CHAPTER I

INTRODUCTION

BACKGROUND AND RATIONALE

Gibbons (*Hylobates spp.*) are small arboreal apes found throughout the tropical rainforests of South and Southeast Asia, which includes Thailand, Indonesia and Malaysia. The family Hylobatidae is represented in Thailand by three species, *Hylobates lar (H. lar), H. pileatus* and *H. agilis*. The white-handed gibbons (*H. lar)* and the pileated gibbons (*H. pileatus*), the two main gibbon species in Thailand, are declining in number due to habitat loss and poaching [1,2]. Gibbons are categorized as protected wild animals in Thailand, as they are at high risk of extinction due to the increase in illegal hunting. The Wildlife Fund of Thailand obtained information from interviews with poachers, which indicated that approximately 1,600 to 2,000 gibbons die in the illegal pet market each year [2].

Within the last few years, hundreds of confiscated and abandoned gibbons have been handed over to the authorities of the Royal Forest Department (RFD). It is estimated that a total number of around 2,500 gibbons are kept in captivity in Thailand [2]. One strategy for re-establishing a viable wild gibbon population is the reintroduction of selected captive gibbons to a depleted protected area. Before a reintroduction and restocking program can be implemented, special attention must be paid to the issue of infectious diseases, which may interfere with the health and reproduction of the wild gibbon population and may constitute a public health risk to humans. Therefore, it is not only important to prevent disease spread within the population of captive gibbons, but also to be aware of the potential for zoonotic diseases spread between captive gibbons and humans, as well as other wild primates. Among the human viruses tested, gibbon sera cross-reacted with human hepatitis B virus (HBV) antigens[3]. A survey of captive gibbons revealed that approximately 50% of the animals were positive for at least one marker of HBV infection[4-5].

HBV is a small, double-shelled virus in the Family *Hepadnaviridae*. HBV contains partially double stranded DNA of approximately 3,200 bases, which represents numerous antigenic components, including hepatits B surface antigen (HBsAg), hepatitis B core antigen (HBcAg) and hepatitis B early antigen (HBeAg)[6]. Infection with HBV produces manifestations ranging from asymptomatic to persistent infections, which may lead to chronic liver disease and hepatocellular carcinoma. The World Health Organization estimated the number of HBV carriers would be about 400 million by the year 2000. The high mortality of HBV infection, approximately one million deaths per year, indicates the extent of the global health problem posed by this virus[7].

The HBV genome contains four overlapping open reading frames including polymerase (*P*), surface (*S*), core (*C*) and *X* genes. The *P* gene encodes a polymerase enzyme with both reverse transcriptase and DNA polymerase activity. The viral envelope protein consists of three components : major, middle and large proteins translated from the *PreS1, PreS2* and *S* genes, respectively. The *C* gene represents the nucleocapsid that encloses the viral DNA, while the *X* gene encodes two proteins that serve as transcription transactivators, which aid in viral replication. The *S* and *C* genes have upstream regions termed *PreS* and *PreC* [6].

The earliest event of HBV infection is attachment and entry steps of virions into host cells. Following receptor binding, virions deliver their nucleocapsids to the cytoplasm and these then translocate to the nucleus. Genomic DNA is matured to the covalently closed circular DNA (cccDNA) form which represent the replication of HBV[8]. Study of the viral surface proteins showed that this region plays an important role in viral entry and selected specific host infections. Most of the work has been done on the viral envelope components that may be involved in viral-host cell interaction. PreS protein participation in cellular receptor binding was confirmed by the study of antipeptide antibodies to PreS1 which block adherence of HBV particles to HepG2 cells [9] and the binding of HBV virions to human liver plasma membrane fraction is blocked by monoclonal antibodies to the HBV PreS1 domain [10]. Unfortunately, neither experiment is able to relate binding to productive infection. Until now, no established cell line supports HBV infection. Only primary human hepatocytes can be successfully infected with HBV *in vitro*[11,12] eventhough these cells are very difficult to obtain.

HBV is also found in several other species of nonhuman primates, for example chimpanzee (*Pan troglodytes*)[13], gorilla *(Gorilla gorilla)*[14] and orangutan (*Pongo pygmaeus*) [15]. There are three main modes of transmission : through a mixing of blood products, through sexual contact and perinatal routes from mother to fetus [16]. Although everyone is at risk for HBV contact, risk is increased based on certain behaviors. Due to the close contact between humans and captive animals, the cross transmission of HBV between humans and gibbons should be considered. Experimental transmission of human viruses to gibbon has been reported [17], although there is no evidence of gibbon HBV transmission to humans. However, the fact that both viruses can replicate in chimpanzees after subcutaneous inoculation may indicate the ability of viral cross transmission in various species. Comparison of the surface protein structure and *in vitro* human hepatocyte HBV infection experiments may predict for cross-species viral transmission.

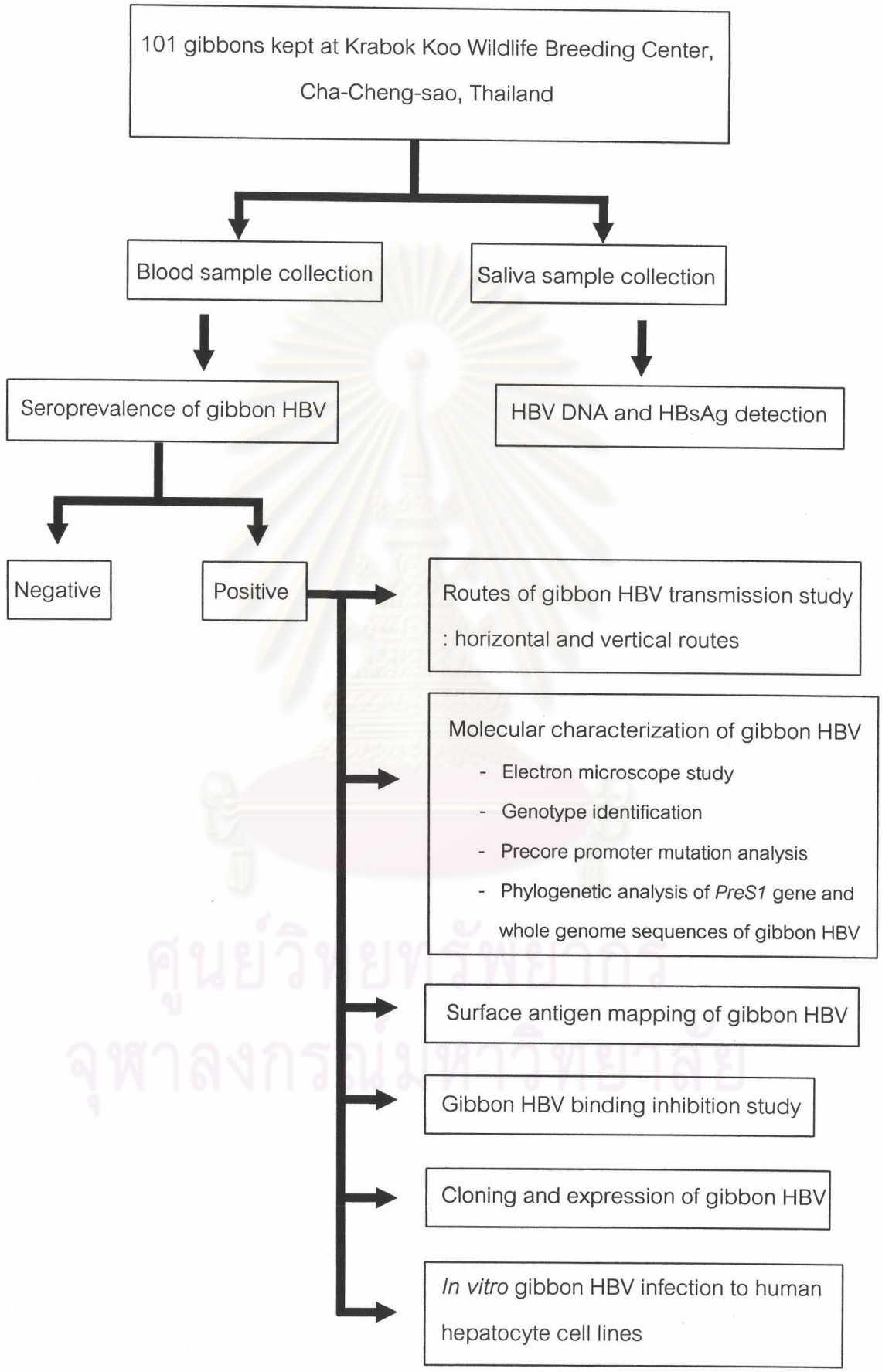
Hypothesis

Due to the ability of both gibbon and human HBV to infect a chimpanzee host, it is expected that the viral characteristics and surface antigens of both viruses, especially the viral binding regions PreS1, should form similar structure and support the cross transmission of both species. Nucleotide sequence analysis and monoclonal antibody mapping studies of the surface protein may identify similarity in conformational structure of both species HBV particles by present the same pattern of monoclonal antibodies binding and indicate the same structure of viral binding region to host cells. Infection of human hepatocyte cells with gibbon HBV may explain the cross-species infectivity of HBV.

Objectives

1. To study general informative of gibbon HBV in detail with respect to seroprevalence, routes of transmission, molecular characterization and to confirm the phylogenetic relationship of non-human primate and human HBV.
2. To study the surface protein structure of gibbon and human HBV by monoclonal antibody mapping.
3. To study the infectivity of gibbon HBV in human hepatocyte cell culture.

Conceptual Framework



Assumption

All animals including in this study are captive gibbons kept at Krabok Koo Wildlife Breeding Center. Historical data and their origin cannot define. Other factors such as bacterial infection in gibbon population were excluded from our analysis. All steps of sample collection were done during the routine check up under permission of the Royal Forest Department's officers.

Limitation

To avoid unnecessary handling or restraint of gibbons by anesthetic agent, the gibbon blood and saliva collection was performed as a part of routine health care program. A small volume of each sample was collected. The follow up blood sampling, repeated collecting and sample taking of young animals were not allowed.

Operational Definition

Hepatitis B virus carrier is defined as HBsAg and HBV DNA positivity, HBV DNA represented the infectious particles circulating in blood. *In vitro* HBV infection of human hepatocyte cell lines will be accepted only when the cccDNA is detected in infected samples.

Expected Benefit

1. Mastering the general information of gibbon HBV comparative with human viruses and measuring prevalence and possible transmission routes of gibbon HBV in Thailand.
2. Studying the surface protein structure of HBV by monoclonal antibody mapping and possible prediction of the viral binding epitope, which plays an important role in cross-species transmission.
3. Development of a model for testing cross transmission *in vitro* of HBV infection in various hosts by human hepatocyte cells.

Research Methodology

1. Sample collection

   Gibbon samples : 101 captive animals at Krabok Koo Wildlife Breeding Center, Cha-Cheng-sao, Thailand.

   Human samples : 34 animal keepers at Krabok Koo Wildlife Breeding Center, Cha-Cheng-sao, Thailand.

   Human positive samples for surface antigen mapping study and inhibition binding HBsAg assay were keep in serum bank at the Viral Hepatitis Research Unit, Chulalongkorn University, and Department of Virology, University Hospital Rotterdam.

2. Process of study
   - blood and saliva collection
   - seroprevalence and blood chemistry test
   - route of gibbon HBV transmission
   - molecular characterization of gibbon HBV
   - surface antigen mapping of gibbon HBV
   - gibbon HBV inhibition binding HBsAg study
   - cloning and expression of gibbon HBV surface protein
   - *in vitro* gibbon HBV infection of human hepatocyte cell lines

3. Data collection and analysis

   Descriptive data analysis was used to calculate the percentage of gibbon HBV serological study comparing with HBV DNA detection and S antigen monoclonal antibodies mapping study.

   To analyse the correlation of ALT level, amount of white and red cells between normal and HBV infected gibbons, T- test analysis and chi square test were performed, respectively. Anova two-way test was used as to study the antigen mapping of gibbon and human HBV.

   Phylogenetic analysis was performed by the PHYLIP package, version 3.57c (J. Felsenstein, Department of Genetics, University of Washington).