever duration prior to visit*						**0.007**
1-2 days	330	168 (50.9)	162 (49.1)	Ref	-	
3 days	244	129 (52.9)	115 (47.1)	1.08	0.78–1.51	
4-7 days	203	131 (64.5)	72 (35.5)	**1.75**	**1.23-2.51**	
Temperature at enrollment*						**0.009**
Below 38.5°c	468	240 (51.3)	228 (48.7)	**Ref**	**-**	
≥ 38.5°c	309	188 (60.8)	121 (39.2)	**1.48**	**1.10-1.98**	
No YF vaccination†* (*ref.* received vaccination)	630	363 (57.6)	267 (42.4)	**1.72**	**1.19-2.46**	**0.004**
Presence of signs and symptoms (*ref.* absence)						
Rash*	84	60 (71.4)	24 (28.6)	**2.21**	**1.34–3.63**	**0.002**
Fatigue*	620	353 (56.9)	267 (43.1)	**1.45**	**1.02-2.05**	**0.040**
Retro-orbital pain**	104	92 (88.5)	12 (11.5)	**7.69**	**4.14-14.30**	**<.001**
Headache*	749	420 (56.1)	329 (43.9)	**3.19**	**1.39-7.33**	**0.006**
Nasal congestion*	21	5 (23.8)	16 (76.2)	**0.25**	**0.09-0.68**	**0.007**
Rhinorrhea*	28	7 (25.0)	21 (75.0)	**0.26**	**0.11-0.62**	**0.002**
Neck pain	11	6 (54.6)	5 (45.5)	0.98	0.30-3.23	0.971
Sore throat	11	4 (36.4)	7 (63.6)	0.46	0.13-1.59	0.220
Cough**	81	28 (34.6)	53 (65.4)	**0.39**	**0.24-0.63**	**<.001**
Nausea & vomiting	285	154 (54.0)	131 (46.0)	0.94	0.70-1.25	0.655
Diarrhea	21	8 (38.1)	13 (61.9)	0.49	0.20-1.20	0.120
Abdominal pain	263	153 (58.2)	110 (41.8)	1.21	0.90-1.63	0.216
Loss of appetite	383	217 (56.7)	166 (43.3)	1.13	0.85-1.50	0.385
Myalgia**	366	227 (62.0)	139 (38.0)	**1.71**	**1.28-2.27**	**<.001**
Arthralgia	521	295 (56.6)	226 (43.4)	1.21	0.89-1.63	0.219

Statistical significance of the frequencies: *p-value<0.05 **p-value<.001

†based on self-report

Table 3. Univariate logistic analyses showing significant indicators and their odds ratios of dengue-positivity during non-outbreak periods, from the facility-based fever surveillance established in Ouagadougou, Burkina Faso, between December 2014 and February 2017

Characteristics	During non-outbreak (n=2152)					
	Total N	N (%) dengue-positive (n=312)	N (%) Non-dengue (n=1840)	Univariate analysis Dengue-positive vs. non-dengue		
				OR	95% CI	p-Value
Age group (years)*						**0.003**
1-14	549	62 (11.3)	487 (88.7)	Ref	-	
15-24	579	74 (12.8)	505 (87.2)	1.15	0.80-1.65	
25-34	630	100 (15.9)	530 (84.1)	**1.48**	**1.06-2.08**	
35-55	394	76 (19.3)	318 (80.7)	**1.88**	**1.31-2.70**	
Gender						
Male	608	94 (15.5)	514 (84.5)	Ref	-	
Female	1544	218 (14.1)	1326 (85.9)	0.90	0.69-1.17	0.426
Under observation** (*ref.* OPD)	52	25 (48.1)	27 (51.9)	**5.85**	**3.35-10.22**	**<.001**
Fever duration prior to visit*						**0.001**
1-2 days	1124	133 (11.8)	991 (88.2)	**Ref**	**-**	
3 days	628	109 (17.4)	519 (82.6)	**1.57**	**1.19-2.06**	
4-7 days	400	70 (17.5)	330 (82.5)	**1.58**	**1.15-2.17**	
Temperature at enrollment						0.285
Below 38.5°c	1691	238 (14.1)	1453 (85.9)	Ref	-	
≥ 38.5°c	461	74 (16.1)	387 (84.0)	1.17	0.88-1.55	
No YF vaccination†** (*ref.* received vaccination)	1353	225 (18.9)	1098 (81.2)	**3.02**	**2.24-4.09**	**<.001**
Presence of signs and						

symptoms (*ref.* absence)						
Rash*	174	35 (20.1)	139 (79.9)	**1.55**	**1.05-2.29**	**0.029**
Fatigue**	1509	250 (16.6)	1259 (83.4)	**1.86**	**1.39-2.50**	**<.001**
Retro-orbital pain**	134	39 (29.1)	95 (70.9)	**2.62**	**1.77-3.89**	**<.001**
Headache**	1858	288 (15.5)	1570 (84.5)	**2.06**	**1.33-3.19**	**0.001**
Nasal congestion	104	15 (14.4)	89 (85.6)	0.99	0.57-1.74	0.982
Rhinorrhea	134	23 (17.2)	111 (82.8)	1.24	0.78-1.98	0.366
Neck pain	49	7 (14.3)	42 (85.7)	0.98	0.44-2.21	0.966
Sore throat	64	7 (10.9)	57 (89.1)	0.72	0.33-1.59	0.414
Cough	364	63 (17.3)	301 (82.7)	1.29	0.96-1.75	0.096
Nausea & vomiting**	620	116 (18.7)	504 (81.3)	**1.57**	**1.22-2.02**	**<.001**
Diarrhea	130	15 (11.5)	115 (88.5)	0.76	0.44-1.32	0.325
Abdominal pain*	647	118 (18.2)	529 (81.8)	**1.51**	**1.17-1.94**	**0.001**
Loss of appetite	687	114 (16.6)	573 (83.4)	1.27	0.99-1.64	0.059
Myalgia*	513	92 (29.5)	421 (82.1)	**1.41**	**1.08-1.84**	**0.012**
Arthralgia	858	131 (15.3)	727 (84.7)	1.11	0.87-1.41	0.409

Statistical significance of the frequencies: *p-value<0.05 **p-value<.001

†based on self-report

Table 4 shows the final set of variables. During both outbreak and non-outbreak periods, dengue-positive patients had increased odds of presenting with rash [outbreak: 2.6 (95%CI=1.5-4.6); non-outbreak: 1.5 (95%CI=1.0-2.4)] and retro-orbital pain [outbreak: 7.4 (95%CI= 3.7-14.7); non-outbreak: 1.4 (95%CI=1.01-1.8)].

Table 4. Multivariate logistic analysis showing significant indicators and their odds ratios of dengue-positivity by outbreak or non-outbreak periods, in the facility-based fever surveillance established in Ouagadougou, Burkina Faso, between December 2014 and February 2017

Characteristics	Multivariate analysis					
	During outbreak (n=777) *ref.* non-dengue (n=349)			During non-outbreak (n=2152) *ref.* non-dengue (n=1840)		
	Dengue-positive (n=428)		p-Value	Dengue-positive (n=312)		p-Value
	aOR	95% CI		aOR	95% CI	
Female (*ref.* Male)	**0.63**	**0.45-0.89**	**0.008**	0.98	0.73-1.30	0.869
Age (years)			0.612			**0.041**
1-14	Ref			Ref		
15-24	1.23	0.73-2.06		1.18	0.80-1.75	
25-34	0.99	0.59-1.64		1.45	0.98-2.14	
35-55	1.24	0.73-2.09		**1.74**	**1.16-2.62**	
Under observation ≤3 days (*ref.* OPD)	**6.01**	**3.33-10.84**	**<.001**	**4.32**	**2.33-8.02**	**<.001**
No YF vaccination* (*ref*. received vaccination)	**1.73**	**1.12-2.68**	**0.013**	**2.42**	**1.76-3.32**	**<.001**
Temperature at enrollment			**0.015**			0.752
Below 38.5°c	Ref			Ref		
≥ 38.5°c	**1.54**	**1.09-2.17**		1.05	0.77-1.44	
Fever duration prior to visit			0.081			0.087

1-2 days	Ref			Ref		
3 days	0.93	0.62-1.41		**1.40**	**1.04-1.89**	
4-7 days	1.53	0.97-2.43		1.25	0.87-1.80	
Presence of signs and symptoms (*ref.* absence)						
Rash	**2.59**	**1.46-4.59**	**0.001**	**1.54**	**1.00-2.37**	**0.049**
Retro-orbital pain	**7.37**	**3.69-14.71**	**<.001**	1.42	0.90-2.25	0.134
Nausea & vomiting	0.75	0.52-1.08	0.117	**1.36**	**1.01-1.82**	**0.042**
Cough	**0.36**	**0.21-0.63**	**<.001**	1.21	0.87-1.69	0.248
Loss of appetite	**0.46**	**0.30-0.71**	**<.001**	0.93	0.69-1.27	0.659
Headache	2.28	0.93–5.62	0.072	1.43	0.90-2.29	0.130
Constipation	1.08	0.23-4.97	0.926	0.52	0.24-1.10	0,087

*based on self-report; aOR = adjusted odds ratio

Discussion

Recent reports of dengue outbreaks in Burkina Faso suggest substantial dengue transmission in this region. However, existing evidence on epidemiological characterization of dengue in Burkina Faso was limited in scope prior to this study. The current study collected population-based epidemiologic data in Ouagadougou during a 27-month period from 2014-2017, including all three months of the 2016 dengue outbreak. Our data demonstrated that dengue is an important cause of febrile illnesses, accounting for one-quarter of non-malarial febrile illness in patients seeking care at CSPSs in the study. This proportion was very high (55%) during the outbreak itself, but even outside the outbreak, a considerable proportion (15%) of non-malarial febrile episodes was dengue-positive. Since then, Ouagadougou has experienced another, larger, dengue outbreak in 2017 (16, 18). Recent outbreaks and the current study indicate that dengue transmission is likely to be underestimated and underdiagnosed in Burkina Faso (16, 18).

Differences between outbreak and non-outbreak periods

The predominant DENV serotype identified from PCR-positive outbreak cases in the study was DENV2. This was consistent to the results of MoH/WHO investigation of the 2016 outbreak where DENV2 was the predominant serotype (16, 17). DENV2 was also the dominant serotype detected in outbreaks in Burkina Faso in 1982 and 1983-1986 (9, 28). The study found DENV3 to be predominant during the non-outbreak period preceding the 2016 outbreak. DENV3 was the dominant serotype in the 2013 outbreak in Burkina Faso (29). A change in predominant DENV serotype may have fueled the outbreak in 2016. Although the current study did not determine DENV strain, DENV2 strains reported from ill French travelers returning from Burkina Faso in November 2016 were nearly identical to a DENV2 strain detected in Burkina Faso in 1983. This suggests that the 2016 outbreak may have been due to an endemic strain of DENV2 circulating in Burkina Faso for 30 years, perhaps maintained partly through a sylvatic cycle (30). More detailed phylogenetic analysis of DENVs from the current study is planned.

Only a quarter of dengue-positive cases received a clinical diagnosis of suspected dengue in this study, with this proportion being only slightly higher during the 2016 outbreak (31% of dengue cases were suspected clinically) compared to outside the outbreak (17%). In the routine care system, clinicians in the CSPS refer to a guideline issued by the Burkina Faso MoH (31), primarily based on the 2009 WHO dengue guidelines. The dengue RDTs

were made available at the CSPSs in the study, but the results of dengue RDT might not have contributed to the clinical assessment, if the results were not made available in time (dependent on patient volume and clinician availability). Dengue RDTs are typically unavailable for routine use in Africa; and many non-malaria febrile etiologies, including dengue, are likely to be under-diagnosed (12, 32). Clinicians in Burkina Faso may need to consider dengue more frequently as a clinical diagnosis, with or without point-of-care assays.

Our multivariable analysis showed differing patterns of signs and symptoms associated with dengue-positivity during the outbreak period compared to non-outbreak periods. Rash was associated with dengue-positivity during both outbreak and non-outbreak periods. Rash is a common sign for dengue and listed in dengue classification in both 1997 and 2009 WHO dengue guidelines (3, 33, 34). However, retro-orbital pain showed increased odds of dengue-positivity only during the outbreak. Retro-orbital pain, also listed in the 2009 WHO case definition, is another common sign associated with dengue-positivity (3, 33, 34). Also, it was suggested that ocular symptoms, including retro-orbital pain, in dengue patients may possibly indicate thrombocytopenic state with increased likelihood of hemorrhage (35). In our data, dengue-positive patients with retro-orbital pain were 5.8 times (95% C.I: 3.5 – 9.6, p<.001) more likely to require observation than dengue-positive patients without retro-orbital pain during the outbreak. During non-outbreak, it also showed a similar pattern with statistical significance, but with a wide confidence interval. Therefore, further information is needed for validation. While hemorrhagic signs were not commonly reported in our data, requiring observation may indicate severity of dengue illness and retro-orbital pain being associated with dengue-positive cases in the outbreak, but not outside the outbreak, may indicate likely severity of dengue illness during the outbreak.

Epidemiologic characteristics of dengue in Ouagadougou

Our data showed a high proportion of individuals 15-40 years of age among dengue-positive cases in the outbreak period (a mean age of 26.8 years in dengue-positive patients). This was also found in the outbreak investigation by the Burkina Faso MoH with WHO where 70% of affected people were 25 years and older, with a mean age of 30 years (16). It suggests that those in the labor force may be impacted, leading to significant economic and social burden (36).

In our data, the adjusted model showed that female sex was associated with decreased odds of dengue-positivity (OR=0.65, 95 C.I= 0.48-0.87) in the outbreak period. This finding was consistent to a finding of another study conducted in Burkina Faso in 2016 where only 23% of cases were female (17) as well as other previous data reporting excess of reported male dengue cases among older adolescents and adults (27, 37). However, such pattern was to the contrary to the finding of the investigation conducted by MoH of the 2016 outbreak. In the MoH investigation, women were more affected than men (16). There may have been differences in terms of gender ratio and demographic profile in the studied populations in previous reports. Thus, more assessment of gender differences for dengue incidence would be necessary to study biological or gender-related association for the risk of dengue in Africa (27).

Adjusted for age and gender, our model found higher odds that dengue-positive cases required observation, compared to non-dengue, during both outbreak (6.0 times) and non-outbreak (4.3 times) periods. Given the substantial proportion of dengue-positive cases among non-malarial febrile illnesses, this suggests that dengue may account for greater utilization of healthcare resources in CSPSs than other etiologies, during both outbreak and non-outbreak periods. As in many other parts of Africa, these primary healthcare centers have limited resources, such as beds (38), and could be especially overextended during outbreaks. Since the study only enrolled patients at CSPSs, the burden on the healthcare system due to dengue inpatients is unclear.

Self-reported YF vaccination was associated with increased odds of dengue-positivity, suggesting predisposition of YF vaccinated individuals to develop symptomatic dengue (39). However, self-reporting may be unreliable due to recall bias, and the study could not confirm YF vaccination using patient records.

Study limitations and strengths

Dengue transmission can vary substantially over time and space. Hence, the generalizability of the current study is limited by enrollment from the five selected CSPSs in the capital during the 27-month study period. We would have missed those community residents with relevant symptoms seeking care elsewhere than study centers, including private providers. In addition, patients with severe illness would have not been enrolled since they would likely have sought care directly at inpatient facilities; and subclinical and mild DENV infections would also not have been detected.

The study surveillance excluded patients with malaria RDT positive results, localizing signs or known/confirmed diagnosis with other diseases, possibly omitting co-infections of dengue with another pathogen. In particular, given the prevalence of malaria in this region, dengue and malaria co-infection may require further investigation. Nevertheless, the available information on co-infections suggests they are uncommon (9, 40-43).

Performance of malaria RDTs, in terms of sensitivity, would depend on local conditions, especially the level of malaria transmission shown to be variable from reported incidence in Ouagadougou (44, 45). There could have been misclassification among non-malarial patients (i.e. false negative results on malaria RDT included in the study being differently classified between dengue-positive and non-dengue groups). Also, this could vary by the level of dengue transmission (i.e. during and outside of the outbreak), leading to differential misclassification.

Our findings were based on outpatients and patients requiring observation, and clinical characteristics may be different for hospitalized patients and individuals with subclinical infections. Also, such findings may depend on other co-circulating pathogens endemic in the area, however our study did not confirm etiologies of non-dengue cases. Therefore, further information on the etiologies of non-dengue febrile cases may be needed to verify which signs are useful in distinguishing non-dengue from dengue illnesses (46).

In our analysis, laboratory-confirmed and probable dengue cases were combined into the dengue-positive group. There may be some limitations with probable dengue being not as certain as lab-confirmed dengue. However, we performed analysis using 3 categories of dengue infection status (lab-confirmed-; probable-; and non-dengue) as part of sensitivity analysis and this yielded similar results (see S2-S4 tables).

Conclusion

Dengue is an important cause of non-malarial fever in Burkina Faso, both during and outside of outbreaks, despite being infrequently suspected by clinicians. Despite the many possible etiologies of febrile illness in this region, limited surveillance and diagnostic capacity will continue to pose challenges to dengue prevention and control. Additional longitudinal studies to better characterize dengue epidemiology and clinical presentation, including in inpatients and for subclinical/mild cases, along with encouraged use of dengue RDTs, would help to inform strategies to approach dengue countermeasures in this region.

Acknowledgments.

The authors thank the patients, doctors and staff of CSPSs of Ouagadougou. Also, we thank the laboratory staff of Centre Muraz. The authors would also like to thank the staff of AGIR, the health authorities of Ouagadougou and Burkina Faso, and particularly the Ministry of Health, for their support in the study execution. Lastly, we would like to thank the DVI team and statisticians and administrative staff at the International Vaccine Institute for their helpful comments during the preparation of this manuscript and support during the studies.

References

1. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerg Infect Dis. 1995;1(2):55-7.
2. World Health Organization. Global strategy for dengue prevention and control 2012-2020. Geneva: World Health Organization; 2012. p. vi, 43p.
3. World Health Organization. Dengue and severe dengue. Fact sheets 2019 2 February 2018 [cited 2019 12 May]; Available from: http://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
4. Messina J, Brady O, Scott T, Zou C, Pigott D, Duda K, et al. Global spread of dengue virus types: mapping the 70 year history. Trends Microbiol. 2014;22(3):138-46.
5. Amarasinghe A, Kuritsky J, Letson G, Margolis H. Dengue virus infection in Africa. Emerging Infectious Diseases. 2011;17(8):1349-54.
6. Kraemer MU, Sinka ME, Duda K, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLife. 2015 Jun 30, 2015 4:e08347.
7. Baba M, Villinger J, Masiga DK. Repetitive dengue outbreaks in East Africa: A proposed phased mitigation approach may reduce its impact Reviews in Medical Virology. 2016 29 February 2016;26(3):183-96.
8. Amoako N, Duodu S, Dennis FE, Bonney JHK, Asante KP, Ameh J, et al. Detection of dengue virus among children with suspected malaria, Accra, Ghana. Emerg Infect Dis 2018 2018 Aug;24(8):1544–7.
9. Ridde V, Agier I, Bonnet E, Carabali M, Dabiré K, Fournet F, et al. Presence of three dengue serotypes in Ouagadougou (Burkina Faso): research and public health implications. Infect Dis Poverty. 2016 2016 Apr;5(5):23.
10. Were F. The dengue situation in Africa. Paediatr Int Child Health. 2012 May;32 Suppl 1:18-21.
11. Ridde V, Carabali M, Ly A, Druetz T, Kouanda S, Bonnet E, et al. The need for more research and public health interventions on dengue fever in Burkina Faso. PLoS Neglected Tropical Diseases. 2014;8(6):e2859.
12. Brah S, Daou M, Salissou L, Mahaman S, Alhousseini D, Iroungou B A, et al. Fever of unknown origin in Africa: The causes are often determined! Health sciences and diseases. 2015;16(2).
13. Gonzalez J, Du Saussay C, Gautun J, McCormick J, Mouchet J. Dengue in Burkina Faso (ex-upper Volta): seasonal epidemics in the urban area of Ouagadougou. Bulletin de la Societe de Pathologie Exotique. 1985;78(1):7-14.
14. Robert V, Lhuillier M, Meunier D, Sarthou J, Monteny N, Digoutte J-P, et al. Yellow fever virus, dengue 2 and other arboviruses isolated from mosquitos, in Burkina Faso, from 1983 to 1986. Entomological and epidemiological considerations. Bulletin de la Societe de Pathologie Exotique. 1993;86(2):90-100.
15. Ministère de la Santé. Rapport d'étape de l'investigation de cas suspects de Dengue dans la région sanitaire du Centre. Ouagadougou, Burkina Faso: Direction de la lutte contre la maladie; 2013.
16. World Health Organization. Dengue fever - Burkina Faso. Disease outbreak news 2016 2016 November 18 [cited 2018 August 18]; Available from: http://www.who.int/csr/don/18-november-2016-

dengue-burkina-faso/en/

17. Tarnagda Z, Cissé A, Bicaba B, Diagbouga S, Sagna T, Ilboudo A, et al. Dengue fever in Burkina Faso, 2016. Emerg Infect Dis 2018 2018 Jan;24(1):170-2.

18. World Health Organization. Dengue fever - Burkina Faso. Disease outbreak news 2017 2017 November 6 [cited 2018 August 18]; Available from: http://www.who.int/csr/don/6-november-2017-dengue-burkina-faso/en/

19. Beatty M, Stone A, Fitzsimons D, Hanna J, Lam S, Vong S, et al. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas Dengue Prevention Boards. PLoS Negl Trop Dis 2010 2010 Nov 16;4(11):e890.

20. Lim J, Carabali M, Lee J-S, et al. Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative. BMJ Open. 2018;2018(8):e017673.

21. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6(8):e1760.

22. Secretariat General. Annuaire statistique 2016. In: sectorielles. Dgdéeds, editor. 03 BP 7009 Ouagadougou 03.: Ministere de la Sante, Burkina Faso; 2017.

23. Beogo I, Liu C-Y, Chou Y-J, Chen C-Y, Huang N. Health-care-seeking patterns in the emerging private sector in Burkina Faso: A population-based study of urban adult residents in Ouagadougou. PLoS ONE. 2014;9(5):e97521.

24. Rossier C, Soura A, Baya B, Compaoré G, Dabiré B, Dos Santos S, et al. Profile: The Ouagadougou Health and Demographic Surveillance System. International Journal of Epidemiology. 2012 June 1, 2012;41(3):658-66.

25. Alm E, Lindegren G, Falk KI, Lagerqvist N. One-step real-time RT-PCR assays for serotyping dengue virus in clinical samples. BMC Infectious Diseases. 2015 2 November 2015;15(493).

26. World Health Organization. Handbook for clinical management of dengue. Geneva, Switzerland: World Health Organization,; 2012.

27. Ankera M, Arimab Y. Male-female differences in the number of reported incident dengue fever cases in six Asian countries. Western Pacific Surveillance and Response Journal. 2011 2011;2(2):17-23.

28. Sang RC. Dengue in Africa. [cited 2019 January 21]; Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.599.6007&rep=rep1&type=pdf

29. Tarnagda Z, Congo M, Sagna T, Ouédraogo C, Nikiéma V, Cissé A, et al. Outbreak of dengue fever in Ouagadougou, Burkina Faso, 2013. International Journal of Microbiology and Immunology Research. 2014;2014(2):101-8.

30. Baronti C, Piorkowski G, Touret F, Charrel R, de Lamballerie X, Nougairede A. Complete coding sequences of two dengue virus type 2 strains isolated from an outbreak in Burkina Faso in 2016. Viruses. 2017.

31. MoH of Burkina Faso. Directive national de prise en charge des cas de dengue au Burkina Faso. In: MoH, editor. Burkina Faso; 2014.

32. Zongo S, Carabali M, Munoz M, Ridde V. Dengue rapid diagnostic tests: Health professionals' practices and challenges in Burkina Faso. SAGE Open Medicine. 2018;6.
33. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention, and control. Geneva: World Health Organization; 2009.
34. Hadinegoro SRS. The revised WHO dengue case classification: does the system need to be modified? Paediatr Int Child Health 2012 2012 May;32(S1):33-8.
35. Chan DPL, Teoh SCB, Tan CSH, Nah GKM, Rajagopalan R, Prabhakaragupta MK, et al. Ophthalmic complications of dengue. Emerg Infect Dis 2006 2006 Feb;12(2):285-9.
36. Lee J, Mogasale V, Lim JK, Ly S, Lee K, Sorn S, et al. A multi-country study of the economic burden of dengue fever based on patient-specific field surveys in Burkina Faso, Kenya, and Cambodia. PLoS Negl Trop Dis 2019 2019 Feb 28;13(2):e0007164.
37. Eldigail MH, Adam GK, Babiker RA, Khalid F, Adam IA, Omer OH, et al. Prevalence of dengue fever virus antibodies and associated risk factors among residents of El-Gadarif state, Sudan. BMC Public Health. 2018 27 July 2018;201818:921.
38. Hospital bed density - World. Thematic Map 2018 January 1, 2018 [cited 2018 October 21]; The map displayed shows how Hospital bed density varies by country]. Available from: https://www.indexmundi.com/map/?t=0&v=2227&r=xx&l=en
39. Guzman JR, Kron MA. Threat of dengue haemorrhagic fever after yellow fever vaccination. Lancet. 1997 June 21, 1997;349(9068):P1841.
40. Wiwanitkit V. Concurrent malaria and dengue infection: a brief summary and comment. Asian Pac J Trop Biomed 2011 2011 Aug;1(4):326–7.
41. Epelboin L, Hanf M, Dussart P, Ouar-Epelboin S, Djossou F, Nacher M, et al. Is dengue and malaria co-infection more severe than single infections? A retrospective matched-pair study in French Guiana. Malar J. 2012;11(142).
42. Magalhães BML, Siqueira AM, Alexandre MAA, Souza MS, Gimaque JB, Bastos MS, et al. *P. vivax* malaria and dengue fever co-infection: A cross-sectional study in the Brazilian Amazon. PLoS Negl Trop Dis. 2014 October 23, 2014.
43. Carme B, Matheus S, Donutil G, Raulin O, Nacher M, Morvan J. Concurrent dengue and malaria in Cayenne hospital, French Guiana. Emerg Infect Dis 2009 2009 Apr;15(4):668–71.
44. World Health Organization. Malaria rapid diagnostic test performance summary results of WHO product testing of malaria RDTs: round 1-8 (2008–2018). Geneva; 2018.
45. Ouedraogo B, Inoue Y, Kambiré A, Sallah K, Dieng S, Tine R, et al. Spatio-temporal dynamic of malaria in Ouagadougou, Burkina Faso, 2011-2015. Malaria Journal. 2018 2 April 2018;17(138).
46. Yoon I-K, Srikiatkhachorn A, Hermann L, Buddhari D, Scott TW, Jarman RG, et al. Characteristics of mild dengue virus infection in Thai children. Am J Trop Med Hyg. 2013 2013 Dec 4;89(6):1081-7.

Supporting Information

S1 Checklist: STROBE Checklist

S2 Table: Demographic and clinical characteristics of patients by dengue infection status from the health facility-based fever surveillance established in Ouagadougou, Burkina Faso

S3 Table: Univariate logistic regression analyses showing significant indicators and their odds ratios between dengue-confirmed and non-dengue cases during the period of outbreak in the health facility-based fever surveillance

S4 Table: Univariate logistic regression analyses showing significant indicators and their odds ratios between dengue-confirmed and non-dengue cases during the period of non-outbreak in the health facility-based fever surveillance

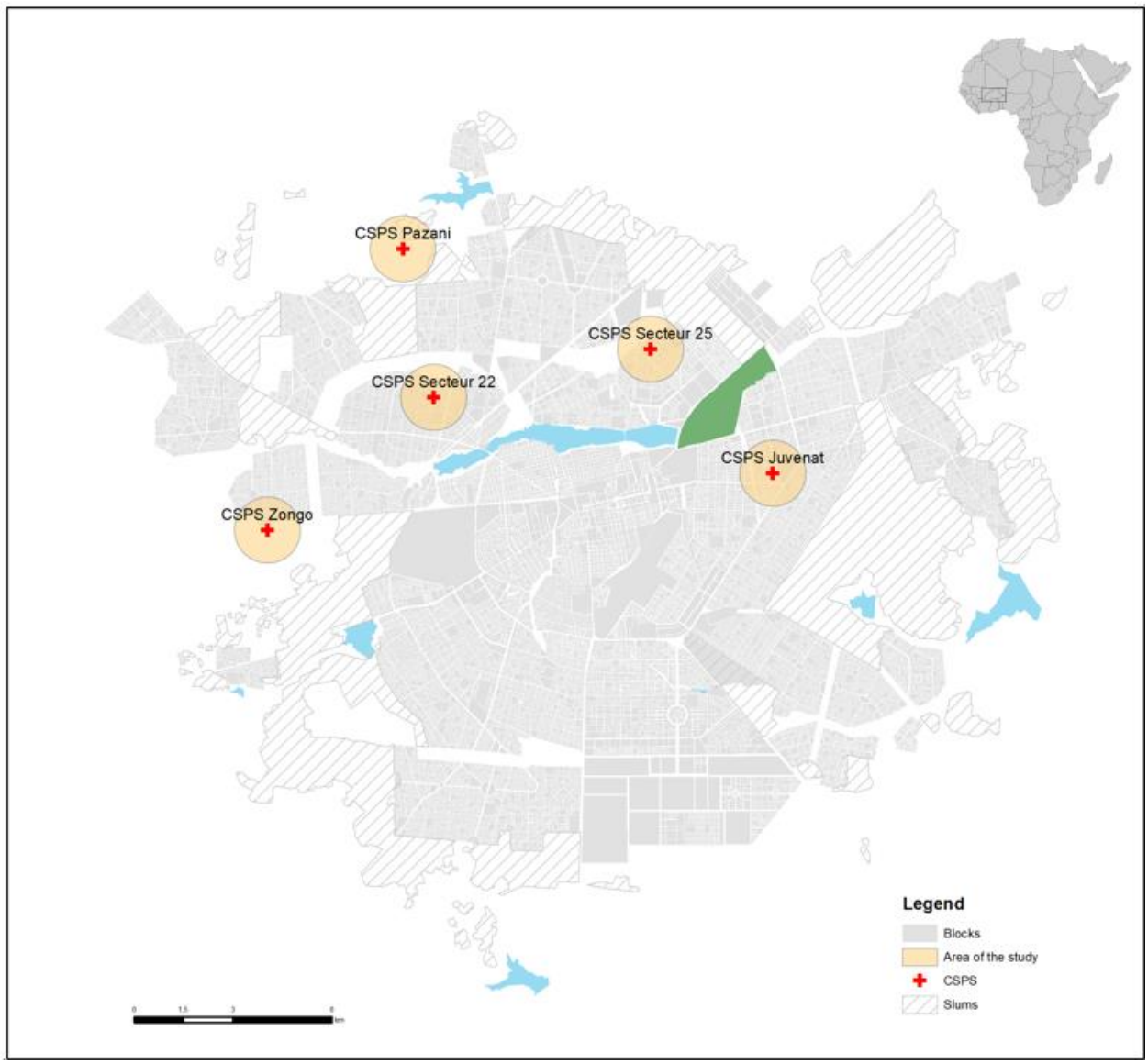


Fig. 1 A map of the study area in Ouagadougou

A map of the study area in Ouagadougou - The map shows the approximate location of the selected facilities of 5 CSPSs (Pazani, CSPS22, CSPS25, Juvenat Fille, Zongo), serving a catchment population of 110,000 residents of Ouagadougou, Burkina Faso.

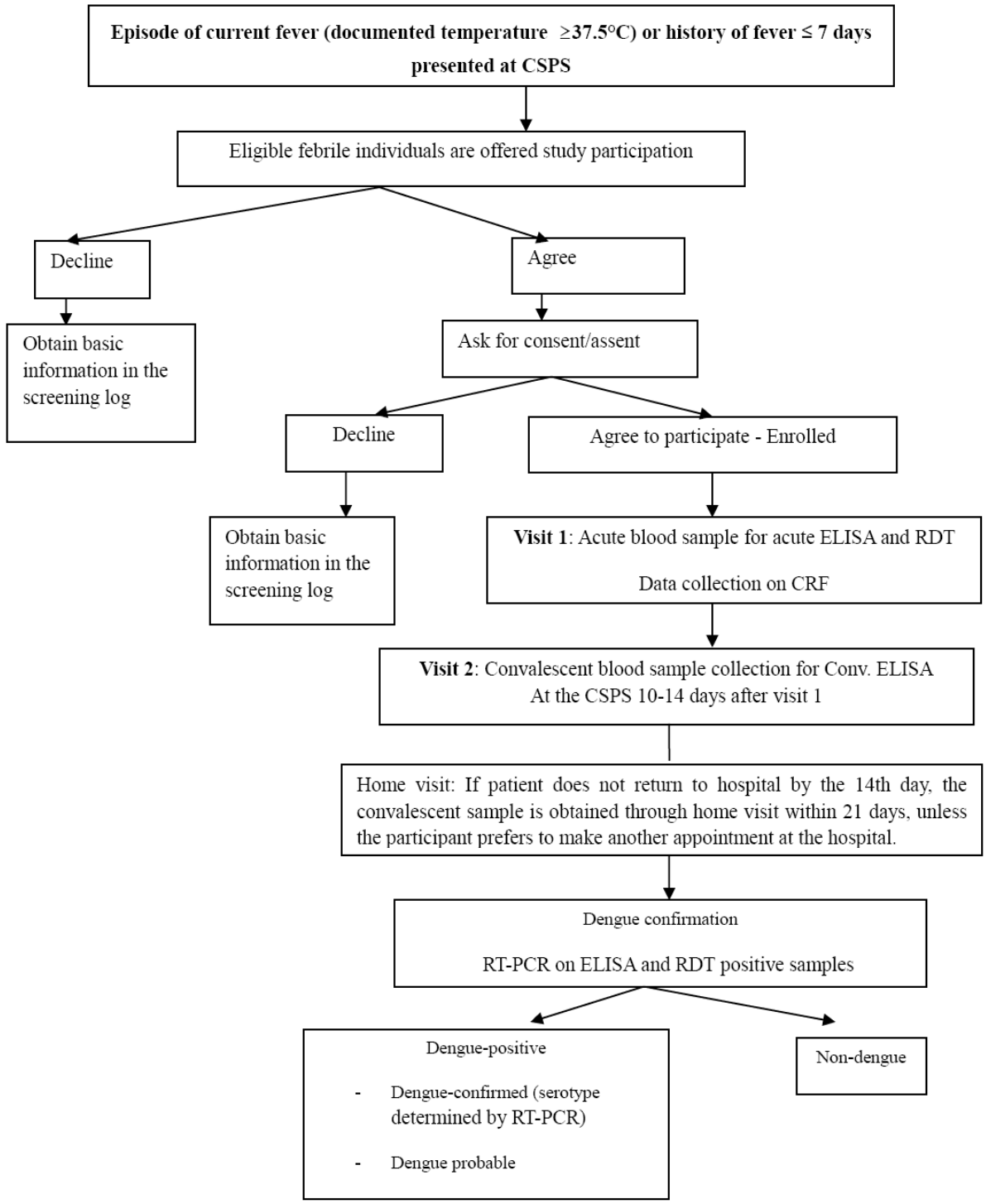


Fig. 2 A chart of patient flow in passive fever surveillance

A chart of patient flow in passive fever surveillance- The chart shows the study flow when a febrile patient presents at the CSPS.

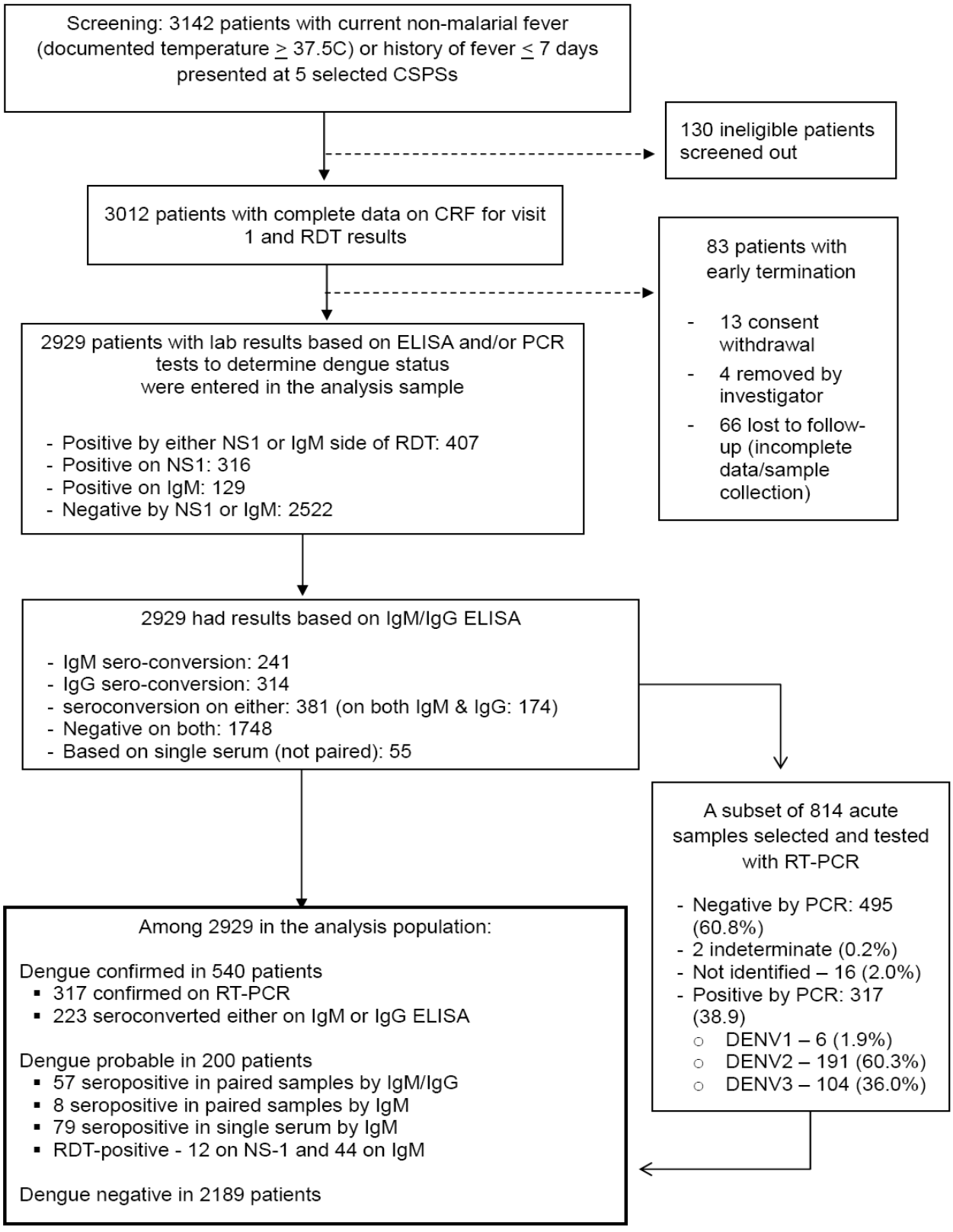


Fig. 3 Flow chart of patient enrollment and lab testing of the samples

The chart shows the flow of patients from screening, enrollment to study participation, with the samples undergoing multiple stages of lab testing for determination of laboratory-based status of dengue infection, as well as how the analysis sample was reached.

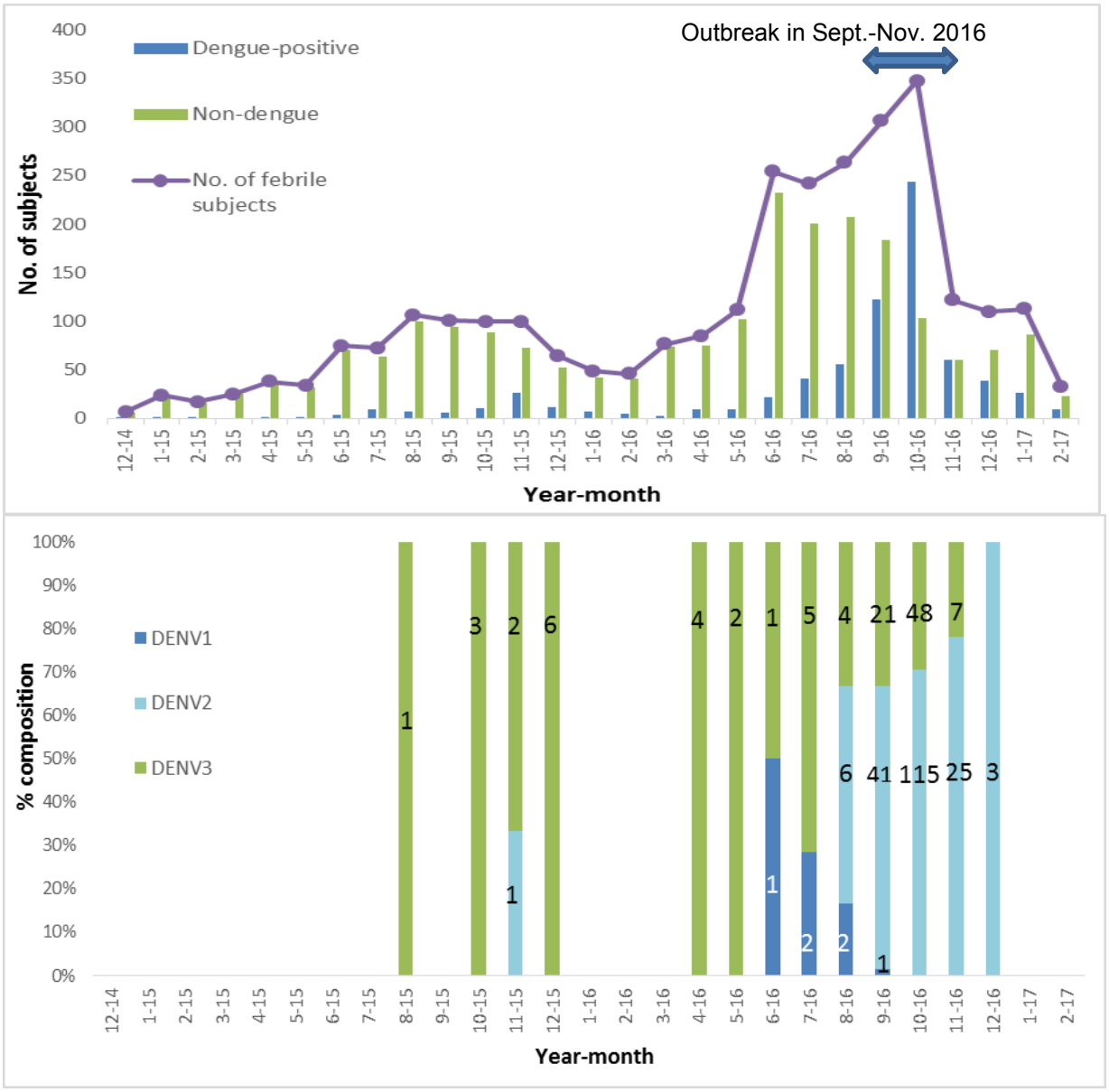


Fig. 4. Monthly distribution of febrile enrollees, dengue-positive and non-dengue cases & monthly distribution of dengue serotypes* in PCR-positive cases

*number of identified serotypes shown in the bars

The figure has two parts: the upper part shows monthly distribution of dengue-positive and non-dengue cases among the enrolled patients; and the lower part shows distribution of serotypes identified (numbers shown in the bars) by month

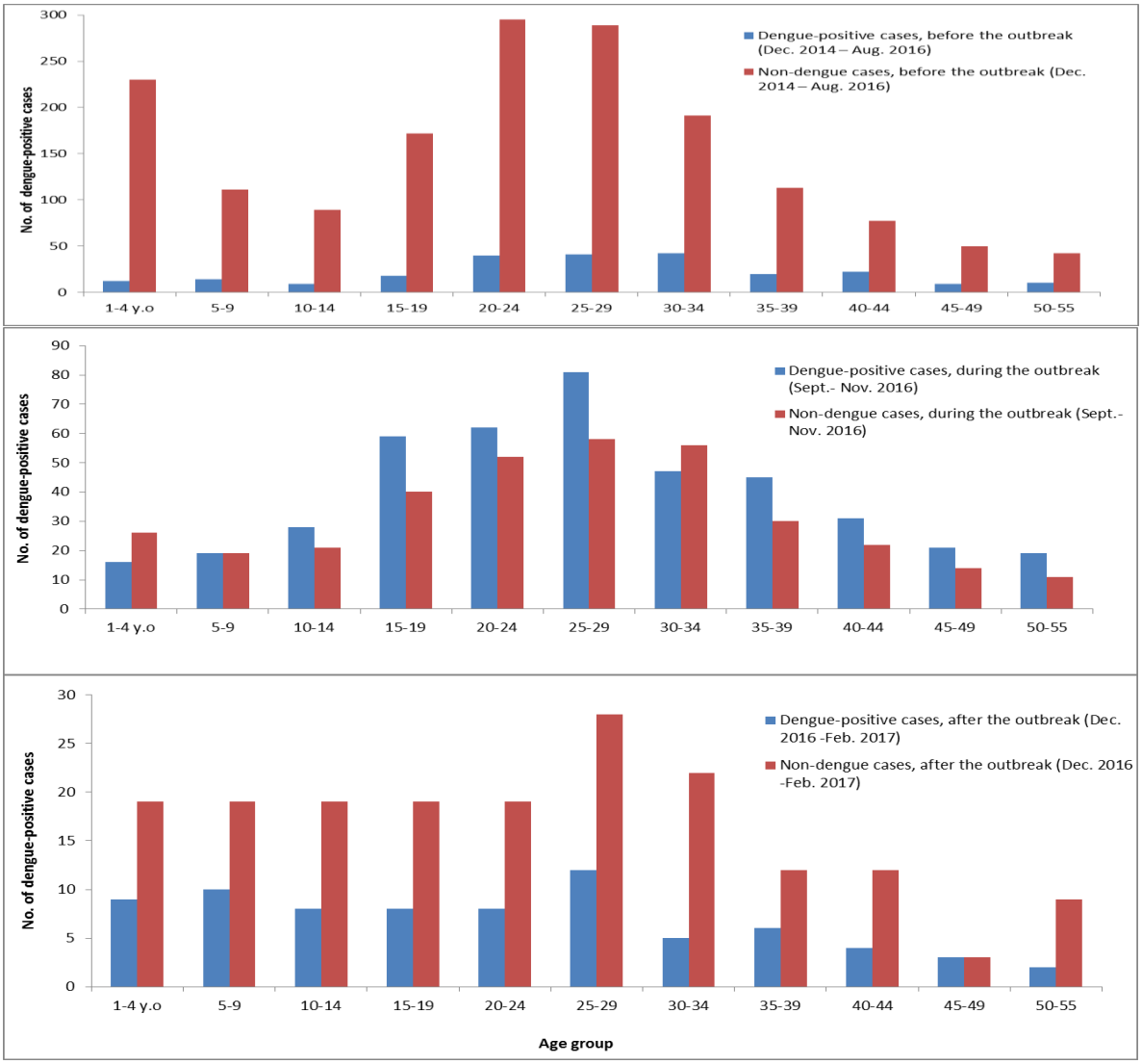


Figure 5. Age distribution of dengue-positive cases before, during, and after the 2016 outbreak

The figure shows age distribution of dengue-positive cases, compared to non-dengue cases, before, during, and after the 2016 outbreak

Chapter 6. Dengue virus seroprevalence and force of infection estimated using repeated serosurveys in Ouagadougou, Burkina Faso

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	LSH1405874	**Title**	Ms.
First Name(s)	Jacqueline Kyungah		
Surname/Family Name	LIM		
Thesis Title	Undocumented burden of dengue in Africa		
Primary Supervisor	Prof. Neal Alexander (associate supervisor: Tansy Edwards)		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Journal of Infectious Diseases
Please list the paper's authors in the intended authorship order:	Jacqueline K. Lim, Mabel Carabali, Ahmed Barro, Desire Dahourou, Kang Sung Lee, Teguewende Nikiema, Jung Seok Lee, Emmanuel Bonnet, Therese Kagone, Tansy Edwards, Paul-André Somé, Jae Seung Yang, In-Kyu Yoon, Valéry Ridde, Neal Alexander, Yaro Seydou
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I am first author. I led the study design and co-developed the protocol with Dr. Mabel Carabali. Some site-specific details were added in collaboration with the key investigators (Y Seydou and V Ridde, as the PI and co-investigator, and M Carabali). I oversaw the ethical approval process, supported study set-up, monitored data collection, performed literature review, performed data cleaning, and conducted statistical analysis including generating SAS code for analysis and all the figures, except for Fig. 1 (by Emmanuel Bonnet, one of co-authors). I wrote the first draft of the manuscript. I, as the first author, led the process of manuscript preparation, revision, and submission. M. Carabali also supported data and sample collection. Yaro Seydou and Valéry Ridde, as the PI and co-investigator, were responsible for study set-up and execution in Ouagadougou. Ahmed Barro, Desire Dahourou, Kang Sung Lee, Teguewende Nikiema, Suk Namkung, Jung Seok Lee, Losseni Kaba, and Paul-André Somé provided support in setting up the study at the sites, in sample and data collection, and data management and analysis. Mee Young Shin, Therese Kagone, and Jae Seung Yang performed laboratory work. Emmanuel Bonnet provided support in data cleaning. Neal Alexander and In-Kyu Yoon provided oversight and guidance on the overall study design, project execution, and specific methodologies, e.g. epidemiology, statistical, and virology and other laboratory methods. Neal Alexander and Tansy Edwards oversaw statistical analysis, and contributed to manuscript preparation.

SECTION E

Student Signature	[redacted]
Date	26June 2019

Supervisor Signature	[redacted]
Date	27 June 2019

Title: Dengue virus seroprevalence and force of infection estimated using repeated serosurveys in Ouagadougou, Burkina Faso

Jacqueline K. Lim[1 2*], Mabel Carabali[1 4], Ahmed Barro[5], Desire Dahourou[3 6], Kang Sung Lee[1], Teguewende Nikiema[3], Jung-Seok Lee[1], Mee Young Shin[1], Emmanuel Bonnet[7], Therese Kagone[3], Losseni Kaba[8], Tansy Edwards [2], Paul-André Somé[5], Jae Seung Yang[1], Valéry Ridde[7 9], In-Kyu Yoon[1], Neal Alexander [2], Yaro Seydou[3]

[1]International Vaccine Institute, Seoul, Korea, Republic of; [2]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine; [3]Centre MURAZ, Bobo-Dioulasso, Burkina Faso; [4]McGill University, Montreal, Quebec Canada; [5]Action-Gouvernance-Integration-Renforcement (AGIR), Program Equité, Ouagadougou, Burkina Faso; [6]Institut de Recherche en Sciences de la Santé, Ouagadougou, Burkina Faso; [7]Institute for Research on Sustainable Development (IRD), CEPED, Université de Paris, ERL INSERM SAGESUD; [8]Centre National de Transfusion Sanguine, Ouagadougou, Burkina Faso; [9]University of Montreal Public Health Research Institute (IRSPUM), Montreal, Canada

*Corresponding author
Jacqueline K. Lim[1 2*]
Research Scientist
Global Dengue and *Aedes*-transmitted Diseases Consortium (GDAC)
International Vaccine Institute (IVI)
SNU Research Park, Gwankak-ro 1
Gwanak-gu, Seoul, Korea 151-191
T: +82-2-881-1340 (Dir) / +82-2-872-2801 (ext. 340)
Email: kalim@ivi.int

30 pages (not counting tables/figures)
Word count of the text: 5958 (not counting the Abstract and tables)
Word count of the abstract: 488

Figures and tables: 2 figures and 5 tables

Abbreviations: AGIR, Action-Gouvernance-Integration-Renforcement; °C, Celsius degrees; CI, Confidence Interval; DCF, Data Collection Form; DENV, Dengue Virus; DF, Dengue Fever; DHF, Dengue Hemorrhagic Fever; DSS, Dengue Shock Syndrome; DVI, Dengue Vaccine Initiative; ELISA, Enzyme-Linked Immunosorbent Assay; ICF, Informed Consent Form; IgM/IgG, Immunoglobulin type M and type G; IRD, Institute for Research on Sustainable Development; IRB, Institutional Review Board

Keywords: Dengue, seroprevalence, asymptomatic, outbreak, Burkina Faso, Africa

Contributor's statement page

All persons designated as authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content. And all the authors contributed in some or all areas of acquisition of funding, conception of the study, collection of data, analysis and interpretation of data, drafting the article, article revision, scientific support, and final approval of the version to be published. The authors meet the criteria for authorship and qualify for authorship of this manuscript.

Financial disclosure: This study was supported by funding from the Bill and Melinda Gates Foundation (grant #: OPP 1053432), as well as from the governments of Sweden, India, and the Republic of Korea. This research project was part of the "Community research studies and interventions for health equity in Burkina Faso" program funded by the Canadian Institutes of Health Research (ROH-115213).
NA and TE were supported by award MR/R010161/1, which is jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 programme supported by the European Union.

Conflict of interest statement: I certify that the authors do not have any relevant financial relationships or potential conflicts of interest to disclose regarding the material discussed in this manuscript.

Abstract

Background: There is limited information on dengue burden in Burkina Faso. To understand the dynamic of dengue virus, we conducted repeated serological surveys and estimated seroprevalence and force of infection in Ouagadougou, Burkina Faso.

Methods: Four consecutive age-stratified serological surveys, with an interval of six months covering the rainy period, were conducted among the same individuals aged between 1-55 years residing in Ouagadougou. To reflect the age distribution of the general population of the district, 80% of the serosurvey samples were from individuals < 35 years-of-age and 20% from adults between 35 and 55 years of age. The enrollment bleed (S1) took place in June 2015, and enrolled subjects were followed in subsequent serosurveys in Dec. 2015 (S2), May 2016 (S3), and in March 2017 (S4). The last two serosurveys covered the 2016 outbreak between September and November. All samples were tested with commercial Panbio Dengue IgG indirect ELISA. Lab results and basic demographic and clinical data were analyzed to determine antibody prevalence, and to calculate force of infection (FoI), the rate at which susceptible individuals become infected, based on sero-conversion between pre-and post-transmission paired sera. The sero-conversion rate up to the enrollment serosurvey was estimated by binomial regression, taking age as the duration of exposure, and assuming that this FoI had been constant over age and calendar time. Then, FoIs between consecutive surveys were estimated, with rate ratios for potential characteristics in association, including age, also being assessed.

Results: Among 2897 at enrollment, 66.3% were IgG positive. At S2, 67.2% were IgG positive among 2387 subjects followed up. At S3, 2215 were followed up and 67.2% were IgG positive. At S4, 67.9% were IgG positive among 1681 subjects followed up. Based on initial seroprevalence, a previous FoI of 5.95% per year was estimated, assumed to be constant over age and calendar time. Of 1269 subjects who stayed in all 4 serosurveys, 438 were IgG-negative at enrollment, with 107 sero-converting over the subsequent study period of almost two years. The annualized FoI was 14% over the rainy season in 2015; 10% over the non-rainy season of 2016; and 20% during the

outbreak in 2016. In those intervals covering the non-outbreak times, older age and self-reported pre-existing conditions were associated with increased rate of sero-conversion. For the interval covering the 2016 outbreak, rates were similar across age.

Conclusion: Overall, we observed a high level of seroprevalence and also high FoIs. The randomly sampled population-based follow-up design provides stronger evidence on DENV transmission in the studied community than previously available data on the seroprevalence and incidence of dengue in Burkina Faso. However, the results are subject to limitations of losses to follow-up, and possible cross-reaction of the IgG ELISA with other flaviviruses. The results could be used to facilitate informed decision-making on implementation of control and preventive measures for dengue, such as vaccine introduction. Nonetheless, additional evaluation across the region would be necessary to assess the generalizability of our findings.

Introduction

Dengue Fever (DF) is a mosquito-borne disease caused by four related but antigenically distinct dengue viruses (DENVs, serotypes 1–4). There has been a dramatic increase in the burden of dengue globally and DF with dengue hemorrhagic fever (DHF) are considered major causes of mortality and morbidity in tropical and sub-tropical countries (1, 2). About 50 to 100 million cases of DF are reported to occur annually worldwide, with 500,000 severe dengue cases requiring hospitalization and 20,000 deaths annually (3-6).

Aedes mosquitoes and dengue cases were documented as early as 1823 in Africa and *Ae*. *aegypti* and *Ae. albopictus* are widely distributed in the continent (7-9). Amarasinghe et al. indicated that dengue cases have been reported in 34 countries in Africa (10). Previously, dengue was not recognized as an important etiology of non-malarial febrile episode in Africa, but there are reports of repeated outbreaks and more studies demonstrate dengue to be a common viral infection (11-13). However, most of the data are from a few countries and studies are often not representative or population-based, often limited to retrospective testing using existing samples or reports from outbreak investigations (10, 12, 14).

There have been multiple outbreaks of DENV in Burkina Faso, the first being reported in 1925 (10). There was an outbreak declared by the Ministry of Health of Burkina Faso in November 2013, and, more recently, in 2016 and 2017 (15-18). The outbreak in 2016, between August and November, reported 1061 dengue RDT positive cases and 15 deaths from Ouagadougou (17, 19). The outbreak in September 2017 was larger, with 9029 suspected dengue cases, 5773 dengue RDT-positive cases, and 18 deaths throughout the country (18). These repeated outbreaks suggest a considerable dengue burden in Burkina Faso.

Despite information from outbreaks, there are not many data available on dengue virus burden in Burkina Faso and Africa, in terms of seroprevalance and force of infection (FoI), the rate at which susceptible individuals become infected (20, 21). In terms of seroprevalence, there was a study based on testing 683 samples from pregnant women and blood donors with a mean age of 25 years, using IgG ELISA, and the estimated prevalence was 26.3% in rural settings and 36.5% in urban settings in

2003-2004 (22). While this is not a representative sample of the general population, it showed similar estimates to seroprevalence estimated in Nigeria, where a study estimated the prevalence of flavivirus infections among 1,816 children and adults in urban and rural areas during the early 1970s using virus-specific hemagglutination inhibition and neutralization testing (23). The prevalence was 45% for DENV-2 infection and higher in urban (48%) than in rural areas (37%) (23).

To better understand the ongoing DENV transmission in Burkina Faso, in terms of seroprevalence and FoI, measured by IgG sero-conversion, we conducted four serological surveys in the same individuals residing in the capital, Ouagadougou, from 2015-2017, in collaboration among Centre MURAZ, University of Montreal, the French Research Institute for sustainable Development (IRD), Action-Gouvernance-Integration-Renforcement (AGIR), and Dengue Vaccine Initiative (DVI). The study served two objectives. First, seroprevalence of dengue virus was measured at enrollment. Secondly, we estimated age-specific annual FoI, measured by sero-conversion. Lastly, the outbreak occurring from September to November, 2016, between serosurveys 3 and 4, allowed identification of demographic and clinical characteristics associated with DENV sero-conversion in the outbreak and non-outbreak periods.

Methods

Study area and population

Selection of the study area was based on previous outbreaks and case reports as well as seroprevalence studies in the literature, modelling studies and the availability of research infrastructure (7, 24, 25). Ouagadougou is the capital city of Burkina Faso in West Africa. The 2016 population was 2.6 million, almost 95% residing in urban settings (26, 27). The rainy season is from May-October, with a mean temperature of 28 °C (82 °F). The maximum temperature during the hot season (March-May) can reach 43 °C (109 °F).

The serosurveys were conducted on residents in a defined catchment population of 100,000 residents of Ouagadougou (Fig. 1). The resident population in Ouagadougou

is very stable with a rate of transmigration of 4.1% per year, and more than 80% of the inhabitants with home ownership (28).

Study design

Four serosurveys were conducted approximately six months apart covering the rainy season. The age-stratified sample of approximately 3000 residents between 1 and 55 years of age reflected age distribution of the general population of the district (24), with 80% of the serosurvey sample under 35 years-of-age and the remainder aged from 35-55 years. Pre-and post-transmission paired sera were tested with a commercial Panbio® Dengue IgG Indirect ELISA (Abbott Diagnostics, United States).

The serological survey enrollment was performed at the household-level. There were 12 districts and 52 sectors in Ouagadougou. For randomization, sectors were randomly selected using previously collected census data. In the selected sectors, households in the chosen sectors were pre-selected, also, randomly. In the selected households, all the eligible household members were offered enrollment. When members of the selected house declined, the study team invited the residents of the neighboring household for the enrollment, to reach the needed sample size. The serosurvey was briefly described to the head of the household. Once the eligible member(s) of the family was identified and study participation is agreed, then informed consent and assent were sought. The consent documents were transported to and stored at AGIR/ Centre Muraz.

Trained phlebotomists performed a blood draw of 3 -5 ml in subjects 1-7 years-of-age and 5-10ml in subjects 8 years and older with aseptic measures using disposable needles and syringes. After blood sample collection, a short interview was conducted and the data collection forms were completed (24). After the IgG ELISA results become available, the study team delivered the results and made arrangement for the follow-up visit. The same procedures were followed with the same family members for the subsequent serosurveys.

Subject eligibility

Individuals who meet the following criteria were considered as eligible subjects:

Inclusion Criteria

1. Age 1- 55 years old
2. Residents of Ouagadougou for more than six months
3. An informed consent from (ICF) obtained from each participant. For those aged between 8 and 17 years, an assent form, plus informed consent from at least one parent or legal guardian.

Exclusion Criteria

1. Individuals with plans to move out of the catchment area during the study period, October 2014- September 2017)
2. Individuals who are willing to provide only a single blood sample (i.e. at only one survey)

Laboratory Testing Algorithm

Samples collected were first transported to the Centre National de Transfusion Sanguine (CNTS), Ouagadougou, Burkina Faso where blood samples were centrifuged and separated into four aliquots of 0.5 – 1 ml serum in cryotubes under sterile conditions, labeled and stored at -70°C freezer. The samples were transported to Centre Muraz and stored at -70°C immediately at their arrival waiting for testing with a commercial IgG ELISA test (Panbio® Dengue IgG Indirect ELISA, Abbott Diagnostics, United States). The laboratory testing procedure was previously described in more detail (24).

The IgG ELISA was used to detect IgG antibodies to dengue antigen serotypes (1, 2, 3 and 4). The IgG cut-off were set to detect levels of IgG present in past dengue virus infections. The index value > 1.1, shown by Panbio unit > 11, indicated evidence of past or recent dengue infection; index value 0.9 – 1.1, indicated by Panbio unit 9 – 11, was classified equivocal (samples require repeated testing); index value < 0.9

indicated by Panbio unit < 9 was considered negative. Dengue status was categorized by the cut-off as positive, equivocal, and negative. For this analysis, sero-conversion of anti-dengue IgG between the pre- and post- transmission was considered to be dengue infection.

Statistical analysis

There are 3 components in the analysis.

1. *Characteristics of subjects by the status of dengue IgG at enrollment*

A descriptive summary of characteristics is presented by the status of dengue IgG ELISA at enrollment (IgG-positive and negative). Age was initially broken down to 8-level categorical variable for descriptive purposes. Yellow fever vaccination history was dichotomized between those who reported having been vaccinated versus those who did not remember or reported no vaccination. Known previous dengue infection was also based on self-report and was dichotomized between those who reported having had dengue versus those who did not remember or reported none. Pre-existing condition was initially a 8-level categorical variable, and was compressed to a dichotomous variable (i.e., any pre-existing condition vs. none reported). Dichotomous variables were also created for various signs and symptoms (presence vs. absence). For nausea and vomiting, patients were asked whether they either had nausea and/or vomiting during their illness.

Occupation was initially 11-level categorical variable, but was later compressed to a 3-level categorical variable: i) student; ii) housewife or retired/unemployed – to indicate those that stay mostly at home; and iii) others, e.g. business owners, public/private sector employees, farmers, service/skilled/unskilled workers, etc. Level of education, initially a 7-level categorical variable, was compressed to a 3-level categorical variable: illiterate or no official education; elementary school; and secondary education or more. Categorical pair-wise comparisons were made across dengue status

(sero-positive versus sero-negative) using χ^2 or Fisher's exact tests. Continuous variables were compared using Student's *t*-test or ANOVA.

DENV infection can occur with any of the 4 serotypes, and infection with one serotype provides long-term protection against the infecting serotype, but not to other three (29, 30). Therefore, individuals who already experienced prior infection of DENV are still susceptible to heterotypic infections (29, 30). However, the IgG ELISA test cannot distinguish between dengue serotypes (29). Therefore, in the analysis, infection refers to sero-conversion with any DENV serotype.

Assuming a constant rate of exposure (μ) to the totality of serotypes — so that μ is the force of infection — the probability of a person sero-converting within the subsequent time t is $1\text{-}e^{-\mu t}$ (31). If μ is assumed constant across ages and calendar time prior to the enrollment serosurvey, then, if person i has age A_i at that serosurvey, t can be replaced by A_i in the above expression. So, if this person's probability of being sero-positive is denoted p_i then we have (31):

$$\log(-\log(1 - p_i)) = \log(\mu) + \log(A_i)$$

Hence, if we apply binomial regression with a complementary log-log link, with log(midpoint of age category) as an offset, we can interpret the intercept as the logarithm of the FoI (31).

2. *Seroconversion and FoI in IgG-negative subjects who contributed to all 4 serosurveys*

In the subset of subjects who contributed to all 4 serosurveys, IgG ELISA results among IgG-negative subjects were followed through S1 to S4 to observe changes in IgG status. S1-S2 covered the non-outbreak rainy season in 2015; S2-S3 covered the non-outbreak non-rainy season in the first half of 2016; and S3-S4 covered the 2016 outbreak. Starting with those IgG negative at enrollment, the proportions of sero-conversion from IgG– to IgG+ were measured over each interval.

FoI was also estimated over each interval, using a similar approach as before. The value of the repeat surveys is that the FoI no longer needs to be assumed constant across ages. Rather, age can be included among other risk factors in the regression, and sero-conversion rate ratios obtained. The logarithm of the time between samples, rather than the logarithm of age, is included as an offset. In the analysis based on paired surveys, only the paired results were considered for sero-conversion. For example, if a subject was IgG negative at S2, and IgG positive at S3, then this was considered sero-conversion between S2 and S3, even if they had been IgG positive at S1.

3. Seroconversion rate ratios

To assess how demographic and clinical characteristics are associated with DENV sero-conversion, and the difference in patterns in the outbreak (S3-S4) vs. the non-outbreak times (S1-S2 and S2-S3), a descriptive summary of demographic and clinical characteristics is presented among IgG-negative subjects at each preceding serosurvey between those who sero-converted (IgG – at the preceding serosurvey turning IgG+ at the subsequent serosurvey) vs. those who remained sero-negative (IgG – at the preceding serosurvey remaining IgG- at the subsequent serosurvey). Chi-squared tests were used to test for differences between sero-status groups.

Furthermore, to assess how different variables were associated with changes in the rate of sero-conversion, demographic and clinical information investigated were: age, gender, neighborhood, level of education, occupation, any known previous infection of dengue, and yellow fever vaccination history, as well as signs and symptoms obtained from subjects based on self-reports for the particular interval. Binomial regression models with the log time of the interval between the surveys as offset were run with a binary outcome of the sero-converted cases vs. those who remained sero-negative in each interval. Changes in the rates of sero-conversion were reported as rate ratios. All analyses were performed using SAS® version 9.4 (SAS Institute, Cary, North Carolina).

Ethical considerations

The study protocol received ethical approvals from the Institutional Review Boards (IRBs) of IVI, the London School of Hygiene and Tropical Medicine, the National Ethical Committee for Health Research of Burkina Faso, and the Ethics Committee of the Centre Hospitalier de l'Université de Montréal (CRCHUM) at University of Montreal. Consent forms were obtained from each participant: If the subject is between 8 to 17 years old, an assent form from the subject and an informed consent from at least one parent or legal guardian were obtained. If subject is 7 years old or younger, an informed consent was obtained from at least one parent or legal guardian. If the subject is >17 years old, an informed consent was obtained from the subject.

Results

Among 3026 enrolled subjects, 2897 subjects had complete demographic (age, gender, and neighborhood) data and laboratory results at enrollment; 129 had incomplete demographic data (Fig 1). The subjects who were enrolled at S1 and followed up at S4, compared to those that were lost and not followed up at S4 were significantly different in terms of: age distribution, younger among the ones that stayed at S4; and residing neighborhood, those stayed at S4 more likely to be residing in Juvenat Fille, Pazani, and Zongo. However, the two groups were similar in terms of gender and yellow fever vaccination history.

Of 2897 subjects at enrollment (S1) in the enrollment bleed conducted in June 2015, 66.3% (n=1920) were IgG positive and 33.7% (n=977) were IgG negative. There were three additional follow-up serosurveys. In the second serosurvey (S2) conducted in December 2015 in 2,109 subjects, 651 (30.9%) were IgG negative and 1417 were (67.2%) IgG positive. In the third serosurvey (S3) in May 2016 in 2,106 subjects, 672 were (31.9%) IgG negative and 1400 were (66.5%) IgG positive. In the fourth serosurvey (S4) in March 2017 in 1,651 subjects, 509 were (30.8%) IgG negative and 1121 (67.9%) were IgG positive.

1. *Characteristics of subjects by the status of dengue IgG at enrollment*

The first part of the analysis was performed on 2897 subjects with complete clinical and laboratory data at enrollment. By IgG status at enrollment, IgG positive subjects were significantly older than IgG negative subjects with the mean age for IgG sero-positive subjects being twice that of the IgG negative subjects (Table 1). There were 82% (171/208) and 65% (268/410) of 1-4 and 5-9 year olds, respectively, were IgG negative and at risk of infection at the start of the study.

Almost all of our subjects were Burkinabe and more female subjects were IgG sero-positive. With the given age difference between the groups, more of sero-negative subjects were student, about 46%, and higher proportions of sero-positive subjects had occupations, such as housewife, business owner, skilled/unskilled worker, private/public sector employees, etc.

In terms of pre-existing conditions, also possibly due to age patterns, cardiovascular-, lung, musculoskeletal-, and gastrointestinal-related pre-existing conditions were more frequently found among sero-positive subjects than in sero-negative subjects. Overall, self-report of previous dengue infection was rare and none of the IgG-negative individual reported having had previous dengue infection.

The seroprevalence by dengue IgG at enrollment by age is shown in Fig. 2. The binomial regression based on IgG positivity by age at enrollment, assuming the force of infection was constant over calendar time, resulted in FoI, shown by the curve in blue, of 5.950% (95% C.I: 5.658-6.242) per year. With increasing IgG positivity with age, by age 11, it shows that about 50% of dengue IgG positivity was reached and by age 26, IgG positivity reached 80%.

2. *Seroconversion and annual FoI in IgG-negative subjects who contributed to all 4 serosurveys*

The second part of the analysis is based on 438 IgG negative subjects among 1269 who stayed in all 4 serosurveys. Cascade by IgG ELISA status throughout the 4 serosurveys is shown in Table 2. Of the IgG negative subjects in S1, there were 33

(7.5%) that sero-converted by S2. Of the subjects that were IgG negative in S1 and S2, there were 10 (2.5%) that sero-converted by S3. Of the subjects that were IgG negative in S1-S2-S3, there were 64 (16.5%) that sero-converted by S4, over the 2016 outbreak.

Using the binomial regression with the log duration of interval as denominator for each interval, FoI was calculated among IgG negative subjects at the preceding serosurvey. For the interval S1-S2, FoI was 14.03%; for the interval S2-S3, 9.90%; and for the interval S3-S4, 20.40% (Table 3). Age-specific annual FoIs were calculated for 5-year age bands. For the intervals S1-S2 and S2-S3, the FoIs was higher for older ages, and the lowest FoI was found in children under 5 years in both intervals (Table 3). For the interval S3-S4, the annual FoI was also high in older ages, but high FoI was also found in those aged 15-19 years (33.5%) and even the lowest FoI was 11.21%, in those aged 30-34 years.

3. Seroconversion rate ratios in intervals with and without outbreak

The 3rd part of the analysis is based on each pair of surveys. For S1-S2 (analysis sample = 1494 subjects), among 455 subjects at risk (i.e. IgG negative at S1), 33 subjects sero-converted and 422 stayed sero-negative. For S2-S3 (analysis sample = 1488 subjects), among 443 subjects at risk (i.e. IgG negative at S2), 23 sero-converted and 420 stayed sero-negative. For S3-S4 (analysis sample= 1401 subjects), among 455 subjects at risk (i.e. IgG negative at S3), 78 sero-converted and 377 stayed sero-negative. Table 4 describes demographic and clinical characteristics of IgG sero-converted, compared to those that stayed IgG negative for each interval.

Proportions of sero-conversion were different by age and pre-existing conditions in S1-S2 and S2-S3 with statistical significance. Proportions of sero-conversion were different by presence of myalgia, for S1-S2, and arthralgia, for S2-S3 with statistical significance. During S3-S4 encompassing the 2016 outbreak, proportions of sero-conversion were different by age and presence of fatigue with statistical significance.

To assess how these variables might be associated in changes in rates of sero-conversion, ratios of rate sero-conversion of dengue IgG antibodies over the intervals were estimated from the binomial regression analysis. Over S1-S2, those 25-55 years

[rate ratios: 4.1 (95% C.I: 1.4-15.0)], compared to children under 5 years, those with self-reported pre-existing conditions [rate ratios: 2.7 (95% C.I: 1.1-5.7)], compared to those without any conditions, and those from Pazani, compared to those from Juvenat Fille [rate ratios: 3.3 (95% C.I: 1.2-12.1)], were associated with increased rate of dengue sero-conversion with statistical significance (Table 5).

Over S2-S3, age of 15 and older, compared to 1-4 years, was associated with increased the rate of sero-conversion, with wide confidence intervals. Having self-reported pre-existing conditions [rate ratios: 3.1 (95% C.I: 1.1-7.5)], compared to those without any conditions, being a housewife, retired/unemployed than being a student [rate ratios: 3.8 (95% C.I: 1.5-11.1)], and presence of arthralgia [rate ratios: 2.5 (95% C.I: 1.0-5.8)], compared to absence, were associated with increased rate of dengue sero-conversion with statistical significance. Over S3-S4, presence of fatigue [rate ratios: 1.6 (95% C.I: 1.0-2.5)] was associated with increased rate of sero-conversion with statistical significance. Presence of loss of appetite in S2-S3, and nausea/vomiting in S3-S4, compared with absence, were associated with decreased rate of sero-conversion with statistical significance.

Discussion

To our knowledge, this is the first study from Africa to present data on population-based seroprevalence and rates of DENV sero-conversion. Also, we were able to longitudinally follow the same enrolled subjects in 4 repeated serosurveys at 6 month intervals to calculate sero-conversion empirically as well as estimate based on binomial regression models.

1. Characteristics of subjects by the status of dengue IgG at enrollment

Overall, it shows that two-thirds of the 2897 enrolled subjects already were IgG positive at enrollment. It was observed that seroprevalence increased with age, reaching 80% IgG positivity by age 26 years in the catchment area population. There

have been speculations of the dengue burden in Africa to be similar to that of the Americas, supported by a largely unrecognized burden in the region and also masked by other illnesses with similar symptomatic presentation (32, 33).

The seroprevalence reported in this study were comparable to areas which are considered to be highly endemic: 61% in individuals 1-65 years in Colombia; 83.1% in individuals aged 15-19 years in Tahiti; 86.6% sero-positive in adults, 18 years and older, in Malaysia; and 68.7% in Salvador, Brazil (34-37). In Recife, Brazil, the seroprevalence measured with IgG ELISA was estimated at 74 and 91%, for areas of high and low socio-economic status, respectively (38). In all these studies, testing was done using IgG ELISA and interpretation should consider the possible cross-reactivity of IgG across different flaviviruses circulating. Nonetheless, hyperendemicity of dengue is well documented in these countries, and we would expect more intense transmission of DENV. However, their estimates of seroprevalence by IgG positivity were not so different from what we observed in Burkina Faso.

With the assumption of the constant force of infection over calendar time used in the binomial regression model, FoI was estimated to be 6% per year based on sero-positivity. The FoI of 6% is generally lower than what was reported previously from other known endemic regions, such as Sri Lanka at 14% (29), Colombia at 8.7% (36) and Recife, Brazil at 5.3 and 17.7% for urban areas of high and low socio-economic status, respectively (38). However, our estimate was higher than those of Nicaragua, in the times of high FoIs in 1997-1998 at 480 per 1000 and 1998-1999 at 555 per 1000 (39) and Thailand at 0.019-0.038/year (40).

2. *Seroconversion and annual FoI in IgG-negative subjects who contributed to all 4 serosurveys*

Based on repeat surveys following up with the same individuals, we empirically measured the proportion of sero-conversion. In our study, there was a subset (n=1269) of enrolled subjects that stayed in all 4 serosurvey serosurveys and following the IgG ELISA results changing over 4 surveys, it was found that 7.5% sero-converted between S1-S2; 2.5% that were IgG negative in S1 and S2 sero-converted by S3; and 16.5%

that were IgG negative in S1, S2, and S3 sero-converted by S4.

In the non-outbreak period, for S1-S3, 43 of 437 IgG negative subjects sero-converted (9.8%) whereas it was 17% in the outbreak period. While the durations differ, this shows the magnitude of DENV transmission, indicated by sero-conversion, during the outbreak. Also, given the IgG results throughout the 4 serosurveys, these are likely primary infections. The majority of the IgG negative subjects were children: 50% of IgG negative subjects at S1, S2, and S3 were children under 10 years. This age distribution of IgG negative subjects also supports that most of these sero-conversions could be primary infections.

In addition to these observed proportions of sero-conversions among IgG negative subjects, annual FoIs were measured by the binomial regression: 14% for S1-S2; 10% for S2-S3; and 20% per year for S3-S4. These estimates provide understanding on how the annual FoI could be as low as 10% if in the non-outbreak and no rainy season (likely to be the peak time of transmission) and can reach as high as 20% in a large outbreak setting, as the one declared in 2016. Unlike the observed proportions of likely primary infections, these show the annual rates of infection, measured by sero-conversion due to DENV, which may include both first infection as well as subsequent heterotypic infection, which will not be distinguished by IgG ELISA. Also, the highest FoI was found during the interval of the outbreak, but what is noteworthy was a substantial level of FoI even during those non-outbreak periods. In terms of repeat surveys, there is limited information available. Nonetheless, these are comparable to what had been reported from Colombia at 8.7% (36), but our estimates are higher than those of Nicaragua (39).

In calculation of the age-specific FoIs, given that seroprevalence increases with age, there was a small number of IgG negative subjects in older age groups and this led to few of these remaining IgG negative subjects sero-converting over the interval, leading to a high FoI. The age groups with number of sero-negative subjects < 5 are 45 years and older, among whom close to 90% of subjects were already sero-positive. Disregarding these age groups, for intervals of the non-outbreak times, at S1-S2 and S2-S3, higher rates were found among adults above 30 years, at rates ranging between 29 to 47% per year. Overall, FoI increased with age in the intervals of the non-outbreak

times. For the interval with the outbreak, FoI was similar across age, with rates ranging between 13 to 34% per year.

Consistent with the findings from the facility-based surveillance, which was conducted in the same catchment area during the same period, adults were more at risk for dengue in Burkina Faso. However, during the outbreak, it was both adults and children at similar risk for dengue in Burkina Faso. It could possibly be due to the serotype change from DENV 3 before to DENV 2 during the outbreak, but this has not been confirmed with the serosurvey samples. Further analyses using neutralization assay on the samples from the current study is planned.

3. Seroconversion rate ratios in intervals with and without outbreak

The 3rd part of the analysis was in the context of cases of sero-conversion in paired serosurveys. Various demographic and clinical variables were assessed to see if they are associated with changes in rates of sero-conversion. Using the binomial regression analysis, it was found that over S1-S2 and S2-S3, older age, compared to age under 5 years, and having self-reported pre-existing conditions, compared to none, were associated with DENV sero-conversion. Over S2-S3, presence of arthralgia, compared to absence, and being a housewife, retired or unemployed, compared to student, most likely due to older age, were associated with DENV sero-conversion. Over S3-S4, rate of sero-conversion was similar across age. And, presence of fatigue was a risk factor of sero-conversion.

It is likely that pre-existing conditions come with age. In our data, the mean age of those that reported pre-existing conditions was 31.2 years whereas the mean age for those that reported none was 20.3 and the difference was statistically significant ($p<.001$ based on t-test). With the rate of sero-conversion increasing with age, having pre-existing condition also showed to be associated with rate of sero-conversion for both intervals S1-S2 and S2-S3, but not in S3-S4.

In terms of symptoms, arthralgia was associated with increased rate of sero-conversions in S2-S3, but for S3-S4 it was fatigue/weakness. Subjects were asked whether they experienced these symptoms in each interval, since the previous survey.

However, we cannot conclude that these associations are causal. Nonetheless, it is interesting that these are persistent signs associated with dengue infection (41, 42). Overall, there were not many symptoms reported during the intervals. However, these subjects are likely to have suffered from subclinical illness with low frequency of fever reported. Also, about 18% at S2, 17% at S3, and 26% at S4 reported that they visited health centers during the interval. Our data cannot validate whether these visits were associated with dengue sero-conversion and self-reports of symptoms were not verified during their visits.

4. Considerations in interpretation and limitations

This study is subject to a number of limitations. Firstly, our results were based on serology using IgG ELISA. Further analyses using neutralization assay is planned, but in this analysis, there is no confirmatory testing applied to verify the IgG results. Due to serological cross-reaction with other flaviviruses, this could result in inaccurate seroprevalence and force of infection estimates as antibodies to other flaviviruses, as well as dengue, could have been detected (29, 43). The most important of other flaviviruses in circulation in Africa could be yellow fever virus (YFV). Also, neutralizing antibodies against YFV generated by YFV vaccine could result in interference with the specificity of DENV IgG ELISA tests (43). In our study, the subjects were explicitly asked whether they had been vaccinated for yellow fever after each interval, and by the 4^{th} bleed, among 2526 subjects, 4% (n=90) answered that they had been vaccinated. While this was based on self-report and there could be recall bias, it was still a low level of YF vaccination. This is different from what was reported as the coverage rate of Expanded Program on Immunization (EPI) at the end of 2007 at 85.3% (44). Also there have been reported outbreaks of YFV in 1998, 2003, and 2004, (44-46). When we investigate this using self-reported YF vaccination history as a proxy for YFV sero-positivity, we did not find any evidence for this effect, indicating that those with YF vaccination were not more likely to be detected by our ELISA (i.e. there was no difference in sero-positivity by IgG ELISA between those that received YF vaccination vs. those that did not). Also, there are some data on cross-reaction between DENV and

YF vaccination, reporting that recent yellow fever vaccination did not affect DENV sero-positivity (43, 47).

For other flaviviruses, such as West Nile virus, Burkina Faso has not reported the presence of WNV (48). However, for Zika virus (ZIKV), Burkina Faso had reported prevalence of ZIKV antibodies in human populations and was classified in 2018 as category 2 country where there is either evidence of virus circulation before 2015 or ongoing transmission that is no longer in the new or re-introduction phase, with no evidence of interruption (49, 50). While the extent to which ZIKV is present in Burkina Faso is unknown, there was a study which followed neutralizing antibodies to ZIKV and DENV in longitudinal serologic specimens in in patients with Zika and DENV from Latin America and Asia (51). The authors reported that cross reactivity was low to DENV in Zika patients and also low to Zika among DENV patients, indicating ZIKV and the DENV serocomplex to be distinct, based on the patterns of antibody cross-neutralization (51). Still, there might be other cross-reacting flaviviruses or arboviruses in circulation in Africa. And, given these viruses at unknown levels as background transmission affecting our measurement of sero-positivity, our results could be overestimates.

Despite these limitations, there were previous studies with findings supportive of its performance in dengue diagnosis. Chungue et al. conducted a study comparing performance of IgG ELISA to that of haemagglutination inhibition test (HI) in detection of dengue antibodies using a baseline serosurvey with age-stratified samples collected from 327 children up to 19 years in Tahiti between April and June 1987 (34). The authors reported that both sensitivity and specificity, as well as agreement between two tests, were good (34). Tahiti is an island in French Polynesia, with circulation of flaviviruses, such as DENV and Zika virus (52-54), and the population would have been exposed to some of the flaviviruses. In addition, Inoue et al. conducted a study comparing dengue IgG indirect ELISA results to HI for diagnosis of secondary DENV infection using 187 samples from patients with known dengue secondary infection, 40 samples of known dengue primary infection, and 44 samples from healthy volunteers (55). The results were also compared to JE indirect IgG ELISA to measure anti-flavivirus IgG as DENV cross-reacts with the Japanese encephalitis virus (55). The authors reported that the results of DENV IgG highly correlated with those of the DENV

HI test and concluded that DENG IgG ELISA could be a simple, rapid, sensitive, and quantitative test to use in the determination of dengue secondary infection (55).

A study conducted in Malaysia, another country with DENV hyperendemicity and circulation of other flavivirus, among 277 healthy adults in a rural district between April and May 2015 (35). The samples were tested for immunoglobulin G (IgG) using the same test as in our study (Panbio® Dengue Indirect IgG ELISA) and were confirmed on a subset of randomly selected samples of IgG-positive sera with the plaque reduction neutralization test (PRNT) (35). The authors found evidence of past infection in 75.5% (209/277) of participants and of these 96 samples were confirmed with the PRNT assay, to show that the detected antibodies were indeed specific to DENV (35). Even in a rural community, there was a high exposure to dengue when measured by IgG indirect ELISA and confirmed by PRNT. While these studies were not conducted in Africa, where there may be a different and unknown composition of flaviviruses in circulation, there are available data in support of IgG indirect ELISA to be well correlated with the results from more confirmatory and better validated tests, such as HI and PRNT.

Also, our estimates are based on results of IgG ELISA, which does not distinguish the serotypes. Therefore, our estimates are rates of sero-conversion due to any serotype.

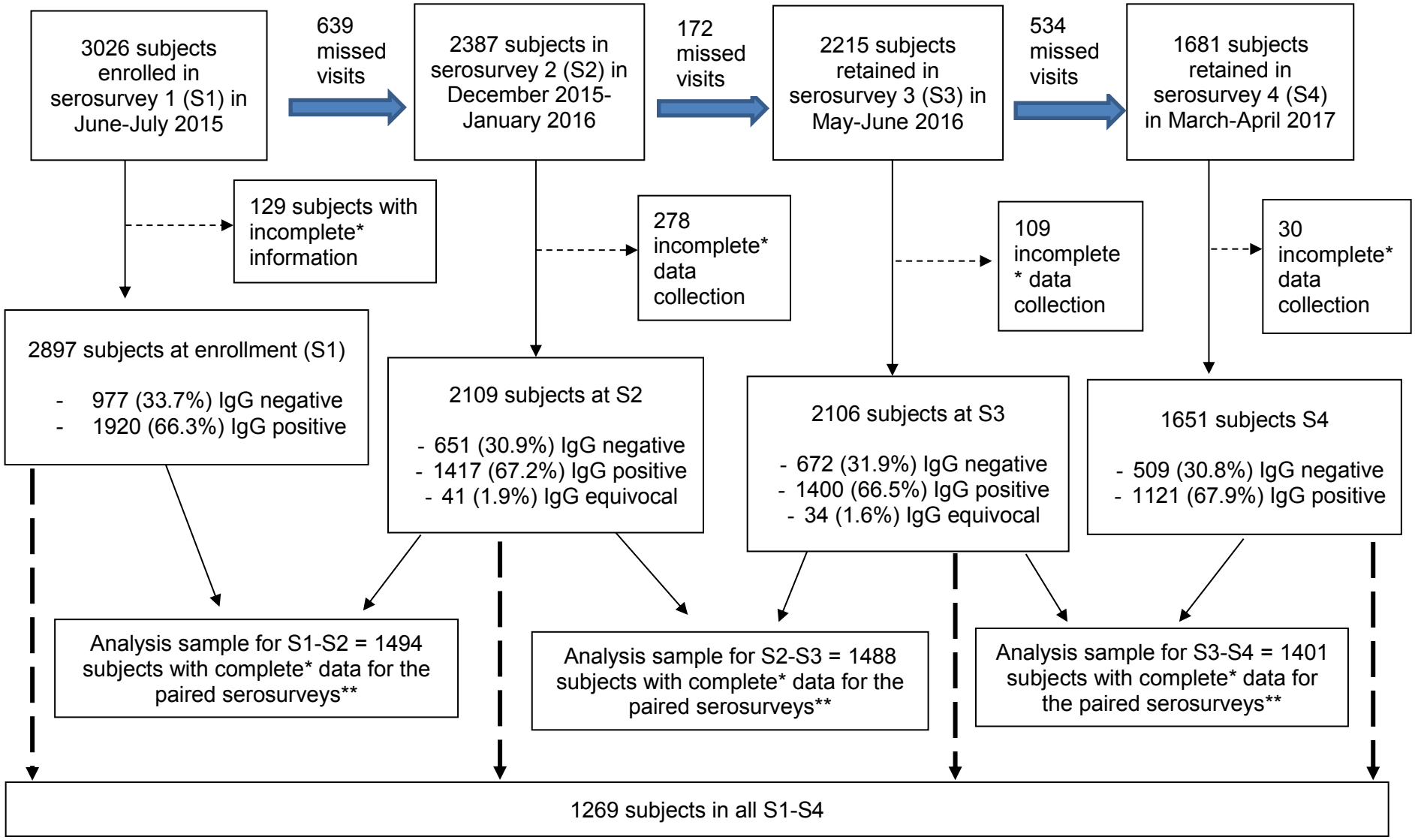
Conclusion

Our estimates of both seroprevalence and FoI showed to be comparable to results of other studies from more dengue endemic countries in the Americas. While repeated outbreaks indicate a considerable level of transmission of DENV in Ouagadougou, the extent of transmission and hyperendemicity of dengue needs to be further verified. Specifically, additional evaluation with confirmatory tests in the general population in the region would be necessary to further confirm and validate our findings.

Burden estimates, such as the seroprevalence and the FoI, are important factors to be considered for evidence-based policy decisions for implementation of interventions for prevention and control, including introduction of vaccines. To our knowledge, there have been no published data on seroprevalence and FoI, even if

measured by sero-conversion, of dengue in Africa. Nonetheless, in absence of other reliable population-based data on dengue in Africa, our data provide practical evidence, despite limitations in interpretation of our estimates based on the IgG ELISA results, and could be used to support decisions and develop strategies for vaccine introduction.

Fig. 1 Flow chart of the subject enrollment



*Lab results and basic demographic information required to be considered complete
**In the analysis based on paired surveys, only the paired results were considered for sero-conversion (i.e. if a subject was IgG negative at S2, and IgG positive at S3, then this was considered sero-conversion between S2 and S3, even if they had been IgG positive at S1)

Table 1. Demographic and clinical characteristics of subjects by the dengue IgG status at enrollment in June-July 2015 in Ouagadougou, Burkina Faso

Characteristics	Total (n=2897) [a]	Sero-positive at enrollment (n=1920) [b]	Sero-negative at enrollment (n=977) [c]	p-value (χ^2 test)
	n (%)	n (%)	n (%)	
Mean age (SD)	22.32 (13.92)	27.02 (13.36)	13.08 (9.74)	<.001
Age group				<.001
1-4 years	208 (7.2)	37 (1.9)	171 (17.5)	
5-9 years	410 (14.2)	142 (7.4)	268 (27.4)	
10-14 years	384 (13.3)	189 (9.8)	195 (20.0)	
15-19 years	379 (13.1)	243 (12.7)	136 (13.9)	
20-29 years	694 (24.0)	560 (29.2)	134 (13.7)	
30-39 years	410 (14.2)	357 (18.6)	53 (5.4)	
40-49 years	264 (9.1)	249 (13.0)	15 (1.5)	
50-55 years	148 (5.1)	143 (7.5)	5 (0.5)	
Female	1741 (60.1)	1218 (63.5)	523 (53.5)	<.001
Ethnicity				0.712
Burkinabé	2871 (99.6)	1906 (99.6)	965 (99.5)	
Others	13 (0.5)	8 (0.4)	5 (0.5)	
Neighborhood				<.001
Sector 22	447 (18.2)	342 (20.5)	105 (13.3)	
Sector 25	510 (20.7)	395 (23.6)	115 (14.6)	
Juvenat fille	517 (21.0)	353 (21.1)	164 (20.8)	
Pazani	433 (17.6)	281 (16.8)	152 (19.2)	
Zongo	547 (22.2)	297 (17.8)	250 (31.7)	
Nioko	8 (0.3)	4 (0.2)	4 (0.5)	

Occupation				<.001
Student	1033 (35.8)	586 (30.6)	447 (46.0)	
Housewife	885 (30.7)	666 (34.8)	219 (22.5)	
Small business owner	163 (5.7)	130 (6.8)	33 (3.4)	
Unskilled worker	153 (5.3)	124 (6.5)	29 (3.0)	
Government official	92 (3.2)	77 (4.0)	15 (1.5)	
Private sector employee	82 (2.8)	66 (3.5)	16 (1.7)	
Merchant	55 (1.9)	46 (2.4)	9 (0.9)	
Retired	53 (1.8)	26 (1.4)	27 (2.8)	
Farmer	49 (1.7)	35 (1.8)	14 (1.4)	
Skilled worker	43 (1.5)	32 (1.7)	11 (1.1)	
Service sector worker	43 (1.5)	34 (1.8)	9 (0.9)	
Education level				<.001
Illiterate	887 (30.7)	596 (31.1)	291 (29.9)	
Literate, but not educated	72 (2.5)	58 (3.0)	14 (1.4)	
1-6 years of school	803 (27,8)	455 (23.8)	348 (35.8)	
7-10 years of school	551 (19.1)	388 (20.3)	163 (16.8)	
11-13 years of school	274 (9.5)	200 (10.4)	74 (7.6)	
University or higher	210 (7.5)	171 (8.9)	39 (3.0)	
Others*	57 (2.0)	39 (2.0)	18 (1.9)	
Pre-existing conditions [d]				
Cardiovascular	113 (4.0)	100 (5.3)	13 (1.4)	<.001
Diabetes	10 (0.4)	7 (0.4)	3 (0.3)	0.817
Lung disease	19 (0.7)	17 (0.9)	2 (0.2)	0.034
Cerebrovascular	27 (0.9)	18 (0.9)	9 (0.9)	0.990
Musculoskeletal	101 (3.5)	82 (4.3)	19 (2.0)	0.002
Gastro-intestinal	193 (6.7)	148 (7.8)	45 (4.7)	0.002
Anemia	10 (0.4)	2 (0.1)	8 (0.80)	0.002
Others	108 (3.8)	79 (4.1)	29 (3.0)	0.139

Self-reported previous dengue				
Yes	13 (0.5)	13 (0.7)	0	<.001
No	2421 (84.7)	1635 (86.0)	786 (82.1)	
Unknown	426 (14.9)	254 (13.4)	172 (18.0)	

Values are N (col. %) unless otherwise noted.

P values based on χ^2 test

[*]religious and other informal education

[a] Total subjects enrolled at S1

[b] Dengue positive on IgG indirect ELISA among the enrolled subjects at S1

[c] Dengue negative on IgG indirect ELISA among the enrolled subjects at S1

[d] Pre-existing conditions are based on self-report by the subjects

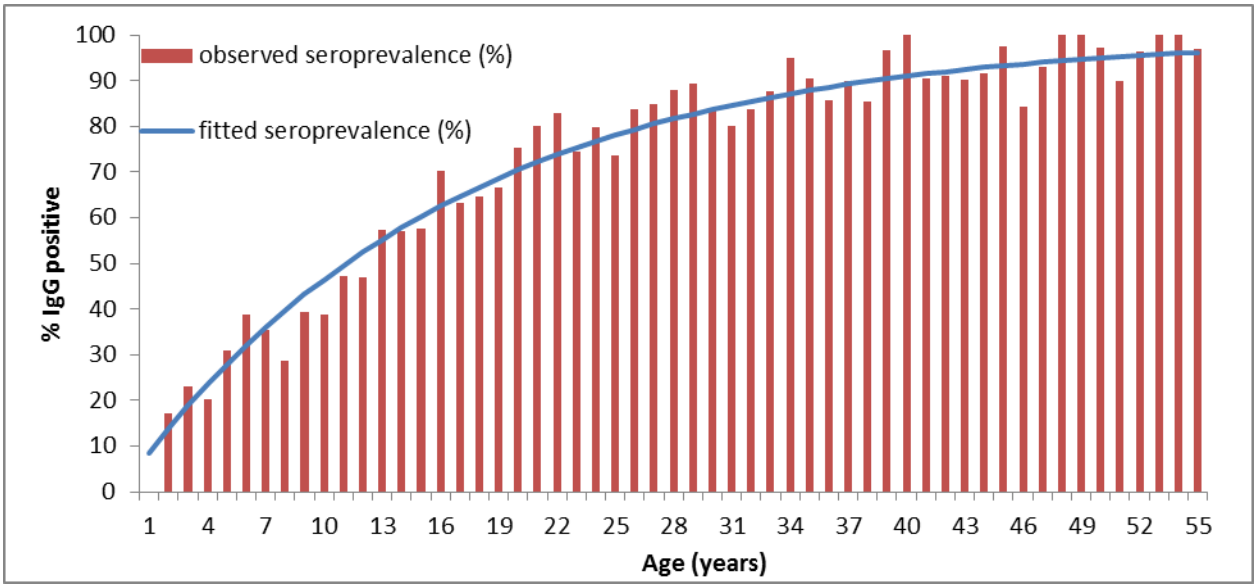


Figure 2. Seroprevalence by dengue IgG by age at enrollment, showing the FoI per year

The figure shows observed seroprevalence at enrollment measured by IgG ELISA (in red) and fitted seroprevalence using FoI (in blue). The FoI per year was 0.0595 (95% CI: 0.05658 – 0.06242), estimated by binomial regression, with the assumption of constant risk across ages and calendar time prior to the enrollment serosurvey, and a complementary log-log link, with log(mid-point of age) as an offset. The intercept is interpreted as the logarithm of the FoI.

Table2 Cascade of ELISA results among 1269 subjects who stayed in all 4 serosurveys

ELISA result N (% of subjects with identified IgG status at the preceding serosurvey) (n=1269)							
S1		S2		S3		S4	
Negative (NEG)	438 (34.5%)	NEG	398 (90.9%)	NEG	387 (97.2%)	NEG	323 (83.5%)
						POS	64 (16.5%)
				POS	10 (2.5%)	NEG	8 (80.0%)
						POS	2 (20.0%)
				Equivocal	1 (0.3%)		
		POS	33 (7.5%)	NEG	6 (18.2%)	NEG	5 (83.3%)
						POS	1 (16.7%)
				POS	25 (75.8%)	NEG	2 (8.0%)
						POS	23 (92.0%)
				Equivocal	2 (6.1%)		
		Equivocal	7 (1.6%)				
Positive (POS)	831 (65.5%)	NEG	22 (2.7%)	NEG	11 (50.0%)	NEG	11 (100%)
						POS	0
				POS	8 (36.4%)	NEG	2 (25.0%)
						POS	6 (75.0%)
				Equivocal	3 (13.6%)		
		POS	797 (95.9%)	NEG	6 (0.8%)	NEG	0
						POS	6 (100.0%)
				POS	788 (98.9%)	NEG	15 (1.9%)
						POS	773 (98.1%)
				Equivocal	3 (0.4%)		
		Equivocal	12 (1.4%)				

Table 3. Number and rates of sero-conversion by age group between surveys

Age group (years)	enrollees with all 4 serosurveys (n=1269)	S1-S2 (between June-July 2015 and December 2015-January 2016)					S2-S3 (between December 2015-January 2016 and May-June 2016)					S3-S4 (between May-June 2016 and March-April 2017), covering the outbreak in 2016				
		Sero-negative at S1	Sero-converted	% sero-converted	Mean duration of exposure (years)	Age-specific annual FoI (95% C.I)	Sero-negative at S2	Sero-converted	% sero-converted	Mean duration of exposure (years)	Age-specific annual FoI (95% C.I)	sero-negative at S3	Sero-converted	% sero-converted	Mean duration of exposure (years)	Age-specific annual FoI (95% C.I)
1-4	95	72	3	4.17	0.513	0.080 (-0.007-0.166)	69	1	1.45	0.427	0.034 (-0.031-0.098)	68	13	19.12	0.859	0.219 (0.114-0.324)
5-9	218	142	7	4.93	0.518	0.093 (0.027-0.159)	140	3	2.14	0.439	0.048 (-0.005-0.101)	136	15	11.03	0.827	0.132 (0.070-0.194)
10-14	193	93	6	6.45	0.514	0.122 (0.031-0.213)	89	2	2.25	0.435	0.051 (-0.018-0.120)	91	16	17.58	0.840	0.206 (0.116-0.296)
15-19	145	53	5	9.43	0.521	0.173 (0.035-0.311)	50	5	10.00	0.446	0.211 (0.047-0.374)	48	14	29.17	0.845	0.335 (0.192-0.478)
20-24	124	28	2	7.14	0.552	0.126 (-0.037-0.289)	25	1	4.00	0.416	0.093 (-0.081-0.268)	25	4	16.00	0.839	0.188 (0.022-0.353)
25-29	139	19	3	15.79	0.546	0.270 (0.010-0.531)	18	1	5.56	0.423	0.126 (-0.105 – 0.358)	18	5	27.78	0.839	0.321 (0.090-0.553)
30-34	81	12	2	16.67	0.535	0.289 (-0.048-0.625)	11	2	18.18	0.439	0.367 (-0.035-0.769)	10	1	10.00	0.887	0.112 (-0.095-0.319)
35-39	74	9	2	22.22	0.512	0.388 (-0.030-0.805)	9	0	-	-	-	9	2	22.22	0.838	0.259 (0.050-0.568)
40-44	69	7	2	28.57	0.527	0.472 (0.002-0.941)	5	0	-	-	-	5	0	-	-	-
45-49	59	2	0	-	-	-	3	2	66.67	0.445	0.915 (0.611-1.220)	1	0	-	-	-
50-55	72	1	1	100.00	0.564	-**	1	1	100.00	0.427	-**	2	1	50.00	0.871	0.549 (-0.169-1.267)
Total	1269	438	33	7.53	0.528	0.140* (0.096-0.185)	420	18	4.29	0.433	0.099* (0.056-0.142)	413	71	17.19	0.857	0.204* (0.162-0.246)

*when constant risk of infection is assumed; **omitted due to small numbers

Table 4. Demographic and clinical characteristics by the status of IgG sero-conversion

	Seroconverted vs. IgG negative between S1-S2				Seroconverted vs. IgG negative between S2-S3				Seroconverted vs. IgG negative between S3-S4			
Characteristics	Total N (n=455)	IgG sero-converted (n=33) [a]	Stayed IgG-negative (n=422) [b]	p-Value	Total N (N=443)	IgG sero-converted (n=23) [a]	Stayed IgG-negative (n=420) [b]	p-Value	Total N (N=455)	IgG sero-converted (n=78) [a]	Stayed IgG-negative (n=377) [b]	p-Value
Age group				**0.003**				**0.002**				**0.037**
1-4	77	4 (5.2)	73 (94.8)		74	1 (1.4)	73 (98.7)		78	13 (16.7)	65 (83.3)	
5-10	138	5 (3.6)	133 (96.4)		138	3 (2.2)	135 (97.8)		146	16 (11.0)	130 (89.0)	
10-14	97	5 (5.2)	92 (94.9)		95	4 (4.2)	91 (95.8)		97	16 (16.5)	81 (83.5)	
15-24	91	9 (9.9)	82 (90.1)		85	7 (8.2)	78 (91.8)		85	23 (27.1)	62 (72.9)	
25-55	52	10 (19.2)	42 (80.8)		51	8 (15.7)	43 (84.3)		49	10 (20.4)	39 (79.6)	
Female	236	21 (8.9)	215 (91.1)	0.160	227	15 (6.6)	212 (93.4)	0.169	237	42 (17.7)	195 (82.3)	0.733
Neighborhood				0.097				0.118				0.072
Juvenat fille	103	5 (4.9)	98 (95.2)		100	3 (3.0)	97 (97.0)		94	24 (25.5)	70 (74.5)	
Nioko	4	0	4 (100.0)		4	0	4 (100.0)		2	0	2 (100.0)	
Pazani	73	11 (15.1)	62 (84.9)		63	0	63 (100.0)		68	12 (17.7)	56 (82.4)	
Zongo	118	6 (5.1)	112 (94.9)		121	10 (8.3)	111 (91.7)		157	17 (10.8)	140 (89.2)	
Sector 22	61	3 (4.9)	58 (95.1)		62	3 (4.8)	59 (95.2)		57	12 (21.1)	45 (79.0)	
Sector 25	96	8 (8.3)	88 (91.7)		93	7 (7.5)	86 (92.5)		77	13 (16.9)	64 (83.1)	
Pre-existing conditions[d]				**0.014**				**0.013**				0.458
No/unknown	404	25 (6.2)	379 (93.8)		396	17 (4.3)	379 (95.7)		402	67 (16.7)	335 (83.3)	
Yes	51	8 (15.7)	43 (84.3)		47	6 (12.8)	41 (87.2)		53	11 (20.8)	42 (79.3)	

Occupation				0.930				**0.016**				0.180
Student	221	15 (6.8)	206 (93.2)		212	6 (2.8)	206 (97.2)		232	34 (14.7)	198 (85.3)	
Housewife or retired/unemployed	106	8 (7.6)	98 (92.5)		106	11 (10.4)	95 (89.6)		105	24 (22.9)	81 (77.1)	
Others (business owner, employees, workers, etc.)	128	10 (7.8)	118 (92.2)		125	6 (4.8)	119 (95.2)		118	20 (17.0)	98 (83.1)	
Level of education				0.921				0.170				0.159
Illiterate or no official schooling	138	9 (6.5)	129 (93.5)		139	11 (7.9)	128 (92.1)		136	23 (16.9)	113 (83.1)	
Elementary school	169	13 (7.7)	156 (92.3)		161	5 (3.1)	156 (96.9)		182	25 (13.7)	157 (86.3)	
Secondary school or more	148	11 (7.4)	137 (92.6)		143	7 (4.9)	136 (95.1)		137	30 (21.9)	107 (78.1)	
Self-reported YF vaccination				0.371				0.469				0.535
No/unknown	445	33 (7.4)	412 (92.6)		429	23 (5.4)	406 (94.6)		427	72 (16.9)	355 (83.1)	
Yes	10	0	10 (100.0)		14	0	14 (100.0)		28	6 (21.4)	22 (78.6)	
Previous dengue [e]				-				0.052				0.865
No/unknown	455	33 (7.3)	422 (92.8)		442	22 (5.0)	420 (95.0)		450	77 (17.1)	373 (82.9)	
Reported yes	0	0	0		1	1 (100.0)	0		5	1 (20.0)	4 (80.0)	
Signs and symptoms [f] (presence)												
Fever	192	15 (7.8)	177 (92.2)	0.694	111	5 (4.5)	106 (95.5)	0.706	218	41 (18.8)	177 (81.2)	0.366
Fatigue/weakness	126	9 (7.1)	117 (92.9)	0.955	108	6 (5.6)	102 (94.4)	0.845	158	35 (22.2)	123 (77.9)	**0.039**
Retro-orbital pain	34	4 (11.8)	30 (88.2)	0.295	26	2 (7.7)	24 (92.3)	0.637	16	1 (6.3)	15 (93.8)	0.239
Headache	248	22 (8.9)	226 (91.1)	0.145	230	9 (3.9)	221 (96.1)	0.207	292	51 (17.5)	241 (82.5)	0.807

Rash	23	0	23 (100.0)	0.397	29	2 (6.9)	27 (93.1)	0.656	18	4 (22.2)	14 (77.8)	0.560
Eye pain	39	5 (12.8)	34 (87.2)	0.161	20	3 (15.0)	17 (85.0)	0.078	28	5 (17.9)	23 (82.1)	0.918
Arthralgia	81	8 (9.9)	73 (90.1)	0.315	79	8 (10.1)	71 (89.9)	**0.029**	81	14 (17.3)	67 (82.7)	0.970
Myalgia	57	8 (14.0)	49 (86.0)	**0.035**	60	3 (5.0)	57 (95.0)	0.943	65	15 (23.1)	50 (76.9)	0.170
Constipation	16	0	16 (100.0)	0.619	16	2 (12.5)	14 (87.5)	0.199	46	5 (10.9)	41 (89.1)	0.234
Diarrhea	52	6 (11.5)	46 (88.5)	0.206	57	1 (1.8)	56 (98.3)	0.338	40	3 (7.5)	37 (92.5)	0.090
Nausea/vomiting	111	11 (9.9)	100 (90.1)	0.215	117	4 (3.4)	113 (96.6)	0.466	102	11 (10.8)	91 (89.2)	0.053
Abdominal pain	123	10 (8.1)	113 (91.9)	0.661	155	7 (4.5)	148 (95.5)	0.638	180	25 (13.9)	155 (86.1)	0.136
Loss of appetite	128	13 (10.2)	115 (89.8)	0.135	179	5 (2.8)	174 (97.2)	0.061	114	23 (20.2)	91 (79.8)	0.321
Neck pain	18	2 (11.1)	16 (88.9)	0.381	17	3 (17.7)	14 (82.4)	0.052	14	1 (7.1)	13 (92.9)	0.481
Sore throat	30	3 (10.0)	27 (90.0)	0.470	28	2 (7.1)	26 (92.9)	0.649	21	3 (14.3)	18 (85.7)	0.722
Nasal congestion	73	4 (5.5)	69 (94.5)	0.630	58	1 (1.7)	57 (98.3)	0.339	74	9 (12.2)	65 (87.8)	0.214
Cough	72	6 (8.3)	66 (91.7)	0.700	71	1 (1.4)	70 (98.6)	0.149	115	19 (16.5)	96 (83.5)	0.838

P values based on χ^2 test

[a] Subjects who sero-converted from IgG negative at the preceding serosurvey to IgG positive at the subsequent serosurvey on IgG indirect ELISA for DENV

[b] Subjects whose sero-status stayed IgG negative at the preceding serosurvey and the subsequent serosurvey on IgG indirect ELISA for DENV

[d] Pre-existing conditions are based on self-report by the subjects

[e] Previous infections of DENV are based on self-report by the subjects

[f] Presence of signs and symptoms based on the subject's self-report during the interval between serosurveys

Table 5. Binomial regression showing ratio of rates of sero-conversion, among residents in Ouagadougou, Burkina Faso

Characteristics	Univariable binomial regression Sero-converted (n=33) vs. IgG negative (n=422) between S1-S2			Univariable binomial regression Sero-converted (n=23) vs. IgG negative (n=420) between S2-S3			Univariable binomial regression Sero-converted (n=78) vs. IgG negative (n=377) between S3-S4		
	Rate of sero-conversion	95% CI	p-Value	Rate of sero-conversion	95% CI	p-Value	Rate of sero-conversion	95% CI	p-Value
Age group			**0.006**			**0.002**			0.038
1-4	Ref			Ref*			Ref	-	
5-10	0.70	0.19-2.84					0.65	0.31-1.37	
10-14	1.00	0.27-4.05		2.25	0.53-9.52		1.00	0.48-2.12	
15-24	1.99	0.65-7.35		**4.55**	**1.37-17.38**		1.77	0.91-3.60	
25-55	**4.11**	**1.37-14.99**		**9.13**	**2.87-34.20**		1.29	0.55-2.94	
Female	1.67	0.84-3.51	0.155	1.83	0.80-4.55	0.166	1.10	0.70-1.72	0.686
Neighborhood			0.153			0.668			**0.133**
Juvenat fille	Ref			Ref			Ref		
Nioko	-			-			0	-	
Pazani	**3.27**	**1.19-12.08**		-			0.66	0.32-1.30	
Zongo	1.09	0.33-3.79		2.95	0.90-13.16		**0.41**	**0.21-0.75**	
Sector 22	1.02	0.21-4.15		1.64	0.30-8.84		0.81	0.39-1.59	
Sector 25	1.82	0.61-6.02		2.67	0.74-12.40		0.66	0.32-1.27	
Self-reported pre-existing conditions			**0.016**			**0.017**			0.433
No/unknown	Ref			Ref			Ref		

Yes	**2.67**	**1.13-5.67**		**3.11**	**1.12-7.48**		1.29	0.64-2.34	
Occupation			0.935			**0.025**			0.177
Student	Ref			Ref			Ref		
Housewife or retired/unemployed	1.11	0.45-2.56		**3.81**	**1.45-11.05**		1.64	0.96-2.75	
Others (business owner, employees, workers, etc.)	1.15	0.50-2.54		1.70	0.53-5.45		1.16	0.66-2.01	
Level of education			0.917			0.191			0.164
Illiterate or no official schooling	Ref			Ref			Ref		
Elementary school	1.19	0.52-2.89		0.39	0.12-1.06		0.80	0.45-1.42	
Secondary school or more	1.15	0.48-2.85		0.61	0.23-1.55		1.33	0.78-2.32	
Self-reported YF vaccination									0.560
No/unknown	Ref			Ref			Ref		
Yes	-			-			1.28	0.50-2.72	
Self-reported previous dengue						<.001			0.897
No/unknown	Ref			Ref			Ref		
Reported yes	-			-			1.14	0.07-5.17	
Signs and symptoms (presence)									
Fever	1.15	0.57-2.28	0.690	0.80	0.26-2.01	0.659	1.25	0.80-1.96	0.319
Fatigue/weakness	0.98	0.43-2.04	0.963	1.08	0.39-2.60	0.871	**1.61**	**1.02-2.51**	**0.037**
Retro-orbital pain	1.75	0.52-4.44	0.296	1.54	0.25-5.26	0.559	0.33	0.02-1.49	0.272

Headache	1.69	0.84-3.62	0.155	0.56	0.23-1.28	0.177	1.07	0.67-1.72	0.791
Rash	-			1.34	0.21-4.56	0.694	1.36	0.41-3.28	0.553
Eye pain	1.98	0.67-4.70	0.160	3.27	0.77-9.56	0.056	1.06	0.37-2.37	0.906
Arthralgia	1.50	0.63-3.18	0.321	**2.52**	**1.02-5.81**	**0.035**	1.04	0.56-1.80	0.888
Myalgia	2.33	0.98-4.95	0.037	0.94	0.22-2.74	0.919	1.53	0.84-2.61	0.140
Diarrhea	1.75	0.65-3.95	0.217	0.29	0.02-1.40	0.231	0.39	0.10-1.04	0.109
Nausea/vomiting	1.56	0.73-3.15	0.228	0.57	0.16-1.50	0.300	**0.55**	**0.27-0.99**	**0.065**
Abdominal pain	1.17	0.53-2.38	0.686	0.78	0.30-1.84	0.591	0.70	0.43-1.11	0.140
Loss of appetite	1.67	0.81-3.33	0.149	**0.39**	**0.13-0.98**	**0.062**	1.28	0.77-2.06	0.318
Neck pain	1.58	0.26-5.22	0.531	3.99	0.94-11.65	0.026	0.39	0.02-1.76	0.349
Sore throat	1.46	0.35-4.09	0.535	1.41	0.23-4.81	0.642	0.82	0.20-2.21	0.743
Nasal congestion	0.70	0.21-1.79	0.508	0.29	0.02-1.38	0.227	0.65	0.30-1.23	0.218
Cough	1.19	0.45-2.71	0.695	0.23	0.01-1.08	0.146	0.95	0.55-1.56	0.832

*age groups 1-4 and 5-9 were merged due to data scarcity in sero-converted subjects

References

1. Gubler DJ. Resurgent vector-borne diseases as a global health problem. Emerging Infectious Diseases. 1998;4(3):442-50.
2. Gubler DJ, Clark GG. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerg Infect Dis. 1995;1(2):55-7.
3. Gubler DJ. Dengue/dengue haemorrhagic fever: history and current status. Novartis Foundation Symposium. 2006;277:3-16; discussion -22, 71-3, 251-3.
4. Halstead S. Pathogenesis of dengue: challenges to molecular biology. Science. 1988 January 29, 1988;239(4839):476-81.
5. World Health Organization. Global strategy for dengue prevention and control 2012-2020. Geneva: World Health Organization; 2012. p. vi, 43p.
6. Singhi S, Kissoon N, Bansal A. Dengue and dengue hemorrhagic fever: management issues in an intensive care unit. J Pediatr (Rio J). 2007 2007 May;83(2 Suppl):S22-35.
7. Messina J, Brady O, Scott T, Zou C, Pigott D, Duda K, et al. Global spread of dengue virus types: mapping the 70 year history. Trends Microbiol. 2014;22(3):138-46.
8. Kamgang B, Ngoagouni C, Manirakiza A, Nakouné E, Paupy C, Mirdad K. Temporal patterns of abundance of *Aedes aegypti* and *Aedes albopictus* (*Diptera: Culicidae*) and mitochondrial DNA analysis of *Ae. albopictus* in the Central African Republic. PLoS Negl Trop Dis 2013 2013 Dec 12;7(12):e2590.
9. Surtees G. The distribution, density and seasonal prevalence of *Aedes aegypti* in West Africa. Bull World Health Organ. 1967;36(4):539-40.
10. Amarasinghe A, Kuritsky J, Letson G, Margolis H. Dengue virus infection in Africa. Emerging Infectious Diseases. 2011;17(8):1349-54.
11. Kiemde F, Spijker R, Mens P, Tinto H, Boele M, Schallig H. Aetiologies of non-malaria febrile episodes in children under 5 years in sub-Saharan Africa. Trop Med Int Health. 2016 Aug. 2016;21(8):943-55.
12. Baba M, Villinger J, Masiga DK. Repetitive dengue outbreaks in East Africa: A proposed phased mitigation approach may reduce its impact Reviews in Medical Virology. 2016 29 February 2016;26(3):183-96.
13. Amoako N, Duodu S, Dennis FE, Bonney JHK, Asante KP, Ameh J, et al. Detection of dengue virus among children with suspected malaria, Accra, Ghana. Emerg Infect Dis 2018 2018 Aug;24(8):1544–7.

14. Kraemer MU, Sinka ME, Duda K, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLife. 2015 Jun 30, 2015 4:e08347.
15. Ministère de la Santé. Rapport d'étape de l'investigation de cas suspects de Dengue dans la région sanitaire du Centre. Ouagadougou, Burkina Faso: Direction de la lutte contre la maladie; 2013.
16. Ridde V, Carabali M, Ly A, Druetz T, Kouanda S, Bonnet E, et al. The need for more research and public health interventions on dengue fever in Burkina Faso. PLoS Neglected Tropical Diseases. 2014;8(6):e2859.
17. World Health Organization. Dengue fever - Burkina Faso. Disease outbreak news 2016 2016 November 18 [cited 2018 August 18]; Available from: http://www.who.int/csr/don/18-november-2016-dengue-burkina-faso/en/
18. World Health Organization. Dengue fever - Burkina Faso. Disease outbreak news 2017 2017 November 6 [cited 2018 August 18]; Available from: http://www.who.int/csr/don/6-november-2017-dengue-burkina-faso/en/
19. Tarnagda Z, Cissé A, Bicaba B, Diagbouga S, Sagna T, Ilboudo A, et al. Dengue fever in Burkina Faso, 2016. Emerg Infect Dis 2018 2018 Jan;24(1):170-2.
20. Beatty M, Stone A, Fitzsimons D, Hanna J, Lam S, Vong S, et al. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas Dengue Prevention Boards. PLoS Negl Trop Dis 2010 2010 Nov 16;4(11):e890.
21. Ridde V, Agier I, Bonnet E, Carabali M, Dabiré K, Fournet F, et al. Presence of three dengue serotypes in Ouagadougou (Burkina Faso): research and public health implications. Infect Dis Poverty. 2016 2016 Apr;5(5):23.
22. Collenberg E, Ouedraogo T, Ganame J, Fickenscher H, Kynast-Wolf G, Becher H, et al. Seroprevalence of six different viruses among pregnant women and blood donors in rural and urban Burkina Faso: A comparative analysis. J Med Virol. 2006 May;78(5):683-92.
23. Fagbami A, Monath T, Fabiyi A. Dengue virus infections in Nigeria: a survey for antibodies in monkeys and humans. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1977;71(1):60-5.
24. Lim J, Carabali M, Lee J-S, et al. Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative. BMJ Open. 2018;2018(8):e017673.

25. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6(8):e1760.
26. Secretariat General. Annuaire statistique 2016. In: sectorielles. Dgdéeds, editor. 03 BP 7009 Ouagadougou 03.: Ministere de la Sante, Burkina Faso; 2017.
27. Ouagadougou. 2019 20 May 2019 [cited 2019 13 June]; Available from: https://en.wikipedia.org/wiki/Ouagadougou
28. Rossier C, Soura A, Baya B, Compaoré G, Dabiré B, Dos Santos S, et al. The Ouagadougou health and demographic surveillance system. International Journal of Epidemiology. 2012 June 1, 2012;41(3):658-66.
29. Tam CC, Tissera H, de Silva AM, De Silva AD, Margolis HS, Amarasinge A. Estimates of dengue force of infection in children in Colombo, Sri Lanka. PLoS Negl Trop Dis. 1 June 2013;7(6):e2259.
30. Egger JR, Ooi EE, Kelly DW, Woolhouse ME, Davies CR, Coleman PG. Reconstructing historical changes in the force of infection of dengue fever in Singapore: implications for surveillance and control. Bulletin of the World Health Organization 2008 March 2008;86(3):161-240
31. Collett D. Modelling binary data. London: Chapman and Hall; 1991.
32. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013 Apr 25;496(7446):504-7.
33. Jaenisch T, Junghanss T, Wills B, Brady OJ, Eckerle I, Farlow A, et al. Dengue expansion in Africa-not recognized or not happening? Emerg Infect Dis. 2014 2014 Oct;20(10):e140487.
34. Chungue E, Marché G, Plichart R, Boutin J, Roux J. Comparison of ImmunoGlobuln G Enzyme-Linked ImmunoSorbent Assay (IgG-ELISA) and Haemagglutination Inhibition (HI) test for the detection of dengue antibodies, prevalence of dengue IgG-ELISA antibodies in Tahiti. Trans R Soc Trop Med Hyg. 1989 1989 Sep-Oct;83(5):708-11.
35. Dhanoa A, Hassan S, Jahan N, Reidpath D, Fatt Q, Ahmad M, et al. Seroprevalence of dengue among healthy adults in a rural community in Southern Malaysia: a pilot study. Infect Dis Poverty. 2018 2018 Jan 16;16(7):1.
36. Carabali M, Lim J, Velez D, Trujillo A, Egurrola J, Lee K, et al. Dengue virus serological prevalence and seroconversion rates in children and adults in Medellin, Colombia: implications for vaccine introduction. Int J Infect Dis 2017 2017 May;58:27-36.

37. Teixeira MdG, Barreto ML, Costa MdCaN, Ferreira LDA, Vasconcelos PFC, Cairncross S. Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. Tropical Medicine and International Health. 2002;7(9):757-62.
38. Braga C, Luna CF, Martelli CM, de Souza WV, Cordeiro MT, Alexander N, et al. Seroprevalence and risk factors for dengue infection in socio-economically distinct areas of Recife, Brazil. Acta Tropica. 2010 March 2010;113(3):234-40.
39. Katzelnick L, Ben-Shachar R, Mercado J, Rodriguez-Barraquer I, Elizondo D, Arguello S, et al. Dynamics and determinants of the force of infection of dengue virus from 1994 to 2015 in Managua, Nicaragua. Proc Natl Acad Sci U S A 2018 2018 Oct 16;115(42):10762-7. .
40. Rodríguez-Barraquer I, Buathong R, Iamsirithaworn S, Nisalak A, Lessler J, Jarman RG, et al. Revisiting Rayong: Shifting seroprofiles of dengue in Thailand and their implications for transmission and control American Journal of Epidemiology. 2014 1 February 2014;179(3):353–60.
41. Seet RCS, Quek AML, Lim ECH. Post-infectious fatigue syndrome in dengue infection. Journal of Clinical Virology. 2007 January 2007;38(1):1-6.
42. Mohd Zim MA, Sam IC, Omar SF, Chan YF, AbuBakar S, Kamarulzaman A. Chikungunya infection in Malaysia: Comparison with dengue infection in adults and predictors of persistent arthralgia. Journal of Clinical Virology. 2013 February 2013;56(2):141-5.
43. Santiago e Souza NC, Félix AC, de Paula AV, Levi JE, Pannuti CS, Romano CM. Evaluation of serological cross-reactivity between yellow fever and other flaviviruses. International Journal of Infectious Diseases. 2019;81:4-5.
44. World Health Organization. Yellow fever in Burkina Faso. Global Alert and Response (GAR) 2008 [cited 2019 May 29]; Disease Outbreak News (DONs)]. Available from: https://www.who.int/csr/don/2008_11_03/en/
45. Collenberg E, Ouedraogo T, Ganamé J, Fickenscher H, Kynast-Wolf G, Becher H, et al. Seroprevalence of six different viruses among pregnant women and blood donors in rural and urban Burkina Faso: a comparative analysis. Journal of medical virology. 2006;78(5):683-92.
46. Mutebi J-P, Barrett ADT. The epidemiology of yellow fever in Africa. Microbes and Infection. 2002 November 2002;4(14):1459-68.
47. da Silva-Nunes M, de Souza V, Pannuti C, Sperança M, Terzian A, Nogueira M, et al. Risk factors for dengue virus infection in rural Amazonia: population-based cross-sectional surveys. Am J Trop Med Hyg 2008 2008 Oct;79(4):485-94.

48. Sule WF, Oluwayelu DO, Hernández-Triana LM, Fooks AR, Venter M, Johnson N. Epidemiology and ecology of West Nile virus in sub-Saharan Africa. Parasites & Vectors. 2018;2018(11):414.
49. World Health Organization. Zika virus (ZIKV) classification table Geneva: World Health Organization; 2018 15 February 2018.
50. Majumder MS, Hess R, Ross R, Piontkivska H. Seasonality of birth defects in West Africa: could congenital Zika syndrome be to blame? F1000Research. 2018;2018(7):159.
51. Montoya M, Collins M, Dejnirattisai W, Katzelnick L, Puerta-Guardo H, Jadi R, et al. Longitudinal analysis of antibody cross-neutralization following Zika virus and dengue virus infection in Asia and the Americas. J Infect Dis 2018 2018 Jul 13;218(4):536-45.
52. Laigret J, Rosen L, Scholammer G. [A dengue epidemic in Tahiti] Sur une épidémie de dengue survenue á Tahiti en 1964. Relations avec les " fiévres hémorragiques " du Sud-Est asiatique. Bulletin de la Société de Pathologie Exotique 1967;60:339-53
53. Saugrain J, Moreau JP, Rosen L. [The dengue epidemic in Tahiti 1971. Evolution of the haemorrhagic tendency and comparison with preceding epidemics] L'épidémie de dengue de Tahiti en 1971. Évolution de la tendance hémorragique et comparaisons avec les épidémies précédentes. Bulletin de la Société de Pathologie Exotique 1973 66(3):381-5
54. Richard V, Paoaafaite T, Cao-Lormeau V-M. Vector competence of French Polynesian aedes aegypti and aedes polynesiensis for Zika Virus. PLoS Negl Trop Dis. 2016 September 21, 2016;10(9):e0005024.
55. Inoue S, Alonzo M, Kurosawa Y, Mapua C, Reyes J, Dimaano E, et al. Evaluation of a dengue IgG indirect Enzyme-Linked Immnosorbent Assay and a Japanese Encephalitis IgG indirect Enzyme-Linked Immnosorbent Assay for diagnosis of secondary dengue virus infection Vector Borne Zoonotic Dis. 2010 2010 Mar;10(2):143-50.

Chapter 7. Discussion, conclusions, and future directions

7. Discussion, conclusions and future directions

Dengue is a major public health problem in tropical and sub-tropical countries and its transmission continues to expand globally (1, 2). Especially in Africa, the magnitude of dengue transmission is largely unknown. In Africa, there are some data obtained from outbreak investigations and also from retrospective testing of previously collected samples. However, there are not many data on the population-based estimates of burden of dengue in the region. Challenges to accurate burden assessment include limitation in terms of diagnostic capacity and surveillance systems to detect and monitor dengue incidence. In addition, many co-existing causes of fever illness with similar symptomatic presentations in Africa further complicate accurate assessment of dengue burden (3). Therefore, the principal aim of this thesis was to address knowledge gaps on dengue burden in Africa, in terms of the proportion of dengue fever among non-malarial febrile episodes, with the focus of outbreak periods, compared to non-outbreak period, as well as seroprevalence and annual rate of DENV infection, based on field studies conducted in Burkina Faso and Kenya. Further discussion is included here based upon chapters 4, 5, and 6 of this PhD thesis, by comparing findings from Ouagadougou and Mombasa in the times of outbreak versus non-outbreak as well as interpreting the results from the surveillance and seroprevalence studies conducted in Ouagadougou.

7.1 Summary of the thesis

Data presented in this thesis support that there is a considerable level of transmission and burden of dengue in Burkina Faso and Kenya. However, given variability of dengue epidemiology over time and by region, more prospective longitudinal studies with further laboratory analyses would be necessary to confirm the conclusions presented in this thesis. In such resource-limited environments in Africa, consideration of preventive and control interventions, such as dengue vaccines, may be premature, especially considering the current status of vaccine development. However, should dengue become a better-recognized major public health problem in Africa, as it is in the Americas and Southeast Asia, these data will facilitate evidence-based decision making for implementation of interventions for dengue prevention and control, including vaccine introduction.

7.2 Burden of Dengue fever at health facilities

Results from the facility-based passive fever surveillance studies in Chapters 4 and 5 suggest that dengue may be an important cause of fever, other than malaria, in Africa. In the surveillance, a quarter of non-malarial febrile patients who sought care at 5 healthcare centers in Ouagadougou, Burkina Faso, were found to be dengue-positive. In Kenya, more than half of non-malarial febrile patients who sought care at 3 health facilities Mombasa were found to be dengue-positive. The majority of the dengue-positive cases had lab-confirmed dengue infections based on paired ELISA and/or PCR, both in Kenya and Burkina Faso. Given the considerable transmission of dengue, these data confirm that dengue is endemic in both Kenya and Burkina Faso.

In both sites, the occurrence of outbreaks during the study enabled us to compare various demographic and clinical indicators of dengue during and outside the outbreak. Proportions of dengue-positive cases among non-malarial episodes were higher in the time of outbreak in both sites, compared to the non-outbreak periods. These support that dengue takes a major cause of AFI after ruling out malaria, especially in dengue outbreaks, but also in non-outbreak times.

There are limited data on comprehensive identification of etiologies of AFI in Africa (4, 5). However, the estimates in chapters 4 in both outbreak and non-outbreak in Kenya were higher, compared to existing data in literature on the proportion of dengue as a cause for AFI after ruling out malaria in Africa (6, 7). For Burkina Faso, the reported estimates were comparable to existing data (4, 8).

Also, the proportion was much higher in Mombasa, than in Ouagadougou, despite the larger outbreak taking place during the study period in Ouagadougou compared to the one in Mombasa. As described in chapters 4 and 5, there were 5 health centers where subjects were enrolled in Ouagadougou, covering a larger catchment area population of 100,000 residents, compared to Mombasa where subjects were enrolled at 3 facilities, of different healthcare provider levels, covering a smaller catchment area population of 70,000 residents. Also, recruitment performed much better in Ouagadougou, throughout a longer study period of 27 months, compared to Mombasa, where recruitment was interrupted with clinicians' strike and high turnover of staff, during a shorter study period of 15 months. These differences led to a smaller number of enrolled subjects in Mombasa, than in Burkina Faso. Without enrollment interruptions and a longer study period, a larger size of enrolled subjects could have resulted in more comparable estimates of proportion of dengue-positivity in Mombasa.

In terms of age, the majority of dengue-positive cases in Kenya were adults between 20-34 years of age. The age group with the highest proportion dengue-positive cases was 20-24 years, followed by 25-34, and 15-19 years. Similarly in Burkina Faso, the majority of dengue-positive cases were older children and adults between 15-35 years of age. The age group with the highest proportion dengue-positive cases was 25-29 years, followed by 20-24, and 35-39 years. What was noteworthy was in both Kenya and Burkina Faso, dengue was not a disease of children. The data supporting that dengue in Africa was more frequent in teenagers and older adults, than in children, were consistent to reports of recent literature on increased mode of age for dengue (9-11).

Outpatient dengue is known to account for the greatest burden of disease, both epidemiologically and economically and, although different levels of facilities were covered in the surveillance studies, both surveillance sites included outpatient as well as hospitalized (under observation) departments (12, 13). Difference between two sites was the numbers of patients requiring hospitalization or observation (i.e. staying in the facility up to 3 days) based on clinical judgement. Six percent of subjects required observation in Ouagadougou and there were increased odds of requiring observation that the dengue-confirmed patients were almost 11 times more likely, compared to non-dengue patients, to require observation at CSPSs. However, in Mombasa, only 2 subjects required hospitalization and both were dengue-positive. With only 2 subjects hospitalized and no DHF or complications reported, it can be concluded that most of the dengue-positive cases in the study in Kenya were mild. Of these two, one had a complete record of admission: no complication was reported, and they spent two days in hospital before discharge.

Clinical responses depend on several factors (14), including age, and exposure to heterotypic virus. There is also evidence from Cuba that expression of certain genes is associated with severity (15). For example, in Kenya, although the current study did not seek to record race or ethnicity, 95% of Kenyans self-identify with an ethnic group associated with the Nilo-Sharan (Nilotic), Cushitic or Bantu language families (16, 17). Bantu ethnic groups are likely to be genetically closer to the West African ancestors of most Afro-Cubans (15). Since there is a large genetic variation between Africans (18), either the same or other genes may be responsible for the lower risk of dengue in black Africans found in Tanzania (19). In any case, such genetic factors, associated with race, may help explain our findings of dengue as mild disease in native residents of Mombasa.

In Burkina Faso, three quarters of patients requiring observation were dengue-positive patients. However, there was no DHF reported and, among patients with medical record

during observation, there was only 1 case of complication, which was reported to be nose bleeding. While it may be speculated that dengue illness, in general, was more severe in Burkina Faso, than in Kenya. However, the data collected were insufficient to verify this and one detail to note is that the CSPSs in Ouagadougou were basic health facilities whereas, in Mombasa, three different levels of healthcare were included in the surveillance with one of them being a provincial level referral hospital. Decision for hospitalization and observation is made by clinicians and there may be differences in criteria for clinical judgement in two sites.

Overall, the data collected did not include any information on severity and requiring hospitalization and observation could be an indicator of severity of dengue illness. Diseases severity may be associated with secondary dengue infection, but the study was unable to collect complete IgM/IgG lab results based on paired sera to determine this (20). Given that we used commercial kits, without quantitative results, the data were unable to calculate IgG/ IgM ratio to differentiate the secondary dengue from primary dengue cases (20).

7.2.1 Clinical diagnosis and its accuracy

In the context of clinical diagnosis of dengue, prior to lab confirmation, 63% of dengue-positive cases were diagnosed with suspected dengue in Kenya. This was higher over the outbreak, but, it was lower than half of dengue-positive cases diagnosed with suspected dengue during the non-outbreak period. In Burkina Faso, the performance of clinical diagnosis was very low. Despite repeated outbreaks and consequently higher awareness of dengue in general, clinicians in Burkina Faso did not appear to consider dengue frequently as a clinical diagnosis, and only a quarter of dengue-positive cases were diagnosed with suspected dengue overall.

One possible reason could be from the inherent differences in the study set-up. In Ouagadougou, the surveillance was launched in 5 basic health centers. However, in Kenya, the three facilities where the surveillance was launched covered low to high levels of healthcare providers, from basic health center to provincial level referral hospital. Clinicians in Kenya could have been more trained with higher awareness of dengue than the staff in CSPS, often nurses and rarely clinicians, in Burkina Faso. Availability of clinicians in addition to nurses as well as possibly heightened awareness in the study in Kenya could have led to the higher likelihood to clinically suspect dengue than in Burkina Faso.

7.2.1.1 Recommendations in terms of clinical diagnosis

Febrile patients in Africa are commonly diagnosed and given presumptive treatment for malaria, although they are often lab-confirmed with other infections (21-23). Especially in resource-limited settings, it is challenging to identify any or all of the infecting pathogens (24). The lack of study of dengue in Africa may have contributed to the low rate of clinical diagnosis of suspected dengue among the patients in the current surveillance study. Given the high proportion of dengue-positive cases among non-malarial febrile episodes, the data in this thesis suggest that clinicians in Kenya and Burkina Faso should consider dengue more frequently as a clinical diagnosis, with or without point-of-care assays.

Furthermore, another suggestion would be to train healthcare professionals, especially in terms of using clinical algorithms for dengue case identification (25). It could be the adoption of the 1997 and/or 2009 WHO dengue case classification criteria for routine reference and use (26). Considering the unknown and possible difference in terms of clinical presentation of dengue in Africa, there may be a need to make necessary modifications to the existing case classifications based on data generated from Africa to make it applicable and sensitive for practical use in the clinical settings in the region. In any case, more systematic use of a clinical algorithm for the clinicians to better identify dengue cases would be recommended.

7.2.2 Use of RDTs for diagnosis of dengue in clinical setting

RDTs were provided as part of the study in both sites. At enrollment, all subjects were tested with dengue RDT. Clinical diagnosis of suspect dengue was made prior to lab-confirmation (ELISA and/or PCR-based) of dengue and, as for RDT results, there may have been cases where the RDT results were not available at the time of clinical diagnosis. This was often the case in high volume of patients and blood collection is done in the lab, rather than by clinicians/nurses in the examination room. So, clinical diagnosis could have been made in the absence of knowledge of the dengue RDT results. Work from chapter four of this thesis reported that less than half of the enrolled febrile patients in Kenya had positive results for NS1 and/or IgM on dengue RDT. In Burkina Faso, only one in every 7 enrolled patients had positive results for NS1 and/or IgM on the RDT kit.

The laboratory algorithm in the surveillance was such that RDT results were used as a screening tool for further testing with RT-PCR of samples showing positive results on dengue RDT and/or IgM/IgG ELISA. Positive result on any side of the RDT kit (NS1, IgM, and IgG) prompted the sample to undergo additional testing with RT-PCR. Thus, the data were

limited to document accuracy in terms of sensitivity and specificity of dengue RDTs, as determination of dengue-confirmation was based on results from PCR testing for which RDT results influenced the selection of samples. Overall, much lower detection rates of RDTs were observed than the advertised performance, in terms of sensitivity and specificity, of the commercial RDT kit (27-30).

7.2.2.1 Recommendations in terms of surveillance

After the 2016 outbreak, dengue was included in the routine national surveillance system for potential epidemic diseases in Burkina Faso. Also, the MoH conducts outbreak investigations at several sentinel health centers (31). Especially during the outbreak in 2017, a laboratory-based arbovirus sentinel surveillance was implemented in November 2017, which was built on existing routine surveillance with enhancement of sample testing, improvements in case reporting as well as in data management (32). There have been outbreaks of dengue-like illness in several locations in Africa, but Burkina Faso is one of the few where dengue is included in the national reporting system. Despite efforts put into implementation of an improved surveillance system, there were some shortcomings, such as limited data management and delays due to procedural requirements (32). In Kenya, there is no ongoing surveillance of dengue, but the Division of Disease Surveillance and Response within the Ministry of Health conducts arbovirus outbreak investigations. In order to understand the burden and extent of transmission in the region, case reporting is critical. Active surveillance would be ideal to understand the true population-based incidence and severity of dengue. However, considering resource limitations, health facility-based surveillance would be a good start to detect cases and monitor epidemics.

In addition, there are other *Aedes* vector-transmitted diseases, such as Yellow fever, West Nile, chikungunya and Zika, and there is continued absence of data on the distribution of these diseases and patterns of transmission of these arboviruses (33). Often, the frequently non-specific clinical presentation makes it difficult to distinguish one from other infectious diseases present in Africa. Also, concurrent infections are usually unrecognized and the extent of co-infections is largely unknown. Therefore, if the resource constraint were not a limiting factor, it would be ideal to establish arboviral disease surveillance, equipped with diagnostic assays for multiple pathogens, including dengue. If so, such surveillance should include case detection using the multiplex rapid diagnostic test for simultaneous detection (commonly trioplex with dengue, Zika, and chikungunya) with confirmation based

on multiplex PCR. Currently, there are efforts to develop point-of-care tests using nucleic acid amplification technologies for improved performance to test for multiple pathogens (34).

In addition to the surveillance of dengue cases in humans, despite financial and infrastructure-wise challenges, it would be more informative to implement an integrated human and vector surveillance (35). As these are vector-borne diseases, entomological surveillance also helps to monitor the risks of arbovirus epidemics (36, 37). There is no comprehensive data on xenomonitoring of Aedes vectors in Africa, but a study was conducted in Brazil to monitor prevalence of infected mosquitoes and to understand the burden of adult and larval/pupae of *Aedes mosquitoes* with DENV (37). The study reported co-circulation of three of the four DENV serotypes and such finding demonstrates the possibility of co-infection cases by more than one serotype as well as with Yellow fever, chikungunya and Zika viruses (37).

Early detection of cases through these surveillance systems will allow timely onset of control interventions and dissemination of outbreak alerts. Especially for dengue, monitoring of cases should be equipped with virus serotyping on a subset of samples, so that such surveillance could provide and possibly prevent severe disease caused by transmission of multiple DENV serotypes.

7.2.3 Diagnostic options for dengue diagnosis

As described in chapter 3, the patients enrolled in the surveillance studies who were febrile, or with a history of fever in the past 7 days, were first tested for malaria using RDT as part of routine practice. Malaria RDT-negative patients were enrolled in the study and tested with dengue RDTs. An acute sample of blood was taken at enrollment at the first visit, and the second blood sample was collected within 21 days of the first visit. All the acute and convalescent samples were tested using IgM/IgG ELISA. The results from IgM/IgG ELISA and RDT were used for selecting samples that would undergo further testing by RT-PCR.

As described in chapter 2 of this thesis, a single method often does not provide a definitive conclusion for dengue diagnosis. There are multiple testing options, based on serologic, molecular, and virus antigen detection as well as combination of these methods (38-40). For serologic testing, such as ELISA, paired samples are needed to monitor change in the antibody level to reach a conclusion. Even so, it may require further analyses by PRNT testing, due to cross-reaction with other flaviviruses. PRNT and molecular methods, such as RT-PCR, while providing more confirmatory results of dengue diagnosis, require some infrastructure, such as equipment set-up and some technical expertise/training. These factors

contribute to challenges in the laboratory aspect, of accurate assessment of dengue burden in Africa.

Given these complexities, in the studies covered in chapters 4 and 5, paired samples were collected and laboratory confirmation for dengue infection was performed according to WHO diagnostic criteria (41). Sero-conversion of anti-dengue IgM and IgG between the acute and convalescent phases and/or RT-PCR positive in the acute serum specimen was considered to be confirmed dengue. A positive IgM serology in single serum and/or positive on NS1 or IgM of RDT in single acute serum were considered probable dengue infection (41). Samples with negative results on RT-PCR and sero-negative results on paired IgM and IgG ELISA results were classified as non-dengue. Also, a positive IgG serology in single serum, with negative results from all other tests, could have been non-recent infection, and was classified as non-dengue.

Such multi-layered testing for diagnosis is ideal, but may only be feasible in a research study setting. For clinical diagnosis, tests that are easy to perform with quick turnaround for results would be more adoptable in resource-limited environments in Africa.

7.2.3.1 Recommendations in terms of dengue diagnosis

While there are various new promising technologies being developed for dengue diagnosis, it will take time and resources for these to be made available in Africa (34). Hence, one way to overcome this problem might be to make the best use of the existing and already established networks. One possible option might be through the African Field Epidemiology Network (AFENET)(42). AFENET supports laboratory capacity development in the Africa region and its scope includes training of laboratory staff, laboratory equipment calibration, and provision of pre-packaged laboratory kits (42). Also, the countries without comprehensive diagnostic capacities should collaborate with international partners and agencies, such as Institut Pasteur or CDC. For example, the Institut Pasteur network includes Senegal, Madagascar, Cameroon, etc. and the CDC global health network includes Kenya, Ghana, South Africa, etc. They are already working in partnership with the governments of selected countries in Africa to build sustainable in-country public health capacity and laboratories of these agencies may serve as regional reference laboratories to perform testing of samples obtained from nearby countries. Based on such collaborative relationships, more data could be generated and countries could work together in preparation against epidemics as well as policy developments for necessary vector control efforts.

7.2.4 Advantages and benefits in using dengue RDTs

In the context of limited information on dengue in Africa, one of the challenges in accurate assessment of dengue burden is diagnostics. Dengue RDTs could be a convenient option for acute dengue diagnosis especially in resource-limited settings. However, as described in chapter two, data in the current literature were limited to demonstrate any economic impact of using RDTs in dengue diagnosis in clinical setting.

The data described in chapters four and five reported that lower than expected levels of accuracy were observed for the dengue RDTs in Burkina Faso and Kenya. However, RDTs, with results generated within 15 minutes, can be simple and user-friendly tools for dengue diagnosis. Despite their compromised sensitivity and specificity, there are benefits of using dengue RDTs (27). Especially as point-of-care tool, these may help to avoid unnecessary treatments leading to cost savings. With this as a hypothesis, the literature review, as reported in chapter 2, was conducted with two search terms, “dengue” and “cost or economic” to explore evidence of benefit of dengue RDTs, specifically with respect to its cost aspect. Two articles were found to describe on the cost aspect of dengue RDT use. Interestingly, these reached opposite conclusions on the cost-effectiveness of dengue RDTs. While one of the two studies reported satisfactory performance of IgM-based Panbio RDT, concluding that it would be cost-effective in endemic setting (43, 44), the other, based on modeling analysis with assumed parameters, reported that a dengue RDT would not be advantageous in terms of cost and effectiveness compared to current practice of antibiotics prescription for AFI (45). Given the lack of evidence, the review concluded that, in spite of growing use and need of dengue RDTs in research and clinical settings, data were limited to demonstrate an economic impact.

While there was no evidence based on empirical data in the existing literature supporting cost savings associated with dengue RDT use, it is an area that needs to be explored and clearly documented. In the studies described in chapters 4 and 5, although not reported in the thesis, there were dengue-positive patients prescribed with antibiotics in both Kenya and Burkina Faso. In Kenya, 56% of dengue-positive, compared to 67% of non-dengue cases, and in Burkina Faso, 61% of dengue-positive, compared to 69% of non-dengue cases, were prescribed with antibiotics. Even though prescription was made prior to lab-confirmation and even in possible absence of RDT results, these are still high proportions of patients with viral infection given antibiotics treatment. With more targeted

treatment prescription, i.e. no antibiotics given to dengue RDT positive patients, there may be cost savings to the patients from avoided antibiotics treatment.

7.2.4.1 Recommendations in terms of dengue RDT use

Dengue diagnostics can be complex and may require multiple testing for accurate confirmation. In Africa, it will be challenging and resource-limited to set-up laboratories with more comprehensive diagnostic capacities. Despite the low detection rates of RDTs observed in the field setting, considering the benefits of dengue RDTs, it would be advised to make dengue RDTs available for more routine use in the clinical setting (46). However, routine use of dengue RDTs among non-malarial fever patients year round may be too resource-consuming, especially considering other pathogens also commonly circulating in the region. Although not in Africa, it has been documented that dengue case numbers rise during the time of rainy season (47, 48). If it is too much of resource consumption to make dengue RDTs available year round, then a more targeted use limited to around the time of the known rainy season will help to monitor dengue cases and capture possible epidemics.

In addition to a recommendation of more limited use covering the rainy season targeting dengue transmission, it is advised to use dengue RDTs in addition to malaria RDTs, as the febrile patients are routinely tested with the malaria tests. This will prevent possible influence of performance of malaria RDT on detection of dengue cases.

7.3 Burden of Dengue at the community level in Ouagadougou

To date, there is no information on dengue burden estimates, especially in terms of population-based seroprevalence and FOI, in Africa. In the same catchment area as the passive fever surveillance in Ouagadougou, there were 4 repeated serosurveys at 6 month intervals following up with the same individuals. As described in chapter 6, the study's aims were to estimate overall prevalence and sero-conversion rates in Ouagadougou and gain understanding on DENV transmission in the community.

In terms of DENV transmission in the community, with subclinical and inapparent dengue, a high level of seroprevalence and high force of infection (FoI), based on IgG seroconversion, were observed in the studied population in Ouagadougou. Benefiting from the repeat surveys following up with the same individuals, FoI reached 20% per year, during the interval covering the 2016 outbreak. IgG ELISA was used for diagnosis in the serosurveys and results should be interpreted with caution resulting from using serologic test with the possible cross-reaction across flaviviruses.

7.3.1 Sero prevalence

Two thirds of the subjects were shown to be IgG positive at enrollment. Seroprevalence increased with age, reaching 80% by age 26 years. While this was measured empirically by IgG positivity, this was comparable to existing data, based on IgG ELISA results, from other endemic countries, such as Colombia, Tahiti, and Brazil (49-52).

7.3.2 FoI measured by seroconversion

The binomial regression, based on IgG positivity by age at enrollment (S1) assuming the force of infection was constant over calendar time and across age groups, led to an estimate of the force of infection of 6% per year, as reported in chapter 6. While this was calculated based on IgG positivity by age at enrollment, this was comparable to findings from Colombia and high SES areas of Brazil, but lower than findings from Sri Lanka and low SES areas of Brazil and higher than estimates from Nicaragua (9, 51, 53, 54).

Again assuming the constant risk of infection across age, but with the mean duration of interval as denominator, rates of infection ranged between 10-20% per year. Given the highest annual rate of infection measured over the interval covering the 2016 outbreak, it was about twice of FoI in outbreak compared to non-outbreak period. Furthermore, age-specific annual rates of infection were reported. For non-outbreak intervals, the rates were higher in adults, older than 30 years, than in children. However, for the interval covering the outbreak, the rates were found to be even across age. This was similar to the age patterns reported for the clinical disease identified in the surveillance reported in chapter 4. In the surveillance, which took place during the same study period in the catchment area population, it was found that the odds of dengue-positivity were greater for adults in non-outbreak period, but there was no difference across age observed in the outbreak period. While there is a major difference that these were clinically ill cases of dengue infections whereas the serosurveys captured transmission in healthy individuals in the community, the age patterns observed in two studies were consistently indicating that age group affected most by dengue, at the healthcare facilities and in the community, was adults in non-outbreak periods.

7.3.3 The Serologic assay used for diagnosis

The serosurveys were based on 4 sets of blood samples collected at 6 month intervals in the same individuals. Only Panbio IgG ELISA results were available and without confirmatory results from other tests, such as PRNT, to estimate the prevalence and rate of infection, measured by seroconversion. In the absence of a gold standard diagnostic, taking advantage of the study feature of repeated surveys before and after the outbreak with a large sample size of about 1,700 subjects, overall and age-specific annual rates of infections were calculated based on seroconverted individuals with the mean duration of interval.

There were concerns of cross-reaction with other flaviviruses. However, as described in chapter 6, there have been previous studies supporting performance dengue IgG indirect ELISA (i.e. the detected antibodies were indeed specific to DENV (49, 50, 55). Also, in terms of other co-circulating flaviviruses cross-reacting with DENV, Burkina Faso has not reported the presence of WNV (56). For Zika virus (ZIKV), Burkina Faso had reported prevalence of ZIKV antibodies in human populations (57, 58). However, possible cross-reaction between DENV and ZIKV has been assessed in a study where neutralizing antibodies to ZIKV and DENV were longitudinally followed in patients with Zika and DENV from Latin America and Asia and reported low cross reactivity between DENV and Zika viruses (59). Still, there might be other cross-reacting flaviviruses or arboviruses in circulation in Africa and our results could be overestimates if these viruses as background transmission affected our measurement of sero-positivity (59). Even with this as weakness, as there are no other existing data on seroprevalence and rate of infection of dengue in Africa, what was presented in chapter 6 is thus far the only available data on intensity of community-based DENV transmission in Africa.

7.3.3.1 Recommendations in terms of seroprevalence and vaccination

The data generated as part of this thesis form an evidence base for decision making on introduction of various strategies, in terms of preventive and control measures. Especially, in terms of implications in the context of dengue vaccines, these data are relevant and much needed. Currently, several dengue vaccine candidates have been in development, and recently, a first dengue vaccine from Sanofi Pasteur was licensed in 2015 in multiple countries in Asia and Latin America. However, this vaccine has variable efficacy and has a restricted indication in dengue-exposed subjects only from 9 years and above, due to increased risk of severe dengue in seronegative subjects (60, 61). More

specifically, WHO issued their position with respect to the use of this first dengue vaccine and the recommendations are: to perform pre-vaccination screening to identify persons with evidence of a past dengue infection; and, if such individual screening is not available, to consider vaccination in areas with recent documentation of seroprevalence rates higher than 80% by the age of 9 years (61). In our studied population in Ouagadougou, the seroprevalence level of 80% is reached by the age of 20 years. By the age of 9 years, the seroprevalence by IgG positivity was below 50%. Therefore, given the current WHO recommendation, dengue vaccine introduction would not be an immediate priority for public health action in Burkina Faso.

Despite substantial transmission of DENV documented in both Kenya and Burkina Faso, there is no supporting evidence of prior immunity with 80% threshold in population 9 years and older. It may be similar in other populations in the region, even if more outbreaks are being reported in Africa. In 2013, due to the uncertainties of the disease burden, especially in Africa, as well as unknown vaccine efficacy with the first vaccine candidate, dengue was one of the vaccine-preventable diseases under consideration for GAVI vaccine investment strategy (VIS), but was not selected after review of the data available at the time. Despite known complications associated with the first dengue vaccine licensed in 2015, efforts continue to develop safe and efficacious vaccines for dengue. Until better options become available in terms of individual screening and/or better vaccines without such complications in use become available, it would be premature to consider introduction of dengue vaccine for a public health use in Africa. In the meanwhile, other preventive and control measures, such as vector control methods, may be considered. Such approaches may contribute to prevention and control of other arboviral diseases also transmitted by *Aedes* mosquitoes (33).

7.4 Symptoms associated with dengue

As described in chapters 4 and 5, surveillance studies captured entire or partial outbreaks in Burkina Faso and Kenya, respectively. This enabled analysis to assess differences in clinical patterns and epidemiology of dengue during the outbreak and non-outbreak periods. Symptoms positively associated with dengue were different between outbreak vs. non-outbreak periods in the two study areas. This may have been due to circulating serotype. There was no serotype change observed in the outbreak, remaining predominantly DENV 2 in Mombasa. DENV 3 was the prevalent serotype outside outbreak in Burkina Faso and it was DENV 2 during the outbreak. For example, in the data from

Kenya, nausea/vomiting was associated more significantly with DENV 2 than other serotypes (p-value = 0.023). There were some data supporting that gastrointestinal signs are associated with DENV 2 (62). However, in a multicountry outpatient-based passive surveillance study in the Americas, individuals infected with DENV3 were reported with a higher prevalence of gastrointestinal manifestations, including nausea/vomiting, compared to individuals with other serotypes (63, 64).

Also, arthralgia was found to be more frequently associated with dengue during non-outbreak in Kenya. Arthralgia has been associated with DENV 2 infection, and there is no clear explanation of why arthralgia was associated with dengue during non-outbreak, but not in the outbreak period, when prevalent serotype was DENV 2 throughout (65). Overall, there are no conclusive data on patterns of clinical manifestation by dengue serotype in existing literature.

Symptomatic presentation may vary significantly by the history and intensity of DENV transmission as it may be different for secondary vs. primary infection, days into illness, and also by viral load (62, 65). With limitation in terms of laboratory analyses, the surveillance data were unable to determine between primary vs. secondary infections. Also, lacking detailed information on virus strain, it is difficult to determine whether there were virological differences affecting clinical presentation, between outbreak and non-outbreak periods.

While the surveillance data did not have indicators of dengue severity, what was different between dengue patients in Kenya vs. Burkina Faso was the number of patients hospitalized in Kenya or requiring observation for < 3 days in Burkina Faso's basic health center setting. There were a total of only 2 hospitalized patients in Kenya. In Burkina Faso, dengue-positive patients were more likely to be requiring observation, compared to non-dengue cases, in both outbreak and non-outbreak periods.

7.5 Limitations

One of the major limitations of this thesis is the limited generalizability of data collected in Mombasa and Ouagadougou throughout Kenya and Burkina Faso, respectively, and ultimately to Africa. Dengue epidemiology is known to be variable over time and by region. Nonetheless, Mombasa and Ouagadougou are major cities of these countries, and the demographics and level of urbanicity of these cities may influence dengue transmission. Given dengue being more of an urban disease, the burden of dengue in large cities, such as Mombasa and Ouagadougou, would presumably differ from rural parts in Africa.

Therefore, the data described in this thesis would be limitedly generalizable to dengue endemic areas in urban setting within Kenya and Burkina Faso.

In terms of dengue prevention and control, even if safe and efficacious vaccines become available, the data would not be able to provide insights as to whether an intervention tool, such as a dengue vaccine, would be needed in rural parts of Africa, and separate studies would be needed to generate evidence to support such decision-making.

An additional limitation includes the diagnostic limitation across the studies in the thesis. Sequencing to obtain strain information would have been ideal for the PCR-positive samples from the surveillance. Also, neutralization assay on a randomly selected subset of samples in the serosurveys would be helpful to validate the IgG positivity to be specific to dengue antibodies. With limited laboratory resources and timeline, such further analyses were not feasible. If the IgG ELISA detected other flaviviruses, the empirically measured estimates of seroprevalence and seroconversions might be overestimates.

The surveillance study excluded patients with malaria RDT positive results, localizing signs or known/confirmed diagnosis with other diseases, possibly omitting co-infections of dengue with another pathogen. In particular, given the prevalence of malaria in this region, dengue and malaria co-infection may require further investigation. Nevertheless, the available information on co-infections suggest they are uncommon (4, 66-69). In addition to malaria, co-infection of dengue and Chikungunya is known to occur in Kenya (70, 71). Also, there was a systematic review of the literature on concurrent detection of chikungunya and dengue viruses and such evidence for concurrent infection of chikungunya and dengue viruses has been reported in Angola, Gabon, Madagascar, Myanmar, Tanzania, and Yemen among countries in Africa, in addition to several other countries in Asia (72). The article concluded that the major limitation in accurate estimation of burden of such co-infection is due to absence of robust lab-based diagnosis. Nonetheless, when this was assessed in our surveillance samples from Kenya, none of the samples were positive for Chikungunya virus. As the study period was only for 15 months, it could have been the time of no chikungunya circulation. Nonetheless, in the literature, such co-infection is reported to be not commonly occurring (7, 73).

Furthermore, due to the study design where patients with malaria RDT positive results were excluded, performance of malaria RDTs, which is known to be affected by local conditions, would be a source of bias (74). Malaria incidence in Ouagadougou is reported to be variable and malaria RDT performance is influenced by parasite density as well as the level of transmission in the population (74, 75). Therefore, depending on the test

performance of the malaria RDT, there could have been misclassification among non-malarial patients (i.e. false negative results on malaria RDT included in the study being differently classified between dengue-positive and non-dengue groups). Also, this could be variable between during and outside of the outbreak (with different intensity of dengue transmission), leading to differential misclassification.

Also, as shown in chapters 4 and 5, the majority of the patients in the surveillance are outpatients and it is possible to have missed patients with severe illness and patients with other mild illness seeking care elsewhere. Therefore, it is possible that the results of chapters 4 and 5 could be influenced by healthcare-seeking behaviour. They pertain to those who are able to seek healthcare at public health facilities, not private clinics. In resource-limited environments, such as in Africa, healthcare-seeking behaviour is closely related to the socioeconomic status (SES) (76, 77). The association between SES and risk of dengue is unclear, but some studies reported differences in seroprevalence and force of infection of dengue in areas of high and low SES (54). As the passive fever surveillance studies in this thesis did not capture those seeking care at private clinics, it would miss those potential subjects from a particular group of socioeconomic status.

By contrast, the serosurveys described in chapter 6 were in randomly preselected residents of the defined catchment area. However, over four bleeds, there was a considerable proportion of lost to follow-up. Though considerable effort was made to follow up on all initially enrolled subjects in subsequent bleeds and, despite the stability supported by the reported level of home ownership, of 3026 subjects initially enrolled, 1681 subjects remained in the study by the fourth bleed at final follow-up over a 22-month study period.

7.6 Alternative strategy and future directions

Based on lessons learnt, there can be alternative approaches to characterize the epidemiology of dengue in Africa. More longitudinal cohort or active surveillance studies would be ideal for assessment of incidence and seroprevalence. Also, this would allow for analysis of severity grade of dengue-positive cases and more comprehensive follow-up to monitor change in the immunity status as well as progression of their illness (hospitalization, entire duration of illness, etc.). Also, comprehensive laboratory algorithm including further analyses would allow more definitive assessment of the burden without influence of possible cross-reaction of other flaviviruses.

Therefore, additional future investigations into the epidemiology of dengue in other locations in Africa, other than Ouagadougou and Mombasa would be beneficial. As dengue

has been recognized as a major global public health problem, efforts have been made to develop effective tools to prevent and control dengue, such as vector control and vaccines. Vaccination could be a good solution to combat this problem, once a safe and efficacious vaccine becomes available. However, to develop strategies on how to introduce such a vaccine, patterns of transmission and epidemiologic characteristics need to be better documented. Especially for dengue, given 4 serotypes and the possibility of subsequent infection with a heterotypic DENV leading to a greater risk of more severe disease (DHF/DSS) with pre-existing immunity to 1 serotype, such burden estimates will be important to make decisions for vaccine introduction (78). For the currently available Dengvaxia®, the vaccine demonstrated higher efficacy in pre-vaccination dengue-seropositive individuals with a higher risk of subsequent more severe dengue in dengue-naïve individuals. The current recommendation is to give this vaccine to dengue-seropositive individuals by performing pre-vaccination screening for past DENV infection(79). If this is not feasible, then vaccine should be considered in areas where the dengue seroprevalence is documented to be > 80% by the age of 9 years (79). Therefore, in addition to identifying high incidence areas, it is important to consider level of seroprevelance of DENV in evaluation of the necessity of a vaccine and strategies for vaccine introduction, once a safe and cost-effective dengue vaccine becomes available.

Therefore, additional studies on seroprevalence and burden would help to better inform policy decisions regarding dengue vaccine implementation. Another direction for future research derived from this thesis is the need for further data on dengue RDTs, in terms of its clinical benefits and economic impact to the individual from cost savings at hospitals, furthermore in terms of possible reduction in antibiotics resistance. These RDTs are easy to use, fast, and relatively inexpensive. From existing literature and the data described in chapters 4 and 5, low sensitivity of the RDT was the concern. In absence of vaccine and with repeated outbreaks, prompt identification of cases and incidence monitoring are critical to reduce morbidity and transmission due to DENV in Africa. As helpful tools for dengue diagnosis especially in resource-limited conditions, more readily use of RDTs should be promoted supported by evidence.

7.7 Conclusions

Data from reported outbreaks in different parts of Africa support a considerable level of transmission of dengue virus in Africa. Nonetheless, accurate information on dengue burden in Africa based on population-based studies was limited. The principal aim of this

thesis was to address the knowledge gap on the magnitude of the dengue problem and generate improved data on dengue epidemiology in Burkina Faso and Kenya. This thesis assessed burden from the symptomatic side of dengue among patients seeking for care at study facilities in the passive health facility-based surveillance in Burkina Faso and Kenya. Work from this thesis has confirmed that dengue fever is an important cause of non-malarial acute febrile illness in study sites. Capturing outbreaks in both sites, the data provided differences in clinical presentation and outcomes of dengue patients between outbreak and non-outbreak periods. Also, the data suggest that clinicians in Burkina Faso and Kenya should consider dengue more frequently as a clinical diagnosis, with or without point-of-care assays. Additionally, transmission of varying intensity has been quantified in other regions, and the findings, based on the repeated serological surveys, seroprevalence at baseline and rates of infection, measured by sero-conversion, are within the range of those places where transmission is high enough to be considered a problem.

Work from this thesis addressed the knowledge gap on the magnitude of the dengue problem in Ouagadougou and Mombasa, in the context of dengue-positive cases among non-malarial febrile episodes as well as community-based seroprevalence and FoI. In conclusion, data generated in the studies of the thesis would be able to facilitate informed decision-making on implementation of control and preventive measures for dengue in the region, including vaccine introduction in the future. However, given the currently available information on dengue burden in Africa and the status of dengue vaccine development, including the only licensed vaccine with restrictions in public health use, consideration of dengue vaccine introduction may not be an urgent priority for Africa. Nonetheless, the data in this thesis will likely serve as a basis for future research to further define the burden of dengue in Africa.

References

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013 Apr 25;496(7446):504-7.
2. Gubler DJ MM. Impact of dengue/dengue hemorrhagic fever on the developing world. Adv Virus Res. 1999;53:35-70.
3. Kiemde F, Spijker R, Mens P, Tinto H, Boele M, Schallig H. Aetiologies of non-malaria febrile episodes in children under 5 years in sub-Saharan Africa. Trop Med Int Health. 2016 Aug. 2016;21(8):943-55.
4. Ridde V, Agier I, Bonnet E, Carabali M, Dabiré K, Fournet F, et al. Presence of three dengue serotypes in Ouagadougou (Burkina Faso): research and public health implications. Infect Dis Poverty. 2016 2016 Apr;5(5):23.
5. Ochieng C AP, Vittor AY, Nyoka R, Gikunju S, Wachira C, Waiboci L, Umuro M, Kim AA, Nderitu L, Juma B, Montgomery JM, Breiman RF, Fields B. Seroprevalence of Infections with Dengue, Rift Valley Fever and Chikungunya Viruses in Kenya, 2007. PLoS One. 2015;10(7).
6. Munyuga K, Ng'ang'a J, Inoue S, Syengo C, Ndege Co, Kwallah A, et al. Co-circulation evidence of dengue virus serotypes at the Kenyan coast in 2014, 2015. Journal of Pharmacy and Biological Sciences. 2016 December 2016;11(6):83-7.
7. Ngoi CN, Price MA, Fields B, Bonventure J, Ochieng C, Mwashigadi G, et al. Dengue and chikungunya virus infections among young febrile adults evaluated for acute HIV-1 infection in coastal Kenya. PLoS One. 2016;11(12):e0167508.
8. World Health Organization. Dengue fever - Burkina Faso. Disease outbreak news 2016 2016 November 18 [cited 2018 August 18]; Available from: http://www.who.int/csr/don/18-november-2016-dengue-burkina-faso/en/
9. Rodríguez-Barraquer I, Buathong R, Iamsirithaworn S, Nisalak A, Lessler J, Jarman RG, et al. Revisiting Rayong: Shifting seroprofiles of dengue in Thailand and their implications for transmission and control American Journal of Epidemiology. 2014 1 February 2014;179(3):353–60.
10. Halstead SB. Dengue in the Americas and Southeast Asia: do they differ? Revista Panamericana de Salud Pública. 2006 1 December 2006;20(6):407-15.
11. Nemg Simo F, Sado Yousseu F, Evouna Mbarga A, Bigna J, Melong A, Ntoude A, et al. Investigation of an Outbreak of Dengue Virus Serotype 1 in a Rural Area of Kribi, South Cameroon: A Cross-Sectional Study. Intervirology. 2018;61(6):265-71.
12. Anderson K, Chunsuttiwat S, Nisalak A, Mammen M, Libraty D, Rothman A, et al. Burden of symptomatic dengue infection in children at primary school in Thailand: a prospective study. Lancet. 2007;369(9571):1452--9.
13. Okanurak K, Sornmani S, Mas-ngammueng R, Sitaputra P, Krachangsang S, Limsomboon J. Treatment seeking behavior of DHF patients in Thailand. . Southeast Asian J Trop Med Public Health 1997;28:351-8.
14. Halstead S. Recent advances in understanding dengue [version 1; peer review: 2 approved]. F1000Research. 2019 31 Jul 2019;8(F1000 Faculty Rev):1279.
15. Sierra B, Triska P, Soares P, Garcia G, Perez A, Aguirre E, et al. OSBPL10, RXRA and lipid metabolism confer African-ancestry protection against dengue haemorrhagic fever in admixed Cubans. PLoS Pathog 2017 February 27, 2017;13:e1006220.
16. Kenya national bureau of statistics. Ethnic affiliation. Census 2009 summary of results 2013 March 22, 2013 [cited 2019 Sept 1]; Available from: https://www.knbs.or.ke/ethnic-affiliation/
17. Campbell MC, Tishkoff SA. AFRICAN GENETIC DIVERSITY: Implications for Human Demographic History, Modern Human Origins, and Complex Disease Mapping. Annu Rev Genomics Hum Genet 2008;2008(9):403–33.
18. Yu N, Chen F, Ota S, Jorde L, Pamilo P, Patthy L, et al. Larger genetic differences within africans than between Africans and Eurasians. Genetics. 2002;161(1):269-74.
19. Boillat-Blanco N, Klaassen B, Mbarack Z, Samaka J, Mlaganile T, Masimba J, et al. Dengue fever in Dar es Salaam, Tanzania: clinical features and outcome in populations of black and non-black racial category. BMC Infect Dis 2018 2018 Dec 12;18(1):644.
20. Changal KH, Raina AH, Raina A, Raina M, Bashir R, Latief M, et al. Differentiating secondary from primary dengue using IgG to IgM ratio in early dengue: an observational hospital based clinico-serological study from North India. BMC Infectious Diseases. 2016;16(715).
21. Molla E. Malaria: What are the Needs for Diagnosis, Treatment and Control? Biology and Medicine. 2016 July 06, 2016;8(6):1000320.
22. Crump JA, Morrissey AB, Nicholson WL, Massung RF, Stoddard RA, Galloway RL, et al. Etiology of Severe Non-malaria Febrile Illness in Northern Tanzania: A Prospective Cohort Study. PLoS Negl Trop Dis. 2013;7(7):e2324.
23. Stoler J, al Dashti R, Anto F, Fobil JN, Awandare GA. Deconstructing “malaria”: West Africa as the next front for dengue fever surveillance and control. Acta tropica. 2014;134:58-65.
24. Maze MJ, Bassat Q, Feasey NA, Mandomando I, Musicha P, Crump JA. The epidemiology of febrile illness in sub-Saharan Africa: implications for diagnosis and management. Clinical Microbiology and Infection. 2018 August 2018;24(8):808-14.
25. Otu A, Ebenso B, Etokidem A, Chukwuekezie O. Dengue fever – an update review and implications for Nigeria, and similar countries. African Health Sciences. 2019;19(2):2000-7.
26. Hadinegoro SRS. The revised WHO dengue case classification: does the system need to be modified? Paediatr Int Child Health 2012 2012 May;32(S1):33-8.
27. Blacksell SD. Commercial dengue rapid diagnostic tests for point-of-care application: Recent evaluations and future needs? Journal of Biomedicine and Biotechnology. 2012 11 February 2012;2012(Article ID 151967).
28. Krishnananthasivam S, Fernando A, Tippalagama R, Tennekoon R, De Man J, Seneviratne D, et al. Evaluation of a

commercial rapid test kit for detection of acute dengue infection. Southeast Asian J Trop Med Public Health. 2015 2015 Jul;46(4):602-10.
29. Hunsperger E, Sharp T, Lalita P, Tikomaidraubuta K, Cardoso Y, Naivalu T, et al. Use of a rapid test for diagnosis of dengue during suspected dengue outbreaks in resource-limited regions. J Clin Microbiol 2016 2016 Aug;54(8):2090-5.
30. Blacksell SD, Jarman RG, Bailey MS, Tanganuchitcharnchai A, Jenjaroen K, Gibbons RV, et al. Evaluation of six commercial point-of-care tests for diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. Clin Vaccine Immunol. 2011 Dec;18(12):2095-101.
31. Ridde V, Carabali M, Ly A, Druetz T, Kouanda S, Bonnet E, et al. The need for more research and public health interventions on dengue fever in Burkina Faso. PLoS Neglected Tropical Diseases. 2014;8(6):e2859.
32. Sanou A, Dirlikov E, Sondo K, Kagone T, Yameogo I, Sow H, et al. Building laboratory-based arbovirus sentinel surveillance capacity during an ongoing dengue outbreak, Burkina Faso, 2017. Health Secur. 2018;16(S1):S103-S10.
33. Weetman D, Kamgang B, Badolo A, Moyes C, Shearer F, Coulibaly M, et al. Aedes Mosquitoes and Aedes-Borne Arboviruses in Africa: Current and Future Threats. Int J Environ Res Public Health. 2018;15(2):pii: E220.
34. Wilder-Smith A, Ooi E, Horstick O, Wills B. Dengue. Lancet. 2019 2019 Jan 26;393(10169):350-63.
35. Fournet F, Jourdain F, Bonnet E, Degroote S, Ridde V. Effective surveillance systems for vector-borne diseases in urban settings and translation of the data into action: a scoping review. Infect Dis Poverty. 2018;7(99).
36. Ouattara L, Sangaré I, Namountougou M, Hien A, Ouari A, Soma D, et al. Surveys of Arboviruses Vectors in Four Cities Stretching Along a Railway Transect of Burkina Faso: Risk Transmission and Insecticide Susceptibility Status of Potential Vectors. Front Vet Sci 2019 May 28;6(140).
37. Medeiros AS, Costa DMP, Branco MSD, Sousa DMC, Monteiro JD, Galvão SPM, et al. Dengue virus in Aedes aegypti and Aedes albopictus in urban areas in the state of Rio Grande do Norte, Brazil: Importance of virological and entomological surveillance. PLoS One. 2018;13(3):e0194108.
38. Centers for Disease Control and Prevention. Testing guidance. Dengue-Testing 2019 May 3, 2019 [cited 2019 May 26]; Available from: https://www.cdc.gov/dengue/healthcare-providers/testing/testing-guidance.html
39. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention, and control. Geneva: World Health Organization; 2009.
40. Muller DA, Depelsenaire ACI, Young PR. Clinical and laboratory diagnosis of dengue virus infection The Journal of Infectious Diseases. 2017 10 April 2017 215(suppl_2):S89–S95.
41. World Health Organization. Handbook for clinical management of dengue. Geneva, Switzerland: World Health Organization,; 2012.
42. Masanza MM, Nqobile N, Mukanga D, Gitta SN. Laboratory capacity building for the International Health Regulations (IHR[2005]) in resource-poor countries: the experience of the African Field Epidemiology Network (AFENET). BMC Public Health. 2010;10:S8 (2010).
43. Mitra S, Choudhari R, Nori H. Performance and cost-effectiveness of immunochromatography based rapid diagnostic test (RDT) kits in diagnosis of dengue infection in resource limited set up. International journal of infectious diseases. 2014;21(Meeting Abstract: 64.018):450.
44. Mitra S, Choudhari R, Nori H, al. e. Comparative evaluation of validity and cost-benefit analysis of rapid diagnostic test (RDT) kits in diagnosis of dengue infection using composite reference criteria: A cross-sectional study from south India. Journal of vector borne diseases. 2016;53(1):30-6.
45. Lubell Y, Althaus T, Blacksell SD, al. e. Modelling the Impact and Cost-Effectiveness of Biomarker Tests as Compared with Pathogen-Specific Diagnostics in the Management of Undifferentiated Fever in Remote Tropical Settings. PLOS ONE 2016;11(3):e0152420.
46. Lwande OW, Obanda V, Lindstrom A, Ahlm C, Evander M, Naslund J, et al. Globe-Trotting Aedes aegypti and Aedes albopictus: Risk Factors for Arbovirus Pandemics. VECTOR-BORNE AND ZOONOTIC DISEASES. 2019;XX(XX):1-12.
47. Nery MCD. Dengue increase likely during rainy season: WHO warns. [News release] 2019 11 June 2019 [cited 2019 Sept 19]; Available from: https://www.who.int/westernpacific/news/detail/11-06-2019-dengue-increase-likely-during-rainy-season-who-warns
48. Pasin C, Halloran ME, Gilbert PB, Langevin E, Ochiai RL, Pitisuttithum P, et al. Periods of high dengue transmission defined by rainfall do not impact efficacy of dengue vaccine in regions of endemic disease. PLoS One. 2018;13(12):e0207878.
49. Chungue E, Marché G, Plichart R, Boutin J, Roux J. Comparison of ImmunoGlobuln G Enzyme-Linked ImmunoSorbent Assay (IgG-ELISA) and Haemagglutination Inhibition (HI) test for the detection of dengue antibodies, prevalence of dengue IgG-ELISA antibodies in Tahiti. Trans R Soc Trop Med Hyg. 1989 1989 Sep-Oct;83(5):708-11.
50. Dhanoa A, Hassan S, Jahan N, Reidpath D, Fatt Q, Ahmad M, et al. Seroprevalence of dengue among healthy adults in a rural community in Southern Malaysia: a pilot study. Infect Dis Poverty. 2018 2018 Jan 16;16(7):1.
51. Carabali M, Lim J, Velez D, Trujillo A, Egurrola J, Lee K, et al. Dengue virus serological prevalence and seroconversion rates in children and adults in Medellin, Colombia: implications for vaccine introduction. Int J Infect Dis 2017 2017 May;58:27-36.
52. Teixeira MdG, Barreto ML, Costa MdCaN, Ferreira LDA, Vasconcelos PFC, Cairncross S. Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. Tropical Medicine and International Health. 2002;7(9):757-62.
53. Tam CC, Tissera H, de Silva AM, De Silva AD, Margolis HS, Amarasinge A. Estimates of dengue force of infection in children in Colombo, Sri Lanka. PLoS Negl Trop Dis. 1 June 2013;7(6):e2259.

54. Braga C, Luna CF, Martelli CM, de Souza WV, Cordeiro MT, Alexander N, et al. Seroprevalence and risk factors for dengue infection in socio-economically distinct areas of Recife, Brazil. Acta Tropica. 2010 March 2010;113(3):234-40.
55. Inoue S, Alonzo M, Kurosawa Y, Mapua C, Reyes J, Dimaano E, et al. Evaluation of a dengue IgG indirect Enzyme-Linked Immnosorbent Assay and a Japanese Encephalitis IgG indirect Enzyme-Linked Immnosorbent Assay for diagnosis of secondary dengue virus infection Vector Borne Zoonotic Dis. 2010 2010 Mar;10(2):143-50.
56. Sule WF, Oluwayelu DO, Hernández-Triana LM, Fooks AR, Venter M, Johnson N. Epidemiology and ecology of West Nile virus in sub-Saharan Africa. Parasites & Vectors. 2018;2018(11):414.
57. World Health Organization. Zika virus (ZIKV) classification table Geneva: World Health Organization; 2018 15 February 2018.
58. Majumder MS, Hess R, Ross R, Piontkivska H. Seasonality of birth defects in West Africa: could congenital Zika syndrome be to blame? F1000Research. 2018;2018(7):159.
59. Montoya M, Collins M, Dejnirattisai W, Katzelnick L, Puerta-Guardo H, Jadi R, et al. Longitudinal analysis of antibody cross-neutralization following Zika virus and dengue virus infection in Asia and the Americas. J Infect Dis 2018 2018 Jul 13;218(4):536-45.
60. Sridhar S, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T, et al. Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy. N Engl J Med 2018 2018 Jul 26;379(4):327-40.
61. World Health Organization. WHO Position Paper on dengue. WHO; 2018. p. 457-76.
62. Thomas L, Verlaeten O, Cabié A, Kaidomar S, Moravie V, Martial J, et al. Influence of the dengue serotype, previous dengue infection, and plasma viral load on clinical presentation and outcome during a dengue-2 and dengue-4 co-epidemic Am J Trop Med Hyg. 2008;78(6):990-8.
63. Halsey ES, Marks MA, Gotuzzo E, Fiestas V, Suarez L, Vargas J, et al. Correlation of serotype-specific dengue virus infection with clinical manifestations. PLoS Negl Trop Dis. 2012;6(5):e1638.
64. Suppiah J, Ching S-M, Amin-Nordin S, Mat-Nor L-A, Ahmad-Najimudin N-A, Low GK-K, et al. Clinical manifestations of dengue in relation to dengue serotype and genotype in Malaysia: A retrospective observational study. PLoS Negl Trop Dis. 2018;12(9):e0006817.
65. Yung C-F, Lee K-S, Thein T-L, Tan L-K, Gan VC, Wong JGX, et al. Dengue Serotype-Specific Differences in Clinical Manifestation, Laboratory Parameters and Risk of Severe Disease in Adults, Singapore. Am J Trop Med Hyg. 2015;92(5):999-1005.
66. Wiwanitkit V. Concurrent malaria and dengue infection: a brief summary and comment. Asian Pac J Trop Biomed 2011 2011 Aug;1(4):326–7.
67. Epelboin L, Hanf M, Dussart P, Ouar-Epelboin S, Djossou F, Nacher M, et al. Is dengue and malaria co-infection more severe than single infections? A retrospective matched-pair study in French Guiana. Malar J. 2012;11(142).
68. Magalhães BML, Siqueira AM, Alexandre MAA, Souza MS, Gimaque JB, Bastos MS, et al. *P. vivax* malaria and dengue fever co-infection: A cross-sectional study in the Brazilian Amazon. PLoS Negl Trop Dis. 2014 October 23, 2014.
69. Carme B, Matheus S, Donutil G, Raulin O, Nacher M, Morvan J. Concurrent dengue and malaria in Cayenne hospital, French Guiana. Emerg Infect Dis 2009 2009 Apr;15(4):668–71.
70. Wasonga C, Inoue S, Kimotho J, Morita K, Ongus J, Sang R, et al. Development and evaluation of an in-House IgM-Capture ELISA for the detection of chikungunya and its application to a dengue outbreak situation in Kenya in 2013. Jpn J Infect Dis. 2015;68(5):410-4.
71. Konongoi S, Orcid X, Nyunja A, Ofula V, Owaka S, Koka H, et al. Human and entomologic investigations of chikungunya outbreak in Mandera, Northeastern Kenya, 2016. PLoS One. 2018;13(10).
72. Furuya-Kanamori L, Liang S, Milinovich G, Magalhaes RJS, Clements ACA, Hu W, et al. Co-distribution and co-infection of chikungunya and dengue viruses. BMC Infectious Diseases. 2016 3 March 2016;2016(16):84.
73. Mugabe VA, Ali S, Chelene I, Monteiro VO, Guiliche O, Muianga AF, et al. Evidence for chikungunya and dengue transmission in Quelimane, Mozambique: Results from an investigation of a potential outbreak of chikungunya virus. PLoS One. 2018 February 7, 2018;13(2):e0192110.
74. World Health Organization. Malaria rapid diagnostic test performance summary results of WHO product testing of malaria RDTs: round 1-8 (2008–2018). Geneva; 2018.
75. Ouedraogo B, Inoue Y, Kambiré A, Sallah K, Dieng S, Tine R, et al. Spatio-temporal dynamic of malaria in Ouagadougou, Burkina Faso, 2011-2015. Malaria Journal. 2018 2 April 2018;17(138).
76. van der Hoeven M, Kruger A, Greeff M. Differences in health care seeking behaviour between rural and urban communities in South Africa. Int J Equity Health. 2012;11(31).
77. Weinick RM, Zuvekas SH, Cohen JW. Racial and ethnic differences in access to and use of health care services, 1977 to 1996 Medical Care Research and Review. 2000;57 (Supplement 1):36-54.
78. Durbin AP, Schmidt A, Elwood D, Wanionek KA, Lovchik J, Thumar B, et al. Heterotypic dengue infection with live attenuated monotypic dengue virus vaccines: Implications for vaccination of populations in areas where dengue is endemic J Infect Dis. 2011 2011 Feb 1;203(3):327–34.
79. World Health Organization. Dengue vaccines: WHO position September 2018. Geneva, Switzerland: World Health Organization; 2018.

Appendix A. Case report form for passive fever surveillance

Appendix A. Case report form for passive fever surveillance

Serial no. of the study ID _ _ _ _

INTERNATIONAL VACCINE INSTITUTE

Clinical findings
Passive Surveillance
V1: Acute blood draw (at the ASH ☐ GH ☐

Inclusion Criteria (if check 'no' for any of the inclusion criteria, STOP)	**Yes[1]**	**No[2]**
1. 1 ≤ age ≤ 55 years old		
2. Patients presenting with fever (≥ 37.5º C) by (any) thermometer or history of fever for < 7 days of duration without localizing sign		
3. Residents of the selected areas of Lambaréné (for longer than 1 year).		
4. Not participated/enrolled in any of the dengue vaccine trial conducted in Gabon		
5. If under the age of 18 years old, informed consent signed by at least one parent or guardian and assent form signed by child aged 7-17 years.		
Exclusion criteria- for the follow-up cohort (if check 'yes' for any of the exclusion criteria, STOP)	**Yes[1]**	**No[2]**
1 Individuals with plans to move out of the catchment area of Lambaréné within the study period		
2. Individuals suffering from known cause or etiology listed in the patient identification SOP.		
Enrollment – Are you already enrolled in the study? (can be re-enrolled if the current illness has had an interval of a minimum of 21 days between the onsets of the previous fever episodes)		

1. Basic information

1.1 Study ID No. F_ _ _ _ _ _ _	1.2 IPD[1] ☐ OPD[2] ☐	1.3 Area __ __
1.4 Date of Visit (DD/MM/YYYY) : _ _/_ _/_ _ _ _	1.5 Date of Birth (DD/MM/YYYY) : _ _/_ _/_ _ _ _	
1.6 Age: __ __ yrs __ __ mo	1.7 Sex: Male[1] ☐ Female[2] ☐	1.8 If female, are you pregnant? Yes[1] ☐ No[2] ☐
1.9 Date of fever onset (DD/MM/YYYY): _ _/_ _/_ _ _ _	1.10 For how many days did you have fever? __ __ days	
1.11 Weight __ __ __ Kg	1.12 Height __ __ __ cm	1.13 Blood pressure __ __ __ / __ __ __ N/A ☐ (Systolic/Diastolic)
1.14 Respiratory rate __ __ /min	1.15 Pulse rate __ __ __ BPM	
1.16 Current temperature __ __ . __ °C	1.17 Tourniquet test <10[1] ☐ 10 -19[2] ☐ >19[3] ☐ N/A[9] ☐	

2. General health conditions

2.1 How difficult was it to perform your daily activities (jobs, schools, household chores, etc) before this visit?

Not at all[1] ☐ Mild[2] ☐ Moderate[3] ☐ Severe[4] ☐ Extreme(cannot perform)[5] ☐

2.2 Pre-existing conditions (check all that apply) Cardiovascular ☐ Diabetes ☐ Lung disease ☐ Cerebrovascular ☐

Musculoskeletal ☐ Gastrointestinal ☐ Renal disease ☐ Anemia ☐ Liver disease ☐ Other ☐ , _____ ☐☐

3. Signs and symptoms at visit 1							
3.1 General	Yes[1]	No[2]	Unknown[9]	**3.5 hemorrhagic**	Yes[1]	No[2]	Unknown[9]
3.1.1 Rash				3.5.1 Epistaxis			
3.1.2 Fatigue/weakness				3.5.2 Gum bleeding			
3.1.3 Alterations to consciousness				3.5.3 Ecchymosis (spontaneous)			
3.2 Head				3.5.4 Petechiae (spontaneous)			
3.2.1 Headache				3.5.5 Hematemesis (spontaneous)			
3.2.2 Retro-orbital pain				3.5.6 Hematuria			
3.2.3 Neck pain				3.5.7 Melena			
3.2.4 Otalgia				**3.6 Signs of shock**			
3.2.5 Nasal congestion				3.6.1 Cyanosis			
3.2.6 Rhinorrhea				3.6.2 Capillary refill >2 sec			
3.2.7 Sore throat				**3.7 Others**			
3.3 Respiratory				3.7.1 Arthralgia			
3.3.1 Cough				3.7.2 Myalgia			
3.3.2 Expectoration				3.7.3 Hepatomegaly			
3.3.3 Dyspnea				3.7.4 Oliguria			
3.4 Gastrointestinal				3.7.5 Jaundice			
3.4.1 Nausea/vomiting				3.7.6 Flushed face			
3.4.2 Diarrhea				3.7.7 Loss of appetite			
3.4.3 Constipation				3.7.8 Convulsion/seizure			
3.4.4 Abdominal pain				3.7.9 Cervical lymphadenopathy (> 0.5cm)			
3.4.5 Ascites				3.7.10 Pleural effusion by Chest X-ray			

4. Medical History							
	Yes[1]	No[2]	Unknown[9]		Yes[1]	No[2]	Unknown[9]
4.1 Previous dengue infection				4.3 Previous Chikungunya infection			
4.2 Previous hospitalization for dengue				4.4 Previous malaria infection			
4.5 If yes to 4.1, date of the most recent dengue infection __ __/__ __ __ __ (MM/YYYY) unknown ☐							
4.6 If yes to 4.2, was the hospitalization based on clinical diagnosis[1] ☐ lab-confirmation[2] ☐ or unknown[9] ☐ ?							
4.7 If yes to 4.2, date of the most recent dengue hospitalization __ __/__ __ __ __ (MM/YYYY) unknown ☐							
4.8 If yes to 4.2, duration of the most recent hospitalization __ __ days unknown ☐							

5. Laboratory findings	Dates of sample collection (DD/MM/YYYY)						
	__/__/____	__/__/____	__/__/____	__/__/____	__/__/____	__/__/____	__/__/____
5.1 Platelets (x 10^3/uL)	____ ND_	____ ND_	____ ND_	____ ND_	____ ND_	____ ND_	____ ND_
5.2 Highest hematocrit (%)	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_
5.3 Hemoglobin (g/dL)	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_
5.4 Leukocytes (x 10^3/uL)	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_
5.5 Neutrophils (%)	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_
5.6 Lymphocytes (%)	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_
5.7 Total protein (g/L)	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_	__ ND_
5.8 Albumin (g/dL)	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_
5.9 AST (u/L)	_____._ND_	_____._ND_	_____._ND_	_____._ND_	_____._ND_	_____._ND_	_____._ND_
5.10 ALT (u/L)	_____._ND_	_____._ND_	_____._ND_	_____._ND_	_____._ND_	_____._ND_	_____._ND_
5.11 Total bilirubin (mg/dL)	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_	__._ ND_
5.12 Urine WBC (HPF)	_____ ND_	_____ ND_	_____ ND_	_____ ND_	_____ ND_	_____ ND_	_____ ND_
5.13 Urine protein	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_
5.14 Urine sugar	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_	Pos[1]_Neg[2]_ND[9]_

6. Clinical diagnosis (check one where appropriate)

6.1 Undifferentiated fever[1] ☐ 6.2 Dengue fever[2] ☐ 6.3 DHF[3] ☐ 6.3.1 If DHF, grade _____ on N/A ☐

6.4 Non-dengue infection[4] ☐ (if check, please choose one from below)

6.4.1 URI[U] ☐ 6.4.2 Influenza[I] ☐ 6.4.3 Bronchitis[B] ☐ 6.4.4 Pneumonia[P] ☐

6.4.5 Otitis media[O] ☐ 6.4.6 Viral syndrome[V] ☐ 6.4.7 UTI[T] ☐ 6.4.8 Diarrheal illness[D] ☐

6.4.9 Chicken pox[C] ☐ 6.4.10 Measles[M] ☐ 6.4.11 Malaria[A] ☐ 6.4.12 Other[R] ☐_____ ☐☐

7. Treatment received

7.1 Antibiotics Yes[1] ☐ No[2] ☐	7.2 Start date (DD/MM/YYYY) __/__/____	7.3 End date (DD/MM/YYYY) __/__/____
7.4 Paracetamol Yes[1] ☐ No[2] ☐	7.5 Start date (DD/MM/YYYY) __/__/____	7.6 End date (DD/MM/YYYY) __/__/____
7.7 Ibuprofen Yes[1] ☐ No[2] ☐	7.8 Start date (DD/MM/YYYY) __/__/____	7.9 End date (DD/MM/YYYY) __/__/____

CRF version No. DVI – P11 Version 1.0 Oct. 2014

7.10 Aspirin Yes[1] ☐ No[2] ☐	7.11 Start date (DD/MM/YYYY) __/__/____	7.12 End date (DD/MM/YYYY) __/__/____
8. Outcome		
8.1 Outcome of this visit: Hospitalized[1] ☐ Returned home[2] ☐ Left against medical advice[3] ☐ Referral[4] ☐		
****Only for hospitalized subject****		
9. Hospitalization		
9.1 Date of admission (DD/MM/YYYY) __/__/____	9.2 Admission diagnosis _____	
9.3 Date of discharge (DD/MM/YYYY) __/__/____	9.4 Discharge diagnosis _____	
9.5 Complication: Yes[1] ☐, define _____ ☐☐ No[2] ☐		

	Yes[1]	No[2]	Unknown[9]		Yes[1]	No[2]	Unknown[9]
9.6 Bleeding manifestations				9.8 Evidence of shock			
9.7 Plasma leakage				9.8.1 Persistently low BP			
9.7.1 Hemoconcentration				9.8.2 No detectable BP			
9.7.2 Anasarca				9.8.3 Cold extremities			

9.9 Treatment	IV fluids[1] ☐	Blood/blood product transfusion[2] ☐	Others[3] ☐, define _____ _____ _____ _____ _____

Hospital charges (during visit 1 ONLY at the facility) Passive Surveillance			
H1 Study ID No. F __ __ __ __ __ __ __ __ __			
H2 In total hospital charges, admission charges: __ __ __ __ __ __ __ __ CFA	Not available ☐		
H3 Laboratory (Test) related charges: __ __ __ __ __ __ __ __ __ CFA	Not available ☐		
H4 Medication (prescription) related charges: __ __ __ __ __ __ __ __ __ CFA	Not available ☐		
H5 Overall hospital charges (H2+H3+H4): __ __ __ __ __ __ __ __ __ CFA	Not available ☐		
H6 The amount of out of pocket payment by the patient: __ __ __ __ __ __ __ __ __ CFA	Not available ☐		
H7 who paid/will pay majority for the services provided at the clinic/hospital?	Parent[1] ☐	Legal guardian[2] ☐	Grandparents/relatives[3] ☐
	Insurance[4] ☐	Neighbor[5] ☐	Other[6] ☐, _____ ☐☐

Clinical findings
Convalescent blood draw (V2)
At the ASH or GH[1] ☐ OR Home visit[2] ☐

1. Basic information		
1.1 Study ID No. F _ _ _ _ _ _ _ _	1.2 IPD[1] ☐ OPD[2] ☐ (during the illness)	1.3 Area _ _ _
1.4 Date of Visit1 (DD/MM/YYYY) _ _/_ _/_ _ _ _	1.5 Date of Follow-up Visit (DD/MM/YYYY) _ _/_ _/_ _ _ _	
1.6 Have symptoms of the illness ended: Yes[1] ☐ No[2] ☐	1.7 Duration of fever _ _ _ days	

2. Signs and symptoms during this illness

2.1 General	Yes[1]	No[2]	Unknown[9]	2.5 hemorrhagic	Yes[1]	No[2]	Unknown[9]
2.1.1 Rash				2.5.1 Epistaxis			
2.1.2 Fatigue/weakness				2.5.2 Gum bleeding			
2.1.3 Alterations to consciousness				2.5.3 Ecchymosis, spontaneous			
2.2 Head				2.5.4 Petechiae, spontaneous			
2.2.1 Headache				2.5.5 Positive tourniquet test			
2.2.2 Retro-orbital pain				2.5.6 Hematemesis, spontaneous			
2.2.3 Neck pain				2.5.7 Hematuria			
2.2.4 Otalgia				2.5.8 Melena			
2.2.5 Nasal congestion				**2.6 Signs of shock**			
2.2.6 Rhinorrhea				2.6.1 Capillary refill >2 sec			
2.2.7 Sore throat				2.6.2 Cyanosis			
2.3 Respiratory				**2.7 Others**			
2.3.1 Cough				2.7.1 Arthralgia			
2.3.2 Expectoration				2.7.2 Myalgia			
2.3.3 Dyspnea				2.7.3 Hepatomegaly			
2.4 Gastrointestinal				2.7.4 Oliguria			
2.4.1 Nausea/vomiting				2.7.5 Jaundice			
2.4.2 Diarrhea				2.7.6 Flushed face			
2.4.3 Constipation				2.7.7 Convulsion/seizure			
2.4.4 Abdominal pain				2.7.8 Cervical node			
2.4.5 Ascites				2.7.9 Loss of appetite			
				2.7.10 Pleural effusion by CXR			

3. Final outcome				
3.1 Final diagnosis _____				
3.2 Outcome of illness:	Recovery [1] ☐	Sequelae [2] ☐	Death [3] ☐	
	Referral to another hospital [4] ☐	Left against medical advice [5] ☐		
3.3 If 'death' checked, was cause of death:	Dengue infection [1] ☐	not dengue-related [2] ☐		
3.4 If 'dengue-related, the main cause of death	Severe bleeding [1] ☐	Fluid overload [2] ☐	Prolonged shock [3] ☐	
	Organ Failure [4] ☐, define _____ ☐☐	Other [8] ☐, define _____ ☐☐		
3.5 Participation terminated early Yes [1] ☐ No [2] ☐	3.6 If yes, date of early termination (DD/MM/YYYY) __/__/____			
3.7 Reason for termination of participation	Study complete [1] ☐	Consent withdrawal [2] ☐	Loss of follow-up [3] ☐	By investigator [4] ☐
3.8 Reason for termination by investigator	Moved out of study area [1] ☐	Other severe medical conditions [2] ☐	Death [3] ☐	Other [4] ☐

Laboratory findings Passive Surveillance							
Dengue testing results							
L1 Study ID No. F__ __ __ __ __ __ __ __							
L2 Date of the acute blood draw (DD/MM/YYYY) __/__/____			Phlebotomist initials __ __ __ __ __				
L3 Date of the convalescent blood draw (DD/MM/YYYY) __/__/____			Phlebotomist initials __ __ __ __ __				
ELISA for dengue	IgM		IgG				
Acute sample	L4 __ __ __ __		L8 __ __ __ __				
Convalescent Sample	L6 __ __ __ __		L10 __ __ __ __				
Acute sample				Convalescent Sample			
	Positive [1]	Negative [2]	Not done [9]		Positive [1]	Negative [2]	Not done [9]
L12 JE rapid							
L13 ELISA DENV IgG				L16 ELISA DENV IgG			
L14 ELISA DENV IgM				L17 ELISA DENV IgM			
L15 RT-PCR							
L18 If RT-PCR positive, serotype by PCR : DENV_____ or N/A ☐							
L19 Interpretation	Non-dengue [1] ☐	Primary DENV infection [2] ☐	Secondary DENV infection [3] ☐	Questionnable [4] ☐			

Appendix B. Data collection form for the serosurvey

Appendix B. Data collection form for the serosurvey

Serial No. in the Subject ID ___ ___ ___ ___

INTERNATIONAL VACCINE INSTITUTE

Clinical findings Serological survey blood draw (bleeding no. __)							
Inclusion Criteria (if check 'no' for any of the inclusion criteria, STOP)						**Yes[1]**	**No[2]**
1. 1 ≤ age ≤ 55 years old							
2. Residents of the selected areas of Lambaréné							
3. Not participated and not enrolled in any of the ongoing dengue vaccine trial in Gabon							
4. If under the age of 18 years old, informed consent signed by at least one parent or guardian and assent form signed by child aged 7-17 years.							
Exclusion criteria- for the follow-up cohort (if check 'yes' for any of the exclusion criteria, STOP)						**Yes[1]**	**No[2]**
1 Individuals with plans to move out of the catchment area of Lambaréné within pre and post sero survey period (~6 months)							
2 Individuals who are willing to provide only a single blood sample not as paired samples							
Enrollment – Are you already enrolled in the study?							
1. Basic information							
1.1 Serosurvey ID No. S _ _ _ _ _ _ _ _	1.2 Sex: Male[1] ☐ Female[2] ☐				1.3 Cluster/Area _ _ / _ _		
1.4 Date of Birth (DD/MM/YY) _ _/_ _/_ _	1.5 Ethnicity: Gabonese[1] ☐ Caucasian[2] ☐ Others[3] ☐ (Others: _____)						
2.Medcal History							
2.0 Date of Visit (DD/MM/YYYY) _ _/_ _/_ _ _ _	2.1 Wt _ _ _ kg			2.2 Ht _ _ _cm			
2.3 Pre-existing conditions	Cardiovascular ☐ Diabetes ☐ Lung disease ☐ Cerebrovascular ☐						
Musculoskeletal ☐ Gastrointestinal ☐ Renal disease ☐ Anemia ☐ Liver disease ☐ Other ☐ , _____							
Please consider history of the subject's lifetime if this is the 1st bleeding, otherwise, record the history of last 6 months.							
	Yes[1]	**No[2]**	**Unknown[9]**			**Yes[1]**	**No[2]**	**Unknown[9]**
2.4 Previous dengue infection				2.5 Previous hospitalization for dengue			
2.6 Known previous chikungunya infection				2.7 Known previous malaria infection			
2.8 If yes to 2.4, date of the most recent dengue infection __ __/__ __ __ __ (MM/YYYY) Don't know[9] ☐							
2.9 If yes to 2.5, was the hospitalization based on clinical diagnosis[1] ☐ or lab-confirmation[2]? ☐ Don't know[9] ☐							
2.10 If yes, to 2.5, what was your clinical diagnosis (check one that apply)	Undifferentiated fever[1] ☐ Suspected dengue[2] ☐ Primary dengue[3] ☐ Secondary dengue[4] ☐ DHF[5] ☐ None of the above[6] ☐						
2.11 Have you visited our study facility with febrile illness in the past 6 months? Yes[1] ☐ No[2] ☐							
2.12 If yes, record the study ID assigned (record multiple, as needed) 1. F_ _ _ _ _ _ _ _ 2. F_ _ _ _ _ _ _ _ 3. F_ _ _ _ _ _ _ _ 4. F_ _ _ _ _ _ _ _ 5. F_ _ _ _ _ _ _ _ 6. F_ _ _ _ _ _ _ _							
Please consider history of last 5 years if this is the 1st bleeding, otherwise, record the history of last 6 months.							
3.1 Have you had fever? Yes[1] ☐ No[2] ☐	3.2 If yes to 3.1, how many times? _ _ times						
Febrile episodes	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐		
3.3 Onset date(MM/YYYY)							

3.4 Duration(days)					
3.5 Admission (yes[1]/no[2])					
3.6 Diagnosis					
3.7 Remark if Dengue suspected					

4. Signs and symptoms during this illness (during the past 6 months)
Please answer below for the episode of dengue-suspected febrile illness. If none of the febrile episodes have been dengue-probable, please answer the below based on the last febrile episode.

4.1 General	Yes[1]	No[2]	Unknown[9]	4.4 Respiratory	Yes[1]	No[2]	Unknown[9]
4.1.1 Rash				4.4.1 Cough			
4.1.2 Fatigue/weakness				4.4.2 Sputum production			
4.2 Head				4.4.3 Breathing difficulty			
4.2.1 Headache				**4.5 Hemorrhagic**			
4.2.2 Retro-orbital pain				4.5.1 Epistaxis			
4.2.3 Neck pain				4.5.2 Gum bleeding			
4.2.4 Earache				4.5.3 Bruise			
4.2.5 Nasal congestion				4.5.4 Petechiae			
4.2.6 Rhinorrhea				4.5.5 Hemitemesis			
4.2.7 Sore throat				4.5.6 Hematuria/melema			
4.3 Gastrointestinal				**4.6 Others**			
4.3.1 Nausea/vomiting				4.6.1 Arthralgia			
4.3.2 Diarrhea				4.6.2 Myalgia			
4.3.3 Constipation				4.6.3 Loss of appetite / anorexia			
4.3.4 Abdominal pain				4.6.4 Flushed face			

4.7 PE: All Normal[1] ☐ Abnormal[2] ☐ , _____

4.8 Blood drawing(5-7cc) Yes[1] ☐ No[2] ☐ , Reason_____

Appendix C. Chapter 2 Supplementary File (Tables 1, 2A, and 2B): Data extraction from descriptive studies on economic/clinical benefit of dengue RDT use

Supplementary File (Tables 1, 2A, and 2B)

Data extraction from descriptive studies on economic/clinical benefit of dengue RDT use

Table 1. Basic information and introduction of the articles

Basic information				Introduction
Authors	**Year of publication**	**Journal**	**Title**	**Context/ study question (relevant to the review question)**
Mitra, Shubhanker; Choudhari, Rajat; Nori, Harshita; et al. &	2016	JOURNAL OF VECTOR BORNE DISEASES &	Comparative evaluation of validity and cost-benefit analysis of rapid diagnostic test (RDT) kits in diagnosis of dengue infection using composite reference criteria: A cross-sectional study from south India	To determine the sensitivity, specificity and predictive value of four commercially available RDTs [Panbio Dengue Duo cassette, Standard Diagnostics (SD) Bioline Dengue Duo, J. Mitra Dengue Day-1 test and Reckon Dengue IgG/IgM] against composite reference criteria (CRC), and compare the cost of the tests
A meeting abstract by Mitra, S.; Choudhari, R.; Nori, H.; et al.(1, 2)		INTERNATIONAL JOURNAL OF INFECTIOUS Meeting Abstract (2014)	Performance and cost-effectiveness of immunochromatography based rapid diagnostic test (RDT) kits in diagnosis of dengue infection in resource limited set up	
Lubell, Yoel; Althaus, Thomas; Blacksell, Stuart D.; et al.(3)	2016	PLOS ONE	Modelling the impact and cost-effectiveness of biomarker tests as compared with pathogen-specific diagnostics in the management of undifferentiated fever in remote tropical settings	To assess the ability of dengue and scrub typhus rapid tests to improve antibiotic targeting in primary care, as compared with testing for elevated C-Reactive Protein (CRP), a biomarker of host inflammation To determine the likely cost-effectiveness of the approaches be as compared with current practice in community care of febrile patients in the rural tropics

1. Mitra S, Choudhari R, Nori H, al. e. Comparative evaluation of validity and cost-benefit analysis of rapid diagnostic test (RDT) kits in diagnosis of dengue infection using composite reference criteria: A cross-sectional study from south India. Journal of vector borne diseases. 2016;53(1):30-6.
2. Mitra S, Choudhari R, Nori H. Performance and cost-effectiveness of immunochromatography based rapid diagnostic test (RDT) kits in diagnosis of dengue infection in resource limited set up. International journal of infectious diseases. 2014;21(Meeting Abstract: 64.018):450.
3. Lubell Y, Althaus T, Blacksell SD, Paris DH, Mayxay M, Pan-Ngum W, et al. Modelling the impact and cost-effectiveness of biomarker tests as compared with pathogen-specific diagnostics in the management of undifferentiated fever in remote tropical settings. PLoS One. March 30, 2016;11(3):e0152420.

Table 2A. Data extracted from the articles

Article number	Authors	Methods									
		Target population	Population size	Age	location	Study design	Cost being evaluated	Comparators	Time horizon	Health outcome	# cases
1	Mitra et al. (including the meeting abstract)	Patients who sought care for AFI at the study hospital in Vellore, India (using stored blood samples)	281 patients with community acquired acute febrile illness	>18 years	Christian Medical College (CMC), Vellore, India	prospective cross-sectional observational study	The cost per test (as per manufacturer's quoted price in India)	used the composite reference criteria (CRC) for diagnosis of dengue-related illness to compare the performance of the four RDTs from 4 manufacturers (Panbio, SD, J.Mitra and Reckon)	September 2012-February 2013	Dengue infections against other cases of proven alternative diagnosis	132 cases of dengue (149 controls)
2	Lubell et al.	Outpatients who sought care with fever	1083 outpatients (among 1938 febrile patients recruited)	5-49 years	three provincial hospitals in the provinces of Salavan, Luang Namtha, and Xieng Khouang in rural Laos	Cost-effectiveness modelling based on data from a hospital-based prospective fever study	cost effectiveness of different testing approaches, including a dengue RDT	the ability of dengue and scrub typhus rapid tests to inform antibiotic treatment, as compared with testing for elevated C-Reactive Protein (CRP)	(based on data collected) May, 2008-December, 2010	A viral/bacterial infection (Influenza, leptospirosis, scrub typhus, dengue, etc.)	156 dengue cases

<table>
<tr><th>Article No.</th><th colspan="6">Methods</th></tr>
<tr><th></th><th colspan="4">Effectiveness</th><th>Preference based (measurement /valuation)</th><th>Estimated costs (resources)</th><th>Currency (price date/conversion)</th><th>Model choice</th><th>Methods</th></tr>
<tr><th></th><th colspan="3">Sensitivity & specificity</th><th>Single study or synthesis-based?</th><th>Note.</th><th></th><th></th><th></th><th></th><th></th></tr>
<tr><td>1</td><td colspan="3"><table>
<tr><td rowspan="2">Manufacturer</td><td colspan="4">Comparison of the performance of dengue RDTs</td></tr>
<tr><td colspan="2">IgM assay</td><td colspan="2">NS1 assay</td></tr>
<tr><td></td><td>Sensitivity (%) (95% CI)</td><td>Specificity (%) (95% CI)</td><td>Sensitivity (%) (95% CI)</td><td>Specificity (%) (95% CI)</td></tr>
<tr><td>Panbio</td><td>97.7 (93.5–99.5)</td><td>87.8 (81.5–92.5)</td><td>NA</td><td>NA</td></tr>
<tr><td>SD Bioline</td><td>64.3 (55.4–72.6)</td><td>96.6 (92.2–98.9)</td><td>20.9 (14.3–28.9)</td><td>97.3 (93.2–99.2)</td></tr>
<tr><td>Reckon</td><td>13.9 (8.6–21.2)</td><td>99.3 (96.2–99.9)</td><td>18.6 (12.3–26.4)</td><td>96.6 (92.2–98.9)</td></tr>
<tr><td>J. Mitra</td><td>36.4 (28.1–45.4)</td><td>68.7 (60.6–76.1)</td><td>27.1 (19.7–35.7)</td><td>92.5 (87.0–96.2)</td></tr>
</table></td><td>Single study</td><td>Comparison of sensitivity, specificity and predictive values of four commercial RDTs was done against CRC</td><td>measurement</td><td>Cost of the study (no costs for the RDT kits -2 manufacturers [J. Mitra and Reckon] provided test kits for testing free of cost and other two tests were part of the routine testing in the study hospital)</td><td>US$</td><td>Four commercially available and most commonly used RDTs were selected for the study, from the following manufacturers: Panbio®(Dengue Duo cassette), Standard Diagnostics Bioline (Dengue Duo), J. Mitra (Dengue Day-1 test), and Reckon Diagnostics (Dengue IgG/ IgM)</td><td>Measuring the performance of the four commercially available and widely used RDTs and comparing sensitivities and specificities against CRC</td></tr>
<tr><td>2</td><td colspan="3">They assumed a sensitivity and specificity of 95% for a dengue RDT when performed on presentation and for Scrub typhus IgM RDTs (based on beta distribution). Authors have also assumed the same baseline accuracy with no cross reactivity with other rickettsial infections.</td><td>Synthesis-based</td><td>-</td><td>valuation</td><td>Data obtained from a previously conducted fever study; cost of resources for the modelling efforts</td><td>US$</td><td>Cost-effectiveness assessment of the tests in primary care setting</td><td>Economic evaluation of diagnostics using a decision tree model and calculating the number of DALYs averted for each strategy using as inputs probability of an antibiotic being effective for the</td></tr>
</table>

	bacterial pathogens, as well as the estimated excess duration of illness and mortality in patients that did not receive an effective antibiotic.

Table 2B. Data extracted from the articles

Article No.	Results				Discussion			
	Parameters	**Incremental costs and outcomes (cost-effectiveness)**	**Uncertainty**	**Heterogeneity**	**Study findings**	**Limitations**	**Generalizability**	**Funding source**
1	Performance (accuracy value) of the RDT kits for diagnosis of acute dengue febrile illness (sensitivity, specificity, PPV and NPV) and different costs of the kits	The cost per test for Panbio, SD, Reckon and J. Mitra is US$ 6.90, 4.27, 3.29 and 3.61	-	seroprevalence of IgG positivity measured in the population using Panbio IgG RDT was lower (49.3%), compared to a previously measured 93% in a household based survey	In dengue outbreak, Panbio IgM capture RDT alone could be a reliable and easily available test for use in resource-limited setting; other 3 RDTs of NS1 assay may not be reliable for the diagnosis of acute dengue infection with low sensitivity. The cost per test for Panbio, SD, Reckon and J. Mitra is US$ 6.90, 4.27, 3.29 and 3.61 respectively.	Comparison of the sensitivity, specificity and predictive values of four commercial RDTs was made against CRC, which may not be the gold standard of dengue confirmation; comparison could not be done with standard ELISA based NS1 or IgM capture assay; further verification of the lab results (due to possible cross-reactivity with other flaviviruses) was not done due to resource constraints	Generalizable in similar settings – where dengue prevalence as high as Vellore, India	NA
2	Sensitivity and specificity of dengue and scrub typhus tests; Mortality rate for bacterial infections in the absence of an effective antibiotic; Years of life lost per death; Cost of RDTs; Cost of a course of antibiotics; Probability of treatment in patients with a negative dengue or scrub typhus test (38% and uniform distribution used)	Median incremental cost (CrI) $1.5 (0.5; 3.2) Median DALYs averted (CrI) -0.006 (-0.301; 0.089) for a dengue RDT. Dengue RDT is dominated by current practice, with a higher cost and fewer numbers of DALYs averted.	A probabilistic Sensitivity analysis was done with relevant distributions, to address uncertainty in most parameters. From the cost effectiveness acceptability curve, it was shown	Variable utility and accuracy of tests, subject to seasonal and spatial heterogeneity, whether used alone or in combination Heterogeneity in fever etiology Variability in terms of the incidence of different	Use of dengue RDTs would lead to a reduction in antibiotics prescription for viral infections, whereas use of scrub typhus RDTs led to a larger proportion of bacterial infections receiving antibiotics. The CRP test performed better than the two tests above in terms of reduction in antibiotic prescription for both viral and bacterial infections. The model showed that the dengue test offers	These simulations use data from a fever study in which enrollees did not have an identifiable pathogen or had multiple pathogens as the cause of illness. For patients with no identifiable cause, implication of the findings is not clear. For patients with multiple pathogens, a positive test result for a specific viral pathogen could mistakenly suggest that no antibiotic is required, when a treatable bacterial infection is also present. There is a need for further clinical studies about these approaches. Another	In cases without an identified pathogen, CRP tests may be an effective tool (albeit imperfect) to guide antibiotic prescription. Overall, the model outputs will have limited generalizability to the broader	The fever study where the aetiology data originated was funded by the WHO WPRO; the Australian Agency for International Development, the Ministry of Foreign Affairs of Japan, and the USAID; the

	Main assumptions and inputs: the differences in resources use are only those related to the diagnostics and treatments; other capital and labour overheads are similar in all strategies; for the costs of tests, a gamma distribution was applied with a mean of $1.5; the cost of a course of antibiotic is estimated at a mean of $0.5; all self-limiting viral infections and treated bacterial infection are associated with a week of ill health with a disability weight of 0.053; bacterial infections that do not receive an appropriate treatment are associated with a further week of illness and a 1% mortality rate; each of these deaths is associated with a mean loss of 45 life-years (one way sensitivity analysis between 20-60); the willingness to pay threshold was set at $1400		that Dengue RDT is associated being cost effective <50% at any value of willingness-to-pay	infections, and baseline antibiotic prescription practices	little or no advantage over current practice (-0.006 median DALYs averted). The scrub typhus averted an average 0.031 DALYs and the CRP test averted 0.017 DALYs per febrile episode. These estimates suggest that either the scrub typhus or CRP testing is likely to be cost-effective, given uncertainty in many model parameters.	limitation is that it did not account for the longer-term societal health and economic costs associated with antibiotic consumption and resistance. It would be necessary to incorporate these aspects into economic evaluation for more comprehensive assessments of the costs and benefits involved.	population of febrile patients.	Foundation for Innovative New Diagnostics (the UK Department for International Development), National Center for Immunization and Respiratory Diseases, US CDC; and the Wellcome Trust

Appendix D. Chapter 4 S1 STROBE checklist

STROBE Statement: Clinical and epidemiologic characteristics associated with dengue fever before and during the 2017 outbreak in Mombasa, Kenya

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1, title; 3, para 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 3, para 2-3)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 5 para 2-3)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 6 para 1)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 8 para 1-2)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 7 para 1-3; page 8 para 2)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (Page 9 para 2)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 10 para 1; Page 11 para 2; page 12 para 2)
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 10 para 1; page 11 para 2-3; page 12 para 2-3)
Bias	9	Describe any efforts to address potential sources of bias (Page 12 para 3; page 24 para 2)
Study size	10	Explain how the study size was arrived at (Figure 2 on page 37)

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (Page 11 para 1-2; page 12 para 2-3)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 11 para 2; page 12 page 2-3)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 11 para 2 - subgroups)
		(*c*) Explain how missing data were addressed (Page 13 para 3; page 24 para 2)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (Not applicable)
		(*e*) Describe any sensitivity analyses (none)

Results

Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (Figure 2 on page 37)
		(b) Give reasons for non-participation at each (Figure 2 on page 37)
		(c) Consider use of a flow diagram (Figure 2 on page 37)
Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 13 para 3; Table 1 page 14)
		(b) Indicate number of participants with missing data for each variable of interest (Table 1, footnote on page 16)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) (Not applicable)
Outcome data	15	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures (Page 13 para 3, Table 1 on page 14)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Page 19 para 1; Page 21 para 1; tables 2, 3A/B, and 4 on pages 18-22)
		(*b*) Report category boundaries when continuous variables were categorized (Page 11, para 1-2; specified in the tables)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (Not

		applicable)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Not applicable)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 22 para 1; page 23 para 1; page 26 para 1, 3)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 29 para 1)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 23 para 2-3; Page 24 para 1; Page 25 para 1-2; Page 26 para 1; Page 27 para 1-2; Page 28 para 1-3)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 29 para 1)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Financial disclosure on pages 30-31)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix E. Chapter 4 S2 Table S1: Data by 3-level dengue-confirmation status

Table S1. Demographic and clinical characteristics by dengue confirmation status (confirmed-, probable dengue, non-dengue) among febrile enrollees of the health facility-based fever surveillance in Mombasa, Kenya in 2016-2017

Characteristics	Dengue-confirmed (n=223)	Dengue-probable (n=72)	Non-dengue (n=187)	Total (n=482)	p-value
Place of enrollment					0.215
CPGH	97 (43.50)	42 (58.33)	94 (50.27)	233 (48.34)	
Tudor	100 (44.84)	23 (31.94)	70 (37.43)	193 (40.04)	
Ganjoni	26 (11.66)	7 (9.72)	23 (12.30)	56 (11.62)	
Mean age (SD)	23.04 (8.28)	24.29 (11.71)	23.14 (13.46)	23.27 (11.05)	0.694
Age group (years)					**<.001**
1-4	4 (1.79)	4 (5.56)	31 (16.58)	39 (8.09)	
5-9	6 (2.69)	4 (5.56)	6 (3.21)	16 (3.32)	
10-14	11 (4.93)	2 (2.78)	6 (3.21)	19 (3.94)	
15-19	38 (17.04)	7 (9.72)	21 (11.23)	66 (13.69)	
20-24	95 (42.60)	29 (40.28)	39 (20.86)	163 (33.82)	
25-34	48 (21.52)	13 (18.06)	44 (23.53)	105 (21.78)	
35-44	17 (7.62)	7 (9.72)	28 (14.97)	52 (10.79)	
45-55	4 (1.79)	6 (8.33)	12 (6.42)	22 (4.56)	
Female	87 (39.01)	30 (41.67)	90 (48.13)	207 (42.95)	0.173
IPD/OPD	1 (0.45)/222 (99.55)	1 (1.39)/71 (98.61)	0/187 (100.0)	2 (0.41)/480 (99.59)	0.296
Fever duration prior to visit (SD)	2.96 (1.86)	2.97 (2.12)	2.84 (1.79)	2.91 (1.87)	0.806
Fever duration, entire illness (SD)*	7.04 (3.78)	6.28 (3.61)	4.91 (2.76)	6.17 (3.55)	**<.001**
Temperature at presentation (SD)	37.87 (0.67)	37.81 (0.63)	37.71 (0.73)	37.80 (0.69)	0.064
Temperature at presentation					**0.031**
Below 38.0°c	132 (59.19)	47 (65.28)	134 (71.66)	313 (64.94)	
≥ 38.0°c	91 (40.81)	25 (34.72)	53 (28.34)	169 (35.06)	
Prev. dengue infection	2 (0.90)	1 (1.39)	3 (1.60)	6 (1.24)	0.426
YF vaccination	115 (51.57)	31 (43.06)	77 (41.18)	223 (46.27)	0.092
Clinical diagnosis					
Suspected dengue	156 (69.96)	30 (41.67)	18 (9.63)	204 (42.32)	**<.001**
Undifferentiated fever	44 (19.73)	32 (44.44)	121 (64.71)	197 (40.87)	
Non-dengue	23 (10.31)	10 (13.89)	48 (25.67)	81 (16.80)	
URI (% of non-dengue)	13 (56.52)	5 (50.00)	27 (56.25)	45 (55.56)	
Malaria	1 (4.35)	0	3 (6.25)	4 (4.94)	
UTI	2 (8.70)	0	2 (4.17)	4 (4.94)	
Pneumonia	0	0	3 (6.25)	3 (3.70)	
Diarrheal illness	1 (4.35)	0	1 (2.08)	2 (2.47)	

Others	6 (26.09)	5 (50.00)	12 (25.00)	23 (28.40)	
Signs and symptoms (presence)					
Rash	27 (12.11)	7 (9.72)	10 (5.35)	44 (9.13)	0.060
Fatigue/weakness	205 (91.93)	64 (88.89)	156 (83.42)	425 (88.17)	**0.029**
Headache	215 (96.41)	67 (93.06)	155 (82.89)	437 (90.66)	**<.001**
Retro-orbital pain	133 (59.64)	33 (45.83)	69 (36.90)	235 (48.76)	**<.001**
Neck pain	70 (31.39)	20 (27.78)	43 (22.99)	133 (27.59)	0.166
Ear pain	19 (8.52)	4 (5.56)	10 (5.35)	33 (6.85)	0.401
Breathing difficulty	1 (0.45)	0	5 (2.67)	6 (1.24)	0.131
Nasal congestion	10 (4.48)	5 (6.94)	26 (13.90)	41 (8.51)	**0.003**
Rhinorrhea	18 (8.07)	9 (12.50)	37 (19.79)	64 (13.28)	**0.002**
Sore Throat	12 (5.38)	5 (6.94)	22 (11.76)	39 (8.09)	0.057
Cough	35 (15.70)	11 (15.28)	48 (25.67)	94 (19.50)	**0.025**
Sputum production	6 (2.69)	3 (4.17)	15 (8.02)	24 (4.98)	**0.044**
Nausea & vomiting	120 (53.81)	31 (43.06)	75 (40.11)	226 (46.89)	**0.017**
Diarrhea	25 (11.21)	6 (8.33)	25 (13.37)	56 (11.62)	0.509
Constipation	10 (4.48)	3 (4.17)	9 (4.81)	22 (4.56)	0.972
Abdominal pain	81 (36.32)	20 (27.78)	55 (29.41)	156 (32.37)	0.220
Nose bleeding	6 (2.69)	2 (2.78)	0	8 (1.66)	**0.041**
Gum bleeding	9 (4.04)	1 (1.39)	0	10 (2.07)	**0.008**
Flushed face	3 (1.35)	3 (4.17)	5 (2.67)	11 (2.28)	0.287
Loss of appetite	155 (69.51)	40 (55.56)	93 (49.73)	288 (59.75)	**<.001**
Myalgia	169 (75.78)	52 (72.22)	114 (60.96)	335 (69.50)	**0.004**
Arthralgia	171 (76.68)	51 (70.83)	104 (55.61)	326 (67.63)	**<.001**

*only among those that reported the end of fever illness (n=309; 156 dengue-confirmed, 43 dengue-probable, and 110 non-dengue cases)

Appendix F: Chapter 5 S1 Checklist: STROBE checklist

S1. STROBE Statement: Clinical and epidemiologic characteristics associated with dengue during and outside the 2016 outbreak identified in health-facility-based surveillance in Ouagadougou, Burkina Faso

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 4)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 6, para 2-5)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 6, para 5)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 7 para 3, page 8 para 1-2, Figure 2)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 7 para 1-2, Figure 1, 3)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (Page 7 para 3)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 8 para 2-3, Page 9 para 1)
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 9 para 1, page 10 para 1)
Bias	9	Describe any efforts to address potential sources of bias (page 25 para 2, page 26 para 2)
Study size	10	Explain how the study size was arrived at (Figure 3)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which

		groupings were chosen and why (Page 8 para 3, page 9 para 1)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 9 para 2, Page 10 para 1-2)
		(*b*) Describe any methods used to examine subgroups and interactions (Page 10 para 2, 4)
		(*c*) Explain how missing data were addressed (Page 10 para 4)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (Not applicable)
		(*e*) Describe any sensitivity analyses (Page 10 para 2- analysis just between dengue-confirmed and non-dengue groups, reported in tables S2-S4)
Results		
Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (figure 3)
		(b) Give reasons for non-participation at each (figure 3)
		(c) Consider use of a flow diagram (figures 3)
Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 10 para 1, Page 11, Table 1)
		(b) Indicate number of participants with missing data for each variable of interest (Page 10 para 4, explained on Fig 3 to have reached a sample size with complete information from variables used)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) (Not applicable)
Outcome data	15	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures (Tables 1, 2, 3)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Tables 2, 3, and 4)
		(*b*) Report category boundaries when continuous variables were categorized (Page 9 para 1, in methods)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (Not applicable)

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (Table S2-S4)
Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 20 para 1)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 22 para 3, page 23 para 1-4)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 20 para 2-3, Page 21 para 1-2, page 22 para 1-2)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 22 para 3)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 3)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix G. Chapter 5 Supporting information

Demographic and clinical characteristics of patients by dengue infection status in the fever surveillance in Ouagadougou, Burkina Faso

- S2 Table: Demographic and clinical characteristics of patients by dengue infection status from the health facility-based fever surveillance established in Ouagadougou, Burkina Faso
- S3 Table: Univariate logistic regression analyses showing significant indicators and their odds ratios between dengue-confirmed and non-dengue cases during the period of outbreak in the health facility-based fever surveillance
- S4 Table: Univariate logistic regression analyses showing significant indicators and their odds ratios between dengue-confirmed and non-dengue cases during the period of non-outbreak in the health facility-based fever surveillance

S2. Table. Demographic and clinical characteristics of patients by dengue confirmation status from the health facility-based fever surveillance established in Ouagadougou, Burkina Faso

Characteristics	Dengue-confirmed (n=540)	Dengue-probable (n=200)	Non-dengue (n=2189)	Total (n=2929)	p-value
Age group (years)					**<.001**
1-4	25 (4.6)	12 (6.0)	275 (12.6)	312 (10.7)	
5-9	26 (4.8)	17 (8.5)	149 (6.8)	192 (6.6)	
10-14	28 (5.2)	17 (8.5)	129 (5.9)	174 (5.9)	
15-19	70 (13.0)	15 (7.5)	231 (10.6)	316 (10.8)	
20-24	85 (15.7)	25 (12.5)	366 (16.7)	476 (16.3)	
25-29	97 (18.0)	37 (18.5)	375 (17.1)	509 (17.4)	
30-34	70 (13.0)	24 (12.0)	269 (12.3)	363 (12.4)	
35-39	53 (9.8)	18 (9.0)	155 (7.1)	226 (7.7)	
40-44	39 (7.2)	18 (9.0)	111 (5.1)	168 (5.7)	
45-49	23 (4.3)	10 (5.0)	67 (3.1)	100 (3.4)	
50-55	24 (4.4)	7 (3.5)	62 (2.8)	93 (3.2)	
Female	333 (61.7)	132 (66.0)	1563 (71.4)	2028 (69.2)	**<.001**
CSPS					**<.001**
Pazani	81 (15.0)	32 (16.0)	400 (18.3)	513 (17.5)	
Zongo	60 (11.1)	31 (15.5)	592 (27.0)	683 (23.3)	
CSPS 22	45 (8.3)	20 (10.0)	240 (11.0)	305 (10.4)	
CSPS25	206 (38.2)	60 (30.0)	502 (22.9)	768 (26.2)	
Juvenat Fille	148 (27.4)	57 (28.5)	446 (20.4)	651 (22.2)	
Under observation ≤3 days/OPD	117 (21.7)/423 (78.3)	18 (9.0)/182 (91.0)	45 (2.1)/2144 (97.9)	180 (6.2)/2749 (93.9)	**<.001**
Mean days, fever duration prior to visit (SD)	2.89 (1.20)	3.03 (1.23)	2.61 (1.22)	2.69 (1.23)	**<.001**
Fever duration prior to visit					**<.001**
1-2 days	233 (43.2)	68 (34.0)	1153 (52.7)	1454 (49.6)	
3 days	162 (30.0)	76 (38.0)	634 (29.0)	872 (29.85)	
4-7 days	145 (26.9)	56 (28.0)	402 (18.4)	603 (20.6)	
Mean temperature at	38.34	38.14 (0.78)	38.03 (0.78)	38.09	**<.001**

enrollment (SD)	(0.75)			(0.78)	
Temperature at enrollment					**<.001**
Below 38.5°c	333 (61.7)	145 (72.5)	1681 (76.8)	2159 (73.7)	
≥ 38.5°c	207 (38.3)	55 (27.5)	508 (23.2)	770 (26.3)	
Mean days, fever duration, entire illness (SD)	4.77 (2.44)	4.57 (2.71)	4.04 (2.46)	4.21 (2.49)	**<.001**
Prev. dengue infection	10 (1.9)	4 (2.0)	2 (0.1)	16 (0.6)	**<.001**
YF vaccination					**<.001**
Received	83 (15.4)	39 (19.5)	824 (37.6)	946 (32.3)	
Not received	457 (84.6)	161 (80.5)	1365 (62.4)	1983 (67.7)	
Clinical diagnosis					
Suspected dengue	144 (26.7)	43 (21.5)	12 (0.6)	199 (6.8)	**<.001**
Undifferentiated fever	379 (70.2)	150 (75.0)	1987 (90.8)	2516 (85.9)	
Non-dengue	17 (3.2)	7 (3.5)	190 (8.7)	214 (7.3)	
URI (% of non-dengue)	3 (17.6)	2 (28.6)	27 (14.2)	32 (15.0)	
Bronchitis	2 (11.8)	2 (28.6)	30 (15.8)	34 (15.9)	
Pneumonia	6 (35.3)	0	21 (11.1)	27 (12.6)	
Viral syndrome	1 (5.9)	2 (28.6)	11 (5.8)	14 (6.5)	
Diarrheal illness	1 (5.9)	1 (14.3)	28 (14.7)	30 (14.0)	
Influenza	1 (5.9)	0	4 (2.1)	5 (2.3)	
Others	3 (17.6)	0	69 (36.3)	72 (33.6)	
Signs and symptoms (presence)					
Rash	73 (13.5)	22 (11.0)	163 (7.5)	258 (8.8)	**<.001**
Fatigue	446 (82.6)	157 (78.5)	1526 (69.7)	2129 (72.7)	**<.001**
Headache	518 (95.9)	190 (95.0)	1899 (86.8)	2607 (89.0)	**<.001**
Retro-orbital pain	103 (19.1)	28 (14.0)	107 (4.9)	238 (8.1)	**<.001**
Neck pain	8 (1.5)	5 (2.5)	47 (2.2)	60 (2.1)	0.556
Nasal congestion	16 (3.0)	4 (2.0)	105 (4.8)	125 (4.3)	**0.044**
Rhinorrhea	21 (3.9)	9 (4.5)	132 (6.0)	162 (5.5)	0.120
Sore Throat	7 (1.3)	4 (2.0)	64 (2.9)	75 (2.6)	0.088
Cough	62 (11.5)	29 (14.5)	354 (16.2)	445 (15.2)	**0.024**
Sputum production	2 (0.4)	2 (1.0)	30 (1.4)	34 (1.2)	0.123

Nausea & vomiting	190 (35.2)	80 (40.0)	635 (29.0)	905 (30.9)	**<.001**
Diarrhea	14 (2.6)	9 (4.5)	128 (5.9)	151 (5.2)	**0.008**
Constipation	6 (1.1)	6 (3.0)	85 (3.9)	97 (3.3)	**0.005**
Abdominal pain	195 (36.1)	76 (38.0)	639 (29.2)	910 (31.1)	**<.001**
Nose bleeding	6 (1.1)	1 (0.5)	10 (0.5)	17 (0.6)	0.198
Gum bleeding	4 (0.7)	1 (0.5)	2 (0.1)	7 (0.2)	**0.021**
Loss of appetite	251 (46.5)	80 (40.0)	739 (33.8)	1070 (36.5)	**<.001**
Capillary refill >2 sec	4 (0.7)	4 (2.0)	19 (0.9)	27 (0.9)	0.218
Alterations to consciousness	2 (0.4)	4 (2.0)	7 (0.3)	13 (0.44)	**0.014**
Myalgia	234 (43.3)	85 (42.5)	560 (25.6)	879 (30.0)	**<.001**
Arthralgia	315 (58.3)	111 (55.5)	953 (43.5)	1379 (47.1)	**<.001**

S3 Table. Univariate logistic regression analyses showing significant indicators and their odds ratios of between dengue-confirmed and non-dengue cases during the period of outbreak in the health facility-based fever surveillance

Characteristics	Total N	N (%) dengue confirmed (n=357)	N (%) Non-dengue (n=349)	Univariate analysis Dengue-confirmed vs. no dengue		
				OR	95% CI	p-Value
Age group (years)						0.095
1-14	129	46 (35.7)	66 (51.2)	Ref	-	
15-24	213	106 (49.8)	92 (43.2)	**1.65**	**1.04-2.64**	
25-34	242	110 (45.5)	114 (47.1)	1.38	0.88-2.19	
35-55	193	95 (49.2)	77 (39.9)	**1.77**	**1.09-2.87**	
Female* (*ref.* male)	484	207 (42.8)	237 (49.0)	**0.65**	**0.48-0.89**	**0.007**
Under observation** (*ref.* OPD)	128	99 (77.3)	18 (14.1)	**7.05**	**4.16-11.96**	**<.001**
Fever duration prior to visit*						**0.011**
1-2 days	330	147 (44.6)	162 (49.1)	Ref	-	
3 days	244	101 (41.4)	115 (47.1)	0.97	0.68–1.37	
4-7 days	203	109 (53.7)	72 (35.5)	**1.67**	**1.15-2.42**	
Temperature at enrollment *						**0.004**
Below 38.5°c	468	195 (41.7)	228 (48.7)	**Ref**	**-**	
≥ 38.5°c	309	162 (52.4)	121 (39.2)	**1.57**	**1.16-2.12**	
No YF vaccination[A]* (*ref.* received vaccination)	630	309 (49.1)	267 (42.4)	**1.98**	**1.34-2.93**	**<.001**
Presence of signs and symptoms (*ref.* absence)						
Rash*	84	48 (57.1)	24 (28.6)	**2.10**	**1.26–3.52**	**0.005**
Fatigue*	620	300 (48.4)	267 (43.1)	**1.62**	**1.11-2.35**	**0.012**
Retro-orbital pain**	104	80 (76.9)	12 (11.5)	**8.11**	**4.33-15.19**	**<.001**
Nasal congestion*	21	5 (23.8)	16 (76.2)	**0.30**	**0.11-0.82**	**0.019**
Rhinorrhea*	28	6 (21.4)	21 (75.0)	**0.27**	**0.11-0.67**	**0.005**
Cough**	81	23 (28.4)	53 (65.4)	**0.39**	**0.23-0.64**	**<.001**

Nausea & vomiting	285	131 (46.0)	131 (46.0)	0.97	0.71-1.31	0.817
Diarrhea	21	5 (23.8)	13 (61.9)	0.37	0.13-1.04	0.060
Abdominal pain	263	127 (48.3)	110 (41.8)	1.20	0.88-1.64	0.255
Loss of appetite*	383	191 (49.9)	166 (43.3)	1.27	0.94-1.70	0.115
Myalgia*	366	189 (51.6)	139 (38.0)	**1.70**	**1.26-2.29**	**<.001**
Arthralgia	521	246 (47.2)	226 (43.4)	1.21	0.88-1.65	0.242
Headache*	749	350 (46.7)	329 (43.9)	**3.04**	**1.27-7.28**	**0.013**
Sore throat	11	3 (27.3)	7 (63.6)	0.41	0.11-1.62	0.204

Statistical significance of the frequencies: *p-value<0.05 **p-value<.001

[A]based on self-report

S4 Table. Univariate logistic regression analyses showing significant indicators and their odds ratios between dengue-confirmed and non-dengue cases during the period of non-outbreak in the health facility-based fever surveillance

Characteristics	Total N	N (%) dengue confirmed (n=183)	N (%) Non- dengue (n=1840)	Univariate analysis Dengue-confirmed vs. no dengue		
				OR	95% CI	p-Value
Age group (years)*						**<.001**
1-14	549	33 (6.0)	487 (88.7)	Ref	-	
15-24	579	49 (8.5)	505 (87.2)	1.43	0.91-2.27	
25-34	630	57 (9.1)	530 (84.1)	**1.59**	**1.02-2.48**	
35-55	394	44 (11.2)	318 (80.7)	**2.04**	**1.27-3.28**	
Female (*ref.* male)	1544	126 (8.2)	1326 (85.9)	0.86	0.62-1.19	0.358
Under observation** (ref. OPD)	52	18 (34.6)	27 (51.9)	**7.33**	**3.95-13.58**	**<.001**
Fever duration prior to visit*						0.196
1-2 days	1124	86 (7.7)	991 (88.2)	Ref	-	
3 days	628	61 (9.7)	519 (82.6)	1.35	0.96-1.91	
4-7 days	400	36 (9.0)	330 (82.5)	1.26	0.84-1.89	
Temperature at enrollment						0.263
Below 38.5°c	1691	138 (8.2)	1453 (85.9)	Ref	-	
≥ 38.5°c	461	45 (9.8)	387 (84.0)	1.22	0.86-1.75	
No YF vaccination [A]** (*ref.* received vaccination)	1353	148 (10.9)	1098 (81.2)	**2.86**	**1.95-4.18**	**<.001**
Presence of signs and symptoms (*ref.* absence)						
Rash*	174	25 (14.4)	139 (79.9)	**1.94**	**1.23-3.06**	**0.005**
Fatigue**	1509	146 (9.7)	1259 (83.4)	**1.82**	**1.25-2.65**	**0.002**
Retro-orbital pain**	134	23 (17.2)	95 (70.9)	**2.64**	**1.63-4.28**	**<.001**

Nasal congestion	104	11 (10.6)	89 (85.6)	1.26	0.66-2.40	0.485
Rhinorrhea	134	15 (11.2)	111 (82.8)	1.39	0.79-2.44	0.249
Cough	364	39 (10.7)	301 (82.7)	1.39	0.95-2.02	0.088
Nausea & vomiting**	620	59 (9.5)	504 (81.3)	1.26	0.91-1.75	0.163
Diarrhea	130	9 (6.9)	115 (88.5)	0.78	0.39-1.56	0.475
Abdominal pain*	647	68 (10.5)	529 (81.8)	**1.47**	**1.07-2.01**	**0.018**
Loss of appetite*	687	60 (8.7)	573 (83.4)	1.08	0.78-1.49	0.647
Myalgia*	513	45 (8.8)	421 (82.1)	1.10	0.77-1.57	0.601
Arthralgia	858	69 (8.0)	727 (84.7)	0.93	0.68-1.27	0.634
Headache*	1858	168 (9.0)	1570 (84.5)	**1.93**	**1.12-3.32**	**0.018**
Sore throat	64	4 (6.3)	57 (89.1)	0.70	0.25-1.95	0.495

Statistical significance of the frequencies: *p-value<0.05 **p-value<.001

[A]based on self-report

Appendix H. Chapter 6 STROBE checklist

STROBE Statement: Dengue virus seroprevalence and force of infection estimated using repeated serosurveys in Ouagadougou, Burkina Faso

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 3)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (Page 4, para 4-5)
Objectives	3	State specific objectives, including any prespecified hypotheses (Page 4, para 5)
Methods		
Study design	4	Present key elements of study design early in the paper (Page 6 para 2-3)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (Page 6 para 1, Page 7 para 1)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (Page 7 para 1-2)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (Page 7 para 3, Page 8 para 1-3, Page 9 para 1-3)
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (Page 8 para 1, 4, page 9 para 1-2)
Bias	9	Describe any efforts to address potential sources of bias (Page 8 para 3, Page 10 para 1)
Study size	10	Explain how the study size was arrived at (Figure 1)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which

		groupings were chosen and why (Page 7 para 3, page 8 para 1-2)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (Page 8 para 1-4, Page 9 para 1-3)
		(*b*) Describe any methods used to examine subgroups and interactions (-)
		(*c*) Explain how missing data were addressed (Page 10 para 1)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (Not applicable)
		(*e*) Describe any sensitivity analyses (none)
Results		
Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (figure 1, page 10 para 1)
		(b) Give reasons for non-participation at each (figure 1)
		(c) Consider use of a flow diagram (figure 1)
Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (Page 10 para 2-5, Page 11 para 1-5, Page 12 para 1-2)
		(b) Indicate number of participants with missing data for each variable of interest (page 11 para 2)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) (Not applicable)
Outcome data	15	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures (Tables 1, 2, 3, 4)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (Tables 3-5)
		(*b*) Report category boundaries when continuous variables were categorized (Page 8 para 1, in methods)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (Not applicable)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		

Key results	18	Summarise key results with reference to study objectives (Page 12 para 3-4, page 13 para 2-3, page 14 para 2)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (Page 15, page 16 para 1)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 13 para 1, 4, Page 15 para 1, 4, Page 16 para 4)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 16 para 3)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (Page 2)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix I. Sample size calculation

Appendix I. Sample size calculation

Sample size calculations for the surveillance and serosurvey were based on standard error or margin of error at a fixed significance level (*Chow, Shao, Wang. (2008). Sample size calculations in clinical research (2nd edition). Taylor & Francis Group.).*

The formula for confidence interval is as below (*Altman D G. (1999). Practical statistics for medical research. Chapman & Hall/CRC*):

$$\bar{x} \pm z * / \frac{\sigma}{\sqrt{n}} = \bar{x} \pm \text{margin of error}$$

Taking the margin of error part of the formula above, we get an equation as below:

$$\text{margin of error} = 0.25p = z * / \frac{p(1-p)}{\sqrt{n}}$$

where $\sigma = p(1-p)$

$z = 1.96$

p = anticipated population proportion (incidence or seroprevalence)

As we wish to have the resulting estimate to fall within 25% of the true proportion with 95% assurance, margin of error is 0.25 x p. This gives relative precision of 75% and this is an acceptable level, considering the gap in evidence for dengue incidence in the study area.

Rearranging it for n, the sample size for precision equals to:

$$n = \frac{\{z^2[p(1-p)]\}}{(p \text{ x } 0.25)^2}$$

Passive facility-based fever surveillance

The incidence of 0.002% per year (column D) was obtained from unpublished data from the local investigator on a sub groups tested for dengue within a previously conducted Malaria study. Unfortunately, population level age-specific incidence reported from previous literature or surveillance was not available and this was used across all age groups. Expansion factor of 21.3 was applied for age under 15 years based on the reported expansion factor (275). For gradual reduction in the value used in adjustment, there was the expansion factor of 10 assumed for age between 15-39 years and no adjustment was applied for those 40 years and older (column E). With 95% assurance, Z-score 1.96, and relative precision of 75%, using the formula above for n, the sample size was calculated per age group, as shown in column J. The sample sizes for age groups 0 to 54 years were added to reach 105623.

A	B	C	D	E	F	G	H	I	J	K
Age group	**Population size**	**% of the national pop**	**Est. incidence**	**Expansion**	**Mean corrected incidence**	**(1-P)**	**1-Relative precision**	**(margin of error) Precision**	**N (sample size for age groups)**	**Total (0-55 yrs)**
0-4	2957423	0.182	0.002105	21.3	0.045	0.955158	0.25	0.011211	1309	105623
5-9	2428944	0.150	0.002105	21.3	0.045	0.955158	0.25	0.011211	1309	
10-14	2084133	0.128	0.002105	21.3	0.045	0.955158	0.25	0.011211	1309	
15-19	1772685	0.109	0.002105	10	0.021	0.978947	0.25	0.005263	2858	
20-24	1460073	0.090	0.002105	10	0.021	0.978947	0.25	0.005263	2858	
25-29	1220504	0.075	0.002105	10	0.021	0.978947	0.25	0.005263	2858	
30-34	1043180	0.064	0.002105	10	0.021	0.978947	0.25	0.005263	2858	
35-39	838019	0.052	0.002105	10	0.021	0.978947	0.25	0.005263	2858	
40-44	662548	0.041	0.002105	1	0.002	0.997895	0.25	0.000526	29135	
45-49	492282	0.030	0.002105	1	0.002	0.997895	0.25	0.000526	29135	
50-54	363812	0.022	0.002105	1	0.002	0.997895	0.25	0.000526	29135	

The serosurvey

Using the same formula above, the sample size for the serosurvey is calculated using the prevalence proportion of 30.4 % from a previous study conducted in Burkina Faso (260).

A	B	C	D	E	F	G	H	I
Age group	**Population size**	**% of the national pop**	**Est. prevalence**	**(1-P)**	**1- Relative precision**	**(margin of error) Precision**	**N (sample size for age groups)**	**Total (0-55 yrs)**
0-4	2957423	0.182	0.3040	0.696	0.25	0.076	141	1548
5-9	2428944	0.150	0.3040	0.696	0.25	0.076	141	
10-14	2084133	0.128	0.3040	0.696	0.25	0.076	141	
15-19	1772685	0.109	0.3040	0.696	0.25	0.076	141	
20-24	1460073	0.090	0.3040	0.696	0.25	0.076	141	
25-29	1220504	0.075	0.3040	0.696	0.25	0.076	141	
30-34	1043180	0.064	0.3040	0.696	0.25	0.076	141	
35-39	838019	0.052	0.3040	0.696	0.25	0.076	141	
40-44	662548	0.041	0.3040	0.696	0.25	0.076	141	
45-49	492282	0.030	0.3040	0.696	0.25	0.076	141	
50-54	363812	0.022	0.3040	0.696	0.25	0.076	141	

Taking 20% non-response rate into consideration per follow-up visits and with 3 additional follow-up in plan after enrollment, the final sample size reached was 2477.

Following the same calculation as in the surveillance, the sample size reached was 141 per age group and when these are added to cover 0-55 years, the total sample size for the serosurvey reached 1548. With lack of seroprevalence data from Burkina Faso, the same prevalence (30.4%) was used in calculation across age. If a higher prevalence estimate (e.g., what we may find for older adults, if data were available) was used, then the sample size will become smaller than what we have here. Therefore, we believe this is a conservative estimate (i.e. bigger sample size than what may be needed) for the sample size needed for the serosurvey.