Imported Infections’ Importance: Global Change Driving Dengue Dynamics

Mikkel B. Quam
To my traveling companion,

who inspires me along

this journey
"The three rules of the Tipping Point—the Law of the Few, the Stickiness Factor, the Power of Context—offer a way of making sense of epidemics."

“There are exceptional people out there who are capable of starting epidemics. All you have to do is find them.”

“In epidemics, the messenger matters: messengers are what make something spread. But the content of the message matters too. And the specific quality that a message needs to be successful is the quality of "stickiness."

“Epidemics are sensitive to the conditions and circumstances of the times and places in which they occur.”

-Malcolm Gladwell, excerpts from his book *The Tipping Point: How Little Things Can Make a Big Difference*
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Abstract

Background Dengue is a significant problem of international health concern. According to the World Health Organization in 2012, globally, dengue is “the most important mosquito borne viral disease” with incidence 30 higher than it had been 50 years ago. While most of the burden of disease associated with dengue is located in areas with a tropical and sub-tropical climate, increasing evidence suggests temperate areas are also at risk. Considering the recent introduction of relevant mosquito vectors into Southern Europe, and increasing numbers of imported dengue via travelers, Europe and other temperate areas may be increasingly at risk for dengue emergence, establishment and local transmission in the foreseeable future.

Methods Recent dengue emergence in Madeira and reemergence in Tokyo underline the hypothesis that passenger air-travel can be an important conduit for the importation of vector-borne disease leading to emergence in naïve areas climatically suitable for dengue transmission, including parts of Europe. Combining information on travel with virus genetic similarity was useful in discerning likely pathways of for the importation of infections. Generalizing information learned from outbreaks in Tokyo and Madeira with global epidemic intelligence, global travel networks, and climate change projections, leads to more refined understanding of the magnitude of dengue infectious imported into temperate areas and these virus introduction events’ potential implications for seeding epidemics in the 21st century.

Results While compared to total travel, imported dengue events and epidemics of dengue outside the tropics are rare, our combined evidence and modeled estimations suggest strongly that epidemic dengue emergence in temperate areas is possible and will continue to increase. We found that global change dynamics including warming temperatures in the much of the northern hemisphere and increasing passenger interconnectivity between areas endemic for dengue and dengue free areas are key mechanisms partly explaining these unprecedented epidemiological transitions.

Conclusion While we calibrated our models on information known about dengue, many elements of the methods and conclusions may increase understanding of the potentially global implications for imported infections of other climate-sensitive infectious diseases’ that may have similar parameters. During 2016 and the years to come, techniques developed in this doctoral research will contribute to models used in risk analysis for vector-borne diseases of interest, including the increasing important potential for imported Chikungunya and Zika viruses into a variety of unexposed areas.

Keywords: Dengue, Zika, Vector-borne Disease, Aedes, Global Change, Climate Change, Viral Evolution, Phylogenetics, Travel, Interconnectivity, Disease Modeling, Madeira, Italy, Japan, Europe
Contributing Publications

The research conducted during this doctoral journey has contributed to four publications:


*The 4th manuscript is under review.

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Introduction and Background

Overview

The 21st century offers a plethora of new opportunities for the globalization of ideas, individuals, and infections, propelled by increasing interconnectivity and global environmental change. In our modern world, the time between persons from disparate places diminishes for more and more of the globe’s population each year, given the rise of passenger air-travel. Simultaneously, we are observing unprecedented rates of environmental transformations in terms of population growth, urbanization, and climate change. Both the changes in travel and changes in climate, like many other aspects of global change, are changing at different paces in different places and for different persons. So too are dynamics of dengue—increasing at an unprecedented pace and unequally spread across the globe.

Dengue is a virus, transmitted by Aedes mosquitoes, which most commonly causes mildly symptomatic infections in humans, however, can be potentially fatal (Murray et al., 2013). Generally, dengue is regarded as being a disease endemic to warmer areas of the globe including the tropics and subtropics, where the mosquito vectors are established (Murray et al. 2013). The disease, once called “dandy” or “breakbone” fever is primarily transmitted by Aedes aegypti mosquitoes but also the secondary vector Aedes albopictus which is better adapted to temperate areas of the Asia, the Americas, and increasingly Europe (Murray et al. 2013, Simmons et al., 2012, Ashburn and Craig, 1907). Beginning at least as early as the 19th century in dengue transmission was geographically wide-spread—as evidenced by epidemics recorded in various parts of the Africa, Asia, the Americas, and parts of southern Europe (Murray et al. 2013, (Smart, 1877, Porcher, 1880, Thomas, 1880, Halstead, 2008, Akbar et al., 2012, Simmons et al., 2012). During this time already, as increasing intercontinental sea travel allowed for the swift exchange of goods, services, and individuals, so too began the intercontinental importation of infections through individual travelers, including dengue (Murray et al. 2013, (Smart, 1877, Porcher, 1880, Thomas, 1880, Halstead, 2008, Akbar et al., 2012, Simmons et al., 2012). Although these imported infections spawned important epidemics already during the 19th century (and potentially even earlier), the advent of commercial flight and relatedly increasing interconnectedness of the globalized world during the late 20th and early 21st centuries, are driving unprecedented acceleration and expansion of the global burden of dengue into temperate areas (World Health Organization., 2012, Bhatt et al., 2013, Wilder-Smith et al., 2012). Furthermore, climate change can exacerbate the effects of modern travel on disease spread in increasing the suitability of dengue to be transmitted locally in areas where it has been introduced by infected travelers. Global travel and climate-based modeling are important areas of current research and further investigation is needed to determine the potential risks of dengue emergence or re-emergence in geographical areas like Europe, commonly considered dengue free (Wilder-Smith et al., 2012).
Disease

Host: Dengue Host Dynamics

Although, the natural history of disease transmission itself is not a key focus in this doctoral work, unique features of dengue do provide context to the doctoral work, influence the importance of imported infections, and provide necessary background to the tools used to ascertain potential implications and mechanisms by which global change can drive dengue’s dynamic expansion into uncharted territories. Dynamic behaviors of individuals with human host populations enables the ongoing spread of disease, however, in this section, we focus mainly on the disease itself, and how it can affect an infected individual.

While Benjamin Rush and others have previously described dengue in the 18th Century, this account shows how a corresponding physician described the disease’s peculiarly varied manifestation during the 1880 outbreak of dengue in Charleston, South Carolina, United States of America:

*I was somewhat puzzled at first, as there was much malarial fever existing at the time. I did not regard the disease as epidemic, but rather accidental or sporadic. It disappeared as suddenly as it had invaded, and was of an exceedingly mild type, except in two or three cases, which assumed the form of acute articular rheumatism (Porcher, 1880).*

Clinical signs and symptoms of dengue illness indeed range greatly in severity from patient to patient (and virus to virus); however, usual features include sudden onset of fever, severe headache, muscle ache, joints pain, rashes, leucopenia, and thrombocytopenia (World Health Organization, 2009). Dengue illnesses is currently categorized by WHO as “Dengue” (D), “Dengue with Warning Signs” (DW) or Severe Dengue (SD) -previously divided into Dengue hemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS) (Akbar et al., 2012). Some countries surveillance systems differ in case definitions, categorizations, and diagnostic techniques widely from the current WHO standard definitions, making global predictions and cross-regional analysis more difficult. As with the physician in Charleston who initially was confusing dengue with malaria, clinical heterogeneity among other features can
contribute to dengue's enigmatic epidemiology and burden on health systems. While most infections are benign and mild, an infected patient, if developing severe dengue, experiences escalating vascular permeability; therefore plasma leakage can occur, followed by development of capillary bleeding and ultimately decreases in coagulation functionality (St John et al., 2013). Although the vast majority of cases are never symptomatically apparent; therefore do not display any waning signs, some infected individuals rapidly deteriorate, transitioning into a life threatening state (World Health Organization., 2009). The vascular leakage in SD, as with DHF, can involve bleeding in multiple organs, frequently those within the gastrointestinal tract, and the internal accumulation of excreted fluids (St John et al., 2013). In patients having DSS, DHF at its most severe, the widespread permeability progressively increases and weakens the pulse until tipping where blood pressure declines rapidly, the circulatory system collapses completely (St John et al., 2013). In severe cases, which are rare compared to the total number of dengue infections, the infected host's inability to manage fluids within the vascular systems can result in death.

Following incubation, 3–7 days following the infectious mosquito bite, infection occurs in 3 apparent phases: initial febrile phase, critical phase, and a recovery phases (Simmons et al., 2012). The initial 3–7 day febrile phase is the most common for suspected dengue and includes symptoms of increased body temperature (≥38.5°C), headache, vomiting, myalgia, joint pain, rash, minor hemorrhagic manifestations including petechiae or bruising and sometimes a palpable liver (Simmons et al., 2012). The subsequent 3-7 day critical phase may present vascular permeability, warning signs, or hemorrhagic manifestations, such as severe abdominal pain, further vomiting, tender hepatomegaly, serosal effusions, mucosal bleeding, increased hematocrit, decreased platelet levels, and lethargy (Simmons et al., 2012). The heightened vascular leakage tends to be short and spontaneously return to normal in the 2–3 day recovery phase of swift progress back to full health, except fatigue, which occasionally continues for days to weeks (Simmons et al., 2012).

Importantly regarding host dynamics of dengue, not all infections are symptomatic and even among symptomatic cases, the period of infectiousness starts approximately 2 days before the onset of symptoms and lasts nearly 6–7 days, about 5 days after the initial febrile symptoms (Simmons et al., 2012, Nguyen et al., 2013). Additionally, the period of infectiousness does not begin immediately at the time of the infecting mosquito bite, but rather overlaps with the end of the intrinsic incubation period which is thought to begin about 4 days before onset of illness (Halstead, 2007). Also of epidemiological note, individuals, who sustained dengue infection experience a mixed blessing; they enjoy long-lived serotype-specific immunity, however, paradoxically, it is accompanied by heightened risk of increased severity if infected by another DENV serotype in the future (Simmons et al., 2012).
Agent: Dengue Virus Dynamics

That dengue has its own specific germ, which seems to operate when in the human system in a specific manner, admits of little doubt; and that it is susceptible of transportation and is in some degree infectious, its history seems to sustain. (Thomas, 1880).

The 1943 discovery of the dengue virus may be attributed to Ren Kimura and Susumu Hotta, who first isolated the dengue virus in blood samples from the 1943 epidemic of dengue in Nagasaki, Japan (Hotta, 2001). The infectious agents causing dengue are four distinct viral serotypes of the Flaviviridae family, which also includes yellow fever virus and Japanese encephalitis among others (Halstead, 2008, St John et al., 2013). Dengue viruses (DENV) require mosquitoes to act as vectors for the human transmission cycle to continue, therefore, dengue is regarded as an arthropod-borne virus or arbovirus (Halstead, 2008). While the four single-stranded RNA viruses causing dengue (DENV) can also be involved in sylvatic (non-human primate) transmission between mosquito vector and host populations, human transmission is the primary concern to public health (Simmons et al., 2012). All four DENV serotypes are known to cause epidemics, generally presenting symptomatically for some individually in a range of severity extending from unapparent illness to potentially fatal severe dengue (Halstead, 2008, St John et al., 2013). Identifiable through nucleotide sequencing, genetic distinctions at the base pair level can display the relationships within and between the dengue serotype groups, thereby distinguishing the dengue four viruses, DENV-1, -2, -3, -4 (Halstead, 2008, Guzman et al., 2010). Perhaps, more importantly these sequencing techniques when combined with libraries of past sequences are enabling phylogeography - the classification of distinct virus strains according to their origins and genetic similarities (Guzman et al., 2010). Nucleotide sequencing and phylogenetic analysis techniques enabled the classification of various strains of dengue viruses recently, which allows researcher to better track the viral evolution of dengue over time and space (Diaz et al., 2006).

Dengue viruses can have a low, medium, or high epidemiological impact and/or virulence, according to their characteristics for causing dengue epidemics or disease manifestations having greater severity (Rico-Hesse, 2003). Some strains of DENV have very low noticeable epidemiological
impact, because they are more persistent in sylvatic cycles (transmitted mainly among animal populations, rarely in humans) or cause primarily mild dengue fever epidemics—so-called ‘silent epidemics’ (Rico-Hesse, 2003). Still other genotypes with higher virulence can be associated with much higher case incidence of human cases and epidemics having more severe dengue (Rico-Hesse, 2003). The American genotypes of DENV-2 and DENV-3, showing a comparatively reduced ability to grow in cell cultures and mosquitoes, for example, have been shown to be less virulent than Asian DENV-2 and DENV-3 genotypes (Wilder-Smith et al., 2010, Rico-Hesse, 2007, Rico-Hesse, 2010, Armstrong and Rico-Hesse, 2001).

This highlights the importance of interactions between hosts, vectors, viral agent, and to an extent context and environment, can to a degree govern the epidemiological impact. Dengue viruses, circulating during an outbreak, are at heightened levels within a population, and more likely to undergo random mutations due to more numerous replication without the aid of RNA-dependent RNA polymerase for proof-reading (Wilder-Smith et al., 2010). In this manner, the scale and severity of outbreaks as exemplified by the 1998 DENV-4 epidemic in Puerto Rico can correlate with the genetic diversity of particular strains (Bennett et al., 2010). In Latin America, Asia, and more recently Africa, phylogenetic analysis is used to help categorize dengue viruses dynamics, where those of superior epidemiological impact predominate those strains with lesser impact, particularly after importation of new strains occur (Diaz et al., 2006, Wilder-Smith and Gubler, 2008, Rico-Hesse, 2010) (Sessions, 2013).

Dengue dynamics are driven by ever emerging and evolving lineages of viruses on the micro and macro level; during the course of urban endemic transmission and continent to continent (Bennett et al., 2010, Messer et al., 2003, Guzman et al., 2010). The global distribution of dengue’s various strains signposts human interactions in viral evolution, through virus importation and exportation, because viruses remain relatively geographical stable in their evolutionary histories if not carried by human hosts place to place (Imrie et al., 2006, Simmons et al., 2012). Therefore the multitude of serotypes, genotypes, and strains currently dispersed throughout the tropical regions of the globe are driven by the movements of host and expansion of vectors (Simmons et al., 2012). These movements of virus are possible to occur, when infected mosquitoes or humans travel (while infectious) to new geographic areas, which environmentally supports both vector and susceptible host populations (Murray et al., 2013).

Global change, mainly thorough travel and trade, has contributed since 1970 to observable expansion of the geographical domains associated with the virus serotypes (Guzman et al., 2010, Murray et al., 2013). In 1970, DENV-1 and DENV-2 were mainly confined to circulation in Southeast Asia, central western Africa, and Mexico, while at that time DENV-3 and DENV-4 was chiefly limited to parts of Asia (Guzman et al., 2010). In equatorial regions, since 2004, all DENV serotypes (1-4) are emerging on all continents, however,
as expected, with some spatial temporal dynamics at the local levels (Guzman et al., 2010). Excluding imported cases and the recently past 2012 outbreak in Madeira, Europe appears to be the only WHO region without regular circulation of one or more dengue virus serotype (Bennett et al., 2010). Though this expansion of dengue virus serotypes since the middle of the 20th century has many deminsions, travel related imported infections remains the beating heart circulating virus strains throughout the globe (Bennett et al., 2010). The heritage of individual serotypes and genotypes have become more manageable in recent years with the recent reductions in cost associated with genetic sequencing of dengue virus and advent of GenBank (http://www.ncbi.nlm.nih.gov/genbank/), an annotated and maintained genetic sequence database. Dengue virus sequencing from travelers returning to Europe matched various genotypes from all four serotypes being imported from each continent’s tropical areas, including Africa (Domingo et al., 2011). More recent molecular surveillance confirms the diversity of dengue virus types around the globe, acquired during foreign travel and imported to Europe (Domingo et al., 2011, Dinu et al., 2015).
Vector: Dengue Vector Dynamics

Suggesting that dengue was a vector-borne disease for the first time in recorded history, in last millennia’s Chin-Dynasty medical encyclopedia’s original explanation regarded illnesses resembling dengue as “water poison,” reasoning that it was connected with flying insects, which were associated with water (Gubler, 1998a). This hypothesized speculation of the medical encyclopedia was confirmed around the turn of the 20th century, when the mosquito was directly implicated in dengue transmission to humans (Ashburn and Craig, 1907). Aedes aegypti and later Aedes albopictus were identified as the relevant vectors for dengue transmission to human (Halstead, 2007). Dengue viruses are transmitted into and among human host populations through mosquito vector populations, when an infecting bite from a female, either Aedes aegypti (primary vector) or Aedes albopictus (secondary vector) takes place (Gubler, 1998a, Lambrechts et al., 2010, Nguyen et al., 2013). When biting infected human hosts during the typical 5-7 days of infectiousness or viraemia, the mosquito vectors can themselves acquire the DENV virus, which then is retransmitted via the mosquitoes’ salivary glands following a 10 day extrinsic incubation period in the mosquitoes’ intestinal tract (Guzman et al., 2010, Rigau-Perez et al., 1998). Aedes aegypti, adapted to urban environments, remains the primary vector implicated in many endemic and epidemic transmission events of dengue in the tropics and subtropics, while the more adaptable, but less competent secondary vector, Aedes albopictus, has expanded its geographical range considerably in recent years especially in temperate areas of the United States, Europe, and parts of Asia (Simmons et al., 2012).

Near the dawn of 20th century, researchers explored the means and modality of dengue transmission (Halstead, 2008). Based on an earlier hypothesis, in 1907 Ashburn and Craig published persuasive confirmation based on human volunteer challenge studies that showed mosquitoes to be capable vectors for transporting the infectious agent for dengue from person to person (Ashburn and Craig, 1907). Among their foundational contributions to dengue research, they also described eco-epidemiological features, consistent with current understanding of connections between environmental vector suitability and dengue transmission:
...Its seasonal prevalence; its occurrence along low-lying, moist coast regions, and in the valleys of rivers, most frequently; its rapid diffusion in some localities, and its lack of diffusion in others; its relation to changes in temperature and moisture; its manner of spread from building to building in infected places; its absence in high altitudes where mosquitoes are absent; the presence of multitudes of mosquitoes wherever dengue occurs, and the absence of the disease in regions where mosquitoes are absent or few in number (Ashburn and Craig, 1907).

Entomological features of Aedes mosquitoes can be helpful for designing dengue control interventions and identifying patterns in transmission dynamics which are persistent compared to those that are not. Aedes mosquitoes involves two distinct life stages, immature stages of larvae followed by pupae in water, and adult stage including the laying of eggs and biting which occur after emerging from water. Heavily dependent on breeding site availability, nutrients, humidity, and temperature, the complete maturation cycle completes in 1-2 weeks typically and longevity can range up to 100 days, although is generally much shorter (Halstead, 2008).

**Primary Vector: Aedes aegypti**

Globally, *Aedes aegypti* is the most important vector for driving the incidence of dengue, particularly in urban tropical settings. Originally noted for its role in yellow fever in Africa and the Americas, *Aedes aegypti* and its viruses have adapted remarkably to urban and peri-urban environments (Gubler, 1998b). This unique feature to transmit dengue in urban areas combines with other traits of *Aedes aegypti* to generate the high vector competence and make this vector most ‘domesticated’ mosquito, therefore driving the majority modern epidemic dengue transmission (Gubler, 1998b). Further underscoring this ‘domestication’, *Aedes aegypti* almost never populates areas more than 100 meters from human settlements, largely due to its human preference, daytime and indoor biting behavior, and ability to breed in artificial containers (Hemme et al., 2010, Reiter, 2010). *Aedes aegypti* populations’ symbiotic relationship with urban human populations has turned urban jungles into manufacturing plants for the dengue vector, providing shelter without predators, a revolving menu of susceptible hosts providing blood meals, and abundant adjacent breeding sites. (Reiter, 2010).

Despite adapting from the jungle to the urban landscape, *Aedes aegypti* is more susceptible to temperature variation than the secondary vector, which has impact on its ability to transmit dengue in more temperate and temperature variable areas. In this manner especially the cold and extreme heat, provide a natural environmental or climate driven barrier outside of which *Aedes aegypti* populations rarely thrive, therefore, the vector persists mainly in equatorial regions between the Tropics of Cancer and Capricorn. However, relatively minor temperature increases, even within the suitable range can modify the biting habits of female mosquitoes’ greatly. In warmer
temperatures, while attempting to complete a blood meal, she may bite multiple hosts, as she is apparently disrupted more easily and with greater frequency (Halstead, 2008). In this manner slightly temperature variation can drastically alter the potential of dengue transmission to secondary hosts, as the bites, necessitated to complete the initial blood meal for reproduction, can vary -therefore warming can attenuate the vector’s epidemiological impact even without drastically increasing its population (Halstead, 2008). *Aedes aegypti* has shown itself capable of epidemic transmission of dengue even in more temperate are as evidence by its sustaining a dengue outbreak in 2012, following introduction to Portuguese island of Madeira as earlier as 2005, despite its general predominance in the tropics (Huhtamo et al., 2013). Presently, *Aedes aegypti* populations in the Americas and elsewhere have been identified as the most common vector driving transmission of another flavivirus very similar to dengue, Zika virus (Chouin-Carneiro et al., 2016). In terms of the vector, Zika infections in *Aedes* mosquitoes have recently been shown to have many similarities to dengue, and like dengue also some potential for variations in a variety of host-viral interactions among mosquitoes from different locations around the world (Chouin-Carneiro et al., 2016). The pandemic transmission of Zika virus, currently ongoing in many sites having *Aedes aegypti*, resulted in the World Health Organization declaring a Public Health Emergency of International Concern on February 1, 2016 (Chouin-Carneiro et al., 2016).

**Secondary Vector: Aedes albopictus**

The Asian tiger mosquito, *Aedes albopictus*, by contrast, most typically has a geographical distribution including areas further outside the urban centers and outside the tropics. *Aedes albopictus* populations seem to be more adaptable to breeding sites available in peri-urban, rural, and natural environments, less perturbed by greater variations in environment conditions such as temperature and humidity, and less discriminating in terms of host preference (Lambrechts et al., 2010). Given that *Aedes albopictus* can freely take blood meals from a greater menu of animal host populations, it can even endure in environments independent of human populations. While the coupled relationship between *Aedes aegypti* and urban human host populations creates difficulties as well, the independence of *Aedes albopictus* increases vector surveillance and control difficulties in a completely different manner. Paradoxically but logically in terms of dengue transmission to humans, the fitness of *Aedes albopictus* uncoupled from human host makes it a less specific vector dengue and limits the vectors’ contribution to stable transmission (Reiter, 2010). It is mainly for this reason that *Aedes albopictus* is regarded as a secondary vector for dengue, but still remains very important especially in terms of dengue expansion an inter-epidemic periods and places (Reiter, 2010). Further highlighting potential importance of *Aedes albopictus*, it has been shown to transmit the virus sexually unlike the primary vector and crucially, vertically more effectively than *Aedes aegypti* (Gratz, 2004). These features combine with a much broader ecological niche to christen the secondary vector with the unique role in maintaining endemic virus circulation during periods between epidemics in some settings. Recent rapid
global expansion constitutes serious public health concerns, as the Asian tiger mosquito’s ability to transmit alphaviral, flaviviral and bunyaviral human pathogens combine with a hearty reputation for survival and less predictable biting preferences (Lambrechts et al., 2010).

A suggested modality for the importation and expansion of *Aedes albopictus* into Europe and parts of North America is the global commercial trade of used auto tires which are shipped over sea (Ward and Burgess, 1993, Grist, 1993). Although it has been known to be in vast areas of Asia and Oceania earlier, as early as 1979, *Aedes albopictus* was found to have been imported to Albania (Gratz, 2004). In the early 1980s, *Aedes albopictus* was imported to the United States and Brazil, as discovered initially by Paul Reiter in Memphis, Tennessee in 1981, however, its role in the transmission of dengue was thought to be more limited until facilitating an epidemic in Hawaii in 2005 (Weaver and Reisen, 2010). By laying eggs in the wells of stacked tires, that are eventually distributed by ocean-going transport, whereby pooled water from precipitation enables development into adult vectors, the hearty invasive mosquito species has truly become a world citizen (Knudsen, 1995). Given the maritime trade routes, port areas along the Mediterranean were initially subjected to the greatest threat; however, *Aedes albopictus* populations are also invasive competitors on land and are identified in an ever expanding portions of the eastern United States, Northern Japan, across much of Italy, the Aegean coast, the French Rivera, and even parts of Belgium and Switzerland (Gratz, 2004). Since being introduced in 2004, *Aedes albopictus* has swelled its spatial domain extensively in the Iberian Peninsula particularly along the eastern coastal areas (Bueno Mari and Jimenez Peydro, 2012). Not so long ago, the secondary vector for dengue exhibited its population abundance and highly effective urban transmission of disease emergence following an imported infection of chikungunya fever in Italy (Reiter, 2010).

More recently in the Americas, the vector has been implicated in pandemic transmission of chikungunya, for which it seems to be especially well adapted, and found suitable to transmit Zika virus (Chouin-Carneiro et al., 2016). While an epidemic of a non-dengue alphavirus, the 2007 outbreak of chikungunya in Italy signaled the real public health threat that the Asian tiger mosquito represents in combination with imported infections occurring in many temperate. Observations and climate modeling suggest environmental suitability for *Aedes albopictus* to continue expanding further into areas southern France, northern Italy, coastal Spain, Adriatic coast and western Turkey with increasing suitability in central northwestern Europe and the Balkans (Caminade et al., 2012, Fischer et al., 2013). In the Americas and Asia, the northern expansion of the vector also seems to follow the same trends as observed and modeled for Europe, in part due to warming temperatures, but also the species unique abilities to adapt to various environments and outcompete other species. Combining similar climate-based techniques with information on sea faring traffic in an application of the used tires hypotheses, research have recreated, retrospectively through models, many of past importation events of *Aedes albopictus* around the world (Tatem et al., 2006a).
Environment: Dengue Environmental Dynamics

The dynamics of environment provide an envelope for transmission of environmentally sensitive diseases, such as dengue. Notably suitable environments provide the foundation for all living organisms and in the case of dengue, the persistence of virus transmission cycles. Environmental features govern where and how individual human hosts dwell, spend time, and subsequently become exposed to infectious agents such as dengue through being bitten by infected dengue vectors. Environmental features further mitigate the successful transmission of the viral agent, through a variety of vector dependent parameters, which we explore in this doctoral work in several studies. Beyond that, the environment also enables, promotes, and limits the survival, abundance and behaviors of vector species. Like human hosts, viral agents, and vector populations, environments are dynamic vibrant systems which are distinct place to place and moment to moment. Understanding the dependence of dengue disease transmission associated with environmental conditions and dynamics, enables the potential identification of the spatial temporal limits of the disease.

The mainstay of environmental dynamics related to dengue dynamics are features which promote the infection of non-immune humans with dengue virus by infectious mosquitoes' bites and subsequently infection of non-infected mosquito with dengue virus by biting infectious humans. Environmental conditions can limit or promote the transmission cycle. Although, this doctoral work chiefly involves environmental factors related to climate, other non-climate factors also promote and demote dengue disease dynamics. For instance, recent urbanization in emerging economies often involves disruption of natural environmental for the construction of buildings. During this process, artificial breeding sites for mosquitoes in close proximity to humans and their dwelling may emerge. The phenomenon of urbanization, combining with hastily planned living spaces, and among the socioeconomically, can provide other kinds of idea conditions contributing to an ecological landscape fit for transmission of dengue as was documented in the United States after 19th century outbreaks occurred there:

*The earth had been disturbed in the paving of the road-bed of King Street, an extensive thoroughfare running north and south the entire length of the city; and the special section where the first cases were noticed was not in as good condition as others, being near the marshes, and new streets having been opened there (Porcher, 1880).*
If ideal climate conditions for dengue transmission in terms of rainfall, humidity, temperature, etc. promote the swift reproduction of the vector, accelerate their human biting, increase the viral replication, decelerate their vector mortality, etc., the transmission of dengue can be maximized. Equally, on the other end of the spectrum, less ideal conditions, environmentally, may disable vectors from transmitting virus effectively and efficiently.

*Many observers thought the hot, sultry weather, abundant rains, and general stagnation of air had a special effect in developing dengue as an epidemic. (Holliday, 1880)*

As Dr. Holliday noted, regarding outbreaks in New Orleans, Louisiana (USA) so agreed numerous other scholars at the time experiencing outbreaks in around the Southeast United States and Caribbean islands (Porcher, 1880). More recently elsewhere in the region, in Trinidad, West Indies, dengue fever incidence was shown to be associated with climate, especially precipitation, which defined in reoccurring patterns a dengue transmission season (Chadee et al., 2007). Since then, the associations have been quantified and refined such that we now can identify better than temperature plays key roles in adult vector survival, viral replication, and infective periods (Carrington et al., 2013a, Carrington et al., 2013b, Liu-Helmersson et al., 2014, Delatte et al., 2009, Lambrechts et al., 2009, Wilder-Smith and Gubler, 2008, Reiter, 2001, Gubler et al., 2001, Patz and Reisen, 2001). For these reasons, increases of temperature may result in increased survival and or migration of vectors into previously non-endemic geographic areas outside the tropics (Liu-Helmersson et al., 2014, Hales et al., 2002). As the proliferation of Aedes mosquitoes is climate dependent, climate or meteorological environmental dynamics when contributing can potentially provide useful information in designing dynamic predictive models of dengue disease. Specifically, weather variability and climate change related modelling has been used shown to predict and project dengue vector activity, disease potential, outset of epidemic, and case incidence through a variety of modelling approaches (Liu-Helmersson et al., 2014, Bouzid et al., 2014, Fischer et al., 2014, Gubler et al., 2001, Patz and Reisen, 2001, Earnest et al., 2012, Wu et al., 2007, Hii et al., 2009).
Epidemiology and Expansion

Global Epidemiology of Dengue and Burden of Disease

The World Health Organization (WHO) labeled dengue as the globe’s most important mosquito-borne viral disease, due to the half of the world people living in countries having dengue activity (Guzman et al., 2010, World Health Organization, 2012). Within the second half of the 20th century, the WHO identified unparalleled intensification (30 fold) in the number of dengue cases, with modeled global burden of disease estimations as high at 390 million infections annually (World Health Organization, 2012, Bhatt et al., 2013). Although the modern observed geographic expansion of dengue is multi-factorial, global travel, changes in climate, virus evolution, vector distribution, population growth, urban development, and commercial trade all have contributed (Wilder-Smith and Gubler, 2008, Astrom et al., 2012). The factors involved may act specifically toward dynamics of the hosts, viruses, vectors, and or environments or more often the nexus of all of the above.

The global distribution of dengue is expansive even given most conservative and crudely calculated estimates from WHO member state based reporting of some 50-100 million annual cases in over 100 countries (Simmons et al., 2012, World Health Organization, 2012, Bhatt et al., 2013). There continues to be widespread discrepancies on the proportion of global population at risk for dengue. In terms of geographical presence and absence, the most thorough and rigorous present evidence-based consensus suggests dengue occurs in 128 countries, although previous reports of the Centers for Disease Control of the United States of America (CDC) and the WHO claim 36 fewer countries (Brady et al., 2012).

Although global research interests sharpened focus on dengue lately, the empirical distribution of dengue remains enigmatic (Brady et al., 2012). Nevertheless, the literature concurs that dengue is an increasing global health concern in terms of both geographical expansion and endemic intensity (Van Kleef et al., 2010, Gubler, 2011, Guzman et al., 2010). On all continents with the exception of Antarctica, recent history has record of dengue outbreaks (Halstead, 2008). Endemic or epidemic in nearly every tropical country, dengue is truly a global threat particularly within the isotherms about the equator (Guzman et al., 2010). Given the significant expansion during the last few decades, current consensus mapping indicates 128 countries have strong evidence to suggest the present of dengue at widely variable levels of transmission (Bhatt et al., 2013, Brady et al., 2012).

The continent of Asia, especially within WHO region Southeast Asian, has well documented endemic and epidemic dengue transmission in countries ranging from Indonesia to Pakistan (Brady et al., 2012). The presence of dengue is confirmed in 85% of countries across Oceania (Brady et al., 2012). Especially in the last three decades the dengue incidence is well documented within the
Americas, from southern Brazil extending northward until the southern border of United States, (Brady et al., 2012). In Africa, despite the lowest levels of available surveillance data, and thus uncertainty regarding incidence, the same group indicates good evidence for presence of dengue in 58% of countries with greatest consensus in coastal countries in eastern and western Africa (Brady et al., 2012). Recent epidemics suggest gross under quantification of what may be substantial ongoing and increased risk for dengue transmission, especially considering the climatic suitability and the difficulty with obtaining accurate surveillance data even for known conditions in Africa (Sessions et al., 2013, Chepkorir et al. 2014, Simmons et al., 2012).

While no European countries were expressly listed as having dengue transmission activity in the 2010 consensus mapping, occasional indigenous transmission events have occurred (Brady et al., 2012). Dengue transmission was detected in 2010 in France and Croatia, following the importation and expansion of Aedes albopictus along the Mediterranean coast in the 1990s (World Health Organization. Regional Office for Europe., 2011, Medlock et al., 2012, Gjenero-Margan et al., 2011). Of more recent and greater health concern, autumn 2012 saw a multi-week outbreak of dengue transmission on the island of Madeira, Portugal involving more than 2000 cases (Alves et al., 2013). While transmission was limited to only a few cases in Croatia and France, the Madeira epidemic resulted in exportation of cases to other European destinations, luckily to-date, none of which spawned further autochthonous transmission (Epidemiological update: Outbreak of dengue in Madeira, 2013).

The most recent consensus approach approximates 3.97 billion people are ‘at risk’ or reside in counties of endemic dengue transmission, contrasted with CDC and WHO claims of 30% and 54% or 2.05–3.74 billion, respectfully (Brady et al., 2012). These estimates are based on the population living in areas known and/or suspected to have dengue activity. Estimates of dengue incidence have commonly been derived from the population ‘at-risk,’ such that the most common incidence approximation cited in the literature and communicated by WHO is 50–100 million infections per year (Bhatt et al., 2013). These estimations do not directly reflect reported cases of infections, but rather extrapolate infections based on the proportion of dengue cases, which were severe dengue manifestations like DHF and DSS in the late 1980s (Bhatt et al., 2013). Rather unsurprisingly, the WHO has called for strengthened global surveillance investment to gain better estimates of dengue burden of dengue (World Health Organization., 2012).

Dr. Peyre Porcher in his extensive account of the 1880 epidemic in Charleston, South Carolina, prophetically describes the enigmatic nature of dengue disease surveillance stating, “it is difficult to calculate the number who have suffered, as very many have not employed a physician (Porcher, 1880).” Similarly in their landmark publication in Nature, Bhatt et al. estimate that clinically apparent dengue infections only account for approximately a quarter of the total number of infections (Bhatt et al., 2013). In this manner, based on
a comprehensive literature review of over 2800 articles, the total annual incidence for 2010 is estimated to have been 390 million cases of infection globally (Bhatt et al., 2013). Largely because Bhatt et al. is also able to include clinically ‘inapparent’ infections, these estimations is more than double the largest previous estimations of dengue infection incidence conducted in 2009 by Beatty et al. and nearly eight times higher than that found by TDR in 2006 (Bhatt et al., 2013). The areas of greatest uncertainty are in countries of very high population, like China and India, and in countries with less developed disease surveillance and reporting systems, like much of sub-Saharan Africa (Bhatt et al., 2013).

Given the difficulty in defining where and how much dengue exists, defining is equally elusive (Stanaway, et al. 2016). The burden of dengue can hardly be separated from its clinical management and control methods; or better stated, the lack thereof. Unfortunately, despite the great expansion of dengue to include half the world population at risk, limited options for treatment are available and dengue control has proven exceedingly difficult. The WHO recognizes that swift clinical and laboratory diagnosis is essential and should be followed by properly supervised intravenous rehydration, to grapple with the epidemiological features of dengue and support dengue control from a perspective of clinical management (World Health Organization., 2012). Regrettably fluid therapy, which if properly administered prevents critical organ failure, remains the only management strategy for patients and clinicians as there are not presently approved antiviral medications to treat or vaccines to prevent dengue infections in most places (Simmons et al., 2012) (Guzman and Harris, 2015). Fluid therapy seeks to replace the minimum fluid needed to maintain cardiovascular function, whether through oral rehydration in less severe situations, isotonic solutions in severe cases, or more rarely blood transfusions in life-threatening cases, as each risks the development of fluid overload which can also be problematic (Simmons et al., 2012, Guzman and Harris, 2015).

**Dengue Global and Regional Expansion**

Given the recent increasing global trends of dengue infections, many experts hypothesize that dengue will continue to increase in the future, both in geographic expansion, incidence and reporting to WHO (Wilder-Smith et al., 2010, Wilder-Smith and Gubler, 2008, Astrom et al., 2012, Hales et al., 2002). This sub-chapter will highlight and elaborate on some of the potential factors that drive dengue activity. These important factors, which have lead and are expected to continue to contribute to dengue’s global expansion, include factors of climate, global interconnectivity, and human settlement as described below.

Mean temperatures are predicted to rise globally and climate variability is likely to increase in many parts of the globe, according to the Intergovernmental Panel on Climate Change (IPCC, 2007). Such environmental regime shifts may generate climatic and environmental
circumstances favorable to the proliferation of Aedes species in areas that are currently non-endemic (Colon-Gonzalez et al., 2013). Climatic similarity with areas endemic for dengue and conducive climates of many currently non-endemic suggest that both Aedes aegypti and Aedes albopictus may establish or re-establish in the near future (Reiter, 2010, Oki and Yamamoto, 2012).

The distinct role climate change may be playing in the recent resurgence of dengue remains indeterminate, but is an active area of current modeling research (Van Kleef et al., 2010, Louis et al., 2014, Naish et al., 2014). While some authors, argue that climate change can be a driver of infectious diseases, others suggest climate may not be the main driver for dengue expansion (Barclay 2006). Beebe et al. concluded rather than due to climate change itself, the an amplified risk of Aedes aegypti range expansion in Australia was due to the installation of domestic water storage containers as a response to persistent regional dryness (Beebe et al., 2009). Moreover, in warmer parts of the United States, from the 18th – early 20th centuries, dengue and yellow fever caused multiple epidemics, which were finally controlled with industrialization and modernization, not due to a change in climate (Reiter, 2001, Reiter, 2010).

Temperature is known to influence adult mosquito vector survival, dengue viral replication and dissemination, infective periods, biting rates, and the probability of bites being infectious (Wilder-Smith and Gubler, 2008, Reiter, 2001, Gubler et al., 2001, Patz and Reisen, 2001, Brady et al., 2014, Liu-Helmersson et al., 2014). Rising temperatures could increase survival and/or mediate the expansion vectors into geographic areas outside the tropics, which were previously non-endemic (Hales et al., 2002, Hemme et al., 2010). Given that the proliferation of Aedes mosquitoes is climate dependent to some extent, climate or meteorological factors can potentially provide useful information in predictive models (Banu et al., 2014). Models utilizing weather variables have been shown to successfully predict dengue activity (Gubler et al., 2001, Patz and Reisen, 2001, Earnest et al., 2012, Wu et al., 2007, Hii et al., 2009).

In the Southwest Pacific, a study conducted suggested that global temperature increases observed over 40 years was associated with increased risk of dengue outbreaks (Banu et al., 2011). Increased transmission of dengue has been shown in some climate change studies to be due to higher temperatures, humidity, and precipitation associated with changes in climate (Hales et al., 2002, Hii et al., 2009, Souza et al., 2010). These findings support hypothesis that observed climatic changes, in the form of increased average global temperature and increased humidity, could theoretically increase the epidemic potential of dengue (Russell et al., 2009, Van Kleef et al., 2010). Hales et al. predicted that approximately 5 billion to 6 billion people would be living in areas at risk of dengue transmission by 2085, based on long-term average vapor pressure prediction, along with climate change and population projections (Hales et al., 2002). This compares in the study’s analysis to only 3.5 billion people, or 35% of the global population would be at risk for dengue
in 2085, if climate change did not occur as projected or were not taken into the model (Hales et al., 2002).

**Globalization, Travel, and Trade Factors**

While climate change unaccompanied may not be a comprehensive and sufficient causal factor in the contemporary expansion of dengue, wider 'global change' may be (Sutherst, 2004). The 'global change' framework broadly includes numerous factors of the modern world scaffolding the expansion of vector-borne communicable diseases (Sutherst, 2004). Current contributors to the swift modern increases of vector-borne communicable disease include globalization factors, such as commercial trade and personal travel, accompanying vector accommodating tendencies in contemporary human dwelling and progressively suitable climate conditions in more and more area (Gubler, 2011). The influences of expansive assess to global mobility, both for vector and human populations, may be the most critical variable to explaining the recent escalation in dengue transmission (Gubler, 2011).

Climate, in combination with human behavior factors, may synergistically facilitate and rationalize the mechanism through which potential for introductions or reintroductions of dengue into non-endemic zones at borders with areas of endemic transmission (Brady et al., 2014, Bhatt et al., 2013). For other areas, further away from the tropics, even though slight expansion due to climate change may have large consequences for human-vector-virus interactions, they may pale in comparison to factors of globalization, which may govern the very coexistence of vectors, humans, and virus. Globalization is a major contributor, but also result of modern global economic development, cultivating a global ecosystem of exchange (Gubler, 2011). The contemporary global reality is one of worldwide passenger travel and intercontinental goods exchange (Wilder-Smith and Gubler, 2008).

In the last six decades passenger air travel exploded, increasing by a factor of 40, increasingly to and from dengue endemic areas (Gubler, 2011). The foremost driving force in increased global transmission and expansion of dengue is held to be infected travelers (Gubler, 2011, Russell et al., 2009). Current-day transport, especially flying, accounts for importation of dengue by surpassing natural obstacles of long distance and relatedly travel time, which had previously limited extension from endemic areas into non-endemic areas (Gardner et al., 2012). A recent model identified routes on which importation of dengue was an increased risk using a geospatial distribution of transmission via passenger air travel between networked nodes (Gardner et al., 2012). Heightened risk routes between the United States and Latin America, and also between Europe and Asia, were classified correlated with geographical expansion of the secondary dengue vector, *Aedes albopictus* (Gardner et al., 2012). All four dengue virus serotypes are circulating in some areas due to intercontinental air travel between areas within the tropics introducing and re-introducing virus of different serotypes (Gubler, 2011,
Wilder-Smith and Gubler, 2008). Furthermore, some speculate the ideal urban breeding ground is combined with a global distribution point at some of the overcrowded airports located in the tropics (Gubler, 2011, Gardner et al., 2012).

As members of global community increasingly trade and travel worldwide, they occasionally transport the excess baggage of communicable and vector-borne diseases (Gubler, 2011). Not only travel, but also trade drives the expansion of dengue transmission and through imported dengue infections. *Aedes aegypti* and *Aedes albopictus*, can accompany international transport of cargo and goods, especially via commercial sea shipment (Banu et al., 2011). The introduction of exotic American mosquito varieties into Italy, linked to commercial sale and sea transport of used auto tires across the Atlantic has been implicated in other vector-borne disease epidemics (Banu et al., 2011, Sutherst, 2004, Napoli et al., 2012). Global tire transport played a major role in the expansion of this major public health threat over the last few decades; expectations are that emerging markets’ increasing automobile consumption will continue this trend into the future, given the vectors’ over-wintering ability, suitability to breed and survive sea travel within water collected in a tire (Reiter, 1998). In addition, developing local travel networks between rural and urban areas via increasing road infrastructure, collectively with dwindling access to diagnostics and surveillance may act as a silent conduit for rural dengue transmission (Mahabir et al., 2012, Vong et al., 2010, Chaparro et al. 2014). The local spatial dynamics and patterns of localized travel have also been showed to influence dengue transmission clustering and cycles (Sharma et al., 2014). The important function of human involvement in constructing favorable ecologies for dengue transmission, in addition to climate environmental factors, is being increasingly considered and modeled in current research (Russell et al., 2009, Astrom et al., 2012, Louis et al., 2014, Naish et al., 2014).

*"That is the paradox of the epidemic: that in order to create one contagious movement, you often have to create many small movements first."

-Malcolm Gladwell
Vector Borne Disease Modeling

Modeling is an area of increasing public health interest, as technology, data, and knowledge has become available to meet global demand for better health security (Keeling and Rohani, 2007). More accurate predictions of the risks infectious diseases may pose are now able to be informed by both mathematical and statistical computerized modeling techniques (Reiner et al., 2013). Vector borne diseases are among the most difficult infectious diseases to generate predictive mathematical models because of their multi-factorial patterns of transmission, involving a plethora of disease- and environment-specific factors (Sutherst, 2004). Even within vector borne diseases, dengue is notoriously problematic computationally for global modeling due to the volume of data required and intricacy of the models used to describe spatial and temporal intersections (Keeling and Rohani, 2007, Hay et al., 2013). Dengue models therefore generally focus on limited aspects of the disease, limited geographical areas, or single points in time (Keeling and Rohani, 2007). Mathematical models are built on assumptions from a given theoretical framework and parameters, which are used in tuning models to better describe or fit observational data (Reiner et al., 2013).

Due to the shortcomings inherit in modeling and the complexity of dengue, models may focus on specific components of dengue transmission such as the environmental suitability of the vector to survive, or the climate dependent competence of the vector to transmit the virus (Racloz et al., 2012). Other more global approaches to dengue modeling seek to amalgamate large volumes of knowledge available from the literature, surveillance reporting, observational studies, and routine data to suggest the spatial extent of the disease or to estimate the incidence of dengue infections (Brady et al., 2012, Bhatt et al., 2013). Still other modeling techniques are used to generate more prospective potential of vector or disease importation and eventual emergence by integrating factors of globalization, commercial trade, and human movements (Tatem et al., 2006a, Huang et al., 2012). This sections discusses and describes the modeling techniques and current published literature most important for current understanding of modeling the current risk of dengue emergence in temperate areas, particularly in Europe.

Mathematical Modeling of Infectious Disease

Mathematical modeling of infectious disease seeks to describe the phenomena of the given disease through a series of casual pathways or according to a theoretical framework, either descriptively in the case of past events or prospectively in the case of future events (Keeling and Rohani, 2007). Public health research in the area of vector borne disease expanded beyond empirical studies into modeling quite early in an effort to ascertain the effectiveness of control mechanisms and predict public health problems before they become epidemics (Smith et al., 2012). In public health today, geospatial-mapping augments modeling of disease activity, in order to describe existing patterns of disease risk geographically. This can be helpful in understanding the
biological mechanisms or statistical relationships that enable disease transmission, which thereby aid in predicting future scenarios of geographical expansion or heightened incidence such as epidemics (Bouzid et al., 2014, Stevens and Pfeiffer, 2011).

Although computation has improved vastly over the last 100 years, modeling mosquito vector disease transmission is a far more seasoned art, which began around the turn of the 20th century, when Sir Ronald Ross started to model malaria dynamics (Reiner et al., 2013). In his 1916 description of modeling, Ross differentiated between the a priori and a posteriori methods:

[In a priori modeling] we assume a knowledge of the causes, construct our differential equations on that supposition, follow up the logical consequences, and finally test the calculated results by comparing them with the observed statistics, [whereas with the a posteriori method], we commence with observed statistics, endeavor to fit analytical laws to them, and so work backwards to the underlying cause (Smith et al., 2012).

In the 1950s and 1960s, Macdonald refined Ross’s original assumptions and framework to develop a series of assumptions commonly described as the Ross-Macdonald mathematical model(s) for mosquito-borne disease transmission, upon which theory much of today’s modeling is still based (Smith et al., 2012, Reiner et al., 2013). The basic theoretical framework of the Ross-Macdonald model simplifies the transmission cycle in mosquito borne disease in four stages as follows:

• Blood-borne disease agents are transmitted to a susceptible host from vectors through infectious mosquito bites;
• The disease agent then matures and replicates at least enough within the newly infected host such that the host becomes infectious, or has sufficient pathogen blood density for onward transmission
• A susceptible (non-infected) mosquito becomes subsequently infected through feeding on the infectious host;
• The disease agent then matures and replicates at least enough within the newly infected mosquito such that the mosquito becomes infectious or has sufficient saturation of the pathogen in the salivary glands or mouth for onward transmission (Smith et al., 2012).

Perhaps in response to global expansion of dengue, mathematical modeling, particularly of mosquito-borne disease, has increased significantly in the literature during the last four decades, with the vast majority of publications newer than 2005 (Reiner et al., 2013). Mathematical modeling continued to expand in the later half of the 20th century and the beginning of the 21st century to include Geographical Information System (GIS) mapping of vector and disease areas to estimate and illustrate the spatial extent of vector borne disease using climate and ecology data, where more empirical surveillance methods were impracticable (Racloz et al., 2012). In light of the clear contributions of globalization and human movements to recently observed
expansions in vector borne disease’s zones of transmission, newer models are integrating travel parameters to predict disease emergence (Tatem et al., 2006c). Combinations of elements of multiple theoretical frameworks are not uncommon in the development of mathematical models, particularly in the case of predicting vector borne disease emergence dynamics (Reiner et al., 2013). Mathematical modeling allows complex phenomena to be described in a logical quantifiable structure upon, which better public health decision can be made (Pinho et al., 2010). In 2010, decision makers of the World Health Organization meet to discuss the role of mathematical modeling in the eventual implementation strategies of the much anticipated dengue vaccine (World Health Organization. Dept. of Immunization Vaccines and Biologicals., 2011). While to-date no vaccine has been approved for the prevention of dengue, mathematical modeling of dengue is an increasingly important area and remains highest on the research agenda after malaria, as evidenced by a recent systematic review of 388 mosquito-borne disease models (Reiner et al., 2013).

Environmental Drivers Modeling of Disease

Climate based suitability indexes and models range widely in their mathematical complexity, however, most models seek to describe the potential geographical range within which vectors are expected to survive, their respective population sizes, and their relative propensity to transmit dengue (Colon-Gonzalez et al., 2013). Generally climate dependent dengue vector modeling considers the impact of rainfall, temperature, and seasonal patterns on the temporal and spatial distribution of vector populations (World Health Organization. Dept. of Immunization Vaccines and Biologicals., 2011). Crude models focus purely on the mosquito’s climate dependent life cycle exclusively, while others focus more on the organisms’ competence to act as vector of the disease. Literature from various model’s suggest within temperate areas, warmer area with adequate rainfall, such as parts of southern and coastal Europe, to be at least seasonably “suitable” to support vector populations of Aedes Aegypti or Aedes Albopictus, both of which have been imported and observed, albeit the latter to a far greater extent than the former (Patz et al., 1998, Reiter, 2010, Reiter, 2001, Caminade et al., 2012, Schaffner et al., 2013, Brady et al., 2014).

Mathematical modeling of infectious disease epidemics began over a century ago to look at factors, which enable mosquitoes to act as vectors transmitting blood-borne disease agent to humans (Reiner et al., 2013). In their most basic form, such modeling holds that individuals undergo the infectious disease transmission dynamic according to the following stages: from “susceptible” to “infectious” to “recovered,” or as it commonly abbreviated SIR –model (Smith et al., 2012). The assumptions built into these early models (and those to follow) included the idea that epidemics of some diseases were related to population densities of humans and mosquitoes, the quantity of infectious and infected mosquitoes, mosquito-biting tendencies, and mosquitoes’ lifespan or survival rate (Smith et al., 2012). As it was eventually discovered that many
of these elements depend on climate factors, contemporary modeling vector-
borne disease increasingly integrates meteorological data to describe areas of
dengue suitability, vector suitability, and the vectors’ epidemic potential
(Lambrechts et al., 2010, Pinto et al., 2011, Massad and Coutinho, 2012, Brady
et al., 2014, Liu-Helmersson et al., 2014, Campbell et al., 2015).

**Modeling Disease Distribution and Incidence**

Although not yet able to operate a real-time model, in 2013, Bhatt et al.
provided a comprehensive novel approach to integrate data from multiple
sources and describe the global incidence of dengue infections with a high
degree of spatial specificity (Bhatt et al., 2013). Continuing efforts published
by co-authors Hay et al. and Brady et al., this body of work described the total
number of infections worldwide in 2010 to have been approximately 390
million (Brady et al., 2012, Bhatt et al., 2013, Hay et al., 2013). This
estimations was derived from a three-step methodology involving a thorough
systematic review of dengue occurrences, a boosted regression tree model of
dengue risk, and cohort study based non-parametric Bayesian hierarchical
model to associate risk with incidence (Bhatt et al., 2013).

Unlike many previous attempts to describe the total incidence of dengue,
these methods utilized a cartographical methodological approach to model
incidence, which initially consisted of a systematic literature review and the
compilation of case reporting data (Bhatt et al., 2013). Peer-reviewed articles
determined 84.6 % of the total 8309 data points organized according to the
magnitude of dengue activity and mapped via latitude and longitudinal
coordinates onto the areas of dengue occurrence extracted from the text
(Bhatt et al., 2013). The cases were organized according to the area of dengue
transmission; in other words imported cases were assigned to the country
where the illness was contracted and autochthonous cases were assigned to
the lowest specific administrative level feasible (Bhatt et al., 2013).

The observed occurrence database was combined with mathematical
modeling of “dengue probability” based on 8 ‘explanatory co-variant’ risk
parameters (Bhatt et al., 2013). The model sought to better describe the
intricate nature of dengue transmission mathematically by expanding
environmental suitability beyond climatic and into socioeconomic factors
important for dengue dynamics including public health infrastructure,
poverty, and urbanization (Bhatt et al., 2013). Unlike the methodologies of
the Ross-Macdonald model, the modeling suite used 336 boosted regression
trees (BRT) statistical analysis models to generate probability of dengue
occurrence globally with a high degree of spatial precision (Bhatt et al., 2013).
By inclusion of temperature, precipitation moisture, urbanization, and
poverty, this modeling more holistically demonstrates area having a suitable
ecology for dengue transmission including vector, virus, and human host
drug and utilizing data available on the global scale (Bhatt et al., 2013). In
so doing, this novel approach transcends past mathematical models of dengue
suitability, which are focused primarily on climate-based factors known to influence vector competence in virus transmission (Bhatt et al., 2013).

Bhatt et al. augmented the systematic literature review of the newly defined spatial extent of dengue transmission, and the probability of dengue occurrence BRT modeling with 54 serological cohort studies to determine and relate the regionally specific incidence of dengue infections in the population (Bhatt et al., 2013). Due to the inclusion criteria of serological cohort study only, Bhatt et al. where able to make more comprehensive determinations about infection incidence rates, being able to identify both infections which presented with clinically ‘apparent’ symptoms and ‘inapparent’ or non-symptomatic infections (Bhatt et al., 2013). These finding were subsequently used to construct Hierarchical Bayesian linear model relating probability of dengue occurrence to both ‘apparent’ and ‘inapparent’ infection incidence (Bhatt et al., 2013). This model was combined with the cartographical models previously described to generate the published estimates of dengue infection incidence rates by country in 2010 (Bhatt et al., 2013). While this does not directly explain risk of introduction nor future trends geographically or temporally, this inclusive body of work has shed new light on the total incidence of dengue during 2010. Reliable and comprehensive estimations on dengue infection rate around the world are vital in modeling the risk of travel-related importation of dengue.

Modeling Global Interconnectivity and Air Travel

The role of travel and trade in the distribution of vector borne disease is a growing area of research (Schaffner et al., 2013). Global interconnectivity plays a vital role in the recently observed invasive expansion of the dengue vector, *Aedes albopictus*, the viral dynamics trending towards hyperendemicity in various parts of the tropics, and increasing importation of dengue in Europe (Guzman et al., 2010, Reiter, 2010, Wilder-Smith and Gubler, 2008, Napoli et al., 2012, Odolini et al., 2012). Mathematical modeling is currently being used to structure the role played by global interconnectivity, particularly flight transport, in disease importation in a number of different applications (Huang et al., 2012, Tatem et al., 2006c, Sessions et al., 2013, Quam et al., 2014, Wilder-Smith et al., 2015, Semenza et al., 2014, Lourenco and Recker, 2014).

As earlier as the 1930s, air traffic was found to be responsible for the unintended importation of exotic mosquitoes varieties along other insects, however sea going transport is currently most often associated with vector invasion dynamics (Tatem et al., 2012, Tatem et al., 2006a). Air passenger flow and/or commercial trade routes are vital components, which differentiate this type of modeling from those discussed in earlier sections. Various studies and models have been published, which seek to model the importation-related risk and public health impact of modern transportation linking areas of endemic disease activity to non-endemic areas, which either lack the necessary environment parameters, vectors, or pathogens for
transmission (Tatem et al., 2012, Huang et al., 2013, Huang et al., 2012, Goubar et al., 2009, Gardner et al., 2012, Tatem, 2009, Seyler et al., 2009, Tatem and Hay, 2007). Most such modeling approaches used flight transport either as a proxy for interconnectedness between regions or as the primary conduit of disease importation. The sources and precision of transport data, even on commercial flight, varied widely between the studies, as does the degree of complexity associated. Generally modeling are constructed based on the paradigm that disease contracted while travelling can be imported if the traveler is still infectious upon return (Seyler et al., 2009, Quam and Wilder-Smith, 2015, Lopez et al., 2016, Nunes et al. 2014, Semenza, et al. 2014).

The most comprehensive approaches currently published, integrate detailed disease occurrence and potential occurrence information on both sides of travel with detailed travel data and spatial on travel routes to generate risk predictions or descriptions (Huang et al., 2012, Gardner et al., 2012, Goubar et al., 2009). The vast majority of dengue importation models involve a multistage theoretical framework. Most begin methodologically by defining the geographic extent of dengue either simply by using routine sources such as the CDC Yellow Book list of countries or more intricately by generating a model to spatially define areas of endemic dengue activity (Gardner et al., 2012, Tatem et al., 2012, Huang et al., 2012, Seyler et al., 2009). Then some estimate or incorporate a parameter related to the incidence within defined areas of endemic activity, such as country level incidence based on WHO regional office reporting (Seyler et al., 2009, Gardner et al., 2012). Presumably, due to the high degree of variability in surveillance techniques and reliability limitations of such global incidence data sources, the modeling efforts of Tatem et al. and Huang et al., producers of the VBD-air tool, did not include an incidence parameter in their model(Huang et al., 2012, Tatem et al., 2012, Huang et al., 2013). Rather their model included a climatic similarly index called Climatic Euclidean Distances (CEDs), which has been the basis for previous interconnectivity studies (Huang et al., 2012, Tatem and Hay, 2007). The final stage of the common framework is based on a parameter of traveler (or trade) volume, which largely consisted of direct flights scheduled passenger capacity along the routes of interest (Gardner et al., 2012, Tatem et al., 2012, Huang et al., 2012, Seyler et al., 2009).

Most studies had some additional parameters or variation in the models presented in the literature to account for additional dimensions of dengue risk including the presence of suitable vector populations and/or modeled transmission dynamics. For this reason, the quantitative risk generated by each model differed in unit, magnitude, and external comparability. Despite the variety of models, parameters and quantitative importation risks, they produced, the conclusions in the literature were compatible and complementary, often presenting conclusions in terms of predicted cases of importation or ranked routes of highest risk (Tatem et al., 2012, Gardner et al., 2012).
Materials and Methods

Overview

The studies included in this doctoral work involved a number of materials and methods from various quantitative analytical disciplines and combined several modeling frameworks. At the core of these studies were the following objectives aimed at generating greater understanding of the occurrence and importance of imported infections of dengue in temperate areas:

- To dissect spatial and temporal origins of novel epidemic transmission events (studies 1 and 3, retrospective analyses)
- To model recurring patterns of dengue importations related to travel and/or climate (studies 1, 2, 3, and 4, both retrospective and prospective analyses)
- To generalize models to assess potential for importation driven epidemics in non-endemic areas driven by global change dynamics (studies 2 and 4 prospective analyses)

Two of the studies contained in this doctoral work look specifically at two recent epidemic transmission events of dengue fever, which occurred in temperate climates. The first, upon our research culminated into study paper 1, was an outbreak of dengue fever on the island of Madeira in 2012. Madeira is an autonomous island region of Portugal, which is a popular tourist destination in Atlantic Ocean, lying about 1000 km west south west of continental Europe (Sousa et al., 2012). The second event which we analyzed in great detail was the first outbreak of dengue in Japan in approximately 70 years, taking place in Tokyo in 2014 (Arima et al., 2014). Through various techniques, we assessed factors, which may have contributed to the outset of these unique transmission events, which went on to become outbreaks.

Our analysis included several techniques of modeling to suggest likelihood of time of introduction of virus and the location where the infected individual likely contracted the infection. We extensively used travel (study 1) and climate data (study 3) along with available information on the genetic similarity (studies 1 and 3) of the specific dengue virus circulated during these outbreaks to assess potential pathways for the induction of virus. Furthermore, our analyses of these events importantly enabled us to generate knowledge of the temporal spatial suitability for introduction events like the capacity for an infected traveler to drive epidemic dengue activity in new areas. Largely the phylogenetic analysis of the imported virus compared to other virus sequences available in international databases, allowed us to validate and better confirm specific hypothesis generated by the imported infections and environmental conditions’ modeling exercises to be consistent with the importation event, which occurred in the case of the two outbreaks dissected in studies 1 and 3.

In our studies, which focused attention on Italy (study 2) and Europe (study 4), we were able to describe and project the potential magnitude for such
imported infection events, and analyze what potential for local outbreaks of
dengue may exist in temperate areas, particularly under global change
dynamics. Specifically, we modeled the separate effects of climate change
(study 4) and increased air-passenger travel (study 2) departing areas
endemic for dengue activity in these studies. The following chapter will outline
the materials and methods used in the four studies of the doctoral work to
reach these objectives, describe the models developed and utilized, and detail
the types and sources of data, which drove these models to produce the
studies' results and findings.

The study approach of this doctoral work involved systematically developing,
assembling, and applying techniques to assess the potential for novel
emergence of dengue in temperate areas to be instigated by imported
infections among passenger air-travelers.

Elements of the methodological process included:
• Considerable review of literature to develop theoretical framework
• Acquisition of routine reporting data, country specific surveillance data,
  vector surveillance data, modeled data on geographical extent of dengue
  incidence, and air passenger travel data
• Data cleaning and analysis
• Synthesis of ideas, information, and available data
• Utilization of existing tools, and frameworks
• Development and refinement of emerging mathematical models and
  procedures for integrating existing data
• Application of models through calculations and analysis conducted using
  programming, statistical, and cartographical software
• Development of results' tables, maps, charts, figures, videos, and
  contribution for decision support tools

The review of literature coupled with expert knowledge networks within the
research team aided in building the modeling theoretical framework, choice
of sites, choice of data to be harvested and knowledge on how to operationalize
available resources. The development of a theoretical framework generated
tools to more systematically critically analyze factors that can contribute to the
potential emergence of dengue in temperate areas.

There were prospective and retrospective portions of the doctoral work. The
retrospective studies (studies 1 and 3) aimed to leverage the rare occurrence
of dengue in temperate areas to dissect how epidemic dengue managed to
emerge, from where the virus might have been imported, and what global
environmental or human dynamics contributed to the commencement of the
outbreaks. In an effort to understand the main contributing factors for
emergence of dengue in new places, this thesis had two arms: one focusing on
estimating the occurrences imported infections (paper 2) and the other
focusing on potential onward transmission implications of imported dengue
infections (paper 4). These prospective portions of the study sought to harness
some lessons learned from the association models based on the retrospective
analysis of epidemics and other knowledge from the review of scientific literature to generate short term predictions of imported infections and long term projections of potential epidemic emergence in key temperate areas of potential risk for dengue transmission in the near future.

To estimate the likelihood for imported infections to drive novel dengue emergence, we developed an importation model based on travel and model-based on environmental drivers of dengue, which could each generate spatial temporal outputs and eventually be combined.

The importation model involved integrating information air-passenger travel with information of dengue occurrence, such as national and international surveillance data, or available modelled estimations on dengue infections in over 160 countries of disembarkation. Although many types of models exist based on environmental drivers of dengue, we decided to employ and further refine a mathematical model of vectorial capacity to estimate the temperature-dependent dengue epidemic potential in temperate areas of Europe and Japan.

The models developed to integrate routinely available data, like previous models of traveler-related imported infections identified from the literature review, included three keys components:

• Destinations’ suitability for disease transmission
• Origins’ level of disease activity
• Volume of travel from disease endemic origin to disease suitable destination

Although the studies presented in this doctoral work are based in only a few locations, the generalization of this modeling framework seems appropriate for other locations and potentially other kinds of infections, noting that not all parameters and model elements could be conserved. The studies included only investigated outbreaks in specific destinations of the Portuguese Island of Madeira, and Tokyo, Japan and generalized applications for Rome, Italy and Europe at large to explore both theoretically and observable suitability for importation driven local dengue transmission (Cignini et al., 2008, Medlock et al., 2012, Alves et al., 2013, Kutsuna et al., 2015). These sites were chosen based on their respective resulting categorization of dengue potential emergence, the availability of data on air passenger travel, and current dengue or projected vector presence.
Theoretical Framework

Figure 1: Host-Agent-Vector-Environment: H.A.V.E. Transmission Tetrahedron

Figure 1 above shows the Host-Agent-Vector-Environment Transmission Tetrahedron. This theoretical framework illustrates the multidimensional elements necessary to be present and interacting simultaneously to “H.A.V.E.” dengue transmission. The green base of the tetrahedron represents a dengue suitable environment. The red right side of the tetrahedron illustrates the necessity for an appropriate mosquito vector population to be present and support within the environment. The yellow left side of the tetrahedron displays the agent, any of four dengue virus serotypes, which act as the disease causing pathogens in dengue transmission. The blue back side of the tetrahedron representing the host population allows for the completion of dengue transmission cycles, assuming temporal and spatial interaction with the other three elements aforementioned.

All four components, host, agent, vector, and environment, have unique dynamics and are interrelated drivers of dengue transmission, which if an individual or combination of elements is changed may enable or disable the transmission of disease. The H.A.V.E. dengue transmission framework has the following combinations of potential factors for dengue transmission, which
reflect the conditions missing for transmission to become possible. In the case of temperate areas, imported infections can introduce novel viruses coinciding with environmental conditions which influence disease dynamics particularly related to vector interactions. If the puzzle pieces all come together in time and space transmission of dengue could emerge in areas, where it had not before, as was the case in Madeira 2014 (Study 1) and Tokyo 2014 (Study 3).

<table>
<thead>
<tr>
<th>H</th>
<th>A</th>
<th>V</th>
<th>E</th>
<th>Potential for transmission if...</th>
<th>Elements</th>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>Conditions remain similar</td>
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</tr>
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</tr>
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<td>+</td>
<td>0</td>
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<td>Host settlement expansion &amp; virus importation</td>
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<td>0</td>
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<td>No risk of establishment</td>
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</table>

*Shaded areas indicate scenarios most pertinent to the included studies

Temperate Areas Modeling Site Selection

A portion of the review of literature was particularly focused on the introduction of dengue into temperate areas and especially Europe. This included particular focus to the articles, which studied, modeled, described, or displayed the suitability for dengue transmission and vector populations specifically within temperate places, including parts of North America, Asia, and individual areas of Europe. These articles informed the degree and categorization of potential risk for dengue emergence within individual countries and regions. From this analysis the specific studies sites for study were chosen.

To identify and categorize areas according to the theoretical framework for emergence of dengue, articles with specific information temperate regions were examined in detail, with priority to topics of mosquito surveillance, climate related vector born disease expansion, and traveler related importation of disease. According to their suitability and recorded observations of features determined necessary for novel emergence of dengue, counties’ risk potentials were categorized. Literature review was the chief mechanism for this determination and verification; however, it was also informed by secondary data sources on climatic conditions, geographical features, and other published entomological information. This was especially true in obtaining mapping data on the presence and climatic suitability for survival and expansion of relevant dengue vectors in Europe. Consensus of vector surveillance, literature, climate data, and previous maps from multiple sources were used to develop a map of for both Aedes albopictus and Aedes aegypti introduction, establishment and suitability in Europe according to district administrative level using QGIS and later ArcGIS Softwares.

The Portuguese Island of Madeira was selected due to the population of Aedes aegypti found there since 2005, climatic suitability, and its observed recent epidemic of dengue hypothesized to be of imported origin (Frank et al., 2013). Similarly, in light of the 2014 outbreak of dengue, Tokyo, Japan was chosen. Rome, Italy was selected as a destination of interest for the risk modeling, due to the evidence based consensus of Aedes albopictus vector activity, relative climatic suitability for dengue transmission, and large volume of international flight traffic (Burdino et al. 2012). Lastly, we opted to expand the application of methods used for modeling the environmental suitability for dengue emergence to other destinations in Europe. We sampled a number of metropolitan areas to study the variation within temperate areas under past, present and projected future environmental conditions. Our choice of cities sought to maximize some of the range of potential exposure to importation-driven outbreaks, therefore, we included some comparison areas with dengue transmission, to temperate areas currently with greater and lesser environmentally conducive to epidemic transmission, some already having relevant vector populations and many of which having large international airports.
Model Selection and Design

Travel Drivers’ Model

One basic tenant of this doctoral work based on the theoretical framework, was that persons travelling from locations where disease transmission occurs, can pose a risk of importation to places where of disease does not occur, if individuals are infected before they depart and infectious at some point after they arrive. We hypothesized that the potential of infected travel driving dengue dynamics in new areas would depend initially on the intensity of dengue activity in the departure country and secondly on the amount of travelers going from the country with dengue activity to the new area.

Initially, in study 1, we developed and applied very simple index for assessing the comparative likelihood of an imported infection of disease coming from a specified place where that disease was measure during a specified time. The practicality of this importation index was that it provided a simple means for comparing potential origins of an epidemics’ index infection(s), which could be mined globally in a similar manner from individual countries surveillance systems. By applying data on travel, one could generate metrics on the potential for traveler based importations of infections and assess to a degree the likelihood for introduction event which could lead to vector borne disease emergence. Most importantly, despite its coarseness, this method was also able to be applied as temporal model to some degree, enabling the synergistic qualities of the seasonal patterns of travel and those of seasonal disease dynamics to emerge in the model.

In this preliminary model, we defined importation index for an area as:

\[ ID = IR \times TV, \]

where ID was the calculated importation index, IR was the case incidence rate of dengue in the country or area where the travelers’ departed and TV was the volume of persons traveling to the destination, where dengue was potentially imported. Importantly the incidence should be scaled according to the total population of the area and time period during which travelers depart. In the application of this model in study 1, we utilized it to generate output in both annual and monthly time horizons at the country level, however, also applications of the model at lower administrative levels or time intervals may be possible, limited by collection of data on dengue incidence and travel.

The model itself, although incredibly simple, rests on the complexity and quality of the data which is input to it. The initial importation index was useful in development of comparative metrics for the importation potential that one area may pose toward another in terms of relative origin of imported cases, however, did little to approximate the total number of potential imported infections. For the retrospective analysis, as was the objective of study 1, this simplified model enabled relative discrimination between areas from which potentially infected travelers may arrive in Madeira, however, estimating the
actual number of imported infections in a setting where there had not yet been an outbreak required a more refined approach.

To calculate the potential for dengue emergence based on the intersections between data on dengue incidence in endemic areas, vector presence in temperate areas, seasonal vector suitability and possible virus importation via air passenger travel to Rome, Italy, a simplified risk of importation model was developed for use in study 2. For satisfying the objectives of study 2, we set out to develop a more refined version of the importation index model to better quantify estimates of the total exposure to imported infections of dengue that a given location has. We also wanted to do this in a manner, which allowed for some year-to-year dynamics in both dengue incidence and travel patterns to inform prospective predictions for how imported infections might be changing in the near future. Crucially in this updated Importation Model ($R_I$), we wanted to refine the simple model to one which described the likelihood that a traveler from an endemic country was actually infectious for dengue during or after the travel to the temperate area.

The model was designed to estimate the number of importations of dengue virus via air passengers from dengue-endemic countries to Rome, Italy, who were viremic at the time of travel or post arrival. Our refined importation model in its most straightforward terms computes an estimation of the potential infections imported to Rome ($R_I$) by multiplying three factors together as defined by the following formula

$$R_I = T \times I \times P,$$

where $R_I$ estimated infected travelers importing dengue infections within a given time period (governed by the input data temporal resolution) to the destination airport, based on the volume of passenger air-travelers ($T$) arriving from a given dengue-endemic area with the estimated per-person incidence ($I$) of dengue infection and temporal probability ($P$) of dengue infection overlapping with travel. The model estimated output is the number of infections, which would be imported theoretically by air-passenger within a defined period of time. Datasets on infection incidence were associated with datasets on travel to calculate the potential imported infection ($R_I$) posed by each county to the destination airport (Rome), by quarter, and by year using this model. Although having some unique features in its applications and units of measures, the initial and refined travel models remained conceptually consistent with numerous other studies on the subject (Huang et al., 2012, Tatem et al., 2012, Gardner et al., 2012, Seyler et al., 2009).

Two key improvements were made in this refined model compared to the initial model. Firstly, the $I$, in this model, represents infection incidence rate per person in a given area (or country in the application in study 2), where $IR$, in the previous model, was the national case incidence per 100,000 persons living in a given country. The distinction between the two measures of the departing locations’ intensity of dengue activity, is between reported cases and estimated infections—more than anything is a trade off involved in the choice of input data. In terms of the output, dengue infections consistently occur
more commonly than do cases get reported for multitude of reasons, so the refined method will have higher numbers. Using infections also highlights the fact that dengue disease emergence through importation events could be sparked by individuals, who do not meet standard case definitions, seek healthcare, or even exhibit symptoms. Cases, as used in the initial model, are more conservative and traceable, given that cases are reported and infections are model estimated. The second major distinction was the addition of the temporal probability factor, $P$. This factor integrates the several human dynamics of dengue disease into the model. Ascertaining the actual intrinsic incubation period in human host can be difficult due to ethical concerns. Early human volunteer studies offered some insight suggesting a range from 3-10 days, averaging around 5-6 days was between an infecting mosquito bite and the onset of symptoms (Nishiura and Halstead, 2007, Halstead, 2007). Unfortunately, discerning the precise duration and distribution of viremia has been hampered by diagnostic tool, however, some studies suggest 7 days, of which 4-5 days could occur after the intrinsic incubation period (Nguyen et al., 2013, Seyler et al., 2009). Therefore, our model considers imported dengue infections possible if an infecting mosquito bite occurs within the 10 days proceeding travels (intrinsic incubation period, ~5.5 days added to the remaining viremia, ~4.5 days). If the reporting period for infections in the model is annual, as was the case with study 2, then $P = 10$ days/365 days.

The calculations for $R_I$ and quantities $T$, $I$, and $P$ assume:
- Travelers and non-travelers have equal susceptibility to become infected
- All persons within areas have equal likelihood of contracting an infection
- All times within a period have equal chances of infection
- The summed length of incubation and infectiousness is normally distributed about a 10-day mean following an infecting mosquito bite
- Travel is unrelated to onset of infection or illness, i.e. symptoms do not reach a level of severity to dissuade individuals from traveling
- Historical multi-year trends in disease and travel continue in the near future

**Environmental Drivers’ Models**

Another basic tenant of this doctoral work based on the theoretical framework, was that imported infections only posed a series threat to public health in terms of disease emergence if the environmental conditions were such that onward transmission and eventually epidemics could subsequently occur. We hypothesized that the epidemic potential for temperate areas could be quantified using deterministic mathematical models based on environmentally sensitive vectorial capacity calculations. Parameterization for these deterministic models was based on extensive literature review conducted by the research team. We sought to find temperature-sensitive measurements based on laboratory experiments for various entomological traits important the quantifying the vectors’ ability to transmit dengue. The generation of a temperature dependent vectorial capacity model allowed us to better generalize laboratory measurements of individual dengue transmission dynamics related to both vectors, agents, and host based on environmental
conditions. This allowed for the development of models which can help to describe the potential spatial and temporal dynamics governing the possibility and intensity of dengue transmission even in places, where one of more elements needed for transmission is still missing. For novel transmission events such as that in Madeira in 2012 and Japan in 2014, as well as those in temperate areas yet to come, these models were particularly appropriate.

For study 3, we utilized a model developed by Liu-Helmersson and colleagues previously published model of temperature-dependent relative vectorial capacity to describe the temporal dengue epidemic potential for Tokyo during the 2014 outbreak (Liu-Helmersson et al., 2014). Vectorial capacity or daily reproductive number is a concept originally introduced in 1964 by WHO researcher Garret-Jones to describe malaria transmission (Massad and Coutinho, 2012, Smith et al., 2012). This model highlights the entomological features of vector-borne diseases by integrating the mosquito dynamics more into the Ross-Macdonald based SIR model (Smith et al., 2012). Based on the assumptions that the appropriate mosquito vector population was present and dengue virus was circulating in human hosts and/or mosquito vectors, vectorial capacity quantifies the probable magnitude of transmission, which mosquitoes can drive as vectors, which can vary according to environmental conditions (Smith et al., 2012). Specifically, vectorial capacity describes the vectors’ success at spreading disease by determining the mean quantity of secondary cases generated each day by one infected person amongst a fully susceptible population during his/her infectious period (Liu-Helmersson et al., 2014). We used relative vectorial capacity (rVc) for Aedes vectors to quantify the dengue epidemic potential based on temperature dependent parameters in Japan. Our calculations followed a modified Ross-MacDonald/Garret-Jones model to determine the vectorial capacity relative to the vector-to-human population ratio. The relative vectorial capacity was defined by the following formula:

\[ rVc = \frac{a^2 b_h b_m e^{-\mu m n}}{\mu m}, \]

where \(a\) is the average daily vector biting rate, \(b_h\) is the probability of vector to human transmission per bite, \(b_m\) is the probability of human to vector infection per bite, \(n\) is the duration of the extrinsic incubation period, and \(\mu m\) is the vector mortality rate (Liu-Helmersson et al., 2014). All of these parameters effecting the vectors’ ability to transmit dengue, like many of those impacting mosquito development, have been shown to vary according to temperature, both in terms of mean temperature and daily temperature variation for Aedes aegypti (Liu-Helmersson et al., 2014, Alto and Juliano, 2001, Almeida et al., 2005, Lambrechts et al., 2011, Descloux et al., 2012, Brady et al., 2014, Carrington et al., 2013a, Carrington et al., 2013b).
Figure 2 above shows the temperature dependencies of 5 parameters as calculated for the relative vectorial capacity model applied in study 3 based on Liu-Helmersson and colleague’s models, as published in 2014. The parameters are as follows: \( a \) is the average daily vector biting rate, \( b_h \) is the probability of vector to human transmission per bite, \( b_m \) is the probability of human to vector infection per bite, \( n \) is the duration of the extrinsic incubation period, and \( \mu_m \) is the vector mortality rate. The laboratory studies leading to the development of this model were conducted for *Aedes aegypti* in various sites (Scott et al., 2000, Lambrecht et al., 2011, Briere et al., 1999, Watts et al., 1987, McLean et al., 1974, Focks, et al., 1995, and Yang et al., 2009).

The information provided by modeling relative vectorial capacity using, these climate dependent parameters aid in determining the daily reproductive number, or the number of new secondary cases per infectious person needed to sustain transmission cycles assuming that the ratio between female mosquitoes and susceptible humans is constantly equal to one. In this manner,
relative vectorial capacity describes some of the dynamics of the ecological-based potential for epidemic transmission and/or suitability for dengue establishment in naïve areas, once introduced, however, relative vectorial capacity cannot describe the likelihood of the introduction of virus or nor in anyway take into account the abundance of vector, which are prerequisites assumptions of the model.

In our more general application of the environmental drivers’ model, in study 4 for multiple locations within Europe, we opted to refine the relative vectorial capacity model in several ways. Namely, we sought to better take into account the role that mosquito abundance can play and calculate the climate sensitive vectorial capacity. We also wanted to calculate the vectorial capacity not only based on *Aedes aegypti*, but also the secondary vector *Aedes albopictus*, which is widespread in some parts of Europe, particularly in the south. To do this, we found literature supporting some parameter changes and procedures for estimating for the temperature dependence of the female mosquito to susceptible human ratio based on temperature sensitive mortality (Lambrechts et al., 2010, Delatte et al., 2010, Brady et al., 2014, Liu-Helmersson et al., 2014). For the latter, we found that we could tune the relative vectorial capacity model according to this ratio, such that the temperature variable ratio of mosquitoes to human would be maximally 1.5 under optimal conditions. Figure 3 shows how these refined models including parameter changes could influence the output metrics for a range of scenarios in terms of mean temperature and diurnal temperature range.

**Figure 3: Comparison of Relative Vectorial Capacity with Vectorial Capacity for *Aedes aegypti* and Vectorial Capacity for *Aedes albopictus***

![Figure 3](image)

Figure 3 shows the overall similarity in the various vectorial capacity models in terms of patterns of temperature dependence, however, one should note that the intensity of the *Aedes aegypti* based model is clearly greatest. The yellow color in all three model variations indicates the threshold condition of 0.2, which corresponds to a basic reproductive number of 1, assuming individuals have 5 days of infectiousness as Liu-Helmersson and colleagues have done (Liu-Helmersson et al., 2014). Using this same threshold, the original relative vectorial capacity model used in study 3 for Japan has greater similarity to the vectorial capacity model for albopictus in terms of overall scale and appearance, however, less similarity in terms of the range of mean temperatures and diurnal temperature range combinations resulting in above
threshold environmental conditions which would theoretically permit the commencement of epidemic transmission.

The calculations of relative Vectorial Capacity and Vectorial Capacity assume:
• Sufficient virus, mosquito, and host populations are present and interacting
• Temperature is the main environmental driver of once above is satisfied
• Spatial and temporal smoothing of data do not interfere with the model
• Infectious period is on average 5 days per infected individual
• Vector parameters from the laboratory studies hold true in temperate areas
• Historical, present, and future data are comparable for long-term analysis

**Phylogenetic Analysis Models**

Multiple sequence alignment was possible to aid in the investigations of the imported dengue strains to Madeira and Japan in studies 1 and 3, respectively. Once samples from early cases in each outbreak had been collected and sequenced by members of our research team, among others, we sought to link the implicated sequence to those deposited in GenBank. This comparison, harnessing tools of 21st century big data sharing tools, enabled us to assess the genetic similarities and differences to further suggest the most probable origin and to a lesser degree timing of the imported case. Colleagues used a fast Fourier transform in MAFFT software for the multiple sequence alignment modelling (Katoh et al., 2002). To generate a maximum-likelihood phylogenetic tree RAXML and FigTree v1.4.0 softwares were used at a robustness of 1000 bootstrap replications (Stamatakis et al., 2005, Rambaut, 2012). These phylogenetic sequencing and models efforts were explored as a mechanism to validate parts of the findings of the initial models on travel and environmental conditions, which precipitated the 2012 and 2014 outbreaks of dengue in Madeira and Japan, respectively. The constraints on the usefulness of this methodology like the models above are primary to do with the data used for input; however, given that such is available, the model itself is relatively simple.

The input data for phylogenetic analysis is based on the molecular genetic sequencing of all or part of the genome of a specific sample of dengue virus, isolated from an infected patient. Generally, with dengue whole genome sequencing may be desirable, but more often the envelope protein or E gene is prioritized. This is because some evolutionary modulation in envelope protein amino acid changes has been evidenced to accompany endemic and epidemic emergence, suggesting the virus itself may have a role to play as well as mosquito hosts interactions (Wang et al., 2000). Wang and colleagues demonstrated that domain III may play a role in this viral adaptation to naive hosts, whether mosquito or human, through their analysis of modifications to the envelope protein, postulated to correlate with endemic and/or epidemic emergence (Wang et al., 2000). It is now hypothesized that increased efficiency of transmission of dengue within vectors and hosts shift the evolution of dengue disease towards greater virulence, such as strains responsible for epidemics (Rico-Hesse, 2010). For these reasons, the
catalogue of available envelope protein sequences in GenBank is vast compared to full genomes sequenced. For study 1, the level of analysis was a sequence of 454 base pairs, which were compared to the analogous proteins sequence from outbreaks and transmission events around the world. For the confirmations used in study 3, the sequences of envelope protein isolated from the outbreak were critically assessed for there base-pair level differences with 3,282 other unique DENV1 sequences in the GenBank depository maintained by the National Center for Biotechnology Information. As an international resource, the dengue depository in GenBank provided information on the time (usually year) and place (usually country) where the virus was isolated and had been discovered to be in transmission, which lends this model technique well to validate the other temporal spatial dynamic models which were part of this doctoral work.

The phylogenetic analysis calculating similarity between virus strains assume:
• Strain(s) recovered from outbreaks are representative of transmission there
• GenBank databank used for comparison has exhaustive related sequences
• Base pair differences occur through viral evolutionary mutations
• Cataloging these variations indicate the time and/or place where ancestral virus strains were most likely last circulated
Data Selection

Travel data

Travel data were collected, analyzed and integrated in order to model the potential for imported infections of dengue to arrive selected destinations, where local dengue transmission was uncommon. As explained above, we selected the cities of Funchal (Madeira), Portugal, Rome, Italy, and Tokyo, Japan as the air-passenger travel destinations within studies 1, 2, and 3. To obtain quantitative data on global air passenger interconnectivity between Rome and Madeira and countries with good consensus on dengue activity, we partnered with Bio.Diaspora, now BlueDot in Toronto, Canada. Traveler information used in the retrospective analysis of outbreak in Japan was retrieved from Japanese authorities (Japan National Tourist Organization, 2014). The flight data Bio.Diaspora contributed was retrieved from International Air Travel Association (IATA) datasets through their partnership to access to the comprehensive air-passenger travel information collected from approximately 4,000 IATA airports globally. We cleaned, organized and analyzed annual International Air Travel Association (IATA) data for 2005-2012.

Figure 4: BioDiaspora User-Interface for Direct Flights Routes to Rome

We elected to partner with Bio.Diaspora and utilize the IATA data because it is the most robust and complete source on international air travel available at the passenger level. Unlike other more accessible data sources we initially selected and those selected in previous publications on the topic, we were able to access a greater range of years for which data at this level of detail was available on a global scale through Bio.Diaspora. This also allows us to continue the relationship with BlueDot and integrate models into an existing platform and framework for communicating imported disease risk with potential decision makers, already partnering with the organization. For cost-
effectiveness, privacy, security, and data volume, the data obtained at the individual passenger level from IATA is received anonymized, and aggregated monthly. The format of data collection conducted by IATA began in 2005 and is available to our partners at BlueDot as recent as 2015. For applications of these models in emergent situations, although not used in our analysis, some additional short term predictions datasets are available from IATA, however, these predictions datasets have lesser certainty, lower resolution, and higher costs.

Some data sources tend to only have passenger capacity or information on schedule flight routes, rather than data on actual individuals’ travel. IATA data, on the other hand, contains passengers’ full travel itineraries. This means from the first airport of the journey to airport at the final destination. While certainly ground transport and sea transport information is less often contained in IATA itineraries, most travels begin and end their journey relatively near these airports. This level of detail allowed for the more comprehensive analysis of all air passengers from the beginning to end of their flight journey unless they had single layovers of more than 24 hours. This is a significant advantage compared to other flight tracking data, which generally can only look at one segment of a journey at a time, scheduled flight routes capacity, or registries (tourist, visa or customs), which differ nation to nation and airport to airport. From the IATA datasets, only those full flight itineraries with final destinations as Funchal – Madeira Airport (FNC) or Fiumicino – Leonardo da Vinci International Airport (FCO), the primary airports of the Portuguese Island of Madeira and Rome, Italy, were selected for studies 1 and 2, respectively. For the analysis of the outbreak in Japan, we needed to use data collected by tourism offices and expected slightly diminished data quality, mainly in terms of spatial resolution. With these data sources, determining the actual airport of departure and arrival for travelers is possible only for some originating areas and destinations, where others are spatially aggregated.

Furthermore, we only retained travel itineraries to FNC, FCO, and Japan starting in those countries identified as having “good consensus” for dengue activity as described in the next section. Once cleaned and sorted this data became a novel crucial input component to the model for estimating risk of dengue importation and emergence. Using R, GIS programs, and Microsoft Excel, we managed and explored the data, generated tables, graphics, trends and projections for the travel analysis components of studies 1, 2, and 3.

**Dengue Infections in Departure Countries**

The risk of importation of dengue to temperate areas, like Europe and Japan, depends on the dengue endemicity in the countries that export dengue via air travelers. Studies 1 and 2 directly employed the use of quantitative information on dengue occurrences to model potential for having imported infections, while studies 3 and 4 utilized information on locations having dengue activity but quantified environmental drivers influencing the potential
for outbreaks more explicitly. In all studies, “Good consensus” for dengue activity, as determined by recently published literature, was the selection criteria for departing countries to be included (Brady et al., 2012). This consensus study made extensive use of published literature and surveillance sources around the globe to determine a comprehensive list of countries having dengue activity. To be conservative in our estimates, we excluded countries having merely marginal positive consensus on dengue activity. To obtain global quantitative data on annual dengue infections in dengue endemic countries, data was sought from a number of sources. Where available for Study 1, data on reported episodes of clinically apparent dengue was obtained from online governmental disease surveillance portals, particularly in countries having highest interconnectivity with the destination of interest, the Portuguese Island of Madeira. Such data, with higher temporal resolution, was analyzed to observe trends and describe seasonality within countries of higher endemic activity, where available. This was possible, unfortunately only for the minority of countries and only for reported cases, as opposed to infections, thus only applied in Study 1.

For global infection incidence needed in study 2, we ultimately retrieved from published estimations by Bhatt et al., 2013, after routine sources including WHO regional offices dengue reporting were determined to be highly inconsistent and gross underestimations of actual dengue infection incidence. For Study 2, the origins’ level of disease activity was quantified as the country-level estimated incidence of infections in 2010, which were scaled annually to WHO reporting to model year-to-year variations. Data were obtained following initial publication in April 2013 for 139 countries; however, we excluded countries having marginal consensus about their dengue activity (Brady et al., 2012, Bhatt et al., 2013). This data was harvested from supplementary publications from the Bhatt et al. studies, which estimated country level incidence using a three stage cartographical modeling approach described in the literature review (Bhatt et al., 2013). Unfortunately, due to the methods, employed the authors indicated that the highest temporal resolution available for these infection estimations were annual, precluding the used of more seasonally refined analyses, which we desired.

**Environmental Conditions in Arrival Countries**

Over the course of this doctoral work, many different sources for data on environmental conditions related to dengue suitability were explored. Ultimately, due to the use of a parameter based deterministic model based on literature review of laboratory-based entomological studies, we opted to use temperature data in our models from several sources depending on the scope and objective of the analysis. The decision to use weather and climate data was mainly driven by the availability of data and meeting our objective to generate models useful for both description and prediction in places where dengue had not recently or ever been transmitted locally. Weather data, although somewhat difficult to manage and access in some ways, is collected using somewhat comparable tools in many locations around the world. While not exhaustively standardized nor universally available at infinitely high
temporal or spatial resolution, weather data, in particular temperature is one of the most universal environmental parameters which is quantified, and recorded in time and space. This quality combined with the emerging discipline of global climate change modeling lends the use of temperature to be especially useful for historical, present-day, and future projections of the environmentally sensitive dynamics, including time-space models of potential for dengue outbreaks.

Although not in the published data for study 1 or study 2, we examined the environment conditions leading to the outbreak in Madeira in 2012 during subsequent retrospective investigation and the refinement and development of the models used in study 4, which also explicitly included Rome. Where as study 1 modeled and analyzed travel to dissect the origins of the Madeira outbreak, Study 3 modeled and analyzed environmental conditions to investigated the origins of the Tokyo outbreak.

For the model inputs on environmental conditions, we harvested meteorological data for study 3 and study 4, from several data sources. Even if temperature, seems like a standard measurement, in fact, it can vary quite a bit, as can the datasets storing it. Some datasets serve some purposes better than other data sets. This can depend on the resolution of data which is important for the analysis being conducted, both temporally and spatially. It also greatly depends on the nature of the model to which it will be applied. Lastly, like other decisions in choice of research methodology, it depends on the purpose this dataset is to serve. In purely statistical models for predicting short term risk in a specific area such as a city, for instance, high temporal and spatial resolution along with precision may be very important, while accuracy may not be, reproducibility in other areas, and availability of long term forecasts may be less important. For deterministic mathematical models projecting potential shifts in disease patterns under climate change scenarios for large areas, generalizability over space and time become higher priorities, as a result accuracy should be optimized, even if precision would be compromised in aggregation and smoothing algorithms. Given that we used a mathematical model in a variety of spatial and temporal applications, we also used a variety of datasets and explored the use of many more.

Firstly, for high temporal resolution meteorological observation stations data, we utilized observations of temperature time-series known as Met Office Integrated Data Archive System dataset (MIDAS). This dataset is useful for gaining access to up to 8 observations per day in thousands of observations stations around the world for various parameters, including ambient air temperature. However, MIDAS also has its drawback in some applications given that observation data always is subject to missing data, different starting times, other data quality issues, and most importantly unequal distributions of stations, which makes this dataset less useful generally for spatial applications. Unfortunately, the nature of observations stations data is that it is spatially confined to the location(s) where it is collected; not all places have stations, nor the same density of stations. For places where data is collected, observation data is one of the best options in terms of accuracy and temporal
refinement. For this reason, with Tokyo in study 3 and several cities in study 4, including Funchal (Madeira), we have used MIDAS dataset for recent time periods, from about 1-10 years in the past. This data set is particularly useful in the retrospective analysis of the two outbreaks, where we can apply the model to highly temporally resolved data for loci of epidemics.

As we seek to apply models to other locations and longer time periods, we also opted to use two types of gridded observation datasets in addition to the MIDAS data. Gridded observation datasets are processed products based on re-analysis of numerous stations’ time series datasets in order to spatially and temporally fill in gaps. Since these products smooth observations data in time and space, some important information is lost while other important information is gained. Rather than multiple observations per day, only mean, minimum, and maximum temperature are generally available in either daily, monthly, yearly or even multi-year aggregations. The advantage of these dataset can be spatially generalization, as we desired in studies 3 and 4, however, at the cost of spatial resolution. In generating data for grids over larger extent, than point observations used in station datasets, these products treat large areas as homogenous in meteorological parameters, such as temperature. This resolution loss can also occur temporally. With parts of studies 3 and 4, we used the Climatic Research Unit (CRU) datasets, which are monthly product at the 0.5 degrees’ latitude by 0.5 degrees’ longitude resolution. The CRU dataset is a foundation for long term historical models and climate change models, but not as swiftly updated nor as highly resolved as another gridded observation-based dataset, E-OBS. For more recent periods in the European extent for study 4, we opted this alternative dataset which offers more often updated daily products at 0.25 degrees’ latitude by 0.25 degrees’ longitude resolution.

In study 4, we also preformed projections analysis of dengue epidemic potential under climate change scenarios. For these analysis, we used datasets, which were part of the ISIMIP project. These are a specific selection of 5 models outputs, corresponding Global Circulation Models (GCMs) to the CMIP5 initiative for Representative Concentration Pathways (RCPs) of carbon forcing under climate change. For this analysis, we used all four RCPs (2.6, 4.5, 6.0, 8.5) for the following GCMs: NorESM1-M, MIROC-ESM-CHEM, IPSL-CM5A-LR, HadGEM2-ES and GFDL-ESM2M. Our model input for the climate change scenarios were therefore 20 datasets, each having a monthly time series each year of the 21st century at 0.5 degrees’ latitude by 0.5 degrees’ longitude resolution. These climate change projection products were based upon and most similar in resolution and format to the CRU dataset, which was chosen for the historical analysis and therefore offered greater continuity to the overall modelling endeavor. We opted for the use of a widely used multi-model mean in the ISI-MIP dataset to counteract some of the uncertainty in different possible outcomes of each model.
Limitations of the Materials and Methods

In modeling, the notion of a perfect model, without limitations, is analogous to a perfect map, which is scaled 1:1—its perfection is only matched by its complexity, immensity, and utter uselessness to the user. The modeling conducted as part of this thesis, like all comparable modeling approaches published in previous studies, has some limitations, but sought to simplify and predict the complex dynamics governing the potential for dengue transmission in Europe. Like any map, which is scaled, the model’s formula itself, the theoretical framework upon which it is based, the evidence base for vector-borne disease contributors in Europe, the choice and validity of data on vector surveillance, travel interconnectivity, and global dengue activity are each individually subject to limitations, assumptions, errors, and oversimplification. At each point in the process, care was taken to seek out a range of options, which were critically analyzed to include the best-known parameters, proxies and data.

The choice of data on global incidence of infections, while the best currently available, is based on 2010 modeling, making it 5 years behind the current status and subject to its own range of limitations and confidence intervals. Utilizing the newest list of countries with known dengue activity, which expands on prior lists by some 20 countries or so, also may prove to be a limitation. This inclusion, however, does not impact the major findings of the study, as these new nations have relatively low dengue incidence estimates. Similarly the flight data, while far more comprehensive and accurate than other sources, still has limitations and was only harvested for a minority of the total European destinations, which may be at some level of risk. The IATA data was so rich and detailed that its analysis expended greater resources and computing power on a large scale than other comparable datasets. The duration of stay following any particular itinerary was not available, which made calculated risk of emergence far more difficult, as well as capturing travelers who had stops of longer than 24 hours.

The model, which integrated the incidence data and travel data, applied a temporal probability of being infected at time of travel which assumed all people within a given country, at all times during the year are equally likely to be infected with dengue and are equally likely to be travelling to Europe. These assumptions, while necessary for the model’s simplicity, clarity, and global applicability, neither account for correlations between flight travel and socio economic status, nor the correlation between poverty and dengue infection, nor for spatial distribution of dengue infections nor travelers within dengue endemic countries. Despite these limitations, the findings of this novel simplified model add significant knowledge to the risk of dengue importation air-travel may pose to Europe and open new areas for further research.

One of the largest assumptions of this study is the assumption that ongoing dengue transmission with *Aedes albopictus* is actually possible in Europe. It also assumed viremic persons to be the essential mode of introduction of
dengue virus into Europe. Another major assumption, which may limit these studies, is the notion that flight travel based interconnectivity poses the greatest risk for importation. The role of sea-faring transport of infectious passengers may also be heavily involved in importing infections of dengue to remote islands or coastal areas of Europe, including Madeira. Similarly, the notion of infected mosquito populations being introduced into Europe by freight or otherwise was not directly accounted for within the model. Furthermore, the environmentally driven models were based on temperature dynamics alone, not full life cycle and ecological niche models, so the assumption of vector presence at the micro-scale would be violated more often than met.

Lastly the accuracy and precision of climate data, especially climate change data at the micro-level poses a great limitation, which is perhaps most noticeable in coastal areas. We have attempted to mitigate for this limitation where possible through sensitivity analyses and alternate date sources as well as multi-model means, however, some intrinsic smoothing biases due to geographical features are not homogeneously spread within larger (lower resolution) gridded datasets such as the ISIMIP climate change datasets and the CRU historical climatologies.

**Ethical Considerations**

Due to the modeling nature of this doctoral work based on the existing data analysis, no formal internal review board process was conducted expressly for this study; however, where applicable, ethical considerations were still taken with regard to the handling of the data received and communication of results. At least in theory, data, similar to that captured by IATA, is collected and marginally analyzed in near real-time to achieve numerous industry specific needs. However, largely due to security related considerations, such data is not currently made immediately available for public health analysis. While expense and volume of data also contribute to the delay of data for public health modeling, ethical considerations regarding in-flight security also cannot be completely excluded. National authorities make some similar data available as estimates or projections once aggregated and personal information has been removed. The secure partially cloud-based servers of Bio.Diaspora were used to generate data queries of full travel passenger itineraries, which are anonymized by IATA before integration into the data warehouse. Although this data is anonymized when received, privacy and ethical considerations were observed, as the data is licensed for the public health purposes of Bio.Diaspora and its partners—not for other commercial or personal enterprises. Climate and weather data along with genetic sequencing data is not always publically available; however, the data used in these studies was made available on depositories accessible to researcher and to an extent the public for generally non-commercial purposes. As the identifying and proprietary features of these sources of data was not longer present at the time of analysis, the security of these data was not required to be as great as the flight data.
Results and Findings

Overview

Here we present the selected findings of the investigations and data analyses which were conducted during the four studies of the doctoral work. The initial section describes the application of resulting HAVE transmission tetrahedron theoretical framework and categorizations for countries within Europe following the substantial review of available literature. The results are then focused on the potential for imported infections of dengue arriving in selected locations via passenger air travel. The final sections focus on output of the modally related to environmental conditions. Finally, results of the genetic sequencing analysis in the form of phylogenetic trees of the virus strains isolated from two outbreaks is presented.

Theoretical Framework:

Dengue is a multidimensional global public health concern. The comprehensive review of literature unveils that the emergence of dengue transmission cycles is reliant on the simultaneous establishment of susceptible hosts (H), disease causing agents (A), and viable vector populations (V) in an environment (E), suitable for each to survive and interact with the others. To visualize and conceptualize the multi-dimensional nature of dengue transmission dynamics, this thesis investigation developed the H.A.V.E. dengue transmission tetrahedron. Each facet of this four sided structure represent the necessary presence of a susceptible vertebrate host population, an appropriate mosquito vector population, the circulation of dengue virus(es), and conducive environmental conditions, which promote repeated exposure and interaction between all three populations. The transmission tetrahedron, displayed in the figure previously, was a theoretical framework to understand the individual and collaborative elements, which contribute to dengue transmission in endemic areas and understand the extent of dengue emergence possible in areas currently dengue-free. The application of this theoretical framework for transmission of dengue and thereby elements involved in emergence of the disease was the basis for the categorization of potential emergences areas within Europe display in the table on the following page.

A systematic evaluation of every country in Europe was conducted and tabulated according to the H.A.V.E. framework. Areas with established vector populations within close proximity to large metropolitan settlements, seaports, and large volume airports were considered to be at the highest relative potential for dengue emergence and are identified with shading in Table 3.
<table>
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<th>V</th>
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Shaded areas indicate countries with heightened potential for dengue emergence

Table 2: Application of H.A.V.E. Framework to Countries of Europe to Identify Potential Areas of Novel Dengue Emergence

47
The environmental suitability of much of Europe was found to be especially conducive for the proliferation and expansion of the invasive *Aedes albopictus* mosquito, the secondary vector for dengue. The primary dengue vector, *Aedes aegypti*, however, was generally projected to be limited to the warmer islands in and around southern Europe and the Mediterranean coastline, however, it has rarely been observed there recently. Actual observed vector populations of *Aedes albopictus* and *Aedes aegypti*, however, are currently much more limited than their climate suitable zones of potential expansion, and than the areas, where if established, they could drive dengue epidemics.

The red most areas of the map displayed in the figure below show areas where vector populations have been established; i.e. most of Italy, southern France, southeastern Spain, Portuguese Island of Madeira (lower left corner), parts of southern Greece, and the eastern Caspian. The dark orange and light orange areas have seen the introduction of vectors, either *Aedes aegypti* or *Aedes albopictus*, respectively. The yellow areas are environmentally conducive for *Aedes albopictus* further expansion. Sustained cooler temperatures, more arid climates, and/or higher elevations were generally thought to climatically limit the vectors potential expansion in Europe especially notable in parts Scandinavia, the United Kingdom, Ireland, areas within the Iberian Peninsula, and the Alps. These areas of lessened potential for dengue emergence are shaded green on the map.

**Figure 5: Map of Dengue Vectors in Europe**

Figure 5 depicts Europe according to climatic suitability, vector introduction, and vector establishment of dengue vector mosquitoes *Aedes albopictus* and *Aedes aegypti*, based on consensus from various climatic suitability modeling publications and ECDC vector surveillance network VBORNET.
Models Applications

Retrospective Travel Model: Madeira 2012

To describe the origins of the viral importation spawning dengue transmission on the Portuguese Island of Madeira and eventually the 2012 outbreak, we conducted retrospective modeling using a simplified importation index model. This model combined metrics on dengue activity at air passengers’ departure country with the total volume of passengers arriving in Madeira’s international airport in Funchal (FNC). Early on in the outbreak and corresponding investigation, it had been suggested that the 2012 outbreak of dengue in Madeira was of South American origin, which prompted further investigation into modes of DENV importation to Madeira including flight.

During the eight years between the known establishment of dengue vector Aedes aegypti in 2005 and the 2012 outbreak of dengue, there were approximately 200,000 air-passengers arriving at Madeira’s Funchal Airport (FNC) from at least 50 countries with dengue activity around the world.

The initial modeled indices, quantifying the importation index (ID) are summarized in Table 3 corresponding to the travel model conducted initially in study 1. For this analysis, the formula used is ID = IR x TV, where ID is the annual importation index, TV is traveler volume (annual travelers to Madeira), and IR is the annual incidence rate/100,000 inhabitants at the country, where individuals embarked on their travel to Madeira (departure country).

Data on the incidence of dengue for this analysis was taken from World Health Organization (WHO) regional offices reporting of dengue case incidence and incidence rater. PAHO countries including Venezuela, Brazil, Aruba, Costa Rica, Colombia, Panama, Mexico, Ecuador, Paraguay, Puerto Rico, Guatemala, and Bahamas were available for a number of years including 2012. SEARO data was used for countries of Thailand, Sri Lanka, and India and WPRO data was used for countries Singapore, Vietnam, and Philippines. WHO Regional Office for Africa did not publish dengue incidence data by country, therefore such not available at the WHO regional level. This was true for the following AFRO countries which were investigated: Angola, Cape Verde, Senegal, Mozambique, Reunion, Nigeria, Guinea-Bissau, Cameroon, Mauritius, Ghana, and Madagascar.

The methodologies and data sources used for the development of this table are those that were applied in study 1 using the simplified importation index (Quam and Wilder-Smith, 2015, Wilder-Smith et al., 2014).
<table>
<thead>
<tr>
<th>Country of Embarkation</th>
<th>TV</th>
<th>IR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ID&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venezuela</td>
<td>15884</td>
<td>174.86</td>
<td>27.77</td>
</tr>
<tr>
<td>Brazil</td>
<td>4676</td>
<td>295.33</td>
<td>13.81</td>
</tr>
<tr>
<td>Thailand&lt;sup&gt;c&lt;/sup&gt;</td>
<td>214</td>
<td>116.35</td>
<td>0.25</td>
</tr>
<tr>
<td>Aruba</td>
<td>11</td>
<td>653.92</td>
<td>0.07</td>
</tr>
<tr>
<td>Philippines&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19</td>
<td>172.15</td>
<td>0.03</td>
</tr>
<tr>
<td>Mexico</td>
<td>20</td>
<td>149.11</td>
<td>0.03</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>4</td>
<td>487.46</td>
<td>0.02</td>
</tr>
<tr>
<td>Colombia</td>
<td>9</td>
<td>215.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Paraguay</td>
<td>3</td>
<td>585.39</td>
<td>0.017</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>4</td>
<td>345.6</td>
<td>0.013</td>
</tr>
<tr>
<td>Panama</td>
<td>37</td>
<td>37.36</td>
<td>0.01</td>
</tr>
<tr>
<td>Singapore&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17</td>
<td>86.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Ecuador</td>
<td>6</td>
<td>123.39</td>
<td>0.007</td>
</tr>
<tr>
<td>Sri Lanka&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>206.95</td>
<td>0.004</td>
</tr>
<tr>
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<td>84.81</td>
<td>0.003</td>
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<tr>
<td>Guatemala</td>
<td>3</td>
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<td>0.002</td>
</tr>
<tr>
<td>India&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>4.17</td>
<td>0.00004</td>
</tr>
<tr>
<td>Bahamas</td>
<td>4</td>
<td>1.46</td>
<td>0.00006</td>
</tr>
<tr>
<td>Angola</td>
<td>1281</td>
<td>n/a</td>
<td>n/a</td>
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<td>Cape Verde</td>
<td>352</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Senegal</td>
<td>122</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Mozambique</td>
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<td>Reunion</td>
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<td>n/a</td>
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<td>n/a</td>
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<td>Cameroon</td>
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<td>n/a</td>
</tr>
<tr>
<td>Mauritius</td>
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<tr>
<td>Ghana</td>
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<td>n/a</td>
</tr>
<tr>
<td>Madagascar</td>
<td>4</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

<sup>a</sup> Annual incidence per 100,000 inhabitants using World Health Organization (WHO) and Regional Office websites.

<sup>b</sup> Annual importation index units in traveller incident cases per year

<sup>c</sup> Where only incidence was available, 2012 population was taken from www.indexmundi.com.

Table 3; Dengue-endemic countries: the annual number of air travelers to Madeira, annual incidence rate of dengue infection, and annual importation index into Madeira in 2012
The table below shows the top ten dengue-endemic origin countries according to the total volume of flight to Madeira during 2011 and 2012 and related likelihood of importing dengue based on the more refined model which estimated \( (R) \) potential threat posed to Madeira, in terms of the theoretical number of infected individuals per departure location per year anticipated to arrive at FNC. This refined \( (R) \) model therefore included country’s estimated annual infections and the temporal probability that infection and travel coincide consistent with the methodologies applied in study 2, however for retrospective analysis of the outbreak primarily discussed in study 1 (Bhatt et al., 2013, Quam et al. 2015, Wilder-Smith et al., 2014).

<table>
<thead>
<tr>
<th>2011 Air Travel to FNC Potentially Importing Dengue</th>
<th>2012 Air Travel to FNC Potentially Importing Dengue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Departing Country</strong></td>
<td><strong>Arrivals</strong></td>
</tr>
<tr>
<td>Venezuela</td>
<td>15,403</td>
</tr>
<tr>
<td>Brazil</td>
<td>4,657</td>
</tr>
<tr>
<td>Egypt</td>
<td>1,397</td>
</tr>
<tr>
<td>Angola</td>
<td>541</td>
</tr>
<tr>
<td>Thailand</td>
<td>195</td>
</tr>
<tr>
<td>Maldives</td>
<td>144</td>
</tr>
<tr>
<td>Cape Verde</td>
<td>84</td>
</tr>
<tr>
<td>Mozambique</td>
<td>85</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>36</td>
</tr>
<tr>
<td>Reunion</td>
<td>44</td>
</tr>
<tr>
<td>39 country total</td>
<td>23,044</td>
</tr>
</tbody>
</table>

Table 4: 2011-2012 Annual incoming air passengers to Madeira (FNC) from dengue endemic countries and corresponding refined importation model estimating quantity of infections expected to be imported \((R_i)\).

The data analyzed from IATA showed routinely over 85% or more of the incoming passengers at FNC from dengue endemic countries started either in Brazil or Venezuela. Both countries are highly endemic countries for dengue activity as both annual models clearly identified. These two countries consistently had the greatest potential for being the origin of imported infections, which may have lead to the 2012 outbreak in Madeira. There were also over 4000 passengers in 2011 and 2012 originating in areas of Africa including Angola and Egypt, which have more recently been identified as having dengue activity. However, the modeled potential for imported infections, reflecting the notably lower incidence of dengue in those areas and decreased interconnectivity with Africa leading up to the 2012 dengue epidemic in Madeira compared to the South American countries.

Seasonal trends in both disease incidence and flight patterns were computed using the model to determine the temporal likelihood of importation for Brazil.
and Venezuela for study 1. Both countries had similar travel patterns with bimodal peaks in July and December, with the notably more travelers arriving between June and September. Over the eight years 2005–2012, since Madeira has had a dengue vector population, Venezuela averaged over four times as many passengers to Madeira as Brazil and has seen an increase in the annual total air passenger volume flying to Madeira of 65.9% over the same period.

Figure 6: Map of air travel volume by country and city from dengue-endemic countries to Madeira, 2012

Figure 6, above, shows that most of the air travel from Venezuela originated from Caracas on the only direct flight to Madeira from a South American dengue endemic country, whereas in Brazil’s passengers most travel originated from Sao Paulo and Rio de Janeiro and had at least one layover, as can be seen in the map from study 1 based on IATA data we analyzed with partners at Bio.Diaspora (Wilder-Smith et al., 2014).

Both Brazil and Venezuela have seasonal peak of interconnectivity with Madeira during the month of July, however distinctly different peak seasons of dengue activity. While the seasonal trends in travel were similar between the two South American countries, dengue activity varied between them widely according to their reporting of dengue cases to the governmental surveillance ministries. Online reports in epidemiological bulletins and surveillance data portals display notable seasonality of dengue episodes with both Brazil and Venezuela. The Brazil data displayed temporal trends
indicating the greater incidence of reported cases of dengue in April and December, while the Venezuelan data indicated peak dengue incidence in August and September. The combination of travel and dengue incidence combined in the original Importation Index model from study 1 at the monthly time scale is depicted in the figure below (Wilder-Smith et al., 2012):

**Figure 7: Monthly importation index for dengue virus by travelers to Madeira from Venezuela and Brazil, January–December 2012**

Figure 7 illustrates the monthly importation index for dengue virus by travelers to Madeira from Venezuela and Brazil, January–December 2012. During February, March, April, and May, Brazil posed slightly higher threat of dengue importation to Madeira than Venezuela, which posed much higher threat during remaining summer, autumn, and winter months. During August 2012, the monthly $ID$ (initial model) and $R_I$ (refined model, not display) both peaked, estimating an importation index of around 6. Using the refined model, the August 2012 peak corresponds to at least 12 dengue-infected persons were to have arrived at FNC from Venezuela, about 3 of which would be suspected to have apparent infections, 9 inapparent or asymptomatic infections.

For Brazil the monthly $ID$ (initial model) and $R_I$ (refined model), which combined both factors of travel and estimated infection incidence by month, reaches its modest annual climax in the month of April, whereas Venezuela reaches a much higher maximum in August. Using just the monthly metrics for the months of July, August and September, the index reflects a 10-fold greater importation index for Venezuela compared with Brazil. These findings strongly suggest, based on the importation indices, that the most likely source of importation of dengue to Madeira in late summer 2012, was an infected person traveling from Venezuela just before the outbreak’s first reported case on October 3, 2012. The attenuation and spread of virus by active the *Aedes aegypti* mosquito population would eventually cause an epidemic, having over 2000 cases which peaked in the week of November 9, 2012.
Retrospective Environmental Model: Tokyo 2014

In light of the 2014 outbreak of dengue and an imported case of dengue in Germany in a traveler, returning from Japan in 2013, we aimed to conduct meteorological analysis of the environmental conditions and dengue epidemic potential for Tokyo and the islands of Japan (Kobayashi et al., 2014). We hypothesized and investigated that environmental factors combined with human movements were contributing to this re-emergence of dengue, as this was the first dengue outbreak in Japan in over 70 years. Detected in late August 2014, Japanese authorities report 160 people contracted dengue near Tokyo, Japan as of October 31, 2014 (Arima et al., 2014). We calculated temperature-dependent relative vectorial capacity based on the initial environmental drivers’ model as previously described by Liu-Helmersson and colleagues. The modelled calculations and maps generated described the environmentally driven potential for dengue outbreaks to begin and persist, given the introduction dengue virus into viable vector populations, under the assumption of a constant female mosquito to susceptible human population ratio of 1. Our resultant analysis of multiple environmental conditions leading up to the Tokyo outbreak is summarized in the following figure from study 3.

Figure 8: Conditions Facilitating the Dengue Outbreak in Tokyo in 2014

Figure 8 shows the modelled relative vectorial capacity calculations’ output as a time series for year 2014 along with other environmental conditions contributing to increased dengue epidemic potential in advance of the recent dengue outbreak in Tokyo. The models greatest success was its ability to assimilate temporally the environmentally driven potential for dengue outbreaks to begin and persist, given the introduction dengue virus into viable vector populations. The relative vectorial capacity entered the threshold for
epidemic potential as the Aedes vector activity was beginning for temperate part of Japan in early May, then peaked at the same time as the initial case reports began (Kobayashi et al., 2014). The outbreak was relatively short-lived and subsided completely in the same week at the relative vectorial capacity dropped below the 0.2 threshold theoretically corresponding to a basic reproductive number of 1.

In light of the relative vectorial capacity model’s success at quantifiably identifying temporal dynamics of dengue epidemic onset based on environmental drivers, we developed and applied the model spatially in conjunction with our retrospective analysis of the 2014 Tokyo outbreak. As part of the study 3 investigation we also found increasing relative vectorial capacity in Tokyo in the recent years, compared to long-term averages and found this also to be true elsewhere in Japan. Warmer than average temperatures corresponded to elevated epidemic potential which were particularly apparent in the late-spring, summer, and early-autumn of 2014 for Tokyo, Japan. In analysis conducted to accompany study 4, we verified that this was also the case for the outbreak in Madeira in 2012. Our spatial generalizations of the relative vectorial capacity are summarized in the map in the next figure.

For the initial analysis we used meteorological observation data from Tokyo (2005-2014) from MIDAS and then generalized the model using gridded climatologies for the Korean Peninsula, China, and Japan (2004-2013) from CRU. As can be readily seen in both analyses on the 2014 time series and the decade of spatial analysis for the islands of Japan and surrounding mainland Asian regions, the seasonal period in temperate areas having greatest potential for dengue epidemics to begin is from May to October, with a notable peak around July and August. There is also a north south gradient, which tends to very closely mimic the northern progress of Aedes mosquito activity, especially considering, invasive secondary species, Aedes albopictus, is now found in all but the northern most reaches of Japan (Kobayashi et al., 2014). Throughout the studied decade or so, summer and early autumn presented the greatest dengue epidemic potential continuously, however, with notable year to year variation. This retrospective analyses suggested that the climatic suitability for dengue could be modeled using the relative vectorial capacity methodology and that current environmental conditions were sufficient to drive outbreaks in some areas of Asia in recent times largely dengue-free, like Guangzhou, China, and Tokyo, Japan (Shen et al., 2015, Lai et al., 2015, Lin et al., 2016). Although seasonally limited, both of these areas had higher relative vectorial capacity in 2014 and experienced historic dengue outbreaks (Huang et al., 2016).
Figure 9: Relative Vectorial Capacity (rVc) In Japan 2004-2013 by Month
**Phylogenetic Confirmation: Madeira 2012 and Tokyo 2014**

We set out to investigate the outbreaks of dengue in more temperate, previously dengue-free areas of Madeira and Tokyo in terms of the conditions which potentially lead to their onset and the origins of the imported infections. To aid in the validation of our models, we employed genetic sequencing techniques and built phylogenetic trees. These techniques helped to discern the most closely related fellow strains of DENV-1 virus catalogued in GenBank by other researcher investigating previous dengue transmission in locations around the world.

Our findings of the DENV-1 sequences strongly corroborated our models finding in both the Madeira and Tokyo outbreaks, which lead us to further generalize these models in accessing the potential for importation driven outbreak of dengue in places where outbreak have not yet occurred. In the case of the Madeira outbreak, the genetic sequencing of virus base pairs used for the analysis indicated that this particular virus was a DENV-1 strain within Genotype V from South America. From the sequencing comparison with those available in GenBank, the Madeira strain was determined at that time to have greatest similarity with those from Colombia (GQ868570) Brazil (JN713897) and Venezuela (JN819415), three neighboring countries in South America. The strongly confirmed the likely pathway of virus introduction being a viraemic passenger traveling from Venezuela as the travel models had suggested. Later, in more detailed analysis of the full envelope gene, our fellow co-authors were able to conduct more extensive molecular sequencing based investigative analysis, which even more strongly underlines similarities between virus isolated from Venezuela during 2012 and the dengue virus circulated in the 2012 outbreak in Madeira, further validating Venezuela as the probable origin (Franco et al., 2015).

Similarly, consistent with others findings upon both initial and more extensive investigations, our team’s investigation into the most likely sources of the Tokyo 2014 outbreak through phylogenetic analysis showed virus circulating in China and Indonesia in the 2013 to be most similar, followed shortly by other strains circulating in Singapore since 2004 and Vietnam since 2008. This supported our suspicions that China is the most likely origin of the Japan virus importation considering the proportion of travelers arriving from China and the timing of the outbreak corresponding with dengue activity in China, which we also saw in our temperature-driven relative vectorial capacity model (Lai et al., 2015, Lin et al., 2016). Combined with knowledge of the historic outbreak of dengue co-occurring in 2014 in Guangzhou, China, and relatively normal dengue activity in the other areas implicated by the genetic sequencing, we were able to surmise that our environmental and travel models correctly identified the environmental anomalies, leading to higher than normal in dengue epidemic potential in the region, which combined with travel to sustain local transmission in Tokyo, once introduced (Huang et al., 2016).
Figure 10: Phylogenetic Tree of Virus Isolated from Madeira in 2014

Figure 11: Phylogenetic Tree of Virus Isolated from Tokyo, Japan in 2014
Prospective Travel Model: Rome 2005-2020

Rome, Italy is home to one of Europe’s busiest airports, accommodates one of largest volumes of international tourists, and in recent decades, also hosts a sizable population of the invasive secondary mosquito vector for dengue, *Aedes albopictus*. The Fiumicino – Leonardo da Vinci International Airport (FCO) is therefore at heightened risk for potential emergence of dengue as result of air-travel related importation as compared with some other areas in Southern Europe. Our data showed that between 2005-2012, FCO saw arrivals from over 7.3 million passengers coming from 100 different countries with good consensus on dengue activity (Brady et al., 2012). Modeling projections using the refined travel model ($R_t$) suggest of these circa 7.3 million passengers, correspond in an estimated 18,840 (12,880-27,432) infections imported (passenger infected with dengue at their time of travel) over the eight-year study period, of which as many as at least three-fourths would be expected to have completely ‘inapparent’ infections (Bhatt et al., 2013). The dynamics of dengue, travel, and imported infections are interrelated in the model, therefore, not static country to country, year to year, or quarter to quarter. In the next figure, one can see how the nations contributing most dengue infections in the study period trend over 2005-2012 according to the model.

Figure 12: Estimated Imported Dengue Infections to Rome, 10 Top Exporters

The findings reflected trends observed by clinical researcher and also varied over time as global change dynamics influencing dengue also had impacts on the models output in terms of estimated imported infections of dengue arriving in Rome, Italy (Pierro et al., 2011, Burdino et al., 2011, Lagi et al., 2014). During the eight years in the retrospective portion of study 2, both
global dengue activity and travel from dengue-endemic areas to Rome was growing, however, not equally in all places, nor was travel always evenly distributed throughout the year. In very few places was the dengue activity static year-to-year over the study period 2005-2012, in terms of annual cases reported, from which infections were scaled according to modeled estimates developed by (Bhatt et al., 2013). Similarly, macro-level travel dynamics changed seasonally and increased year by year but at different rates in terms of the number of passenger departing endemic countries headed for Rome.

The total aggregated mean number of importations per year from all country combined increased from 1,634 (1,096–2,014) in 2005 to 3,256 (2,318–4,553) in 2012. Our projections based country-level regression analysis at the quarterly travel level for each country showed that this would continue to increase in the future, as show in the next figure which aggregates at imported infections at the global level. Already by 2012, the estimated imported infections had grown by 100 percent over the 2005 baseline, and as displayed, the trend continues such that the 2020 estimations over 300% of the original. Notably, year after year the seasonal boom in arrivals from dengue endemic areas to Rome was quarter 3, July to September, which alone expects 1,512 (1,088 – 2,173) of the 5,465 (3,963 – 7,824) imported infections 2020.

Figure 13: Future Trends of Estimated Imported Dengue Infections to Rome

Figure 13 shows trends in global arrivals and estimated importations of dengue infections into Rome’s Fiumicino-Leonardo da Vinci International Airport (FCO) from an exhaustive list of dengue-endemic departure countries. The total volume of air travelers into FCO from these countries with known dengue activity is plotted quarterly with circles corresponding to the left axis. The corresponding total dengue virus importation (RI) based on the estimated number of infections (asymptomatic and symptomatic) imported to Rome is
plotted in black quarterly using the right axis. The lower bounds of the credible interval (0.025 to mean value) and the upper bounds (mean value t 0.975) are shown as gray lines.

Figure 14: Determining Variables and Output of the Model Estimated Imported Dengue Infections to Rome, 2005-2012
Figure 14 maps the determining variables for estimated importations of dengue infection into Rome's Fiumicino-Leonardo da Vinci International Airport by country. The depict the comparative contribution to the estimated Imported Infections (modeled RI) over the entire retrospective study period 2005-2012. Variable \( T \), based on travel data analysis, is displayed in the top map which shows the scaled intensity of air travel global interconnectivity with Rome. Variable \( I \), based on estimated per-person incidence of dengue infection (asymptomatic and symptomatic), is displayed in the middle map, which depicts the intensity of dengue activity in the endemic departure countries. The combinations of \( T \) and \( I \), displayed in the bottom map displays the contributions to the model-generated Introduction Model (RI) values, which estimate the quantity of imported dengue infections to Rome. All countries’ values have been scaled as a percentile, where those areas in white (first percentile) represent fewest air travelers, lowest dengue incidence, and least potential for being the source of an imported dengue case to Rome. Darkest areas (99th percentile) represent areas with relatively greatest potential for dengue exportation to Rome, due to simultaneously elevated dengue incidence and relatively higher air travel to Rome.

**Prospective Environmental Model: Europe 1901-2099**

In our study 4, we sought to apply our refined environmental conditions model for temperature dependent vectorial capacity to the European extent to calculate the likelihood that an imported infection could actually produce onward epidemic transmission. In other words, we modeled the potential that those estimated imported infections predicted to arrive in Rome, Italy (study 3), or to Funchal, Madeira (study 1) would be able to actually spawn a novel outbreak of dengue. The generalization of this model and refined of the methodology to better reflect the daily reproduction number (\( R_0 \)) by including more the temperate-dependent ratio of mosquito to humans was influenced by the results of respective analysis conducted in study 3. In this more general, application, we again used two different gridded datasets for historical analysis, which we compared to weather station data sources a sensitivity analysis, as well as 20 datasets from climate change projections. We have generated a time-space model using the EOBS daily gridded data set for years available, particularly the recent decade. In this endeavor, we created daily outputs of dengue epidemic potential using the vectorial capacity model for both species in each location in Europe in grids, which are approximately 25 km by 25 km. We also generated a longer historical and projected time-space model for the vectorial capacity for both *Aedes aegypti* and *Aedes albopictus* the period 1901-2099 at the monthly temporal resolution under 4 Representative Concentration Pathways for 5 Global Circulation Models of projected climate change. These datasets were available at less fine resolutions of ~50 km X ~50 km. Given the massive amount of inputs and outputs associated with this undertaking, we have described only a small selection in this section, however, we created the infrastructure for the wider general application to be shared with partnering researchers and ultimately contribute to decision making tools, such as the E3 Network of the European Centre for Disease Prevention and Control (ECDC). Further reading and
viewing of our finding from the application of the prospective environmental model for Europe can viewed in the manuscript for study of this doctoral work.

**Figure 15**: Dengue Epidemic Potential in Areas with *Aedes* Vectors 2006–2015

Figure 15, above displays the monthly aggregated output of the most recent decade of highly resolved daily gridded observations for the European extent in areas to have known *Aedes* vectors, introduced, established, and potentially active. The EOBS dataset was used to generate a time-series of fluctuations throughout the day in based on interpolation of daily temperature means, minima, and maxima to drive the temperature dependent vectorial capacity model for *Aedes albopictus*. Areas depicted in yellow to red have higher vectorial capacity than 0.2, which corresponds to a basic reproduction number high enough (1) for an outbreak to commence, therefore have necessary epidemic potential for an imported infection of dengue to be of public health importance. Under global climate change, the implications of imported infections could become more problematic in the future than they have been in the past, as exemplified by the 10 selected European European cities in the following figure.
Figure 16: Seasonality of Dengue Epidemic Potential, Past, Present and Future in 10 European cities for *Aedes aegypti* (A) and *Aedes albopictus* (B).

Figure 16 shows a three decade average vectorial capacity by month for 3 different periods: Past (Fig. 16i), Current (Fig. 16ii), Future (Fig. 16iii-vi) under four different projected climate scenarios or emission pathways (RCPs). CRU- and ISIMIP gridded (0.5 X 0.5 degrees) temperature data were used. For each emission scenario, outputs were averaged over the climate change modelsto give a multi-model mean output. One can note the distinction between *Aedes* species clearly, as well as, increasing intensity and duration of the above threshold epidemic potential in most cities. Under higher emission RCPs, this effective increase in vectorial capacity is happening sooner and with greater intensity as Europe like many temperate areas is project to be warmer and more suitable to vector-borne disease transmission.
Figure 17: Europe’s Dengue Epidemic Potential at the end of the 21st Century, Two Pathways for Two Aedes Vectors in 2090-2099

Figure 17, above applies the methodologies from Figures 15 and 16 on the previous pages to map Europe’s seasonal dengue epidemic potential at the end of this century under two distinct climate futures; Representative Concentration Pathway 8.5, previously referred to as the “business as usual” scenarios and Representative Concentration Pathway 2.6, sometimes called “green growth” for both species. Dengue outbreaks potentially threatening Europe could be greatly mitigated if climate change mitigation were achieved through lessened greenhouse gas emissions, as compared in the figures’ the inner (more greenhouse gas emissions) and outer columns (less greenhouse gas emissions). The seasonal decade aggregations were defined as follows: Winter: December–February; Spring: March–May; Summer: June–August; Autumn: September–November. Clearly the models distinguish between the two Aedes species in terms of the intensity of vectorial capacity, with primary vector species Aedes aegypti on the left and secondary vector species Aedes albopictus on the right. One should note that unlike the maps in Figure 15 above, this projection model generalization was conducted for dengue epidemic potential in all areas of Europe independent of their historic or current vector populations. While expansion of the Aedes vectors’ ecological domain into wider swaths of Europe is likely to happen in the future, and already happening in many places for the invasive species, Aedes albopictus, this model assumes vector are already established and models the vectors success in driving epidemic disease transmission. These findings illustrate the epidemic impact of Aedes albopictus further expanding, would potentially drive more outbreaks; however, that widespread establishment of Aedes aegypti would pose yet greater threats at the end of the 21st Century.
Discussion

Dengue is a multidimensional global public health concern. Concerns about the potential emergence of dengue into currently uninfected countries are justified in light of the 2012 outbreak of dengue in Madeira, the 2014 outbreak of dengue in Tokyo, Japan, local transmission of dengue in France and Croatia in 2010. Global change dynamics involving increased interconnectivity via air-passenger travel and alterations in environmental conditions due to climate change are reinforcing the concern for high resource setting in temperate areas more than ever before (Parreira and Sousa, 2015). The studies contained in this doctoral work set out to investigate and model the factors that potentially contribute to the emergence of epidemic dengue transmission as result of imported infections in temperate areas; that is to discern imported infections’ importance.

Specifically, in studies 1 and 3, we conducted extensive retrospective analyses of two imported infection driven outbreaks of dengue in temperate areas to dissect the spatial and temporal origin of the novel introduction of virus, which precipitated epidemics in Madeira (2012) and Tokyo (2014). We employed both travel- and environmental- drivers’ models to recreate the patterns of conditions facilitating the outbreaks and validating our findings using phylogenetic analysis of virus strain envelope gene sequences. Once we tested and validated our models in the retrospective analyses in studies 1 and 3, we improved our models and generalized their application prospectively to access how global change dynamics of increasing air passenger travel and climate change could drive epidemic dengue transmission in new areas following imported infections. Our findings correctly identified the most probable sources and time periods of virus introduction via infected traveler(s) and retrospectively anticipated the commencement of the subsequent outbreak(s). In the generalized prospective applications, our models successfully replicated observable trends in disease importation and epidemic potential the selected temperate areas chose for studies 2 and 4.

Theoretical Framework:

The comprehensive review of literature unveiled that the emergence of dengue transmission cycles is reliant on the simultaneous establishment of susceptible hosts (H), disease causing agents (A), and viable vector populations (V) in an environment (E), suitable for each to survive and interact with the others. To visualize and conceptualize the multidimensional nature of dengue transmission dynamics, the H.A.V.E. dengue transmission tetrahedron was developed and applied to the countries of Europe.

The environment acts as a container, which varies the suitability for and the stability of the interactions between hosts, agents, and vectors, both temporally and spatially. The other three elements, hosts, agents, and vectors, whose geographical extent and timing for interactions are environmentally
dependent, are those most directly involved in disease transmission. Interactions between human (or rarely primate) hosts, any of four dengue virus serotype agents, and mosquito vectors, either *Aedes aegypti* or *Aedes albopictus*, result in transmission dengue cycles, which include the following:

- naïve vectors biting infectious hosts;
- vectors becoming infected;
- agents replicating within the vectors;
- vectors becoming infectious;
- infectious vectors biting naïve hosts;
- hosts becoming infected;
- agents replicating within the hosts;
- host becoming infectious;
- and naïve vectors biting infectious hosts to restart the cycle.

The dengue virus is the most geographically and temporally confined element of the H.A.V.E. transmission model. Agents require host or vector for replication in addition to suitable environmental conditions. Furthermore, for transmission to occur in a previously dengue free area, DENV must be introduced. Here again, host and vector populations’ introductions must proceed the viral agent, if they are to establish ongoing transmission cycles. Considering this, virus strains are hypothesized to move around the world via movements of their infectious hosts or infected populations of vectors. In both case, human driven factors play a role including globalization, urbanization, travel, and trade. The introduction and subsequent establishment of virus is the keystone for dengue emergence in naïve areas, which already have a suitable environment, established vector population, and numerous susceptible hosts. Therefore, imported infections are at the heart of this doctoral work.

Host populations must also be present for transmission of dengue. Without such, although vertical transmission of dengue within vector populations is possible, it is less likely to sustain dengue emergence in the long-term, especially within temperate areas, where the viral development within the mosquito, extensive incubation period is longer (Lambrechts et al., 2010). Hosts can include non-human primate populations, however, generally the literature is more focused on human hosts, which when infected can develop dengue. Human host populations, although globally distributed, are settled with and ever changing heterogeneous density. The role of both susceptible and infected hosts is key to the novel emergence of dengue, as host movements allow for exposure of naïve hosts to infected vector population and naïve vector populations to new virus strains. This is especially complicated in that the vast majority of infections are not clinically symptomatic; therefore infection may be spread geographically through the movements of asymptomatic hosts (Bhatt et al., 2013, Tatem et al., 2012, Chastel, 2012).

The H.A.V.E. transmission tetrahedron is a novel approach, rooted in the literature review, to define the conditions necessary for the transmission of vector-borne disease, specifically dengue. In this manner, as a theoretical
framework for transmission, it was well suited to describe how novel transmission could come about in new areas like Europe. The theoretical framework allowed for determining, which areas and countries are closest to meeting the requisite conditions for transmission and therefore prioritizing further investigation. Areas determined to have less elements necessary for transmission were defined as having less potential for dengue transmission and therefore not further investigated. Among those countries scored to have highest relative potential for the emergence of dengue transmission in Europe, the virus, vector, or environment was most often the missing element, each being heavily time sensitive. Categorization according to the theoretical framework crystallized the impact of the importation of the dengue virus, given that the “Agent” element was largely absent from most European nations, included those having all other preconditions for transmission. That being said, vectors and conduciveness are also missing from some places, which have a number of imported cases each year. The coincidence of all four elements in time and place as determined in retrospective studies is the true driver of transmission.

The novel emergence of dengue in naïve temperate areas is therefore resultant of the introduction of both dengue virus and dengue vectors to host populations in an environment suitable for subsequent transmission. Within this framework, it is generally suspected that vector populations once introduced or initially imported must become then established given environmental, climatic, and seasonal conditions allow for vector breeding, feeding, and proliferation. Meeting these conditions likely requires regular feeding on a susceptible host population settled in close proximity. Once the vector population is established within an area; dengue virus must be introduced for autochthonous transmission to emergence. This may occur either through the importation or expansion of infected vectors or viremic vertebrate hosts. Viremic human host movements such as international flight travel can result in the importation of dengue virus strains into non-endemic areas. This route of introduction is hypothesized to pose risk in Europe and is quantifiable. Given all other necessities for transmission are fulfilled in a given area, such instances of introduction can result in dengue epidemic emergence and theoretically eventually lead to ongoing endemic dengue transmission.

Basically, all humans in all areas of Europe are susceptible hosts, as there is currently no dengue transmission in most parts in Europe. With the exception of the Madeira outbreak in 2012 and extremely limited cases of autochthonous transmission of dengue in France and Croatia in 2010, the occurrence of DENV in Europe has been confined to imported cases since an outbreak in Greece in 1928 (Chastel, 2009, Gjenero-Margan et al., 2011, La Ruche et al., 2010, ECDC, 2013). Hence, dengue viruses are considered currently absent in all areas of Europe except Madeira. While absent currently, global interconnectivity and commercial exchange contribute to frequent importation of both infectious cases and vector populations (Arya and Agarwal, 2010, Allwinn, 2011, Schaffner et al., 2013).
Vector populations, mainly of the secondary vector *Aedes albopictus*, were present at least seasonally in a number of European localities, largely in southern Europe as indicated in the previously display map. The environmental suitability for dengue transmission in Europe is certainly less than in the tropics, but remains high for many areas where vector populations persist. The environmental potential for surviving *Aedes albopictus* populations to be competent epidemic vectors for dengue in Europe, however, has now been quantified in terms of vectorial capacity. Areas of highest risk for dengue emergence were defined as those with host and vector populations in close contact, large volumes of international exchange and travel, long periods of environmental suitability for mosquito vector activity, and sufficiently high vectorial capacity.

Pursuant to this line of reasoning, Europe's southern coastal areas are found to be areas to be most threatened by imported infections of dengue, particularly along the Mediterranean, where there are high volumes of seasonal travelers and shipping trade routes convergence on relatively dense human settlements with more temperate climates, many of which have *Aedes albopictus* populations. Among the areas most prone to dengue emergence, after those areas where importation driven autochthonous transmission has already occurred is Rome, Italy, which has the Mediterranean's most active international airport and is situated within an area known to have a long established dengue vector population of *Aedes albopictus*. Within Europe, Madeira is the site of highest current risk for dengue emergence, having all four elements present as indicated by seasonal transmission of during autumn 2012. Tokyo like much of Japan and parts of coast China as realized in recent outbreak of 2014, may also be increasingly at risk for the ongoing local transmission and/or re-emergence of dengue, given the environmental envelop, presence of vector, high density of settlements, and high degree of interconnectivity with endemic parts of southeast Asia (Huang et al., 2016, Lai et al., 2015, Lin et al., 2016). With Rome, China, Japan, and Madeira, before the dengue outbreak, all elements necessary for transmission other than the dengue virus are present. Virus importation through viraemic travelers was been linked to the onset of transmission cycles of novel dengue virus strains in other settings and outbreaks of other arboviral diseases in Italy, most recently Chikungunya in 2007 (Burdino, 2011).

**Global Change and Imported Infections**

Air travel and general interconnectivity between temperate areas and tropical low and middle income countries where dengue persist is on the rise in the 21st century (Jelinek et al., 2002, Lai, et al., 2014, Knope et al., 2013, Jelinek et al., 2009). This is result of many economic and technological advancements, as are most facets of global change—and also like many other global change dynamics, the reduction of time and distance between societies, can involve some drawbacks for some places, while affording advantages to others. The internationalization of infectious diseases spread through global
network of air-travelers is one such potential side effects of development and globalization.

To estimate and model the likelihood for imported infections of dengue to arrive at temperate destinations which could support transmission, air-travel was regarded as the most likely conduit for importing vector-borne disease consistent with previous investigations conducted in other studies (Huang et al., 2012, Tatem et al., 2012, Huang et al., 2013, Seyler et al., 2009, Gardner et al., 2012, Napoli et al., 2012). Previous studies vary widely in approaching the application of the global network of air travel. Some used the global air travel network and climate dynamics to describe the viability importation with a focus on vectors importation (Tatem et al., 2006a, Tatem et al., 2006b, Tatem et al., 2006c, Tatem and Hay, 2007, Tatem, 2009). Other studies focused strictly on specific direct flight routes of travel and/or the corresponding passenger capacity of those routes (Gardner et al., 2012, Huang et al., 2012, Tatem et al., 2012, Huang et al., 2013, Seyler et al., 2009, Napoli et al., 2012). Most models combined this information with some measure of disease occurrence, usually based on reported cases to national and international authorities or another modeled metric of risk based on surveillance data. This doctoral investigation sought to synthesize novel data sources and to innovate on the limitations and shortcomings in previous models, including ours wherever possible. Our refined travel model theorized that the potential for importation of dengue via infected air passenger travel is dependent on two main input factors, for which data can be harvested, volume of travel from endemic areas \( T \) and incidence of dengue in those departure areas \( I \), and one constant representing the probability that travel and infection co-occur \( P \).

For the travel variable, unlike previous models, for the most part we were able to use actual passenger full flight itineraries data from IATA, rather than scheduled flight routes, passenger capacities, or tourism-based proxies to drive our imported infections metrics. Using this data provided more accurate and robust indications of actual travelers’ movements during the duration of a journey, rather than just direct routes, which were identified using other models. This IATA data was also available for years 2005-2012 making this model not only more current than past studies (at the time), but also giving the data a much broader span than past studies, in addition to the added richness of showing actual passengers’ movements. Being simultaneously more up to date, rich in detail, and broad enough to compare 8 years of data allowed this study’s model a greater ability to look at both the seasonality and multi-year trends of flight travel and create meaningful forward projections. In this manner, unlike other measurements of air-passenger travel, all arriving passengers could be linked with their departure location and counted by the model regardless of multiple flight transfers. The ongoing partnership with Bio.Diaspora (now known as BlueDot, www.BlueDot.global) was essential for enabling us to operationalize the models used in studies 1 and 2 and remain important as we work together to identify new applications of this research for public health decision making.
The second factor necessary for modeling risk of importation was the incidence of dengue in those areas, from which the travelers originate. Harvesting data from recent modeling approaches, study 2 especially integrated total projected infection incidence at the country level for all countries with good consensus on dengue activity according to recent studies (Brady et al., 2012, Bhatt et al., 2013). This model integrated the most up-to-date consensus findings on the geographical extent of dengue, including more than 20 additional countries, which had not been included previously by the WHO and CDC maps. Furthermore, this model used harvested data from recently published modeling estimates of the global incidence of infection rather than incidence of disease. In doing this, rather than using routine data, which only includes reported cases of dengue, both symptomatic and asymptomatic infections were used to establish the likelihood of that a given person was infectious. The usage of such a global data set on total incidence of dengue infections was a novel and richly detailed comprehensive approach compared to past studies, which limited their measures of endemic dengue activity to WHO reported incidence, county-level surveillance data, and/or sentinels like traveler-imported case (Seyler et al., 2009, Gardner et al., 2012, Napoli et al., 2012).

This model also included a constant third factor to describe the temporal probability that a certain proportion of travelers were infected before travel and would be infectious during some point after there arriving in the destination. While a similar factor appeared in some models, but not in others, which seek to quantify the risk of introduction of dengue, none included importation potential beyond viraemia (Seyler et al., 2009, Gardner et al., 2012, Huang et al., 2012, Tatem et al., 2012, Huang et al., 2013). This model innovated beyond previous models by describing the probability of becoming infectious post arrival, therefore contributing to risk of importation of dengue, as a 10-day window around their time of travel. This accompanying factor of the model was determined to be 10 days as this is the total average time between an infecting mosquito bite and the end of viraemia (Nishiura and Halstead, 2007, Halstead, 2007, Nguyen et al., 2013, Seyler et al., 2009). Arrival on any day during this 10-day period, much of which is the internal incubation period, therefore before the onset of symptoms, could result in an importation of infection. Numerically this was calculated to be 10 days of 365 days considering that the incidence data harvested was on an annual basis. Including this factor allowed for the unit of the model estimated risk of importation to be potential imported infections.

Retrospective Analysis

On October 3, 2012, two autochthonous dengue cases were reported on the autonomous island region of Madeira, with symptoms onset beginning on September 26, 2012 (ECDC, 2013). One week later, the Institute of Health and Social Affairs (Instituto de Administração da Saúde e Assuntos Sociais, IASAÚDE) announced 18 confirmed cases of dengue infection and 191 additional probable cases, making this Madeira the first outbreak of dengue
transmission in Europe since Greece in 1928 (ECDC, 2013). This outbreak, with over 2000 cases during the first four months, brought attention to the plausibility of dengue outbreaks in Europe as result of importation and prompted further research into the threat dengue introduction and emergence poses in Europe and other temperate areas (Schaffner et al., 2013). The 2012 outbreak in Madeira therefore provided an ideal proof of concept for the modeling developed during this doctoral work.

Madeira, following the discovery of *Aedes aegypti* populations in 2005, became among the most susceptible areas in Europe for dengue transmission, taking into account the climatically suitable environmental conditions, and the presence of susceptible hosts (Almeida et al., 2007, Rogers and Hay, 2012). Investigating air-travel patterns to Madeira therefore presents a reasonable approach to explore the most likely country of origin for the introduction of dengue viruses—via viremic travelers—to Madeira (Lourenco and Recker, 2014).

The majority of these were contributed by travelers originating in Venezuela, with more than half occurring in the four-month period immediately prior to the onset of the outbreak. As the outbreak in Madeira was first reported at the beginning of October 2012, taking the extrinsic and intrinsic incubation time of the *Aedes* mosquitoes into account, the most likely time of importation into Madeira would have been between July and September 2012. Using the monthly risk for importation modeling estimates for July, August and September in 2012 Venezuela’s contribution is at least ten times higher than that of Brazil, both of which are an order of magnitude higher than any other country. Using these data from 2012 for both countries to generate and apply 2012 seasonal trends, the models reflected compelling evidence that the highest modeled risk of importation of dengue in 2012 came from Venezuela during July and August. The modeled findings estimate a peak in the number of imported infections during this period and strongly suggested that Venezuela is the most likely source of importation of dengue to Madeira in late summer 2012, briefly before the outbreak’s first reported case. Although the threshold of imported infections necessary to create an outbreak is not yet known, nor directly proven by these findings, the country of origin seems in line with dengue virus introduction from flight-based importation of infected passenger.

Given dengue transmission suitable climatic conditions and known activity of *Aedes aegypti* in Madeira begins during this time, such heightened risk of importation may have been sufficient to sustain the epidemic, which emerged in autumn 2012 and explain the potential for the initial cases identified in late September 2012 (Lourenco and Recker, 2014, ECDC, 2013, Almeida et al., 2007, INdS, 2013, Frank et al., 2013). The mid to late summer 2012 emergence of dengue transmission is also consistent with our findings based on climate dependent modeling of vectorial capacity in Madeira, consistent with others environmentally driven R0 models (Lourenco and Recker, 2014). Our findings confirm that prior years, which had even greater total flight
Based exposure such as 2008–2010, were met with less suitable climatic conditions for the vectorial capacity of *Aedes aegypti* population on Madeira. These findings are further underscored by the relatively higher population counts of *Aedes aegypti* populations in what limited vector surveillance was conducted in Madeira (INdS, 2013). These findings showed the season most conducive to adult vector activity to be in late summer and autumn in Maderia and the number of adults found in mosquito traps to have almost doubled in 2012 compared to the two previous years (INdS, 2013). Furthermore, Funchal, the urban municipality, which contained the international airport, was the area where the dengue vectors were first found in 2005; was home to the highest vector density of *Aedes aegypti* and most numerous breeding sites in 2011 and 2012; and the site of the vast majority of cases according to spatial epidemiological investigation (Alves et al., 2013, INdS, 2013, ECDC, 2013, Sousa et al., 2012).

Early speculation during the investigation of the outbreak suggested that the origin of the dengue virus strain circulating on Madeira, confirmed to be a DENV-1 serotype, was of South American origin (Alves et al., 2013, Huhtamo et al., 2013, ECDC, 2013, Sousa et al., 2012, Cnops et al., 2014, Franco et al. 2015). Evidence from the genetic sequencing supports the notion that Venezuela is the most likely country of origin for the strain of dengue circulated in Madeira during the 2012 outbreak (Franco et al, 2015, Wilder-Smith et al. 2014). The virus sample sequenced from Madeira was compared with existing dengue sequences in GenBank to further strengthen the importation models’ findings. The dengue sequencing done showed that the virus is most closely related to the South American strains, in particular those from Venezuela. Studies conducted by others on cases of dengue imported from Madeira to other European destinations support this finding, albeit with less strain specificity than the phylogenetic typology investigation conducted in study 1 (Alves et al., 2013, Huhtamo et al., 2013).

**Prospective Analysis**

The model was retrospectively in Madeira and prospectively in Rome, Italy, to describe the potential risk for future importation of dengue based on current and recent trends in both air-passengers arrivals and the incidence of dengue within the countries from which they departed. Rome, Italy, according to the framework, has all necessary elements for transmission except the disease causing agent, dengue virus (Gobbi et al., 2014). The relevant vector population of *Aedes albopictus* expanded into Rome as early as 1997 after being introduced in the early 1990s to other parts of Italy (Medlock et al., 2012, Schaffner et al., 2013). Due to environmental suitability, including climatic factors and plentiful urban breeding sites in the built environment, the well-adapted invasive species has thrived in Rome after establishing there quickly (Caputo et al., 2012).

The third quarter of each year corresponded with the peak in travel and the beginning of dengue vector activity in Rome as indicated by the review of
literature (Caputo et al., 2012, Napoli et al., 2012). Our analyses show that the second and third quarters of the year (April-September) are at highest importation risk based on the triple threat of the high season for travel to Rome, vector activity in and around Rome, and incident of dengue infections in the main countries of origin.

The seasonal findings of the model were supported empirically by past studies of dengue importation into Italy over the period 2008-2011 (Gobbi et al., 2012, Burdino et al. 2011, Napoli et al., 2012, Lagi et al., 2014). These data showed repeatedly an increase in the number of important cases of dengue, during August and September and increasing during most recent years of 2010 and 2011 over 2008 and 2009 (Napoli et al., 2012). Similar to the findings of these doctoral studies, this dengue importation study found the case importation peaks to coincide with the activity period of *Aedes albopictus* during the period of July 15 through November 15, such that the overall risk posed by importation was attenuated during summer months known to have more frequent travel into Italy (Napoli et al., 2012).

Despite, 7.3 million passengers from dengue endemic departures traveling to Rome, corresponding to an estimated 18,840 (12,880-27,432) infections imported between 2005 and 2012, local dengue transmission have not yet commenced. While conducive for the emergence of dengue in the future, this threat does not yet seem to have been realized in Rome or elsewhere in Italy. This indicates that the threshold of infectious hosts necessary for *Aedes albopictus* to transmission dengue is still largely unknown. Although the importation of dengue to Italy is much higher than that of chikungunya, the importation of chikungunya resulted in autochthonous transmission in the year 2007 (Vega-Rua et al., 2013, Napoli et al., 2012, Burdino et al., 2011). This is most likely because *Aedes albopictus* is more susceptible to chikungunya viruses compared to dengue viruses (Vazeille et al., 2008, Moutailler et al., 2009, Tomasel and Schlagenhauf, 2013). Further research in particular modeling is needed to determine the thresholds for *Aedes albopictus* populations and number of infected humans to cause autochthonous transmission of dengue in Southern Europe.

**Global Change and Dengue Epidemic Potential**

Given that introduction of dengue virus seem potentially to occur frequently and in high number, we further hypothesized the the importance of estimated imported infections of dengue would be chiefly determined by the potential for onward transmission in a previously dengue free area. In other words, we were interested in when, where, and in what quantities the imported infections were arriving, because this would impact the likelihood of environmental conditions facilitating further spread of disease, or much more likely the termination of transmission. Global change dynamics are also influencing observed and likely future alterations in environmental conditions which can impact the transmission of dengue in several ways. We explored the bio meteorological impact of ambient temperature on dengue vectors’
potential for spreading dengue. Ultimately, informed by many studies previously conducted, we developed methods for the generalization of a temperature-dependent mathematical model of dengue epidemic potential based on the calculation of vectorial capacity for both *Aedes* vectors.

**Retrospective Analysis**

Japan, a temperate country, experienced the first major dengue outbreak in 2014 (Arima et al., 2014, Kojima, 2015). The outbreak occurred in Tokyo, with most cases traceable to the Yoyogi Park, and resulted in 160 dengue cases between August and October 2014 (Kojima, 2015). For an outbreak to occur in a previously dengue-free country, several converging factors are necessary including the introduction of the vector and its establishment based on favorable ecological and climate conditions, plus importation of the dengue virus.

We investigated the origin of the dengue outbreak in Japan in 2014 based on travel interconnectivity, phylogenetic analysis and the environmental drivers for dengue epidemic potential in terms of the initial relative vectorial capacity model. Given suitable vectors, susceptible populations, and increasing interconnectivity with endemic areas of Asia, Tokyo’s 2014 outbreak may foretell subsequent autochthonous dengue activity, making it a prime candidate for applying the environmental drivers model in both specific and more generalized terms. When we applied the model and combined it with other meteorological factors, for the retrospective analysis of Tokyo during 2014, the temporal alignments indicated that the dengue epidemic potential model was capable of anticipating the timing of outbreak. The model succeeded in describing the environmentally driven potential for dengue outbreaks to commence and sustain as long as an introduction dengue virus into viable vector populations proceeded it. Like had been the case with Madeira, the peak of the relative vectorial capacity occurred immediately before the initial cases, while the introduction of virus and beginning of the period above the threshold for epidemic potential started a few months earlier. Despite being relatively few cases and only of few weeks in duration, the relative vectorial capacity dropped below the 0.2 threshold in the same week as the final cases illness onset was reported. The onset and the conclusion to the epidemic highly corroborate the threshold condition of 0.2 corresponding to a basic reproductive number of 1.

Given this success, we applied the relative vectorial capacity, we liberally applied the model spatially in conjunction with our retrospective analysis of the 2014 Tokyo outbreak. This allowed for us to make other important connections within the region and particularly to identify the seasonal patterns of the dengue epidemic transmission window, when later used in our prospective analyses. Nearby China experience similar environmental dynamics during 2014 and was at the time experiencing a large scale epidemic in at the same time; apparently transmitting a DENV-1 strain close genetically to the one circulated in the Tokyo outbreak (Lai et al., 2015, Lin et al., 2016).
We substantiated the environmental drivers’ hypothesis through phylogenetic analysis, which showed strong similarity between the index cases strain of dengue and stained previously sequenced in Guangzhou, China, Singapore and Bali, Indonesia. Consistent with others’ findings, of those dengue strains on record from Asia, the virus circulated in Japan in 2014 was particularly close genetically to one isolated from Guangzhou, China in 2013 (Kutsuna et al., 2015). The similarity was very strong between the DENV-1 strain introduced and one sequenced in the previous year in Guangzhou, China, where an outbreak with 45,000 cases also occurred in 2014, however, based on the phylogenetic analysis alone, Singapore and Bali (Indonesia) cannot be fully ruled out (Huang et al., 2016).

**Prospective Analysis**

The environmental drivers’ models we used to calculate dengue epidemic potential had temperature variable durations of extrinsic incubation, biting, mosquito mortality, mosquito to human infection probability and human to mosquito infection probability. Due to the models’ deterministic nature, mathematical construction and the general availability of temperature data in a variety of formats, we had the opportunity to apply the model to current, historical, and projected future scenarios. This allows us to create time-series modelled estimation of dengue epidemic potential all over Europe, and other parts of the world, limited largely by our data inputs and the accuracy of the laboratory based parameters on which the model was based.

The greatest advantage of this modeling approach was the ability to utilize global environmental change projections models of climate change scenarios. We were able to project how the importance of imported infections arriving in temperate areas might change in the near and far future, with regards to likelihood of outbreak. In other modelling endeavors, this had not been done with the same degree of rigor and quality of data analysis for so many scenarios, time periods, and dengue vectors. Most other models were statistical risk models applied to coarse temporal resolution datasets for climate change futures; however, some have also taken on more comprehensive analysis of ecological niches of disease transmission under multiple scenarios, time-periods and vectors (Campbell et al. 2015, Bouzid et al., 2014).

Our interest in investigating the importance of imported infection demanded both time and space components of the model to be present in the outputs— not lost in pre aggregation. Data management and data quality therefore became paramount elements of the model building protocols. Our product, however, due to this prioritization can contribute along side the importation models to aid in decision making about the potential impact of the growing number of infections of dengue imported into temperate areas. Furthermore, knowing the time-space potential for epidemics currents, past and future can stimulate targeted interest and necessary support for vector surveillance endeavors, vector control measures, dengue notifications/reporting, clinical
preparedness and emissions’ mitigation efforts. Our results strongly encourage vector control and mitigation of greenhouse gas emissions in order to reduce the public health threat imposed by imported infections of dengue in Europe.

Potential Alternative Applications and Future Directions

Since beginning the studies involved in this doctoral work, several important infectious disease pandemics have been fueled by imported infections including those causing three separate Public Health Emergencies of International Concern (PHEIC) as declared by the World Health Organization since 2014. In 2014, unanticipated resurgence of polio and later Ebola epidemics occurred in areas, where the agents were believed to have been already eliminated. These events captivated global attention because they constituted tangible global public health threats, given that imported infections potentially spawning local outbreaks were only a simple plane ride away. February 1, 2016 would see the WHO’s third announcement of a PHEIC in as many years—this time for the ongoing pandemic of Zika virus. Pandemic transmission of novel corona virus in the middle east, large scale outbreaks of chikungunya in the Americas and some of the worst dengue epidemics in years, did not attract the same level of international concern, but are also highly relevant. While neither Aedes vectors nor climate change singlehandedly drive all these pandemics in isolation, the frameworks and methods developed here could certainly be applied, with modifications, where necessary. The potential applications with the most conservation of methods and materials would be the assessment of potential epidemic outbreaks of arboviral diseases spread by infected travelers, such as chikungunya and Zika virus, both spread by Aedes mosquitos. Based partially on components of this doctoral work, the skills gained during these these investigation, and the network of fellow researchers, we have been able to lend new insights to the quantification of disease dynamics, epidemic potential, and other imported infections of current importance including not only dengue, but also polio, Ebola, and most recently Zika (Quam et al., 2014, Wilder-Smith et al., 2015, Lopez et al. 2016, Massad et al., In Press). These alternative applications highlight the demand to adapt current and develop new methodologies, models, and theoretical constructs to address pressing global public health threats as well as opportunities to model the implementation strategies for newly developed vaccines. Leveraging spatial temporal analysis models, like those described here, to anticipate real-time imported infections’ importance in the context of dynamic global change, could cultivate proactive assessment of global disease spreading networks and assess the context specific public health preparedness needs for today, —tomorrow, —and well into the future.
Conclusion

These studies provided an important foundation to the investigation of the potential for emergence of dengue in Europe. The specific emphasis of this study was on the development and application of novel mathematical models for quantifying potential risk of dengue importation posed by commercial air-passerger movements into Europe, specifically Rome, Italy and the Portuguese Island of Madeira. Secondly, we sought to quantify the importance of imported infections in terms of likely spread in temperate areas using the example of Tokyo, Japan and expanding to a spatial temporal model for Europe in the 21st Century. Leading up to the application of these models to European destinations, new knowledge has been contributed to the research base in numerous ways. Additionally, our investigations have brought to light the need for further exploration into the areas of Europe at heightened potential for emergence and the dynamics, which govern novel dengue emergence in temperate area.

The application of an original theoretical framework for dengue emergence in naïve areas was the foundation of this study. As result of the extensive review of literature, this novel theoretical framework was developed and applied to the countries of Europe to prioritize subsequent investigation in areas with greatest potential for future dengue transmission. Following from this theoretical framework, new vector maps were synthesized, and tables describing theoretical scenarios of dengue emergence under different conditions were hypothesized. Applying the novel Host-Agent-Vector-Environment (H.A.V.E.) Transmission tetrahedron, which defined the minimum parameters for dengue emergence in naïve areas, highlighted virus importation as the most pertinent risk for dengue emergence in Europe, particularly southern Europe. Due to virus importation being a common risk factor, areas of highest potential risk were selected due to their interconnectivity with dengue endemic areas, largely through large international airports and/or frequent sea traffic.

The application of this model to the case of the Portuguese Island of Madeira and to Rome, Italy was an important initial step in understanding the dynamics, which govern dengue importation via air-travel into Europe and how these related to the possible emergence of dengue in novel settings. With the case of Madeira, the model provided a potential explanation for the origins and timing of the 2012 outbreak. With Rome, where there has not yet been transmission of dengue, the model successfully indentified trends of dengue case importation. With Japan, the dengue epidemic potential models successfully identified the environmental envelope for the 2014 outbreak there. This combined with travel analyses and molecular phylogenetic epidemiology, also provided key information about the origin of the imported infections which precipitated the outbreak in Tokyo. Finally, we applied our models on vectorial capacity to better generalize the epidemic potential for dengue in Europe, past, present and future under climate change.
Further research into the dynamics of dengue transmission in novel areas remains needed, however, applying this newly developed theoretical framework, the imported infection model, and temperature-dependent vectorial capacity model could be a valuable initial steps in generating decision making tools. In light of the current Zika outbreak going on in the Americas, researchers and policy-makers are likely to find lessons learned from dengue research particularly useful, especially during this period of uncertainty, while so much about Zika remains unknown. Future directions of these studies may well include the integration of these models into existing temporal-spatial dynamics repositories to assess near real time potential for arbo-viral importation driven outbreaks in Europe and other temperate areas such as North America, China, and Japan.

Particularly in areas of heightened risk, additional and ongoing vector surveillance and strengthened dengue surveillance are recommended foundations to prevent dengue transmission in Europe. In areas of highest risk for imported infections with established vector populations, this remains of utmost importance. Vector control measures are necessary to prevent and mitigate for potential dengue transmission; however, entry screenings on arriving passengers are unlikely to be a useful and cost effective solution for dengue in Europe. Additionally, our findings suggest strong support for mitigating climate change to stem the tide of epidemic potential for dengue in Europe during the 21st Century. Until an effective vaccine can be developed and fully approved, focus on dengue control should remain a high priority in endemic areas and disease and vector surveillance should remain high priorities in naïve areas to prevent dengue transmission and diminish the importance of imported infections.
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With Peace and Gratitude,

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