**TWENTY YEARS OF DENGUE SURVEILLANCE AT A MINISTRY OF HEALTH HOSPITAL IN KAMPHAENG PHET, THAILAND, 1994-2013**

by

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**ABSTRACT**

Dengue virus (DENV) is a vector-borne viral disease transmitted by the *Aedes aegypti* and *Aedes albopictus* mosquitos. The majority of dengue cases present as asymptomatic to mild febrile illness. Only a small percentage of cases manifest more severe symptoms. Currently, dengue is endemic in over 100 countries, and five different continents, making about 40% of the world’s population at risk for dengue transmission. This represents a global public health risk.

Long-term observational studies provide valuable insights into overall dengue epidemiology. This paper presents an analysis of dengue cases presenting to a Ministry of Health provincial hospital in Kamphaeng Phet, Thailand during the twenty-year period from 1994-2013. Data was analyzed from 12,200 hospitalized patients with suspected DENV infection and confirmed by Nested PCR and enzyme-linked immunosorbent assay (ELISA).

Exploratory data analysis was performed and several long-term trends of dengue disease were identified. Chief among those findings was an increase in mean age for both primary and secondary infections. Co-circulation of multiple serotypes in multiple years was also found.

Thailand is an important setting for studying DENV transmission due to the hyper endemic status of dengue, and the presence of a strong surveillance. Further research within Thailand is needed to continue to understand the relationship between geography and dengue incidence. These results may increase understanding of dengue in other countries with similar changes in transmission and population demographics that may now or soon be occurring.

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**1.0 INTRODUCTION**

The purpose of this essay is to evaluate trends of dengue incidence over time in a hospital in Kamphaeng Phet, Thailand. The goal of this was to identify information for this region of Thailand to see if there was anything unusual. There have been many different studies looking at Bangkok over the years, but there has not been a study looking at a rural area in Thailand for over 10 years.

* 1. **Dengue**

Dengue virus (DENV) is the causative agent of dengue disease1. Dengue is a global tropical disease that has the capability to affect half of the world’s population, because they live in areas where the vector lives. Due to dengue’s wide spread and ever-changing dynamic, it is of global health concern. While dengue disease is not new, today’s ever changing world with its increasing globalization and travel gives it new opportunities to expand its reach and increase infections, giving it a new resurgence and new importance on the global agenda. One recent case in point is the 2015-2016 outbreak of Dengue on the big island of Hawaii, a location not previously affected by dengue for years.

**Virus**

Dengue, Yellow Fever, West Nile, and Japanese Encephalitis (JEV) all belong to the family of viruses known as *Flaviviradae,* genus *Flavivirus1.* DENV a positive single-strand RNA virus12. Its genome is approximately 11 kb in length12. The reading frame has three structural proteins and seven non-structural proteins12.

DENV has four antigentically distinct serotypes (DENV-1, -2,-3,-4)1. The four serotypes share major antigens within the disease, as well as between 62-67% of their genomes7. This is approximately the same degree of relatedness that different West Nile virus shares with Japanese encephalitis12. Due to the four different serotypes causing almost identical symptoms and circulating in the same ecological niche, they are considered the same disease12.

There is ample evidence that suggests that dengue was originally found in monkeys. It was thought to be a part of the sylvatic transmission between non-human primates and mosquitoes12. Forest-dwelling *Aedes* mosquitos have been identified as vectors in the jungles of both Africa (DENV2) and Southeast Asia (DENV1-4), involving mainly non-human primates7. The vectors there are phylogenetically distinct from the urban *Aedes* mosquitos, although these sylvatic strains of DENV may rarely cause disease in humans7.

DENV is thought to have made the jump to humans in the vicinity of the Southeast Asian jungles. Research done in the 1950s, demonstrated high levels of anti-DENV antibodies in both rural humans and non-human primates, even with the disease absent. The antibodies were found to be present in 50% of rural children in Malaysia, compared to only 3-9% in children in urban cities in the region like Singapore or Kuala Lumpur. This is worth noting because at that time, *A.aegytpi* was only found in urban, costal cities, and *A.albopictus* was found in coastal and inland rural areas.

Using molecular techniques, the progenitor of today’s current DENV serogroups are thought to have evolved about 1000 years ago7. Analysis of phylogenetics of both sylvatic and human strains suggest that each serotype jumped from non-human primates to humans separately from different sylvatic ancestors. Emergence of DENV serotypes in humans is thought to have happened relatively recently, about 125-320 years ago depending on the serotype7. Most phylogenies have DENV4 as the most divergent genome from the other serotypesand have DENV2 as the second most different serotype7. DENV1 and DENV3 have been found to be the two serotypes most closely related7.

Although the four serotypes of dengue are classified as one disease, there are large differences in virulence and severity. DENV4 is usually seen as the most clinically mild serotype, with the smallest percentage of cases having severe disease7. DENV2 and DENV4 are seen as having increased severity of disease if they are secondary infections7. DENV1 and DENV3 are thought of to cause more severity in primary infections then DENV2 or DENV47. In general, DENV2 is thought of as the serotype most associated with DHF, followed by DENV1 and DENV37.

**Epidemiology**

Dengue is one of the most common vector-borne diseases found in humans. It is thought of as the most important international arboviral disease. Although it is seen as a global disease, numerically, there are disagreements about the frequency of dengue in different populations. According to the World Health Organization (WHO, 2012), an estimated 390 million people in 128 countries are at risk of dengue viral infection2. Another study states that 390 million infections happen each year, with a 95% confidence interval of 284-528 million2. Of those 390 million infections, 96 million will have some clinical symptom of the disease, with a confidence interval of 67-136 million2.

Even with dengue being found in all WHO regions, only member states in 3 regions-the Americas, South-East Asia, and Western Pacific- annually report their case numbers. In 2008, the regions had over 1.2 million cases total, almost 2.4 million in 2010, and over 3 million in 2013. 2.35 million cases of dengue were reported in the Americas alone in 2013. Three million is 3.125% of the 96 million with clinical symptoms and 0.769% of the of the expected 390 million infections each year. These numbers are gross underestimates of the burden of dengue, mostly due to incomplete reporting systems of several countries. The lack of reporting, and lack of seeking medical care keep country reporting numbers low.

Dengue is an infectious disease, and like many infectious diseases, the true disease burden on populations from dengue are hard to find and quantify. Dengue is often misdiagnosed, as its initial clinical symptoms are difficult to distinguish from other viral febrile diseases3. Reports put 50-90% of dengue cases are subclinical or asymptomatic5,7. People infected either show no symptoms or their symptoms are so mild or non-differentiable from other febrile illnesses that people do not think to seek out medical care4,5.

Reporting of dengue is also hindered by the differences in national health systems3. Outbreaks that happen in remote areas are less likely to be counted, because of the lack of health care in rural areas3. When health facilities exist, the facilities might have poor case management or no laboratories to correctly diagnose or confirm dengue infection4.

Historically, dengue has been known to authors for centuries. The first mention of dengue was found in a Chinese medical encyclopedia from 992 A.D., originally published by the Chin Dynasty (265-420 AD) centuries earlier then being formally edited later on6,7. In this encyclopedia, the dengue-like symptoms were referred to as ‘water poison’ whose spread was associated with flying insects6. In 1635, the West Indies had an epidemic that simulated dengue in the disease course and spread. Central America also had a similar epidemic in 16996. From 1780 in Philadelphia until 1945 in New Orleans, dengue epidemics were typical in the United States6.

For many years, dengue was spread to other locations worldwide through shipping vessels6. The shipping vessels allowed the primary mosquito access to transportation and humans for transmission, allowing the mosquitos and virus to slowly be introduced to the world6. During World War II, dengue rapidly expanded globally, as well as after, being facilitated through the movement of military troops. More soldiers then ever before were being dispersed inland into the jungle instead of staying on the coast6. They were utilizing modern transportation within and between countries, bringing the disease to areas it had never reached before6. Due to the rapid transportation, rapid urbanization, and population growth in post-World War II, South East Asian countries soon became hyperendemic for dengue6.

Despite the fact that dengue has been around for centuries, technical advancements did not allow for the laboratory isolation of DENV until World War II7. When it was first found, only two serotypes were identified, named DENV1 and DENV2 respectively7. Serotypes three and four were found when the urban centers in the Philippines and Thailand were faced with a severe dengue outbreak in 1953-19587,8. Due to the majority of large DHF outbreaks happening in cities, it has come to be thought of as a primarily urban disease8.

Over the last forty years, dengue has become a global concern. Before 1970, there were only nine countries that had experienced severe dengue cases6. Today, there are over 120 countries2,6,7. It is an expensive burden, both in labor and economic costs. Estimates of Disability Adjusted Life Years (DALY) due to dengue vary, as the main burden of the disease lies with children.

A 2009 estimate puts a global figure of 700,000 DALYs lost due to dengue annually6. A study done in 2005 examined the costs of dengue, in days hospitalized, work days lost, and financial costs in eight countries across Asia and the Americas 9. The study found that for patients who were able to ambulate, their average length of illness was 11.9 days, 5.9 of those days with fever9. For those hospitalized, average illness length was 11.0 days9. If the patient was a student, they lost 4.2 days of school if they were ambulatory, compared to 5.6 if they were hospitalized9. For people working, patients lost 6.6 days of work compared to 9.9 if they were hospitalized9. Unweighted costs per case were $514 2005 International dollars for ambulatory patients9. For hospitalized patients, it was $1,3949. Weighted by the official numbers of deaths and cases by setting, the mean cost of a dengue reported case was $1,031 in the eight countries9. However, there was a significant difference between the two continents. In Asia, the cost of a reported case was $2,005, compared to $759 in the Americas9. This is a 2.7-fold difference in cost9.

Unlike South East Asia, the Americas appeared to have dengue under control following World War II. During the 1940s, the Pan American Health Organization (PAHO) started a yellow fever disease control campaign6. Due to a large mosquito elimination as part of the campaign, dengue was restricted in transmission throughout the continent6. The yellow fever campaign lasted until the 1970s.6 However, subsequentially, the incidence of dengue increased throughout the 1980s, bringing levels of dengue in the Americas back up to pre-campaign numbers in 19956.

Even though there was a lull of dengue transmission from the 1940s to 70s, dengue in the Americas today is increasing. Presently, almost all countries in the Americans have all four serotypes circulating with indigenous dengue transmission6. The only two countries without indigenous transmission are Uruguay and Chile4. Along with the hyper endemicity, the number of cases of DHF have increased more than eightfold4. Local transmission in the United States has been found, particularly in Florida and Hawaii6,10. Since September of 2015, Hawaii has been having an outbreak of locally-acquired dengue on the big island of Hawaii10.

Dengue has not been a problem in Europe for decades, with the last reported outbreak being in Greece between 1926-284,6. No dengue transmission was reported in the region until the 1990s6. Presently,evidence of local transmission has been noted in France and Croatia4. Further, mosquito vectors are found in large parts of southern Europe. In 2012-2013, a major outbreak occurred on the island of Madiera4,6. During the summer of 2014, 370 confirmed cases of both dengue and chikungunya were reported in mainland France4.

The impact of dengue in the African continent is unclear, largely due to inconsistent reporting by countries4,6. Despite the lack of reports, there is evidence to suggest that dengue outbreaks are increasing in size and frequency6. There was a large dengue outbreak in 2009 in the Cape Verde region6. There is evidence that from 1960 to 2010 that 22 African countries had either sporadic cases or outbreaks6. In a 2011 study, disease transmission was found to be endemic in 34 countries, with all four serotypes being present4,6. With malaria being such a significant illness in Africa, over 70% of ‘febrile illnesses’ are likely to be misdiagnosed and treated as malaria6. The commonality of multiple febrile illnesses along with lack of good diagnostics most likely contribute to infrequent diagnosis of dengue4.

In the WHO Eastern Mediterranean region, large epidemics have been reported since the 1990s4. Only three of the four serotypes have been reported in the region4. Smaller outbreaks with multiple serotypes of DENV are being reported more frequently6. A major risk factor for these epidemics if the increased travel and trade in the region, with large immigrant work-forces and pilgrims to holy sites4. As urbanization increases, so do outbreaks. In Saudi Arabia, there were 4,411 dengue cases in 20134. This was a fourfold increase from 20124.

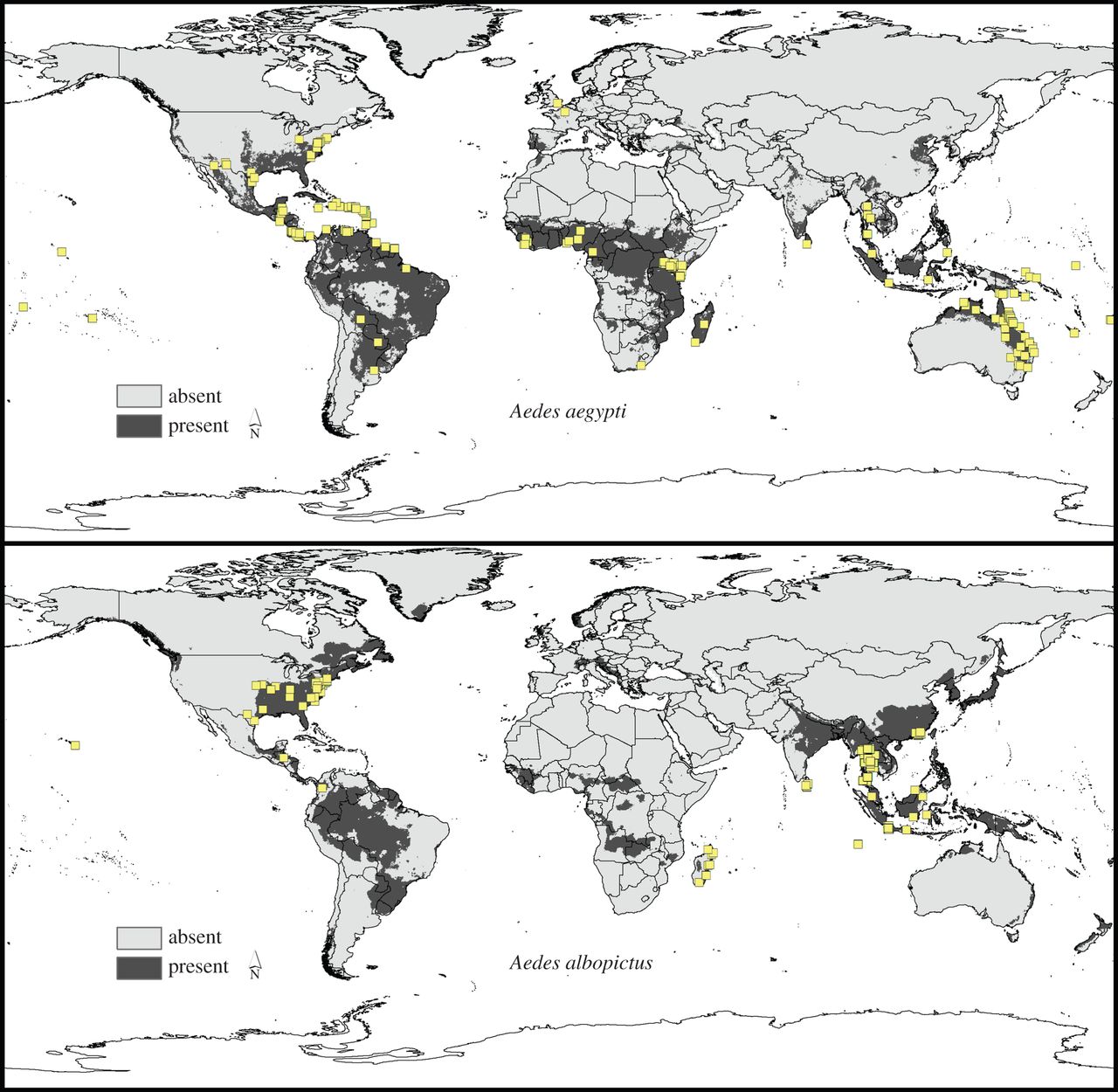
The Western Pacific region accounts for a large proportion of the global dengue burden6. After a 25-year absence, two dengue serotypes were reintroduced to the Pacific Islands from the Americans in 1964 and 19716. The following decade had all four serotypes from Asia gradually introduce themselves to the population6. Due to the mixing of serotypes from both America and Asia, the island nations in the Pacific show a particular susceptibility to dengue and severe dengue outbreaks6. Over the past decade, the number of reported cases has continued to increase in the Western Pacific. In the Asian sub region of the Western Pacific, all four serotypes have been found in these high disease-burden settings. In the Pacific sub region, 91% of cases come from French Polynesia, New Caledonia, Vanuatu, and Australia.

Of the 3.9 billion people exposed to dengue, 75% live in Asia-Pacific2,6. 1.3 billion or a third of the total dengue exposed population, live in ten dengue endemic countries in Southeast Asia6. In the region, dengue is a leading cause of hospitalization and death in children6,8. In 2009, all countries except for the Democratic People’s Republic of Korea had indigenous dengue cases6. Epidemics occur regularly on 3-5 year cycles, with the number of cases and their severity increasing6,8. Eight countries have hyperendemic levels of dengue, and most have severe dengue as endemic as well6. Rates of severe dengue are 18 times higher in the Southeast Asia region compared to the Americas region6.

**Vector**

The *Aedes* mosquito is the vector for dengue. The virus is spread principally by the *Aedes aegypti7. A.aegypti* is believed to originate from Africa7. It spread globally through the use of slave and trading ships from the seventeenth to nineteenth centuries7. In Asia and other parts of the world, dengue epidemics were found to correspond with the spread of *A.aegypti7*. Epidemics and mosquitos first appeared in port towns and slowly made their way inland via waterways7. It is now a domesticated mosquito. It prefers to lay eggs in artificial containers, stay inside, and bite humans instead of animals7.

The secondary vector is *Aedes albopictus,* another urban dwelling mosquito*7.* Even though *A.aegypti* is the driver of dengue, *A.albopictus* is the superior larval competitor11. *A.albopictus,* or the Asian tiger mosquito, orginates from Southeast Asia12. Due to global travel and trade, this mosquito has spread to all continents but Antarctica12. It has a global distribution that is a little more north then *A.aegypti* as seen in Figure 111.

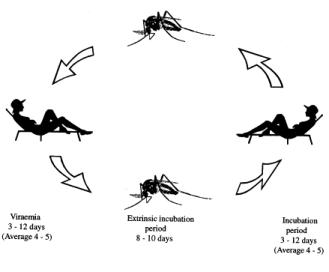


**Figure 1. Present Mosquito Distribution**

**Top: *Aedes aegypti* Bottom: *Aedes albopictus***

Mosquito distributions are highly dynamic and respond quickly to new opportunities. New opportunities can come in the form of overcoming dispersal barriers to colonize new areas, such as hitching rides in shipping vessels11. Other opportunities are ready expansion into new areas as conditions positively change for mosquito biology, which is happening as climates change11. Finally, there are dynamic interactions and potential competitive exclusion between the two species. In cases such as Australia, *A.albopictus* has been introduced, but not established in four states11. Meanwhile *A.aegytpi* has been established in Australia11.

The urban dwelling of the *Aedes* mosquitos is a large part of the DENV transmission. The virus is not transmitted person-to-person, but vector-to-person-to-vector2. There are no important intermediate hosts. Those people who become infected are both primary carriers and viral multipliers2. When a person is experiencing viremia, or high virus load, and is bitten by a mosquito, the mosquito may become infected for life2. Viremia typically happens four to five days after infection, but can last up to twelve days2. The infected mosquito spends 4-10 days in an incubation period with the virus2. After the period, it will bite a new host and will continue the transmission cycle (Figure 2)2.



**Figure 2. Dengue Transmission Cycle**

**Clinical**

In 2008, the WHO stated that “dengue is one disease entity with different clinical presentations and often with unpredictable clinical evolution and outcome”13. This statement highlights that dengue is difficult to identify in clinical-based surveillance systems. Dengue infections present with a wide spread of symptoms and clinical presentations that range from asymptomatic to self-limiting febrile illness to severe dengue12.

Dengue is a self-limiting disease, meaning most of the time it will resolve itself with or without specific treatment, and has no long-term harmful effect on a person’s health13. Usually, dengue will only last a week, but, in some populations, the symptoms may stay longer. After an incubation period of 4-7 days, the illness begins and is followed by three phases: 1) febrile, 2) critical, and 3) recovery13,14.

The febrile phase is the initial phase, and usually lasts 2-7 days. The onset is marked by a sudden high-grade fever, and if often accompanied by flushing, rashes, fatigue, severe joint and muscle pains, and severe headaches14. Some patients have symptoms in the gastrointestinal area with anorexia, nausea and vomiting13. A full blood count should be done at the first visit to check the hematocrit, or red blood cells, levels and platelets in the blood13. Low platelets and high viremia are present during the febrile stage.1,13

The critical phase starts when the fever breaks, usually on days 3-7 of illness. Progressive white blood cell reduction happens followed by platelet reduction13. The blood capillary walls increase in permeability, shown with rising hematocrit and decreasing platelet levels13. For 24-48 hours, a period of clinically significant plasma leakage occurs, and then resolves13. Depending on the degree of plasma leakage, fluid buildups between the lungs and chest as well as the abdomen can be detected. In a small percentage of cases, symptoms may progress to internal bleeding and shock, which are hallmarks of severe dengue, DHF, and dengue shock syndrome (DSS)13.

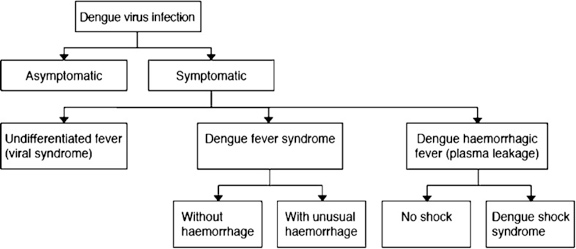
Diseases progression depends upon how patients do with capillary permeability. If the patient survives the critical phase, they move on to the recovery phase. This phase is marked by the gradual reabsorption of fluid lost during capillary permeability for 48-72 hours13. General well-being and appetite improve, gastrointestinal symptoms stop, and blood levels return to normal13. If too many intravenous fluids have been delivered, respiratory distress can happen in either the critical or recovery phase, and is associated with pulmonary edema or congestive heart failure13.

If the patient does not do well in the critical phase, they can progress to severe dengue. Severe dengue is defined by one or more of the following: 1) plasma leakage that may lead to shock and/or fluid accumulation, with or without respiratory distress 2) severe bleeding 3) severe organ impairment13.

Shock happens as dengue vascular permeability increases and the amount of liquid in the blood decreases during the critical phase after the fever breaks. Warning signs usually come before this happens. Initially the patient will have a normal systolic and rising diastolic blood pressure, increased heart rate, cold extremities and delayed capillary refill time do to constricted peripheral blood vessels. The patient is usually conscious and lucid. Later, both pulse and blood pressure suddenly disappear. The prolonged shock and oxygen deficiency can lead to multi-organ failure.

Major bleeding is usually associated with profound shock. This is because there is a combination of shock, oxygen deficiency, rising blood acidity, and platelets decreasing13. This then leads to multiple organ failures and abnormal clumps of blood clots inside blood vessels using up clotting factors so that bleeding can happen elsewhere. The only way major bleeding would not be associated with shock is if an infected person took aspirin, ibuprofen or anything other then acetaminophen13.

In 2009, the WHO revised its case definitions of dengue. Earlier case classifications had dengue fever (DF), DHF (grades 1 and 2) and DSS (DHF grades 3 and 4)(see Figure 3) 13,15. The top figure shows the classification system from dengue viral infection to different components of DF, and DHF. The bottom figure highlights the signs and symptoms and laboratory tests for DF and DHF, and the different stages of DHF. The main difference between DF and DHF were the presence of thrombocytopenia with concurrent haemoconcentration. There were numerous limitations of this classification system. First, it was based on Thai children, which was not universally representative of all dengue patients, especially as more adults and adolescents are stricken with dengue. Second, the classifications required repetitive clinical tests, which can be difficult for cash-strapped countries to perform15. Finally, the tourniquet test was integral to the case classifications, but didn’t effectively determine between DF, and DHF, as well as dengue and other febrile illnesses15.

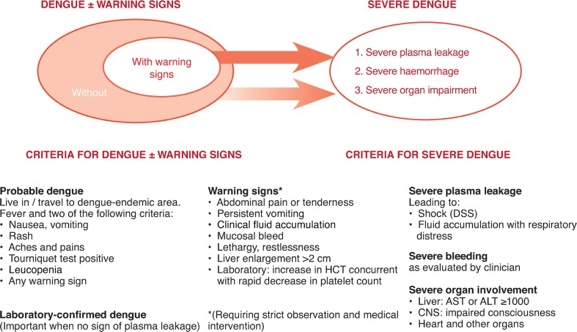




**Figure 3.** **1997 WHO Classification of Dengue Virus Infection**

**Top: General Classification Bottom: Grades Of DF/DHF/DSS**

As a result, the WHO formed a study to evaluate the limitations of the 1997 criteria in all age groups in Southeast Asia and Latin America15. The results of this review formed the basis of the new 2009 WHO classification system. The new system classified clinical dengue into three different categories based on severity: dengue without warning signs, dengue with warning signs, and severe dengue (see Figure 4)15. The new classification is more sensitive to capture severe disease then the earlier guidelines. A multi-center study showed that even without strict DHF criteria applied, the earlier classification could not classify 14% of cases, compared to 1-6% in the 2009 system15. The new classification is found to be better with triage and clinical management of dengue, reporting during surveillance and endpoint measurements for clinical trials15. There has been some confusion over changes to the classification system. This can be remedied by giving healthcare workers more training in the new system, as well as better dissemination of these criteria.



**Figure 4.** **2009 WHO Dengue Case Classification**

**Primary and Post-Primary Infections**

If DENV infections occur in someone who has not had previous exposure to a flavivirus, they will produce a primary infection. For a primary infection, anti-dengue virus antibodies, initially the IgM class, will slowly evolve1. The molar ratio of IgM to IgG is high, usually greater than 1.8:1.0, for at least three weeks1. During a primary infection, the antibodies will slowly develop to relatively low titers.

There is lifelong immunity from a specific serotype of dengue that has caused previous infection. However, after a short period of cross-protection, infection by different DENV serotypes are possible. A secondary dengue antibody response happens when a person with prior exposure to one more more flaviviruses, is exposed to a DENV infection1. The exposure to other viruses can be through natural infection or immunization. Secondary infections produce IgG antibodies as memory responses1. The antibodies arrive early and rapid rise, usually during the febrile phase1. There is an IgM component as well, and the IgM to IgG ratio is less than or equal to 1.81. There is some thought that the IgM response reflects the number of new epitopes found by the new infecting virus1. People with Japanese encephalitis vaccinations who then are infected with DENV develop more IgM antibodies then someone with a second DENV infection1.

People with secondary, or post-primary, infections with DENV usually have a much more severe disease progression. This is one of the defining factors of dengue. The majority of asymptomatic or mild cases are usually from primary infections. The more severe forms of DF, DHF, and DSS have a higher probability of occurring if a patient is having a second, third or fourth dengue infection with a different serotype. DHF and DSS occur pretty much solely in two groups: children and adults with secondary DENV infections and infants born to dengue-immune mothers.

The main accepted hypothesis as to why this happens is the theory of antibody-dependent enhancement (ADE) of the disease. The ability of virus-specific antibodies to enhance DENV and other flavivirus infections was first recognized *in vitro* in the 1960s16. The model states that anti-DENV IgG-actively acquired from a previous DENV infection or passively acquired from maternal-fetal transfer in mothers- enhances DENV infection of Fc receptor-bearing cells under particular conditions in vivo16. ADE increase viremia and triggers a host immunological cascade that causes more severe disease16. There is also evidence that suggests that the dengue-specific memory T-cells may be reactivated by the new DENV infection17. The T-cell effector mechanisms can expand to high levels, and could result in direct and indirect effects causing increase vascular permeability17.

**Dengue in Thailand**

As in other developing tropical and subtropical countries, Thailand has demographic characteristic and geographical conditions that favor dengue transmission. At present, it is experiencing rapid population growth and rural-urban migration. It also has densely populated urban areas that are suitable for *Aedes* mosquito larval habitats18.

Thailand had its first official case of dengue in 1949, with its first major outbreak of DHF in 1958, in Bangkok19. By the early 1960s co-circulation of all of the dengue serotypes were occurring in Bangkok19. In the late 1970s, dengue was widespread and DHF became the leading cause of hospitalization and death among Thai children18,19. In 1987, there was a ‘mega-outbreak’ of dengue, with over 174,000 cases reported, and an incidence of 325/100,000 per total population, 819 out of 100,000 in children under 15, and 1007 deaths18,19. After the outbreak, cases of dengue were relatively stable until two other large outbreaks, in 1997 and 199818. From 1985-1999, Thailand had an average of 69,000 reported cases per year18. In Thailand, dengue epidemiology is characterized by cyclical epidemic activity.

The epicenter of dengue in Thailand is Bangkok, the largest urban center in the country. Thereafter, dengue spreads spatially out to the rural areas of Thailand. According to a study done by Cummings et al, waves of dengue travel out of Bangkok at a speed of 148km per month20. Of 72 provinces in the country, 65 provinces are either right with or follow Bangkok, in a repeating, spatial-temporal travelling wave20. Depending on how far away a place is, this location might have as much as ten months’ advance notice of a coming dengue epidemic. The only parts of the country that do not correlate well with Bangkok are border regions20. These areas might follow a different epicenter originating from another country.

Thailand started a surveillance system to report dengue starting in 195818. The national surveillance system for DHF started in 1972, DF was added in 1994, initiated by the Bureau of Epidemiology in 1972, later the Thai Ministry of Public Health (MoPH) in 197418. Reports are collected from hospital and ambulatory patients at health facilities across the country. The surveillance system collects information from all government hospitals, and some private hospitals and clinics. All confirmed cases must be reported, but suspected cases are up to the physician’s discretion to report18. The reporting form used in this system collects demographic data –age, sex, day of onset, and locality where occurred-classified municipalities or ‘other/rural’ areas18.

Dengue incidence in Thailand follows patterns of high and low incidence both monthly and yearly. Incidence in the cold months of December and January is low, and starts to increase between April and June during the dry hot months18. Epidemic peaks are usually 2-4 weeks before the rains arrive, which varies across the country, but can be anytime between June and September18. There does not seems to be a relation between the magnitude of epidemics and rainfall, though they do coincide18. The rainy season usually ends in October, but can last into November.

Dengue is considered a disease of the young in Thailand, with most cases occurring in people aged 5-24 years18. This age group represents a third of Thailand’s population. In 2002, the age group with the highest incidence went from the 5-9 group to those ages 10-1418. General shifts have been seen starting in the 1980s of dengue disease that see a switching from young children to people over 1519. Most of the severe cases are found in individuals 5-14 compared to those 15 or older18,19.

* 1. **Objectives**

The objectives of this analysis are to determine what the trends over time for dengue serotypes are for this rural section of Thailand over the twenty years of 1994-2013. There has been no study published about long-term trends in this region before.

* The primary objective is to analyze samples from a provincial hospital in Kamphaeng Phet, Thailand, and determine serotype frequency and how many serotype specific outbreaks occur over time.
* The secondary aim is to look at the type of disease infections type over time, to see if there is an increase in primary cases being hospitalized.
* The third aim is to look at average age of individuals affected by dengue over time. Previous studies in Bangkok have shown an increase in the average age. This analysis looks to see if the results remain consistent in rural areas.
* The fourth aim was to see if there was regression model that might help explain the increase in average age, and the difference in dengue serotypes.

**2.0 METHODS**

**2.1 Sample Selection**

The analysis in this study was approved by the Institutional Review Board of the University of Pittsburgh. This evaluation is based upon a surveillance of dengue in Kamphaeng Phet Province. The 12,200 observations of suspected dengue cases were reported on site in Kamphaeng Phet, Thailand.

Kamphaeng Phet is the main city in Kamphaeng Phet province, in the Northern Region of Thailand. The province is 8,607.49 sq. km large. In the province 5,537 sq. km are used for farmland, 2,027 sq. km are forested area, and 1,043 sq. km are classified as non-agricultural areas21. It has 730,000 people, with a city population of 35,000 in 201421. Almost 600,000 people, or 82% of the population, in the province are over age 1521. Males made up 49% of the provincial population, and females made up 51% in 201321. There are 221,300 households in the provinces, with an average monthly income of 18672 baht ($593)21.

For this study, data were obtained from the public health surveillance samples of hospitalized dengue cases collected from 1994-2013 at Kamphaeng Phet Provincial Hospital (KPPH). KPPH is a 410-bed MoPH secondary level general hospital in Kamphaeng Phet, Thailand, making it the primary hospital for the province. It has twelve different wards, including men, women, and children. In 2013, it served over 380,000 outpatients and over 40,000 inpatients.

Acute and convalescent blood samples were drawn from clinically suspected dengue inpatients at KPPH. These samples were tested for evidence of DENV infection at the Armed Forces Research Institute of Medical Sciences (AFRIMS) laboratories in Kamphaeng Phet and Bangkok.

From 1994-2009, KPPH used the 1997 WHO dengue classification guidelines. The guidelines placed dengue into undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF), and fever that may lead to dengue shock syndrome (DSS). Due to the variability of symptoms, the clinical definition of dengue was not appropriate without laboratory confirmation. Probable DF cases were defined as acute febrile illness with two or more symptoms such as headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations or leukopenia22. Probable DF cases also had supportive serology or occurrence at same location and time as other confirmed dengue cases22.

For DHF, by definition, four symptoms must be present; a fever lasting 2-7 days, hemorrhagic tendencies, thrombocytopenia, and evidence of plasma leakage22. DSS, by definition, must have all four criteria for DHF, plus evidence of circulatory failure22. Provisional diagnosis of DHF/DSS could also come from indicators of high fever of acute onset, hemorrhagic manifestations, hepatomegaly and shock22. While there are four grades of DHF, with grades III and IV being DSS, there was no grading in the analyzed data.

Primary infection is defined as a first DENV infection in an individual. It was determined serologically by nested PCR or ELISA and defined as having an IgM/IgG ratio of greater than 1.8. Post-primary infection refers to any DENV infection after a primary infection. This was also determined serologically and defined by a IgM/IgG ratio of less than or equal to 1.8.

**2.2 Statistical Analysis**

Analysis was completed using SAS as a data organizer and test generator. Chi-square, regression, and ANOVA analysis was used to determine significance for mean age, serotypes, and interactions. For the analysis, cases were assessed overall as all cases collected by surveillance and by two subgroups of cases with nested PCR confirmed dengue and cases with ELISA confirmed dengue.

All cases were analyzed for demographic characteristics using SAS. Samples were categorized by gender, age, serotype, and disease status to determine the characteristics of each of the four groups.

For the first and second objectives of analyzing dengue by serotype, outbreaks and disease infections, frequency tables and chi-square tests were done. Tests were done to test the average mean age increase over five year intervals, for both primary and secondary infections. ANOVA and regression was then used to see if there is a difference in mean age between the serotypes to prove the fourth aim.

Relationships between mean age, disease severity and serotype, and outbreaks of serotypes were found. Regression and ANOVA were also performed to test the interaction of year and serotype on age.

**3.0 RESULTS**

**3.1 Descriptive Epidemiology**

**3.1.1 Demographics**

The demographic characteristics of the study sample were assessed as the first step in the analytic process. Of 12,200 suspected dengue cases, 5398 (44.25%) were tested with nested PCR and 8268 (67.77%) were tested with ELISA. 5193 (42.56%) cases were tested with both, with the number of cases tested for each test being nearly equal most years. Over the twenty years of study (1994-2013), dengue frequency did not differ by gender, with 50.9% of males compared to 49.1% of female cases. There were slightly more slightly more males tested using PCR versus more women using ELISA. The average age of dengue cases for all data was 14.53 years, with a standard deviation of 11.43 years. (Table 1)

Next, the reported cases were examined to identify the specific serotype of dengue involved. Of the cases with serotype information, 35% were classified DENV-1 or DENV-2., 21% were classified as DENV-3 and 8.66% were classified as DENV4.

**3.1.2 Trends Over Time**

Figure 5 shows the breakdown of incidence of dengue by serotype by year. An outbreak was determined by AFRIMS to be a year with over 100 cases or more related to one serotype. Over the 20 years of examination, there were 13 years with an outbreak. DENV-1 and DENV-2 serotypes were identified in seven years of outbreaks, with four related to the two serotypes simultaneously.

DENV-1 was the predominant serotype from 1997-2009. DENV-2 was seen as a co-dominant strain from 2001-2004 and again in 2008-2013. In 2001, a large DENV-1, DENV-2 and DENV-3 epidemic occurred, having over 2000 cases total. In 2013, another large DENV-3 epidemic occurred, with over 1200 cases. The only DENV-4 outbreak was in 2004.

In the seven years without outbreaks, the number of dengue cases ranged from 150-430 per year. This gives a rough idea of what the endemicity of dengue is in Kamphaeng Phet.

**Table 1. Baseline Characteristics of KPPH Dengue Cases, 1994-2013**

|  |  |  |  |
| --- | --- | --- | --- |
|  | All Data (N=12,200) | Nested PCR Data (N=5398) | ELISA data (N=8268) |
| **Gender** |  |  |  |
| Female | 5933 (49.1%) | 2673 (49.6%) | 4091 (51.3%) |
| Male | 6214 (50.9%) | 2722 (50.4%) | 4029 (48.7%) |
| Missing | 53 | 23 | 43 |
| **Age (Years +/- SD)** | 14.53 +/- 11.43 | 13.59 +/- 8.29 | 13.39 +/- 8.64 |
| **IgRatio** | 1.21 +/- 3.66 | 1.40 +/- 4.13 | 1.32 +/-4.14 |
| **Serologic Response** |  |  |  |
| Primary | 417 (3.42%) | 258 (4.78%) | 417 (5.06%) |
| Secondary | 7215 (59.14%) | 4339 (80.38%) | 7194 (87.23%) |
| Unknown | 4568 (37.44%) | 801 (14.84%) | 636 (7.71%) |
| **PCR Result** |  |  |  |
| DENV1 | 1909 (15.65%) | 1909 (35.34%) | 1841 (22.27%) |
| DENV2 | 1885 (15.45%) | 1885 (34.89%) | 1800 (21.77%) |
| DENV3 | 1136 (9.31%) | 1136 (21.03%) | 1103 (13.34%) |
| DENV4 | 468 (3.84%) | 468 (8.66%) | 456 (5.52%) |
| DENVx2 | 4 (0.03%) | 4(0.07%) | 3 (0.04%) |
| NEG | 6172 (50.59%) | 0 | 2787 (33.71%) |

**Figure 5. Serotype Over Time with Confirmed Nested PCR Cases**

**3.2 Classification of Infection**

Identified dengue cases were next examined to distinguish primary from secondary cases using the ELISA dataset. Overall, only 5.06% of dengue cases were identified as primary infection, while 87.23% were identified as secondary infection. The distribution of primary and secondary infections differed by serotype. While there was a statically significant relationship between infection and serotype, there was no statistical significance found between primary and secondary for each serotype.

Over the twenty years, DENV-4 had the largest proportion of secondary to primary infections, with a ratio of 14:1, followed by DENV-2. DENV-1 had the smallest proportion (Table 2).

**3.2.1 Primary Infection**

Figure 6 has the breakdown of primary infection by year and dengue serotype. There were 1085 cases of acute primary infection in this analysis. The majority of the cases were DENV-1, followed by cases that were negative for nested PCR making the serotype of the case undetermined, then DENV-3. There were only 29 cases of DENV-4 for the entire period. Outbreaks of primary infections were identified in 2001 (236 cases), 2011 (136 cases), and 2013 (107 cases). The lowest number of primary cases in a year was 1996, with 8.

**3.2.2. Secondary Infection**

There were 6766 identified cases of secondary infection. The predominant serotype was DENV-2, followed by DENV-1. Over a third of the cases tested negative for PCR, but were identified with ELISA as having acute secondary infection. The three years with the largest number of secondary infections were 2001, (1315 cases), 2013, (651 cases) and 2008 (546 cases). The lowest number of cases in a year was in 1996, with 103 cases. (Figure 7)

**Table 2. Number of Dengue Cases by Year**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Year | DENV1 | DENV2 | DENV3 | DENV4 | DENVx2 | NEG PCR | NOT DONE | Total |
| 1994 | 8 | 4 | 8 | 10 | 0 | 42 | 265 | 337 |
| 1995 | 6 | 24 | 23 | 4 | 0 | 74 | 58 | 189 |
| 1996 | 19 | 8 | 13 | 0 | 0 | 52 | 60 | 152 |
| 1997 | 151 | 27 | 42 | 7 | 2 | 185 | 94 | 508 |
| 1998 | 36 | 48 | 100 | 0 | 0 | 223 | 7 | 414 |
| 1999 | 43 | 101 | 57 | 13 | 0 | 232 | 34 | 480 |
| 2000 | 19 | 81 | 4 | 0 | 0 | 136 | 3 | 243 |
| 2001 | 334 | 366 | 141 | 11 | 0 | 1156 | 18 | 2026 |
| 2002 | 111 | 102 | 34 | 7 | 0 | 534 | 85 | 873 |
| 2003 | 48 | 85 | 3 | 31 | 0 | 197 | 1 | 365 |
| 2004 | 21 | 61 | 4 | 105 | 0 | 145 | 0 | 336 |
| 2005 | 46 | 82 | 3 | 43 | 0 | 150 | 0 | 324 |
| 2006 | 173 | 4 | 34 | 55 | 0 | 157 | 0 | 461 |
| 2007 | 248 | 21 | 31 | 30 | 1 | 117 | 0 | 448 |
| 2008 | 279 | 117 | 80 | 78 | 1 | 317 | 0 | 872 |
| 2009 | 39 | 97 | 28 | 5 | 0 | 266 | 0 | 436 |
| 2010 | 33 | 152 | 63 | 6 | 0 | 319 | 0 | 573 |
| 2011 | 195 | 262 | 61 | 33 | 0 | 559 | 0 | 1110 |
| 2012 | 45 | 144 | 26 | 11 | 0 | 549 | 1 | 776 |
| 2013 | 55 | 61 | 382 | 19 | 0 | 761 | 0 | 1278 |
| Total | 1909 | 1885 | 1137 | 468 | 4 | 6171 | 626 | 12200 |

**Table 3. Proportion of Primary to Secondary Cases**

|  |  |  |  |
| --- | --- | --- | --- |
| Nested PCR | Acute Primary Inf | Acute 2nd Inf | Total |
| DEN1 | 383 (22.2%) | 1344 (77.8%) | 1727 |
| DEN2 | 159 (9.67%) | 1485 (90.33%) | 1644 |
| DEN3 | 192(18.6%) | 840 (81.4%) | 1032 |
| DEN4 | 29 (6.67%) | 406 (93.33%) | 435 |
| DENx2 | 0 | 4 | 4 |
| ND | 29 (11.15%) | 231 (88.85%) | 260 |
| NEG | 292 (10.68%) | 2443 (89.32%) | 2735 |
| Total | 1084 | 6753 | 7837 |

DEN1 1:3.5 DEN2 1:9.34 DEN3 1:4.375 DEN4 1:14 ND 1:7.96 NEG 1:8.34

**Figure 6**. **Acute Primary Infection by Year**

**Figure 7. Acute Secondary Infections by Year**

**3.3 AGE**

Next, dengue incidence was examined in eight different age groups, >1, 1-4, 5-9, 10-14, 15-19, 20-39, 40-59, and 60 and older. The two subsets of data were analyzed to see if there was any significant difference for age groups in dengue incidence by age. The group with the largest percentage of cases was the 10-14 age group, with 33% of cases. Infants (<1) had the smallest percentage of cases (0.7%), followed by persons 60 years and older (0.11%). An increase in the mean age was seen across four five-year intervals for both primary and secondary infections (Table 4).

**Table 4**. **Average Age Over Time**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Year Interval** | **N Obs** | **Mean** | **Lower 95% CL for Mean** | **Upper 95% CL for Mean** | **Median** | **P-value** |
| **1994-1998** | **540** | 8.34 | 8.03 | 8.64 | 8.30 | <.001 |
| **1999-2003** | **1591** | 10.95 | 10.65 | 11.26 | 10.10 | <.0001 |
| **2004-2008** | **1555** | 14.16 | 13.77 | 14.56 | 12.80 | <.0001 |
| **2009-2013** | **1716** | 17.18 | 16.72 | 17.64 | 15.20 | <.0001 |

When the 20-year study was separated into 5 year intervals of time (1994-1998, 1999-2003, 2004-2008, 2009-2013), the mean age for primary infection was found to increase for each successive interval, 5.105, 5.84, 9.22 and 9.58. A slow rise is seen in the first ten years, followed by a larger jump in subsequent years. (Table 5). Using GLM to look at mean age and the five-year time intervals, there was no difference in mean age between the 1994-1998 and 1999-2003 intervals, as well as the 2004-2008 and 2009-2013 intervals. However, mean age for primary dengue infection was statistically different for all other intervals.

There was a different pattern regarding secondary infections for the five-year intervals. The mean age for each successive interval was 8.67, 11.31, 14.51, and 17.27 (Table 5). There is a consistent three-year jump in mean age every interval. The last interval from 2009-2013 was skewed right. This makes sense because that interval had 5 of the 6 cases of dengue in the 60 and older age group. Using GLM in SAS it was determined that the mean age was statically significant between all of the the five-year time intervals.

After the mean age for the intervals was determined to be statistically significant, ANCOVA using GLM in SAS was used to determine if there was a difference between the four different dengue serotypes in mean age as well. Adjusting for year and classification of dengue, there was a statistical difference in mean age between all four dengue serotypes. The average age of a person with DENV-1 was 10.88, 13.00 years with DENV-2, 11.89 with DENV-3 and 12.6 years with DENV-4. There was no statistical difference found for years that had more than one serotype co-circulating.

**Table 5. Average Age Over Time by Infection Type**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Year Interval** | **N Obs** | **Mean** | **Lower 95% CL for Mean** | **Upper 95% CL for Mean** | **Median** |
| **1994-1998** |  |  |  |  |  |
| Primary | **48** | 5.10 | 4.17 | 6.04 | 5.10 |
| Secondary | **481** | 8.67 | 8.35 | 8.98 | 8.60 |
| **1999-2003** |  |  |  |  |  |
| Primary | **92** | 5.83 | 4.99 | 6.67 | 5.95 |
| Secondary | **1453** | 11.31 | 10.99 | 11.62 | 10.40 |
| **2004-2008** |  |  |  |  |  |
| Primary | **56** | 9.22 | 7.19 | 11.25 | 9.00 |
| Secondary | **1325** | 14.51 | 14.08 | 14.92 | 13.00 |
| **2008-2013** |  |  |  |  |  |
| Primary | **62** | 9.59 | 7.78 | 11.39 | 9.45 |
| Secondary | **1080** | 17.26 | 16.78 | 17.82 | 15.11 |

**4.0 DISCUSSION**

The co-dominance in dengue incidence between DENV1 and DEVN2 serotypes for multiple years represents a departure from what has been reported before in Thailand18. Historically, both Bangkok and Kamphaeng Phet have found one serotype is always dominant over the other three. The large number of dengue outbreaks from 1994-2013 was unusual due to a three to five-year cyclic pattern in Thailand. This pattern may be accounted of difference in age at onset over time, and different dengue serotypes, year, and the interaction between the two variables.

**4.1 Summary**

The results of this observational study of dengue show that all four DENV serotypes circulated continuously in Kamphaeng Phet from 1994-2013, causing both acute primary and secondary dengue in residents of all ages. The equality of percentage of cases of both DENV1 and DENV2 is a departure from what is seen in the literature for dengue in Thailand. Both Kamphaeng Phet and Bangkok have seen a higher incidence of one over the other in the literature. Clapham found DENV1 to be more prevalent in children in Bangkok over forty years, and Endy found DENV3 to be highest in Kamppheng Phet24,25. This departure may be due to a smaller sample size and different population demographics than Clapham25. Clapham was looking at only children in Bangkok, at a level three MOPH pediatric hospital. The Endy et al finding in 1998-2000 represents a much smaller sample size then KPPH’s cases, looking only at school-age children24. This equality of cases between DENV1, which is more virulent as a primary infection, and DENV2, which is more virulent as a secondary infection, may be one reason why there have been more outbreaks in recent years.

The number of outbreak years was startling to see. Usually dengue is cyclic with outbreaks every three to five years in Thailand. DENV3 incidence seemed to peak every twelve years. DENV1 seemed to follow an outbreak every 3 years after the end of the last one, but that outbreak could last for one to three years.

The number of consecutive years of 100+ cases of the same serotype either says that the number of cases used to recognize an outbreak is too low, or that there is an alternate explanation not examined in this paper. After an outbreak was finished, serotype numbers went back down to endemic levels of approximately 10-35 hospitalized cases a year. It would be better to look at incidence rates, rather than just the number of cases. Unfortunately, population data for the entire time-period was not available to calculate incidence rates.

Of the five years with the highest total number of cases, four of the years had two serotypes in an outbreak. In the 2001 outbreak, three serotypes were involved. 2013 had the second highest amount of cases, but only had DENV3 in an outbreak. The large amount of DENV3 cases (largest amount of cases for a single serotype for a single year for the entire 20 years) could be because of the re-emergence of DENV3. DENV3 is seen to be more virulent in primary infections then other serotypes. The large case load might also be explained by this infection being the third or fourth infection for people, which usually does cause more severe dengue symptoms.

Breaking the acute primary and secondary infections down by year gave an idea of what percentage of the cases were primary or secondary. The largest number of primary cases each year come in the same years that outbreaks of either DENV1 or DENV3 happened. Further analysis could determine if the DENV1 and DENV3 cases at KPPH are more virulent as primary infections then the other serotypes.

While mean age of dengue infection has been increasing in Thailand over the twenty years of this analysis, there was no explanation in the literature to account for such large mean age increases in the five-year intervals. The mean age and standard deviation for all infections was similar to the secondary infections, due to secondary cases being over 80% of the confirmed cases compared to 5% of the confirmed cases being primary infections. Using regression, year in study, co-circulating year, and dengue serotype accounted for 17% of variation for age.

The consistent three-year mean age increase for every five-year interval is not something that has been seen before. Each age group was tested for the mean age within their age group for each five-year interval to determine if there was any variation in each five-year interval that might explain the consistent increase. There were no statistically significant findings for the different age groups, just a larger number of adults having secondary dengue. From 1994-1998, no adult over 39 had secondary dengue. By 2009-2013, there were 54 in the 40-59 age range, and 5 in the 60+ age categories.

One possible reason for the increase in adult population infection is that the last five years had DENV3 reintroduced to the population after a long period of low prevalence. Another reason may be that the genotype strains being introduced are not the common Asian DENV strains people are used to. Genotypes of the four different dengue serotypes are different around the world. A genotype that traveled to Thailand from the Americas due to globalization might cause higher viremia in a patient than an Asian genotype, possibly leading to higher rates of hospitalization due to increased severity. Another potential reason is that as Thailand’s population is getting older. As people get older, co-morbidities and a waning immune system makes them potentially susceptible to a strain they might have had when they were younger27.

The increase in the age at primary infection over time does not have a straight-forward explanation. Thailand has seen an increase in the average age of dengue cases, but its average age of a case jumped from the 5-9 to the 10-14 age group in 200219. There should not have been such a large increase in the mean age of primary infection cases from 1999-2003 to 2004-2008. While there were three outbreak years from 1999-2003, including the large outbreak in 2001, the interval from 2003-2008 had multiple outbreak years as well. Looking at primary infection by age for all suspected cases, not just the confirmed cases, shows us a similar jump. The median age is 6.00 for the first decade, before it jumps to 9.2, and lowers to 9.0 for the final five years.

A possible reason for the large increase in mean age at infection is a large decrease in force of infection. After the 2001 outbreak, DENV-1 and DENV-3 were not as prevalent. The secondary infections also jumped, lending credence to the force of infection possibly playing a part in the increase of average age. Birth rates may also have an effect as well. In 2004, there was a crude birth rate of 10/1000; In 2012 it was 8.79/1000. In the first part of 2004, 522,000 of 726,000 people in Kamphaeng Phet were over 15 years of age. In 2013 there are almost 599,000 of 727,500 people. In nine years, the population over 15 has increased by 10%. A lower birth rate and an aging population are thought to decrease the number of susceptible individuals.

**4.2. Limitations**

The analysis in this report may be influence by several limitations. Most importantly, the analysis is based upon hospitalized data, which doesn’t show the entire scope of dengue in the Kamphaeng Phet Province. Another important limitation is the possibility of double dipping. The de-identified data only told the age at time of visit. A person could be in the database more than once, in multiple age and serotype categories. Dengue infection usually gives around 6 months cross-protective effect against other serotypes, depending on the infecting serotype. A person could potentially be hospitalized twice in a year for dengue, making the hospital look like it had more people show up then there were.

A third limitation is the lack of data on dengue severity in the samples. Dengue fever and dengue hemorrhagic fever data was available from 1994 to 2006, with 3501 dengue cases having a diagnosis of severity over that time period. After that, there were no records found with the DF/DHF diagnosis, most likely because Thailand was a site of early testing of the new 2009 WHO dengue classification. The records until 2007 do see a higher proportion of DHF cases then DF, because these are hospitalized cases. The ratio between the DF and DHF cases ranges from 1:1.8 to 1:5.4. The year with the largest ratio discrepancy was 1998, which was not a large outbreak year, but 40% of the cases were DHF. 2001 had a ratio of 1:3.11, which was around the average ratio.

91% of infant cases were DHF. There was nothing significant for the other age groups, with the older ages having a slightly higher percentage of DHF cases, which comes with multiple exposures to dengue. The assumption is that most adults are experiencing secondary infections, possibly their third or fourth.

These analysis results were not consistent enough to be looked at as a confounding factor for the later part of the twenty years, making it harder to compare that time period from KPPH to Bangkok, or other parts of Thailand. It also doesn’t make it possible to prove a decrease in force of infection.

**5.0 PUBLIC HEALTH SIGNIFICANCE**

Dengue is one of most important vector borne infectious diseases in the world. There is no doubt that it is of critical public health significance. As the majority of the world population now lives in cities in the tropics, compared to rural areas, it creates more people for the *Aedes* mosquito to infect. Shifting global climate has allowed both *A.aegypti* and *A.albopicuts* to expand to almost all areas in the lower and middle latitudes. The expansion increases the amount of people at risk of dengue. An estimate from the WHO suggests that around 4 billion people live in an area at risk for dengue. As climate changes, sea levels rise, and global warning continues, this number is expected to increase.

Another worrying issue is the spread of the other *Aedes* species able to transmit. *A.albopictus* is the secondary vector, but studies say it is the superior larval species then *A.aegypti.* Like *aegypti,* it prefers humans and urban areas. It is a much more incredibly aggressive mosquito then *aegypti* and its habitat is able to reach farther north, and still expanding*.*

Global travel is making it easier for diseases to spread quickly to different locations from more distant places. The Hawaii dengue outbreak started from an infected traveler from South America, and it has flourished. This infection may become more established among the mosquito population within the Hawaii population, and from there can travel more readily to other parts of the United States and world.

**6.0 CONCLUSION**

Dengue is a disease that has been around for years, but has been increasing in prevalence and importance for years. Detailed and ongoing observation of this disease is important to understand how it is spreading and what patterns there are. This observational data is important to give a comprehensive overview of Kamphaeng Phet.

This study demonstrated serotype cycling over the years in Kamphaeng Phet that has not been well documented previously for long periods of time. DENV-1 and DENV-2 were equally dominant for a period of time, and DENV-4 has never reached the same peaks incase numbers of other serotypes. An almost 12-year cycle for DENV-3 has been found. In recent years, all four serotypes have been more consistently circulating, usually resulting in co-dominant outbreak years.

An updated analysis of trends in Kamphaeng Phet is timely for understanding what may happen in the future. Thailand is an important setting for studying DENV transmission due to it’s hyperendmicity and strong surveillance system. Description and understanding of how this dengue epidemiology has developed and interacted with population changes over twenty years is important for other countries who might be or are currently facing the same challenges Thailand has.

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