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Geographic dynamics of viral encephalitis in Thailand

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Abstract

This study involves an ecological analysis of the climatic, geographic and seasonal patterns of clinically reported Viral Encephalitis (VE) in Thailand from 1993 through 1998 using a geographic information system to investigate regional and seasonal differences in disease incidence. It is the first study to describe and analyze the seasonal and spatial distribution of VE in Thailand nation-wide. 3,829 cases of VE were clinically diagnosed during the study interval. Spearman rank correlations of temporal, spatial and geographic variables with disease incidence were performed. Disease in the north-northeastern portion of Thailand demonstrates positive seasonal correlations with previously known ecological variables (*e.g.* temperature and humidity) that affect arboviral disease patterns, but there are no seasonal variations of disease in the southern-central region. Furthermore, the spatial distribution of VE differs from previously known patterns of arboviral disease, with a higher amount of clinical disease in areas that are cooler, higher in elevation, and have lower relative humidity. Previous studies have shown that there is a higher Japanese Encephalitis (JE) proportion of VE in the northern areas of Thailand despite the high antibody prevalence of animal reservoirs in the central and southern areas. The lack of seasonal variation of VE in the southern-central region suggests that a high incidence of non-arboviral diseases which mask or mimic JE activity may be present in this locale. However, many other variables likely contribute to the unique and different ecologies of human disease in Thailand, including vector host preference, regional extremes in temperature and humidity, socio-economic level, proximity of animal reservoirs to human hosts, population resistance, and agricultural technologies. Given these diverse factors, active etiologic surveillance is necessary in a variety of geographical settings in order to provide physicians with information necessary for disease prevention and clinical management of VE in Thailand.

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Table of Contents

1. Introduction 1

2. Specific Aims and Hyptothesis 15

3. Materials and Methods

3.1 Data collection, fieldwork, and personal contribution 17

3.2 Disease incidence, clinical definition, and JE antibody testing 19

3.3 Seasonal disease variance 22

3.4 Climate and geographic data; study area 23

3.5 Statistical analyses 27

3.6 Building the GIS database 28

4. Results

4.1 Temporal analysis and disease incidence 29

4.2 Spatial variation and geographic correlation 39

4.3 Mortality 44

5. Discussion 47

6. Appendix (Ratchaburi hospital pilot surveillance) 61

7. References 63

1. Introduction

Viral encephalitic diseases have become a worldwide problem, often re-emerging in formerly endemic areas or emerging in new locations. Japanese Encephalitis (JE) is one of the leading causes of viral encephalitis worldwide with approximately 35,000 to 50,000 new cases each year.[1] JE is also extremely prevalent in much of the southeast and east Asian continent and archipelagoes. However, other flaviviruses such as dengue and a wide spectrum of viral pathogens also contribute to encephalitic disease. In addition, viral encephalitis (VE) is reemerging in previously controlled areas in Thailand. Although vaccination and mosquito control measures have been implemented which have decreased the overall incidence and mortality, recent data indicate that VE remains a serious problem that still results in significant morbidity and mortality. The ongoing VE endemic necessitates systematic, epidemiological studies and new approaches of controlling the disease.

VE is a major cause of disease in Asia and is endemic nationwide in Thailand, where 3,829 cases of VE have been clinically diagnosed from 1993 through 1998. JE virus is one of the major etiologic pathogens responsible for VE in Thailand and is especially common among children. Severe cases present with central nervous system infection, such as headache, convulsions, muscle spasms and loss of consciousness, and may result in severe neurologic sequelae in as many as 30% of individuals.[2-5] Approximately 30 percent also may lead to death, making JE a major childhood disease in Thailand. There are several common clinical characteristics of JE that are useful in assessing patient morbidity and clinical outcome. Common symptoms and signs of JE infection include nausea, vomiting, headache, drowsiness, stupor, muscle weakness,

alternation of consciousness, fevers, nuchal rigidity, convulsions, lethargy, upper motor neuron signs (*e.g.* Babinski reflex), abnormal respiration and rarely hypertension. Common peripheral lab findings include leukocytosis with left-shift on complete blood count, CNS lymphocytosis (in 76% in one study of Thai children with JE), increased protein (in 59 to 84% of patients), and white cells in CNS (ranging from 3 to 4,200 in the Thai study).[6,1] Brain imaging may show diffuse white-matter edema and electroencephalogram often reveals diffuse Delta-wave activity indicating thalamic involvement.[6]

Despite the severity of clinically presenting JE, historical studies suggest that more than 90 percent of individuals infected with JE may be asymptomatic. Researchers now estimate the subclinical to clinical ratio of JE infection is approximately 25:1 to 1,000:1.[7] In vaccinated populations, however, the ratio of subclinical to clinical infections is approximately 2,000,000:1, which is 2,000-80,000 times higher than for unvaccinated populations.[8] However, large scale studies on the etiology of viral encephalitis in Southeast Asia are lacking, and it is difficult to determine the exact number of new JE cases per year.

JE is caused by a spherical, single-stranded RNA flavivirus that is transmitted by mosquito vectors from pig or bird reservoirs to humans. The *Culex tritaeniorlynchus* mosquito is largely responsible for JE transmission in Thailand, although other species capable of carrying the disease, such as *Culex vishnui, C. pseudovishnui, C. gelidus, C. fuscocephala, C. quinquefasciatus, C. pipiens pallens, C. bitaeniorlynchus*, and *C. annulirostris*, *Aedes togoi, A. japonicus, A. vexans nipponii, Anopheles annularis*, and *A. vagus,* have been identified.[9-12] Mosquito vector abundance in suburban Bangkok has

been shown to be greatest in the monsoon season (May - October) and lowest in the dry season (January - February), with 96% of JE isolates collected from mosquitoes trapped during the monsoon season.[13] Despite the seasonal fluctuations of mosquito vector abundance and JE seropositivity, however, there is little seasonal variation of human JE cases in the planes of central Thailand. Research conducted during the 1980's indicates that JE virus has been hyperendemic in the northern region of Thailand, with more than 65% of the population showing seroconversion to JE antibodies.[14] This suggests that a majority of the population had past exposure to JE virus. In contrast, studies of JE in southern Thailand suggest that there is low prevalence in humans, despite the fact that these regions are marked by very high seroprevalence in pigs (74%), with seroconversion taking place in 70% of pigs within two weeks of birth.[15] The reason that there is high pig seroprevalence but a lower incidence of human clinical cases in the south is not well understood. Vector host preference and the proximity of animal reservoirs to human populations may contribute significantly to these findings (see discussion for full details).

In 1989, a widespread vaccination campaign against JE virus in Thailand was implemented in an attempt to reduce the incidence and mortality of viral encephalitic disease. Several JE vaccines are available which have shown success in preventing acute encephalitic infections. An inactivated mouse-brain derived vaccine is used in inoculation of most travelers from the United States and Europe, although other forms of attenuated vaccines are available in Asia.[16] The ongoing prevention program, which began in the formerly hyperendemic northern region and later expanded to other provinces, is associated with an overall decrease in VE cases.[1, 17] However, a leveling of disease incidence from 1993 to 1998 is cause for concern, given the resources invested in

preventative vaccination programs and the lack of widespread etiologic investigation of encephalitis in Thailand (Figure 1). The high cost of the vaccines and the multiple injections required to guarantee effectiveness have forced prevention programs to look for a more cost-effective and feasible way of vaccinating individuals at risk.[18]

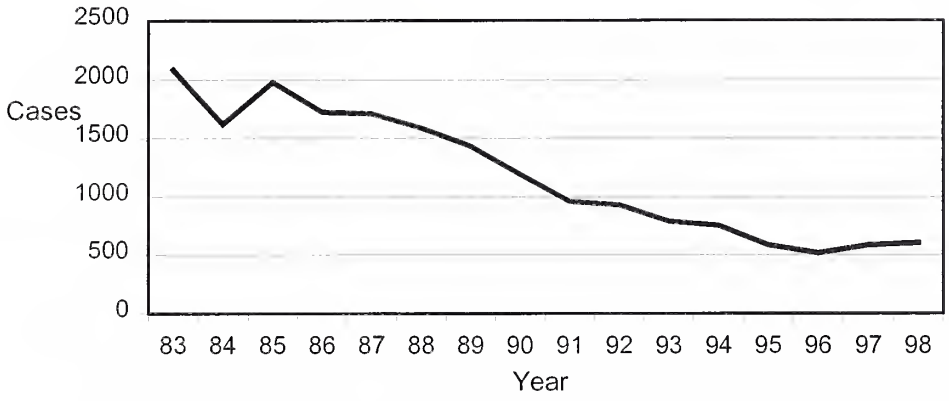

Figure 1. Encephalitis cases in Thailand by year, 1983-1998

In addition, recent studies of HIV-infected children in Bangkok have shown that these individuals are less responsive to multiple doses of JE vaccine than those without HIV.[19] Although the literature on JE vaccine and lasting immunity in HIV positive individuals is sparse, studies of other vaccines indicate that HIV positive and immunocompromised patients have less protective immune responses following standard vaccine protocols. For example, the Committee on Infectious Diseases and the Committee on Pediatric AIDS report that there are unpredictable and suboptimal responses to MMR vaccine in HIV positive patients, and measles antibody titers decline faster in HIV positive patients than in uninfected, immunocompetent patients.[20] It has also been reported that HIV infected children respond inadequately to booster.[21] However, a recent study by Melvin and Mohan[21] shows that children may respond to measles, tetanus toxoid and *Haemophilus influenza* type b vaccines appropriately if they

are receiving highly active anti-retroviral therapy and have intact immune function. The high prevalence of HIV in Thailand[22] (estimated to be from 1 to 4 percent in various populations during the late 1990's) may be responsible, in part, for the puzzling plateauing of disease incidence in Thailand despite the vaccination campaing of the 1990's. A significant portion of patients who received the JE vaccine may have been immunocompromised and did not mount an adequate antibody response.

Dengue viruses are also responsible for encephalitis in Thailand, and may result in a significant amount of clinically presenting VE. Dengue, a flavivirus and mosquito-borne illness with similar ecological characteristics to JE, has been documented to cause encephalitis in southeast Asia. Kankirawatana *et al.*[23] have identified eight serologically confirmed dengue cases from 44 pediatric patients with clinically diagnosed VE in Bangkok. All of these patients had been admitted with encephalitic-like symptoms commonly associated with JE. Solomon and colleagues report in a study of 378 patients hospitalized with suspected CNS infection in a southern Vietnamese hospital that 16 (or 4.2%) of patients were infected with dengue viruses versus 1.4% of matched hospital controls.[24]

Many other arboviruses are capable of causing encephalitis, and may account for regional, seasonal and epidemiological differences in clinical VE. Both mosquitoes and ticks have been identified as vectors of these viruses. For example, Alphaviruses (Togaviridae) spread by *Culex, Culiseta* and *Aedes* vectors in North and South America include Eastern equine, Western equine and Venezuelan equine encephalitidis. Flaviviruses of the West Nile complex that include St. Louis, Japanese, Murray Valley, West Nile, Ilheus, and Rocio encephalitidis have been identified worldwide, with West

Nile and Japanese encephalitis being common in Asia.[25] (West Nile had not yet been isolated from humans in Thailand.) Murray Valley encephalitis has been identified in Australia and New Guinea, but may have spread to Thailand and the Indochinese peninsula given that studies of mosquito dispersal suggest that the spread of arboviral disease within the Pacific region is common. In addition, it is believed that Malaysia was the origin of a common JE proto-virus.[26] Researchers suggests that airborne dispersal of *Culex* vectors from Southeast Asia may have recently introduced JE to northern Australia, despite the large distances of water between these two locales.[27] If JE has been introduced to the Australian continent via airborne vector dispersal, it is possible that diseases native to Australia, or that have developed significant genetic mutation there, have spread to Southeast Asia. However, the prevailing wind conditions may make mosquito dispersal more difficult in the retrograde direction.

Other mosquito-borne viral encephalitidis are caused by Bunyavirusus (California, La Crosse, Jamestown Canyon, Snowshoe Hare, Tahyna, and Inkoo encephalitis) and a closely related Phlebovirus, Rift Valley, from East Africa.[25] There are multiple tick-borne encephalitis complexes that also can cause central nervous system disease, but currently only the mosquito-borne viruses (JE, West Nile) have been identified in Southeast Asia.

In addition to mosquito-borne diseases, encephalitis is often caused by non-arboviral pathogens that are responsible for a portion of human disease in Thailand. For example, encephalopathic symptoms have been associated with infectious diseases such as herpes viruses, influenza, measles, mumps, Epstein-Barr virus, echoviruses, adenovirus and enteroviruses, including polioviruses.[28-33] Herpes simplex encephalitis is

an extremely common cause of encephalitis and aseptic meningioencephalitis. In the western hemisphere, herpes simplex virus I encephalitis occurs in approximately 2 to 4 per 1,000,000 individuals per year and is the most common fatal viral CNS infection for patients of all ages.[34] Herpes simplex II CNS infections are also common in neonates who come in contact with maternal genital secretions at delivery. Despite a large amount of literature on herpes simplex viruses in western countries, very little has been published about the incidence and morbidity in Asia, with no papers on HSV encephalitis specifically in Thailand. Two studies looking at the percentage of HSV infection in patients presenting with encephalitic illness have been done in northern India. In one study, authors report that 14 of 18 patients ranging in age from 4 to 65 years with clinical evidence for HSV encephalitis had HSV antibodies in the CSF.[35] Another study looked at the proportion of HSV-1 IgM antibodies in 90 patients presenting with acute viral encephalitis. The analysis revealed that only 1.1% of acute viral encephalitis cases in Uttar Pradesh were due to HSV-1 viral infection.[36] The reason the percentage of acute viral encephalitis due to HSV is much lower in South Asia is unclear, but it is likely that there is a higher prevalence of other viral pathogens, such as JE or dengue, in this region that "dilutes" the HSV proportion. Enteroviruses are also very common causes of viral encephalitis in the western hemisphere, although the burden of disease is not known in southeast Asia.

Non-viral diseases, both infectious and non-infectious, may mimic the encephalitis caused by herpes simplex and enteroviruses. For example, listeriosis, tuberculosis, cryptococcus, toxoplasma, fungal infections, systemic lupus erythematous, hematomas, vascular disease, and other illnesses have been identified in the literature.[37,25]

Many of these diseases have a more insidious onset when compared to acute viral encephalitis and there are often other clinical signs, symptoms and patient histories (*e.g.* a history of HIV disease) that lead clinicians to work up these alternate etiologies. Bacterial and parasitic infections such as rickettsial encephalitis, eosinophilic myeloencephalitis and amoebic meningoencephalitis can also present with abnormal neurologic symptoms similar to JE, but these diseases do not make up a large portion of clinically reported encephalitic disease in Thailand.[38-40]

The annual proportions of encephalitis cases serologically confirmed as JE from 1977 to 1988 in Malaysia ranged from 18 to 60 percent, which suggests that a large amount of disease is caused by non-JE pathogens.[41] Malaysia borders the southern region of Thailand, and the geography and climate of southern Thailand is more similar to peninsular Malaysia than to the northern parts of the country. For this reason, the percentage of JE in southern Thailand may be more like that of Malaysia than the hyperendemic north. The emergence of new encephalitic viruses such as Nipah virus and the reemergence of neurovirulent strains of enterovirus 71 have recently been detected in Malaysia and may already be causing illness in southern Thailand, decreasing the proportion of JE infection.[42]

Fluctuations in temperature and relative humidity have been shown to affect the abundance, competence and vector infection rates of mosquitoes that are capable of carrying arboviral disease responsible for viral encephalitis and related syndromes. These climate variables have traditionally been thought to influence the seasonal variations of arboviral disease incidence in human populations. Temperature effects on mosquitoes and viral activity have been studied in the greatest detail, but many studies

have inconsistent findings due to the wide range of mosquito species and viruses that they carry. Most studies have shown that temperature is correlated with extrinsic incubation periods of viruses and, as a result, increased viral transmission.[43] Cornel *et al.*[43] report that *Culex univittatus* mosquito longevity increased inversely with incubation temperature, and that the number of days for 100 percent of viral fed mosquitoes to become infected with West Nile virus decreased with increased temperature (ranging from 58 days at 14 degrees C to 15 days at 30 degrees C). Another study on West Nile virus showed that increased temperatures correlated with increased infection rate of viral fed *C. pipiens*; 86 percent of mosquitoes became infected in 32 days at 18 degrees C, whereas 100 percent of mosquitoes became infected in 4 days at 30 degrees C.[44]

Temperature and variations in field conditions have an impact on the developmental rates of *Aedes aegypti* mosquitoes, which is the leading vector for dengue viruses and therefore potential sources of viral encephalitis infection. Tu-Lin *et al*[45] report that mosquito development time from larval hatching to adult maturity decreased with increasing water temperature due to increased rates of development. However, survival percentage of mosquitoes peaked around 25 to 30 degrees C but quickly decreased at temperatures over 30 degrees, suggesting that extremes in temperature decrease mean percentage survival. The authors also show that mosquito breeding and development containers that were shaded (amongst foliage and under trees) and had more available organic matter had faster developments and better immature survival rates.[45] In another study of *Aedes aegypti*, mosquitoes were allowed to feed on dengue 2 infected rhesus monkeys at different temperatures. Researchers concluded that the vector infection rate ranged from 25 to 75 percent at all temperatures, but that the incubation

period (number of days mosquitoes were allowed to feed on infected monkeys) was responsible for the variation in infection rates.[46]

In addition to temperature, humidity has also been shown to influence mosquito infectivity and viral transmission. Thu *et al.*[47] report that the dengue virus positivity of *Aedes aegypt* reflected distinct patterns in temperature and relative humidity. Virus was detected via mosquito head squash and direct fluorescence antibody assays in adult male mosquitoes from the rainy season of Yangon (23-30 degrees C, 90 percent relative humidity) and from Singapore (24-31 degrees C, 87 percent relative humidity). However, no mosquitoes tested positive from the cold season of Yangon (16-33 degrees C, 65 percent relative humidity) or the hot season of Yangon (25-37 degrees C, 66 percent relative humidity). The authors conclude that mosquitoes kept during the rainy season or seasons with high relative humidity had significantly higher dengue virus titres.[47]

Rice paddy cover has also been linked to variations in arboviral disease-carrying mosquitoes and infected livestock in Asia. For example, serum specimens from swine from the Ryukyu islands (the southern most province of Japan, and a sub-tropical archipelago) from 1985 to 1989 showed that JE virus transmission was more prevalent during June and July (warmer and wetter season), with sporadic cases identified during all other months except for February and March (cooler and dryer).[48] In addition, only a few mosquito adults and larvae were collected from Miyako island, where there are no rice paddies, whereas many were collected from Ishigaki island, an area with a high density of rice paddies and standing water. The authors admit, however, that other environmental and geographic conditions of the two islands are very different, and viral

transmission was very low on both islands.[48] These differences may play a role in the variations of vector abundance noted in the study. Other factors have been described to influence the abundance of Japanese encephalitis carrying mosquito vectors in Indian rice fields, although wide spread geographic variations between areas with different amounts of rice field cover were not explored. However, both biotic and abiotic variables contribute to vector abundance, such as seasonal patterns of predator and algal abundance, amount of phytoplankton, paddy height, water temperature, dissolved oxygen content, ammonia nitrogen levels, and nitrate oxygen levels.[49, 50] Increased paddy height, ammonia nitrogen, and dissolved oxygen were associated with decreased abundance, whereas nitrate nitrogen level and increased water temperature were associated with increased abundance.[49]

Despite the experimental studies on the impact of climate and agricultural variables on vector infectivity and abundance as discussed above, few large-scale studies have looked at how these variables influence the seasonal and spatial variation of human infection, and none focus on the entire geographic region of Thailand. One experimental study of the infection of rhesus monkeys in controlled and variable climatic conditions has been helpful in linking vector infectivity and primate infection, however. In the first experiment, rhesus monkeys were exposed to *Aedes aegypti* mosquitoes for 25 days at temperatures of 20, 24, 26, and 30 degrees C (the mosquitoes were previously exposed to a dengue 2 virus infected monkey at the same controlled temperatures). Only the mosquitoes in the 30 degree chambers transmitted virus to the monkeys.[46] Relationships between human cases of JE infection and climatic conditions in Nagasaki, Japan have also been examined to identify seasonal patterns of epidemics from 1950 to 1979. This

study showed that the number of JE cases significantly decreased with increased precipitation during the summer months, and cases increased with increases in temperature (approximately 24 to 28 degrees C).[51] However, the study did not examine the role of relative humidity or variations in agricultural land cover, which have been linked directly to vector infectivity.

Studies using a Geographic Information System (GIS) have been used successfully in analyses of vector-borne pathogens to understand the spatial and temporal dynamics of disease in relation to ecological variables and to build predictive models.[52, 53] GIS is a powerful tool that enables climatic and geographic information to be integrated into analyses of disease occurrence; it has not been extensively implemented in studies of VE in Thailand thus far. Despite the lack of use in the medical literature, GIS is simply a tool to present and analyze data that are linked to spatial coordinates. Ricketts[54] elegantly describes GIS in medicine and public health as, "a simple extension of statistical analyses that join epidemiological, sociological, clinical and economic data with references to space. A GIS system does not create data, but merely relates data using a system of references that describes spatial relationships". Others describe GIS simply as, "a computer-based system for automating, storing, manipulating, and displaying mapped information and data."[55]

GIS began as an analytieal tool used extensively in marketing, urban planning and geology, and has now been adapted for clinical and epidemiological problem solving with the availability of user friendly software packages. GIS has been used to make predictive models of infection status in human and non-human populations that use climate and geographic variables. One study of Sin Nombre virus used GIS to help

predict deer mouse infection rates in Walker River Basin, Nevada and California predicted the presence of infection with 80 percent accuracy.[56] GIS has also been used to show how disease vectors (such as *Ixodes* ticks) interact with environmental factors such as land cover, temperature, soil composition, etc.[57] Using these types of variables, landscape ecologies can help explain the spatial and temporal epidemiology of disease. For example, Wilson[58] found that temperature, humidity, vertebrate host presence, distance to infested sites, and host immunity all helped explain the spatial and temporal variation in the distribution and abundance of *I. scapularis* ticks in North America. This study also showed that each geographic and ecological variable had a different effect on the spatial and temporal tick variation. For example, a variable such as temperature had a high importance in explaining the spatial distribution of ticks (patch size), but a relatively lower importance in explaining the seasonal variation of tick abundance.

Specific spatial analytical tools have also been applied to vector-borne disease epidemiology. Mapping of disease rates has been extremely useful when used with GIS because different disease patterns may exist at different spatial scales, and these patterns can be compared with geographic variables at those scales. (Figure 2 shows examples of how disease information can be mapped with geographic variables). Linking statistical analyses to mapped data enables researchers to: locate areas of disease outbreak or epidemic; find correlations between disease and geographic variables (*i.e.* disease carrying mosquitoes and temperature); predict future occurrence of disease by building statistical models to describe multiple correlations between geographic variables and disease; and help public health workers to maximize health care distribution (including cost and accessibility).

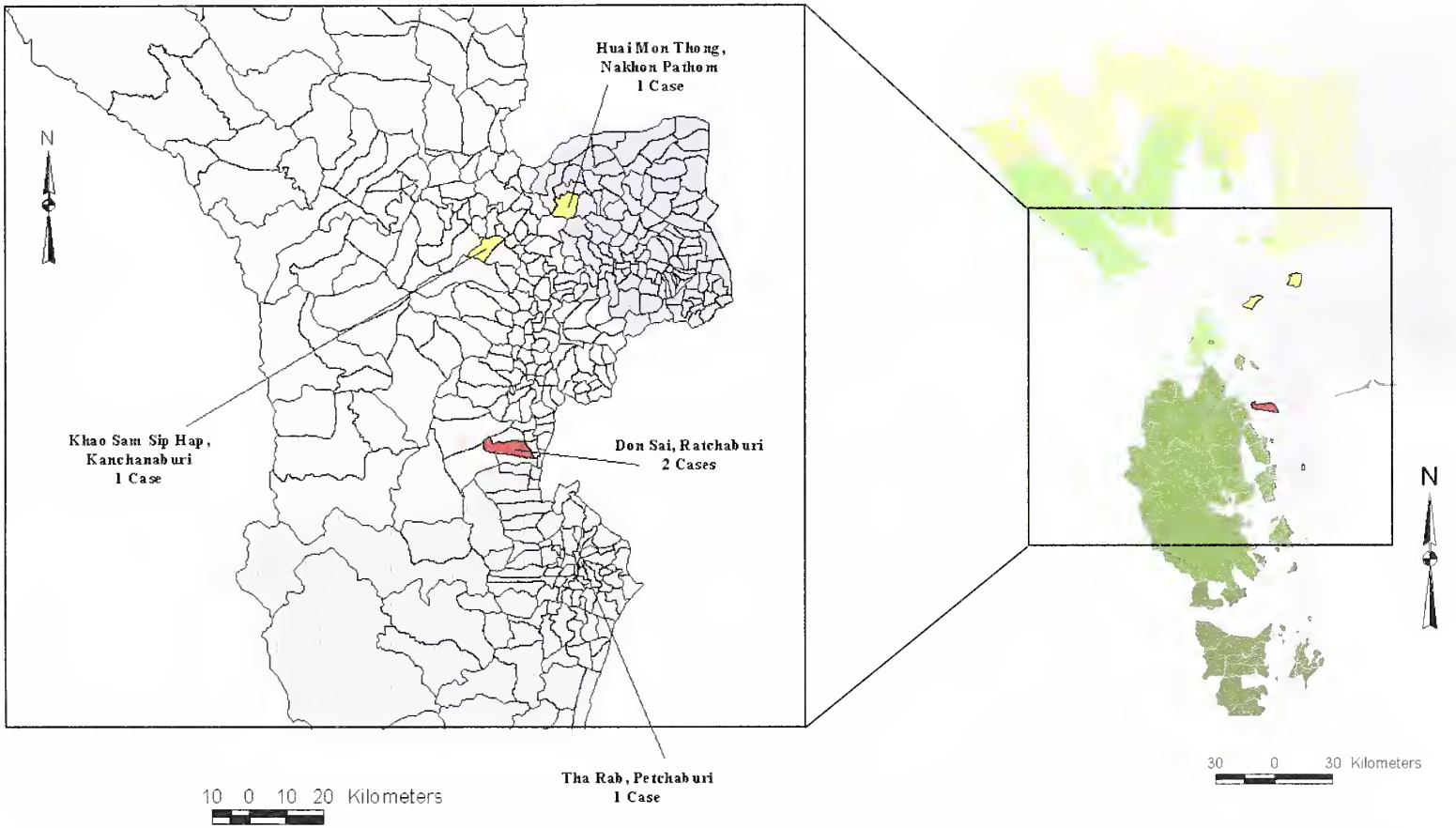

Figure 3 Clinical Encephalitis and Degree of Forestation

Figure 2. Examples of disease mapping with geographic variables as part of a geographic information system. The map on the left shows the political boundaries of individual Ratchaburi districts with the number of new VE cases during summer of 2001represented as shades of yellow and red. The map on the right shows western Thailand with disease overlaid on a chloropleth map of degree of forestation (with darker green representing more densely forested areas.)

2. Specific aims and hypothesis

The present study involves an ecological analysis of the climatic, geographic and seasonal patterns of reported VE in Thailand from 1993 through 1998 using a geographic information system to investigate temporal and spatial differences in disease incidence. Given previously known regional differences in clinically diagnosed VE, it is likely that ecological variables, including temperature, relative humidity, rainfall, elevation, rice field cover, and agricultural land cover, will influence the seasonal and geographic distribution of human disease. More specifically, the seasonal (temporal) distribution of VE should significantly correlate with increased relative humidity, rainfall and temperature. In addition, a spatial analysis of the geographic distribution of disease should reveal significant correlations between human disease and relative humidity, temperature, annual rainfall, and the amount of agricultural land and rice field cover. However, because of regional variations in climate, geography and agricultural use in Thailand, different patterns of disease incidence should be seen in different ecological regions.

The central and southern regions of Thailand, which are associated with a lower proportion of JE and mosquito-borne pathogens seen in previous studies, should have weaker seasonal and spatial associations with the geographic variables, however. This observation is contrary to the finding that pigs, an animal host of JE, have very high seroprevalence in the central Areas of Thailand. However, vector host preferences, distances of disease reservoirs from human populations, and the sanitation and mosquito control efforts centered largely around urban population of Bangkok and its vicinity may explain, in part, the lower human VE incidence in this region.

The morbidity and mortality of VE by age group, month, and province is also described. It is expected that a higher percentage of clinical disease will be found in children, given that they have less of a chance of acquired JE immunity from previous infection. Furthermore, the age distribution of case fatality should be similar to disease incidence given the severity of clinical VE, despite diverse etiologies. A pilot surveillance study has also been initiated at Ratchaburi provincial hospital in central Thailand, in order to ascertain the feasibility of future prospective studies designed to decipher the etiology of VE (proportion of JE, dengue and other pathogens) and presenting clinical signs in different ecological Thai regions.

3. Materials and methods

3.1 Data collection, fieldwork, and personal contribution

This study is based on research I proposed, initiated and completed as a Wilbur Down's International Health Travel Fellow and involves an analysis of clinically diagnosed encephalitis in Thailand from January 1993 to December 1998. I spent approximately three months in Thailand, based primarily at the Research Center for Emerging Viral Diseases of Mahidol University in Salaya (20 kilometers west of Bangkok), and used the majority of this time acquiring and converting the pre-existing viral encephalitis data files from the Thai Ministry of Public Health into an extensive GIS database. I expanded the MOPH's data to include geographic location and age group of all cases from 1993 to 1998 and the month in which each case was reported. A significant amount of time was spent converting raw test files into a fully integrated, hierarchical database. In addition, I made trips to the Thai weather and survey bureaus to acquire raw climate data for the study interval, and I incorporated the raw data into my newly created arbovirus database to create the Geographic Information System. Faculty and staff at Mahidol University played an integral role in connecting me with the appropriate government and provincial resources.

Approximately one-third of my time was devoted to regional fieldwork, including hands-on experience with the examination of children in Ratchaburi province who had been diagnosed with viral encephalitis. Trips to provincial hospitals allowed me to assess the consistency and quality of the clinical VE diagnosis and reporting methods used by the MOPH and to gain hands-on experience with the clinical evaluation and care of encephalitic disease. I also made several trips into rural areas of central Thailand to

collect adult and larval mosquitoes from local villages to provide the ever-expanding GIS database with regional vector information that was lacking from the database (Figure 3). I also made a visit to Songkhla, in southern Thailand, to help develop relations with physicians and doctors at the Prince of Songkhla University who were interested in joining future studies on the regional etiologies of clinical encephalitis throughout different geographic locales. Upon returning to the United States, I designed and carried out all statistical and data analyses discussed below on the VE GIS database using SPSS and the Arcview Spatial Analyst extension software from the Environmental Systems Research Institute. Antibody testing by the MOPH of samples from reported cases and the piloting of VE surveillance at Ratchaburi constitute the only study components not designed or implemented by myself.

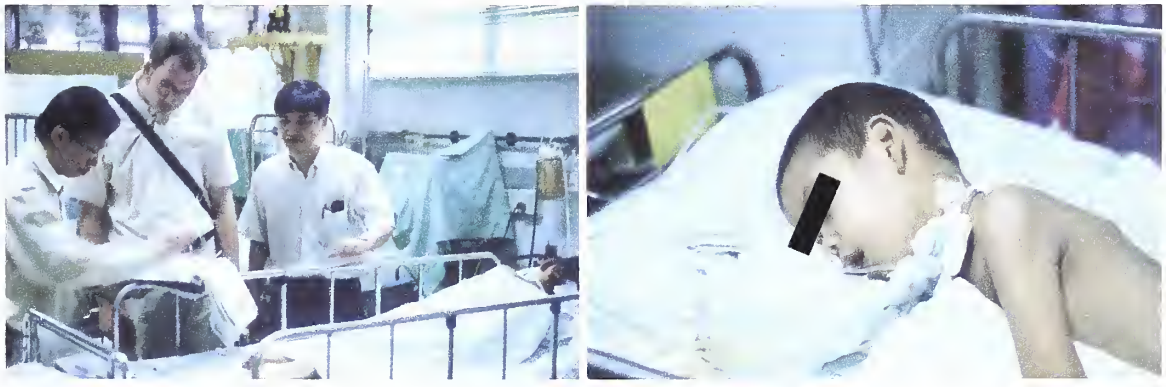

Figure 3a. Examining VE patients at Ratchaburi Hospital with Dr. Sombat Hutchaleelaha

Figure 3b. Collecting mosquito larvae from water jars and trapping live Culex sp. in a farm house.

3.2 Disease incidence, clinical definition and JE antibody testing

Monthly encephalitis data at the provincial level were provided to me by the Ministry of Public Health (MOPH), Nonthaburi, Thailand, and population data were provided by the Department of Local Administration, Ministry of the Interior, Thailand. Clinical data were collected by provincial hospital physicians and reported to the MOPH using case inclusion guidelines based on findings from the patient history, physical exam and laboratory data. Physicians actively used the criteria to rule out non-viral pathogens and non-infectious etiologies. Patient screening was based on subjective criteria such as the presence of confusion, alteration of consciousness, fever, nausea, headache, muscle spasms and seizures. In addition, a history of patient contact with herpes viruses (such as a parent with recent clinical symptoms of herpes simplex viruses type 1 or 2) and history of JE vaccination (and if so, the number of doses) were collected. Information collected from the physical exam included Glasgow Coma scores, presence of stiff neck, alteration of consciousness (defined, such as slow verbal responses, drowsiness, etc.), muscular/extremity weakness and other abnormal neurologic signs (such as a positive Kernig or Brudzinksi sign). Laboratory data collected included the complete blood count and a cerebral spinal fluid profile, including cell count and differential, protein content, and glucose content (including the ratio of cerebral spinal fluid to peripheral blood glucose). Latex agglutination tests and bacterial culture and sensitivity assays were also conducted for available cerebral spinal fluid samples. Other information was also collected at some centers looking at electroencephalograms, head CT scans and survival/morbidity outcomes, including post-recovery coma scores and physical findings.

A limitation with the reporting of VE cases is that each clinician made a diagnosis of viral infection based on a combination of data mentioned above. The availability of laboratory tests, physical findings and clinical information varied for each locale. In addition, the actual diagnosis of encephalitis was based on the clinical judgment of the practicing physician, and the inclusion criteria outlined above acted as a guideline rather than a rigid set of inclusion criteria. However, critical analysis of these data is essential given that it has been used to guide vaccination programs and disease control measures country-wide.[59] According to Whitley and Gnann[25], laboratory confirmation of the cause of brain infections is of prognostic value, but is only of therapeutic value for a limited number of pathogens. Furthermore, etiologic diagnosis is difficult, and a clinical diagnosis of a viral cerebral spinal fluid infection is often necessary for patient care and etiological investigation.

In addition to clinical identification and reporting of VE cases, selected case serum and CSF samples were sent by provincial hospital physicians to the MOPH for viral diagnosis using capture ELISA (enzyme linked immunosorbent assay) for JE and dengue IgM. IgM ELISA has been in use since the early 1980's and was shown to have a 73 percent sensitivity and an approximate specificity of 100 percent.[59] However, anti-JE IgM is known to cross-react with anti-dengue IgM, which makes it difficult to distinguish active or recent JE infection from active or recent dengue virus infection. In order to solve this problem, Innis and colleagues[60] developed an assay often used for JE infection currently that uses a ratio of anti-dengue and anti-JE antibody measurements. A ratio of anti-dengue to anti-JE IgM of less than 1.0 indicates acute JE infection, whereas a ratio of over 1.0 usually indicates acute dengue infection.[59] A 96-well plate is sensitized

overnight with goat anti-human IgM, and IgM is captured onto plates followed by 50HA JE antigen. Horseradish peroxidase conjugated anti-flavivirus and substrate are then added, which produces a measurable colorimetric result. A binding index is then defined as: [the optical density of sample minus optical density of a negative control] divided by [the optical density of weak positive control minus optical density of a negative control]. This index is then multiplied by 100; a result of 40 units or more is positive.[59, 60]

The data from these investigations were obtained, and have been made available by, the Thai MOPH and are discussed below. There were two major limitations with JE antibody testing by the MOPH however. First, samples were sent on the basis of availability and the clinicians need for a viral diagnosis, and therefore did not represent a randomized section of the clinical VE disease population. Also, because timing of sample collection was not recorded, it is impossible to determine whether patients with clinical symptoms had active or recent infection.

In order to understand the clinical manifestations and specific etiologies of VE in regional locales, I helped initiate a pilot study with the pediatrics department of Ratchaburi hospital (Ratchaburi is a western province in the Central region of Thailand). Active surveillance of children presenting to Ratchaburi hospital's pediatric ward with VE began in 2001. Patients were screened using the MOPH clinical criteria for viral encephalitis (see above) and flavivirus (JE and dengue) samples were sent to the MOPH for testing. Enrollment of patient and data collection were began by Dr. Sombat and Dr. Vitaya of Ratchaburi hospital through the summer of 2001 and will be continuing based on an active surveillance model streamlined by myself. Only six patients were able to be enrolled during the first half of 2001 during the Ratchaburi pilot study of children

presenting with clinical VE, and because of the small sample size, statistical manipulations could not be done, and trends could not be recognized. Preliminary clinical and laboratory data on these individuals is presented in Tables G and H in the appendix.

3.3 Seasonal disease variance

In order to investigate temporal variations in encephalitis cases, I designed a system for assigning months to seasonal disease groups was derived using mean values for all provinces and regions from 1993 to1998 and based on seasonal VE patterns identified in Thailand in prior studies.[3] The month with the highest number of mean cases (July) was assigned as the centroid of the high disease case group, which consisted of June, July and August. The three months with the lowest number of mean cases were assigned to the low disease group (December through February) with the remaining months being assigned to two transitional disease group's (March to May and September to November) (see Figure 4).

Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Low Disease Group 1			Transitional Group 1			High Disease Group			Transitional Group 2		

Figure 4. Visual representation of the four disease groups used in ANOVA

I used a single-factor Analysis of Variance (ANOVA) to investigate seasonal variation among the four geographic regions of Thailand. Encephalitis case data were transformed (using $\log_{10}$) to stabilize variance in the data and to adequately conform to the requirements of ANOVA. However, graphical presentations of monthly disease cases were created with non-transformed data for ease of interpretation. F values and probability of differences among means (P) were calculated for each region in order to

identify statistically significant seasonal fluctuations of disease cases. Post-hoc significance testing for each seasonal classification was adjusted using the Bonferroni procedure in order to decrease the probability of calculating random significant correlations.

3.4 Climate and geographic data and study area

Thailand is comprised of 76 provinces that are commonly divided into four geographical regions (Figures 5 and 6): north (17 provinces), northeast (19 provinces), central (26 provinces), and south (14 provinces). The northern region of Thailand is characterized by a cool, dry season (November to February) with temperatures averaging 24 °C; a hot season from March to May marked by mean a mean temperature of 29 °C; and a humid, rainy season from June to November with rainfall averaging 186 mm. In the southern, northeastern and central regions of Thailand, temperatures are more consistent, with an annual mean temperature of 28 °C, and a monsoon season lasting from May through November. Northern Thailand is a mountainous region with forested areas surrounding sporadic agricultural fields. The land cover in the northeast is predominately transplanted rice fields, field crops, and sporadic forested and mountainous regions surrounding a regional plateau. Central Thailand consists of a relatively flat plane comprised predominantly of rice fields and other agricultural crops such as sugar cane. The south is a long peninsular region with karst terrain and many palm plantations.

Geographic and climatic variables were chosen to reflect known vector patterns of arbovirus transmission discussed previously. Mean monthly temperatures, relative humidity and rainfall data were provided by the Meteorology Department, Ministry of Communications, Bangkok. Temperature and humidity data were collected from 74

weather stations and rainfall data from 583 stations located throughout the provinces. Elevation and land-use data were obtained from a national digital database compiled by the Royal Thai Survey Department and the Department of Land Development (Thailand on a Disk 1990).

Mean elevations and ratios of land- use cover were derived from raster data. Mean values for each province were obtained by averaging values of discrete grid cells interpolated from point sources of the provided raster data. The amount of agricultural land (including rice, sugar cane, beans, potatoes, fruit, etc.) as well as the more specific rice field criterion (including transplanted and broadcasted paddies) were used in the geographic analysis as ratios of the total area of each province.

I converted climate data (temperature, relative humidity and rainfall) into grid themes from point sources, and average values were extrapolated for each province for spatial and temporal analysis. The northern, northeastern, southern and central regions used in this study represent traditional political, cultural and geographic distinctions, and are grouped into north-northeastern and south-central regions.

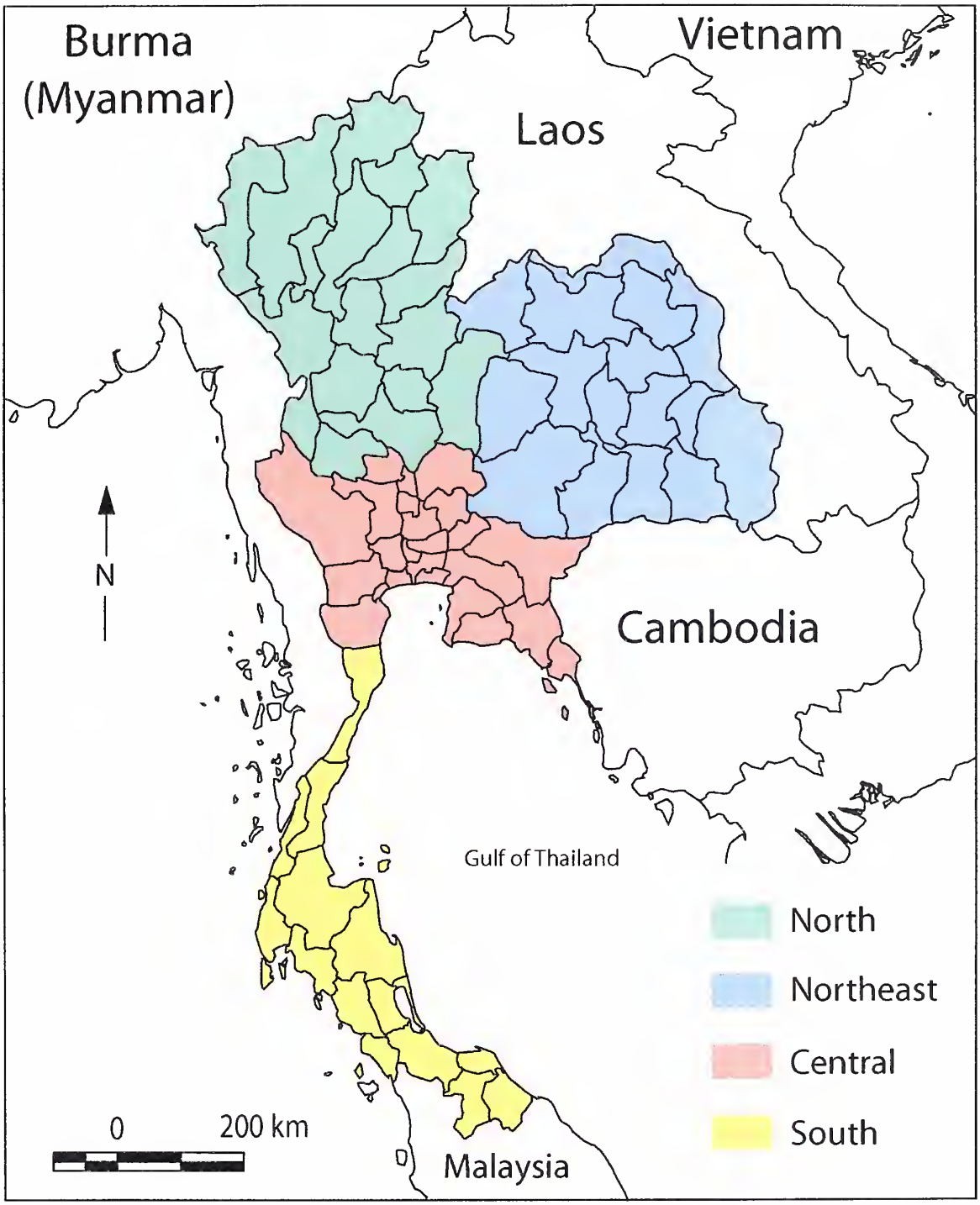

Figure 5. Map of Thailand showing individual provinces within four geographic regions.

Mountainous region typical of northern Thailand.

Rice fields and agricultural land typical of central Thailand.

Coastal area of Songkhla, southern Thailand.

Figure 6. Photographs characteristic of the northern, central and southern regions of Thailand.

3.5 Statistical analyses

Correlations of disease incidence with seasonal and provincial climatic variables were calculated using the Spearman rank method (represented by the correlation coefficient r_s). A rank correlation was chosen over linear correlation (e.g., Pearson) because a precise linear relationship between individual variables and disease incidence had not been identified. In addition, ranking continuous data enables monotonic associations to be evaluated, which is preferable when only sporadic data are available for certain areas. For example, some provinces contain only one temperature and humidity reporting station, and the individual value does not represent the entire provincial area. By ranking climatic and disease variables, only increases and decreases in the value of these data were taken into consideration; actual values were not included in correlation analyses. The rank number for the variables, rather than the variables themselves, were used. Arithmetic means of data from climate reporting stations for each province were used because further interpolation of climate data points adds an additional, unnecessary manipulation when monotonic correlations are used. In order to reduce interpolation error in analyses, provinces that had no climatic data available were excluded from each respective correlation because inclusion of provinces with no data could have lead to significant error in the estimation of climatic variations. Spatial analysis of temperature, rainfall and humidity was done using values for individual years over the study interval in order to increase sample size and statistical integrity. Elevation and land-use data used in this study were constant, and were correlated with total disease incidence from 1993 to 1998. P values were then calculated in order to determine the statistical significance of each correlation, using an alpha level of 0.05.

3.6 Building the GIS database

The disease and geographic variables used in this analysis were integrated into a geographic information system to allow for the graphical and statistical representation of linked data files. Provincial level data interpolation, smoothing and graphical representation were done using ArcView software (version 3.2, Environmental Systems Research Institute) with the Spatial Analysis software extension. An ArcView GIS system involves a project, or the parent file that stores views, tables, charts or layout attributes. A project view allows the researcher to display, overlay or compare one or more themes. A theme is simply a variable that has been transformed into spatial or mapped data. Spatial themes used in this analysis included temperature, disease incidence, relative humidity, rice paddy cover, agricultural land cover. Tabular data (without spatial mapping) may also be included in the project, and the variables can be linked for ease of statistical analysis (see above). Charts may also be produced to represent data in a non-spatial form as well. The layout function of a project enables different views, tables and charts to be presented in one displayable or printable form. (See Figure 7 for a schematic of the ArcView/ArcInfo project.)

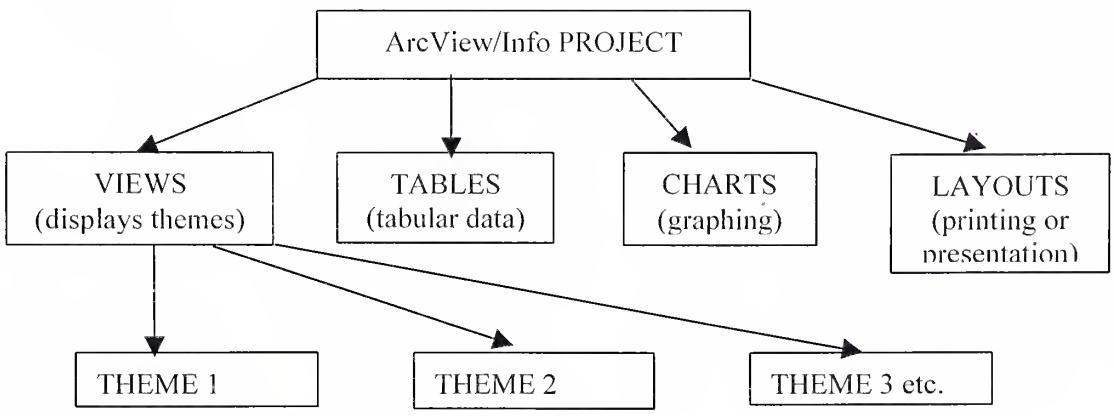

Figure 7. Schematic diagram of an ArcView/Info project showing how multiple data themes can be created and viewed from linked data (in multiple tabular and visual formats).

4. Results

4.1 Temporal analysis and disease incidence

From January 1993 to December 1998, 3,829 cases of clinically diagnosed VE were reported to the Thai Ministry of Public Health (788, 752, 584, 516, 585, and 604 for consecutive years beginning in 1993). Given that the change in incidence (cases per 100,000 persons) from year to year is very small, there is no significant change in the long-term trend of VE incidence during the six year interval (Figure 8). Of the 3,829 cases, 3,777 included the month in which the disease was recorded; the latter were used in temporal correlation analysis. The number of new cases for each month by region is listed in Table A.

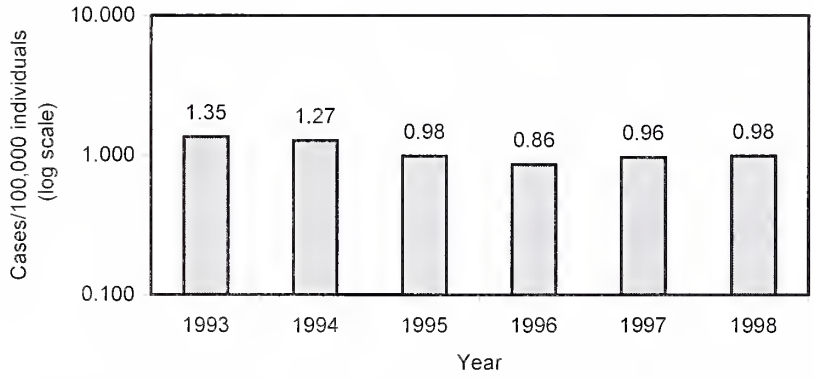

Figure 8. Semi-log plot of incidence of clinically diagnosed viral encephalitis in Thailand, 1993-1998. The number above each year indicates disease incidence rate (cases/100,000).

3,810 cases included an age group in which the disease was recorded, and the number of new cases for each group by region is listed in Table B. VE in Thailand is more common in children and teenagers than in the adult population, which is consistent with previous research on age distribution of JE. This age distribution makes VE a particularly important pediatric infectious disease in southeast Asia.

Table A. Number of new clinical VE cases per month by geographic region, 1993 - 1998.

Region	Month[a]												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
North	59[b]	40	53	95	96	135	186	172	92	94	77	53	1152
	(0.49)[c]	(0.33)	(0.44)	(0.79)	(0.80)	(1.13)	(1.55)	(1.44)	(0.77)	(0.78)	(0.64)	(0.44)	(9.62)
Northeast	74	78	91	91	90	143	157	128	110	112	72	52	1198
	(0.36)	(0.38)	(0.44)	(0.44)	(0.43)	(1.69)	(0.76)	(0.62)	(0.53)	(0.54)	(0.35)	(0.25)	(5.77)
N/NE Total	133	118	144	186	186	278	343	300	202	206	149	105	2350
	(0.41)	(0.36)	(0.44)	(0.57)	(0.57)	(0.85)	(1.05)	(0.92)	(0.62)	(0.63)	(0.45)	(0.32)	(7.17)
Central	51	62	74	75	67	89	100	75	72	170	61	46	879
	(0.27)	(0.33)	(0.39)	(0.39)	(0.35)	(0.47)	(0.53)	(0.40)	(0.38)	(0.56)	(0.32)	(0.24)	(4.62)
South	63	51	44	32	39	48	50	44	49	50	47	31	548
	(0.81)	(0.66)	(0.57)	(0.41)	(0.50)	(0.62)	(0.64)	(0.57)	(0.63)	(0.64)	(0.60)	(0.40)	(7.05)
S/C Total	114	113	118	107	106	137	150	119	121	157	108	77	1427
	(0.43)	(0.42)	(0.44)	(0.40)	(0.40)	(0.51)	(0.56)	(0.44)	(0.45)	(0.59)	(0.40)	(0.29)	(5.33)
Total	247	231	262	293	292	415	493	419	323	363	257	182	3777
(all regions)	(0.41)	(0.39)	(0.44)	(0.49)	(0.49)	(0.70)	(0.83)	(0.70)	(0.54)	(0.61)	(0.43)	(0.31)	(6.34)

[a]Each column represents data for all years 1993-1998.
[b]Total number of new cases per month during the study interval.
[c]Number of new cases per 100,000 population per month during the study interval (calculated using the mean regional population for the years 1993-1998).

Table B. Number of new clinical VE cases according to age group and geographic region, 1993 - 1998.

Region	Age Group[a]												
	<1	1-2	3-4	5-6	7-9	10-14	15-24	25-34	35-44	45-54	55-64	65+	Total
North	32[b]	94	93	122	145	242	158	122	90	32	35	31	1196
	(0.27)[c]	(0.78)	(0.78)	(1.02)	(1.21)	(2.02)	(1.32)	(1.02)	(0.75)	(0.27)	(0.29)	(0.26)	(9.99)
Northeast	60	81	86	93	143	225	156	126	86	68	47	27	1198
	(0.29)	(0.39)	(0.41)	(0.45)	(0.69)	(1.08)	(0.75)	(0.61)	(0.41)	(0.33)	(0.23)	(0.13)	(5.77)
N/NE	90	175	179	215	288	467	314	248	176	100	82	58	2394
Total	(0.28)	(0.53)	(0.55)	(0.66)	(0.88)	(1.43)	(0.96)	(0.76)	(0.54)	(0.31)	(0.25)	(0.18)	(7.31)
Central	52	66	59	84	108	180	103	88	57	25	29	27	878
	(0.27)	(0.35)	(0.31)	(0.44)	(0.57)	(0.95)	(0.54)	(0.46)	(0.30)	(0.13)	(0.15)	(0.14)	(4.62)
South	29	37	42	43	78	110	78	45	23	19	15	19	538
	(0.37)	(0.48)	(0.54)	(0.55)	(1.00)	(1.42)	(1.00)	(0.58)	(0.30)	(0.25)	(0.19)	(0.25)	(6.93)
S/C Total	81	103	101	127	186	290	181	133	80	44	44	46	1416
	(0.30)	(0.39)	(0.38)	(0.47)	(0.70)	(1.08)	(0.68)	(0.50)	(0.30)	(0.16)	(0.16)	(0.17)	(5.29)
Total	173	278	280	342	474	757	495	381	256	144	126	104	3810
	(0.29)	(0.47)	(0.47)	(0.57)	(0.80)	(1.27)	(0.83)	(0.64)	(0.43)	(0.24)	(0.21)	(0.18)	(6.40)

[a]Age group in years of age
[b]Total number of new cases for each age group during the study interval.
[c]Number of new cases per 100,000 population during the study interval (calculated using the mean regional population for the years 1993-1998).

Figure 9 shows that distribution of VE cases clinically identified from 1993 to 1998 in Thailand for 16 different age groups (data from the Ministry of Public Health, Nanthaburi, Thailand). During the study interval, 61 percent of all encephalitis cases were in children under the age of 15. The chances of presenting with VE significantly decreases with age; the lowest risk groups are those over 45 years of age. There were no discernable regional differences between age groups.

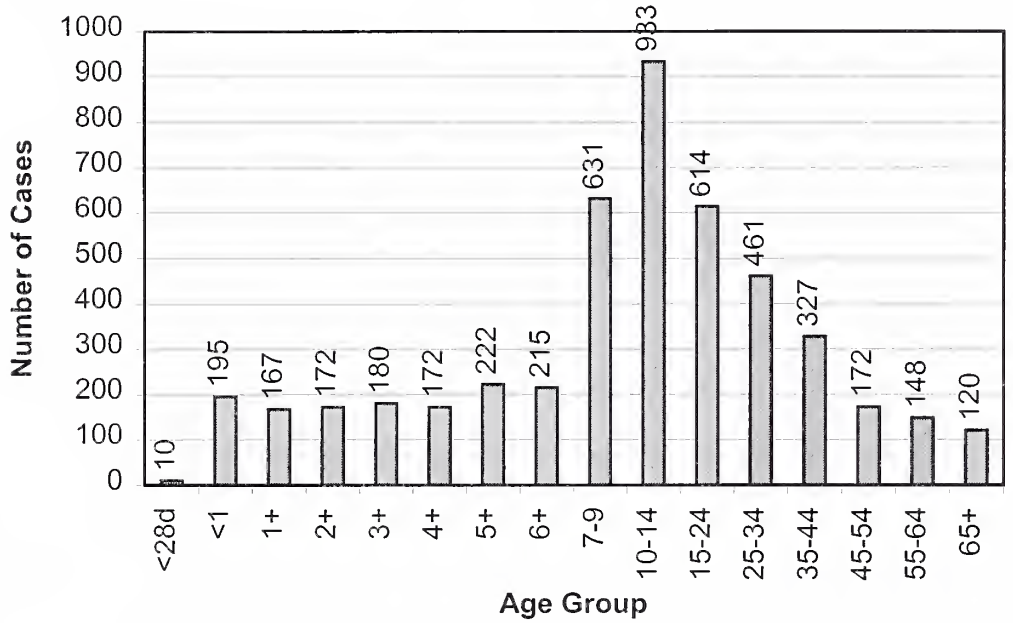

Figure 9. Age distribution of clinically presenting VE in Thailand, 1993-1998.

From 1993 through 1998, the greatest number of newly diagnosed, clinically presenting VE cases in Thailand were recorded during July (493 new cases during the six-year interval), with the lowest number recorded during December (182 new cases). However, when the number of new cases for each month is examined for the four separate Thai geographic regions, different regional temporal fluctuations of disease cases are seen (Figure 10). The number of new cases peaked in July (186) and was lowest

in December (53) and March (53) for the northern region. The number of new cases also peaked in July (157) and was lowest in December (52) for the northeast region. However, the south and central regions exhibited a different pattern of disease, with the greatest number of cases reported from the months of October (107) in the central region and January (63) in the southern region. Encephalitis incidence in the north and northeast regions had remarkable seasonality with similar patterns for both regions, whereas no seasonality was noted for the south and central regions (Figure 10). Because of the similarity in seasonal distribution, north and northeast regions were grouped together for subsequent temporal and spatial analysis, as were south and central regions.

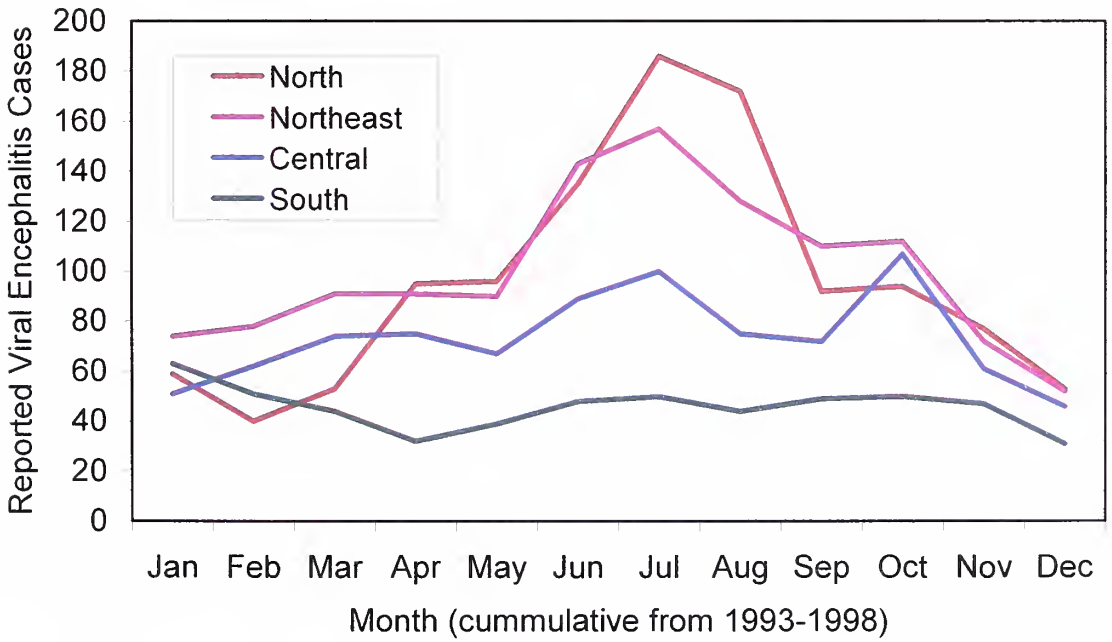

Figure 10. Number of reported viral encephalitis cases by month from 1993 through 1998.

Table C. Monthly, seasonal encephalitis disease group analysis of variance by region from 1993-1998.

	Seasonal Group ANOVA[a]	
Region	F[b]	p value
North	**21.928**	**< 0.001**
Northeast	**10.293**	**< 0.001**
Central	**4.299**	**0.008**
South	0.879	0.457
North/Northeast	**29.111**	**< 0.001**
South/Central	1.81	0.154

[a]Single factor ANOVA was performed using the four seasonally defined encephalitis incidence case groups (high, low, two transitional) with $\log_{10}$ data transformations.
[b]F = Fisher test ratio.

ANOVA of monthly groupings of encephalitis cases revealed significant mean differences between the four seasonal groups (high, two transitional, low) in the northern, northeast, and central regions of Thailand that validate the observations stated above. The north-northeastern compiled area had a significant mean difference between seasonal groups ($F = 29.111, p < 0.001$), whereas the south/central compiled area had no significant mean difference between groups ($F = 1.810, p = 0.154$). Table C lists the complete ANOVA results for the four geographic regions and two compiled areas.

Post-hoc testing revealed that there were significant differences between high and low seasonal incidence groups in northern and northeast Thailand, and that the low and high seasonal groups were significantly different from both transitional groups which were not significantly different from each other (pos-hoc testing results are in Table D).

Table D. Post-hoc test significance between monthly, seasonal disease group analysis of variance by region, 1993-1998.

Geographic Region	Group[a]	Group	Significant Difference[b]	*p* value[c]
North	Low	Trans. 1	**Yes**	**0.036**
		High	**Yes**	**<0.001**
		Trans. 2	**Yes**	**0.002**
	Trans. 1	High	**Yes**	**<0.001**
		Trans. 2	No	1.000
	High	Trans. 2	**Yes**	**<0.001**
Northeast	Low	Trans. 1	No	0.115
		High	**Yes**	**<0.001**
		Trans. 2	No	0.114
	Trans. 1	High	**Yes**	**0.015**
		Trans. 2	No	1.000
	High	Trans. 2	**Yes**	**0.016**
Central	Low	Trans. 1	No	0.143
		High	**Yes**	**0.010**
		Trans. 2	**Yes**	**0.033**
	Trans. 1	High	No	1.000
		Trans. 2	No	1.000
	High	Trans. 2	No	1.000
South	Low	Trans. 1	No	1.000
		High	No	1.000
		Trans. 2	No	1.000
	Trans. 1	High	No	1.000
		Trans. 2	No	0.763
	High	Trans. 2	No	1.000

[a]Seasonally defined encephalitis incidence groups include Low (December-February), Transitional 1 (March-may), High (June-August), Transitional 2 (September-November).
[b]Significant differences between groups calculated from Bonferroni adjusted disease group mean differences.
[c]Significant at the 0.05 alpha level.

In the central region, however, a significant mean difference was obtained only between the high and low seasonal group and the low and the two transitional seasonal groups. There was no mean difference between the high incidence and either transitional group, which indicates that there is no statistically defined temporal variation in encephalitis cases in the nine-month interval from March to the end of November. As expected from the ANOVA, there were no post-hoc mean differences between groups in the southern region and south-central combined area (Table D).

Visual inspection of disease cases, temperature, and relative humidity graphs by month from January 1993 to December 1998 suggests that there is an association between climate and disease in the north-northeast combined areas of Thailand (Figures 11, 12). The relationship between climatic variables and encephalitis cases is not precisely linear, however; peaks in monthly temperature precede peaks in disease by two months which, in turn, precede peaks in monthly relative humidity by two months.

Unlike the north-northeast combined areas, there is no seasonal variation in disease cases in south-central Thailand; the plots of monthly encephalitic cases look more like random noise than a repeating temporal pattern. However, there are mild seasonal fluctuations in mean temperature and relative humidity which suggests that the epidemiology of disease in south-central Thailand is different than in the north-northeast regions (see Figures 13, 14).

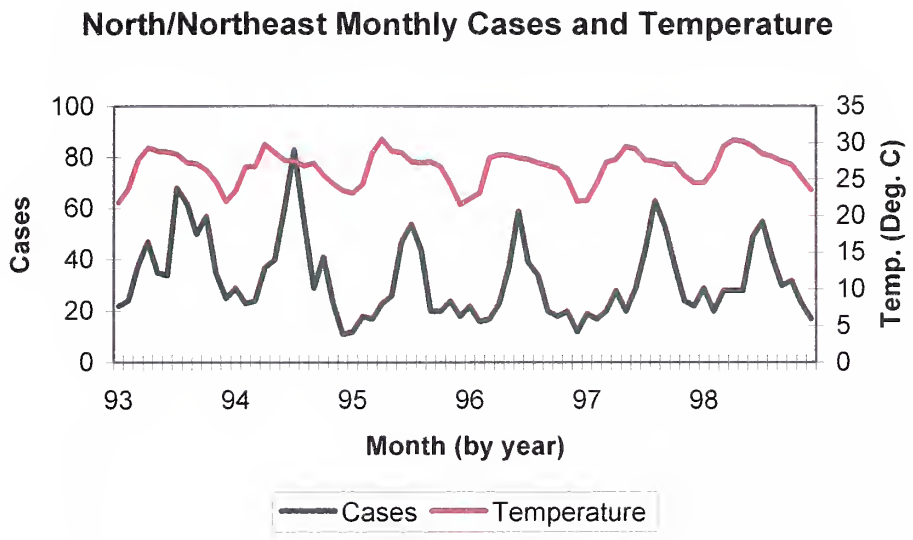

Figure 11. Time series plots of encephalitis cases and monthly temperature in north-northeast Thailand, 1993-1998.

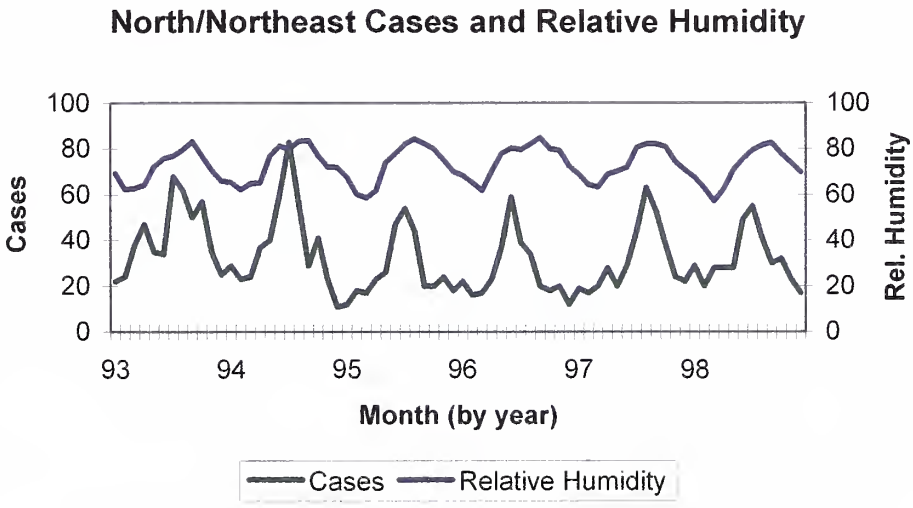

Figure 12. Time series plots of encephalitis cases and relative humidity in north-northeast Thailand, 1993-1998.

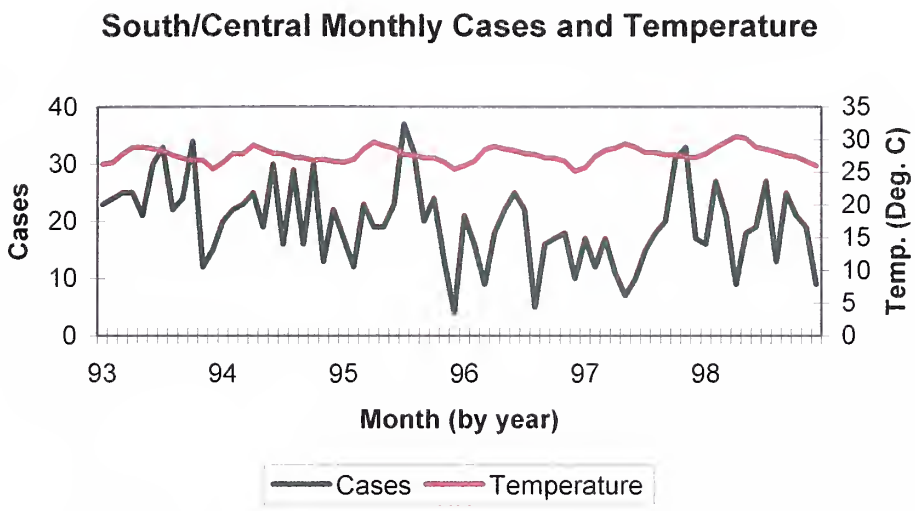

Figure 13. Time series plots of encephalitis cases and monthly temperature in north-northeast Thailand, 1993-1998.

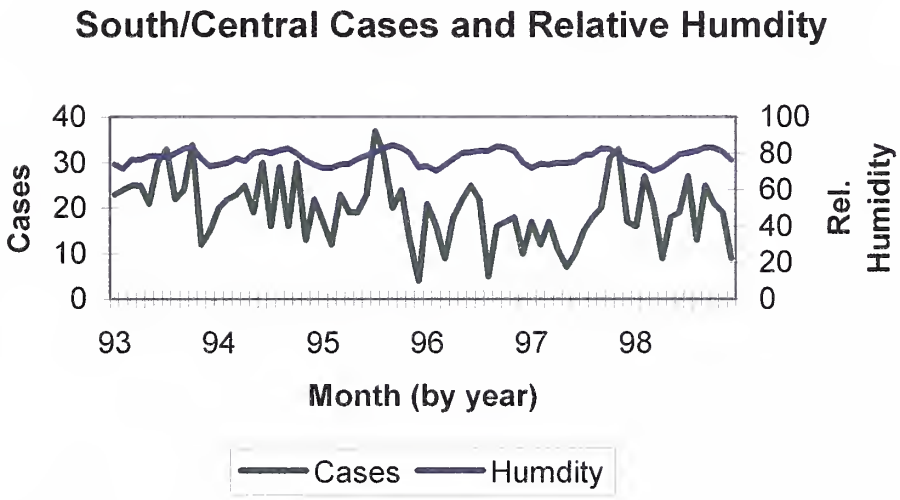

Figure 14. Time series plots of encephalitis cases and relative humidity in south-central Thailand, 1993-1998.

Correlations of disease cases with mean monthly temperature, relative humidity and monthly rainfall were performed for each region as well as for the combined north-northeast and central-southern areas (r_s values for combined data are listed in Table E). Statistically significant positive correlations between climate variables (temperature, humidity and rainfall) and disease cases were obtained in the north-northeast area of Thailand, whereas only one significant correlation, between humidity and encephalitis cases, was observed in the central-southern area.

Table E. Temporal (monthly) correlation of climate variables and encephalitis cases for the north-northeast and south-central areas of Thailand, 1993-1998.

Climate Variable	Disease Correlation			
	North-Northeast		Central-South	
Temperature	**Yes**[a]	**(r_s = 0.460, P < 0.001)**[b]	No	(r_s = 0.114, P = 0.340)
Relative Humidity	**Yes**	**(r_s = 0.525, P < 0.001)**	**Yes**	**(r_s = 0.343, P = 0.003)**
Rainfall	**Yes**	**(r_s = 0.617, P < 0.001)**	No	(r_s = 0.174, P = 0.145)

[a]Spearman correlation of monthly disease cases significant at the 0.05 alpha level, yes = statistically significant, no = not statistically significant.
[b](r_s = Spearman rank correlation coefficient, P value)

4.2 Spatial variation and geographic correlation

The following maps (Fig. 15) represent the incidence of VE in Thailand by province, 1993-1998. It is evident from Figure 14 that the prevalence of VE is greater in the northern region of Thailand and less in the northeast and central areas. The incidence of clinical cases within each province is fairly consistent for each year from 1993 to 1998. The spatial distribution of disease on a provincial level is unique, and correlations

between encephalitis incidence and geographic and climatic variables reveal regional patterns.

Figure 15. Incidence of viral encephalitis in Thailand, 1993-1998.

Correlations between disease incidence of each province and geographic variables, including elevation, annual rainfall and land-use, differed between the north-northeastern and south-central areas of Thailand. Spearman correlation coefficients (r_s) with the associated P values are listed in Table F. A moderately strong positive monotonic correlation was observed for elevation and disease incidence in the north-northeast, whereas no significant correlation was found for the south-central region. A significant negative monotonic correlation was observed for agricultural land-use and rice field cover and disease incidence in the north-northeast. No significant correlations between disease incidence and land-use (agricultural land and rice field cover) were identified in the south-central region. These associations suggest that a higher incidence of clinically diagnosed VE in the north-northeast region is associated with a higher elevation, lower temperature and smaller amount of rice field cover and agricultural land.

Analysis of climate variables revealed a weak negative correlation between mean annual temperature and disease incidence at the provincial level over the six year study interval in the north-northeast region and no significant correlation in the south-central region. No significant correlations were obtained for relative humidity in the north-northeast and south-central regions or for rainfall at the provincial level.

Table F. Spatial correlation of geographic variables and encephalitis incidence for the north-northeast and south-central areas of Thailand, 1993-1998.

Geographic Variable	Disease Correlation			
	North-Northeast		Central-South	
Elevation	**Yes**[a]	**(r_s = 0.689, P < 0.001, N = 36)**[b]	No	(r_s = 0.218, P = 0.176, N = 40)
Rice Field Cover	**Yes**	**(r_s = -0.534, P < 0.001, N = 36)**	No	(r_s = -0.283, P = 0.077, N = 40)
Agricultural Land	**Yes**	**(r_s = -0.617, P < 0.001, N = 36)**	No	(r_s = -0.264, P = 0.100, N = 40)
Temperature	**Yes**	**(r_s = -0.218, P = 0.004, N = 168)**	No	(r_s = -0.133, P = 0.148, N = 120)
Relative Humidity	No	(r_s = 0.070, P = 0.377, N = 162)	No	(r_s = 0.169, P = 0.065, N = 120)
Annual Rainfall	No	(r_s = -0.080, P = 0.260, N = 198)	No	(r_s = 0.032, P = 0.632, N = 232)

[a]Spearman correlation of regional disease incidence (cases/100,000 population) significant at the 0.05 alpha level, yes = statistically significant, no = not statistically significant.

[b](r_s = Spearman rank correlation coefficient, *P* value, N = number of provinces included in each analysis multiplied by the number of years in the study interval.)

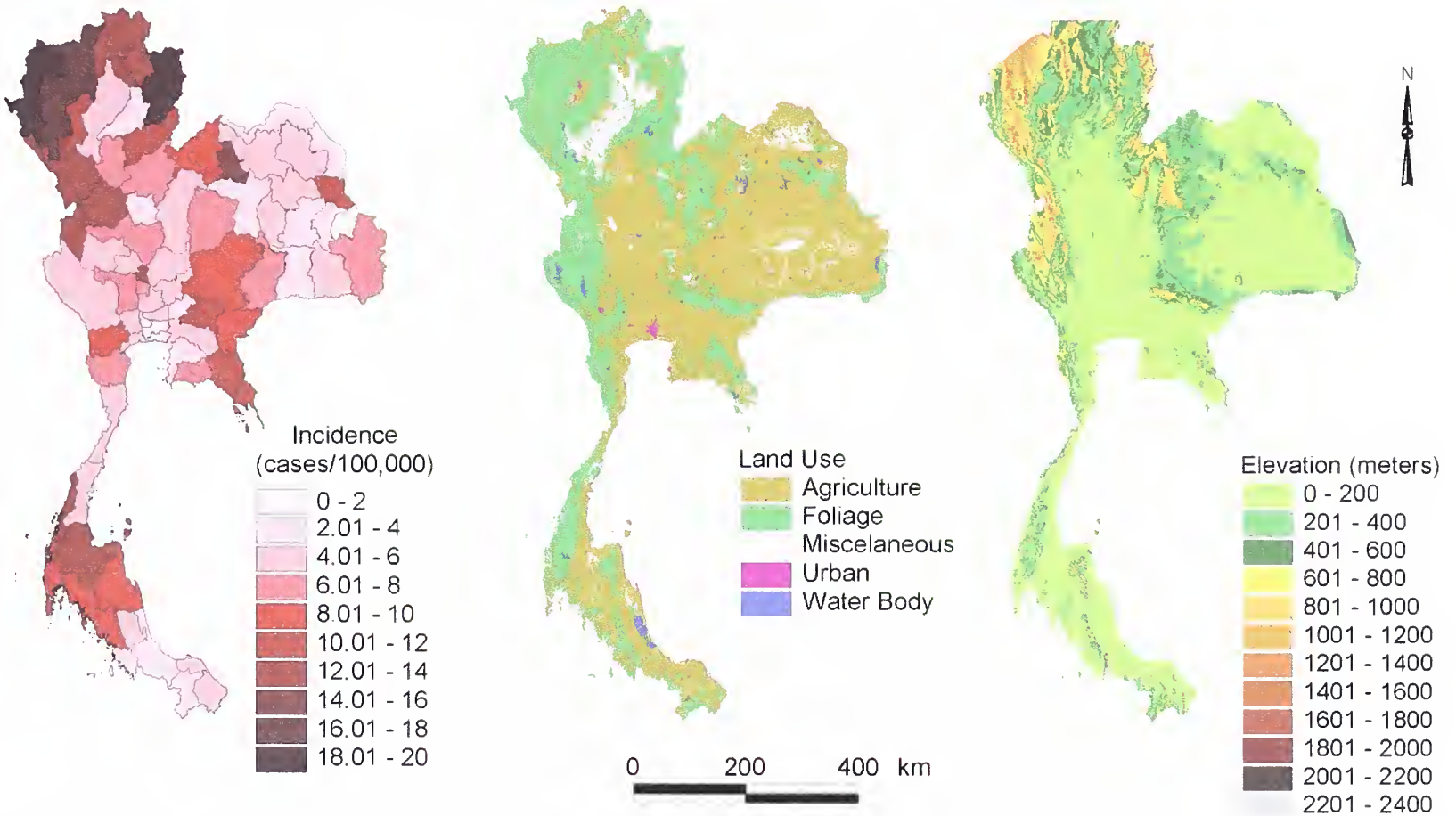

Figure 16. Maps of clinically diagnosed viral encephalitis incidence (cases/100,000 population) in Thailand by province, 1993-1998. Corresponding geographic criteria shown include land-use and elevation.

4.3 VE mortality

Over 400 deaths resulted from clinically presenting encephalitis from 1993 to 1998 in Thailand, and deaths are more common in children and teenagers than in the adult population. A similar percentage of infected individuals die in all age groups. For example, 58 percent of all encephalitis cases were in children under the age of 15. The chance of dying from VE significantly decreases with age, and the lowest risk groups are those over 45 years of age. Figure 17 shows the distribution of VE deaths from 1993 to 1998 in Thailand for 16 different age groups (data from the Ministry of Public Health, Nanthaburi, Thailand).

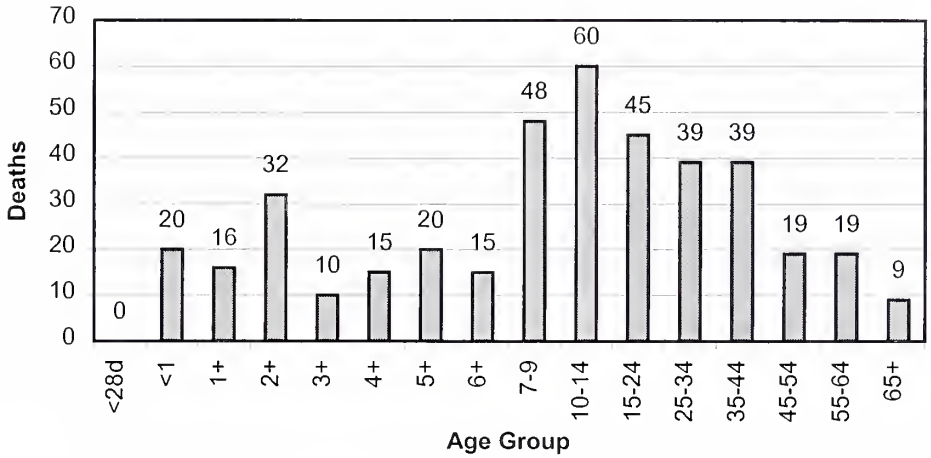

Figure 17. Age distribution of VE deaths in Thailand, 1993-1998.

Statistical analysis shows that there is no regional variation in disease mortality when the deaths per infected individuals are calculated. However, some provinces had very high average VE case fatality rates from 1993 to 1998. For example, Amnat Charoen (50%), Phuket (46%), Phrae (29%), Chonburi (28%), Buri Ram (26%), Pathum Thani (24%), Kanchanaburi (24%), Uthai Thani (22%), Rayong (21%), and Champon (20%) provinces all have fatality rates equal to or greater than 20 percent.

Figure 18 shows the deaths/cases for each province in Thailand from 1993-1998:

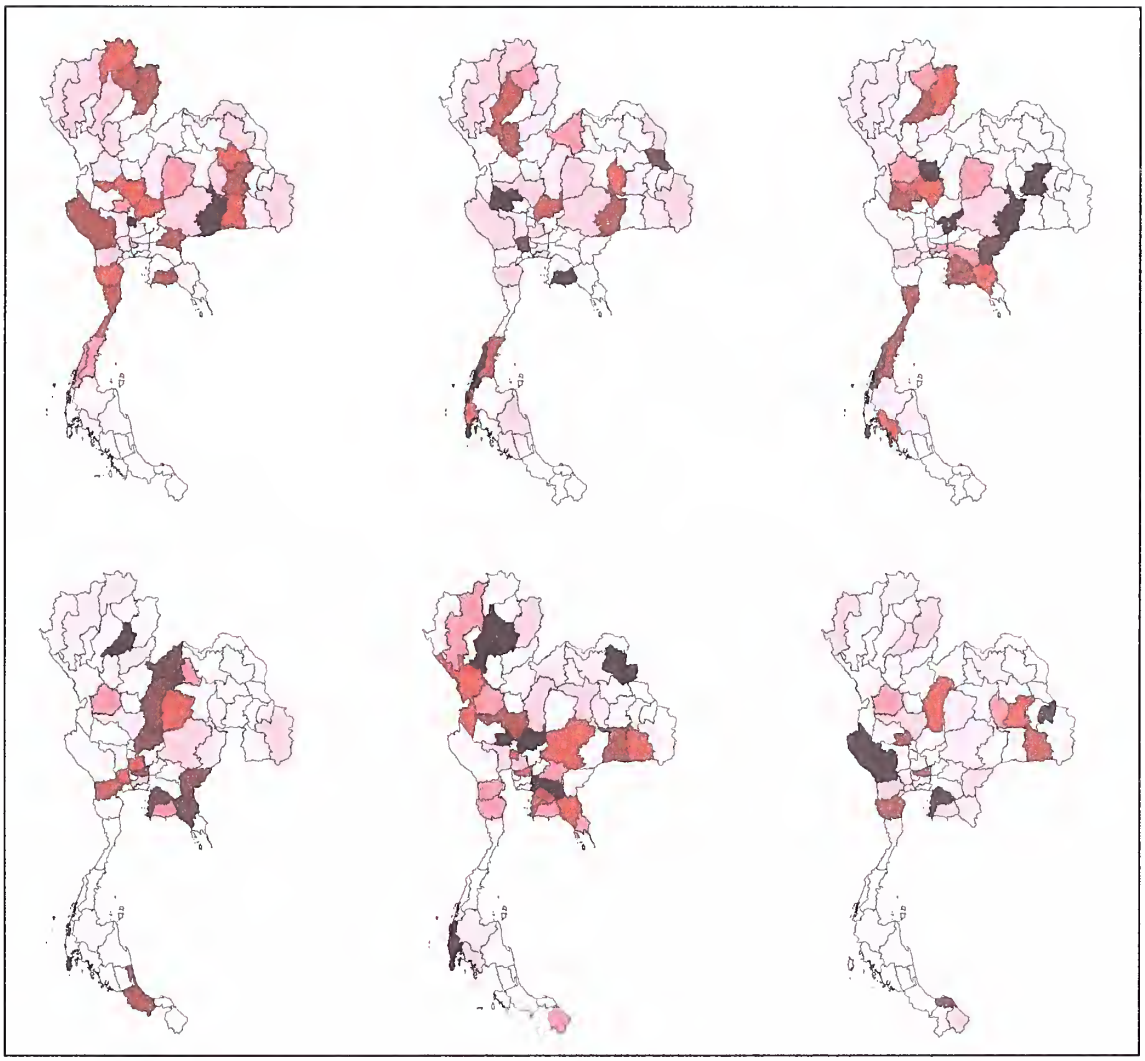

Figure 18. Deaths/infected individuals from viral encephalitis in Thailand, 1993-1998.

The spatial distribution of case fatality is very different from the incidence of clinical disease (see Figure 16); a lower mortality is seen in the northern Thailand, the area with the highest number of new cases each year. These mortality rates are alarming and

indicate that VE remains a serious, lethal disease in southeast Asia. The unique differences between disease incidence and mortality, as well as the serious nature of viral encephalitic illnesses, necessitate future epidemiological surveillance and etiologic investigation in order to reduce human deaths from VE.

5. Discussion

This is the first study to show large-scale, provincial associations between ecological variables and clinical viral disease incidence of VE. In addition, this study shows that traditional concepts of ecological variables such as rice-field cover, temperature, relative humidity and rainfall do not necessarily correspond to the spatial distribution of VE that prior statistical models might predict. Rather, inverse relationships between disease incidence and the proportion of rice-field cover, agricultural land, and temperature suggest that there are etiologic, climatic, host-immunity, and many other factors that influence human disease incidence. Prior studies have predominately focused on how specific viral vectors (e.g. mosquitoes) respond to specific environmental stimuli, but are not sufficient to explain temporal and spatial disease variations.

This study also highlights the importance of implementing relatively simple methodology that can be applied to the clinical syndrome of encephalitis, in order to understand the full scope of the temporal and spatial distribution of clinically diagnosed disease. For example, the monotonic correlations with mean monthly temperature and relative humidity indicate an association between disease incidence and climate. However, these correlations may only be valid in regions where disease and climate exhibit significant seasonal fluctuations. Fluctuations in temperature, relative humidity and rainfall in the southern and central regions of Thailand are smaller than in the north and northeast, with temperatures reaching 27 °C during the cooler months (December through February).

Although deriving seasonal correlations and linear regression models is useful in helping researchers understand annual fluctuations in disease incidence, special care must be taken when extrapolating from these models to explain other trends. This is especially true when applying seasonally derived associations to explain large-scale disease prevalence or long-term patterns. For example, temporal associations of disease, mean temperature, relative humidity and rainfall are not valid at the national level and provincial scale because there are no strong, significant correlations with climate in the north-northeastern or south-central areas of Thailand. However, the lack of correlation at a provincial and regional level does not necessarily undermine the internal validity of seasonal analyses, although statistical manipulation should be done with caution.

In addition, some researchers prefer to smooth spatially mapped data using a Bayesian model.[61, 62] The Bayesian smoothing principal uses mathematical manipulations based on Bayes theorem in order to decrease border bias. Border bias refers to the fact that data collected by province or other political areas are grouped into artificially drawn areas that have no connection to the disease process being studied. Because areas with "free edges" (that is, edges not touching other artificially contrived areas) are not influenced by surrounding data, some claim that a bias is created in these areas that either underestimate or overestimate the disease incidence in statistical analysis. Beysian models can correct for these boundary biases and take into account the possibility that neighboring districts may be more alike than distant ones. However, this method of data smoothing is artificial in itself and may have its own confounding influence on analysis. In addition, many clinicians are unfamiliar with these complex

techniques and may not know how to interpret manipulated data. I prefer to keep spatial data as unrefined as possible.

The seasonal correlations of climate and disease in this study are moderate at best, and no one variable is completely adequate in explaining temporal patterns of infection. Multivariable linear analyses have been used to create predictive models using climate data, but information provided from these studies does not shed light on causation of disease. In addition, complex statistical analyses and seasonal correlations have not been very useful in predicting epidemics, and there is a need for statistical analyses that can be easily interpreted and applied by the health care community.

Geographic analysis of elevation and land use provides insight into the regional variations of VE in Thailand. The positive correlation of elevation and negative correlation of both agricultural field and rice paddy cover with disease incidence in the northern and northeastern regions suggest that a higher incidence of VE is associated with higher altitude and a smaller density of agricultural land cover. These findings are surprising given the increased prevalence of JE-carrying mosquitoes during the rainy season found in previous studies of central Thailand. No geographic or climatic variables exhibit significant correlation with disease in the south-central area of Thailand, which suggests that the spatial patterns of encephalitic disease between the northern-northeastern and southern-central areas are different.

Several explanations for these unique ecological variations in VE in Thailand may be postulated, even though concrete data do not exist at this time to validate these theories. It is likely that the amount of contact between animal reservoirs of flavivirus such as JE (*e.g.* pigs and chickens), the mosquito vectors, and humans is greater in the

more rural northern and northeastern regions of the country (Figure 18). Greater interaction between disease carrying vectors (*i.e.* number of insect bites) and their human hosts would explain a higher proportion of new cases. In addition, agricultural methods common in the border areas of northern and northeastern Thailand, but uncommon in the more populated central and southern regions, may also contribute to the unique disease patterns. For example, slash-and-burn farming (Figure 19) is practiced much more frequently along northern border areas, associated with higher densities of ethnic minority groups. The slash-and-burn farming methods are likely associated with a decreased use of molluskacides (to kill schistosomal carrying snails) that are ubiquitous in the more industrialized central region of Thailand. These molluskacides are thought to be harmful to developing mosquito larvae, although specific studies have not yet been carried out to prove this hypothesis (Nitatpattana, personal communication 2001).

The lower incidence of human VE cases in central and southern regions of the country conflict somewhat with the previously mentioned animal study by Gingrich and colleagues that showed a very high JE prevalence and seroconversion rate in pigs in the areas surrounding Bangkok.[13] That study did not look at human cases, however, and the greater seroprevalence of pig to human cases may be partially explained by vector host preference in addition to the geographic differences in human--animal proximity discussed above. For example, Mwandawiro *et al.* identified heterogeneity in vector preferences of JE hosts in Chiang Mai, Thailand.[63] They discovered that mosquitoes with previous exposures to either cows or pigs would selectively feed on the animal they were originally exposed to when given and choice of pigs or cows in a controlled setting. For example, mosquitoes originally exposed to pigs would selectively feed on pig blood

when given a choice between pigs and cows. It is not known, however, what impact this mosquito preference has on the geographic distribution of human disease, but mosquitoes first raised in contact with pig or bird populations may selectively feed on these hosts rather than on the human population.

Figure 18. Typical rural Thai household and farm. The chicken coups and adjoining wall of the household's pigsty are shown on the right. The animals (flavivirus reservoirs) are kept directly adjacent to the stilted house.

Figure 19. The photograph on the left shows slash-and-burn farming typical of the northern border areas. Note the proximity of the house to standing water (mosquito spraying and control measures are more sparse in these areas). This technique contrasts with the broadcasted and transplanted rice paddy farming technologies practiced in more industrialized areas of the country (right), where aquatic molluskacides are commonly used to control schistosomiasis.

Extremes in temperature and humidity may also have contributed to the unique spatial distribution of VE seen in this study; the overall greater amount of rainfall and extreme high temperatures may decrease larval survival in central and southern Thailand, and therefore decrease disease carrying adult mosquitoes. It has been previously reported[47] that temperatures exceeding 30 degrees C are associated with a lowering of mosquito density, and this may have contributed to the decreased overall incidence of disease in central and southern Thailand. However, it has been previously postulated that number of adult mosquitoes may be increased through an increased survivorship resulting from shortened larval breeding periods associated with greater temperatures.[3] In addition, large amounts of rainfall may also decrease mosquito vector abundance by "flushing out" their breeding sites.[3] Of course, extremely low amounts of rainfall are associated with decreased mosquito breeding as well, given that the larval stage needs a certain amount of standing water to survive. A limitation of the ranked correlation analysis used in this study is that extremes of temperature were treated as outliers in the statistical calculations.

It may be postulated that vector abundance and human bite activity are greatest when temperature and humidity are high, but not when either are at ecological extremes. The time series plots (Figures 11, 12) of temperature, relative humidity and disease cases in northern-northeastern Thailand show an overall positive correlation with the seasonal (not spatial) increases in temperature and humidity and human disease. However, there is a slight time-lag between the peaks of disease and peaks of high temperature and humidity. More specifically, the number of encephalitis cases peaks slightly after the peaks in temperature and slightly before the times of maximum relative humidity. This

suggests that disease incidence falls off just after the highest temperatures are recorded, and the time-lag may reflect decreased vector abundance with extreme temperatures. On the other hand, the adult vector population may peak shortly before extremes of humidity.

The time-lag may also represent a natural delay between intervals of mosquito development and the clinical presentation of human disease. More specifically, the two-month difference between peaks in temperature (which is associated with higher mosquito survival and higher vector infectivity of VE) and peaks in human disease, may be explained by the fact that there are several weeks required for potential vectors to undergo maturation, reach maximal infectivity, and be exposed to the human population. For example, studies of *Aedes aegypti* have shown that the average time from larval deposition to adult maturation is approximately 7.2 days at 35 degrees C with a 93% percent survival rate.[45] Furthermore, studies of *Culex* mosquitoes have shown that the period of time between adult maturation to maximal vector viral load of West Nile virus in the vector population is approximately 11 to 15 days at 26-30 degrees C.[43]

In addition mosquito maturation and infectivity, the two-month time-lag is also influenced by the incubation period of disease in humans. Flaviviruses that cause VE (JE, dengue, West Nile) all have approximately one to two week incubation periods that are often asymptomatic or accompanied by mild, generalized symptoms such as malaise. Given the five-week period required for vector maturation, infectivity and incubation in the human population, it may be predicted that the peaks of human disease would follow peaks in ideal ecological settings of disease by over one month, which are seen in this study. The precise timing of vector maturation and onset of human cases is not completely understood in Thailand, and this retrospective study is unable to provide

sufficient information as to the direct relationship between vectors and disease. In addition, a significant portion of VE is not caused by arboviruses, which confounds the precise temporal relationships of disease; this will be discussed in detail later.

There may also be significant differences between the seasonal lifecycle of JE-carrying vectors in northern Thailand and the warmer southern and central regions. Several theories have been proposed to explain these differences. For example, mosquito populations may decrease during the colder months in the north, and JE may be seasonally reintroduced from warmer areas in the south which have year-round *Culex* abundance.[64] Other theories suggest that JE virus persists in vertebrate and invertebrate hosts.[64] However, lack of temporal disease variation in southern and central Thailand cannot be explained by climatic factors alone. For example, seasonal fluctuations in JE-infected mosquitoes seen in a previous vector study in the central provinces of Bangkok and Kanchanaburi do not correspond with the lack of seasonal disease variation in the regions observed in this study.[13]

A limitation of using clinical data with limited etiologic information is that the regional differences in viral etiology that may explain, in part, the spatial and temporal patterns of encephalitis observed in the northern and southern areas of Thailand are not known. JE has traditionally been treated interchangeably with clinically presenting VE in Thailand. However, laboratory testing by the MOPH of non-random subsets of VE cases suggests that a large proportion of VE cases are of non-JE etiology. In 1997 and 1998, serologic testing for JE viral antibodies was performed by the MOPH on reported cases of VE in Thailand. Of 178 samples collected in 1997, conclusive results were obtained from 57, and 29 of those samples (50.9%) were positive for JE.[65] In 1998, 137 conclusive

results were obtained from 201 collected samples, and 76 of those cases (55.5%) tested positive.[66] However, these results cannot be generalized to the entire clinically presenting VE population given the lack of randomization during the testing process; they therefore only represent a minimum proportion of JE exposure among clinically reported VE cases. Pathogens that are commonly associated only with encephalitic disease may cause clinically diverse nervous system disease, a fact that confounds the etiologic investigation of VE. For example, Solomon *et al.*[67] have identified JE virus in 12 of 22 children (55%) that presented to Vietnamese hospitals with acute flaccid paralysis, a syndrome commonly associated with poliomyelitis.

Non-arboviral pathogens do not express the same epidemiological dynamics as JE and may account for the lack of disease seasonality observed in central and southern Thailand. A study of JE in Vietnam shows seasonal variation, with epidemic peak infection presenting in July, which corresponds to the type of variations seen in northern Thailand.[68] A similar study in Nepal also shows marked seasonal variation of JE infection.[69] These studies, along with identified temporal peaks in disease carrying mosquitoes, suggest that JE normally exhibits seasonal variation in Thailand as well. However, we propose that the temporal pattern is masked by a higher proportion of other viral pathogens in the central and southern areas of Thailand. For example, an outbreak of a novel paramyxovirus, Nipah virus, in peninsular Malaysia from September 1998 to June 1999 in several pig farming villages was originally thought to have been caused by JE. Over 200 people were infected, many presenting with neurologic symptoms associated with encephalitis, such as loss of consciousness, brain-stem disorders, hypotonia and hyporeflexia.[70-73] It is possible that pathogens such as Nipah virus or

enterovirus 71 exist in southern Thailand, which is in close geographic proximity to peninsular Malaysia; these may account for a portion of non-arboviral encephalitic disease. Other viral pathogens that may present with central nervous system manifestations, such as enteroviruses, herpes viruses, mumps, measles and influenza, should be considered possible causes of clinically diagnosed encephalitis as well. As mentioned previously, JE antibody prevalence in peninsular Malaysia ranges from 18 to 60 percent, which is less than the 80 percent seroprevalence found in northern Thailand, strengthening the argument that there are greater percentages of non-JE pathogens causing human disease in central and southern regions of the country.

These etiologic variations may also explain why a higher case fatality rate is not seen in the northern provinces of Thailand – areas with higher incidences of clinical VE. It is possible that a higher proportion of JE in northern Thailand over the past 30 years has created a host population with partial immunity to disease. These individuals may have a more limited or mild form of JE than first-time hosts in the central southern areas. However, the case fatality rate for VE is low overall, and few individuals die from disease each month. The low incidence of death from VE makes it difficult to identify statistical differences in regional and seasonal mortality, and the unexpected variations in case fatality may be a result of the relatively few number of deaths recorded in this study.

In addition to variations in disease etiology, geography, and vector dynamics, other factors such as socioeconomic status, host genetic resistance, vaccine use, and human migration may significantly impact the geographic patterns of disease incidence. However, this information does not exist in the data set used in this study. An epidemiological study of the 1991 St. Louis Encephalitis epidemic in Jefferson County,

Arkansas, revealed that living in a low income household was a risk-factor for disease.[74] Although not statistically significant when adjusted for confounding variables, a study of JE in Henan province, China, during the mid 1990's revealed higher disease incidence in families with low income and education levels.[75] These studies suggest that socioeconomic status may play a role in the geographic distribution of VE in Thailand, although the extent to which it plays a role in the regional dynamics is not known. However, the border areas of northern-northeastern Thailand are comprised of a high percentage of low-income populations (including ethnic minorities from the mountainous areas). These areas have some of the highest incidence of viral encephalitis in Thailand (see Figure 16).

Inherited resistance to flaviviruses has been identified in wild-type and laboratory mice on several alleles of chromosome 5 with the common gene locus referred to as *Flv*.[76, 77] However, the exact mechanism of flavivirus resistance (including JE), the human expression of this allele, and the impact on the geographic distribution of disease are not well understood. In addition to innate resistance which influences viral replication (such as *Flv*), specific and non-specific immunities, such as genetic control of phagocytic uptake and cell killing and receptor binding to foreign antigens, also play a role in the host defense against viral infections.[77] It is likely that there are variations in population flavivirus resistance that contribute to the decreased incidence of VE in central and southern Thailand. However, studies of genetic resistance, which are potentially useful in understanding population level infection patterns, have not been done.

The existence of multiple strains of JE in Thailand has been debated, and the pathogenic variation and effect on host immunity of multiple strains are not fully understood.[78, 79] Five JE genotypes have been identified and characterized by Solomon and colleagues[26] that include: genotype I which is found predominantly in northern Thailand, Cambodia, and Korea; type II found predominantly in southern Thailand, Malaysia, and northern Australia; type III found in the more temperate regions of Asia, including Japan, China, Philippines, and Taiwan; type IV from Indonesia; and one isolate from Muar, Malaysia which may represent its own genotype. The older genotypes (IV) are endemic to the JE origination locale of Malaysia and Indonesia whereas genotypes I, II and III represent more recently evolved groups that have spread from the Malaysian/Indonesian geographic origination and are linked primarily with large epidemics (Solomon, personal communication 2004). There may be less population resistance to the newly evolved genotypes leading to the epidemic patterns of disease seen with disease caused by newer genotypes. It has also been hypothesized that different genotypes may produce different disease phenotypes with varying virulence. For example, this present study shows that the northern areas of Thailand (thought to be JE genotype I) show more of a seasonal, epidemic incidence pattern, whereas the south and central regions have more of an endemic pattern (thought to be genotype IV). However, in Vietnam, which shows a similar north/south epidemic versus endemic pattern of JE as in Thailand, genotype III have been found in both geographic regions.[80] In addition, models of neurovirulence in mice have not shown significant differences between genotypes.[26]

Encephalitis incidence and geographic variables were correlated on a provincial scale, so these results reflect large-scale trends and not minute variations in spatial dynamics that may have contributed to the unique geographic distribution of disease. Studies have been done on the village level in rural and suburban Thailand, however, which show that dengue and JE distributions on this scale are variable. Strickman *et al*[81] showed that in certain communities dengue and JE infection identified in school children directly correlated with the amount of viral illness in their respective villages, whereas other communities showed a disproportionately larger amount of infection in schools than in the children's respective villages. These variations in school versus home disease incidence show that individuals are susceptible to acquiring flavivirus disease both at home and in the work/school setting, suggesting that small-scale population movements may affect geographic distribution of disease. The important variations in disease patterns seen on the village scale make larger scale studies, such as this one, important in understanding disease on a level that is useful to vaccination and other disease control measures.

A widespread vaccination program was initiated in 1989 by the Thai MOPH that used two doses of killed JE vaccine in children before the age of 3. This program was started in the JE hyperendemic northern provinces, but was expanded to include provinces with encephalitis incidences of more than one per 100,000 in 1994 (36 provinces) and to target first graders in endemic provinces.[59] It is estimated that there is an 84 % vaccination coverage in children 2.5 to 3 years of age.[59] However, given that a large proportion of clinically diagnosed VE is not JE, these vaccine programs are more likely effective in areas with seasonal JE epidemics. The vaccine programs were targeted

to provinces with a high incidence of clinical encephalitis, however, and not to JE specifically. It is likely that the variation in etiology caused a leveling of VE disease since the early 1990's, and that programs designed specifically to target JE without information on the pathogens that are causing clinical disease are insufficient. Vaccination programs are most likely to be effective in regions, such as northern-northeastern Thailand, that have a large amount of JE.

In order to control clinical encephalitis, other prevention measures should be taken, such as mosquito control and aggressive outbreak monitoring and prompt clinical interventions. In addition, active surveillance for viral pathogens other than JE are necessary in order to efficiently direct vector control, clinical management, and pharmacological intervention of VE. Testing and screening for a variety of encephalopathic infectious agents using PCR and immunoassays will provide needed insight into the exact epidemiological dynamics of VE. Our suggested pilot study, which was initiated by physicians at Ratchaburi hospital, proves that it is feasible to carry out large-scale active surveillance of VE in order to better understand the specific etiologies of the disease and how they differ clinically (see Tables G and H in the appendix that show the clinical, diagnostic and laboratory findings of each patient). However, these prospective studies must be done in a variety of geographic regions in conjunction with the currently ongoing projects on vector abundance and infection prevalence in order to fully understand the ecological variations of disease.

6. Appendix: *Ratchaburi hospital pilot surveillance study results*

Table G. Clinical and diagnostic imaging findings of encephalitis patients from Ratchaburi hospital pediatrics department, 2001.

Patient	Presenting Symptoms	Physical Findings	GCS[a]			Treatment	CT Head	EEG	Progress and Outcome
			E	V	M				
1 (11f)[b]	Fever and headache for 5 days, drowsiness for 2 days	Generalized decreased motor strength, and 2+ clonus bilaterally	1	2	2	Dexamethasone, Acyclovir	No edema with normal brain parenchyma	N/D	Placed on respirator for 4 days 2 days post triage, had muscle spasticity and weakness at 3 weeks of hospitalization.
2 (3m)	Fever for 6 days, presents uneonscious with generalized muscle spasms/convulsions	Nuchal rigidity, upgoing Babinski reflexes bilaterally and positive clonus	1	1	2	Dexamethasone, Manitol, Dilantin	Normal parenchyma	N/D	Placed on respirator for 2 weeks after triage, patient died 3 weeks post admission
3 (11m)	Fever, convulsions and in stupor for 2 days, on admission generalized muscle spasms and headache	Nuchal rigidity, Muscle spasms greater on right than left, right upward Babinski reflex	2	3	5	Dexamethasone, Acyclovir	Generalized edema, meningeal swelling	Generalized slow wave encephalopthy	Placed on respirator for 6 days 2 days post triage, patient made a full recovery
4 (3m)	Fever for 3 days, headache, nausea and convulsions for 1 day	Generalized muscle spasms, symmetrical hyperreflexia (3+), right sided clonus and upward Babinski	4	5	6		No edema with normal brain parenchyma	Generalized slow wave encephalopthy	Full recovery three weeks after admission
5 (7m)	Fever and headache for 4 days, drowsy with stiff neck on admission	Clonus and upward Babinski bilaterally, inereased general muscle tone	3	3	5	Dexamethasone, Manitol	N/D	N/D	Full recovery three weeks after admission
6 (9f)	Fever and nausea for 8 days, mother reports recent Herpes infection (unspecified)	Nuchal rigidity and positive Kernig's sign	4	4	5		N/D	N/D	Full recovery three weeks after admission

[a]Glasgow Coma Score: E = eye opening (none = 1, to pain = 2, to loud voice = 3, spontaneous = 4); V = verbal response (none = 1, incomprehensible sounds = 2, inappropriate words = 3, confused or disoriented = 4, oriented = 5); M = motor response (none = 1, extension posturing = 2, abnormal flexion posturing = 3, withdraws to stimuli = 4, localizes = 5, obeys = 6).

[b]Age and gender of patient

Table H. Laboratory findings of encephalitis patients from Ratchaburi hospital pediatrics department, 2001.

	CBC				CSF Profile							CSF	CSF	Serum
Patient	Crit	Platelets	WBC	Differential[a]	Protein	Sugar	BS	RBC	WBC	PMN	Lymph	Culture	Viral Screen	Viral Screen
1	34%	324K	7740	N86%, L12, M2	84	89	154	284	373	0%	100%	Negative	N/R	N/R
2	31	449	21300	N83, L13, M2	84	117	266	34	3	0%	100%	Negative	N/R	JE IgM=134, dengue IgM=16
3	36	257	2400	N87, L5, M6, E1	51	85	124	350	240	0%	100%	Negative	NR	N/R
4	35	537	6600	N40, L54, M3, Bands 2, B1, E1	26	92	155	40	350	14%	86%	Negative	JE IgG<1:20	JE IgG<1:20
5	41	373	10200	N66, L26, M 9	65	69	158	1910	70	0%	100%	Negative	JE IgM=275	JE IgM=165
6	39	408	19700	N70, L26, M4				10	580	0%	100%	Negative	N/R	N/R

[a]N = percent neutrophils, L = percent lymphocytes, M = percent monocytes, E = percent eosinophils, B = percent basophils in peripheral blood.

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