DETECTION OF CHIKUNGUNYA SEROPOSITIVITY IN THAI TRAVELERS TO THE SOUTHERN PART OF THAILAND

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Thematic paper
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DETECTION OF CHIKUNGUNYA SEROPOSITIVITY IN THAI TRAVELERS TO THE SOUTHERN PART OF THAILAND

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ABSTRACT

Introduction: A number of zoonotic and vectorborne viral diseases have emerged in Southeast Asia and the western Pacific. Chikungunya virus is one of them. The recent outbreak was in southern part of Thailand, which is a popular tourist destination.

Methods: we conducted a cross sectional study among the Thai travelers to determine the seropositivity of Chikungunya regardless of symptoms .The study was conducted in national blood center Thai red cross society. We screened the blood donors for the history of travel to the southern part of Thailand verbally, and then through a written questionnaire we assessed the travel itinerary, medical history as well as general knowledge about the disease.

Results: Among the healthy asymptomatic blood donors we found 14%seroprevalence for Chikungunya antibodies using the serological test IgM ELISA

Discussion: In our study only the mean age between the seropositive and seronegative groups was statistically significant. There were no statistically significant characteristics that we can correlate with the seropositivity.

Conclusion: With a 14 % of seroprevalence we can conclude that a great proportion of travelers to the endemic areas can be infected with Chikungunya, though they remain asymptomatic.

KEY WORDS: CHIKUNGUNYA/ SEROPOSITIVITY/ SOUTHERN PART OF THAILAND/ TRAVELERS/IgM

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LIST OF ABBREVIATIONS

Abbreviation	Term
CHIK	Chikungunya
ELISA	Enzyme linked immunosorbent assay
HI	Hemagglutination Inhibition
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MOPH	Ministry of public health
NSAID	Non steroidal anti inflammatory drugs
PCR	Polymerase chain reaction
RT-PCR	Real time polymerase chain reaction
SEARO	South East Asia regional office
SIADH	Syndrome of inappropriate secretion of antidiuretic hormone
UNWTO	United nations world tourism organization
WBC	White blood cell
WHO	World health organization

CHAPTER I
INTRODUCTION

Chikungunya Fever is caused by the Chikungunya Virus. It is transmitted by Aedes mosquitoes. Clinically this disease is very similar to Dengue fever, but unlike Dengue hemorrhagic fever it is not fatal. The Virus was first isolated in 1953Tagayanka, now Tanzania (Nero, 2008). The first reported case of Chikungunya in Thailand was recorded in 1960 (Thaikruea et al, 1997).The recent outbreak of Chikungunya was in mid 2008 four Southern provinces of Thailand, thus it is listed as notifiable disease in Thailand since November 2008 (MOPH Thailand).

Fever, severe joint pain, headache, transient maculo papular rash and fatigue are the main symptoms of Chikungunya. Though not fatal, long term persisting disabling joint pain causes significant morbidity. Currently there is no specific drug treatment available for this disease. Diagnosis is based on either RT-PCR or serology (IgM or IgG). IgM is used to detect the acute disease whereas IgG may persist for years (Pialoux et al, 2007).

Recent outbreaks in India and Reunion Island in the Indian Ocean have renewed interest in the virus (Nero, 2008). Due to the outbreaks in the popular tourist destinations, the tourism Industry was substantially dented. As for Thailand the Southern Part is a popular tourist destination and an outbreak of Chikungunya was reported not that long ago. It becomes more serious issue as the asymptomatic person also can spread infection, especially in the regions with the presence of vectors.

We prospectively analyzed the serum samples of the Thai travelers with a history of travel to the Southern part of Thailand and stayed there at least a week and who came for blood donation in The National blood center, Thai Red Cross society. The aim of the study was to detect the seropositivity among the travelers to the areas, where there was a recent outbreak.

CHAPTER II
OBJECTIVES

1. To determine the prevalence of Chikungunya seropositivity in travelers to the Southern part of Thailand.
2. To assess the relationship between the time spent at the Chikungunya affected regions and seropositivity.

CHAPTER III
LITERATURE REVIEW

3.1. Definition

Chikungunya the word used both for the virus and the disease means “to walk bent over” in the African dialect Swahili or Mekonde (Enserink, 2006). The virus was first isolated from blood of a febrile patient in Tanzania in 1953 (Chahar et al, 2009). Clinically resembling Dengue fever, Ckikungunya is a relatively new disease to mankind. Chikungunya virus is an arbovirus belonging to the genus Alphavirus (Togaviridae, family). It has a single stranded RNA genome (Pialoux et al, 2007). The Alphavirus group comprises of 28 viruses, six of which can cause human joint disorders, Chikungunya is one of them (Strauss et al, 1994). Phylogenetic study on E1 gene sequences grouped Chikungunya virus isolated worldwide into three genotypes; Asian, East/Central/South African, and West African (Powers et al, 2000).

3.2. Epidemiology

Chikungunya outbreaks occur in endemic and epidemic patterns. The endemic pattern is prevalent in Africa, whereas the epidemic form occurs mainly in urban areas of Asia (Nero, 2008). Since its first outbreak in 1953, Chikungunya infection has been documented in Burma, Thailand, Cambodia, Vietnam, India, Srilanka and Philippines (Laras et al, 2005). In 2006 an outbreak of Chikungunya was reported from a number of islands in the Indian ocean (The Comoros, Mauritius, The Seychelles, Madagascar, Mayotte, and The Reunion).Epidemics were also reported from Philippines in 1954,1956 and 1968, Sumatra, Java, Timor, Sulawesi and Moluccas Islands in Indonesia between 1982-1985. 25 outbreaks were reported from Indonesia between 1999-2003. In 2006 out of the population of 770,000, there were 265,000 clinical cases reported from Reunion, with 337 deaths (Charrel et al, 2007). The same year 1,400,000cases were reported from India (Ravi 2006).

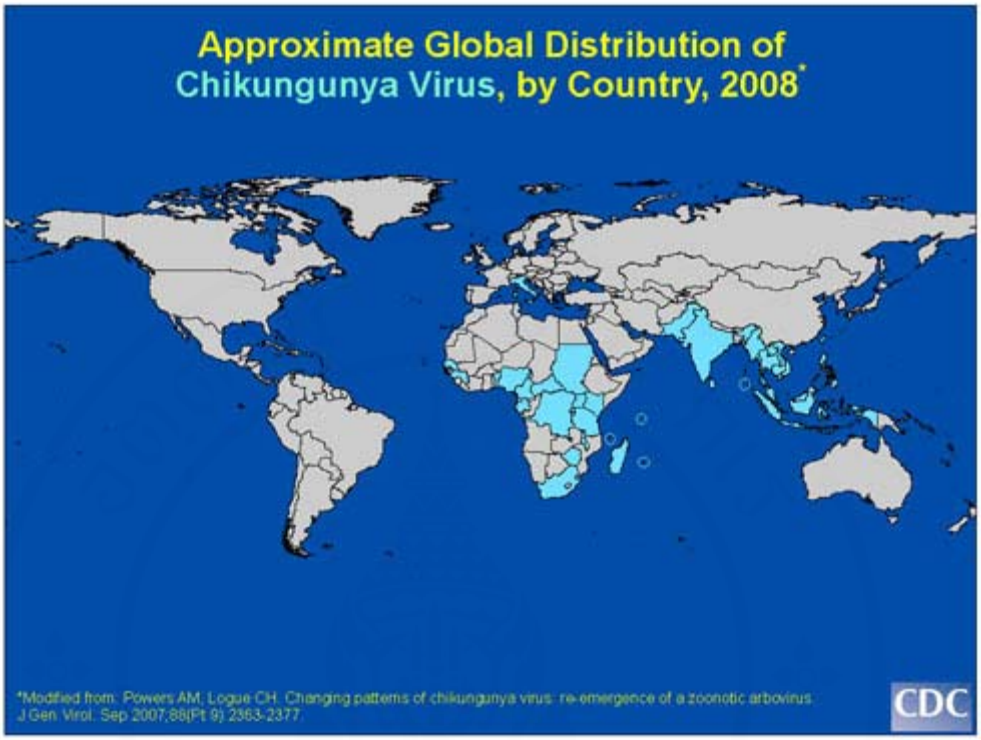

Figure: 1 The global distribution of Chikungunya virus

The first serologically diagnosed case in Thailand was in 1960. There were outbreaks in Khon Kaen, Nakhonsithammarat, and Nong Khai provinces all during rainy seasons (Thaikruea et al, 1997). The recent outbreak was in 2008 involving Yala (488 cases), Songkhla (3629), and Narathiwat (6371), after which the disease has been listed as notifiable disease since November 2008 (MOPH Thailand, 2009).

Three case definitions were described:

1. Suspected case

   Fever with at least two of the following symptoms

   a. Arthalgia
   b. Rash
   c. Myalgia
   d. Headache
   e. Retro orbital pain

2. Probable case

Suspected case with

a. Normal platelets and WBC<5000
b. Epi- linkage with confirmed cases

3. Suspected case with Chikungunya virus laboratory confirmed by PCR, HI or IgM.

Table 1: Reported cases from Southern provinces of Thailand from January till May 2009

Province	**January**	**February**	**March**	**April**	**May**	**Total**
Surat thani	0	0	0	1	0	1
Nakhon si thammarat	0	7	2	0	0	9
Trang	2	0	0	92	1	95
Phattalung	0	2	3	7	0	12
Krabi	1	0	1	3	0	5
Phuket	0	0	1	3	0	4
Pattani	875	1214	442	210	0	2741
Yala	177	354	342	666	0	1539
Narathiwat	2628	2644	874	242	0	6388
Songkhla	1626	1353	775	3389	9	7152

. Source MOPH, Thailand 2009.

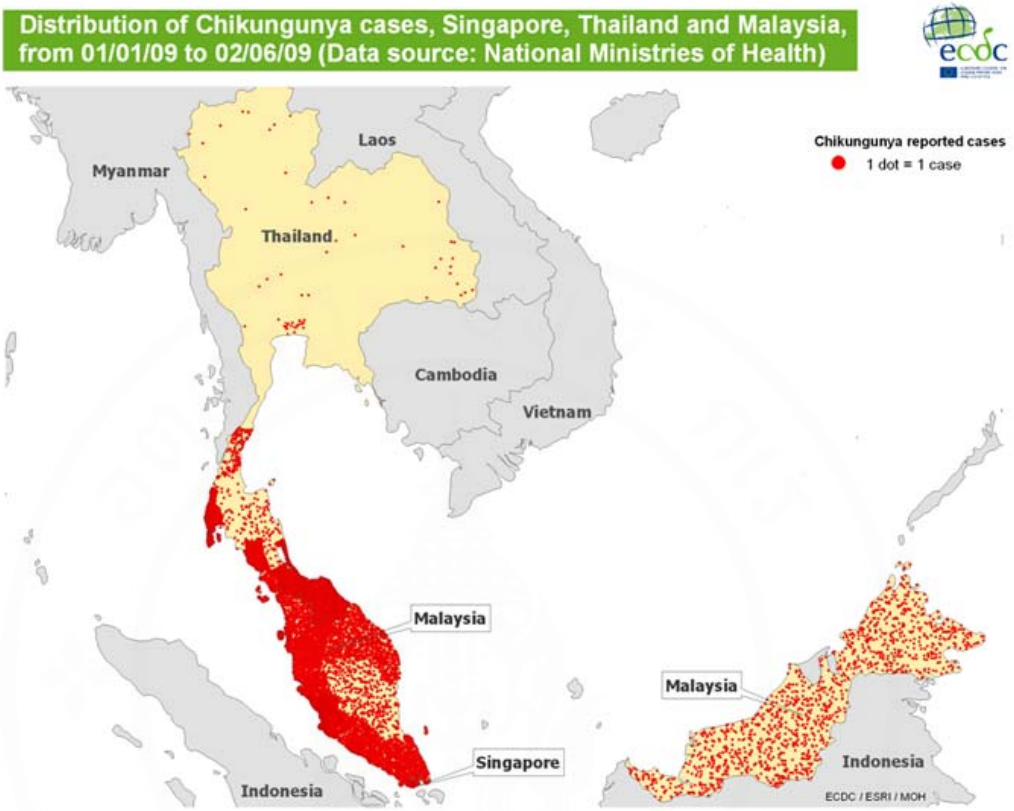

Figure 2: Chikungunya in Thailand and neighboring countries

3.3. Mode of transmission and vectors

Chikungunya fever is transmitted to humans by the bite of infected mosquitoes. Chikungunya virus is maintained in the human population by a human-mosquito-human transmission cycle (Pialoux et al, 2007). In Asia and the Indian Ocean the main Chikungunya vectors are *Aedes aegypti* and *A. albopictus* (Zeller, 1998). A larger range of Aedes species (*A. furcifer, A. dalzieli , A. vittatus, A. fulgens, A. luteocephalus, A. vigilax, A. camptorhynchites)* transmit the virus in Africa, and *Culex annulirostris, Mansonia uniformis*, and anopheles mosquitoes have also occasionally been incriminated (Lam et al, 2001).

A. albopictus has a wide geographical distribution, and can survive in both rural and urban environments (Pialoux et al, 2007). The mosquito eggs are highly resistant and can remain viable throughout the dry season, giving rise to larvae and

adults the following season. Humans act as reservoirs of the virus during epidemic periods. Otherwise, the reservoirs are rodents, birds, and monkeys.

3.4. Clinical features

After infection with Chikungunya virus, there is a silent incubation period lasting 2-4 days on average (range 1-12 days) (Lam et al, 2001).Clinical onset is abrupt, resulting in acute debilitating febrile illness, characterized by severe joint pain and rash (WHO/SEARO, 2008). High fever may result, often accompanied by chills and rigors, the fever may subside and recur, showing a saddleback pattern similar to Dengue (Rajapakse et al, 2010). When patients have only febrile symptoms it is very difficult to differentiate between Dengue and Chikungunya. Though hemorrhaging is rare, Nimmannitya and colleagues have reported hemorrhagic fever in Chikungunya virus infected patients in Thailand.

Arthralgia can be intense, is often polyarticular and symmetrical involving knees, elbows, ankles and small joints, and also the sites of previous injuries (Powers et al, 2000). These symptoms may last from a week to several months or even years, and are often accompanied by myalgia (Brighton et al, 1983). Skin involvement is present in about 40-50 % cases, and consists of:

a) A pruriginous maculopapular rash predominating on the thorax

b) Facial oedema or

c) In children a bullous rash with pronounced sloughing, and

d) Localized rash with petichiae and gingivorrhagia (mainly in children)

Asymptomatic infection probably occurs, but its prevalence is not known. The ratio of symptomatic to asymptomatic infection in Thailand is 2:1 (Bureau of epidemiology, MOPH Thailand). Generally Chikungunya is not considered life threatening, but it has a prolonged morbidity. Rajapakse and colleagues have recently reported the atypical manifestations of Chikungunya, and have divided systematically:

1.Neurological (encephalopathy/encephalitis/meningoencephalitis, seizures, neuropathy, Guillain-Barre syndrome, cerebellar syndrome)

2.Cardiovascular (myocarditis/pericarditis, heart failure, unstable blood pressure, arrhythmias, ischemic heart disease/myocardial infarction)

3.Renal (nephritis, acute renal failure)

4.Skin (maculopapular eruption, pigmentation, penoscrotal ulcers, bullous dermatosis)

5. Ocular (optic neuritis, iridocyclitis, episcleritis, retinitis)

6. Neonatal infection with vertical transmission (ante-partum foetal deaths, meningoencephalitis, disseminated intravascular coagulation)

7. Other possible associations (pneumonia, respiratory failure, hepatitis, pancreatitis, SIADH, hypoadrenalism)

Pre-existing co-morbidity appears to increase the likelihood of most of the complications listed above. The incidence of atypical manifestations appears to be higher in patients aged 65 years and above. Patients above 40 years of age are more likely to develop severe disease (Economopoulou et al, 2009).

3.5. Diagnosis

Recently developed molecular techniques have made detection of Chikungunya virus more reliable. Viral isolation which is based on inoculation of mosquito cell cultures, mosquitoes, mammalian cell cultures, or mice could also be used, but this method is associated with biohazard risk, which is tedious, time consuming and expensive (Pialoux et al, 2007). Even though it has some disadvantages the gold standard for the diagnosis of Chikungunya is viral culture. It has an advantage of detecting a wide range of viruses. The procedure is quite lengthy, and it takes 1-2 weeks for the result. This test should be performed at BSL-3 labs to reduce the risk of viral transmission. However this procedure is seldom routinely carried out as these facilities are not available in only selected centers only.

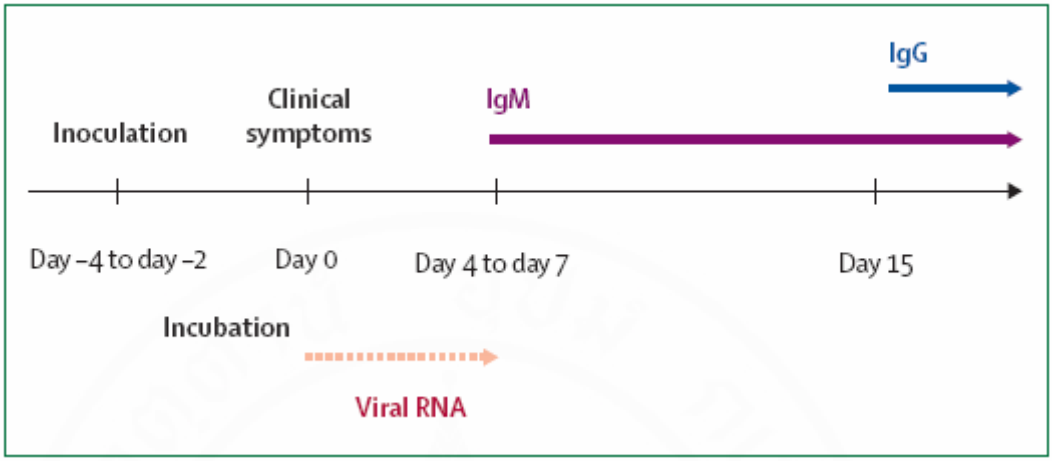

Figure 3: Biological diagnosis of Chikungunya (Pialoux et al, 2007)

Apart from the virus isolation the main diagnostic methods are genomic detection by PCR based methods and serological tests (IgM or IgG). These days RT-PCR is the method of choice for early detection and confirmation of virus in Chikungunya. The RT- PCR is 10 times more sensitive than a conventional block based PCR and could detect as low as 20 copies of RNA transcript (Edwards et al, 2007). It needs sophisticated equipment and skilled personnel to perform this test.

So the most practical test especially in developing and under developed countries is antibody based IgM. The sensitivity and specificity of IgM ELISA is 95% and 97% respectively. IgM is detectable after an average of 2 days by ELISA (1-12 days) and persists for several weeks to 3 months (Jyoti et al, 2009). Whereas IgG is detected in convalescent samples (day 15 onwards) and persists for years. IgM/ IgG – ELISA have lower sensitivity for the detection of infection in majority of Chikungunya patients during the acute phase of infection.

In acute infection the sensitivity of antigen detection has the sensitivity of 95%, which is higher than IgM (20%) or IgG (25%) (Kashyap et al, 2010). Antigen detection gives a positive confirmatory result in early phase of the disease. It is also helpful in prodromal and subclinical stage. Antigen detection is done by enzyme linked immunosorbent assays, whereas antibody detection is done using serological tests IgM, and IgG, ELISA and MAC- ELISA.

IgM capture MAC ELISA is the most commonly used test, but the cross reaction with other Alphavirus antibodies limits the MAC- ELISA as confirmatory test (Kashyap et al, 2010). Serodiagnosis of Chikungunya relies on the demonstration of fourfold increase in anti Chikungunya IgG titre by MAC-ELISA, between the acute and convalescent phase sera, but obtaining paired sera is not always possible and practical, so alternatively IgM antibodies specific to Chikungunya in acute phase sera is used in cases of inability to obtain paired sera (Parida et al, 2008). The antibody based IgM ELISA is found to be cost effective, but as it takes relatively longer time for the patient to develop antibody, has less implication for early clinical diagnosis and patient management. It is a useful tool particularly in economically weaker countries.

A study done by Parida and colleagues in India compared the various methods of laboratory detection of Chikungunya. The findings are as follows

3.6. Management

Currently there is no specific treatment available for treatment of Chikungunya fever. Treatment is therefore purely symptomatic. Paracetamol and non-salicylate analgesics and NSAIDS could be used (WHO/SEARO, 2008).

Cyclooxygenase inhibitors have been used in higher doses to effectively control arthalgia in some cases (Taubitz et al, 2007).Synergistic efficacy was reported between interferon-α, and ribavirin on Chikungunya virus in vitro (Briolant et al, 2004). A trial in southern Africa failed to confirm the efficacy of chloroquine on arthalgia (Savrino et al, 2006). In addition to the drug therapy bed rest and fluids are recommended. There is currently no commercial vaccine available for Chikungunya virus, though some candidate vaccines have been tested in humans.

3.7. Prevention

Because specific treatment and vaccines are not available, all control efforts should be directed against mosquitoes. It is important to maintain in-house and surrounding

hygiene and sanitation in order to eliminate their breeding grounds. This is the most effective way to control the disease (WHO/SEARO, 2008).

The control of both adult and larval mosquito populations uses the same model as for Dengue and has been relatively effective in many countries and settings. The use of DDT has been effective in controlling *A. aegypti*, but not *A. albopictus*. These mosquitoes have varying resistance to insecticides (Nero, 2008).

Early surveillance is necessary to identify outbreaks and to imply public health measures to lessen the severity of the outbreaks. For example in India a multidisciplinary team was deployed in February 2006, to assist local health authorities in improving public health measures, including strengthening of arbovirus surveillance, clinical management of cases, vector control and social mobilization (WHO, outbreak news, 2006).

3.8. Chikungunya and travellers

According to the world tourism barometer the international tourist arrivals are expected to increase in 2010 by 3-4 % in comparison to 2009, in which 4% decline was observed and the number of tourist arrivals fall to 880 million (UNWTO, tourism barometer 2010). If we compare the present international tourist arrivals with that of 1950 the numbers are unbelievable. In 1950 the number of international tourist arrivals was 23.5 million, and the year 2008 this number was 924 million.

Due to the unfavorable global economy the worlds tourism industry too suffered, which now is moving towards the positive way. In the year 2008 South East Asia and Asia observed a growth of 4 % each. Around 188 million people traveled to Asia and the Pacific. In recent years Asia Pacific and Africa outnumbered other regions in terms of travel growth (Chen et al, 2008).

The reason for travel may be various, pleasure, work, scientific and educational purposes, humanitarian aid, religious trips as well as various other reasons. No matter whatever is the purpose the number of tourists is on the rise. The sphere of travel has enlarged over the years and travel patterns have become ever more complex. The Indian Ocean islands, India, Malaysia are popular tourist

destinations. An estimated 1.5 million people travelled form Madagascar, Mauritius, Mayotte, Reunion, and the Seychelles to European countries in 2004.

Travel pattern influences disease outbreaks. Disease would reach nearby cities first, but also distant cities with high air travel volumes. Naturally the travelers to Chikungunya affected areas are prone to get the infection and spread it to their hometowns, in presence of vectors. In the year 2005 the Indian Ocean islands observed an outbreak of Chikungunya, the numbers of cases were dramatically rising, before affecting a million people in India. In 2006, in the United Kingdom the number of Chikungunya seropositive samples among the tourists returning from the disease affected regions increased by 15 times (Edwards et al, 2007)

A study conducted in France by Hochedez and colleagues from March 2005 to February 2006, among the patients with symptoms of Chikungunya virus infection after travelling to any island of southwest Indian Ocean (Comoro, Mauritius, Reunion etc), serologically confirmed Chikungunya in all 22 patients. 19 (86%) were symptomatic while abroad and 3 (14%) were symptomatic within 3 days of return (Hochedez et al, 2006).

Another study conducted in Indonesia by Porter and colleagues between December 1998 and February 1999 on individuals who reported to the community health center with the symptoms consistent with Chikungunya fever. Total 317 patients were included from three subdistricts of Suratmajan, Gowok and Bausasran .Chikungunya was confirmed in 74% patients from Suryatmajan,in 81% from Gowok and in 33% from Bausasran subdistricts by IgM and IgG ELISA (Porter et al, 2004).

Eisenhut and colleagues conducted a study among 670 Germans working overseas from 1987-1993, who have spent at least 4 months abroad. Detection of Chikungunya was tested by IFA. Antibodies to Chikungunya was detected in 9 out of 670 (1.3%) of the participants (Eisenhut et al, 1999). The highest seroprevalence to anti Chikungunya IgG was found in the aid workers returning from Benin (5.7%), followed by the workers returning from Thailand (5.5%).

In 1999 in Malaysia 51 confirmed cases were detected in urban area near the capital (Mackenzie et al, 2001). The major symptoms were fever (2-5 days), severe back pain, transient maculopapular rash on the trunk and the limbs (2-3 days). About 80% of the patients had some sort of joint symptoms.

A study conducted in France by Hochedez and colleagues serologically confirmed Chikungunya in 22 symptomatic patients (Hochedez et al, 2006). They confirmed Chikungunya infection by immunocapture ELISA derived from a yellow fever test by using a goat anti-human immunoglobulin (IgM) antibody. The study was conducted from March 2005 to February 2006, but the study was conducted only among the individuals who had symptoms consistent with Chikungunya infection. Median duration of travel was 21 days, and they had a recent history of travel to any island in the Southwest Indian Ocean.

Another study in Germany conducted among 69 travelers who developed signs and symptoms compatible with Chikungunya fever after returning home from the countries involved in the epidemic. Among the 69, 20 cases were confirmed by serological analysis, PCR and/or cell culture (Taubitz et al, 2007). All the 20 confirmed cases had history of flulike symptoms with fever and arthralgia. 69% of the patients had joint pain for more than a month and 13% had it for more than six months. Apart from arthralgia, no other serious complications were noted. From the four patients who presented to the clinic during the first week of illness, viral RNA was detected. Similarly from the blood of 2 patients of the four, virus was isolated. Chikungunya specific IgM or IgG were detected from all the patients.

The reason of travel may be various, but the travelers to Chikungunya affected regions are prone to get the infection and spread it to their hometowns in presence of the vectors. Previously mentioned study in France showed the infection in all 22 symptomatic travelers. Another study in 3 subdistricts of Indonesia confirmed infection in 33-81% of patients. A study in Malaysia confirmed the joint symptoms in majority (80%) of symptomatic patients. A study in Germany confirmed Chikungunya infection in 5.5% of asymptomatic travelers returning from Thailand.

The confirmation of Chikungunya was higher in symptomatic patients than the asymptomatic individuals. The lowest percent of detection was 33% from the study in Indonesia where as the highest was 100% from the study in France. So the range of detection in symptomatic patients was 33-100%. Where the range of detection in asymptomatic persons was lower than the symptomatic patients, the lowest being 0% in a study in Germany.

The Southern part of Thailand which is popular tourist destination had a recent outbreak of Chikungunya. The outbreak was observed in October 2008 in Narathiwat. The outbreak tended to spread in the adjacent provinces one month apart. From Jan 1st to June 16th 2009 28,056 cases were reported. According to the bureau of epidemiology MOPH Thailand a study conducted among 117 confirmed cases of Chikungunya, 77 (66%) were symptomatic and 40 (34%) were asymptomatic. The outbreak was reported from Chonburi, Chantaburi, Rayong and Uthai Thani in the mid 2009. In a country with the presence of vectors, and the possibility of the asymptomatic individuals to spread infection, it becomes important to know the prevalence among the Thai travelling population to the affected areas.

Table 3:

Countries where people have become infected with Chikungunya virus

Benin	Mayotte
Burundi	Myanmar
Cambodia	Nigeria
Cameroon	Pakistan
Central African Republic	Philippines
Comoros	Reunion
Congo, DRC	Senegal
East Timor	Seychelles
Gabon	Singapore
Guinea	South Africa
India	Sri Lanka
Indonesia	Sudan
Italy	Taiwan
Kenya	Tanzania
Laos	Thailand
Madagascar	Uganda
Malawi	Vietnam
Malaysia	Zimbabwe
Mauritius	

Table source: CDC, 2008 This list does not include countries where only imported cases have been documented

CHAPTER IV
MATERIALS AND METHODS

Study Design

This study is a cross sectional study

Study Site

The blood sample required for the study was collected at the national blood center Thai Red Cross Society, Bangkok, Thailand. It is a central blood bank of Thailand. The sera were separated at the research and development department Queen Saovabha Memorial Institute. Chikungunya antibodies were tested at the central laboratory, faculty of tropical medicine.

Study Duration

The data collection was done from 11th January 2010 till 8th March 2010

Study Population

Healthy Thai blood donors of both genders aged 18-60 years, weighing more than 45 kg, who have travelled to the Southern part of Thailand and spent at least 7 days within past three months and returned at least 2 days back.

Inclusion Criteria

We enrolled healthy Thai blood donors meeting the criteria for blood donation set by Thai Red Cross Society:

Age 18-60 years

Excellent health, with body weight over 45 kg.

No previous history of hepatitis or jaundice

No history of malaria for past three months, no sexually transmitted diseases, infectious diseases, persistent cough, hemoptysis, hemophilia, blood related diseases, asthma, allergy, epilepsy, long-term skin diseases, kidney diseases, heart problems, diabetes, thyroid problems

No considerable weight loss

No behaviors of sexual promiscuity, no record of drug use

Those who have not received blood for last six months

Female donors not pregnant or menstruating

Individuals with history of travel to the Southern part of Thailand or following four provinces: Chantaburi, Chonburi, Rayong and Udon Thani, and spent at least 7 days within past 3 months and returned at least 2 days back. Volunteering to participate in the study and signed written informed consent

Exclusion Criteria

Individuals who had previously been infected by Chikungunya

Residents of Southern part of Thailand for last two years

Residents of Chantaburi, Chonburi, Rayong and Udon Thani for last one year

Individuals who have travelled to South Asia or Africa within last year

Sample Size

The following formula was used to calculate the sample size.

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

Where:

N= expected sample size

a= statistically sampling error (5% or 0.05)

Z=confidence coefficient

p=proportion of disease interested (3% or 0.03)

e=precision of estimation (2.9 or 0.029)

The Ministry Of Public Health has reported that prevalence of Chikungunya fever at Southern part of Thailand is 0.48% (update report on Oct 30, 2009). Since the reported data is based only on symptomatic cases, around 80-90% of asymptomatic cases were not reported. If the assumed population prevalence is 3%(0.03), the estimated effect size is 2.9%.

According to the formula given above:

$N= (1.96)^2 (0.03) (1-0.03) / (0.029)^2$

=133 cases

Operational Definition

The individuals were defined as travelers if they have not resided in The Southern part of Thailand for past two years or in Chantaburi, Chonburi, Rayong and Udon Thani for one year but have travelled to the Southern part of Thailand or the above mentioned four provinces and spent at least 7 days. Travelers to the four provinces mentioned above were excluded from the study as these provinces had recent outbreak and it might affect the outcome of the study.

The persons who had visited south Asia or Africa within the last one year were not included to avoid false interpretation. As South Asia and Africa are known to be Chikungunya endemic area, persons with history of travel to these areas were excluded from the study, as the aim of the study was to determine the Chikungunya seropositivity in Thai travelers to the Southern part of Thailand.

Patient enrollment

The study was conducted in the national blood center Thai Red Cross society. All the participants were healthy volunteer blood donors of Thai nationality. The principal investigator and a supporting staff were stationed at the entrance of the

blood donation room. While the donors waited for their turn to donate blood, few verbal questions, such as have they been to the Southern part of Thailand and spent there at least seven days within the last three months were asked in order to screen the eligibility of the participants. If the answer was positive they were invited to participate in the study. After the participant agreed to participate in the study he/she were handed a three page written questionnaire in Thai/English, which contained 11 questions regarding the travel itinerary, medical history and general knowledge about Chikungunya**.** The details of questionnaire are listed below. After the participants filled the questionnaire, they were asked to sign a single page written informed consent and were handed a single page information sheet in Thai. After the process of documentation the participants were handed a 5 ml test tube marked at 3 ml. During the process of blood donation the staffs from the blood bank collected 3 cc of blood without making an extra venepuncture. The details of questionnaire are as follows:

1. Demographic profile: age, sex
2. Duration of stay in the areas with recent Chikungunya outbreak
3. Purpose of visit
4. Location of stay i.e. the rural or urban areas
5. Previous knowledge about Chikungunya, mode of transmission
6. History of contact with Chikungunya patients during the travel
7. Past history of Chikungunya, Dengue
8. Development of fever during the travel
9. If had fever whether or not had the symptoms characteristic to Chikungunya, like headache, rash, myalgia, joint pain, retro orbital pain etc

All the participants who met the inclusion criteria and signed a written informed consent were enrolled in the study.

Data Collection

Data collection was done from the blood donating volunteers that meet the inclusion criteria. Three cc of blood was collected from each participant during the process of blood donation, without making an extra venepuncture. The obtained blood sample was then centrifuged at 3000 rpm in the room temperature for five minutes to separate the

serum. The separated serum was stored in the temperature of -30 degree Celsius. Thereafter rapid test ELISA kit was used to detect IgM antibodies against Chikungunya. The procedure of antibody detection was performed in the central laboratory, Faculty of Tropical Medicine, Mahidol University. The frozen samples stored at the research and development department of QSMI was transferred to the central laboratory of the Faculty of Tropical Medicine maintaining the cold chain. We used SD Bioline Chikungunya IgM test kit, which is a solid phase immunochromatographic assay for rapid qualitative detection of IgM antibodies to Chikungunya in human serum, plasma, or whole blood. The sensitivity and specificity of this test is 97.1% and 98.9 % respectively (packet insert, SD Chikungunya IgM, 2010). The frozen serum was thawed to obtain room temperature. 1drop (50 microliter) of serum was dropped into the sample well and a drop of assay dilutant was added into the sample well. The test results were interpreted in 10 minutes. The data was transferred to our case record form as in appendix 1. Further the data was entered and stored in computer using Microsoft Excel program, before transferring into SPSS for analysis.

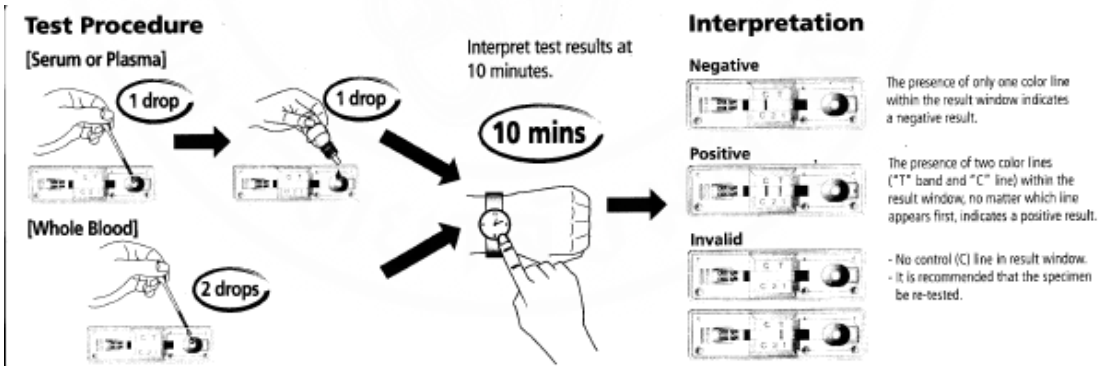

Figure: 4 SD Bioline Chikungunya IgM test

Data Analysis

Data were analyzed Using SPSS for windows (SPSS Inc, Chicago, IL). Data from the Microsoft Excel files will be retrieved to the SPSS database for further analysis. Normality was tested using Kolmogorov-Smirnov test. For data summarization descriptive statistics was used. For quantitative data, student T test and Mann-Whitney –U test were uses for comparison. All the p-values were 2-tailed tests

and the statistical significance was set at 0.05. If the observed values were less than 5 Fisher's exact test was used to determine the p-value, if it was more than five, chi-square test was used.

Ethical considerations

The study has been approved by the Ethical Committee of the faculty of Tropical Medicine, Mahidol University. The approval to collect the blood sample was further obtained from the director of national blood center Thai Red Cross society. As the study was done among the Thai population the patient information sheet and the written consent were in Thai language. The questionnaire was both in Thai and English languages. Two paramedics from QSMI assisted the principal investigator in the process of data collection. The participants were informed about the purpose of the study, the risks and discomforts regarding the process of drawing the blood. In case of any adverse events, the participants were given the telephone number and e-mails of the principal investigator and the major advisor.

CHAPTER V
RESULTS

We enrolled 130 participants for the study. During the data cleaning process one patient was found to have history of Chikungunya 3 months prior to the day of sample collection. She was excluded from analysis. Therefore only 129 participants were considered eligible for analysis. As expected the excluded participant was seropositive for Chikungunya.

The objective of this cross sectional study was to determine the prevalence of Chikungunya seropositivity in Thai travelers to the Southern part of Thailand. To detect the infection within the past three months from the date of data collection, we used serological test IgM. The solitary study site was National blood center Thai red cross society, Bangkok, Thailand.

The result is divided into three parts:

1. General characteristics, travel history and characteristics, past medical history and knowledge about the disease
2. Serological laboratory results
3. Comparison and analysis between the seropositive and seronegative groups

4.1. Descriptive statistics of Characteristics

Of the eligible 129 participants, 67 (51.9%) were male and 62 (48.1%) were female. The age distribution was not normal; their mean age has been 33.02 years and it ranged from 18 to 60 years. According to the behavior the participants are divided into three age groups: 56 (43.4%) belonged to a age group of 18-30 years old. This age group was considered as likely to indulge in adventurous activities. 60 (10.1%) fall into the age group of 31-45 year old. This group is considered to be a

mixture of adventure travelers and conventional travelers. 13 (10.1%) travelers fall into the age group of 46-60 years old, basically they are considered as conventional travelers with a speculation that they would less prefer the adventure.

All the general characteristics are represented in table 4. Travel characteristics are summarized in table 5, past medical history in table 6 and the knowledge about the disease in table 7.

All the travelers included in this study were exclusively Thai nationals. As per the inclusion criteria we included the travelers with a history of travel to the southern part of Thailand for at least 7 days. So the minimum duration of travel was 7 days with the maximum of 180 days. The mean duration of travel was 22.33 days.

To make the analysis simple and easy to understand we divided the duration of travel into three categories. The first category is the duration of 7 days, 45 (34.9%) travelers spent 7 days in the Southern part of Thailand. 70 (54.3%) travelers spent 8-30 days, whereas 14 (10.9%) travelers spent more than 31 days .This is important to see whether the duration of stay in the Chikungunya affected areas influenced in seroconversion.

Of the 129 travelers 47 (36.4%) stayed in the rural area during their stay in the Southern part of Thailand, similarly 74 (57.4%) travelers stayed in the urban area, whereas 8 (6.2%) travelers stayed in both the rural and urban areas.

As far as the purpose of travel to the Sothern part of Thailand is concerned 23 (17.8%) were tourists, 31 (24%) traveled for business purposes, 49 (38%) traveled to visit friends or relatives, whereas 4 (3.1%) had educational purpose for the visit and 22(17.1%) traveled for various other purposes.

Of 129 travelers 16 (12.4%) didn’t hear about Chikungunya prior to the participation in this study. 113 (87.6) had heard about the disease.

Similarly 95 (73.6%) of participants knew correctly how Chikungunya is transmitted. 2 (1.6%) thought that the disease is transmitted by consumption of contaminated food or beverage, 2 (1.6%) believed that the disease is transmitted by close contact with the infected person, whereas 29 (22.5%) did not have any knowledge about the mode of transmission, and 1 (0.8%) participant thought that the disease is transmitted through mosquito bite as well as by the contact with infected person.

105 (81.4%) of the participants did not stay in contact with the infected person during their stay in Southern part of Thailand, whereas 24 (18.6%) had history of contact with the infected person.

113 (87.6%) participants did not have past history of dengue infection. 14 (10.9%) participants had history of dengue infection in the past. The most recent dengue infection among the travelers was 4 years back and the oldest infection was 20 years back. 2 (1.6%) of the travelers were not sure whether they had dengue infection or not.

15 (11.6%) of the travelers had history of fever during their stay in Southern part of Thailand, whereas 114 (88.4%) travelers were afebrile during that period.

Out of the fifteen person who gave the history of fever during their stay in Southern part of Thailand 2 (1.6%) gave the occurrence of rash along with the fever, similarly 3 (2.3%) of the febrile travelers said they had joint pain, 7 (5.4%) had muscle pain . 12 (9.3%) gave history of headache, and 3 (2.3%) of the travelers gave positive history of retro orbital pain.

8 (6.2%) of the travelers visited Prachuap kirikhan, 3 (2.3%) went to Chumphon, 1 (0.8%) to Ranong ,14 (10.9%) visited Surat thani, 2 (1.6%) traveled to Phangnga, 16(12.4%) went to Phuket, 15 (11.6%) had been to Nakhon si Thammarat, 4 (3.1%) went to Krabi, 3 (2.3%) visited Phattalung, 14 (10.9%) visited Songkhla, 1 (0.8%) went to Pattani, 2 (1.6%) had been to Yala, 5 (3.9%) went to Trang, 10 (7.8%) traveled to Phectburi. 31(24%) had visited to more than one province. Satun and Narathiwat were not visited by the travelers included in this study.

Table 4: General characteristics of 129 travelers to the Southern part of Thailand

General characteristics (n=129)	Frequency	Percent
Age in years		
18-30	56	43.4
31-45	60	46.5
46-60	13	10.1
Mean,SD:33.02,8.758		
Range:18-60		
Gender		
Male	67	51.9
Female	62	48.1

Table 5: Travel characteristics of 129 travelers to the Southern part of Thailand

Travel characteristics	Frequency (N)	Percent (%)
Duration of travel		
7-14 days	82	63.6
15-21 days	17	13.2
22-30 days	16	12.4
>30 days	14	10.9
Mean,:22.33 days, SD:31.457 Range:7-180 days		
Area of stay		
Rural	47	36.4
Urban	74	57.4
Both	8	6.2
Purpose of travel		
Tourism	23	17.8
Business	31	24
Visit friends or relatives	49	38
Educational purpose	4	3.1
Others	22	17.1

Travel characteristics	Frequency	Percent
Province Visited		
Phectburi		
Prachuap kirikhan	10	7.8
	8	6.2
Chumphon	3	2.3
Ranong	1	0.8
Surat thani	14	10.9
Phangnga	2	1.6
Phuket	16	12.4
Nakhon si Thammarat	15	11.6
Krabi	4	3.1
Phattalung	3	2.3
Songkhla	14	10.9
Pattani	1	0.8
Yala	2	1.6
Trang	5	3.9
More than 1 province	31	24

Table 6: Medical characteristics of 129 travelers to the Southern part of Thailand

Medical Characteristics	Frequency (N)	Percent (%)
Previous history of dengue infection		
No	113	87.6
Yes	14	10.9
Not sure	2	1.6
History of fever during travel		
No	114	88.4
Yes	15	11.6
Associated Symptoms with fever		
Rash	2	8
Joint Pain	3	11
Muscle pain	7	26
Headache	12	44
Retro orbital pain	3	11
History of contact with infected person during travel		
No	105	81.4
Yes	24	18.6

Table 7: General Knowledge about Chikungunya

General knowledge	Frequency	Percent
Heard about Chikungunya		
No	16	12.4
Yes	113	87.6
Mode of Transmission		
Bitten by mosquito	95	73.6
Consuming contaminated food or drink	2	1.6
Contact with infected person	2	1.6
Don't know	29	22.5
By mosquito and contact with infected	1	0.8

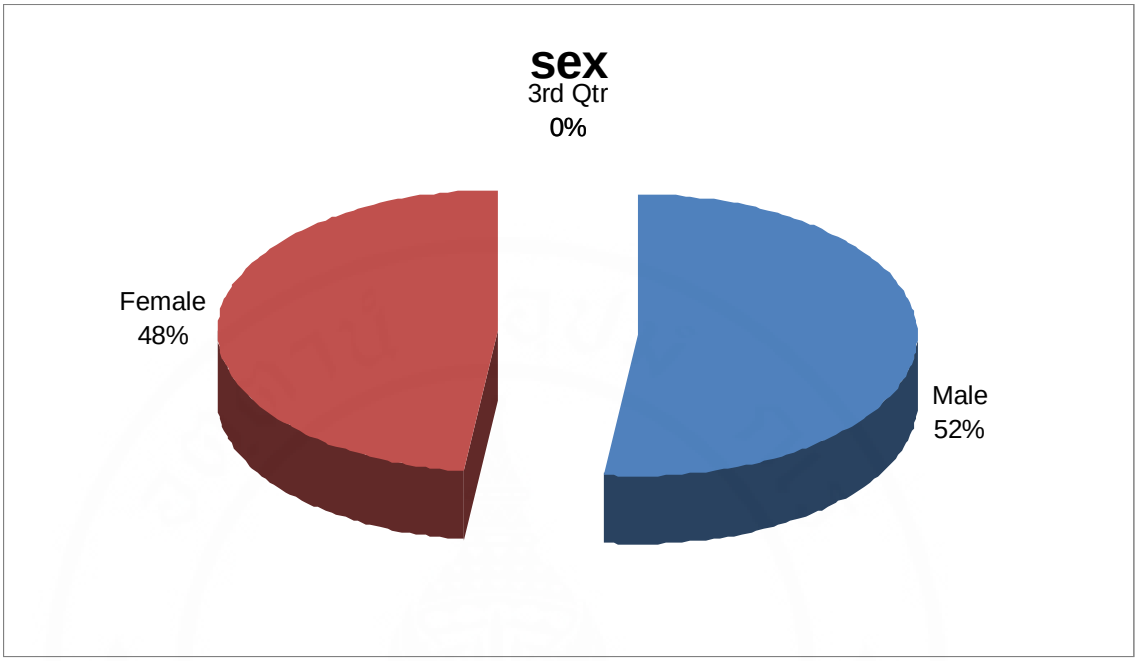

Figure 5: Gender distribution

Of the 129 participants 62 (48%) female and 62 (52%) male

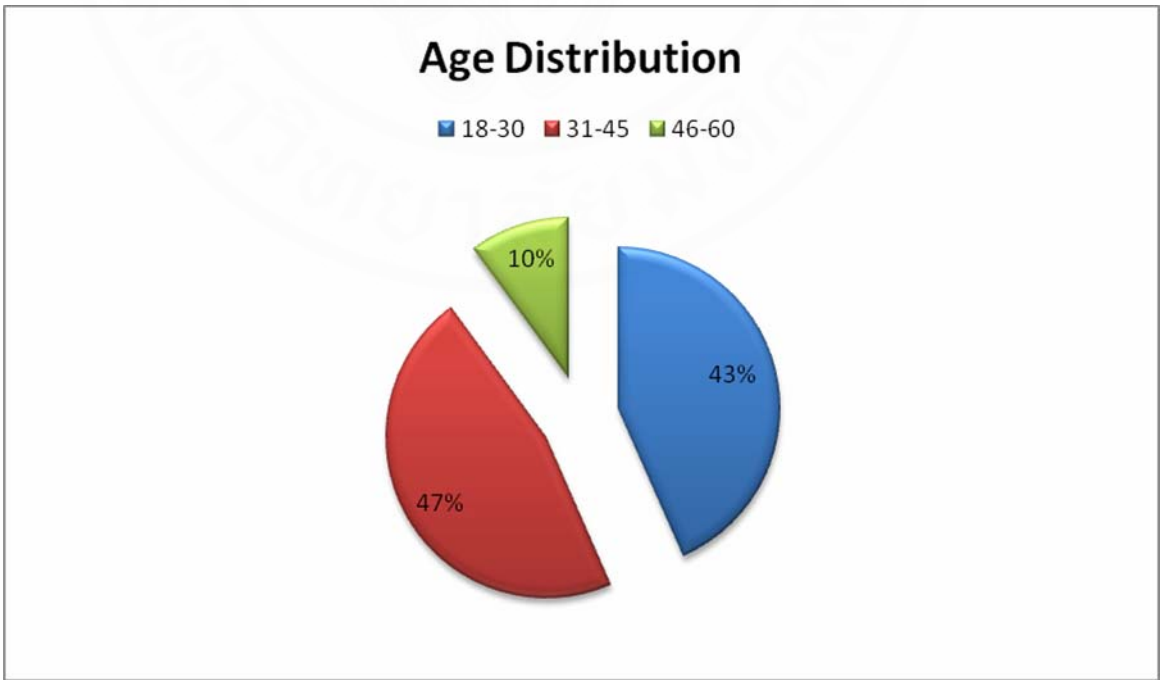

Figure 6: Age Distribution

The age group of 18-30 years consisted of 56 (43.4%), 31-45 years had 60 (46.5%) and 46-60 years consisted of 13 (10.1%) participants.

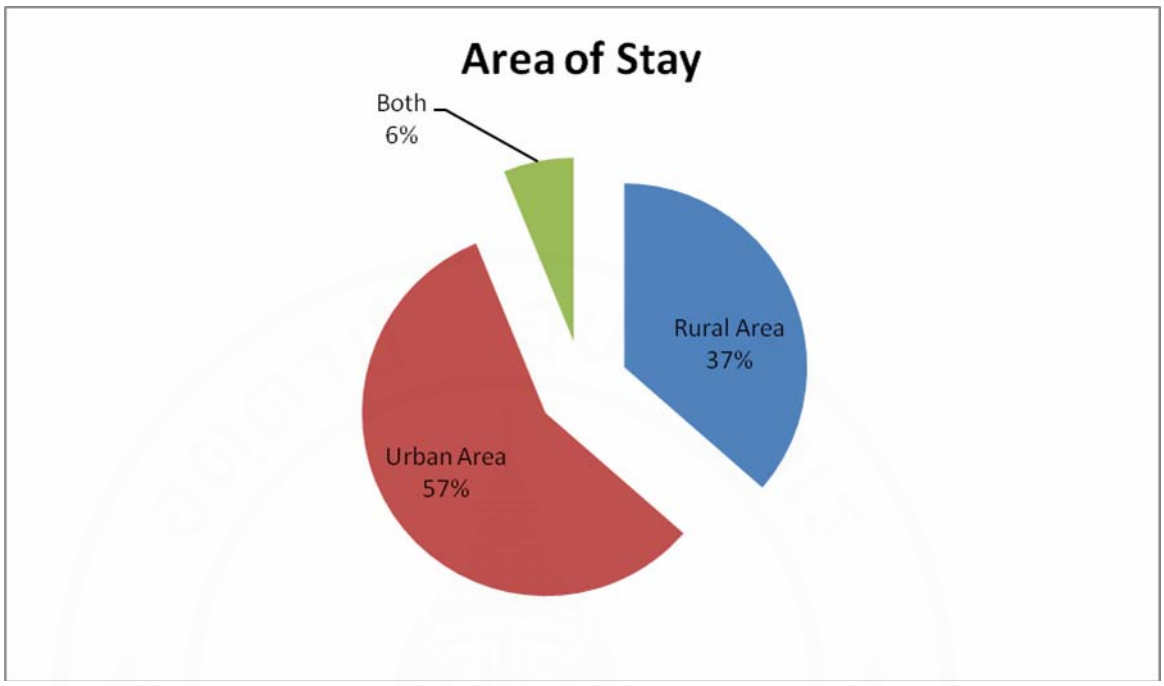

Figure 7: Area of stay

47 (36.4%) travelers stayed in the rural area while 74 (57.4%) stayed in the urban area and 8 (6.2%) stayed both in rural and urban area.

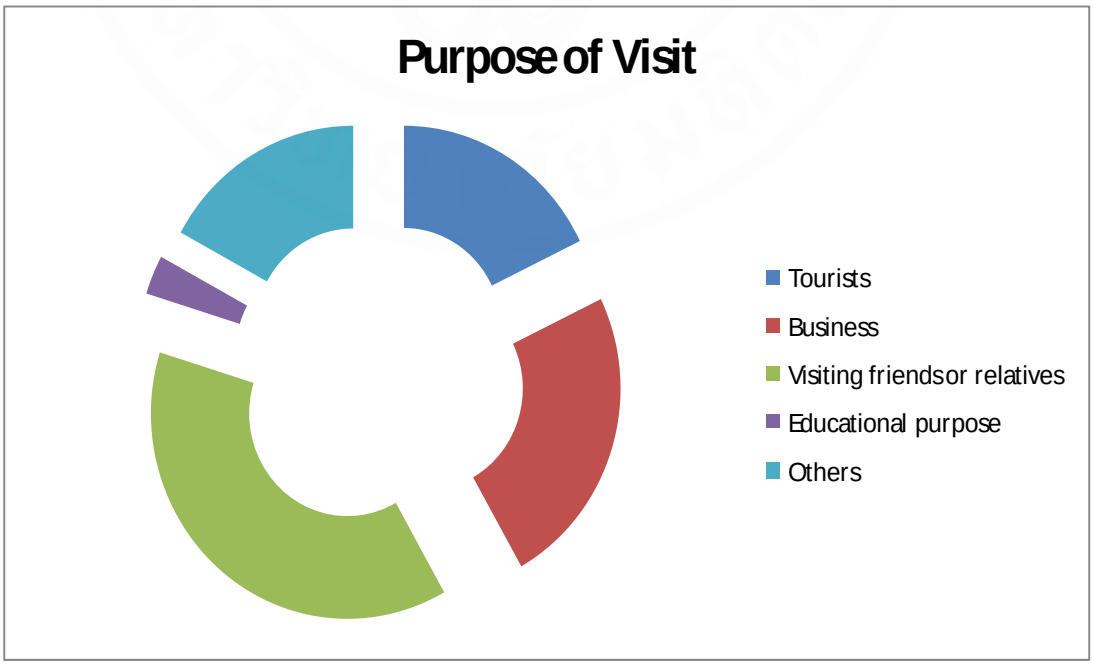

Figure 8: Purpose of Visit

23 (17.8%) were tourists, 31 (24%) traveled for business, 49 (38%) traveled to visit family and friends, 4 (3.1%) had educational trip and 22 (17.1%) had other purposes.

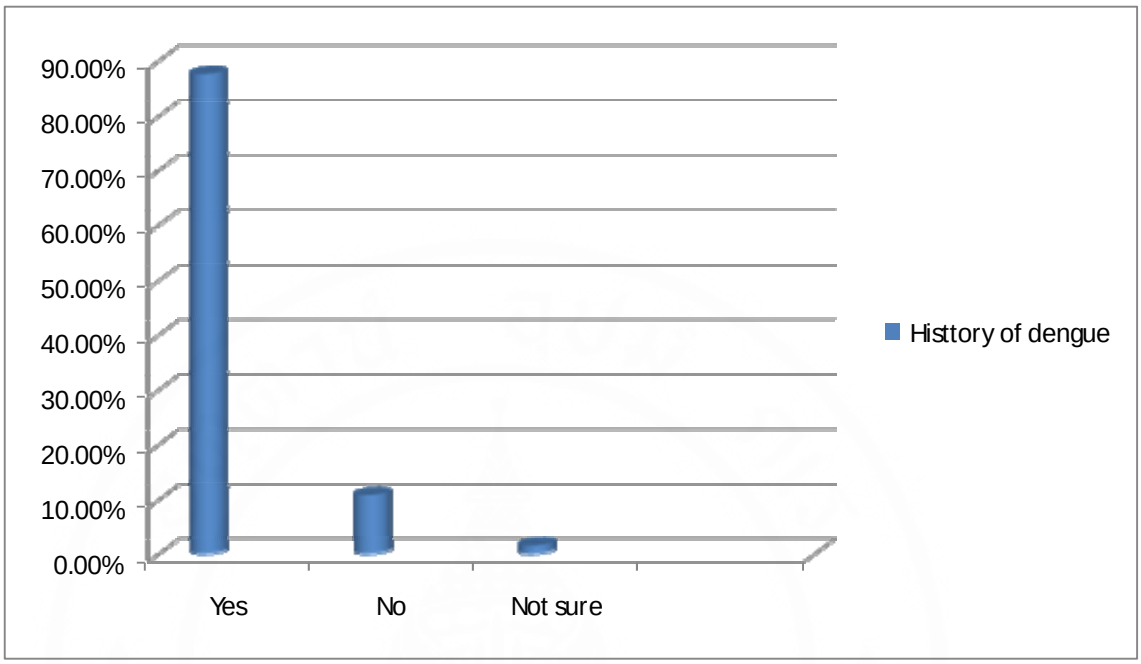

Figure 9: History of Dengue

Among the 129 participants 113 (87.6%) did not had past history of dengue fever, whereas 14 (10.9%) had history of dengue fever, and 2 (1.6%) were not sure whether they had dengue fever or not.

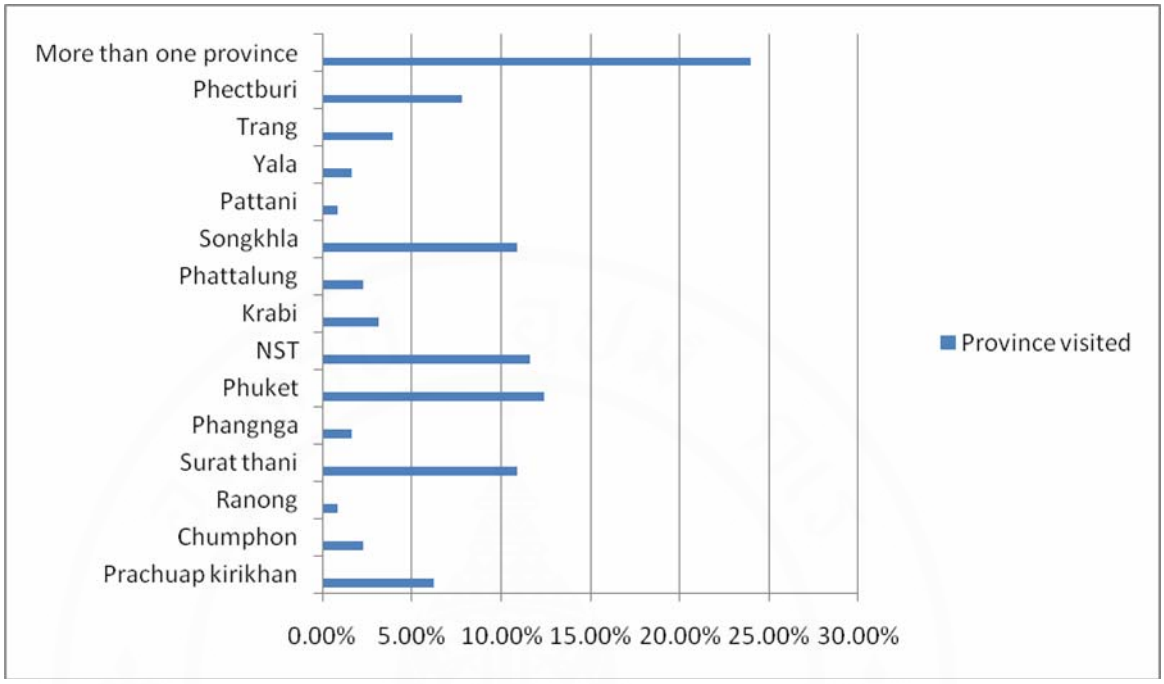

Figure 10: Province Visited

Among the 129 participants, 10 (7.8%) traveled to Phectburi, 8 (6.2%) to Prachuap Kirikhan, 3 (2.3%) to Chumphon, 1 (0.8%) to Ranong, 14 (10.9%) to Surat thani, 2 (1.6%) to Phangnga, 16 (12.4%) to Phuket, 15 (11.6%) to Nakhon si thammarat, 4 (3.1%) to Krabi, 3 (2.3%) to Pattalung , 14 (10.9%) to Songkhla, 1 (0.8%) to Pattani, 2 (1.6%) to Yala, 5 (3.9%) to Trang and 31 (24%) of the participants traveled to more than 1 province.

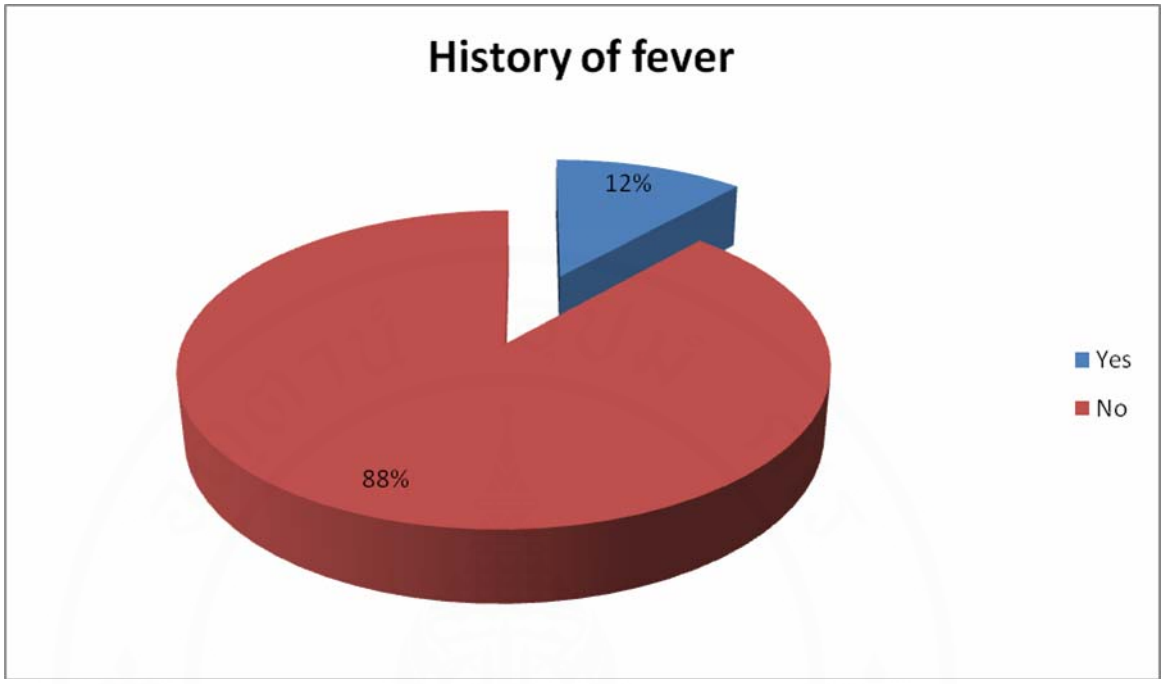

Figure 11: History of fever

114 (88.4%) of the participants were afebrile, and 15 (11.6%) had fever.

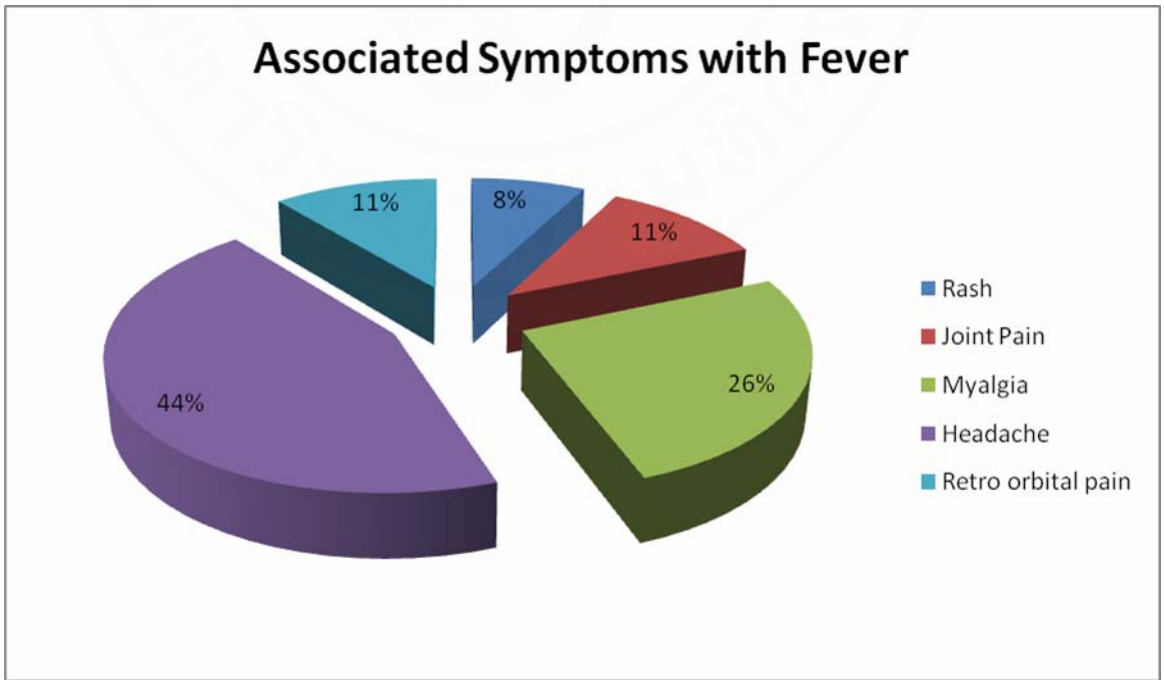

Figure 12: Associated symptoms with fever

Among the febrile participants 2 (8%) had rash, 3 (11%) and joint pain 7 (26%) had muscle pain ,3 (11%) had retro orbital pain and 12 (44%) had headache.

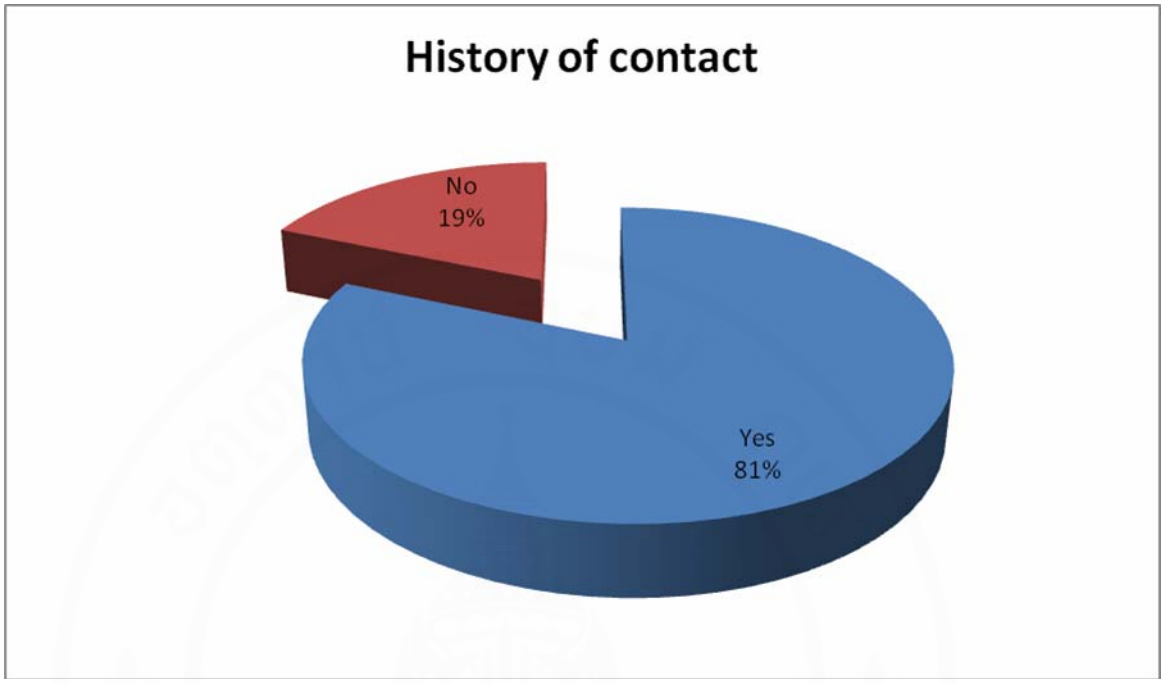

Figure 13: History of contact with Chikungunya infected person

105 (81.9%) did not stay in contact with the infected person whereas 24 (18.6%) gave history of contact with Chikungunya infected person.

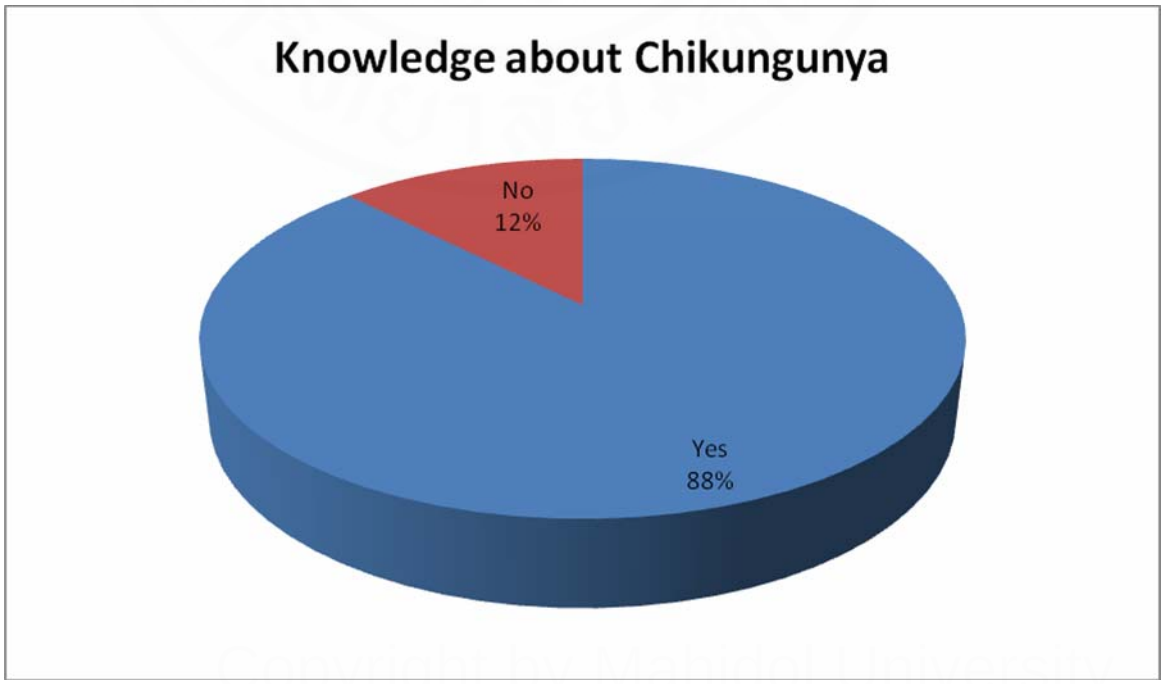

Figure 14: Knowledge about Chikungunya

16 (12.4%) did not hear about the disease and 113 (87.6%) knew about it.

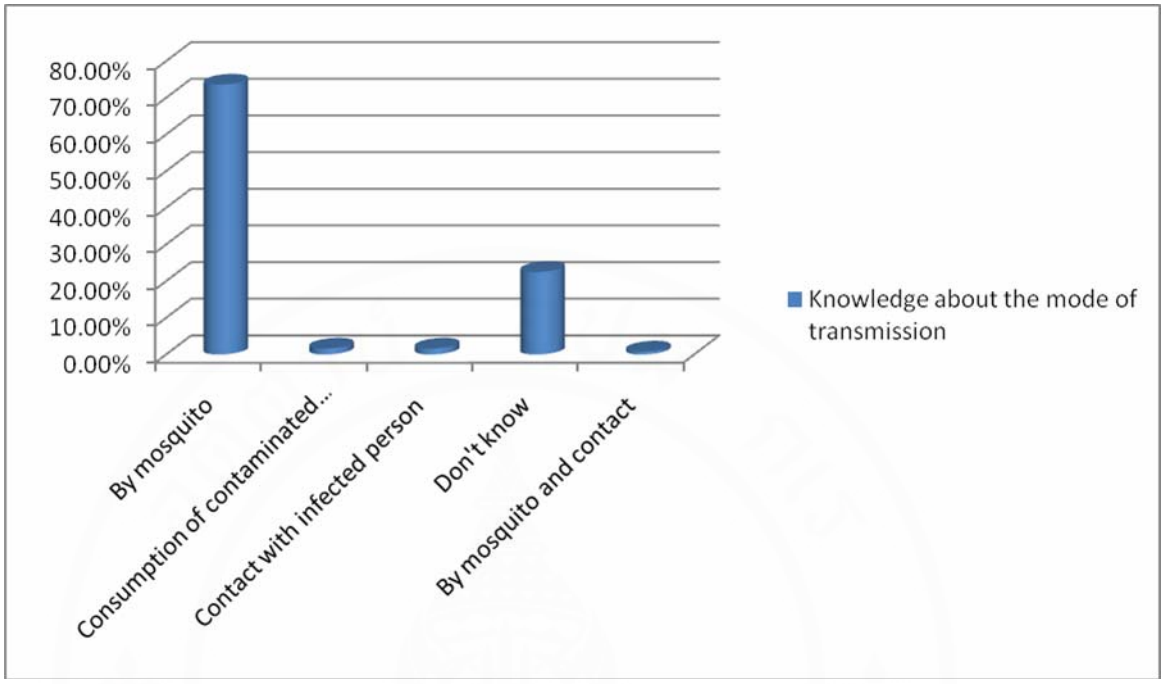

Figure 15: Knowledge about the mode of transmission of Chikungunya

Among the 129 participants 95 (73.6%) thought that the disease is transmitted through mosquito bite, 2 (1.6%) thought it is through contaminated food or beverage, 2 (1.6%) thought it is by contact with the infected person , 29 (22.5%) did not know how it is transmitted whereas 1 (0.8%) thought it is transmitted, both through the mosquito bite and wit the contact with infected person.

4 .2. SEROLOGICAL LABORATORY RESULTS:

Of the 130 initially enrolled participants 1 seropositive participant was excluded from the analysis due to positive history of Chikungunya 3 months prior to the enrollment to this study. Thus 129 participants were considered eligible for analysis. Out of the 129 participants 18 participants were found to be seropositive.

The seroprevalence is **14% (18/129)**

We will compare the different characteristics which might have influence in the seropositivity in the next section, but in this section we will see the duration of travel and its relationship with seropositivity.

Table:8 Duration of travel in seropositive and seronegative groups

Duration of stay	**Seropositive (n=18)**	**Seronegative (n=111)**	**P value**
Mean	16.28	23.32	0.696
SD±	19.348	32.96	

The p value being 0.696 it is statistically not significant.

Comparison of the seropositive and seronegative groups

In this section we will compare the characteristics of the seropositive and seronegative group for any significant relationship of the data.

Table 9: Comparison between the seropositive and seronegative groups

Variables	Seronegative N= 111	Seropositive N=18	P value
Age			
Mean	31.89	40	< 0.001†
SD±	8.095	9.671	
Sex			
Male	59 (53.2%)	8 (44.4%)	0.493
Female	52 (46.8%)	10 (55.6%)	
Travel duration			
Mean	23.32	16.28	
SD±	32.96	19.348	0.381‡
Area of stay			
Rural	40 (36%)	7 (38.4%)	
Urban	64 (57.7%)	10 (55.6%)	1.000*
Both	7 (5.4%)	1 (5.6%)	

Variables	Seronegative N= 111	Seropositive N=18	P value
Purpose of travel			
Tourism	19 (17.1%)	4 (22.2%)	
Business	29 (26.1%)	2 (11.1%)	
Visiting friends or relatives	39 (35.1%)	10 (55.6%)	0.415*
Educational purpose	4 (3.6%)		
Others	20 (18%)	2 (11.1%)	
Previous history of dengue			
Yes	13 (11.7%)	1 (5.6%)	
No	96 (86.5%)	17 (94.4%)	0.771*
Not sure	2 (1.8%)		
History of fever			
Yes	14 (12.6%)	1 (5.6%)	0.693*
No	97 (87.4%)	17 (94.4%)	

*Fisher's exact test

†Mann Whitney test

‡ student t test

CHAPTER VI
DISCUSSION

We found 14% seroprevalence among the Thai travelers to the Southern part of Thailand. The secondary objective of our study was to assess the possible relationship between the time sent at the Chikungunya affected region and seropositivity, but we could not detect any relationship between the two above mentioned factors.

We conducted our research in Bangkok, in a blood donation facility, where only the healthy people came for blood donation. Recently sick people refrained from blood donation for a certain amount of time .So we might not have the exact figure of the people who were sick whether with Chikungunya infection or any other infection during the visit to the southern part of Thailand or soon after the trip. This number of 14% might have been higher.

As our study was cross sectional, we assessed the participants only after their return from the trip. We do not have their sero status before the travel. We don't know whether the participants were sero naïve or already seropositive before the trip. Here in this study we just presumed that our study population was naïve. We don't have any data to support or to rule out the fact that Chikungunya is not present in Bangkok or its vicinity. As we know in these areas the vector of the disease is present, so we cannot rule out the possibility of existence of Chikungunya in Bangkok and its vicinity, where most of our study participants resided. They might already have been seropositive before the travel to the southern part of Thailand.

In a country like Thailand with presence of vectors of Chikungunya infection *Aedes albopictus* and *A. aegypti* the findings of our study might be beneficial in differentiation of Chikungunya infections with clinically similar list of diseases. We focused on the Thai internal travelers to the southern part of Thailand where there was a recent outbreak and is considered as a Chikungunya affected region. According to the Thai MOPH the highest number of cases was recorded during the months of

January –March. Paradoxically the reported cases were higher in the dry winter season in comparison to the rainy season, which is a breeding season for the mosquitoes. Our study was conducted in the months January-march which co-incided with the months of high incidence of Chikungunya cases from the previous year in the Southern part of Thailand.

Out of the 18 seropositive participants only one participant gave the history of fever with symptoms compatible with Chikungunya infection, and one participant had the symptoms without fever. In contrast to the data from the bureau of epidemiology MOPH, Thailand regarding the ratio of symptomatic to asymptomatic infection which is 2:1, our study detected the ratio of almost 1:10.The cross sectional survey in a village in the Southern part of Thailand, in August 2008-March 2009, among 521 people by the bureau of epidemiology MOPH Thailand found 10% of asymptomatic infection. Similarly the same bureau found 34% of asymptomatic cases from 117 confirmed cases of Chikungunya from a village in Southern part of Thailand. A study conducted by Maria L Moro and colleagues in North-Eastern Italy demonstrated 18% completely asymptomatic individuals. This different data about the ratio of asymptomatic infection might depend on the geographical location, the particular species of the vector. As our study was conducted among the healthy Thai blood donors with excellent health, our findings of asymptomatic infection might have been higher in comparison to previous findings.

The seroprevalence of 14% among the travelers to the Southern part of Thailand was quite higher than we expected, where the prevalence of Chikungunya fever is 0.48% (MOPH, Oct 2009). Considering the fact that we enrolled only the healthy Thai volunteers, regardless of the symptoms the results obtained were higher than the expectation. We used Chikungunya IgM rapid test kit, which provides the preliminary test result, to confirm Chikungunya infection ELISA, PCR, tests are recommended by the manufacturer of the kit we used. The increasing age was directly proportional to the seropositivity according to Watanaveeradej and colleagues (Watanaveeradej et al, 2006). The mean age of our participants was 33.02 years and 61% of the participants were from the age group of 31-45 years, this factor might have influenced in obtaining the higher seroprevalence.

One of the factors contributing to our results might be the inability of the sick people to come for volunteer blood donation, in fact the results might have been different from what we obtained.

The incubation period of Chikungunya virus is 2-12 days (usually 3-7 days) (CDC, 2008). We assessed the presence of fever and associated symptoms during the travel duration to the Southern part of Thailand, but we did not assess for the symptoms which might have developed immediately after their return from the trip.

The closest to our study was a study by Eisenhut and colleagues in 1999, among the German overseas volunteers. They detected the seroprevalence of Chikungunya virus in 5.5% of the subjects returning from Thailand. IgG was tested using immunoflurescence assays to detect the Chikungunya antibodies (Eisenhut et al, 1999). The sample size of 36 subjects was comparatively lesser than our sample size of 130. They too have assigned the subjects regardless of symptoms. The minimum duration of stay in the endemic area in this study was 4 months. The mean duration of stay was 37.7 months.

33% of infants born between 1998 and 2000 from uncomplicated pregnancies were found to be seropositive for Chikungunya virus by antibody screening (Watanaveeradej et al, 2006).The increasing maternal age was directly proportional to the seropositivity. This suggested that the several endemics of Chikungunya must have occurred in Thailand, resulting in persistent circulation of Chikungunya virus. Even the study group was different to ours their findings was significantly higher.

A study by Chahar and colleagues concluded that co infections with Chikungunya and dengue viruses occur in areas where these two viruses co circulate (Chahar et al, 2009). For our study we didn't check for the markers of dengue infection. As per our CRF, out of 18 valid seropositive cases, only 1subject had history of dengue in the past, that too not recent.

Though not fatal, Chikungunya infection can cause a prolonged debilitating arthralgia. Clinically Chikungunya infection is very difficult to differentiate with dengue infections, especially when the patients have the febrile symptoms only. Unlike dengue infections hemorrhage is rare in chikungunya infection. Clinically often

misdiagnosed the incidence of Chikungunya fever could be much higher than the reported cases. Chikungunya virus infection regardless of symptomatic or asymptomatic is believed to provide lifelong immunity. Chikungunya fever at first has to be differentiated from dengue infection. Clinical manifestations of these two diseases are similar especially when the patients have only febrile symptoms. Presenting symptoms of early stages of typhoid fever, flulike illness, mono or poly articular septic arthritis, acute food poisoning should also be considered as differential diagnosis. For the prompt diagnosis and management of the diseases resembling Chikungunya, to rule out or confirm Chikungunya, is an important step for the clinicians.

General Characteristics

The study participants were exclusively Thai nationals; natives from the Southern provinces were excluded from the study unless they lived for more than two years outside the Southern provinces. The residents from Chantaburi, Rayong, Chonburi and Udon thani were also excluded from the study as these four provinces had the recent outbreak.

All the participants were volunteer blood donors from the National blood center, Thai Red Cross society. The age range was 18-60 years with a mean age of 33.02 years. The duration of travel ranged from 7 days to 180 days with a mean duration of 22.33 days. Majority of the travelers stayed in well developed urban areas. The main purpose of travel was to visit friends or relatives followed by business purpose and tourism. Almost 90% travelers had heard about Chikungunya and almost 75%of the participants knew the correct mode of transmission of Chikungunya.

Only about 10% of the travelers developed fever during the visit to the Southern part of Thailand, with a very few people having associated symptoms consistent with Chikungunya. The general characteristics of the seropositive travelers are represented in table 11.

Case No.	Sex	Age	Duration	Area Of Stay	Contact with Chikungunya infected person	Purpose	Fever	Constitutional Symptoms
5	F	41	14 days	Urban	No	Business	No	No
13	M	46	15 days	Rural	No	Business	No	No
45	M	43	7 days	Urban	No	Tourism	No	No
49	M	40	15 days	Rural	No	Visit F/R	Yes	Yes
50	F	41	7 days	Rural	No	Visit F/R	No	No
52	M	25	14 days	Rural	No	Visit F/R	No	No
57	F	60	8 days	Rural	Yes	Tourism	No	No
68	F	35	7 days	Urban	No	Other	No	No
72	F	45	7 days	Rural	No	Tourism	No	No
74	F	29	7 days	Urban	No	Visit F/R	No	Yes
79	M	46	8 days	Urban	No	Other	No	No
87	M	36	10 days	Urban	No	Visit F/R	No	No
101	F	24	20 days	Urban	Yes	Visit F/R	No	No
105	M	31	15 days	Rural	No	Visit F/R	No	No
117	F	41	7 days	Urban	No	Visit F/R	No	No
118	M	42	90 days	Urban	No	Visit F/R	No	No
121	F	39	12 days	Rural	No	Visit F/R	No	No
123	F	58	30 days	Urban	No	Tourism	No	No

Table:9 General characteristics of the seropositive participants.

The Details about the seropositive participants

The mean age of the participants who were seropositive was 40 years, ranging between 24 to 60 years, with a standard deviation of 9.67. Among the seropositive participants 10 (55.6%) were female and 8 (44.4%) were male. Seven (38.9%) of them stayed in the rural area and ten (55.6%) in urban area, one (5.6%) stayed both in rural and urban area. 4 (22.2%) of the participants travelled as tourists, 2 (11.1%) travelled for business purpose,10 (55.6%) travelled to meet friends and relatives, and 2 (11.1%) travelled for various other purposes. 15 (83.7%) of the 18 seropositive participants knew about Chikungunya prior to the participation in this study, whereas 3 (16.7%) didn't know about the disease. 14 (77.8%) of them knew correctly about the transmission of the disease, but 4 (22.2%) didn't know about it. 3 (16.7%) had history of contact with the person infected with Chikungunya, whereas 15 (83.3%) had not.17 (94.4%) did not have fever during the travel and 1 (5.6%) had fever.

Limitations

The study had some of its limitations, which are as follows:

a) Although the reported cases from the previous year were at the peak during the winter months of January-March, the breeding season of the mosquitoes is rainy season. If the study would have been conducted in the rainy season ,we assume that we might have obtained different results.

b) As the study design was cross sectional , we had assessed the participants only once. We did not know whether they were sero naïve before the travel.

c) The assessment of the symptoms was done only during the travel to the southern part of Thailand, we don't have data of symptoms immediately after the return from the trip.

Recommendation s for further studies

It would be beneficial to have some additional information from the seropositive participants. Knowing the sero status before the travel would be beneficial. For that purpose a Longitudinal study might be beneficial. . As mentioned previously the results might be different if the study would be conducted during the rainy season. The study site chosen for this study itself restricts the participation of elderly and pediatric population; future studies should include these populations to observe the effect of age in the outcome of the study.

CHAPTER VII
CONCLUSION

Our study is first of its kind detecting the seroprevalence of Chikungunya regardless of symptoms, among the internal travelers from Thailand. The closest to our study is a study by Eisenhut and colleagues from Germany. The result of their study is quite lower in comparison to our study. They detected a seroprevalence of 5.5%, whereas we found the seroprevalence of 14%. Similarly the findings of Taubitz and colleagues of 33% , among the symptomatic German travelers was higher than ours. However we consider that the finding of our study of 14% seroprevalence cannot be ignored.

Even though we detected a relatively high prevalence, we could not detect any statistical significant characteristics except for the age. Any association between the characteristics and seropositivity could not be explained. Nevertheless we can conclude that travelers can be infected with Chikungunya infection after the travel to the endemic areas, even though they remain asymptomatic.

Without the presence of efficient vaccination or specific chemoprophylaxis the only way of prevention is to protect from mosquitoes using various preventive measures.

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APPENDICES

APPENDIX A

Participant Information Sheet

Title of Research: Detection of Chikungunya Seropositivity in Thai travellers, to the Southern part of Thailand.

Principal Investigator: Dr. Dipendra Sharma
Faculty of Tropical Medicine, Mahidol University
Tel: 080-081-3207. E-mail: pyak4u@yahoo.com

Purpose of the study: To determine the prevalence of infectivity among the travellers to the Chikungunya affected areas, as well as the relationship between the time spent at those areas and infectivity.

You have been invited to participate in the study "Detection of Chikungunya Seropositivity in Thai Travellers to the Southern part of Thailand. It is important that you read the following explanation of this study. As a volunteer blood donor you have come to the national blood centre which is one of the best equipped facilities in the country. 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

For the completion of study we require 130 participants. You will be asked to fill out a three page questionnaire which usually takes 5 – 10 minutes, with no follow up. It will assess asking for general data about you and your recent travel history and your travel

itinerary. 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 remain. 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 Chikungunya antibodies, which will determine whether or not you had been infected with Chikungunya infection within last 3 months. For your information Chikungunya infection can occur without any signs and symptoms. In Thailand more than one third of the infection occurs without any signs and symptoms. The leftover blood specimen will be destroyed after the study.

Risks and Discomforts

Blood donation is one of the commonest medical procedures. The medical personnel here are well qualified and experienced. The procedure of blood donating is associated with the discomfort of having a needle stick and bruising at the site of injection. Some unusual events such as fainting, dizziness and swelling of the puncture site may occur. Another potential risk such as thrombophlebitis occurs rarely. Risk of an infection at the puncture site is small. Most of these events are mild and resolve spontaneous recovery. You will be closely monitored throughout the whole procedure and if necessary provided with the appropriate medical care by the staff of the centre, not depending upon whether or not you participate in this study. For our study we will require extra 3 ml. of blood from each participant, for which no extra intervention is required. It is not supposed to cause any additional inconvenience for you. 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 and benefit

Participation in this study is voluntary; there will be no negative consequences if refusal to participate this study. Your questionnaire will remain confidential and anonymous. You will not get any direct benefit from this study, but by participating you will be contributing for a scientific research.

Informed Consent

I have been invited to participate in the study "Detection of Chikungunya Seropositivity in Thai travellers to the Southern part of Thailand". 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. Dipendra Sharma
Faculty of Tropical Medicine, Mahidol University
Tel: 080-081-3207. E-mail: pyak4u@yahoo.com

Or my supervisor;
Dr. Watcharapong Piyaphanee
Faculty of Tropical Medicine, Mahidol University
Tel: 089-8917913. Email: tewpe@mahidol.ac.th

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.

In case of any adverse event arising from blood donation procedure, I will receive standard medical treatment from the National blood bank and the investigator's team 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.

…….. ……...
Signature of Participant Date

……..
Signature of Investigator

เอกสารชี้แจงผู้เข้าร่วมการวิจัย

ชื่อการวิจัย การศึกษาหลักฐานการติดเชื้อชิกุกุนยาในนักท่องเที่ยวไทยที่เดินทางไปยังภาคใต้ของประเทศไทย

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โทร. 089-8917913

สถานที่วิจัย ศูนย์โลหิตแห่งชาติ สภากาชาดไทย และโรงพยาบาลเวชศาสตร์เขตร้อน

ผู้ให้ทุนวิจัย คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล

วัตถุประสงค์ของการวิจัย เพื่อศึกษาความชุกของการติดเชื้อโรคชิคุนกุนยาในนักท่องเที่ยวไทยที่เดินทางไปยังภาคใต้ของประเทศไทย

ท่านได้รับการเชิญชวนให้เข้าร่วมโครงการ “การศึกษาหลักฐานการติดเชื้อชิกุกุนยาในนักท่องเที่ยวไทยที่เดินทางไปยังภาคใต้ของประเทศไทย” โปรดอ่านเอกสารชี้แจงผู้เข้าร่วมวิจัยนี้ให้ละเอียด ก่อนยินยอมเข้าร่วมการศึกษาวิจัย ท่านจะได้รับทราบข้อมูลถึงขั้นตอนการทำวิจัย วัตถุประสงค์กระบวนการ ความเสี่ยงความไม่สุขสบาย ผลประโยชน์ที่จะได้รับ ของการศึกษาวิจัยครั้งนี้ รวมถึงสิทธิของผู้เข้าร่วมที่สามารถถอนตัวจากการศึกษาวิจัยเวลาใดก็ได้

รายละเอียดขั้นตอนการวิจัย

อาสาสมัครที่สมัครใจเข้าร่วมคือผู้มาบริจาคเลือดที่ ศูนย์บริจาคโลหิตแห่งชาติ สภากาชาดไทย การศึกษานี้ใช้ระยะเวลา 3 เดือน โดยรับอาสาสมัครในโครงการทั้งสิ้น 130 คน ผู้สมัครใจเข้าร่วมโครงการจะถูกขอให้กรอกแบบสอบถาม 3 หน้า โดยใช้เวลาประมาณ 5-10 นาที แบบสอบถามจะประเมินข้อมูลทั่วไปเกี่ยวกับการเดินทาง, การเดินทางในภาคใต้ในครั้งล่าสุดของท่าน เมื่อท่านเข้าร่วมวิจัยแล้ว เจ้าหน้าที่จะขอแบ่งเลือดที่ท่านบริจาคเป็นจำนวน 3 มิลลิลิตร (ml) เพียงครั้งเดียว เพื่อเก็บตัวอย่างส่งตรวจที่คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล เพื่อตรวจวิเคราะห์หาหลักฐานการติดเชื้อชิกุกุนยา (Chikungunya antibody) ว่าท่านเคยได้รับการติดเชื้อชิคุนกุนยาในช่วงเวลาที่ผ่านมาหรือไม่ ซึ่งผู้ที่ได้รับเชื้อชิกุกุนยาอาจไม่แสดงอาการใดๆเลยก็ได้ สำหรับประเทศไทยมากกว่า 1 ใน 3 ของผู้ที่ได้รับการติดเชื้อชนิดนี้จะไม่แสดงอาการใด ๆ

ข้อมูลส่วนตัวของท่านจะถูกเก็บรักษาไว้ ไม่เปิดเผยต่อสาธารณะเป็นรายบุคคล สำหรับตัวอย่างเลือดที่ทำการตรวจวิเคราะห์แล้ว จะถูกทำลายหลังจากการศึกษาวิจัยเสร็จสิ้น

ความเสี่ยงและความไม่สุขสบาย

การบริจาคเลือดเป็นส่วนหนึ่งของกระบวนการแพทย์ และบุคลากรที่ให้การพยาบาลเป็นผู้ที่มีประสบการณ์ และมีคุณสมบัติเหมาะสม กระบวนการบริจาคเลือดเป็นกระบวนการที่ไม่สุขสบาย เนื่องจากการถูกแทงเข็มเข้าเส้นเลือดดำและการผิวหนังช้ำบริเวณที่ถูกเข็มแทง ซึ่งอาจเกิดความผิดปรกติบางอย่างได้ เช่น เป็นลม เวียนศรีษะ หน้ามืด และรอยช้ำบริเวณที่ถูกเข็มแทง และรวมไปถึงการอักเสบหรือติดเชื้อของเส้นเลือดซึ่งพบได้น้อยมากสำหรับการศึกษาวิจัย ซึ่งอาการต่างๆเหล่านี้สามารถหายเองได้ การศึกษาครั้งนี้เจ้าหน้าที่ของศูนย์บริการโลหิตแห่งชาติจะเจาะเลือดอาสาสมัคร 1 ครั้ง ซึ่งผู้วิจัยจะขอแบ่งเลือดปริมาณ 3 มิลลิลิตรเพื่อใช้การวิจัย โดยไม่มีมีค่าชดเชยใดๆ ให้กับอาสาสมัคร และหากอาสาสมัครเกิดการปวด บวม อักเสบ เนื่องจากการเจาะเลือดครั้งนี้ ท่านจะได้รับการรักษาตามมาตรฐานโดยไม่มีค่าใช้จ่ายใด ๆ ทั้งสิ้น

สิทธิและประโยชน์ของท่าน

การเข้าร่วมการศึกษาวิจัยครั้งนี้เป็นความสมัครใจ การปฏิเสธการเข้าร่วมจะไม่มีผลกระทบใดๆต่อตัวท่าน แบบสอบถามของท่านจะยังคงถูกเก็บเป็นความลับและไม่ถูกเปิดเผยต่อสาธารณชน ท่านจะไม่ได้รับผลประโยชน์โดยตรงจากการศึกษาวิจัยนี้ แต่การเข้าร่วมวิจัยครั้งนี้ท่านจะเป็นผู้ให้ข้อมูลที่เป็นประโยชน์สำหรับการวิจัยทางวิทยาศาสตร์การแพทย์

ข้าพเจ้าได้อ่านรายละเอียดในเอกสารนี้ครบถ้วนแล้วจึงลงลายมือชื่อยินยอมเข้าร่วมวิจัย

ลงชื่อ.....

(.....)

วันที่.....

หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย

(Informed Consent Form)

วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้า.....ขอแสดงเจตนายินยอมเข้าร่วมโครงการวิจัย เรื่อง **“การศึกษาหลักฐานการติดเชื้อชิกุกุนยาในนักท่องเที่ยวไทยที่เดินทางไปยังภาคใต้ของประเทศไทย”** โดยข้าพเจ้าได้รับทราบรายละเอียดเกี่ยวกับที่มา จุดมุ่งหมายในการทำวิจัย ประโยชน์ที่คาดว่าจะได้รับของการวิจัย รายละเอียดขั้นตอนต่าง ๆ ที่ต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยง/ความไม่สะดวกสบายที่อาจจะเกิดขึ้นจากการเข้าร่วมการวิจัย ตลอดจนแนวทางป้องกันและแก้ไขหากเกิดอันตรายขึ้น โดยข้าพเจ้าได้อ่านข้อความที่มีรายละเอียดอยู่ในเอกสารชี้แจงผู้เข้าร่วมการวิจัยโดยตลอด อีกทั้งยังได้รับคำอธิบายและตอบข้อสงสัยจากผู้วิจัยเป็นที่เรียบร้อยแล้ว

ข้าพเจ้าจึงสมัครใจเข้าร่วมในโครงการวิจัยนี้

หากข้าพเจ้ามีข้อข้องใจเกี่ยวกับขั้นตอนของการวิจัย หรือหากเกิดผลข้างเคียงที่ไม่พึงประสงค์จากการวิจัยขึ้นกับข้าพเจ้า ข้าพเจ้าจะสามารถติดต่อกับ นายแพทย์ดิบเพนดรา ชาร์มา คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล ถนนราชวิถี เขตราชเทวี กรุงเทพ 10400 โทรศัพท์ 0-2354 -9100 ต่อ 1428 โทรสาร 0-2354-9168 โทรศัพท์มือถือ 080-0813207

หรือที่อาจารย์ นายแพทย์ วัชรพงศ์ ปิยะภาณี (ที่ปรึกษาการวิจัย) คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล ถนนราชวิถี เขตราชเทวี กรุงเทพ 10400 โทรศัพท์ 0-2354 -9100 ต่อ 1428 โทรสาร 0-2354-9168 โทรศัพท์มือถือ 089-8917913

หากข้าพเจ้าได้รับการปฏิบัติไม่ตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าจะสามารถติดต่อกับฝ่ายเลขานุการคณะกรรมการจริยธรรมการวิจัยในคน ชั้น4 อาคารเฉลิมพระเกียรติฉลองสิริราชสมบัติครบ 60 ปี คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล เบอร์โทร 0-2354-9100 ต่อ 1524,1525

ข้าพเจ้าได้ทราบว่าผลการศึกษานี้อาจถูกนำไปตีพิมพ์หรือนำเสนอเพื่อประโยชน์ทางการศึกษา อย่างไรก็ตามข้อมูลส่วนตัวของข้าพเจ้าจะไม่ถูกเปิดเผย และข้าพเจ้าทราบดีว่าการเข้าร่วมการศึกษาเป็นไปโดยสมัครใจ ข้าพเจ้าสามารถถอนตัวหรือองดเข้าร่วมการวิจัยได้ทุกเมื่อ โดยจะไม่มีผลกระทบต่อการบริการและการรักษาพยาบาลที่ข้าพเจ้าจะได้รับต่อไปในอนาคต

ถ้ามีผลข้างเคียงใดๆที่เกิดขึ้นจากการบริจาคเลือด ข้าพเจ้าจะได้รับการรักษาตามมาตรฐานการแพทย์จากศูนย์บริการโลหิตแห่งชาติและคณะผู้วิจัย

ข้าพเจ้าได้เข้าใจข้อความในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และหนังสือแสดงเจตนายินยอมนี้โดยตลอดแล้ว จึงลงลายมือชื่อไว้

ลงชื่อ..... ผู้เข้าร่วมการวิจัย วันที่.....
(.....)

ลงชื่อ.....ผู้วิจัย วันที่.....
(.....)

ลงชื่อ.....พยาน วันที่.....
(.....)

Date _____

Number _____

แบบสอบถามสำหรับผู้บริจาคเลือด เพื่อการศึกษาเกี่ยวกับโรคชิคุนกุนยา

Questionnaire for blood donors about the study in Chikungunya

1. เพศ ☐ ชาย ☐ หญิง
   Sex Male Female

2. อายุ _____ ปี
   Age Yrs

3. ช่วงระยะเวลาที่พักอยู่ทางภาคใต้ของประเทศไทย (ตั้งแต่ตอนล่างของจังหวัดเพชบุรี)
   ……..…… (กี่วัน / เดือน)
   Duration of stay in the Southern part of Thailand (Below Phetburi)……..…… (days/months)

4. วัตถุประสงค์ของการไปเยี่ยม ทางภาคใต้ของประเทศไทย
   Purpose of your visit to the Southern part of Thailand
   ☐ ท่องเที่ยว ☐ ธุรกิจ ☐ เยี่ยมเพื่อน หรือ ญาติ
   Tourism Business Visiting friends or relatives
   ☐ เกี่ยวกับการศึกษา ☐ อื่นๆ……..……
   Educational purpose Other, please specify…….

5. ช่วงระยะเวลาที่คุณไปเยี่ยมทางภาคใต้ของประเทศไทย ส่วนใหญ่คุณอาศัยอยู่ที่ใด ?
   During your visit to the Southern part of Thailand, the majority of your visit you stay in
   ☐ (นอกเมือง) ☐ (ในตัวเมือง)
   Rural area Urban area

6. คุณเคยได้ยินเกี่ยวกับโรคชิคุนกุนยามาก่อนหรือไม่
   Have you ever heard about Chikungunya disease before?
   ☐ ไม่เคย ☐ เคย
   No Yes

7. คุณคิดว่าโรคชิคุนกุนยาสามารถติดต่อผ่านทางใด

To best of your knowledge, you can get Chikungunya disease by…

☐ การติดเชื้อจากการถูกยุงกัด
Being bitten by infected mosquito

☐ การปนเปื้อนของอาหาร และ เครื่องดื่ม
Took contaminating food and drink

☐ การสัมผัสใกล้ชิดกับผู้ติดเชื้อ
Contact closely with the infected person

☐ ไม่ทราบ
I don't know

8. คุณเคยพัก หรือสัมผัสผู้ที่เคยถูกวินิจฉัยว่าติดเชื้อชิคุนกุนยาระหว่าง เดินทางไปทางภาคใต้ของประเทศไทย หรือไม่

Have you stayed or have a close contact with the person who had been diagnosed with Chikungunya infection during your trip to the Southern part of Thailand?

☐ เคย
Yes

☐ ไม่เคย
No

9. คุณเคยถูกวินิจฉัยว่าเป็นโรค เหล่านี้ หรือไม่

Have you ever been diagnosed with any of the following diseases?

1. ชิคุนกุนยา ☐ ไม่เคย ☐ เคย ระบุ ระยะเวลา..... ☐ ไม่แน่ใจ
Chikungunya No Yes _____ (specify when) Not Sure

2. ไข้เลือดออก ☐ ไม่เคย ☐ เคย ระบุ ระยะเวลา..... ☐ ไม่แน่ใจ
Dengue No Yes _____ (specify when) Not Sure

10. คุณเคยมีไข้ระหว่างที่พักในภาคใต้ของประเทศไทย หรือไม่

Did you develop fever during your stay in the Southern part of Thailand?

☐ เคย กรุณา ตอบข้อ 10.1
Yes, please answer no. 10.1

☐ ไม่เคย กรุณาข้ามไปข้อที่ 11
No, please go to no. 11

10.1 ช่วงที่มีไข้คุณมีอาการเหล่านี้ด้วยหรือไม่ ?

During the episode of fever, did you have any following symptoms?

- มีผื่น ☐ ไม่มี ☐ มี
  Rash No Yes
- ปวดข้อ ☐ ไม่มี ☐ มี
  Joint pain No Yes
- ปวดเมื่อยกล้ามเนื้อ ☐ ไม่มี ☐ มี
  Muscle pain No Yes
- ปวดหัว ☐ ไม่มี ☐ มี
  Headache No Yes
- ปวดบริเวณตา ☐ ไม่มี ☐ มี
  Pain behind your eyes No Yes

11. กรุณากรอกจังหวัดที่คุณได้ไป และจำนวนวันที่คุณอยู่ ช่วงที่คุณไปท่องเที่ยวทางภาคใต้ของประเทศไทย

Please write in the following box the province visited and days spent during your recent travel to the southern part of Thailand.

จังหวัดที่ได้ไป Province Visited	ระยะเวลาที่อยู่ (วัน) Days Spent

BIOGRAPHY

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