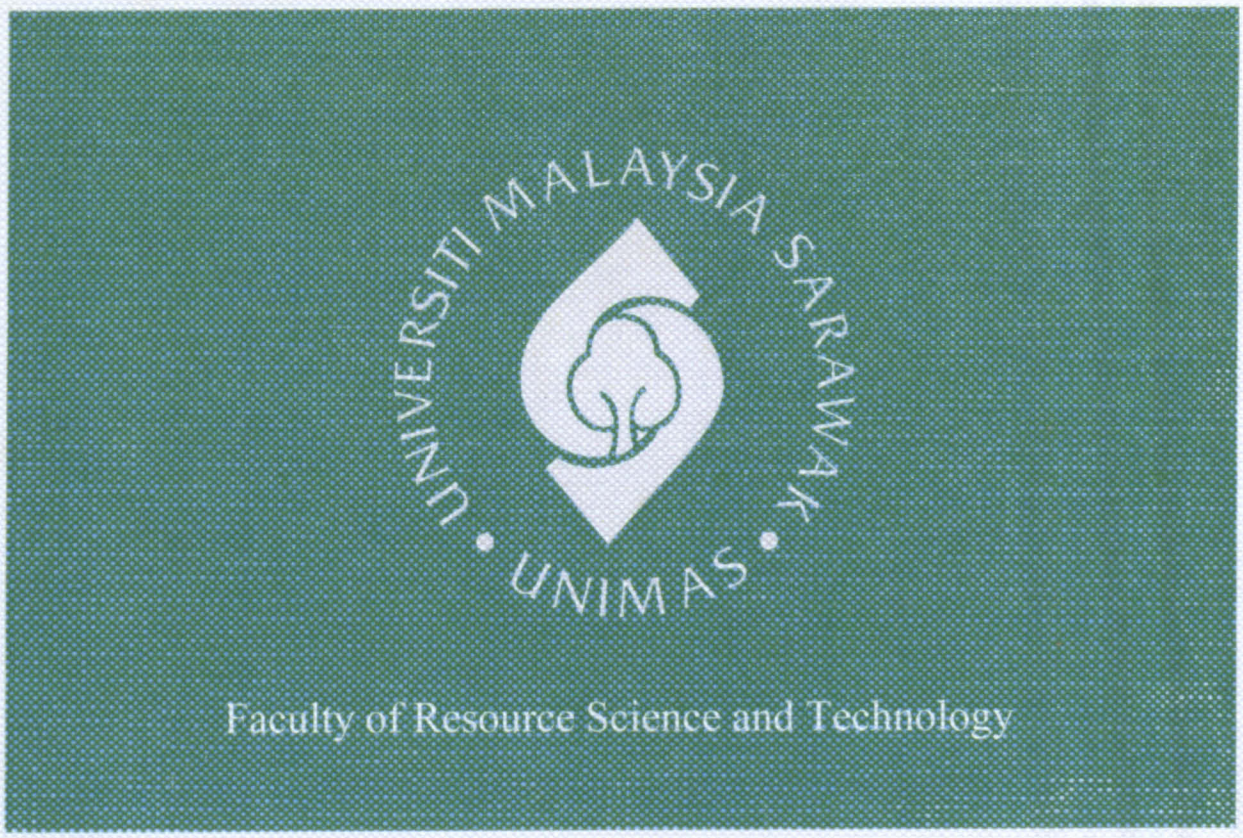

HANTAVIRUS SEROPREVALENCE AND ECTOPARASITE SURVEILLANCE OF RODENTS IN SELECTED FORESTED AREAS IN SARAWAK

Lee Wei Bin

(36648)

Bachelor of Science with Honours
(Animal Resource Science and Management)
2015

UNIVERSITI MALAYSIA SARAWAK

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Final Year Project Report ☑

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PhD ☐

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Hantavirus Seroprevalence and Ectoparasites Surveillance of Rodents in Selected Forested Areas in Sarawak

Lee Wei Bin

A dissertation submitted in partial fulfilment of the Final Year Project II (STF 3014) course

Supervisor: Dr. Chong Yee Ling

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Science and Animal Resource Management
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11 Jun 2015

Declaration

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university of institution of higher learning.

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Hantavirus Seroprevalence and Ectoparasites Surveillance of Rodents in Selected Forested Areas in Sarawak

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ABSTRACT

Hantavirus is a rodent-borne virus that categorised under the family *Bunyaviridae*. The research on hantavirus was only initiated after the Korean War in 1950s and is only being intensively studied in a few countries such as Korea and China. However, the study on hantavirus among rodent population in Malaysia is still minimal. A total of 50 rodents comprising five genus and nine species from five localities, namely, Kampung Sebayor, Kampung Serian Ulu, Samunsam Wildlife Sanctuary, Samajaya Nature Reserve, and Santubong National Park were examined. *Sundamys muelleri* was the predominant rodent species examined. Seven species rodents were found to be infested with at least one of the seven species of ectoparasite recovered. The following ectoparasites was successfully isolated and known as important vectors for the transmission of zoonotic diseases: *Ixodes granulatus, Haemaphysalis* sp. 1, *Laelaps sedlaceki, L. aingworthae, L. echidninus, Laelaps* sp.1 and *Hoplopleura sp.* The total prevalence of ectoparasites infestation rate were 38.00%, 12.00%, 64.00%, 14.00%, 4%.00, 8.00%, and 2.00% for *I. granulatus, Haemaphysalis* sp. 1, *L. sedlaceki, L. echidninus, L. aingworthae, Laelaps* sp. 1, and *Hoplopleura* sp. 1 respectively. The results of Simpson's Index of Diversity (1-D) were 0.2786, 0, 0,3324, 0.4624, and 0.1690 for Kampung Sebayor, Kampung Serian Ulu, Samunsam Wildlife Sanctuary, Samajaya Nature Reserve, and Santubong National Park respectively. The comparison of species diversity for ectoparasites on rodents of five localities was computed by using diversity t-test and the result shown to be significant. The result of Enzyme-linked Immunosorbent Assay (ELISA) test for prevalence of hanvavirus was shown to be negative out of 50 serum samples tested. As a conclusion, data from this research is important for enhancing the knowledge of hantavirus among resident in Malaysia and prevention measures should be taken.

Key: Zoonotic disease, prevalence, Simpson;s Index of Diversity (1 – D), Diversity t-test, ELISA

ABSTRAK

Hantavirus merupakan virus yang dibawa oleh tikus. Hantavirus dikategorikan bawa keluarga Bunyaviridae. Kajian tentang hantavirus hanya dimulakan selepas Peperangan Korean yang berlaku pada 1950-an. Hantavirus telah dikaji dengan intensif di Negara Korea dan China. Walaubagaimanapun, kajian selidik tentang hantavirus di Malaysia masih pada peringkat mula. Sebanyak 50 tikus yang terdiri daripada lima genus dan Sembilan spesies telah ditangkap dari lima lokaliti seperti Kampung Sebayor, Kampung Serian Ulu, Samunsam Wildlife Sanctuary, Samajaya Nature Reserve, dan Santubong National Park. Spesies tikus yang terbanyak ditangkap dan diuji ialah Sundamys muelleri. Ektoparasit berikut telah berjaya didapati dan merupakan vektor yang akan menyebarkan penyakit zoonotik: Ixodes granulatus, Haemaphysalis sp. 1, Laelaps sedlaceki, L. aingworthae, L. echidninus, Laelaps sp. 1 dan Hoplopleura sp.1. Kelaziman untuk kegerumuni ektoparasit ialah 38.00%, 12.00%, 64.00%, 14.00%, 4%.00, 8.00%, and 2.00% for I. granulatus, Haemaphysalis sp. 1, L. sedlaceki, L. echidninus, L. aingworthae, Laelaps sp. 1, and Hoplopleura sp. 1 respectively. Keputusan untuk Simpson's InDEX of Diversity (1 – D) were 0.2786, 0, 0,3324, 0.4624, and 0.1690 for Kampung Sebayor, Kampung Serian Ulu, Samunsam Wildlife Sanctuary, Samajaya Nature Reserve, and Santubong National Park respectively. Perhubungan antara kegeruminian ektoparasi bagi lima sampling lokasi adalah penting. Keputusan daripada ELISA telah menunjukkan kelaziman untuk hantavirus adalah negatif daripada 50 sampel darah. Konklusinya, data darpada kajian selidik ini adalah penting untuk meningkatkan ilmu pengetahuan tentang hantavirus kepada seluruh masyarakat Malaysia supaya langkah-langkah berjaga dapat dilaksanakan.

Kata kunci: Penyakit zoonotik, kelaziman, Simpson's Index of Diversity (1 – D), Diversity t-test, ELISA

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List of Abbreviations

ANDV	Andes virus
DOBV	Dobrava virus
ELISA	Enzyme-linked Immunosorbent Assay
HFRS	Haemorrhagic fever with renal syndrome
HPS	Hantavirus pulmonary syndrome
HTNV	Hantaan virus
IgG	immunoglobulin G
NE	Nephropathia epidemica
PUUV	Puumala virus
SEOV	Seoul virus
SNV	Sin Nombre virus
THAIV	Thailand virus
TPVM	Thottapalyam virus
TULV	Tula virus
PAST	Paleontological STatistics
HB	Head and body length
T	Tail length
E	Ear length
HF	Hind foot length
WT	Weight

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1.0 Introduction

Hantavirus contains several groups of RNA viruses which is categorised under family *Bunyaviridae*. Hantavirus is mostly carried by rodents and able to cause two severe respiratory infections such as Hantavirus pulmonary syndrome (HPS) and hemorrhagic fever with renal syndrome (HFRS) which are transmitted to human via aerosols or direct contact with infected rodents (Zhengqiang *et al.*, 2013). For more information, HFRS affects approximately 200,000 people each year, predominantly in Asia. Although Hantavirus were recognized in Asia, research only initiated after the Korean war in 1950s, in which thousands of United Nation (UN) soldiers were affected by HFRS (Lam *et al.*, 2001).

The genus *Hantavirus* can be grouped into two main groups, which are Old World and New World Hantaviruses. Hantaviruses such as Amur virus, Seoul virus and Hantaan virus (HTNV) are the example of Old World Hantaviruses. On top of that, they are the most important species, with lethality rates up to 15% in Asia. Other examples of Hantaviruses that reside in Europe are Dobrava virus (DOBV), Tula virus (TULV), and Puumala virus (PUUV) (Muranyi *et al.*, 2005). The Thottapalyam virus (TPVM), which was isolated from insectivores *Suncus murinus* in 1960, was the only known species which is indigenous to India. Furthermore, Puumala virus (PUUV) is said to be responsible for the Nephropathia epidemica (NE), which is a milder variant of HFRS, with mortality rates of 0.1%. On the other hand, in early 1990s, the first pathogenic New World Hantavirus or Sin Nombre virus was discovered in the Four Corners region of United States. As the time goes on, numerous pathogenic New World Hantaviruses were identified and characterized.

Pârasites are an essential component of a healthy ecosystem and are known for their role in shaping the community and population structure of hosts. The distribution of parasites among a host population is often characterised by heterogeneity with a small number of

hosts harbouring the majority of parasites (Altize *et al.*, 2003). This situation is caused by host as well as parasite-related properties and environmental factors which will make the host more susceptible to parasites (Wilson *et al.*, 2001). The host-related factor often shows a sex-biased pattern. Normally, male harbours more parasites than females. This is because larger hosts are able to sustain larger parasite populations as they represent larger resources. This statement was supported by Moore and Wilson (2002) & Krasnov *et al.* (2005), since in most mammal species, male has a larger size compared to female. Thus, they will have higher parasite loads. Besides, testosterone are said to be another factor that contribute to higher parasite load in male vertebrates (Klein, 2004). So, this research was carried out to study the seroprevalence of hantavirus and the species diversity of ectoparasites on rodent around Sarawak. Also, we wish to know the medical implication of hantavirus as well as ectoparasites towards residents in Sarawak.

1.1 Research statement

- The research on Hantavirus was only initiated after the Korean War in 1950s and is only being intensively studied in a few countries such as Korea and China. However, the study on Hantavirus among rodent population in Malaysia is still minimal. Penang, Perlis, and port Klang were positive for HTNV when tested by immunofluorescence (Lim *et al.*, 1985). The result shows that 17 out of 252 (6.7%) of rodent samples in Penang and 14 out of 87 (15.91%) of rodent samples in port Klang was infected by hantavirus respectively, but there is still no record of hantavirus infection in rodent population around Sarawak.
- Ectoparasites can cause diseases or act as vector transmitting parasitic zoonoses. Ectoparasites can be categorized into five main groups, Mesostigmate (mites), Prostigmate (chiggers), Acarina (ticks), Phthiraptera (louse) and Siphonaptera (fleas). The common ectoparasites identified in Peninsular Malaysia were *Laelaps* nuttali, *L.* echidninus, *L. sculpturata, Ixodes granulatus* and *Haemaphysalis* sp (Paramasvaren *et al.*, 2009). However, there is limited data on the ectoparasites and their medical importance around Sarawak.

1.2 Objectives

- To study the seroprevalence of Hantavirus in rodents population around Western Sarawak
- To study the prevalence of ectoparasites in rodents from several selected forested areas around Western Sarawak
- To compare the species diversity of rodent ectoparasites among five different sampling sites.

2.0 Literature Review

2.1 Hantavirus

Hantavirus is a type of rodent- and insectivore- borne RNA viruses. It is categorised under genus *Hantavirus* which comprise of the virus family *Bunyaviridae* (Muranyi *et al.*, 2005). There are five genera in *Bunyaviridae* family, which are *Orthobunyavirus, Nairovirus, Phlebovirus, Tospovirus* and *Hantavirus*. Hantavirus is single-stranded, negative sensed RNA viruses with genome consists of three segments named S (small), M (medium), and L (large) that are code for nucleocapsid protein (NP), surface envelope glycoprotein G1 and G2, and the RNA-dependent RNA polymerase, respectively (Muranyi *et al.*, 2005). Several species are known to cause human diseases within Europe. They are Dobrava virus (DOBV), Puumala virus (PUUV), Tula virus (TULV), Saaremaa virus (SAAV) and Seoul (SEOV) viruses. On top of that, Andes (ANDV) and Sin Nombre (SNV) are the predominant Hantavirus species which responsible for serious human disease (Vaheri, 2008). A recent study that had been carried out by Pattamadilok *et al.* (2007) reported that Thailand virus (THAIV) is responsible for HFRS cases in Thailand.

2.1.1 Hantavirus Viral Transmission and Infection

2.1.1.1 Viral transmission between rodents

Transmission of Hantavirus between rodents' population can happen via several ways. According to Calisher *et al.* (2006), intraspecific transmission of Hantavirus occurs through contact with saliva during aggressive encounters such as biting and fighting. Intramuscular injection is a more effective way in transmitting Hantavirus than aerosolized route. In natural rodents' population such as Norway rats, deer mice and bank voles, body mass and physiological maturity is predicted to be another reason of being infected by their associated Hantavirus. This is because the aggressive behaviour such as biting and fighting

is associated with sexual maturity. So it may increase the likelihood of exposure to Hantavirus (Childs *et al.*, 1988). On top of that, hormonal changes associated with puberty may increase the susceptibility to Hantavirus infection. Also, according to Child *et al.* (1988), male has higher rate of getting infected by Hantavirus. This is due to higher testosterone in male that will reduce natural killing cell in mice and reduce the expression of macrophage and monocyte, making it easier to be infected (Garcia-Gomez *et al.*, 2012). Besides, according to a research done recently, ectoparasites such as gamasid mites and trombiculid are naturally infected with Hantaan virus and the infected mites can transmit virus to rodent through biting (Yu, & Tesh, 2014). However, Yu and Tesh (2014) believed further research is needed to confirm the mite-Hantavirus association.

2.1.1.2 Viral transmission to humans

Transmission of Hantavirus to human can happen via inhalation of aerosolised rodent excreta, particularly urine. On top of that, direct contact of virus contaminated materials with mucous membrane or broken skin and a bite from the infected rodents is also a successful way of transmitting the virus (Lee & Van der Groen, 1988). In addition, staying together in a room with active infected rodents may pose the greatest risk for human infection as well.

2.1.2 Host of Hantavirus

The main host of Hantavirus is rodents. Hantaviruses live inside rodent without harming their host while continue their life cycle by multiplying and shedding in the rodent's excreta. Serological evidence of Hantavirus infection was shown in dogs and pigs as well. Nevertheless, it is not clear that these species are actually infected or just a spill over hosts which is another term for secondary host (Hart, & Bennett, 1999). So, each Hantavirus has a single predominant natural reservoir and has their own murid rodents' host. Interestingly,

the phylogenetic interrelationships among viruses and their predominant host show remarkable concordance (Vapalhati *et al.*, 2003). Hantavirus has been identified in subfamilies of rodents. They are found mostly on multiple species of subfamilies Murinae, Arvicolinae, Sigmodontinae, and only in a single species for the order Soricomorpha (*Suncus murinus*). Murinae is the largest number of Old World rodents and are the reservoirs for HNTV, DOBV, SAAV, SEOV, and Amur virus. Besides that, reservoirs for PUUV are Arvicolinae which is responsible to the mild HFRS in Europe and Prospect Hill virus in United States. Furthermore, Sigmodontinae are the largest group of New World rats and are the hosts of SNV and other New World viruses.

2.1.3 Global Prevalence of Hantavirus Infections

Hantavirus infections are distributed all around the world, causing substantial cases of human diseases, making it a global public health problem. Figure 1 shows the geographic distribution of HPS and HFRS worldwide. Bi *et al.* (2008) reported that there are about 150,000 to 200,000 patients with HFRS are subjected to treatment each year through the world. In America, approximately 200 cases of HPS are reported annually. Even though the number of HPS cases reported is significantly smaller than that of HFRS, the average case fatality is still as high as 40% (Lednicky, 2003).

There is a strong evidence of hantaviral infections in Israeli, Kuwait, Laos, Malaysia, Philippines, Vietnam and the China Hong Kong Special Administrative Region via serological investigation (Bi *et al.*, 2008). Other than that, hantaviral infection of rodents in Cambodia was also had been distinguished, however there has been no report of human infections (Reynes *et al.*, 2003).

In Korea, study on seroprevalence of Hantaan virus (HTNV) in rodents was conducted in 18 cities from year 2005 to 2007. Another research was initiated during year 2008 to 2010

on three islands and four mountains (Lim *et al.*, 2012). In the sampling collection, most of the rodents captures was *Apodemus agrarius* which compromise of 80.1% of the total captured species in all trapping sites. The result shows that the overall prevalence of HTNV antibodies was 26% (162 out of 629). They concluded that the seropositive individuals were more recurrent in cities (32.2%, n=410) than on islands (14.0%, n=57) or mountains (13.6%, n= 162) (Lim *et al.*, 2012). The prevalence of HTNV antibodies was higher in males (29.2%, *n*=250) than in females (22.3%, *n*=305). In this research, they concluded that HTNV is extensively circulated throughout South Korea (Lim *et al.*, 2012). So, intensive research on hantavirus in Malaysia has to be carry out to raise the awareness of resident in Malaysia about the medical importance of hantavirus.

In Thailand, two out of 53 (3.8%) *Rattus norvegicus* trapped in Nakhon Pathom Province were seropositive for Hantavirus antibody. In Singapore, a serological research was conducted on the laboratory white rats antibodies to Hantavirus, resulting 143 out of 329 (44%) of the rats were seropositive by the indirect immunofluorescent antibody test. However, none of the rats had hantaviral antigens in lung tissues (Wong *et al.*, 1988). So, it was suspected that hantavirus might spread to Sarawak as well.

In Malaysia, rodent populations around port Penang, Perlis and port Klang were positive for HTNV when tested by immunofluorescence (Lim *et al.*, 1985). The result shows that 17 out of 252 (6.7%) and 14 out of 87 (15.91%) of rodent samples was infected by Hantavirus respectively. While in Kelantan, three samples out of 119 human serum samples were tested positive for Hantavirus (Lam *et al.*, 2001). Nevertheless, no research on hantavirus was done in Sarawak.

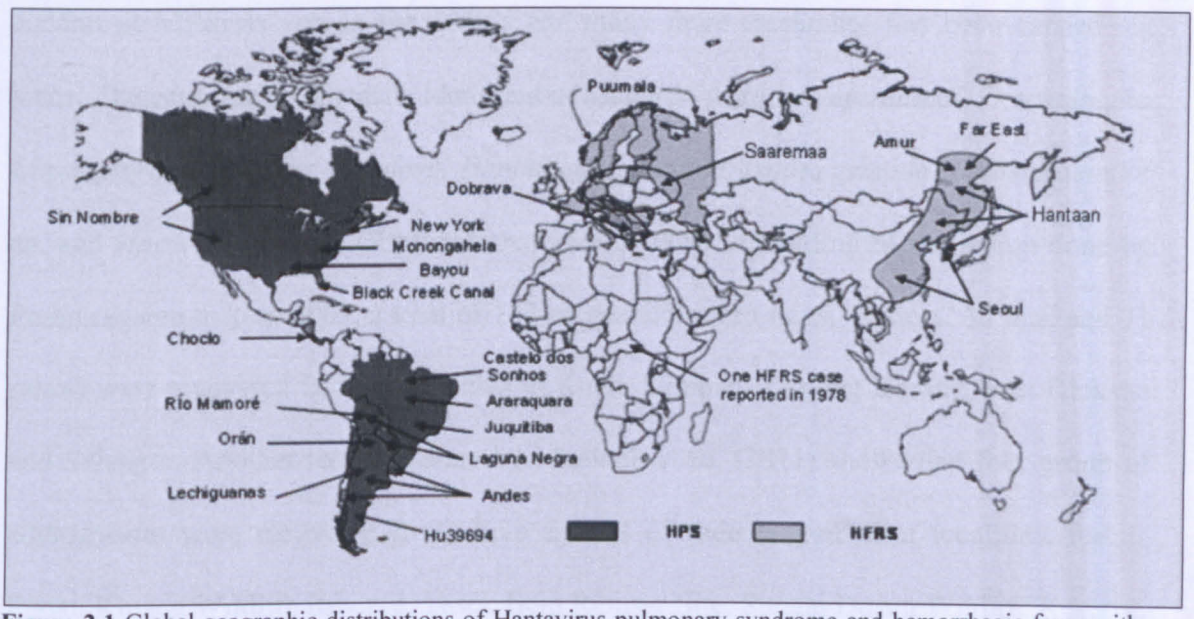

Figure 2.1 Global geographic distributions of Hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome. **Source: Bi, Formenty & Roth (2008)**

2.2 Ectoparasites

Parasites are known as organisms that feed on other living things. Usually, parasites get their nutrients from the host until the host is weakening, and, in some cases, death. After that, they will return to natural and start to find another host. The relationship between parasites and host are known as parasitism. Ectoparasites are a diverse and highly adapted group of animals that infest the external body surface of vertebrates (Hanafi-bojd *et al.*, 2007). Ectoparasites are arthropods that either cause diseases or act as vector transmitting parasitic zoonoses such as babesiosis (Singleton *et al.*, 2003), plague and others. Ticks are well-known vectors of various pathogenic organisms, such as protozoa, rickettsiae, bacteria and viruses. These organisms can cause serious and life-threatening illnesses in humans and animals (Chul-Min *et al.*, 2006). Ectoparasites recovered from rodents are categorized into five main groups, Mesostigmate (mites), Prositgmata (chiggers), Acarina (ticks), Phthiraptera (louse) and Siphonaptera (fleas). Many researches have been conducted around the globe to study the prevalence of parasitic infection in the wild rat population. This included Taiwan, USA and Egypt. The first record of parasitic infection in

rodents at Malaysia was in the 1930's and many more researches had been carried out since. The common ectoparasite identified in Malaysia were *Laelaps nuttali*, *L. echidnanus*, *L. sculpturata*, *Polyplax spinulosa*, *Hoplopleura pacifica*, *Ixodes granulatus*, *Dermacontor* sp. and *Haemophysalis* sp (Paramasvaren *et al.*, 2009). According to a research done by Paramasvaren in year 2009, a total of 147 ectoparasites (15 ticks, 31 fleas, 30 lice, and 71 mites) were recovered from the rodents in Kuala Lumpur, Tanjung Karang, Port Dickson and Selangor. Another research done by Madinah *et al.* (2011) shows that four group of ectoparasites were recovered from three species of rodents from four localities, that is Endau Kluang Wildlife Reserve, Tasek Bera Ramsar Site, Sungai Dusun Wildlife Reserve, and Lata Bujang Krau Wildlife Reserve. The ectoparasites retrieved were three species of ticks, seven species of mesostigmatid mites, three species of chiggers (larval trombiculid mites) and one species of listrophorid mites. As a whole, both hantavirus and ectoparasites are able to transmit deadly diseases to human. Thus, this explains the importance of this research was to learn the medical implication of both hantavirus and ectoparasites towards Malaysian.

3.0 Research Methodology

3.1 Sampling Sites

All sampling activities were conducted under the permission of Sarawak Forest Department. These were several forested areas around Western Sarawak that had been chosen as my sampling sites. They were Betong Serian Ulu (1°24′0″N 111°31′0″E) Santubong National Park (01°44′N 110°20′E), Samajaya Nature Reserve (1°31′21″N 110°23′28″E), Kampung Sebayor (1°28′30″N 110°26′12″E), and Samunsam Wildlife Sanctuary (1°55′0″N 109°36′0″E). Kampung Serian Ulu, Betong is a division located between three main rivers, Batang Lupar, Batang Saribas and Batang Kelaka. The covering area of Betong is approximately 4,180 km^2. Betong is divided into three broad topographical regions, which is coastal region, midland region and the inland region. The coastal region is mainly made up of peat swamp. The inland region is mountainous with steep terrain. The Santubong Peninsula covers approximately 38 km^2 and is located 25 km north of Kuching city. The mountainous part of the Santubong Peninsula is covered with mixed dipterocarp rainforest. Mangrove swamp and palm swamp areas are situated in the southern areas and along the Sarawak River. Kampung Sebayor is a village situated in Kota Samarahan area. The village is surrounded by mangrove swamp. The forest that was selected around Kampung Sebayor is of secondary forest that has been modified from a rubber tree farm. However, the chief village of Kampung Sebayor told us that the rubber tree farm has been abandoned long time ago. Samunsam Wildlife Sanctuary is the oldest sanctuary in Sarawak. Samunsam was made to protect the habitat of proboscis monkeys that live there. The wildlife sanctuary is on a flat, undulating terrain with no more than 25 meters above sea level, with the Samunsam River flowing through it. The vegetation comprises mangrove and lowland rainforest. The lowland rainforest can be further classified as riverine rainforest, tropical health forest or kerangas, and mixed dipterocarp

forest. The Samajaya Nature Reserve covers an area of approximately 38 hectares. Samajaya situated within the suburb of Tabuan Jaya. It consists mostly of karangas forest.

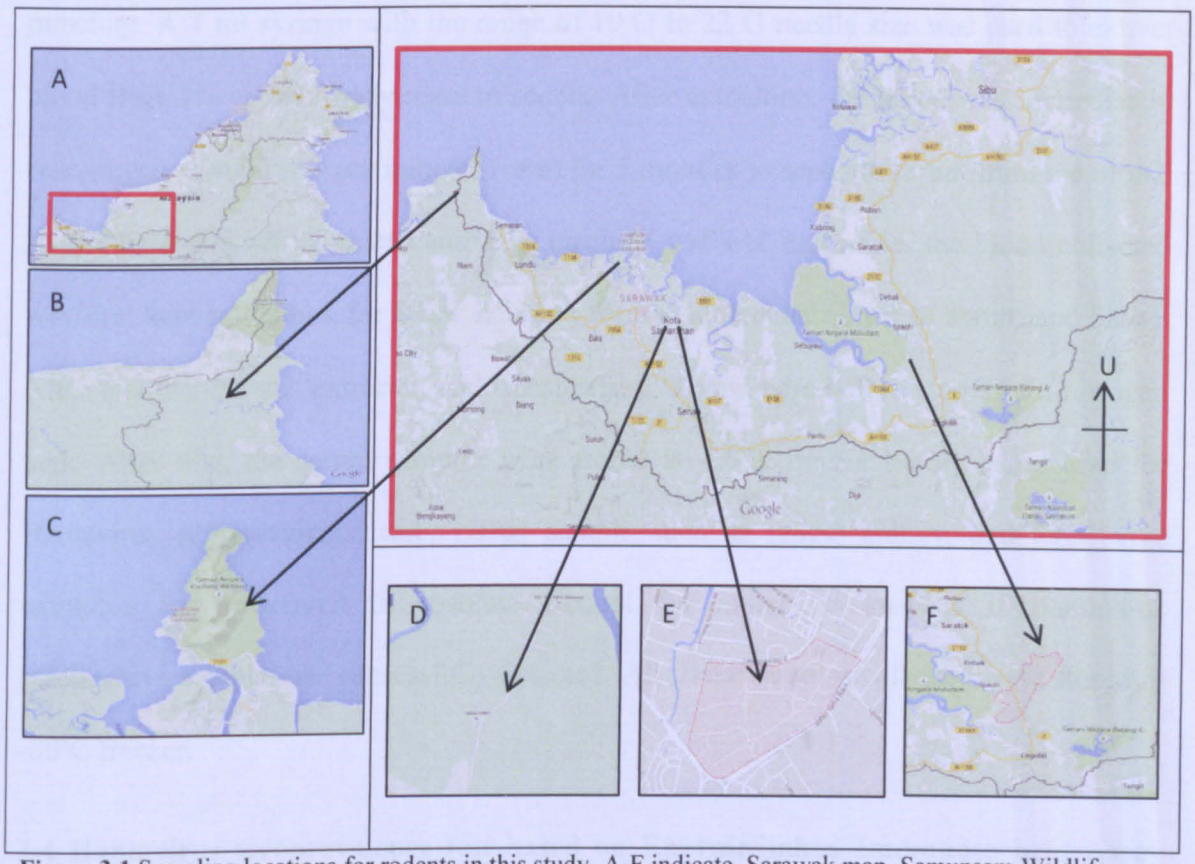

Figure 3.1 Sampling locations for rodents in this study. A-F indicate, Sarawak map, Samunsam Wildlife Sanctuary, Santubong National Park, Kampung Sebayor, Samajaya Forest Park and, Kampung Serian Ulu, Betong respectively (Source: Google Map, 2015). Red box indicate the enlarged version of Western Sarawak map.

3.2 Rodent Collection

A total of 50 to 100 cage traps baited with various strong flavoured baits such as banana, pineapple and salted fish were used to capture rodents. Baited cage traps were checked every five to six hours. Captured rodents were anaesthetised in a plastic bag using cotton wool soaked in chloroform. After that, morphometric measurements of the head-body (HB), tail (T), ear (E), hind foot (HF) and weight (WT) were recorded. Each captured rodents' sex and species was determined as well by using keys and illustrations developed by Payne *et al.* (1985) by measuring morphological measurements and observing physical appearances such as colour of fur and structure of tail.