Spatio-temporal dynamics of dengue and chikungunya

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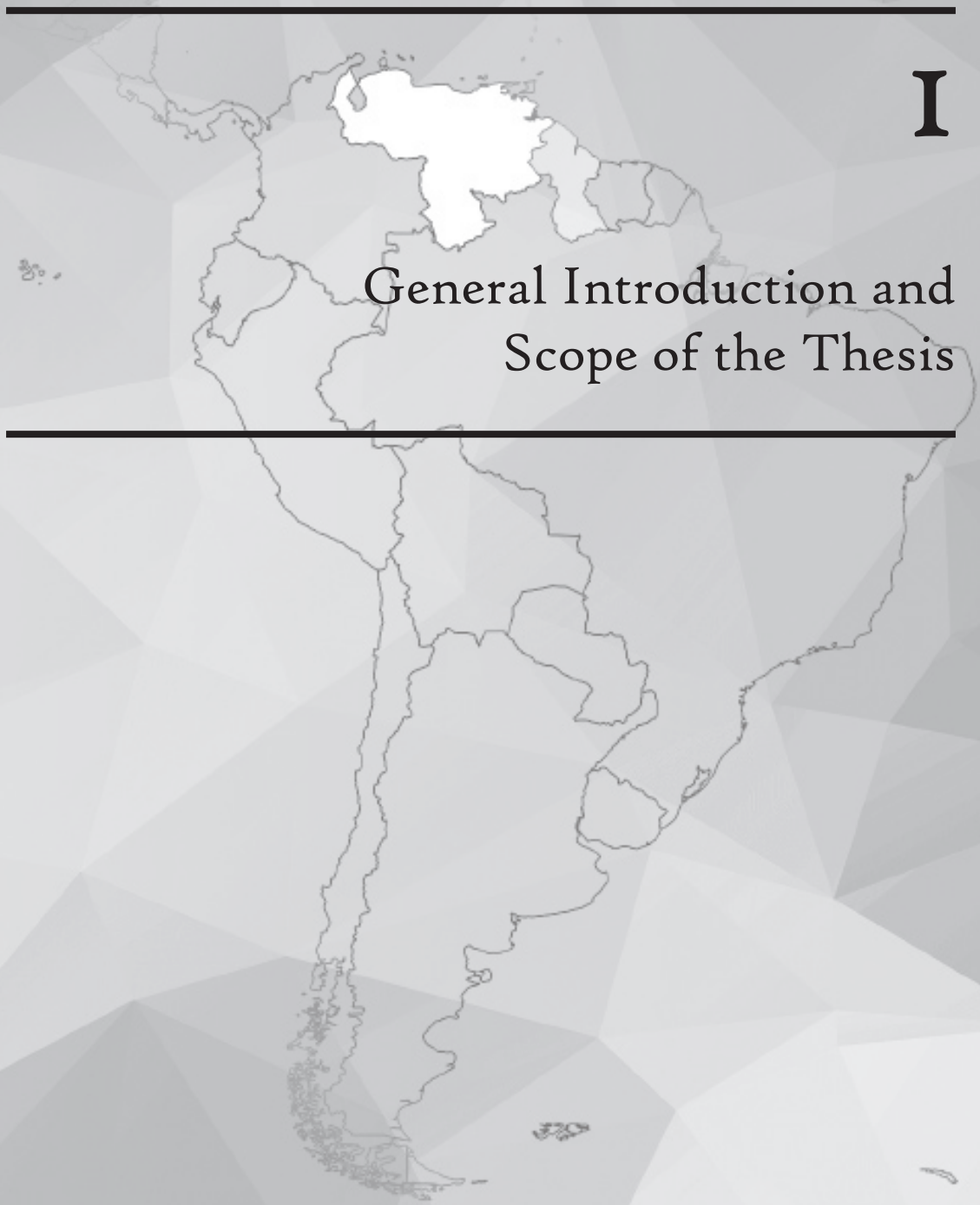
I

General Introduction and Scope of the Thesis



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GENERAL INTRODUCTION

Viruses that are transmitted by arthropod vectors (arboviruses) are spreading either gradually or in explosive (re)-emergent epidemics becoming an increasing threat to global public health [1,2]. In the last 4 years, the two major and devastating epidemics that struck the Americas were caused by arboviruses: chikungunya and Zika viruses. In parallel, dengue virus (another arboviral disease) is endemic in the region with an increasing spread to areas that were formerly unaffected. All three arboviruses are transmitted by the same mosquito, *Aedes aegypti*, with a potential role for *Ae. albopictus*. The control of these arboviral infections has been a challenge, as these difficulties emerge from several factors of different nature (political, institutional, ecological, socio-economical, behavioural). Globally, there is an increasing call to change how surveillance and control of dengue and other *Aedes*-borne diseases (such as chikungunya and Zika) are currently performed, since the classical approach has proven ineffective in reducing the morbidity and mortality caused by these infections. The research described in this dissertation attempts to add relevant knowledge that will contribute to a more integrated approach for arboviral surveillance and control.

The majority of the near 500 known arboviruses belong to one of five families: *Bunyaviridae*, *Flaviviridae*, *Reoviridae*, *Rhabdoviridae*, *Orthomyxoviridae* and *Togaviridae* [3,4,5]. The last twenty years have been marked by important outbreaks caused by two main arbovirus families: *Flaviviridae* and *Togaviridae*. Relevant viruses from the *Flaviviridae* family are dengue, West Nile, yellow fever, Zika (ZIKV), St Louis encephalitis and Japanese encephalitis. The *Togaviridae* family comprises chikungunya, Mayaro, Venezuelan equine encephalitis and Western equine encephalitis viruses. The families *Flaviviridae* and *Togaviridae* are common causes of febrile diseases with different severity outcomes ranging from short and long-term physical or cognitive impairment to death.

Dengue and chikungunya viruses are distributed across several countries belonging to Asia, the Pacific, America, Africa and the Caribbean (Figure 1) and are transmitted in urban and peri-urban settings by infected females of the day-biting mosquitoes, *Ae. aegypti* and *Ae. albopictus* [1]. But recently, in Europe, autochthonous transmission of dengue and chikungunya has been reported in countries such as France [6], Portugal [7,8] and Italy [9,10,11], revealing the expansion of these diseases to novel and unexpected areas posing a new threat for temperate climatic zones. In the Americas, numerous factors are related to the current increase of dengue incidence, and the recent introduction of chikungunya and Zika viruses. Amongst the most important ones are the uncontrolled urbanization, unreliable public services such as water supply, electricity, sewers and waste disposal [12,13]. Furthermore, the progressive deterioration of the public health institutions responsible of the vector control programmes, and the lack of funding and political will have been contributing to the increase of vector density [14,15]. To add more, globalization, the intensification of human movement and climate change have also played a role in the fast spread of dengue [16,17,18].

Dengue is currently the most important arboviral disease in humans with over half of the world's population living at risk and present in 128 countries [18, 19,13,20,21]. Over the last 50 years, a 30-fold increase in disease incidence has been observed [22]. Individuals infected with dengue can be asymptomatic or show symptoms that range from mild febrile disease to severe illness with plasma leakage, multiorgan failure, and death [23,24]. The DENV comprises four distinct serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) and numerous genotypes, being the "Asian" genotypes of DEN-2 and DEN-3 frequently associated with severe disease accompanying secondary dengue infections

[25]. In 2013, it was estimated that 390 million dengue infections occur every year [26], of which 96 million show symptoms, with children being the most affected [24]. The rest of the 294 million cases (75%) were defined as inapparent dengue infections where individuals present very mild or asymptomatic disease but can potentially transmit DENV [27,28].

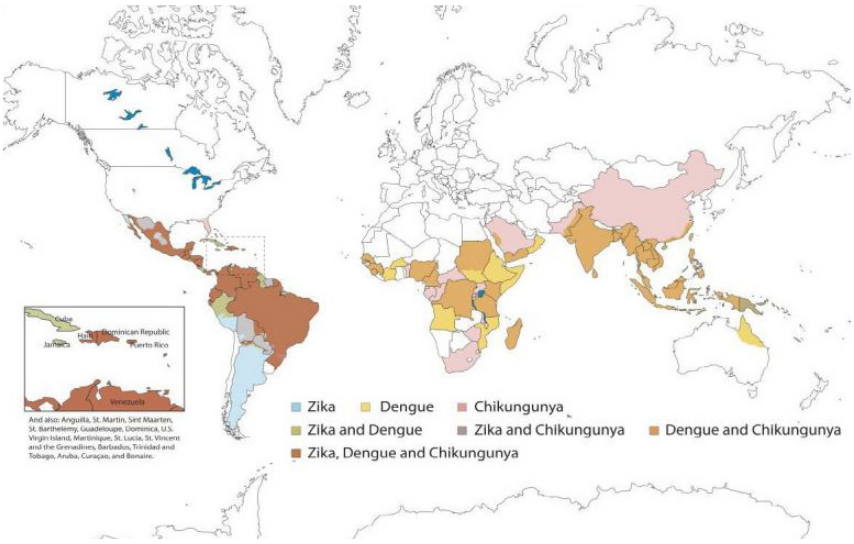

Figure 1.- Estimated global distribution of dengue, chikungunya and Zika. Source : Patterson *et al*, 2016 (https://doi.org/10.5811/westjem.2016.9.30904).

Chikungunya virus, an alphavirus that was first described in 1950 in Tanzania, is responsible for explosive outbreaks in Africa, the Indian Ocean islands, Asia, Europe, and the Americas [29]. From October 2013 onward, when the first autochthonous case was reported in Saint Martin Island, CHIKV rapidly expanded across immunologically naïve populations from the Americas, including countries of South and Central America, and later North America. Nowadays, autochthonous transmission of CHIKV is confirmed to be in 48 countries belonging to the Americas and the Caribbean region. Since the introduction of CHIKV in the Americas, an estimated 2 million suspected cases have occurred [30,31]. Chikungunya infection is mainly symptomatic and is characterized by fever, rash and debilitating arthralgia [32,33,34,35]. In areas where dengue is widespread, the two diseases may be difficult to disentangle without laboratory diagnosis given their common symptoms (fever, muscle pain, headache, fatigue). For both viruses (DENV and CHIKV), disease prevention and control are subjected to education, sanitation and vector control programs.

Dengue in Venezuela

Until 1989, dengue in Venezuela was limited to hypoendemic epidemics with the circulation of a single serotype [36]. Hereafter, the introduction of dengue serotypes had happened sequentially. Although the presence of dengue fever was documented since 1828 [37], it was not until 1964 that an epidemic of dengue was reported in Venezuela (18,315 cases), due to the (re-)introduction of previously non-circulating dengue serotype 3 (DENV-3) strain and coinciding with an increased spread and densities of *Ae. aegypti* [38,39,36,40]. Between 1969 and 1970, an epidemic was confirmed due to the entry of serotype 2 (DENV-2, American genotype). After this last epidemic,

a 7 years epidemiological silence elapsed until serotype 1 (DENV-1) was introduced in 1977. The previous serotype introduction caused a massive epidemic in several countries including Venezuela [41]. Later in 1981, serotype 4 (DENV-4) is confirmed to be the cause of a new dengue epidemic in the Americas; however, in Venezuela, the epidemic had a slight impact in the population [40]. Meanwhile, Cuba was reporting a massive first epidemic of dengue hemorraghic fever (DHF) due to the introduction of a new strain of DENV-2 from Southeast Asia [42]. By the end of the 1980s, this new pathogenic genotype of DENV-2 (genotype III) reached Venezuela causing the first DHF epidemic in the country with 12,220 cases, of which 3,108 (25.4%) were DHF [43,44,45]. From this moment, Venezuela became a dengue hyperendemic country with the co-circulation of serotypes DENV-1, 2 and 4 and with the establishment of the hemorrhagic and severe forms of the disease. Finally, in the year 2000, DENV-3 was re-introduced, causing a large epidemic. By the end of the year 2001, 83180 cases were reported, with an attack rate of 337.7 per 100000 inhabitants [40,46]. From this year on, the co-circulation of the 4 dengue serotypes and the persistent occurrence of DHF and severe dengue cases were established throughout the country, with especially high rates among infants [36,47,48].

Since the year 2000, the disease is one of the Neglected Tropical Diseases (NTDs) of major public health importance in Venezuela, which have been showing an upward trend of the magnitude and frequency of epidemics. The disease has caused a heavy burden in the already deteriorated national health care system, leading to an important increase in rates of morbidity and mortality across the country. The last decade was marked by important dengue epidemic years (2007, 2009-2010, 2013, 2014), with the biggest occurring in 2010 when more than 120,000 cases were reported [46]. The incidence of dengue in Venezuela has exhibited a steady increase since the early 1990's [46,49,50]. In 2015, Venezuela was considered to be a country with high human development (Human Development Index, HDI=71◦), yet, the country has been unable to effectively apply a reliable program for dengue and other mosquito-borne disease prevention and control, showing an increasing trend of Vector Borne Diseases (VBD) [51,46,52]. Deficits in public services such as the infrequent services in water supply and electricity have forced the Venezuelan population to store water intradomiciliary, promoting and maintaining persistent breeding conditions for *Aedes* mosquitoes throughout the year, and making difficult for the limited, -and now, non-existent-, vector control programs to achieve some degree of effectiveness. Furthermore, despite the high HDI in Venezuela, the current proportion of people living in poverty is as high as 87% [53]. Studies conducted in the country found that poverty-related socioeconomic factors increase the risk for dengue infection [54]. Currently, since costs of renting a household suitable for a family are not affordable, this situation had favoured more crammed living conditions and deprivation supporting dengue transmission [54,55].

The emergence of new arboviruses like CHIKV and ZIKV together with persistent transmission of dengue have generated a heavy burden on the population and health systems of the Americas in the last years [56]. In Venezuela, the local transmission of CHIKV and ZIKV was detected for the first time in 2014 and of 2015 respectively, after which these viruses spread quickly throughout the immunologically naive population. The latter affected remarkably several aspects of the Venezuelan population at that moment, which produced high rates of work and school absenteeism and drove a hospital crisis collapsing the health system. The introduction of these new viruses and the concomitant dengue epidemics, have shown the ability of arboviruses to expand rapidly across these regions causing large national epidemics. Moreover, these epidemic events have also revealed a worrying reality about how unprepared are the health systems in Venezuela when it

comes to handle infectious disease emergencies and exposes the importance of improving the current conditions of the management of dengue and other infections transmitted by *Ae. aegypti*. We could in fact categorize the health situation of Venezuela with the term "blue marble health", which describes nations with wealthy economies, showing unexpectedly high prevalence and incidence rates of neglected tropical diseases [57].

Dengue and chikungunya transmission cycle

The full life cycle of dengue involves the insect vector, *Ae. aegypti,* a highly domesticated mosquito, and humans as the host. It is transmitted from human-to-mosquito-to-human through the bite of an infected *Aedes* mosquito [58,59], and unlike other *Aedes*-borne diseases, DENV does not require an enzootic cycle for the maintenance of epidemic transmission in humans. The DENV transmission sylvatic cycle does exist in jungles of Africa and Asia in non-human primates, but this virus is phylogenetically distinct to the urban cycle of dengue involving *Ae. aegypti* (Figure 2) [58,60].

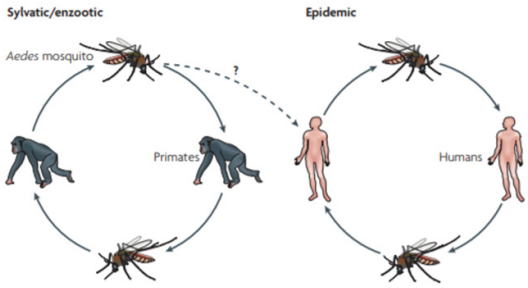

Figure 2.- Transmission of dengue viruses. Source: Whitehead *et al*., 2012 (https://www.nature.com/articles/nrmicro1690). Reprinted from [60] with permission from Springer Nature.

The sylvatic (enzootic) cycle of CHIKV in Africa involves non-human primates with the virus being transmitted by an ample range of forest-dwelling *Aedes* spp. mosquitoes, occasioning infrequent human cases and small outbreaks [61]. However, like dengue, an enzootic amplification is not essential for CHIKV when humans are involved, as these are the main amplifying host for this pathogen (Figure 3) [62,63,64].

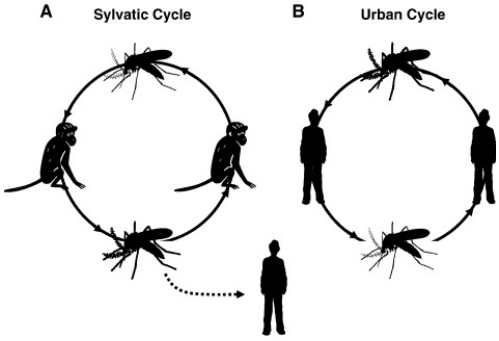

Figure 3.- Transmission of chikungunya virus. Source: Petersen *et al*., 2010 (http://www.tmreviews.com/article/S0887-7963(09)00082-0/fulltext). Reprinted from [61] with permission from Elsevier

Transmission of DENV and CHIKV from the human host to mosquitoes requires that multiple biological factors have to occur in time and space. Typically, the cycle involves a series of events that start with the mosquito taking its blood meal, replication of the virus inside of the mosquito and finally, upon a subsequent feeding event, the transmission of the virus to a new susceptible host [59]. The cycle can be divided in two main parts: The extrinsic incubation period (EIP) or the time taken by DENV/CHIKV to complete its development in the mosquito vector *Ae. Aegypti*; and the intrinsic incubation period (IIP) which starts after a human has been bitten by an infected mosquito, and is defined as the time period taken by DENV/CHIKV to complete its development in the human host. It is noteworthy that infected mosquitoes can continue transmitting DENV/CHIKV to other human hosts for the rest of their life spans (3-4 week period). This can have great implication for disease control, and highlights the importance of an efficient vector control program to avoid VBD to spread further.

Surveillance and control of *Aedes*-borne diseases

Disease surveillance is essential for monitoring disease trends and detecting outbreaks, providing public health officials with the information needed to timely detect and manage disease occurrence. Successful surveillance activities require a series of standardized steps (e.g.: data collection/detection, checking, reporting, opportune diffusion) in order to build an early and accurate response towards disease prevention and control [65]. Currently, one of the weaknesses of dengue surveillance systems relies on the fact that disease control is usually the first option, rather than prevention, suggesting that disease surveillance data is scarcely used for purposes other than disease cases reporting. Certainly, new methodologies for dengue surveillance have been applied to improve data collection (computer-based data collection, health-Geographic information systems (GIS), spatial statistics, mathematical predictive models), enhancing decision making to prioritize resources allocation and help to measure the impact and cost-effectiveness of control activities [66,67,68]. Nonetheless, in the Americas and Venezuela, dengue surveillance relies mainly on passive surveillance, lacking an active surveillance program. The latter is an important factor to anticipate the beginning of an epidemic [69]. Passive surveillance aims to monitor epidemiologic trends in diseases, but in dengue endemic countries, passive monitoring is often delayed due to the overload of the health system during epidemics [69,70]. To add more, there is a general delay in case reporting, ranging from 1.5-3 weeks after patient presentation at a health centre. Since control measures are activated based on the disease reports, this delay has an important impact on the effectiveness of vector control for dengue and other *Aedes*-borne diseases.

Currently, and in the absence of a vaccine, the prevention or reduction of dengue and other *Aedes*-borne virus transmission depends entirely on controlling the mosquito vector or the interruption of human–vector contact. The control of *Ae. aegypti* targets mainly the immature stages of the mosquito (source reduction, environmental management, application of larvicides) and the adults (fumigation with insecticides) [71,72]. In the last decades, *Aedes* control has developed towards the use of novel technologies that complements the classic approach of vector control. These new technologies include suppression of mosquito populations, genetic techniques and population replacement methods that make mosquitoes refractory to arboviral infections [73,74,75]. Despite that the majority of endemic countries make efforts to apply the classic tasks of mosquito control, these measures seem not to be sufficient and effective due to delays in vector control during epidemics, scarce or absent inter-epidemic vector control programs and intermittent spraying tasks applied mainly outside of the households with scarce indoor residual spraying usage [76].

Additionally, these programs are often obstructed by weak surveillance capacity, lack of funding and a limited understanding of the value and cost-effectiveness of the control measures pre-outbreak [72,77].

Similarly, in Venezuela, the surveillance of VBD such as dengue, is passive, with very scarce reports of active surveillance, which is mainly produced by research projects that aim to estimate the real burden of the disease in this endemic country [54,78,79]. The country has not been able to effectively control dengue, -and more recently nor chikungunya nor Zika-, during the last 15 years [46], mainly due to a relaxation in the application of vector control measures and surveillance programs. The vector control measures that are applied in recent years, are not different from other countries. In general, the programs aim to reduce the immature stages of the mosquito by providing larvicides and to reduce sources that enhance mosquito breeding sites. The adult stage of the mosquito is often controlled using fumigation with insecticides [72]. However, this measure has proven ineffective because it does not cover the entire house, and fumigation campaigns are often activated when the number of dengue cases is already increasing exponentially having a reduced effect on mosquito populations. Likewise, the vector control institutions in Venezuela, have a series of strategies for mosquito control that are not implemented on a regular basis, but rather in response to detected clinical cases which are implemented after several days from infection onset, or during epidemic periods, using massive insecticide applications designed to stop the disease from spreading further. More recently, the increase of arboviral diseases in Venezuela can be explained not only because of the reduction in dengue environmental management activities (education and sanitation programmes, surveillance, diagnostics); but also, as a result of economically deprived chemical and biological control programs (due to significant shortage of pesticide supplies) [50].

The alarming increase of dengue in Venezuela in the last decade can be attributed to a series of factors that concomitantly influence the occurrence of this diseases. Climatic, environmental and socioeconomic factors play an essential role in the spatial spread and the temporal persistence of dengue, affecting the physiology/ecology/biology of both virus and vector [55,80,81]. Additionally, in the Venezuelan context, socio-economic and political factors have clearly influenced the worrisome outcome of dengue in the Venezuelan population [52,82]. Since 2000, the Venezuelan government has been progressively shortening the investments in healthcare infrastructure, among others. The deep political and economic instability favored the limitation of funding and resources in several government offices/ministries, causing a collapse of the healthcare, public health and public services systems, leaving aside essential tasks of the prevention and control of dengue and other *Aedes*-borne diseases, such as reliable vector control program and urban sanitation tasks [50,52,82,83]. In the last 4 years, the country increasingly lacks reliable medicine and food supply systems, which together with an untrustworthy health care system, resulted in a more vulnerable population to a wide range of diseases, including malaria, Chagas disease, leishmaniasis, and schistosomiasis [52].

Application of Geographical Information Systems in health

Classic epidemiology involves three important features: time, person and place [84]. Furthermore, the traditional model for infectious disease (epidemiologic triangle) consists of an external *agent*, a susceptible *host*, and an *environment* that, if suitable, brings the host and agent together [85]. All the variables shaping health related factors, such as pathogens and host distributions, social

connections and the built and natural environment have an important geography context [86]. Therefore, "place", or the spatial component is a relevant feature in dengue and other infectious diseases. Likewise, "time", is an important variable since the pattern of occurrence of diseases exhibit dynamics that change over time. Some of these changes occur regularly, while others are somehow unpredictable. In the case of dengue and other *Aedes*-borne diseases, the pattern of occurrence is seasonal, and this seasonality is related with climate variables such as temperature and precipitation [87,88], that favor the maintenance of mosquito breeding sites. Together, seasonal and spatial changes can promote the increase in vector and host populations, facilitating the contact between these elements, and therefore, pathogen and disease amplification [89].

Seasonal and temporal changes drive variability on the distribution of vector and host. These changes are defined as heterogeneity, which is the variability of a property of a system in space and time [90,91]. It has been largely described that spatial heterogeneity is a common aspect of VBD such as dengue and chikungunuya, where disease tends to be concentrated in a small proportion of the epidemiological landscape, contributing disproportionally to the overall transmission [91,92]. Such disease concentration can be manifested in a small group of households, villages, or particular regions that we could denominate "hotspots" where the infection risk is substantially higher than areas around or nearby [93]. Another relevant concept is nidality (from the latin "nest"), defined as the place where pathogen transmission occurs, and where the pathogen occurrence is associated with specific landscapes that favour its amplification [94]. From these two concepts- "Heterogeneity" and "Nidality"- new disciplines have risen, called "Spatial epidemiology" or "Landscape epidemiology" [68,89,92,94].

Both "Landscape epidemiology" and "Spatial epidemiology" assume that interaction and co-existence of a susceptible host and a competent vector will arise only in suitable environments and ecological conditions, favoring the establishment of a focus of infection [89]. Such disciplines combine disease ecology and landscape ecology to better understand the spatial aspects that can affect epidemiological processes across a disease's geographical range and the spatial interactions involved [95]. Based on this approach, Geographic information systems (GIS), remote sensing, and spatial statistics (cluster identification, hotspots, space-time interactions) are tools that are used to analyse and integrate the spatial component in epidemiology of VBD into research, surveillance, and control programs, as this arboviral infections exhibit spatial patterns that arise from underlying variation in environmental conditions that can be shown on maps [92,96]. These techniques describe and analyze the spatial variations of disease occurrence (incidence, cases) and mosquito and human distribution, taking into account different variables (Figure 4). For example, by using these techniques, it is possible to identify villages at high risk for VBD transmission (malaria, dengue, chikungunya, Zika) and correlate this with variables of different nature that may be enhancing disease transmission, such as climatic (precipitation, temperature), socio-economic (poverty, lack of public services, living conditions), behavioural (human movements), and demographic variables (population density) [97]. Furthermore, these techniques may help the prediction of dengue epidemics at different spatial scales [98].

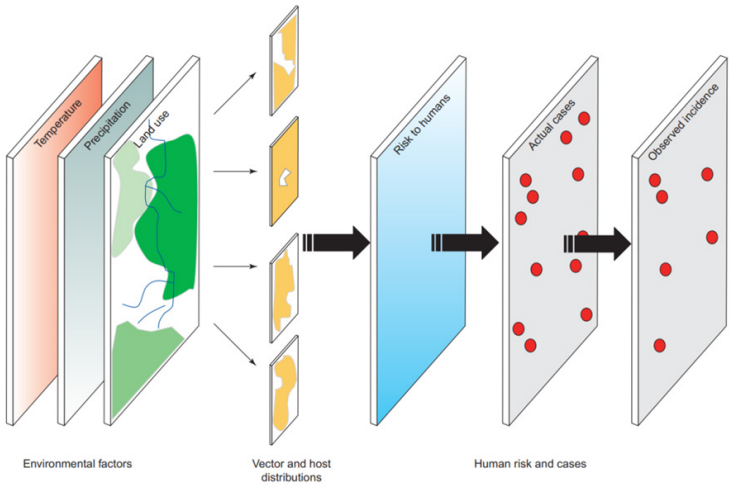

Figure 4.- Conceptual model of the relationship between environmental factors that influence disease and observed incidence of that disease in humans. Source: Ostfeld *et al.*, 2005 (http://www.cell.com/trends/ecology-evolution/fulltext/S0169-5347(05)00071-6). Reprinted from [68] with permission from Elsevier.

Arboviral diseases: a multifactorial disease complex

Dengue is a complex disease due to the intricate interaction between the elements involved in its transmission: human host, the mosquito vector (*Ae. aegypti*) and the virus. Within a suitable environment this interaction is more likely to favor disease establishment. The transmission patterns of dengue are influenced by several factors as shown in Figure 5. These factors modulate the transmission dynamics of chikungunya as well. In such complex interactions, it is likely that changes in the bionomics of one of these disease components will have an important impact on disease transmission. For example, in dengue, serotypes (and genotypes) may differ in virulence, which may affect the degree of disease severity [99]. On the other hand, changes in mosquito density may influence disease transmission patterns, since the relationship between mosquitos and susceptible host is important in determining the infective biting rate and transmission of arboviruses such as dengue and chikungunya [100].

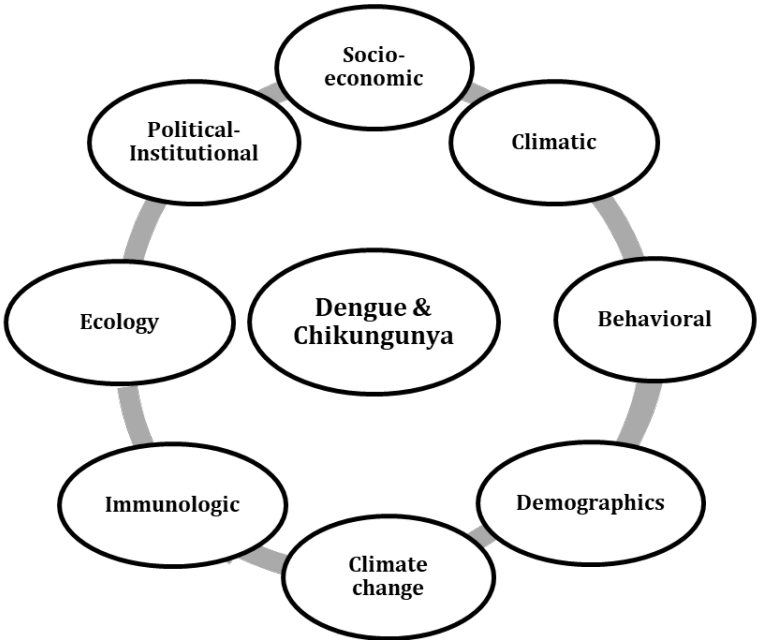

Figure 5.- Main factors associated to the epidemiology of dengue, chikungunya and other related *Aedes*-borne diseases.

Based on the above, we explain here some of the factors involved in the increase of the incidence of arboviral diseases worldwide:

1) Uncontrolled urbanization: One of the main determinants of VBD transmission is population growth. The rapid migration of people to cities can lead to overcrowding and can generate slums-like neighbourhoods. Nowadays, population density had shown an accelerated growth, that causes public services and sanitation programs to lag. In such conditions, poverty-related socioeconomic factors arise, which had been pointed as risk factors for dengue and chikungunya disease [54]. This is because in such settlements, services like garbage collection and running water are deficient or not available, favouring mosquito breeding sites [18]. The latter is related to the domestic behaviour of *Ae. aegypti*, with a short flight range that does not exceed 100 meters if the conditions around it are given for oviposition, therefore, contributing to the overall persistence of DENV and other *Aedes*-borne diseases in endemic regions [101].

2) Globalization and human movement: At large geographical scales (e.g., continents), the constant movement of infected human hosts, enhanced by the intense communication by air and land travel, has determined that arboviruses reach regions that were previously unaffected showing, like dengue, a notorious geographical expansion in the last 50 years. At small geographical scales, transmission is powered by social connections due to routine movements (e.g.: work, high-school, university, relatives and neighbours houses) allowing the virus to disperse among these common places [102].

3) Vector control and inefficient public health policies and disease surveillance: In the absence of available commercial dengue vaccines or antivirals, the control of dengue transmission is limited to the application of mosquito control measures. Nonetheless, vector control of *Ae. aegypti* have proven to be unsuccessful in some areas of high risk for mosquito-borne diseases [18], probably due to the rigor with which it is necessary to apply and maintain the vector control measures. The ideal panorama would be one where vector control measures are used for prevention instead of applying vector control measures after the occurrence of dengue cases. There is evidence that fogging after or during the outbreak has little impact on the spread of the disease [103]. Nevertheless, efforts to create an integrated framework for dengue prevention are currently being discussed. These would look for the best combination of the current vector control measures with novel techniques that are under development, and together with the reinforcement of public services, education programs and vector-source reduction campaigns [76,104].

4) Climate and climate change: Vector-borne diseases are especially affected by climate. Climate together with socio-economic factors and human density exert a strong influence in the increase of DENV and CHIKV transmission rates. The strongest climate drivers of these mosquito-borne diseases are rainfall and temperature, factors that affect the ecology of *Aedes* mosquitoes and the virus. Additionally, some studies have associated the DENV periodic outbreaks with global inter-annual climatic variations such as El Niño Southern Oscillation (ENSO) [80,88,105]. Temperature and rainfall influence mosquito reproduction and mortality rates, the blood feeding frequency of the *Aedes* female and the EIP of the virus which in turn, determine the degree of human exposure to that infection (Figure 6). This does not exclude the great implications that immunological and socio-economic factors have on disease transmission [81,106].

A weather forecast is an added value to *Aedes*-borne diseases surveillance. Since climate variables directly affect the survival rate of mosquitos, it is possible to predict major changes in weather and

relate them with changes in dengue transmission, which could lead to the design of a climate-health preparedness program [22,107]. Climate change may also affect the geographic range and incidence of dengue through effects on human and natural ecosystems, such as water storage, land use, and irrigation [22]. Beyond that, prospective niche models have predicted that under the climate change scenario, the geographic expansion of *Ae. aegypti* to new regions is highly likely, since the vector will overcome the geographical dispersal barriers and establish itself in new areas [101,108].

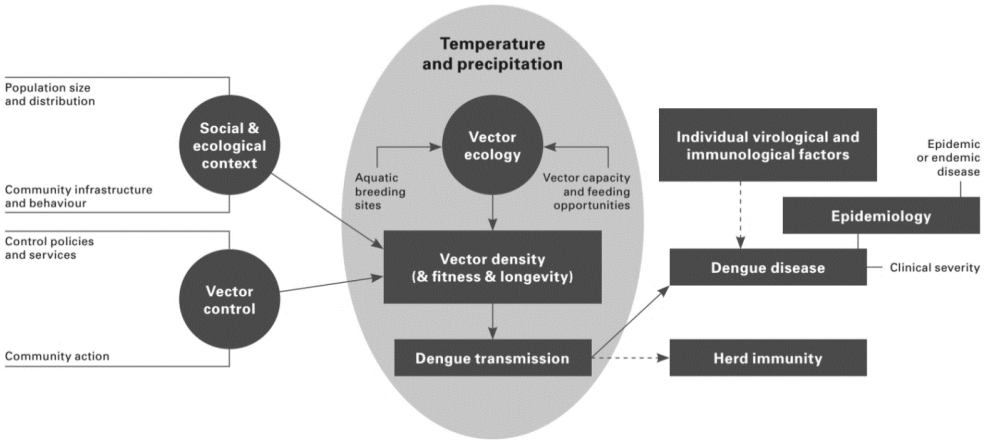

Figure 6.- Interaction of meteorological and other determinants of dengue transmission cycles and clinical disease. Source: World Health Organization and World Meteorological Organization (2012). Available at: http://www.who.int/globalchange/publications/atlas/report/en/

To overcome the difficulties in dengue and other mosquito-borne control, some techniques and policies can be applied. One approach would be the coupled actions of the classical approach of epidemiology, public health surveillance tasks, with the application of spatial epidemiology and climate preparedness. This approach may allow the rapid and efficient identification of areas of disease risk, and the timely application of prevention and control tasks.

SCOPE OF THE THESIS

The research described in this dissertation attempts to identify and characterize determinants of dengue and chikungunya disease and transmission relevant for surveillance and control. The investigation focuses on the dynamics and epidemiology of DENV and CHIKV in highly endemic areas of northern Venezuela, with special emphasis on **i)** the spatial and spatio-temporal patterns of disease prevalence/incidence and their determinants, **ii)** the influence of climate fluctuations on temporal patterns of dengue and **iii)** the effect of human population movement as an important driver of chikungunya and dengue dispersion. To complement the main research, other factors influencing dengue disease control were characterized such as the individual's health-seeking behavior; preferences in healthcare center access; knowledge, attitudes and practices; and predicting factors for dengue versus other febrile illnesses (Addendum).

Main Research

This section presents the core chapters of this dissertation. Here, we explore the advantage of applying hotspot identification to better understand the underlying risk factors that favor the persistence of areas of high dengue prevalence (**Chapters 2 & 3**). Furthermore, the different spatial transmission patterns at greater geographical levels (**Chapter 4**) and the relationship between climate variables and dengue (**Chapter 5**) were also investigated. Finally, we characterize the introduction of chikungunya virus in Venezuela and its explosive spreading patterns (**Chapter 6**).

***Chapter 2.- Spatial Analysis of Dengue Seroprevalence and Modeling of Transmission Risk Factors in a Dengue Hyperendemic City of Venezuela*:** In this chapter, we investigated if dengue cases (defined as serologically positive individuals) were spatially aggregated (hot spots) and if there were socio-economic and behavioral factors determining this clustering. If so, targeting the identified hotspots and their associated risk factors would result in more cost-effective surveillance and control measures. To this end, we spatially stratified dengue seroprevalence in three selected neighborhoods with high reported dengue incidence. A prospective community-based cohort study was set up, and 2,014 individuals in the neighborhoods of Caña de Azúcar (sectors 1 and 2), Cooperativa, and Candelaria in Maracay were recruited in the year 2010. The identification of hot spots (at block and household level) of dengue seroprevalence and the risk factors associated with these clusters were performed using local spatial statistics and a regression modeling approach, respectively. Focalizing dengue control measures during epidemic and inter-epidemic periods to disease high risk zones at focal levels may significantly reduce virus transmission in comparison to random interventions.

Chapter 3.- Dengue inapparent infections and space-time analysis of dengue seroprevalence in Maracay city, northern Venezuela: Based on the results from **chapter 2**, we hypothesized that dengue hotspots persist in time and space, making these clusters ideal targets for dengue control. Therefore, this study focalized on the identification of space-time clusters and disease hotspots of recent dengue infections during a period of 4 years in three previously selected neighborhoods described in **chapter 2**. Given that a proportion of these dengue cases were inapparent infections, we also wondered to what degree hotspots were constituted solely of inapparent infections. Dengue seroprevalence was determined in all surveys using a capture dengue IgM enzyme-linked immunosorbent assay (ELISA). In surveys 1 and 3, seropositive individuals with no recollection of having had fever or other symptoms suggestive of in the previous three months, were considered inapparent dengue cases. Hot spots at household and block level were identified using local spatial

statistics. The results derived from this prospective community-based cohort study (**Chapters 2 & 3**) may show a clearer panorama regarding the space-time distribution and socio-economic determinants of dengue transmission in this area of study.

Chapter 4.- Spatial heterogeneity and persistence of dengue incidence in the north central region of Venezuela: Understanding the spatial heterogeneity of dengue is crucial for control because it allows to reveal some of the factors underlying such spatial spread pattern and help to understand how the infection moves in space and time**.** Previously, in **chapter 2 and 3**, we explored the spatial determinants for dengue transmission at local level (household, blocks, neighborhood). In **chapter 4**, we aimed at testing the hypothesis of a geographical and temporal persistence of dengue at higher spatial levels and identifying possible factors related to this persistence. Using national and regional surveillance data, we first identified space and space-time clusters of dengue incidence, and analyzed the persistence of dengue by civil parish. Additionally, a phase analysis was performed to explore which regions tend to lead and first show an increase in dengue cases reporting. Later, we explored which disease determinants may be related with the particular transmission disease pattern that was found in the areas under study.

Chapter 5.- Warmer temperatures produced by El Niño promote periodic major outbreaks of dengue in Venezuela: In Venezuela, the tendency of dengue epidemics to increase in size and frequency in the last decades are of particular concern. To ascertain this observation, we first quantified the periodicity of dengue incidence in time-series of data to search for particular annual (short-term) and inter-annual (long-term) cycles. We then posed the question of the possible influence of climate variability on temporal changes of dengue transmission. To answer this query, we analyzed the association between annual and inter-annual cycles of dengue with climate variables (minimum and maximum temperatures and precipitation) and El Niño Southern Oscillation (ENSO). Understanding the inter-annual variability in the dynamics of DENV can provide useful insights for disease programs and allow the development of more integrated surveillance and early warning systems to predict disease risk in response to changes in climate.

***Chapter 6.-Spatial dynamics of chikungunya transmission in Carabobo, Venezuela: The first six months of the epidemic*:** In 2014, chikungunya caused one of the most explosive epidemics ever reported in Venezuela (and in the Americas). In **chapter 6**, we describe and quantify the spatial and temporal events following the introduction and propagation of chikungunya into an immunological naïve population from the urban north-central region of Venezuela during 2014. To achieve this, the general spatial trend of chikungunya cases across the study area was developed using Trend Surface Analysis (TSA), a global surface fitting methodology, while the space-time clusters of chikungunya transmission were analyzed using spatial statistics. Understanding the introduction and propagation range in space and time of the initial epidemic wave of CHIKV within the complex urban landscape setting of Venezuela will give us important knowledge on the dynamics of new arbovirus and help manage future threats of new or emerging diseases operating under similar epidemiological conditions.

Addendum: Additional Research

As mentioned earlier, the transmission of dengue is determined by several factors that act together. Consequently, the efforts to control diseases such as dengue and chikungunya, include several disciplines such as epidemiology, sociology, ecology, medicine and immunology among others that aim to answer the most urgent matters related to the prevention, control and medical effects

of arbovirosis like DENV and CHIKV. The Addendum encompasses chapters and research performed in collaboration with peers belonging to the same research group. These topics explored first, the behavior towards health seeking (**Chapter 7**), attendance to healthcare centers (**Chapter 8**) and the Knowledge, Attitudes and Practices (KAP) concerning dengue (**Chapter 9**). Secondly, the identification of parameters to differentiate between dengue and other febrile diseases at the early stage of the disease was also explored (**Chapter 10**).

Chapter 7.- Health Seeking Behaviour and Treatment Intentions of Dengue and Fever: A Household Survey of Children and Adults in Venezuela: In this chapter we analyzed the patterns of health seeking behavior (HSB) in individuals exposed to high dengue incidence aiming to improve early attendance to health centers and medical care by the individuals suspecting dengue disease.

Chapter 8.-Accessing Healthcare in Venezuela: a Community based Study on Health Centre Preferences in the Case of Dengue and Fever: Accessing healthcare in Venezuela can be nowadays a very complex situation. Patients tend to visit different health care centres seeking for quality in medical attendance and supplies availability. In order to improve the understanding of such complicated situation, this study focused on the assessment of the intended health care attendance and perceived motivations/barriers for access to care in the case of fever and dengue in a high dengue transmission urban area.

Chapter 9.- Knowledge, Attitudes and Preventive Practices regarding Dengue in Maracay, Venezuela: Dengue is a viral mosquito-borne disease and it is widespread throughout tropics and sub-tropical areas, affecting more than 100 countries, with an estimated number of annual infections of 390 million. Risk factors of this disease are influenced by variations in climate, urbanization and quality of vector control measures. Specifically, in vector control, social mobilization and community behavioral changes are of crucial importance. In order to improve dengue control of communities exposed to endemic dengue transmission, we identified the factors influencing community dengue preventive practices by describing Knowledge, Attitudes and Practices (KAP) concerning dengue, and investigating determinants of personal protection against mosquitoes and mosquito breeding site elimination.

Chapter 10.- Decision Tree Algorithm that differentiates dengue from other febrile illnesses at the early stage of the disease: The acute phase of dengue begins with fever and non-specific symptoms that are frequently indistinguishable from the initial phase of other febrile illnesses (OFI). For secondary dengue infections, patients are more likely to progress to dengue with warning symptoms or severe dengue if early care and precautions are not taken in a timely fashion, making dengue early differential diagnosis, one of the important steps in dengue disease management. In this chapter we focused on the identification of parameters that could differentiate dengue from OFI at the early stage of the disease resulting in a decision-tree algorithm that uses clinical features and routine laboratory tests.

Chapter 11.- Summarizing Discussion: Summarizes the results and discusses the most relevant conclusion of this thesis.

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