SEROPREVALENCE OF DENGUE ANTIBODIES AMONG FOREIGN TRAVELERS IN SOUTH EAST ASIA

EMANUEL LUTTERSDORFER

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF CLINICAL TROPICAL MEDICINE
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2009

Thematic paper
entitled

SEROPREVALENCE OF DENGUE ANTIBODIES AMONG TRAVELERS IN SOUTH EAST ASIA

.....
Mr. Emanuel Luttersdorfer, M.D.,
Candidate

.....
Mr. Watcharapong Piyaphanee, M.D.,
Dip. Thai Board of Internal Medicine
Dip. Trav. Med., MFTM RCPS (Glasgow)
Major Advisor

.....
Prof. Teerapong Tantawichien, M.D.,
Dip. Thai Board of Internal Medicine
Co-Advisor

.....
Mrs. Prapimporn Shantavasinkul, M.D.,
Dip. Thai Board of Internal Medicine
Co-Advisor

.....
Assist. Prof. Udomsak Silachamroon,
M.D., Dip. Thai Board of Internal
Medicine, Dip. Thai Board of Pulmonary
Medicine
Co-Advisor

.....
Mr. Noppadon Tangpukdee,
Ph.D. (Tropical Medicine)
Co-Advisor

.....
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies
Mahidol University

.....
Assist. Prof. Wirach Maek-a-nantawat,
Dip. Thai Board in Allergy and Clinical
Immunology
Chair
Master of Clinical Tropical Medicine
Faculty of Tropical Medicine
Mahidol University

Thematic paper
entitled

SEROPREVALENCE OF DENGUE ANTIBODIES AMONG TRAVELERS IN SOUTH EAST ASIA

was submitted to the Faculty of Graduate Studies, Mahidol University
for the Degree of Master of Clinical Tropical Medicine
on
March 30, 2009

.....
Mr. Emanuel Luttersdorfer, M.D.,
Candidate

Prapimporn Shantavasinkul
.....
Mrs. Prapimporn Shantavasinkul, M.D.,
Dip. Thai Board of Internal Medicine
Member

Usa Thisyakorn
.....
Prof. Usa Thisyakorn, M.D.,
Dip. Thai Board of Pediatrics
Chair and External Examiner

Udomsak Silachamroon
.....
Assist. Prof. Udomsak Silachamroon M.D.,
Dip. Thai Board of Internal Medicine
Dip. Thai Board of Pulmonary Medicine
Member

W. Piyaphanee
.....
Mr. Watcharapong Piyaphanee, M.D.,
Dip. Thai Board of Internal Medicine
Dip. Trav. Med., MFTM RCPS (Glasgow)
Member

N. Tangpukdee
.....
Mr. Noppadon Tangpukdee,
Ph.D. (Tropical Medicine)
Member

.....
Prof. Teerapong Tantawichien, M.D.,
Dip. Thai Board of Internal Medicine
Member

B. Mahaisavariya
.....
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies
Mahidol University

P. Singhasivanon
.....
Assoc. Prof. Pratap Singhasivanon,
M.B.B.S., D.T.M.&H. (Bangkok),
Dr.P.H. (Epidemiology)
Dean
Faculty of Tropical Medicine
Mahidol University

ACKNOWLEDGEMENTS

My special gratitudes go to my major advisor who acted as my guiding light though the process in organizing my thoughts as far as it has been able to put them down to paper in an acceptable form. Also my very special gratitude to the staff of the QSMI clinic. Without its help in collecting the data and especially the commitment of one person in particular, Dr. Piyada, I would never have been able to collect the number of cases I did finally. Thanks to Dr. Noppadon my work on the statistics has been unexpected fun. Also my gratidude for Prof. Teerapong making all the morning ward rounds at the Culalongkorn University an exciting event. Last but not least my sincere thanks go to all the persons who have somehow been involved in the creation of this study and who haven‘t been mentioned here yet!

Emanuel Luttersdorfer

SEROPREVALENCE OF DENGUE ANTIBODIES AMONG FOREIGN TRAVELERS IN SOUTH EAST ASIA

EMANUEL LUTTERSDORFER 5138420 TMCT/M

M.C.T.M.

THEMATIC PAPER ADVISORY COMMITTEE : WATCHARAPONG PIYAPHANEE M.D. MFTM RCPS, Prof. TEERAPONG TANTAWICHIEN M.D., PRAPIMPORN SHANTAVASINKUL M.D., NOPPADON TANGPUKDEE D. Phil. (Tropical Medicine), Assist. Prof. UDOMSAK SILCHAMROON M.D.

ABSTRACT

Introduction: Dengue virus infections are today‘s most common cause of arboviral diseases in the world. Dengue is endemic in most of the tropical and subtropical countries of which many are popular tourist destinations. In 2006 23% of all dengue cases in the South East Asian region have been accounted to Thailand. **Methods:** We conducted a descriptive cross sectional study among foreign travelers (n=172) to Thailand to determine the seroprevalence of dengue antibodies in order to look for a possible exposure to the dengue virus during their trip. Furthermore we asked them to answer a questionnaire to search for predisposing factors as well as to rule out possible cross reactions caused by their vaccination history. The study participants have been recruited at two travel clinics in the Bangkok Metropolitan area. Their sera have then been analyzed for seroprevalence of antibodies against dengue and Japanese B encephalitis, also to rule out cross reactivity, by using a MAC-ELISA test. **Results:** We found 6.4% to be positive for antibodies against the dengue virus. By taking only that cases into account, which have been likely to have gotten exposed to the dengue virus during their current trip a seroprevalence of 3.5% would result. **Discussion:** Both values are well within the range of the results of other investigations. In our study we couldn‘t show any statistically significant correlations to age or gender like in similar previous studies. **Conclusions:** As long as there is no vaccination available for clinical use and considering the fact that many of the travelers who took part in our study had more than one stay in a dengue endemic area and therefore are prone to a considerably more dangerous secondary infection doctors all over the world should be aware of the existence of dengue as a major cause of fever in the returning traveler.

KEY WORDS : DENGUE / SEROPREVALENCE / TRAVELER / THAILAND

67 pages.

CONTENTS

Page

ACKNOWLEDGEMENTS ..... **iii**

ABSTRACT ..... **iv**

LIST OF TABLES ..... **vii**

LIST OF FIGURES ..... **viii**

LIST OF ABBREVIATIONS ..... **ix**

CHAPTER I INTRODUCTION **1**

1.1. Dengue in Thailand 1

1.2. Dengue and the traveller 2

CHAPTER II LITERATURE REVIEW **4**

2.1. Introduction 4

2.2. Virus and Vector 5

2.3. Pathogenesis and Clinical Manifestation 7

2.4. Diagnosis 8

2.5. Dengue and the traveler 10

2.6. Why this study is being conducted? 12

CHAPTER III MATERIALS AND METHODS **13**

3.1. Study design 13

3.2. Study area and population 13

3.3. Sample size calculation 14

3.4. Inclusion criteria 15

3.5. Exclusion criteria 15

3.6. Sampling 15

3.7. Research instruments 15

3.7.1. Serological tests 16

3.7.2. Questionnaire 21

3.8. Data analysis 22

3.9. Ethical considerations 23

CONTENTS (cont.)

Page

CHAPTER IV RESULTS 24

4.1. Descriptive statistics of characteristics 24

4.2. Laboratory results 36

4.3. Comparison of the subgroups 38

CHAPTER V DISCUSSION 40

5.1. General characteristics 41

5.2. Interpretation of conspicuous laboratory results 41

5.2.1. Dengue 42

5.2.1.1. Duration of stay less than 5 days 42

5.2.1.2. Duration of stay 5-28 days 43

5.2.1.3. Duration of stay >28 days to 6 months 44

5.2.1.4. Duration of stay > 6 months 44

5.2.2. Japanese B encephalitis 47

5.2.2.1. Duration of stay less than 5 days 47

5.2.2.2. Duration of stay 5-28 days 48

5.2.2.3. Duration of stay >28 days to 6 months 48

5.3. Limitations 52

5.4. Recommendations for further studies 53

CHAPTER VI CONCLUSION 54

6.1. General 54

6.2. Outlook 55

REFERENCES 56

APPENDIX 59

BIOGRAPHY 66

LIST OF TABLES

Table		Page
1	Laboratory diagnosis of dengue fever	10
2	General characteristics of 172 travelers to Thailand	27
3	Travel characteristics of 172 travelers to Thailand	27-28
4	Medical characteristics of 172 travelers to Thailand	29-30
5	Laboratory results	37
6	Subgroup analysis	38-39
7	Dengue seropositivity over the duration of stay	45
8	Summary of conspicuous cases (Dengue)	45
9	Corrected number of dengue antibody seropositive individuals	46
10	Summary of conspicuous cases (JE)	49
11	Japanese B encephalitis seropositivity over the duration of stay	50

LIST OF FIGURES

Figure		Page
1	DHF Endemic Countries, SEA Region 2007	1
2	Seasonal trend of dengue cases (1990-2007)	2
3	Countries at risk of dengue transmission 2007	4
4	Genome of the dengue virus and its polyprotein processing	6
5	Algorithm for the determination of a past Flavivirus infection	19
6	Algorithm for the determination of an acute Flavivirus infection	20
7	Study Sites	31
8	Age Distribution	31
9	Sex	31
10	Origin	32
11	Area of stay	32
12	Purpose of travel	32
13	Backpacker	33
14	Previous visit of a dengue endemic area	33
15	Previous diagnosis of a dengue infection	33
16	Purpose of visit to the clinic	34
17	Probable dengue infection (clinically)	34
18	Usage of insect repellent	34
19	Knowledge about dengue	35
20	Vaccination status	35
21	Laboratory results	37
22	Dengue seropositivity over the duration of stay	46
23	Japanese B encephalitis seropositivity over the duration of stay	50

LIST OF ABBREVIATIONS

Abbreviation of Symbol	Term
DF	Dengue fever
DHF	Dengue hemorrhagic fever
JE	Japanese B encephalitis
MACELISA	IgM and IgG capture enzyme linked immunosorbent assay
WHO	World Health Organization
YF	Yellow fever

CHAPTER I
INTRODUCTION

1.1. Dengue in Thailand

The Dengue virus infection is becoming one of the world's major emerging infectious diseases. The estimated annual occurrence is 100 million cases of dengue fever. Dengue is endemic in most tropical and subtropical countries many of these countries being popular tourist destinations. In 2003 only 8 countries in South East Asia Region reported dengue cases, which changed rapidly in 2006 when 10 out of eleven countries in the region reported dengue cases (Bangladesh, Bhutan, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand and Timor-Leste) [1].

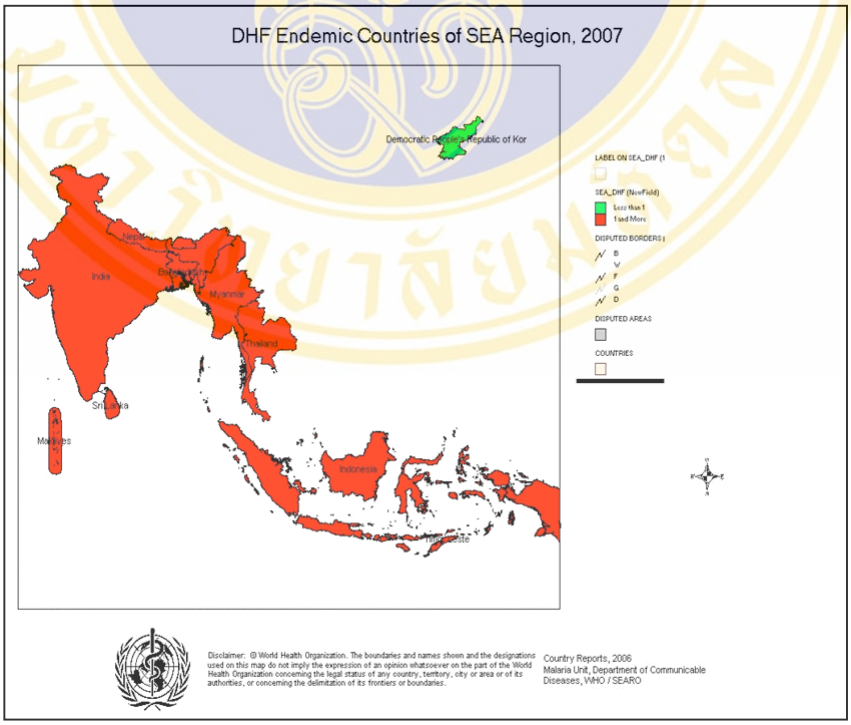


Figure 1: DHF Endemic Countries, SEA Region 2007 [http://www.who.int]

In 2006 23% of all the dengue cases in the South East Asian region have been accounted to Thailand while back in 2003 it had the maximum number of dengue

infections among all South East Asian countries. Dengue hemorrhagic fever in Thailand was first reported in 1949 and its first outbreak in 1958 with 2158 cases and 300 deaths. Currently it is endemic in this country. In 2000 17,582 of DF/DHF cases have been reported with the highest number of reported cases from the central region, followed by the northeastern, the northern and the southern region of Thailand. All four dengue serotypes could be isolated and the principal vector in this region is the *Aedes aegypti* mosquito. For the last ten years the case numbers have always been above 10000 while the case fatality rate has been 1% [1]. This developments as well as a steady increase in the numbers of tourists who visit Thailand every year make dengue an important issue not only for local health authorities.

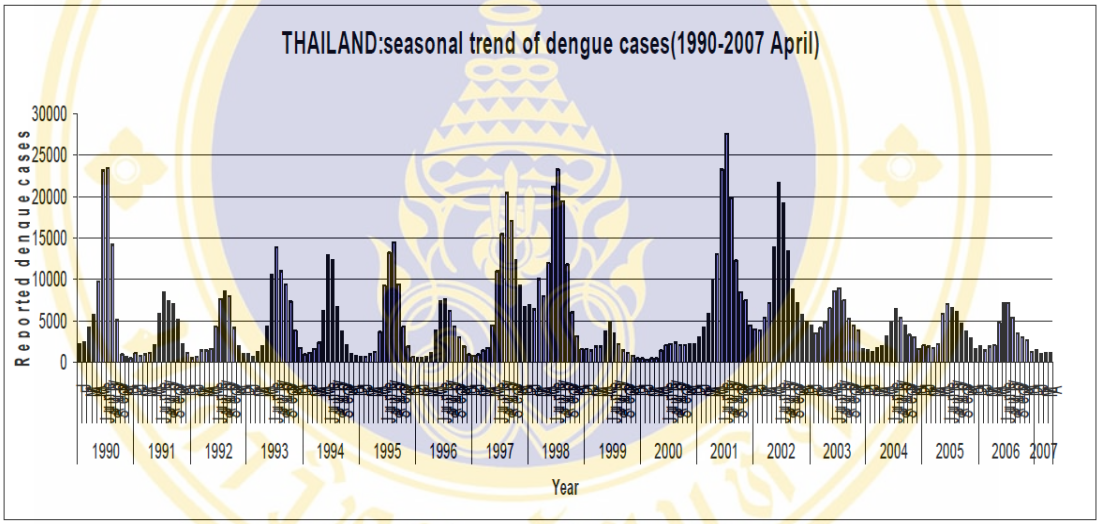


Figure 2: Seasonal trend of dengue cases (1990-2007) [http://www.who.int]

1.2. Dengue and the traveler

The season of maximum transmission starts in July and lasts until September falling together with the season most travelers from Western countries use to make their summer vacation [1]. Dengue has a short incubation time (5-7 days on the average). That means, that travelers may get sick while still on their trip. Although some studies showed, that the duration of stay in an endemic area is positively correlated with an increased risk of a Dengue infection, Dengue should not be looked at as a disease of long-term travelers. The virus itself belongs to the family of Flaviviridae (single stranded, non-segmented RNA viruses) as well as the viruses

causing Yellow fever, Japanese encephalitis, Tick borne encephalitis and West Nile fever. It has four serologically distinct serotypes (DEN-1, DEN-2, DEN-3, DEN-4). Infection with one serotype results in immunity only to this serotype. For this reason a human can be infected up to four times. Studies from areas of endemicity suggest that 14%–87% of all dengue infections cause only a few and mild clinical symptoms [2, 3, 4]. A recent study being conducted on 149 long distance travelers at a travel vaccination centre in Frankfurt/Main revealed that 8.7 – 19.5% (depending on the test, which had been used) of the participants showed to be seropositive for IgG antibodies against DF, though these numbers still include people who have been vaccinated against other Flavivirus transmittable diseases before, which could lead to cross – reactions [5]. Excluding them seropositivity ranges from 3.4 – 13.4%. Test for IgM is supposed to be negative early in the disease and becomes positive after 4-5 days after the onset of symptoms. For an elevated level of IgG antibodies it can be assumed a Dengue infection to have occurred in the past although cross – reaction, which could either result from an exposure to another Flavivirus or a vaccination has to be carefully ruled out. Still there is no safe and effective vaccine available. The Pediatric Dengue Vaccine Initiative, the World Health Organization, industry, the US military and several governments of affected countries are collaborating and currently phase 3 vaccine efficacy trials are being conducted in countries where dengue is endemic including Thailand. So the only action people can take to prevent themselves from an infection is to prevent themselves from mosquito bites. In contrary to the *Anopheles* mosquito, which usually feeds during the nighttime the *Aedes* mosquito prefers feeding during the daytime and the risk of getting bitten is higher in the urban than in the rural area.

CHAPTER II
LITERATURE REVIEW

2.1. Introduction

Dengue virus infections have become one of the worlds major emerging infectious diseases. Today they are the most common cause of arboviral diseases in the world [6]. According to the current maps provided by the WHO, the disease appears almost everywhere in the tropics as shown in figure 3 and it's vector (*Aedes aegypti*) is also prevalent outside tropical regions such as the southern parts of the United States, the southern parts of Europe and Northern Australia.

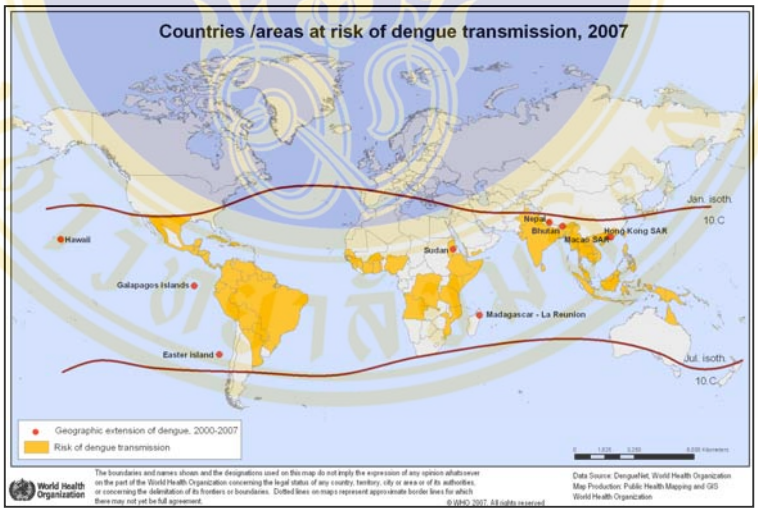


Figure 3: Countries at risk of dengue transmission 2007 WHO GIS

The Americas, South East Asia, the Western Pacific Region as well as Africa and the Eastern Mediterranean may be considered as endemic with the major disease burden in the first three regions [6]. Every year approximately 50 – 100 million dengue fever and about 250.000 – 500.000 officially reported DHF infections occur, whereas the true incidence is not known [7]. An estimated 2,5 billion people currently live at areas potentially at risk for dengue transmission [8]. In the past years

the incidence rate has grown in all endemic areas and especially in the Americas, while the case fatality rates are higher in the South East Asian region and the Western Pacific region [6]. Contributing factors for the emergence and re-emergence of dengue fever may be the increase in the density and geographic distribution of the vector and the rate and geographic range of the virus transmission, so they are determined by demographic changes (population growth and unplanned urbanization), deterioration of public health systems and mosquito control programmes in the endemic areas [9].

As tropical and subtropical regions are very popular holiday destinations and according to the figures mentioned above, travelers of course are at risk as well. These regions also show the highest growth rates for travelers, which is being reflected in the growing number of tourist arrivals, rising steadily in the past years and from 53.1 million in 2006 to 59.6 million in 2007 [10]. Travel and in particular air travel allows different strains, serotypes and genotypes to spread from one region to another [11].

The first description of the disease referred to as “bilious remitting fever” or “breakbone fever” has been made 1779-1780. It simultaneously appeared in Africa, Asia and North America [12]. Major epidemics took place in long intervals (10 - 40 years), because the introduction of new serotypes into a susceptible population was depending on the survival of vectors during the long periods of transportation via sail vessels between the population centers [13]. After World War II a pandemic with it’s origin in South East Asia spread around the world, while the first epidemic of DHF in South East Asia first appeared in 1953 in Manila and remained in South East Asia until the 1970s [14]. In the year 2000 17,582 cases of DF/DHF have been reported in Thailand [15].

2.2 Virus and Vector

The Dengue virus belongs to the family of Flaviviridae, which prototype is the Yellow fever virus. It is a single-stranded, nonsegmented RNA virus and has four serologically distinct serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) [16]. The virus consists of 3 structural and 7 non-structural proteins (see figure 4) and attaches to the

susceptible cell by either forming a complex with a non-neutralizing IgG antibody using the Fc receptor of macrophages or monocytes or by using a trypsin-sensitive receptor of monocytes [17].

Next to dengue fever there are four other important diseases caused by Flaviviridae: Tick borne encephalitis, Yellow fever, Japanese encephalitis and the West Nile fever [16].

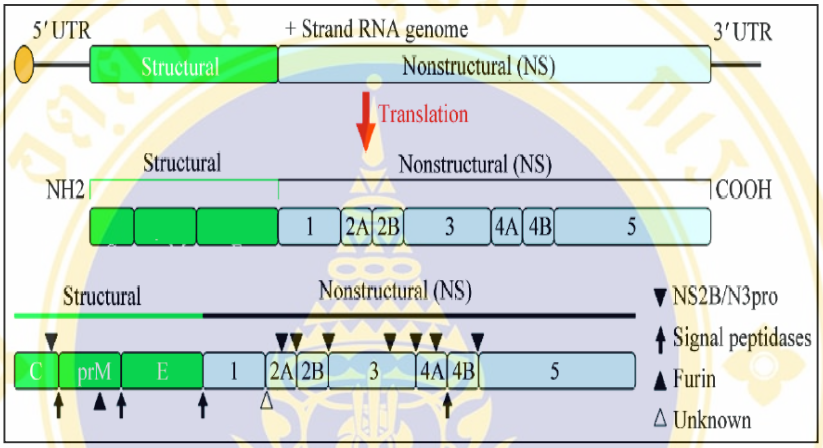


Figure 4: Genome of the dengue virus and its polyprotein processing [17]

All members of the family (more than 60 arthropode – borne viruses) share common antigenic determinants, which results in problems concerning serologic diagnosis [17].

The virus natural hosts are humans, mosquitoes and lower primates. Although the primates show viremia, they do not have any clinically apparent symptoms. Its only natural mosquito hosts are members of the genus *Aedes* and its transmission by *A. aegypti* has been first demonstrated in 1906 [17]. *A. aegypti* is the principal vector and found worldwide in the tropics and subtropics. Because of several reasons this mosquito acts as a very efficient vector. It is highly susceptible to the virus, a daytime feeder, capable of biting several people in a short period for one blood meal and feeds preferentially on human blood [6]. In contrary to other species, the *Aedes* mosquito is well adapted to urban areas. It can breed in clean and stagnant water in containers that collect rainwater, such as tires, pots, buckets, discarded coconut shells, a.s.o. [16]. Indoors the mosquito prefers to rest at dark sites like closets or places under beds [7].

2.3. Pathogenesis and Clinical Manifestation

The infection with any of the four serotypes of the dengue virus causes a variety of symptoms ranging from asymptomatic or mild fever to severe haemorrhages and shock syndrome and even death [18]. The incubation period is usually 4 to 7 days (range 3-14 days), so fever appearing 14 days after a visit to a dengue endemic area or lasting longer than 14 days is very unlikely to be caused by dengue infection [7]. Infection with one serotype results in a long-term immunity to that serotype. Therefore a human can get infected up to four times [18].

The first site of replication for the virus is the regional lymphatic tissue [19] particularly affecting the macrophages as well as dentritic cells [20]. From there it disseminates through the blood and lymphatic vessels to other tissues [19]. Autopsies show effusions and petechial haemorrhages in many organs and similar to the Yellow fever virus infection central necrosis has been found in the liver of dengue patients. The virus has also been identified in the CSF [6].

Pathological examinations have shown perivascular edema, swelling of endothelial cells and an infiltration with mononuclear cells, even though there has no virus or viral antigen been detected. Other findings from extensive pathological surveys from fatal cases of DHF include hemorrhages in the skin, subcutaneous tissues, GI-tract, and the heart while histopathological changes like hemorrhage, dilatation and congestion of vessels and edema of arterial walls are found in the liver, the reticuloendothelial system and the vascular system. Hemorrhagic manifestations in other organs as well as fluid accumulation in body cavities are another common finding [17].

Usually classical dengue fever is characterized by acute febrile illness, frontal headache, retroocular pain, muscle and joint pain, nausea, vomiting and rash. The virus disappears after an average of 5 days, closely related to the time, the fever subsides and there is no carrier status. Mild thrombocytopenia and leucopenia are common. Less frequent are hemorrhagic manifestations like gingival bleeding, gastrointestinal bleeding, epistaxis, petechiae and hypermennorhoea. In over one third of the cases a positive Tourniquet test may be found [7].

Dengue hemorrhagic fever is defined as a febrile disease with major or minor bleeding, thrombocytopenia (less than 10^5/µl) and increase of hematocrit by at least one fifth or decrease of hematocrit by at least one fifth after intravenous fluid therapy, pleural or other effusions or hypalbuminaemia or hypoproteinaemia [21]. It's being associated with all 4 serotypes, while secondary infections especially with serotype 2 seem to result in a more severe disease and DHF being explained by antibody enhancement, a facilitated entry of the virus into its target cells by binding to cross-reactive but non neutralizing antibodies from a previous infection [6]. DHF usually develops at day 3 – 7 of the illness at the time of deferenscence. A further deterioration indicated by narrowing of the pulse curve (< 20mmHg), weak and fast pulse or hypotension indicating circulatory failure and profound shock with undetectable blood pressure correspond with the dengue shock syndrome (WHO Grade III and IV). Mainly the histopathological changes mentioned above may be taken in to consideration for this condition to develop [17].

2.4. Diagnosis

Next to the clinical features, which have already being mentioned above and might also resemble other bacterial, viral or parasitic diseases in this paragraph the laboratory methods are emphasized. Notable changes such as leucopenia, thrombocytopenia are seen in DHF, but are less remarkable in classic dengue fever. To confirm a diagnosis of dengue infection currently viral cultures, PCR and serologic assays are being used [18]. Each method, virological, molecular or serological has its limitations.

Virus isolation in routine diagnosis uses the C6-36 cell line. Concerning sensitivity the direct inoculation into mosquitoes is the best method with rates up to 80% compared with 36% using the cell line. The identification is being performed using a immuno-fluorescence assay with serotype specific monoclonal antibodies. Polymerase chain reaction may not be available in resource-limited settings. Using specific primers it also allows serotype identification as well as genetic strain characterization [22]. Both virus isolation and PCR are dependent on the presence of viral bodies in the body. However, they only remain during the febrile. As soon as the specific antibodies

appear, the virus is cleared rapidly. Serological diagnosis mainly relies on the presence of IgM antibodies in the acute phase of the illness and the rise of IgG antibodies in convalescent sera. 90% of all the patients‘ sera are IgM positive on the sixth day after the onset of symptoms. It remains detectable for an average of 60 days. In areas, where other flaviviruses such as Japanese encephalitis cocirculate diagnosis can be made by measuring their antibody level, too. Considering IgG antibody titer there are differences between the primary and following infections. During primary infection it begins to appear on day 5 after the onset of the symptoms, rises steadily and remains detectable for years. During repeated infections it‘s permanently detectable and the titers will rise rapidly. For that reason the analysis of a paired convalescent IgG sample for people, who lived in dengue endemic areas is necessary. Capture ELISA is the most common used IgM assay. IgG antibodies are measured by ELISA, too [7]. Table 1 shows the differences between a probable and confirmed laboratory diagnosis of dengue [8].

Probable diagnosis	Confirmed diagnosis
At least one of following: • Supportive serology on single serum sample: titre > 1280 with haemagglutination inhibition test, comparable IgG titre with enzyme linked immunosorbent assay, or positive for IgM antibody test • Occurrence at same location and time as confirmed cases of dengue fever	At least on of following: • Isolation of dengue virus from serum or autopsy samples • Fourfold or greater increase in serum IgG (by haemagglutination inhibition test) or increase in IgM antibody specific to dengue virus • Detection of dengue virus in tissue, serum, or cerebrospinal fluid by immunohistochemistry, immunofluorescence or enzyme linked immunosorbent assay • Detection of dengue virus genomic sequences by reverse transcription polymerase chain reaction

Table 1: Laboratory diagnosis of dengue fever [18]

2.5. Dengue and the traveler

One major study carried out between March 1997 and March 2006 among 6957 travelers, who were seeking care at member clinics of the GeoSentinel network because of fever as chief complaint revealed 6% of them having confirmed dengue infection. It should be mentioned, that travelers, who had fever during travelling, which would be a possibility for them having been infected with the dengue virus concerning its minimum incubation time of 3 days, have been excluded. Taking the regional distribution into account, this study shows dengue being responsible for even 18% of all cases with fever [23]. Another study from Germany showed 8.7 up to 19.5% of travelers to be dengue seropositive. Excluding all the patients with vaccination history for Tick borne encephalitis, Yellow fever and Japanese encephalitis the percentage of seropositive travelers would range between 3.4 and 13.4%. In the study Focus (ELISA), PanBio (ELISA) and Progen (IFA) tests have

been used [5]. The results of two prospective studies, one including 104 Israeli travelers with a median stay of 5.3 months in a dengue endemic area and the other including Dutch 447 individuals with a median stay of 4 weeks showed an incidence rate of 6.7 and 2.9% [24,25].

Due to the short incubation time and the presentation as asymptomatic or mild febrile disease most of the dengue fever infections in travelers are likely to be primary infections and many of them might not seek for medical attention at a hospital.

Patients with uncomplicated dengue fever need rest, oral fluid intake to compensate for loss due to vomiting and diarrhea. If they need antipyretics or analgesics prescription of ASS should be avoided so that the platelet function will not be impaired. Still there is no proven use for steroids. If a severe infection is suspected the intravenous route for fluid replacement should be used. Close monitoring of hematocrit, blood pressure, urine output and platelet count is required as plasma leakage in DHF can be very rapid and it insufficient therapy may also progress DHF further into DSS [7].

Until today there is no vaccine against dengue available in clinical practice. Current dengue vaccine development is being conducted by a collaboration of international organizations, governments of tropical countries, private donors and the US military. These days phase 3 vaccine efficacy trials are being carried out in countries where dengue is endemic [26]. Even though vaccination can be implemented in vaccination programmes in these countries within a short period of time, it would still require additional evaluation to determine its usage on travelers.

Travelers to dengue endemic countries may prevent from getting infected by using appropriate clothes to reduce the area of exposed skin, mosquito repellents and aerosol insecticides inside rooms. For healthcare providers in travel clinics it is important to consider dengue infection as a differential diagnosis for people with a recent travel history to a dengue endemic country [7].

2.6. Why this study is being conducted?

First it should be mentioned, that the studies, which have been reviewed above, either they have been designed to determine the seroprevalence or incidence rate have been carried out in centers for travel medicine in traveler‘s countries of origin. That means that none of the studies has been meant to have a cross sectional design to reflect the situation in a region where infections with the dengue virus actually occur i.e. South East Asia for example. Furthermore in this study we will specifically try to determine the influence, which vaccination against yellow fever or Japanese B encephalitis or behavior of the traveler could probably have on the laboratory results. There hasn‘t been a comprehensible and clear approach on how these factors should be coped with in previous studies [5, 24, 25]. To achieve our aim, we also applied some selection, which should help to put the focus on infections, which have been acquired during traveling. We excluded people being born or grown up in dengue endemic countries. In a short summary our study was conducted to give an overview over the current situation for travelers in Thailand to get exposed to the dengue virus. As the prevalence rate in one study conducted in Germany has been ranging from 3.4% to 13.4% we expect our results to be somewhere within this range [5]. Anyways, for the interpretation of our laboratory results we will use a more sophisticated approach also taking additional information about the time passed since the last vaccination against other flaviviruses than dengue and since a potential last stay in a dengue endemic area, which will be asked for in the questionnaire, into consideration.

CHAPTER III
MATERIALS AND METHODS

3.1. Study design

A descriptive cross sectional study among foreign travelers to South East Asia, particularly to Thailand was conducted. Ethical clearance had been obtained from the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

3.2. Study area and population

The study has been carried out at two different locations in the Bangkok Metropolitan area. As first site we chose the travel clinic located at the Hospital for Tropical Diseases on the campus of the Mahidol University on Rajawithi Road. The travel clinic is being frequented by many travelers for getting either advise, vaccination or treatment. The second study site was located at the Queen Saovabha Memorial Institute. This clinic is adjacent to the Bangkok Snake Farm, which serves as a highlight for many foreigners visiting Bangkok and therefore also attracts many travelers. Visitors to the clinic can receive rabies post exposition prophylaxis, surgical treatment for bite wounds as well as pre- and post travel consultation and vaccinations.

Thailand shows a high number of tourist arrivals every year. 2007 14.464.228 people arrived in Thailand from abroad via land, sea or air transport [27]. Suvhanabumi airport in Bangkok serves as a major hub for air traffic in South East Asia. For this reason people from all over the world are expected to arrive here all over the year.

Data collection started on the 11th of November 2008 and was done until the 31st of January 2009.

3.3. Sample size calculation

Based on the method of sample size calculation for determine the incidence rate of the interesting disease, the following formula was used for it's calculation:

$N = Z^2_{\alpha/2}\, p(1-p) / e^2$

Notation:
N = the expected sample size
α = statistically sampling error
Z = confidence coefficient
p = proportion of interesting disease
e = precision of estimation

According to previous studies, seroprevalence of dengue antibody among traveler returned from the tropics was 2-19.5% [18, 5]. Subgroup analysis showed that seroprevalence of dengue antibody of those returned from Asia is higher than Africa. Based on their results, we estimated the prevalence of dengue antibody among travelers in South East Asia equals to 15%.

Statistical sampling error was set as: 0.05
Precision of estimation was set as: 95%

Therefore by using the sample size calculation formula shown above, at least 195 cases were needed for this study.

3.4. Inclusion criteria

Eligible and included were all people above 18 years old, who attended one of the study sites for any reason, being able to self-administer the questionnaire and having signed the informed consent.

3.5. Exclusion criteria

We excluded travelers who had been born or had been grown up in a dengue endemic area according to the WHO GIS World Map (DengueNet) (see figure 3).

3.6. Sampling

The study participants were travelers over 18 years of age, who attended the study sites for any reason. There hasn‘t been any form of compensation to motivate them to take part. Next to fill out the questionnaire the participants were also asked for 3ml of blood being drawn from them, and they had to be approached in person by either the chief investigator or the previously instructed staff. Random sampling has not been performed. Accidental sampling was used instead. All the individuals who agreed to take part and met the inclusion criteria have been included after signing the informed consent form and the patient information sheet.

3.7. Research instruments

For this research work two instruments have been used. A two pages questionnaire and a IgM and IgG capture enzyme linked immunosorbent assay to test a serum sample acquired from the participant for the existence of dengue and Japanese encephalitis IgG and IgM antibodies. The questionnaire has been reviewed and revised by the Committee including advisors and co-advisors and afterwards modified according to their suggestions as well as it has been undergoing the reviewing process

and has been approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University.

3.7.1. Serological tests

Approximately 3 ml of blood have been drawn from each study participant. The sample had then been centrifuged at 3000 rpm for 5 minutes at 25 degree Celsius. The serum then had been stored at minus 30 degree Celsius. The sera were then tested with a IgM and IgG capture enzyme linked immunosorbent assay (MACELISA) for Dengue and Japanese Encephalitis antibodies. The procedure will be described briefly in the following paragraph.

First a 0.018 M Carbonate Buffer with pH 9.0 (±0.1) is being prepared. After the rehydration of lyophilized goat anti-human IgM and IgG it‘s being diluted 1:1400 in the 0.018 M Carbonate Buffer and incubated at room temperature (18˚C to 30˚C) for 18 to 24 hours. For the antibody step all controls and the test specimen are diluted (for sera and plasma at 1:100) in Phosphate Buffer Saline (PBS). As positive controls strong and weak anti-dengue IgM and IgG as well as strong and weak anti-JE IgM and IgG are used. Normal human serum serves as the negative control. First the plate is being washed by rinsing all wells with PBS-T for 6 times and afterwards tapping the plate on layers of towel paper several times to dry the plate. 50µl/well of the diluted control serum and the diluted test serum is being dispersed. Afterwards the plate is being incubated for 2 hours at room temperature (18˚C to 30˚C). In this test pooled dengue antigens are used (DEN-1 HA titer 1:5120 16 units 1:320, DEN-2 HA titer 1:5120 16 units 1:320, DEN-3 HA titer 1:2560 16 units 1:160 and DEN-4 HA titer 2560 8 units 1:320) which are being added to 13.5% NHS (acetone extracted) in PBS 10 ml. Thereafter the plate is being washed avoiding cross contamination by using a Skatron Scan Washer to aspirate the wells at one time. The tips are rinsed twice with Milli Q Water and afterwards rinsed with PBS-T using the procedure mentioned above. Then 50µl of the dengue pooled antigens and the diluted JE antigens are added to the wells of the according plates. Incubation of the plates at room temperature (18˚C to 30˚C) for 2 hours follows this procedure. For the conjugation step 0.5% bovine serum albumin (BSA) in 13.5% NHS (acetone extracted) in PBS and

diluted human anti-Flavivirus IgG-HRP (conjugate) at 1:325 in 0.5% BSA in 13.5% NHS (acetone extracted) in PBS 12.0 ml is prepared. The plates are being washed again using the Skatron Scan Washer and the rinsing with PBS-T as mentioned above. 25µl of diluted conjugate are then added to every well and the plates are being incubated at 35˚C to 37˚C for 1 hour. For the substrate solution o-phenylenediamine, dihydrochloride (OPD) and 50mM citric acid/sodium phosphate buffer is being freshly prepared (30 minutes before use) and added after 6 cycles of rinsing the plates with PBS-T and 2 more cycles of rinsing with PBS as well as tapping. 100µl is dispersed in each well and incubated at room temperature for 15-45 minutes. Whenever the intensity of the color is similar to yellow tip‘s raining/corning the reaction will be stopped by adding 50µl of 4M H_2SO_4 to each well. The optical density (OD) was being measured at a wavelength of 492nm using an ELISA Reader manufactured by Tecan Austria GmbH within 20-30 minutes. The results were then calculated using the following formulas:

A weak positive control is defined as 100 EIA Units

EIA Units = BI x 100

$$\text{Binding Index} = BI = \frac{OD_{av}(test\ sample) - OD_{av}(NC)}{OD_{av}(WPC) - OD_{av}(NC)}$$

A value of IgM ≥ 40 units was considered as a supportive evidence of infection. A ratio of anti-dengue IgM to anti-Japanese encephalitis IgM ≥ 1.0 is typical of a dengue virus infection. A ratio of < 1.0 is typical of Japanese encephalitis virus infection. For dengue virus infection, a ratio of anti-dengue IgM to IgG (if IgM is ≥ 40 units) of 1.8 is typical of primary infection. A ratio of units IgM to units IgG < 1.8 is typical of secondary infection. Only specimens of cerebrospinal fluid with ≥ 40 units of anti-Japanese encephalitis IgM should be classified as coming from patient with “Japanese encephalitis”. Cases with only serum positive for anti-Japanese encephalitis IgM should be diagnosed “recent Japanese encephalitis virus infection”. Cases with anti-dengue IgM ≥ 40 units but not rising should be diagnosed “recent dengue virus infection”. Rising IgM values between acute and convalescent specimens indicated acute infections; IgM would be disappeared after a dengue virus infection usually

within 60 days. A specimen must be obtained at least 3 days after deferverescence without increasing antibody levels to state that there is no evidence of “recent flavivirus infection” (figure 6). Figure 6 has been reproduced from the laboratory manual from the Faculty of Tropical Medicine, Mahidol University.

Below the algorithm for the diagnosis of an acute primary or secondary dengue infection is drawn. As an addition to this scheme the current recommendation from the head of the laboratory responsible for carrying out the tests on the samples is to use a cut point lower than the values in the algorithm to describe an infection in the past (figure 5). Right now there is no consensus on the exact value as the titer decreases over time after an infection has occurred. For this study we assume a titer above 10 to prove that a participant had antigen contact within the last six months. Therefore if a participant shows an IgM titer higher than 10 either for dengue or je he will be considered as positive, otherwise as naïve. Though PCR testing would offer higher sensitivity it would only show positive in the very early stages of infection. IgG Cross-reactivity between dengue and Japanese B encephalitis or Yellow fever may be as high as 17% - 40%. IgM cross reactivity was not found after vaccination [24].

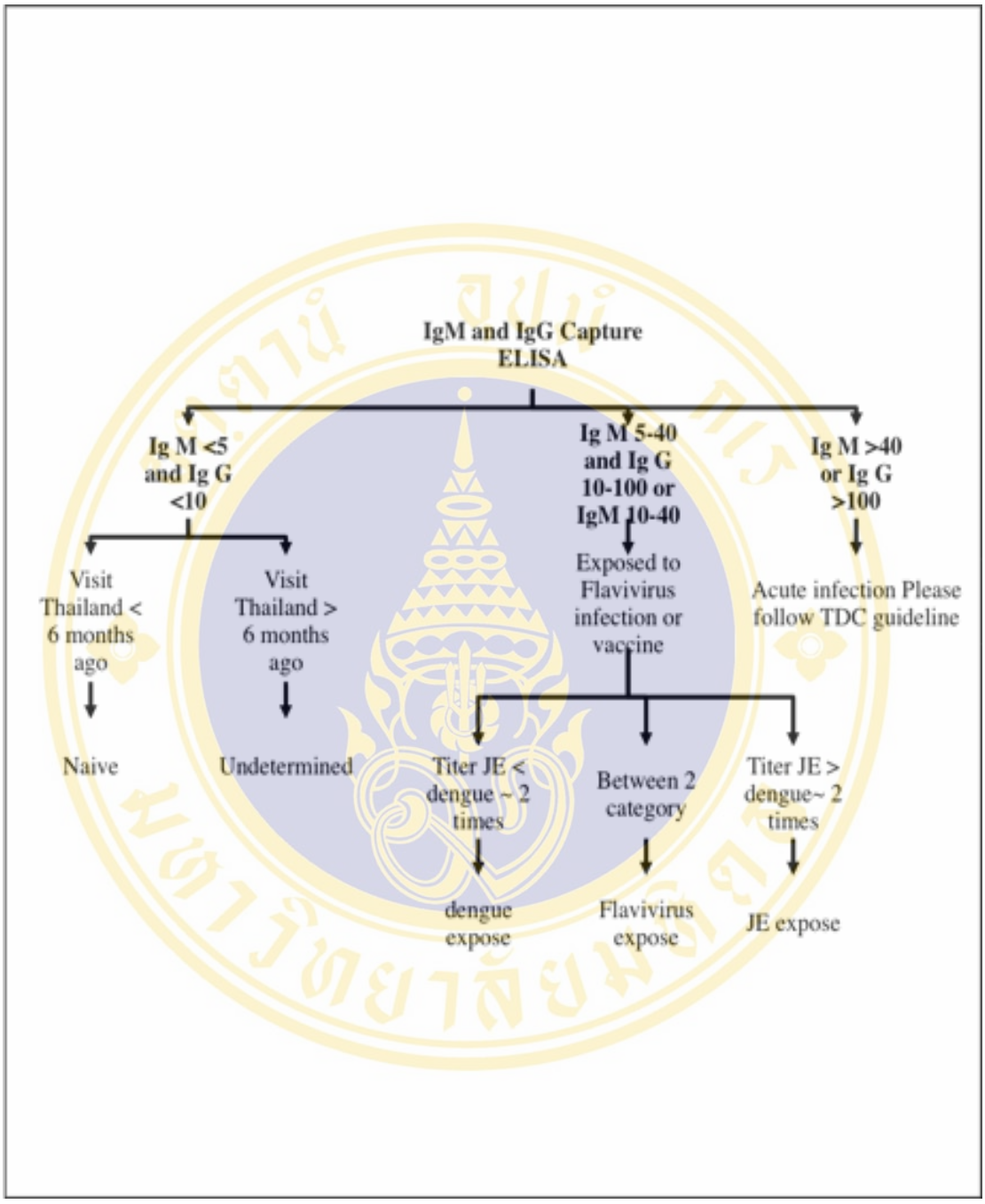
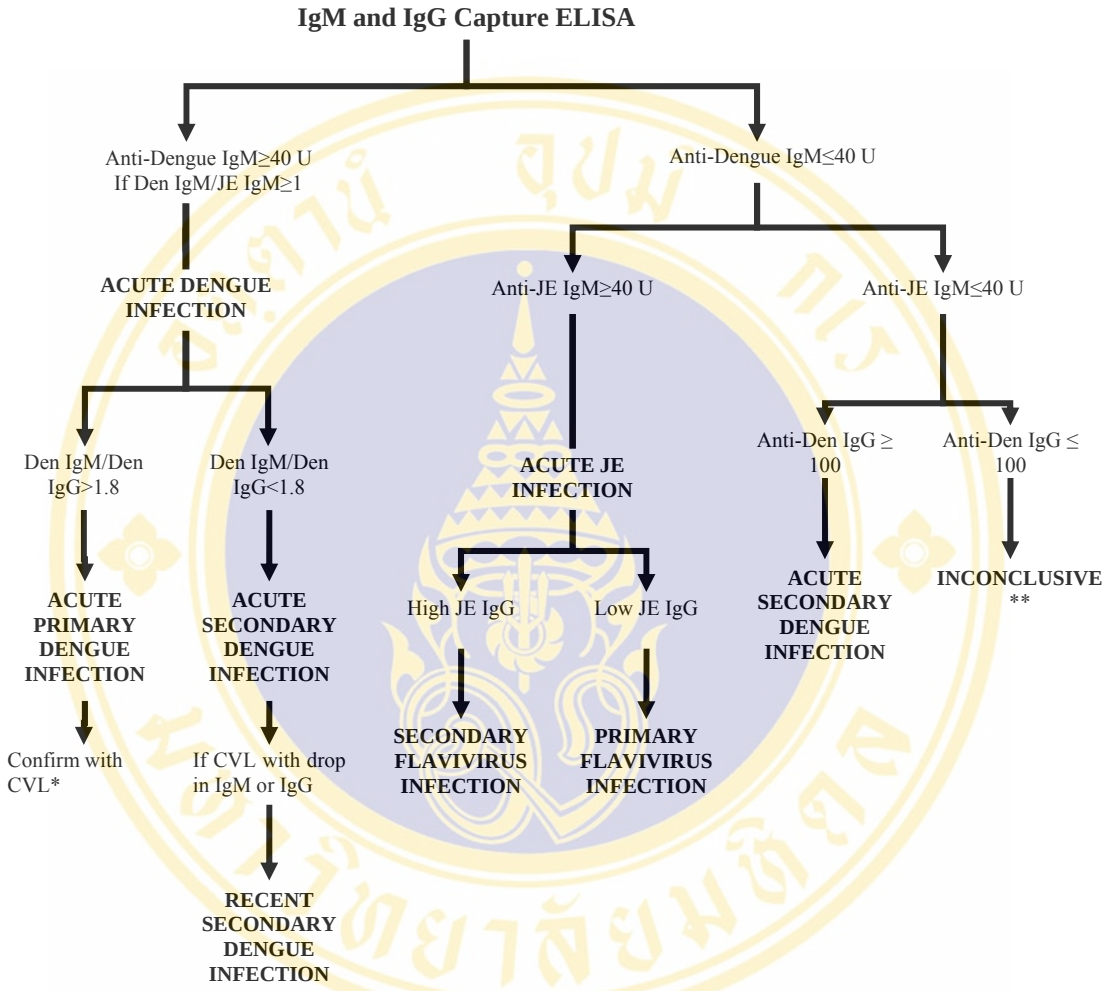


Figure 5: Algorithm for the determination of a past Flavivirus infection

Figure 6: Algorithm for the determination of an acute Flavivirus infection



CVL - Convalescent specimen (collected 2 5-7 days later or 1 7 days after illness onset or '2 3 days after deferveresence)

* confirm with CVL HAI for either 4-fold rise in titer or HAI > 1280 for any serotype

** Need CVL to further assess

6.6 JE IgM ≥ 40 = acute JE infection

6.7 DEN IgM ≥ 40 = acute dengue infection

If neither 1) or 2), then

6.8 4-Fold rise in HAI for any dengue serotype or JE or HAI > 1280 for any DEN or JE antigen = Acute flavivirus infection

6.9 Rise in Den or JE IgG to ≥ 100 units on the second specimen = Acute flavivirus infection

3.7.2. Questionnaire

The questionnaires have been distributed during the time of data collection.

The questionnaire consisted of 14 items. It was divided into:

- Section 1: General characteristics
- Section 2: Travel history and travel characteristics
- Section 3: Possible infection during the trip and/or diagnosed dengue infection in the past
- Section 4: Behaviour regarding protection to exposure including knowledge about the disease and it‘s transmission
- Section 5: Vaccination history

Section 1: General characteristics

Included were the following items: gender, age, nationality and purpose of the trip (tourism, visit friends or relatives, work or others). Close-ended questions had been used for this section.

Section 2: Travel history and travel characteristics

In this section the participant had been asked for a having previous stay in a dengue endemic area, duration of his current trip to South East Asia, if she or he travels as a backpacker (people who haven‘t planned their trip at all and choose their accommodation by the time they arrive at their destination), area of stay (mainly urban or rural).

Section 3: Possible infection during the trip and/or diagnosed dengue infection in the past

To determine a visit to the clinic likely to be caused by an infectious disease the participant first was asked about the reason of her/his visit to the clinic. Furthermore the traveler was asked if he had experienced a period of fever during his

trip accompanied by symptoms, which are typical for a dengue infection. An infection has been suspected to have occurred when the duration of the travel has exceeded 5 days and the traveler described an episode of fever during the current trip for more than 3 days showing at least two of the following symptoms: joint pain, headache, myalgia or rash.

Section 4: Behavior regarding protection to exposure including knowledge about the disease and its transmission

For this section items asking the participant about the usage of insect repellent and her/his general knowledge about dengue have been included.

Section 5: Vaccination history

To rule out possible cross-reactions from the results of the serum sample tests the participant was asked, if and when she/he received vaccinations against other flavivirus infections such as yellow fever, Japanese encephalitis and tick borne encephalitis. These findings have been used for the interpretation of the laboratory results according to the algorithm shown in figure 5. If the participant had not received the recommended booster injections within the specified times (every 10 years for vaccination against yellow fever, every 2 years for the Japanese – B – encephalitis vaccination and every five years for tick born encephalitis vaccination) he has been considered as not vaccinated based on the assumption, that he only would show some kind of antigenic profile within that time.

3.8. Data analysis

All data were reviewed using descriptive statistics such as mean, median, percentage and standard deviation were generated for sample characteristics. Furthermore categorical variables were analyzed using χ^2 test and Fisher‘s exact test, quantitative data using Student – t – test or Mann - Whitney – U test for comparison. All *P* values <0.05 were considered statistically significant and based on univariate analysis.

3.9. Ethical considerations

The study i.e. the research protocol has been approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University. In case the main investigator hasn‘t been present at the study site, because of the fact, there has been more than one site for data collection, the staff had been instructed to answer questions arising during answering the questionnaire. The participants also had the option to contact the main investigator in person by telephone. The purpose of the study had been explained to the participants by handing them out a participant information sheet. They also have been informed about possible risks and discomforts arising from the procedure blood being drawn. It‘s been made clear to them that they had the right to refuse participation in the study at any time without any obligations. They confirmed their will to participate by signing the participant information form as well as an informed consent form. Afterwards the questionnaire was handed out. Their name hasn‘t been used for the analysis. The questionnaires got a number, which linked them to the serum sample and a code for the study site. So the names of the study participants do not appear in any publication and the results are presented as a group only.

CHAPTER IV
RESULTS

The aim of this cross-sectional study was to assess the seroprevalence of travelers to South East Asia. For proof of a recent or past infection and therefore a positive seroprevalence a serological test has been used. The results in conjunction with a history of a recent vaccination against a flavivirus disease have been used for its interpretation. The study sites had been two clinics in the Bangkok Metropolitan area, Thailand.

A total 172 of questionnaires has been distributed during the time of data collection. Of those 172 valid have been obtained, which was 88% of the calculated required sample size of 195 participants.

The results can be divided into 2 parts: 1. General characteristics, travel history, travel characteristics, medical history reviewed by the means of descriptive statistics; 2. Review of the laboratory results; 3. Comparison and analysis for statistically significant differences between the seropositive and the seronegative subgroup;

4.1. Descriptive statistics of characteristics

172 individuals were enrolled into the study. Their age ranged from 18 to 71 years (with a mean age of 37.9 years). 61(35.5%) were below 30 years old, 43(25%) between 30 and 40, 29(16.9%) between 40 and 50 years and 25(14.5) ranged between 50 and 60 years of age. 14(8.2%) had an age of more than 60 years. 64.5%, the majority of the participants, were male. Their mean age has been 39.8 years with a range from 18 to 71 years. 35.5% were females with a mean age of 34.5 years ranging from 19 to 61 years.

All the general characteristics are comprehensively described in table 2, travel characteristics are summarized in table 3 and medical characteristics can be found in table 4.

The participants in this study had 23 different countries of origin. 114 individuals, which equals 66.3%, came from Europe, 15.7% from North America, 11.6% from Australia (including the participants from New Zealand), 4.7% from Asia and 1.7% from Africa. With a minimum of 1 day and a maximum of 6209 days the travelers had a median duration of stay of 21 days. The median duration of stay for tourists is 21 days. The mean duration of stay for travelers visiting friends or relatives is 13 days, 1019 for people who classify themselves as working in Thailand and 744 for those who don‘t put themselves in any of the categories.

9.9% of the participants were enrolled at the Travel Clinic at the Faculty of Tropical Medicine and 90.1% at the Travel Clinic at the QSMI. 73.8% of the study subjects marked tourism as their main reason for staying in South East Asia while 11% visited friends or relatives, 10.5% chose work and 4.7% had other, not further specified reasons for their journey. 76.2% marked their main area of stay to be urban and 23.8% to be rural reflecting the majority of the travelers of all four different subgroups declared to do so. 51.7% defined themselves according to the definitions made above to be backpackers compared to 48.3% who chose not to be. Apparently also 4 (22.2%) of the expatriates described themselves fitting into that category.

73.8% of the study subjects visited a dengue endemic area before their current trip and only 26.2% have never been to the areas defined by the WHO to be dengue endemic. With a median of 12 months since their last stay in a dengue endemic area the minimum and maximum value ranged between 1 and 240 months while only 68 participants were answering this item and 104 values were missing.

Asked about the reason for their visit to the clinic 64.5% were coming for other reasons, which almost all of them defined as a visit to the snake farm adjacent to the QSMI travel clinic. 18% received a vaccination, 15.7% were coming for a general checkup while 0.6% had symptoms related to an infection with the dengue virus and 1.2% came with clinical symptoms not related to a dengue infection. 4.1% had a history of a previously (not in the current trip) diagnosed dengue infection and 1.2% (2 participants) was diagnosed dengue during the current trip.

Of all the participants 4.1% could have probably gotten a dengue infection during their current trip (see table 4 for the definition of the clinical symptoms).

29.7% used an insect repellent during the evening and the nighttime, 14.5% also during the daytime while the majority 55.2% didn‘t use any kind of insect repellent at all.

Of all the travelers participating in the study 59.3% declared to have some knowledge related to the dengue disease before getting informed by the investigators and 40.7% indicated to have never heard before about it.

A total of 39 (22.7%) individuals received vaccination against yellow fever. The highest percentage of people vaccinated against yellow fever with the same continent of origin was applicable to the travelers coming from North America (29.6%) followed by Europeans (25.4%) and Asians (25%). None of the three participants from Africa had received the vaccination. For the vaccination against Japanese – B – encephalitis, which 23(13.4%) out of 172 participants have stated to have received, the volunteers from Asia had the highest vaccination rate of 50% (4 out of 8 participants had received the vaccination against Japanese – B – encephalitis). 33.3% of all African, 14% of all European as well as 7.5% out of all the participants from North America also have been vaccinated against Japanese – B – encephalitis at least one time in their life. Only travelers from Europe reported to have been vaccinated against tick borne encephalitis in the past. Their proportion has been 6.1%. The mean time (in years), which has passed since the vaccine had been administered has been 3.07 for yellow fever, 0.82 for Japanese – B – encephalitis and 2.83 for Tick borne encephalitis. For more convenience most of the findings from above are being visualized in figure 7 – figure 20.

Table 2: General characteristics of 172 travelers to Thailand

General characteristics (n=172)	Frequency	Percent
Study site		
Travel clinic at the Faculty Of Tropical Medicine	17	9.9
QSMI	155	90.1
Age in years		
< 30	61	35.5
30-39	43	25
40-49	29	16.9
50-59	25	14.5
60-69	12	7
>70	2	1.2
Mean ± SD / 37.9 ± 13.3 Range: 18-71		
Gender		
Male	111	64.5
Female	61	35.5

Table 3: Travel characteristics of 172 travelers to Thailand

Travel characteristics (n=172)	Frequency	Percent
Origin		
Europe	114	66.3
North America	27	15.7
Australia, New Zealand	20	11.6
Asia	8	4.7
Africa	3	1.7

Table 3: Travel characteristics of 172 travelers to Thailand (cont.)

Travel characteristics	Frequency	Percent
Area of stay		
Urban	131	76.2
Rural	41	23.8
Purpose of travel		
Tourism	127	73.8
Visit friends or relatives	19	11.0
Work	18	10.5
Others	8	4.7
Backpacker		
Yes	89	51.7
No	83	48.3
Previous visit of a dengue endemic area		
Yes	127	73.8
No	45	26.2

Table 4: Medical characteristics of 172 travelers to Thailand

Medical characteristics	Frequency	Percent
Previous diagnosis of dengue infection		
No	163	94.8
In conjunction with a previous trip	7	4.1
During the current trip	2	1.1
Purpose of visit to the study site		
Others	111	64.5
Vaccination	31	18.0
Checkup	27	15.7
Symptoms, not further specified	2	1.2
Symptoms related to a dengue infection	1	0.6
Probable infection (clinically)*		
Yes	7	4.1
No	165	95.9
Usage of an insect repellent		
No	95	55.2
In the evening	51	29.7
Both	25	14.5
Daytime only	1	0.6
Knowledge about dengue		
Yes	102	59.3
No	70	40.7
Vaccinated against Yellow Fever		
Yes	39	22.7
No	133	77.3

* episode of fever during the current trip for more than 3 days showing at least two of the following symptoms: joint pain, headache, myalgia or rash

Table 4: Medical characteristics of 172 travelers to Thailand (cont.)

Medical characteristics	Frequency	Percent
Vaccinated against Japanese – B – Encephalitis		
Yes	23	13.4
No	149	86.6
Vaccinated against Tick – Borne – Encephalitis (n=172)		
Yes	7	4.1
No	165	95.9

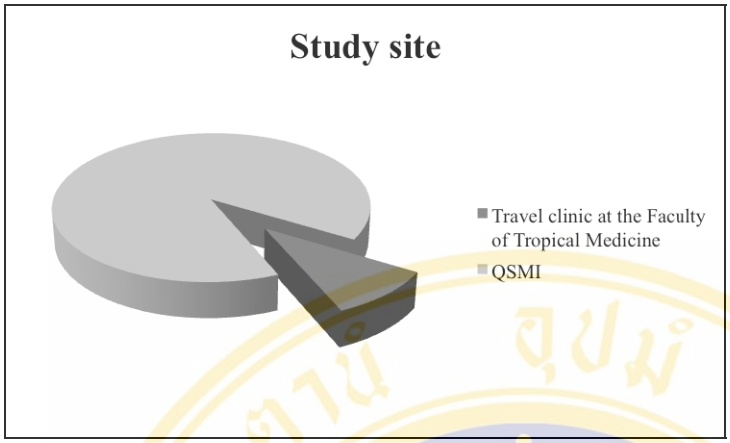


Figure 7: Study Sites

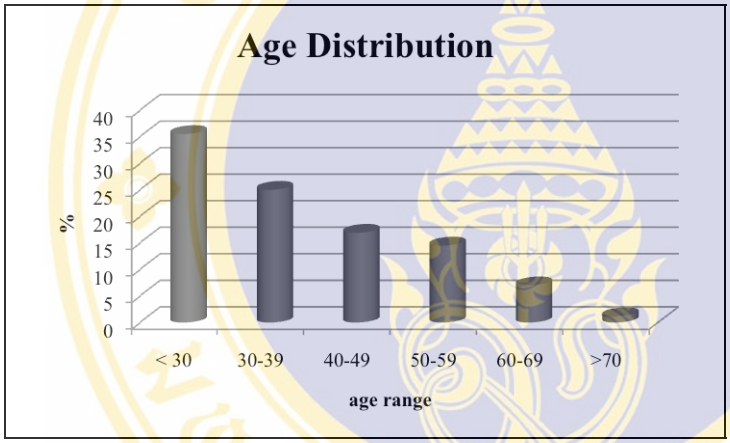


Figure 8: Age Distribution

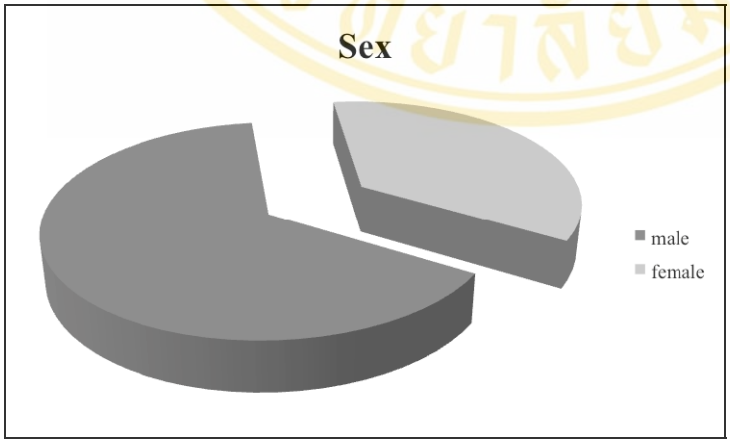


Figure 9: Sex

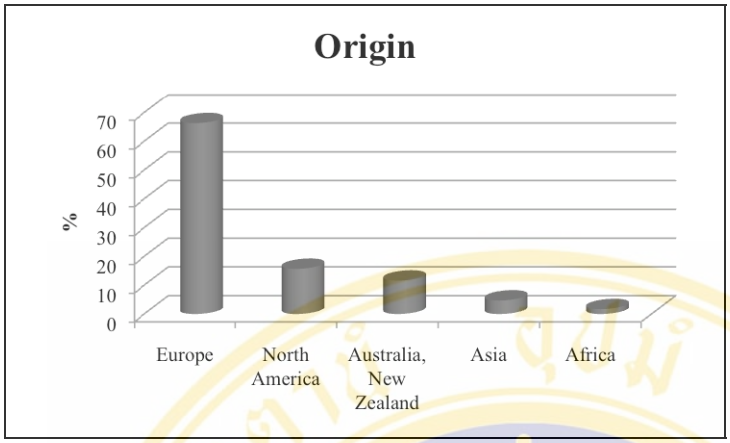


Figure 10: Origin

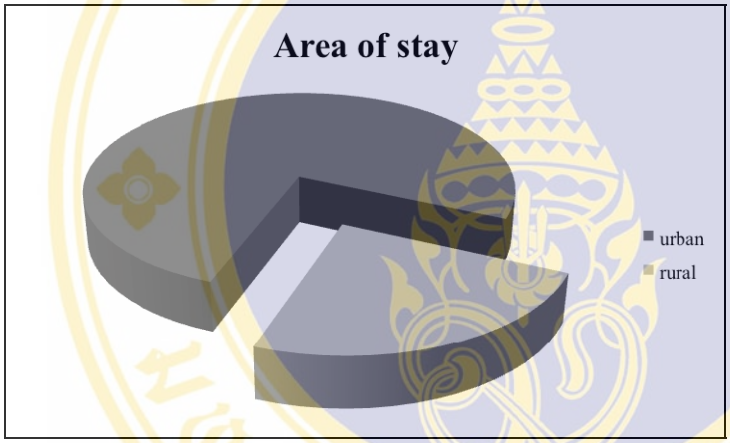


Figure 11: Area of stay

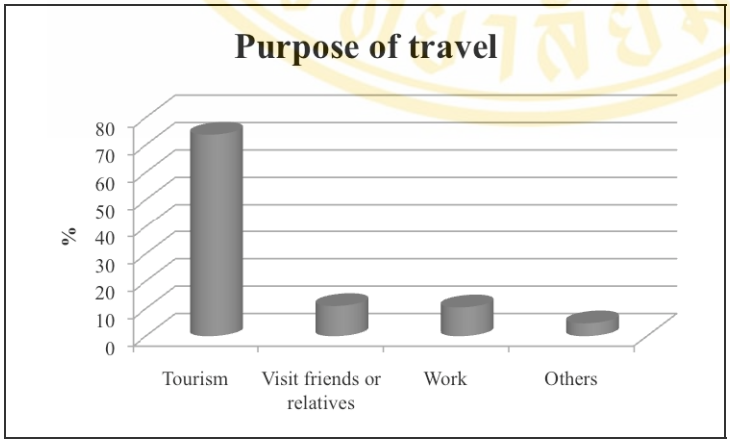


Figure 12: Purpose of travel

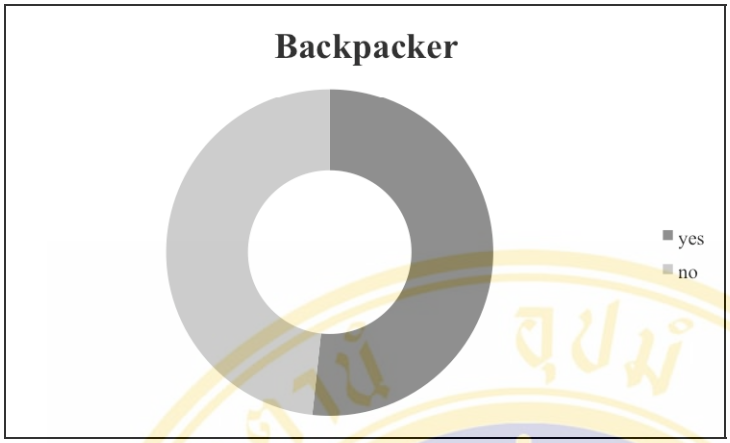


Figure 13: Backpacker

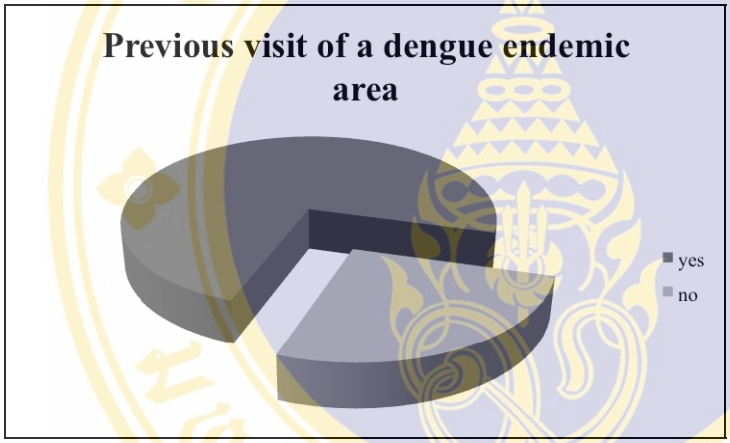


Figure 14: Previous visit of a dengue endemic area

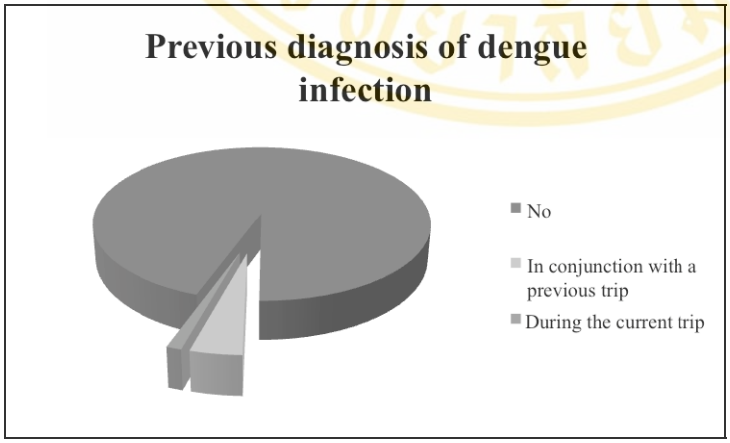


Figure 15: Previous diagnosis of a dengue infection

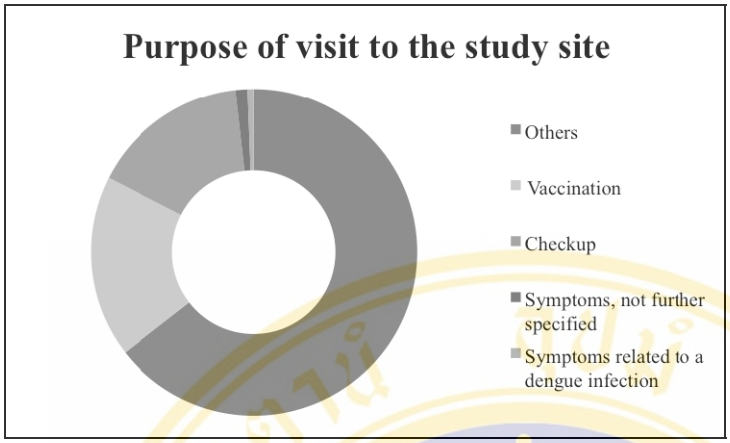


Figure 16: Purpose of visit to the clinic

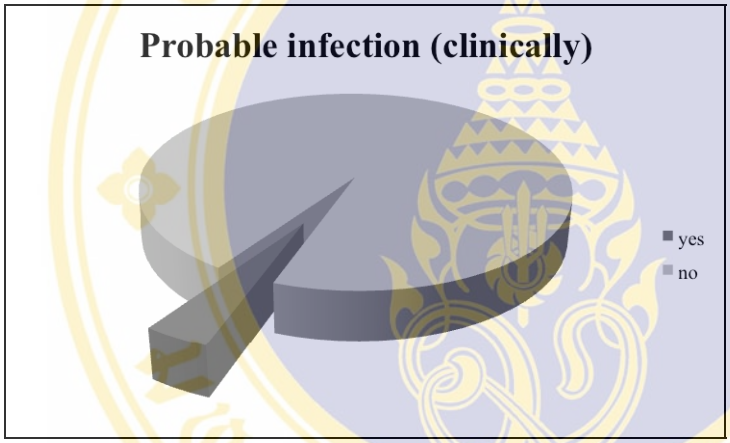


Figure 17: Probable dengue infection (clinically)

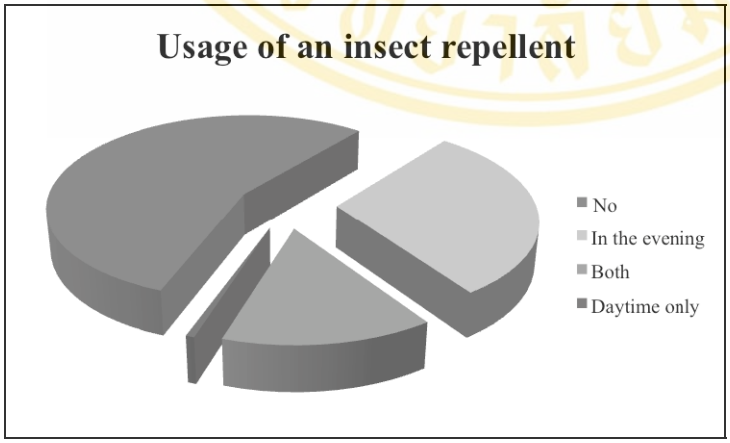


Figure 18: Usage of insect repellent

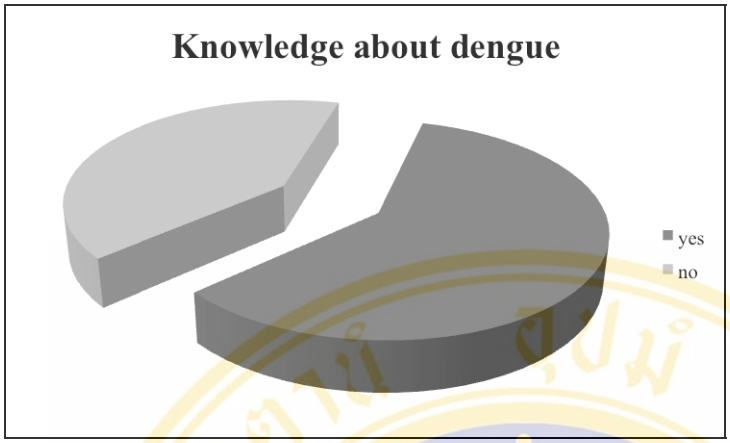


Figure 19: Knowledge about denuge

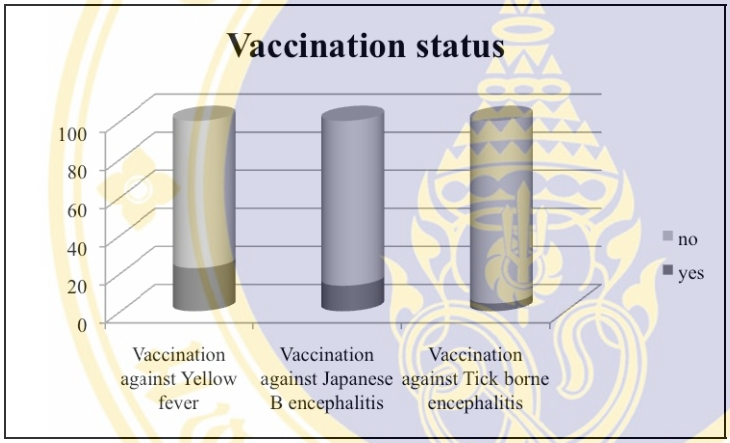


Figure 20: Vaccination status

4.2. Laboratory results - Seroprevalence

None of our participants had laboratory results suggesting having an acute infection with either the dengue or the Japanese – B – encephalitis virus. According to the algorithm which has been introduced in the "materials and methods" section a total of 11 participants showed remarkable laboratory results in a way, that they may be defined as having been exposed to the dengue virus at some time in the past.

Considering a total of 172 participants the seroprevalence is 6.4% (11/172).

When taking a closer look at the cases ruling out possible cross reactions due to vaccination against Yellow fever, Japanese B encephalitis or Tick borne disease as well as all the travelers who stayed less than 5 days in Thailand which would be below the incubation period for the dengue infection the estimated incidence rate over a median duration of stay of 21 days would be 4.2% (6/142). A detailed description of each of these cases will be provided in the "discussion" section.

10 (5.8%) participants showed an elevated titer of antibodies against Japanese B encephalitis in the laboratory results, which qualified them as being exposed to the Japanese B encephalitis virus in the past according to our algorithm. 1 of them (0.6%) could not be directly related to a possible cross reaction due to a recent vaccination against a flavivirus. These results will be shown in table 5 as well as in figure 21.

Table 5: Laboratory results

Laboratory results (n=172)	n	Frequency	Percent
Elevated dengue antibody titer*			
≤ 5 days	30	3	1.7
> 5 - ≤ 28 days	83	6	3.5
> 28 days - ≤ 6 months	47	1	0.6
> 6 months	12	1	0.6
Total	**172**	**11**	**6.4**
Elevated Japanese B encephalitis antibody titer**			
≤ 5 days	30	4	2.3
> 5 - ≤ 28 days	83	2	1.2
> 28 days - ≤ 6 months	47	4	2.3
> 6 months	12	0	0
Total	**172**	**8**	**5.8**

* dengue Ig M 5-40 and Ig G 10-100 or IgM 10-40 and titer JE < dengue ~ 2 times
** JE Ig M 5-40 and Ig G 10-100 or IgM 10-40 and titer JE > dengue ~ 2 times

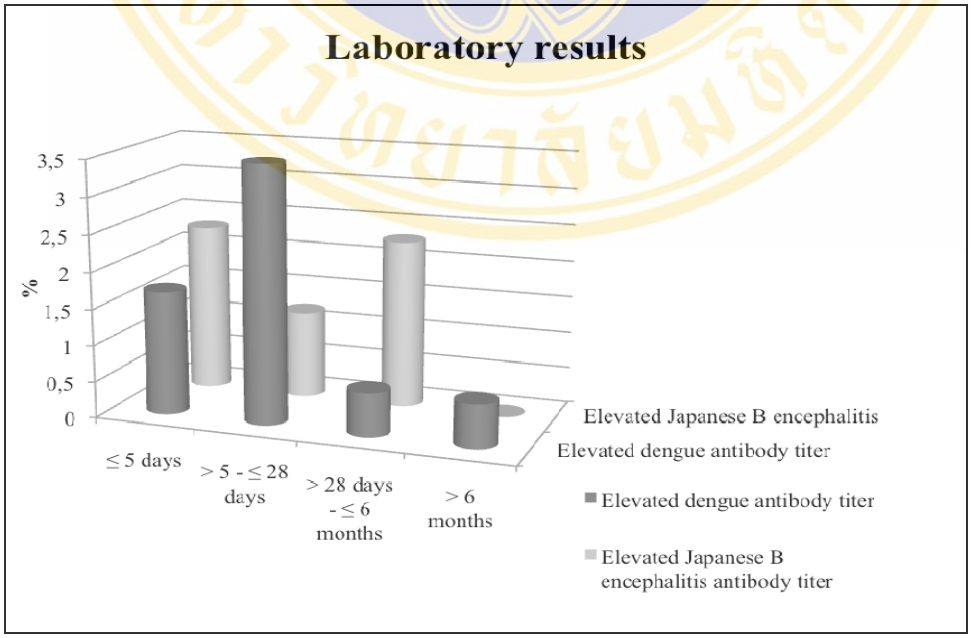


Figure 21: Laboratory results

4.3. Comparison of the subgroups

In the following table a closer look at the characteristics of the seropositive and seronegative subgroup will be taken by putting them into comparison and looking for any significant correlation of data.

Table 6: Subgroup analysis

	Seropositive (n=11)	Seronegative (n=161)	p-value
Age	37*	37.95**	0.819§
Sex (male)	63.6% (7)	64.6% (104)	1.000†
Backpacker (Yes)	54.5% (6)	51.6% (83)	1.000†
Area of stay (Urban)	81.8% (9)	75.8% (122)	1.000†
Possible clinical infection (Yes)	18.2% (2)	3.1% (5)	0.066†
Purpose of travel			0.203‡
Previous visit of a dengue endemic area (Yes)	81.8% (9)	73.3% (118)	0.730†
Purpose of visit to the clinic			0.104‡
Knowledge of dengue (Yes)	63.6% (7)	59% (95)	1.000†
Usage of insect repellent (Yes)	54.5% (6)	55.3% (89)	1.000†
Dengue diagnosed before (Yes)	9.1% (1)	5% (8)	0.456†

Table 6: Subgroup analysis (cont.)

	Seropositive (n=11)	Seronegative (n=161)	p-value
Vaccination against yellow fever (Yes)	36.4% (4)	21.7% (35)	0.273†
Vaccination against Japanese B encephalitis (Yes)	18.2% (2)	13% (21)	0.644†
Vaccination against Tick borne encephalitis (Yes)	0% (0)	4.3% (7)	1.000†

* Mean SD ± 10.44
* Mean SD ± 13.49
* Student t-test
§ Fisher's exact test
† χ^2 test
‡

None of the items has been significantly different between the seropositive and the seronegative group. It should be mentioned, that the number of cases in the seropositive group has been quite small and this may have influenced the outcome of the findings.

CHAPTER V
DISCUSSION

Within the last decades South East Asia became one of the “hotspots” concerning the spread of the dengue infection. This fact in conjunction with the large and still growing number of tourists coming to this region to make vacation converts this major issue for local health authorities to be one of global interest. These developments were leading to an impetus for many researchers in the field of Travel Medicine as well as Tropical Medicine to conduct studies focusing on prevalence and incidence rates of dengue on their patients. All of the major prevalence and incidence studies have in common, that they have been carried out in the tourists‘ countries of origin.

Considering this fact it should be mentioned that dengue infections often tend to get underdiagnosed and thus underestimated [25], because the disease may only resemble the symptoms of a mild febrile disease, which many of the affected individuals not recognize as a potentially severe disease and therefore not seek for medical attention. Furthermore the short incubation time of the disease from 5 to 7 days often drives the patient to look for treatment during his vacation.

To avoid these limitations the study design was aimed to address a population of travelers with a maximum on heterogeneity, which is also being reflected by the chosen study sites. By excluding people who have been born in endemic areas the center of interest was aligned more to the subpopulation of travelers.

All the sera were tested with a well-established MACELISA test. Infections in their early stage should have been ruled out by the questionnaire in which the participant has been asked about symptoms, which are likely to accompany a dengue infection. PCR would offer higher sensitivity but would only show positive in the very early stages of infection. IgG Cross-reactivity between dengue and Japanese

B encephalitis or Yellow fever may be as high as 17% - 40%. IgM cross reactivity was not found after vaccination [24].

5.1. General characteristics

The majority of the study participants originated from Europe and was enrolled while visiting the snake farm or searching for medical attention in the travel clinic at the Queen Saovabha Memorial Institute. Most of them, as stated above, have just been visitors to the snake farm. No kind of randomization has been applied when the participants were asked to join. People below 40 years of age originating from Europe were most prevalent, which fits well with the numbers from the Thai Office for Tourism Development. The vast majority of them had visited an area endemic for dengue before this trip, which we had to consider when making the interpretation of the laboratory results. Unfortunately most of them didn‘t answer, when they have been to such a destination for the last time. Most of our participants regarded themselves as tourists and backpackers and they spent most of their time in well-developed urban areas, which would have been associated with a higher risk of acquiring a dengue infection. The median duration of stay had been 21 days. Only a minority used insect repellent and an even smaller fraction of this minority used it during the daytime despite the fact, that almost 60% stated, that they had knowledge about the disease. Although none of the individuals showed a laboratory result, which would suggest him or her having an acute infection, there has been one case, which has been diagnosed with an acute dengue infection at the travel clinic at the Faculty of Tropical Medicine, Mahidol University.

5.2. Interpretation of conspicuous laboratory results

Below all cases, which have been identified to have abnormally elevated titer levels will be reviewed more detailed. A subdivision into 4 different categories regarding the duration of stay had been chosen to allow an identification of an infection, which might have occurred during the current trip. A summary of these findings will be given in table 7 for the dengue cases and table 9 for the Japanese B

encephalitis cases. Factors influencing the outcome in a way that we chose not to interpret it as seropositive will be summarized in table 8.

5.2.1. Dengue

5.2.1.1. Duration of stay less than 5 days

Case No. 5: The duration of this 31 years old female participant‘s stay in Thailand in this case has been 3 days. The woman originated from US-America and came to visit friends or relatives. Her last stay in a dengue endemic area has been 24 months before this trip. The participant didn‘t report about any history of vaccinations against either Yellow fever, Japanese B encephalitis or Tick borne encephalitis. She didn‘t have any symptoms, which might be related to a dengue infection now or in the past. Dengue IgM titer has been 10. An infection during a previous trip may be likely.

Case No. 175: With a dengue IgM titer of 5 and IgG titer of 11 this participant, a 47 years old male from Australia, met the criteria for a previous dengue infection. He didn‘t have a vaccination history against any of the viruses mentioned above and visited dengue endemic areas before not specifying the time, which has passed since then. When taking part in the study he had been 2 days to Thailand. He came as a tourist and didn‘t report about any dengue related symptoms.

Case No. 201: This 40 years old male from the UK had a duration of stay of 3 days when taking part in our study. He had been to a dengue endemic area previously but not specifying when. He had been vaccinated against Yellow fever 7 years ago. Purpose of his visit has been tourism. He didn‘t complain about any symptoms at all and the laboratory results showed a dengue IgM titer of 12, which might be related to a previous exposure to the dengue virus.

5.2.1.2. Duration of stay 5-28 days

Case No. 13: The participant, a 31 years old German female visiting friends and relatives, has both, an elevated IgM dengue titer (20) as well as an elevated JE IgM titer (19). According to the questionnaire, she came to the Travel clinic with symptoms, likely to be a possible dengue infection. The duration of the current stay has been 18 days. She visited a dengue endemic area 36 months ago. There is no vaccination history against Yellow fever or Japanese B encephalitis. IgG titer for both, dengue and Japanese B encephalitis is not elevated. This case is likely to be a recent dengue infection.

Case No. 121: A 39 years old British expat with a duration of stay of 28 days showing an elevated dengue IgM titer (14). All other values have been within a normal range. Twelve months ago he had been to a dengue endemic area. The participant doesn‘t have vaccination against Yellow fever or Japanese B encephalitis. The patient had been asymptomatic. He has never been diagnosed to have a dengue infection. According to the algorithm, the patient is also likely to have had a dengue infection in the past.

Case No. 143/144: The participants have been a young couple from Austria just returning from a trip to the South of Thailand and a duration of stay of 21 days. The 29 years old female from Austria with a duration of her stay of 21 days. The 38 years old male participant has a vaccination history for Yellow fever 3 years ago and had a stay at a dengue endemic area 24 months ago while the female hasn‘t been vaccinated against Yellow fever or Japanese B encephalitis and doesn‘t mention a previous stay in a dengue endemic area. The reason for their stay had been travelling and none of them showed any symptoms during their stay. According to the algorithm they also might qualify as a dengue infection.

Case No. 148: This participant has been a sixty one year old male from US-America who visited Thailand as a tourist. He came to the QSMI clinic for vaccination and received a Yellow fever vaccination recently. He showed an elevated dengue IgM (26) and IgG (18) as well as an elevated JE IgG (25) titer. He has been to Thailand for 7

days and didn‘t show any dengue related symptoms. As the participant received a vaccination against a Flavivirus quite recently possible cross-reaction cannot be ruled out. This also may be supported by the fact that he didn‘t show any dengue related symptoms.

Case No. 171: This 42 years old participant from Germany spent 21 days in Thailand visiting friends or relatives. The patient didn‘t report about any symptoms and didn‘t have any vaccination history against flaviviruses. He never visited a dengue endemic area before. His dengue IgM antibody titer has been 13. Dengue IgG as well as JE IgM and IgG haven‘t been remarkable at all. Although the IgM antibody titer has been quite low and he didn‘t report travel history to other endemic areas he would be determined having a dengue infection in the past.

5.2.1.3. Duration of stay >28 days to 6 months

Case No. 142: A 28 years old Japanese male expat with a duration of stay of 30 days. He came to the clinic for vaccination against Japanese B encephalitis, the first shot having already taken before. He didn‘t report about any symptoms and his laboratory results showed dengue IgM titer of 11 IgG titer of 12 and JE IgM titer of 8. The participant visited a dengue endemic area before but did not specify when. According to his vaccination history a cross-reaction cannot be ruled out.

5.2.1.4. Duration of stay > 6 months

Case No. 243: This participant has been a 30 years old Belgian nurse working full time in Papua where dengue and Japanese B encephalitis is almost endemic. She has been vaccinated against Japanese B encephalitis 5 years ago and against Yellow fever 4 years prior to her participation in our study. She has been the only participant with a conspicuous laboratory result and a previously diagnosed dengue infection. Dengue IgM titer had been 12, IgG 26, JE IgM 4 and JE IgG 43. These results would fit well with her vaccination history and a previous infection with the dengue virus.

Table 7: Dengue seropositivity over duration of stay

Elevated dengue antibody titer			
Duration of stay	**n**	**Seropositive**	**Probable infection during this trip**
≤ 5 days	30	3	Not applicable
> 5 - ≤ 28 days	83	6	5
> 28 days - ≤ 6 months	47	1	0
> 6 months	12	1	1

Table 8: Summary of conspicuous cases (Dengue)

Case No.		**5**	**175**	**201**	**13**	**121**	**143**	**144**	**148**	**171**	**142**	**243**
Age (yrs)		31	47	40	31	39	29	38	61	42	28	30
Sex		F	M	M	F	M	F	M	M	M	M	F
Duration of stay (days)		3	2	3	18	28	21	21	7	21	30	548
Previous trip to a dengue endemic area		Y	Y	Y	Y	Y	N	Y	Y	Y	N	Y
Vaccination history		No	No	YF	No	No	No	YF	YF	No	JE	YF/JE
Clinical signs of a dengue infection		N	N	N	Y	N	N	N	N	N	N	Y
Dengue	**IgM**	10	5	12	20	14	13	11	26	13	11	12
	IgG	1	11	-2	0	-1	13	12	18	-1	12	26
JE	**IgM**	8	1	8	19	0	9	9	-4	1	8	4
	IgG	2	4	-3	2	2	5	4	25	-1	2	43

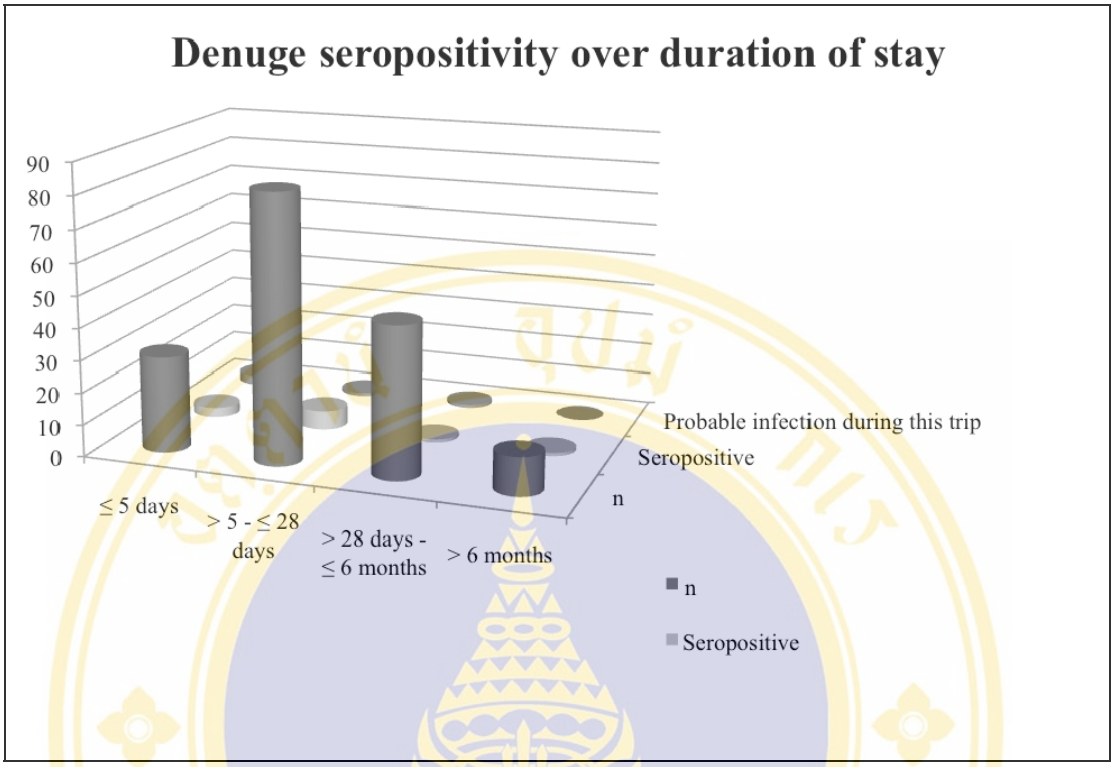


Figure 22: Dengue seropositivity over the duration of stay

Table 9: Corrected number of dengue antibody seropositive individuals

	Numbers excluded	Reason
Duration of stay	**n**	
≤ 5 days	3	A duration of stay less than 5 days cannot be responsible for an infection during the current trip because of an incubation time of at least 5 days
> 5 - ≤ 28 days	1	Recent Yellow fever vaccination
> 28 days - ≤ 6 months	1	Recent Japanese B encephalitis vaccination
> 6 months	0	-

5.2.2. Japanese B encephalitis

5.2.2.1. Duration of stay less than 5 days

Case No. 111: This 28 years old female Canadian tourist reported to have been vaccinated against Yellow fever and Japanese B encephalitis recently which might explain her elevated JE IgM of 18. She has been to Thailand 5 days prior to her visit to the clinic.

Case No. 190: This 43 years old male participant from Denmark has been diagnosed having a dengue infection in a previous trip. He received vaccination against the Yellow fever virus 4 years and against the Tick borne encephalitis 3 years prior to his participation in our study. The duration of travel in this trip has been 3 days. The only remarkable laboratory result had been a Japanese B encephalitis IgG titer of 12. This might most likely be related to a cross-reaction resulting from his dengue infection or the vaccinations he received.

Case No. 239: In this case of a 27 years old female tourist from Japanese the JE IgG titer had been elevated to 13. She came for a medical checkup and didn‘t complain about symptoms at all. She had been vaccinated against the Japanese B encephalitis virus one year prior to her participation in this study. The elevated titer therefore may be related to the vaccination.

Case No. 251: This 22 years old male from the UK had a JE IgM titer of 10. He reported to have received vaccination against both Yellow fever and Japanese B encephalitis one year prior to his visit to our clinic. The duration of his current trip has been 4 days. The laboratory result may be most likely related to his vaccination history and a cross reaction of antibodies.

5.2.2.2. Duration of stay 5-28 days

Case No. 2: The participant, a 26 years old female expat from Sweden, showed an elevated JE IgM titer of 12. She reported to have received vaccination against Japanese B encephalitis virus 6 months prior to her participation in our study, which would explain the result. The duration of her current stay had been 6 days.

Case No. 219: 58 years old male from US-America, staying in Thailand for 21 days. He came to the clinic for vaccination but didn‘t show and history of vaccination against flaviviruses. He didn‘t complain about any symptoms at all. He previously visited dengue endemic areas but didn‘t specify when. The lab revealed an elevated dengue IgG (15) and JE IgG (12) titer. An exposure to a flavivirus might have had occurred in the past.

5.2.2.3. Duration of stay >28 days to 6 months

Case No. 154: A 49 years old male from US-America staying in Thailand for 60 days. He had received vaccination against the Yellow fever virus 4 years ago and showed an elevated dengue IgG titer of 15 which might be related to a previous exposure to the Japanese B encephalitis virus or to a cross reaction related to the Yellow fever vaccination.

Case No. 155: A 23 years old male participant from Japan. He had been vaccinated against Yellow fever recently and showed a JE IgG titer of 14. This might also be related either to an exposure to the Japanese B encephalitis virus or to a cross reaction related to the vaccination.

Case No. 196: This 22 years old Swiss tourist staying in Thailand for 30 days had a Japanese B encephalitis IgM titer of 19. This result will be most likely related to his recent vaccination against this virus.

Case No. 198: The remarkable laboratory result in this case had been an elevated Japanese B encephalitis IgM titer of 11. The participant has been a 55 years old women from Canada who had come to South East Asia for many times and had a duration of 120 days of her current trip. She didn't report to have been vaccinated against Japanese B encephalitis, Yellow fever or Tick borne encephalitis. Therefore this case may not be assigned to a cross-reaction but may reflect a recent exposure to the Japanese B encephalitis virus with an asymptomatic course.

Table 10: Summary of conspicuous cases (JE)

Case No.		**111**	**190**	**239**	**251**	**2**	**219**	**154**	**155**	**196**	**198**
Age (yrs)		28	43	27	22	26	58	49	23	22	55
Sex		F	M	F	M	F	M	M	M	M	F
Duration of stay (days)		5	3	3	4	6	21	60	60	30	120
Vaccination history		YF/JE	YF/TBE	JE	YF/JE	JE	No	YF	YF	JE	No
Dengue	**IgM**	-1	4	5	3	9	2	-2	5	2	1
	IgG	1	9	2	0	0	15	5	7	-1	-1
JE	**IgM**	18	4	3	10	12	0	2	-3	19	11
	IgG	7	12	13	1	7	12	15	14	4	3

Table 11: Japanese B encephalitis seropositivity over duration of stay

Elevated Japanese B encephalitis antibody titer			
Duration of stay	**n**	**Seropositive***	**Probable infection during this trip**
≤ 5 days	30	4	Not applicable
> 5 - ≤ 28 days	83	2	1
> 28 days - ≤ 6 months	47	4	1
> 6 months	12	0	Not applicable

* Seropositivity is defined as JE Ig M 5-40 and Ig G 10-100 or IgM 10-40 and titer JE > dengue ~ 2 times

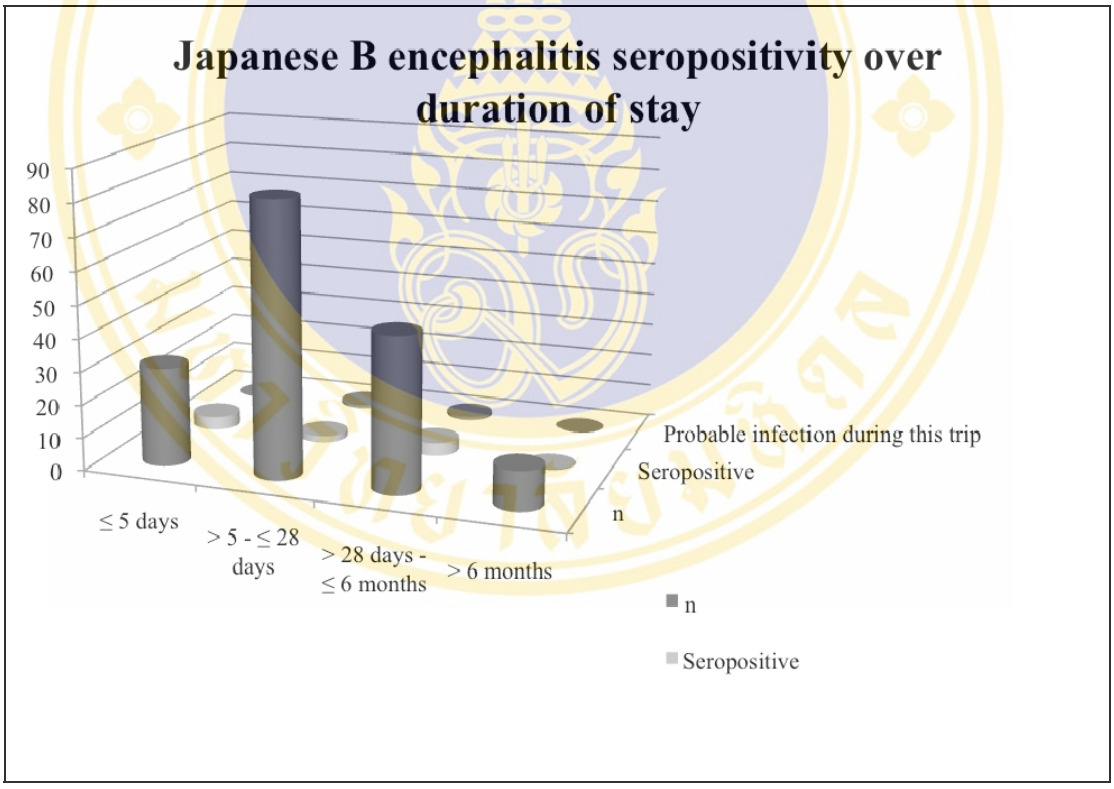


Figure 23: Japanese B encephalitis seropositivity over the duration of stay

Overall the seroprevalence of JE in this study would equal 5.8% (10/172).

Taking all the figures from above into consideration it can be summarized, that the seroprevalence for a traveler coming to Thailand would be as high as 6.4% for dengue antibodies. If we subtract the number of individuals, who might have other

causes for their seropositivity, which, in this study have been a recent vaccination against Yellow fever and against Japanese B encephalitis and can be reviewed in table 8 and those, who probably haven‘t got infected during their current trip, we would get a seroprevalence of 3.5%. What we couldn‘t rule out, especially for the travelers coming from Europe, has been a previous exposure to flaviviruses common there, such as the Central Europe encephalitis or Tick borne encephalitis [25]. Apparently we were not able to say distinctively if a traveler got infected during his current trip or not. The reasons for that circumstance will be highlighted below.

Compared to previous studies from who found a seroprevalence ranging between 3.4% and 13.4% [5] and 8% in symptomatic patients [28] this result doesn‘t differ very much. For the algorithm used for the interpretation we took into account, that most of the individuals would have lost their elevated IgM antibody titer 60-90 days following an infection [16]. Since this study has been a cross-sectional study the incidence rate could not be determined also causing the circumstance not having had our patients available for a follow up and not being able to measure an increase in the IgG titer to prove a secondary infection. One of our participants, a 26 years old female from Switzerland, had been diagnosed with a dengue infection but did not show an elevated IgM or IgG titer at all. The remarkable findings in the laboratory results have been 54% Lymphocytes with 6% Atypical Lymphocytes in the differential blood count and 36% Neutrophils in the CBC. This finding underlines the limitation of the ELISA testing in verifying a dengue infection in its early stage. Also seasonal differences as seen in previous studies [25, 29, 30] would not have been recognized due to our data collection period during the dry season from early November 2008 until early January 2009, while the peak seasons for transmission would be the rainy seasons.

Surprisingly we could not find any statistically significant correlations between factors associated with a higher risk for an infection by other studies [5, 24, 25, 31] like age and gender. Also no correlation between areas of stay, purpose of travel, being a backpacker or usage of an insect repellent could have been shown here. Furthermore symptoms related to a dengue infection according to the definition made above could not be indentified as a reliable predictor.

5.3. Limitations

Several limitations applied to this study and have therefore to be taken into consideration:

a) Due to the fact we were choosing a cross sectional design for the study measuring the seroprevalence in our participants we had to apply certain additional items in the questionnaire as well as looking for a proper way for the interpretation of the laboratory results to make it less likely including individuals, who might have been seropositve before their arrival to Thailand. By looking for the incidence rate we could have avoided this limitation. The reason why that had not been possible will be described in the next point.
b) Time for data collection has been limited due to the design of the study and the necessity to finish within a given period of time of 5 months including 2 months for the data collection.
c) The study had been carried out during the dry season, which might add a bias to the results considering the fact, that transmission of dengue during this season is generally lower than in the rainy season caused by the absence of the *Aedes* vectors.
d) The data collection period coincided with the occupancy of Suvhanabumi airport, Thailand‘s largest airport and the major route for most of the tourists to enter the country. The political disturbances during late November and early December 2008 led in conjunction with the closing of the airport in a severe downturn in tourist numbers and therefore in less people visiting the study sites. Due to these developments we haven‘t been able to acquire the originally calculated number of 195 study participants.
e) By using ELISA based tests for the evaluation of the blood samples early stage infections might not have been recognized due to the lack of antibodies. Besides that the result is also depending on the test, which is being used as showed by Allwinn et al. in 2008 in their dengue seroprevalence study. The results in their study ranged between 8.7 and 19.5%, which is a quite remarkable span.
f) Because of the very small number of positive cases analysis of the data using advanced statistics has not been reasonable and therefore not taken place.

g) The algorithm used for the interpretation of the lab results has been introduced especially for this study only.
h) The exclusion criterion for participants visiting dengue endemic areas before could not be kept due to the difficulty in acquiring sufficient case numbers showing that feature.

5.4. Recommendations for further studies

If it would be possible to circumvent the time limitations we had for our study, it would be interesting only to include participants for whom it can be assumed to be sero - naïve. This could either be accomplished by changing the study design and measuring the incidence rate or by excluding all travelers who ever visited a dengue endemic area before. To circumvent the limitations applying to the serologic and the PCR testing methods considering the limited time both of them would stay positive after exposure to the dengue virus it would be advisable to perform a plaque reduction neutralization test (PRNT) in order to verify if the individual ever got infected with the dengue virus.

CHAPTER VI
CONCLUSION

6.1. General

As far as we know this study is the only one in the recent history with a focus on the seroprevalence of dengue in travelers visiting South East Asia and particularly Thailand. The seroprevalence we have determined in our study is quite similar to the findings from other studies. That might lead to the assumption, that the risk for a traveler coming to this part of the world getting infected with the dengue virus remained largely the same over the past decade. Of course we would have preferred to choose to determine the incidence rate but the time limitation would not have allowed such a study design. To get in contact with the study participant only once made it necessary to develop an algorithm for the interpretation of the laboratory results next to the established one which relies on consecutive blood samples of the patient. By asking for the vaccination history we should have made good effort to rule out the possibility of cross reaction against other flaviviruses influencing our results. The case of a female tourist from Switzerland, which has been mentioned above, reflects once more, that testing for IgM antibodies is not reliable in the early stage of the disease.

For the lack of any statistically significant difference considering the characteristics being asked in the questionnaire between the seropositive and the seronegative subgroup it should be mentioned that this might have been caused by the fact, that the measured seroprevalence had been 6.4% respectively 3.5% and therefore a higher number of participants for the study would have been desirable. Due to these premises the power of the statistical evaluation is certainly limited and conclusions have to be regarded having that in mind. As we could not find any differences there hasn‘t been a possibility to identify protective or non-protective factors. Also findings

from previous studies [5, 24, 25, 31] related to significant correlation between age or gender could not be confirmed in this study.

6.2. Outlook

As there is still no safe and efficient vaccination or chemoprophylaxis available for clinical use the only way for the travelers to protect themselves from an infection would be exposition-prophylaxis during night- and daytime as well. If an infection is being confirmed in a residential area usually space spraying will take place to prevent the further spreading of the virus. Unfortunately all the specific habits and features of the *Aedes* mosquito and especially its intimacy with the humans make it very hard to cope with and eradication currently doesn‘t seem to be an option. Being pushed by many influential organizations and countries the development of an effective dengue vaccine seems to approach the finish line. Luckily the fears, which have been raised about a possible antibody enhancement following the application of a tetravalent vaccine to a previously infected patient haven‘t come true so far [26].

Nevertheless, as long as we have to face this health threat without an option like a vaccine, doctors and especially doctors in countries, which do not show dengue endemicity should be well aware of taking a dengue infection into consideration whenever they encounter a patient with an appropriate history of travel and a febrile disease. This approach should be underlined by the fact, that many travelers have had more than one stay in an endemic area and thus they also may have a secondary infection with all its associated complications.

REFERENCES

[1] The World Health Organization, Regional Office for South - East Asia. Communicable Diseases. Dengue/DHF. http://www.searo.who.int/en/Section10/Section332.htm accessed on the 12.03.2009.

[2] Waterman S, Novak R, Sather G, Bailey R, Rios I, Gubler D. Dengue transmission in two Puerto Rican communities in 1982. Am J Trop Med Hyg. 1985;34:625-32.

[3] Burke D, Nisalak A, Johnson D, Scott R. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg.1988;38:172-80.

[4] McBride W, Mullner H, LaBrooy J, Wronski I. The 1993 dengue 2 epidemic in Charters Towers, North Queensland: clinical features and public health Impact. Epidemiol Infect. 1998;121:151-6.

[5] Allwinn R, Hofknecht N, Doerr HW. Dengue in travelers is still underestimated. Intervirology 2008;51:96-100.

[6] Gibbons RV, Vaughn DW. Dengue, an escalating problem. BMJ. 2002;324:1563-6.

[7] Rigau-Pérez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. Lancet. 1998;352:971-7.

[8] World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control, 2nd edition, Geneva: WHO 1997.

[9] Pinheiro FP, Corber SJ. Global situation of dengue and dengue haemorrhagic fever and its emergence in the Americas. World Health Statist Quart. 1997; 50:161-8.

[10] United Nations, World Tourism Organisation, Facts and Figures, Highlights 2008 edition, http://www.unwto.org/facts/menu.html accessed on the 25.11.2008.

[11] Figueroa R, Ramos C. Dengue virus (serotype 3) circulation in endemic countries and its reappearance in America. Arch Med Res. 2000;31:429-30.

[12] Rush AB. An account of the bilious remitting fever, as it appeared in Philadelphia in the summer and autumn of the year 1780. Medical Inquiries and observations. Philadelphia: Prichard and Hall, 1789:104-17.

[13] Centre of Disease Control, Dengue resource homepage, Current trends and history. http://www.cdc.gov/ncidod/dvbid/dengue/ accessed 25.11.2008.

[14] Gubler DJ. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: Gubler DJ, Kuno G, eds. Dengue and dengue hemorrhagic fever. Wallingford, UK: CAB International, 1997:1-22.

[15] Srisuphanunt M, Sithiprasasna R, Patpoparn S, Attatippaholkun W, Wiwanitkit V.
ELISA as an alternative tool for epidemiological surveillance for dengue in mosquitoes: a report from Thailand. J Vector Borne Dis. 2007; 44:272-76.

[16] Gubler DJ. The global emergence/resurgence of arboviral diseases as public health problems. Arch Med Res 2002;33:330-42.

[17] Henchal EA, Putnak JR. The dengue viruses. Clin Microbiol Rev. 1990 Oct;3(4):376-96.

[18] Wilder-Smith A, Schwartz E. Dengue in Travelers. N Engl J Med. 2005;353(9): 924-32.

[19] Vaughn DW, Green S. Dengue and dengue hemorrhagic fever. In: Strickland GT, ed. Hunter's tropical medicine and emerging infectious diseases: Philadelphia: Saunders, 2000:240-1.

[20] Wu SJ, Grouard-Vogel G, Sun W, Mascola JR, Brachtel E, Putvatana R, Louder MK, Filgueira L, Marovich MA, Wong HK, Blauvelt A, Murphy GS, Robb ML, Innes BL, Birx DL, Hayes CG, Frankel SS. Human skin Langerhans cells are targets of dengue virus infection. Nat Med. 2000;6:816-20.

[21] Pan American Health Organization. Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control. Washington, DC. PAHO Sci Publ 1994 (no 548): 3-20. 49—58, 69—70.

[22] Guzmán MG, Kourí G. Dengue: an update. Lancet Infect Dis. 2002;2(1):33-42.

[23] Wilson ME, Weld LH, Boggild A, Keystone JS, Kain KC, von Sonnenburg F, Schwartz E, GeoSentinel Surveillance Network. Fever in returned travelers: results from the GeoSentinel Surveillance Network. Clin Infect Dis. 2007;44(12):1560-8.

[24] Potasman I, Srugo I, Schwartz E. Dengue Seroconversion among Israeli travelers to tropical countries. Emerg Infect Dis. 1999;5(6):824-7.

[25] Cobelens F, Groen J, Osterhaus A, Leentvaar-Kuipers A, Wertheim-van Dillen P, Kager P. Incidence and risk factors of probable dengue virus infection among Dutch travellers to Asia. Trop Med Int Health. 2002;7(4):331-8.

[26] Edelman R. Dengue vaccines approach the finish line. Clin Infect Dis. 2007;45 Suppl 1:56-60.

[27] Office Of Tourism Development. Tourist Arrivals in Thailand. http://www.tourism.go.th/ accessed on the 30.12.2008.

[28] Settah SG, Vernazza PL, Morant R, Schultze D. Imported dengue fever in Switzerland – serological evidence for a hitherto unexpectedly high prevalence. Schweiz Med Wochenschr 1995;125:1673-8.

[29] Eamchan P, Nisalak A, Foy HM, Careonsook OA. Epidemiology and control of dengue virus infections in Thai villages in 1987. Am J Trop Med Hyg. 1989;41:95-101.

[30] Kuno G. Review of the factors modulating dengue transmission. Epidemiol Rev. 1995;17:321-35.

[31] Jelinek T. Dengue Fever in International Travelers. Clin Infect Dis. 2000; 31:144-7.

APPENDIX

APPENDIX A
PATIENT INFORMATION SHEET

Title of Research: Seroprevalence of Dengue antibodies among foreign travelers in South East Asia

Principal Investigator: Dr. Emanuel Luttersdorfer
Faculty of Tropical Medicine, Mahidol University
Tel: 080-5911947. Email: emanuel@luttersdorfer.com

Purpose of the study: To determine the current percentage of travelers in South East Asia carrying antibodies against the Dengue virus showing a possible recent infection with the agent and possible risk factors associated.

Before agreeing to participate in this research study, it is important that you read the following explanation of this study. This statement describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. Also described are the alternative procedures available to you, as well as your right to withdraw from the study at any time.

Explanation of Procedures

If you agree to take be a participant in this study you will receive a questionnaire. It is essential for our study, that you have not been born in a Dengue endemic area (see countries on the map). 195 participants are required to complete the study. Each participant will be asked to fill out a single questionnaire which usually takes 10 – 15 minutes, with no follow up. It will assess asking for general data about you and your recent travel history as well as your general knowledge about Dengue and the means you use to protect yourself against. You will not be asked to enter your name or your signature in the questionnaire and no sensitive issues will be asked. Your privacy while answering the questionnaire will be remained. If you agree to participate you are requested to sign the informed consent. After completing the questionnaire our personnel will take about 3 ml of blood via a needle inserted intravenously. Your blood will be sent to the Faculty of Tropical Medicine to be tested for Dengue and Japan Encephalitis antibodies. The leftover blood specimen will be destroyed after the study.

Risks and Discomforts

All blood drawn is associated with the discomfort of having a needle stick and bruising at the site of injection, which usually is small and disappears within 7 days. Another potential risk such as thrombophlebitis occurs rarely. Apart from pain at the time of blood drawn, bruise can occur. But it's usually small and disappears within 7 days. Risk of an infection at the puncture site is small and it will be minimized by using experienced nurses and comply with aseptic technique. Pain at the puncture site is usually mild and transient. For this reason analgesia is usually not required. There will be no compensation for you including compensation for pain and suffering, lost wages or the like. However if any severe pain or infection occurs directly

resulting from blood drawn procedure in this study, you will receive standard medical treatment without any charge.

Your Rights

Participation in this study is voluntary; refusal to participate will involve no penalty. Your questionnaire will remain confidential and anonymous.

Agreement

I voluntarily agree to participate in this study. .....

Signature

APPENDIX B
INFORMED CONSENT FORM

I have been invited to participate in the study "Seroprevalence of Dengue antibodies among foreign travelers in South East Asia". I have been informed about the purpose of the study and the procedures. I have completely read and understood the participant information sheet.

I am willing to participate in this study.

If I have any question regarding this study I can contact the principal investigator:

Dr. Emanuel Luttersdorfer
Faculty of Tropical Medicine, Mahidol University
Tel: 080-5911947. Email: emanuel@luttersdorfer.com

If I have not been treated as stated in the participant information sheet I can contact the Ethical Committee as below:

Ethical Committee of Faculty of Tropical Medicine,
4th floor, The 60th Anniversary of His Majesty the King's Accession to the Throne Building
Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400
Tel: 02-3549100-19 ext 1349, 1525 or 02-6435578

I have been informed that the result of this research may be published or presented for academic purposes. However, my personal information will never be disclosed. I have also been informed, that participation in this study is voluntary; refusal to participate will involve no penalty. Each participant is free to withdraw consent and discontinue participation in this project at any time without prejudice from this institution.

Payment for Research Related Injuries

There will be no compensation for the participants including compensation for pain and suffering, lost wages or the like. However if any adverse event arise from blood drawn procedure, standard medical treatment will be given free of charge.
I have read and understood the participant information sheet and the informed consent form. I have had a chance to ask questions that were answered to my satisfaction. By signing this form, I agree to participate in this research study.

Date: .....

…….. ……
Signature of Participant Signature of Investigator

…….. ……..
Participant's name (printed) Investigator's name (printed)

APPENDIX C
QUESTIONNAIRE

Date:.....
Site:.....
Rec. No.:.....

Questionnaire

1. Sex: ☐ male
☐ female

2. Age: .....

3. Nationality: .....

4. Main purpose of this trip: ☐ tourism
☐ visit friends or relatives
☐ work
☐ other (please specify)

5. How long have you been in SE Asia (in this trip): .....☐ days ☐ weeks ☐ months

6. Please describe the area you visited during the majority of your trip:☐ urban
☐ rural

7. Are you a backpacker? ☐ Yes ☐ No

8. Have you ever visited an dengue endemic area before (see map): ☐ Yes ☐ No

9. Purpose of visit to the clinic: ☐ Checkup
☐ Vaccination
☐ Symptoms (please specify): .....
☐ Other (please specify): .....

10. Episode of fever during this trip:
☐ Yes ☐ No

if yes, how many days: .....

accompanied by one or more of the following symptoms: ☐ Yes
☐ No

10.1 Joint pain: ☐ Yes ☐ No

10.2 Headache: ☐ Yes ☐ No

10.3 Myalgia: ☐ Yes ☐ No

10.4 Rash: ☐ Yes ☐ No

11. Use of insect repellent for the majority of the time: ☐ Yes during the ☐ daytime ☐ evening ☐ No

12. Previous history of vaccination: ☐ Yellow fever vaccination Year:.....
☐ Japanese encephalitis vaccin.Year:.....
☐ Tick borne encephalitis vaccin.Year:.....

13. Have you ever been diagnosed as having Dengue infection ?
☐ Yes, in this trip
☐ Yes , but not in this trip
☐ No, I have never been diagnosed before

14. Did you have any knowledge about Dengue before: ☐ Yes ☐ No

Thank you!

BIOGRAPHY

NAME Emanuel Luttersdorfer

NATIONALITY Austrian

DATE OF BIRTH 25th of June, 1976

PLACE OF BIRTH Klagenfurt, Austria

EDUCATION

1995-2003: Medical Doctor, University of Graz; Austria, Faculty of Medicine

2007: Carinthian Association of Emergency Medicine, Klagenfurt, Austria, Diploma in Emergency Medicine

2008: Diploma in Tropical Medicine & Hygiene, Mahidol University, Bangkok, Thailand

2008-2009: Master in Clinical Tropical Medicine, Mahidol University, Bangkok, Thailand (currently enrolled)

WORK EXPERIENCE

2004-2008: General State Hospital Klagenfurt, Austria, Senior House Officer, Dpts. of General Surgery, Internal Medicine, Paediatrics, Dermatology, Obstetrics and Gynecology, Neurology, Neurosurgery, ENT, Dental- and Head

Surgery.

2005: General Public Hospital
Hermagor/Carinthia, Senior House Officer Dept. of Trauma Surgery

PERMANENT ADDRESS

Pöckau 155
A9601 Arnoldstein, Austria/Europe

E-MAIL emanuel@luttersdorfer.com