การจำแนกในระดับโมเลกุล วิวัฒนาการ และการศึกษาการติดเชื้อข้ามสายพันธุ์ในหนูตัดต่อพันธุกรรมที่มีการเจริญของเซลล์ตับจากมนุษย์ของไวรัสตับอักเสบ บี ในชะนีและอุรังอุตัง

นางสาว ภัทรธิดา สงวนหมู่

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาชีวเวชศาสตร์ (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

MOLECULAR CHARACTERIZATION, EVOLUTION AND CROSS-SPECIES TRANSMISSION STUDY IN SEVERE COMBINED IMMUNODEFICIENCY TRANSGENIC MICE WITH HUMAN HEPATOCYTES OF GIBBON AND ORANGUTAN HEPATITIS B VIRUS

Miss Pattaratida Sa-nguanmoo

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biomedical Sciences
(Interdisciplinary Program)
Graduate School
Chulalongkorn University
Academic Year 2009

Thesis Title	MOLECULAR CHARACTERIZATION, EVOLUTION AND CROSS-SPECIES TRANSMISSION STUDY IN SEVERE COMBINED IMMUNODEFICIENCY TRANSGENIC MICE WITH HUMAN HEPATOCYTES OF GIBBON AND ORANGUTAN HEPATITIS B VIRUS
By	Miss Pattaratida Sa-nguanmoo
Field of Study	Biomedical Sciences
Thesis Advisor	Professor Yong Poovorawan, M.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

..... Dean of the Graduate School
(Associate Professor Pornpote Piumsomboon,Ph.D.)

THESIS COMMITTEE

..... Chairman
(Associate Professor Kanisak Oraveerakul, D.V.M. Ph.D.)

..... Thesis Advisor
(Professor Yong Poovorawan, M.D.)

..... Examiner
(Assistant Professor Piyawat Komolmitr, M.D. Ph.D.)

..... Examiner
(Associate Professor Pisit Tangkijvanich, M.D.)

..... External Examiner
(Associate Professor Teeraporn Chinchai, Ph.D.)

ภัทรธิดา สงวนหมู่ : การจำแนกในระดับโมเลกุล วิวัฒนาการ และการศึกษาการติดเชื้อข้ามสายพันธุ์ในหนูตัดต่อพันธุกรรมที่มีการเจริญของเซลล์ตับจากมนุษย์ของไวรัสตับอักเสบ บี ในชะนีและอุรังอุตัง. (MOLECULAR CHARACTERIZATION, EVOLUTION AND CROSS-SPECIES TRANSMISSION STUDY IN SEVERE COMBINED IMMUNODEFICIENCY TRANSGENIC MICE WITH HUMAN HEPATOCYTES OF GIBBON AND ORANGUTAN HEPATITIS B VIRUS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ศาสตราจารย์ นายแพทย์ ยง ภู่วรวรรณ, 155 หน้า.

ไวรัสตับอักเสบ บี นอกจากพบในมนุษย์แล้วยังสามารถพบได้ในสัตว์จำพวก non-human primate ด้วย เนื่องจากจีโนมมีความใกล้เคียงกับไวรัสในมนุษย์ มีการศึกษาพบว่าไวรัสตับอักเสบ บี จากมนุษย์ สามารถถ่ายทอดไปสู่ non–human primate ได้ อย่างไรก็ตามยังไม่มีรายงานการติดต่อข้ามของไวรัสตับอักเสบ บีจากสัตว์ไปสู่มนุษย์ การศึกษาครั้งนี้ได้ทำการตรวจสอบ HBsAg anti-HBs และ anti-HBc ใน non–human primate จำนวน 104 ตัว รวม 12 สปีชีส์ นอกจากนี้ได้ตรวจสอบดีเอ็นเอ หาลำดับนิวคลีโอไทด์ทั้งหมด วิเคราะห์ phylogenetic tree วิวัฒนาการ และทดสอบการติดเชื้อข้ามสายพันธุ์ในหนูตัดต่อพันธุกรรมที่มีการเจริญของเซลล์ตับจากมนุษย์ จากการตรวจสอบพบชะนี 5 ใน 25 ตัวและอุรังอุตัง 7 ใน 54 ตัวเป็นพาหะของไวรัสตับอักเสบ บี ผลการศึกษา phylogenetic tree แสดงให้เห็นว่าไวรัสตับอักเสบ บี จากชะนีและอุรังอุตังจับกลุ่มกันในสปีชีส์ของตนเอง การศึกษาวิวัฒนาการพบว่าไวรัสตับอักเสบ บี ใน non-human primate มีการเพิ่มขึ้นของ population growth rate อย่างรวดเร็วในราวปี คศ. 1750 – 1850 จากการวิเคราะห์พบว่าไวรัสตับอักเสบ บี ในชะนีเกิดขึ้นก่อน ไวรัสตับอักเสบ บี ในอุรังอุตัง และมี evolutionary rate ประมาณ 10^{-4} subs/site/year การทดสอบการติดเชื้อข้ามสายพันธุ์พบว่าไวรัสตับอักเสบ บี จาก อุรังอุตังและชะนีสามารถเพิ่มจำนวนในหนูทดลองที่มีเซลล์ตับของมนุษย์ได้ ผลการศึกษาบ่งบอกทางอ้อมว่าไวรัสตับอักเสบ บี จากสัตว์จำพวก non-human primate สามารถถ่ายทอดไปสู่มนุษย์ได้

สาขาวิชา ชีวเวชศาสตร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

4989684020 : MAJOR BIOMEDICAL SCIENCES

KEYWORDS : HBV / NON-HUMAN PRIMATE / SEROPREVALENCE / PHYLOGENETIC ANALYSIS / CROSS-SPECIES TRANSMISSION

PATTARATIDA SA-NGUANMOO : MOLECULAR CHARACTERIZATION, EVOLUTION AND CROSS-SPECIES TRANSMISSION STUDY IN SEVERE COMBINED IMMUNODEFICIENCY TRANSGENIC MICE WITH HUMAN HEPATOCYTES OF GIBBON AND ORANGUTAN HEPATITIS B VIRUS. THESIS ADVISOR : PROF. YONG POOVORAWAN, M.D., 155 pp.

Hepatitis B virus does not only exclusively infect humans, but also can be found in non-human primates. The genome organization of non-human primate HBV is nearly identical to that human HBV. There are the data on cross-species transmission of human HBVs to the non-human primates. However, a cross-species transmission of HBVs from non-human primates to human has not been yet been elucidated. One hundred and four non-human primates comprising 12 species were subjected to screen for the serological HBsAg, anti-HBs and anti-HBc markers. Subsequently, HBV DNA detection, whole genome characterization, phylogenetic analysis and evolution study were evaluated and analyzed. The cross-species transmission study in severe combined immunodeficiency (SCID) mice with human hepatocytes was performed. HBV infection was detected in gibbon (5/25) and orangutan (7/54). Phylogenetic analysis was performed and analyzed. The gibbon and orangutan viruses clustered within their respective groups. Evolutionary study revealed that the population growth rate of non-human primate HBV started a rapid increase in the effective population size around year 1750 to 1850. GiHBV was occurred before OuHBV and has evolutionary rate approximately 10^{-4} subs/site/year. OuHBV and GiHBV can replicate in chimeric SCID mice with human hepatocytes. The results showed indirect evidence of cross-species transmission of non-human primate to human could be occurred.

Field of Study : Biomedical Sciences Student's Signature Pattaratida Sa-nguanmoo

Academic Year : 2009 Advisor's Signature Yong Poo

ACKNOWLEDGEMENTS

Fist and foremost, I would like to thank my advisor, Prof. Yong Poovorawan. Throughout all my endeavors, your support, guidance, and endless patience have been truly inspirational – “thank you” will never suffice. I also very grateful to Prof. Masashi Mizokami and Prof. Yasuhito Tanaka for their scientific advice and knowledge and many insightful discussion and suggestion. They are my resource for getting my science questions answered. The completion of this dissertation would not have been possible without the supervisory committees: Assoc. Prof. Dr. Kanisak Oraveerakul, Assist. Prof. Dr. Piyawat komolmit, Assoc. Prof. Pisit Tangkijvanich and Assoc. Prof. Dr. Teeraporn Chinchai. Thank you for being such marvellous teachers throughout the years, both in and outside the classroom. I would like to thank Dr. Parntep Ratanakorn, Dr.Suwanna Noppornpanth, Dr. Angkana Sommanustweechai, Ms. Piraya Phakdeeviroj, Ms. Sudarat Baicharoen, the veterinarians and the staffs of the Dusit zoo and Khao Pratubchang Wildlife Breeding Center for plasma and taking care of all those animals.

I also thank Ms. Nutchanart Thawornsuk, Ms. Kesmanee Prianantathavorn, Ms. Preeyaporn Vichaiwattana, Ms. Pitchaya Hacharoen and all staff of the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University for their assistants. I will forever to thankful to Ms. Srunthron Akkarathamrongsin, Ms. Ponsiri Pitchakarn, Mr. Sahut Rajmuengkhwang, Ms. Kanako Tatematsu, Dr. Fuat M. Kurbanov, Mr. Anis Khan, Dr. Shuko Murakami, Dr. Masaya Sugiyama, Dr. Kentaro Matsuura, Dr. Noboru Shinkai, Ms. Hatsue Naganuma, Kusakabe-sensei, Ms. Kyoko Akita, Tsukisaka-san and the member of Department of Virology & Liver unit, Nagoya City University.

Thank you to both the Chulalongkorn University Graduate Scholarship to Commemorate the 72nd Aniversary of His Majesty King Bhumibol Adulyadej and an exchange program fellowship under the program “Strategic Scholarships for Frontier Research Network” of Thailand’s Commission on Higher Education whose funding allowed portions of this dissertation. Finally, many thanks to Dr.Naruemol Jirapanakorn for editing the final draft of my thesis.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF ABBREVIATIONS

aa	=	Amino acid
ADV	=	Adefovir
Ala	=	Alanine
Alb	=	Albumin
ALT	=	Alanine aminotransferase
anti-HBc	=	Anitbodies to HBcAg
anti-HBe	=	Antibodies to HBeAg
anti-HBs	=	Antibodies to HBsAg
Arg	=	Arginine
ASHV	=	Arctic ground squirrel hepatitis virus
Asn	=	Asparagine
AST	=	Aspartate transminase
Au	=	Australia antigen
bp	=	Base pair
C	=	Celsius
cccDNA	=	Covalently closed circular DNA
CeHV-1	=	Cercopithecine herpesvirus 1
ChHBV	=	Chimpanzee hepatitis B virus
DHBV	=	Duck hepatitis B virus
DMSO	=	Dimethyl sulfoxide
DNA	=	Deoxyribonucleic acid
DR	=	Direct repeat
EDTA	=	Ethylenediaminetetraacetic acid
ELISA	=	Enzyme-linked immunosorbent assay
ER	=	Endoplasmic reticulum
ETV	=	Entecavir
ge	=	Genome equivalent
GiHBV	=	Gibbon hepatitis B virus

LIST OF ABBREVIATIONS (continued)

Gln	=	Glutamine
Gly	=	Glycine
GoHBV	=	Gorilla hepatitis B virus
GSHV	=	Ground squirrel hepatitis virus
GTHBV	=	Grey teal hepatitis B virus
HBc	=	Hepatitis B core
HBcAg	=	Hepatitis B core antigen
HBe	=	Hepatitis B e
HBeAg	=	Hepatitis B e antigen
HBIG	=	Hepatitis B immune globulin
HBsAg	=	Hepatitis B surface antigen
HBVs	=	Hepatitis B virus(es)
HBx	=	Hepatitis B x
HCC	=	Hepatocellular carcinoma
HCV	=	Hepatitis C virus
HHBV	=	Heron hepatitis B virus
HPD	=	Highest posterior density
hr	=	Hour
IFN	=	Interferon
Ile	=	Isoleucine
IU	=	International unit
IV	=	Intra-venous
kb	=	Kilo base pairs
l	=	Litre
LdT	=	Telbivudine
Leu	=	Leucine
LHBs	=	Large S protein
LMV	=	Lamivudine

LIST OF ABBREVIATIONS (continued)

Lys	=	Lysine
MDHBV	=	Maned duck hepatitis B virus
MHBs	=	Middle S protein
MHR	=	Major hydrophilic region
min	=	Minute
mIU	=	Milli-international unit
mM	=	Milli molar
NA	=	Nucleotide/nucleoside analogues
nt	=	Nucleotide
ORF	=	Open reading frame
OuHBV	=	Orangutan hepatitis B virus
P	=	Polymerase
PCR	=	Polymerase chain reaction
PEG	=	Polyethyleneglycol
Pro	=	Proline
RIA	=	Radioimmunoassay
RNA	=	Ribonucleic acid
rpm	=	Round per minute
RSGV	=	Ross's goose hepatitis B virus
s	=	Second
SCID	=	Severe combined immunodeficiency
SD	=	Standard deviation
SDS	=	Sodium dodecyl sulfate
sec	=	Second
Ser	=	Serine
SFV	=	Simian foamy virus
SGHBV	=	Snow goose hepatitis B virus

LIST OF ABBREVIATIONS (continued)

SH	=	Serum-hepatitis-related antigen
SHBs	=	Small S protein
SIV	=	Simian immunodeficiency virus
STHBV	=	Stork hepatitis B virus
TDF	=	Tenofovir
Thr	=	Threonine
Trp	=	Tryptophan
uPA	=	Urokinase-type plasminogen activator
UV	=	Ultraviolet
Val	=	Valine
ver	=	Version
vs	=	Versus
WHV	=	Woodchuck hepatitis virus
WMHBV	=	Woolly monkey hepatitis B virus
µl	=	Microlitre
µM	=	Micromolar

CHAPTER I

INTRODUCTION

BACKGROUND AND RATIONALE

Human hepatitis B virus (HBV) is the prototype member of the family *Hepadnaviridae*. It is a spherical enveloped particle containing partially double stranded DNA and RNA dependent DNA polymerase. The majority of infections by this diminutive viral genome affect humans. Hence, various research projects have been aimed at accumulation information on human hepatitis B. In nature, HBV has been found in non-human primate species such as chimpanzees (*Pan troglodytes*) (ChHBV) [1], orangutans (*Pongo pygmaeus*) (OuHBV) [2], wild and captive gibbons (*Hylobates* sp. and *Nomascus* sp.) (GiHBV) [3], gorillas (*Gorilla gorilla*) (GoHBV) [4] and woolly monkeys (*Lagothrix lagothricha*) (WMHBV) [5]. However, information on epidemiology, genome and pathogenicity of non-human primate hepatitis B virus has remained rather limited and mainly been gleaned from captive animals. According to Deinhardt's survey (1976), hepatitis B surface antigen (HBsAg) has been found in chimpanzees, gibbons and orangutans, whereas marmosets (*Callithrix jacchus*), squirrel monkeys (*Saimiri* sp.), baboons (*Papio* sp.) rhesus macaques (*Macaca mulatta*) and vervet monkeys (*Cercopithecus aethiops*) apparently are devoid of both HBsAg and anti-HBs [6]. Up to now, there have been several studies on serological markers of HBV infection in *Cercopithecidae* monkeys [7, 8]. However, all studies showed negative results for serological HBV markers and no attempt at HBV amplification has been successful in this family [8].

Southeast Asia is an area endemic for HBV infection. Several studies have undertaken serological surveys on the families *Pongidae* and *Hylobatidae* to determine epidemiology, phylogenetic relationships and route of cross-species transmission. For examples, Warren et al. have examined 195 orangutans from Borneo and Sumatra [2]. Grethe et al. have investigated 12 gibbons from different parts of Thailand and one gibbon from Vietnam [4]. Noppornpanth et al. have performed studies on 101 captive gibbon from central Thailand [3]. Sall et al. have investigate the

population of pileated gibbon and yellow-cheeked gibbon in the northern and south-western regions of Cambodia and east of the Mekong river [9]. From all these studies, a high prevalence (40-46%) of HBV infection in gibbons and orangutans in this region has become evident.

Upon characterization of the respective nucleotide sequences of human hepatitis B virus was divided into distinct genetic groups. Accordingly, Okamoto et al. differentiated HBV into four genetic groups or genotypes (A, B, C, and D) based on nucleotide differences between sequences of 8% or above [10]. Subsequently, four additional genotypes of HBV (E, F, G and H) have been identified [11-16]. In 2008, a new genotype of human HBV has been proposed (HBV-I) [17-18]. However, some researchers regard this HBV-I genotpes as a new recombinant virus rather than a novel genotype [19]. Recently, a novel genotype J was discovered from a Japanese patient with hepatocellular carcinoma [20]. Genetic characterization is advantageous in that it reveals the relationship among these sequences as well as to the known primate HBV sequences of non-human primates are on a genetic branch separate from other known human HBV sequences [12, 13, 21-23]. Interestingly, based on a comparison of the entire genome, genotype J was the nearest to the gibbon and orangutan HBV [20]. The nucleotide identify for woolly monkey hepatitis B virus (WMHBV) and non-human primate HBVs is 78% and 90%, respectively, in comparison with the human HBV genome [24].

The first outbreak of hepatitis infection occurred in the 1960s among chimpanzee handlers in USA [25]. Three years later, the captive chimpanzees showed symptoms related to the human viral hepatitis [26]. In 1970, sixteen people who had been in contact with 2 imported chimpanzees from Sierra Leone suffered from viral hepatitis infection [27]. Based on these facts, several researchers successfully used chimpanzee as an animal model for researches on human HBV by inoculating the chimpanzees with human sera, saliva, or semen collected from the HBV-infected people [28-32]. Similar experiments have also been performed in other non-human primates such as gibbons [33, 34]. These data confirmed the fact that human HBVs can infect several non-human primates.

Moreover, previous studies showed that non-human primate HBVs can transmit to other non-human primate species. Despite the previous hypothesis of species-specific HBV infection, a geographical basis rather than species association accounting for the distribution of HBV variants has been increasingly recognized. The inoculation of GiHBV to a chimpanzee resulted in the acute hepatitis infection. The virus isolated from the infected chimpanzee was GiHBV too [35]. It seems to be that non-human primate HBVs can transmit to the other non-human primate species. For instance, HBV in orangutans consistently grouped within the gibbon clade in Southeast Asia and similarly, a gorilla sequence (AJ131567) clustered with chimpanzee sequences in central Africa. Lack of strict species-specificity of HBV variants was also reported for a chimpanzee sequence (AJ131575) grouped with a gibbon cluster, and another chimpanzee sequence (AB032431) grouped with human HBV genotype E [8, 9]. These observations support probable interspecies transmission which could be explained by sharing a common habitat in geographic regions with high prevalence of HBV infection, such as Southeast Asia and central Africa. Thus, the more the regions both species inhabit overlap, the higher the probability of cross-species transmission [8,9].

The genome organization of non-human primate HBVs is nearly identical to that of human HBVs. Because of this close similarity, the question of cross-transmission of HBV between species has arisen. There are many data on cross-transmission of human HBVs to the non-human primates. However, a cross-transmission of HBVs from non-human primates to humans has not been reported yet. Using more advanced diagnostic methods, the non-human primates have increasingly been identified as a reservoir of several viruses such as lymphocryptoviruses, Cercopithecine herpesvirus 1 (CeHV-1), Simian immunodeficiency virus (SIV), Simian foamy virus (SFV), and HBVs. Thus veterinarians, zookeepers, or people in close contact with non-human primates may potentially become infected with those virus causing severe diseases. Enhanced awareness of prevalence, genetic relatedness, evolution and cross-species transmission study of non-human primate HBVs will help prevent further spread and cross-transmission of these viruses between humans and non-human primates.

HYPOTHESIS

Due to the fact that physical genome organization of human and non-human primate HBVs is similar and several research projects have demonstrated that human HBVs can be transmitted to non-human primates. It is expected that non-human primate HBVs can also transmit to human. Cross-species transmission study in severe combined immunodeficiency mice with human hepatocytes may explain the cross-species infectivity of non-human primate HBVs.

OBJECTIVES

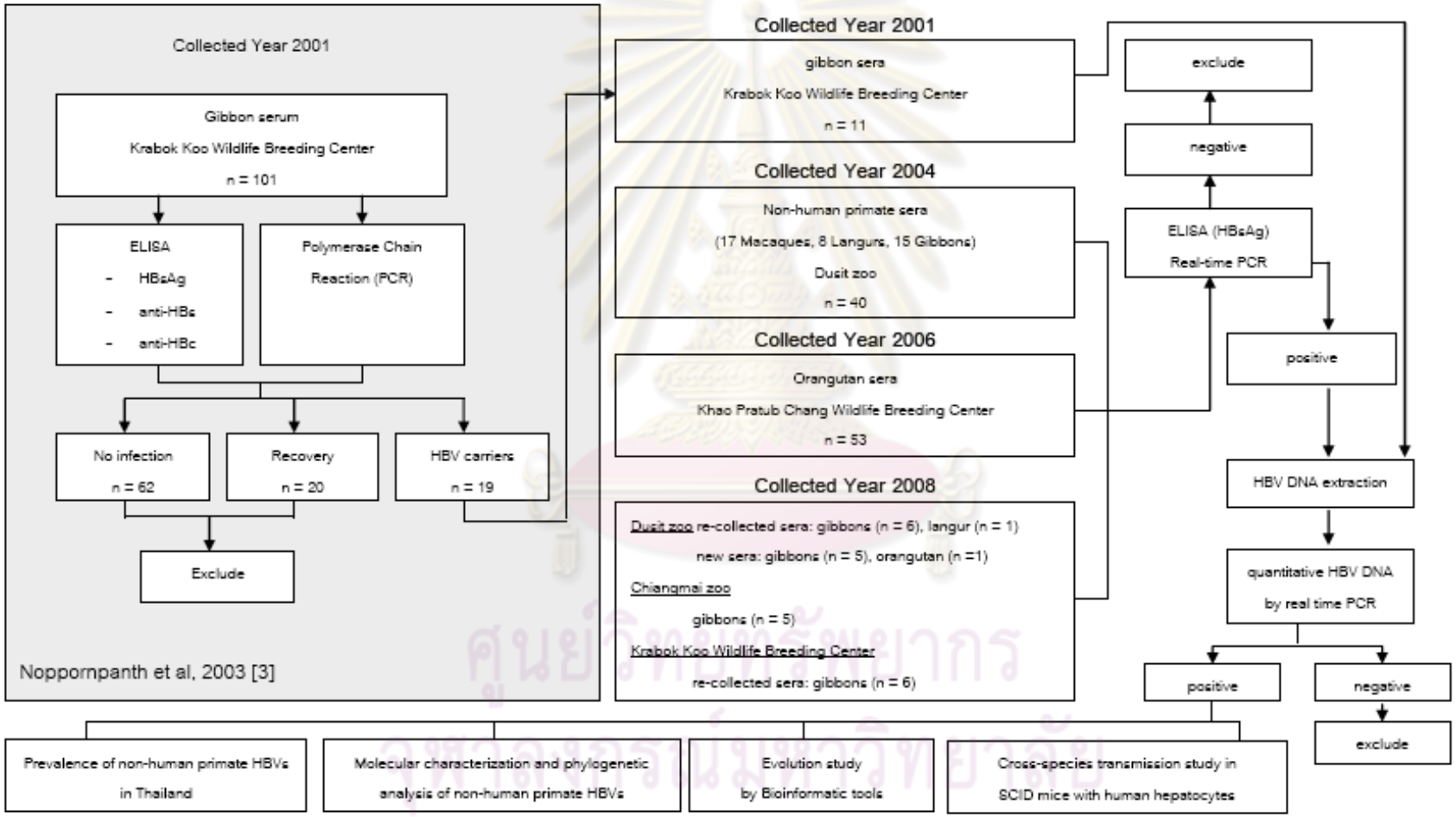
1. To elucidate epidemiology, pathogenicity and potential reservoirs of HBV infection in non-human primates.

2. To sequence the entire genomes of HBVs isolated from the carriers and perform phylogenetic analysis on both viral isolates to investigate their genetic relatedness to the previously identified human and non-human primate strains of HBVs.

3. To contribute the knowledge and information of evolution of non-human primate HBVs.

4. To study the potential of cross-species transmission of non-human primate HBV in severe combined immunodeficiency transgenic mice with human hepatocytes.

CONCEPTUAL FRAMEWORK



ASSUMPTION

All animals including in this study care captive non-human primates kept at Dusit zoo, Bangkok, Chiangmai Zoo, Chiangmai, the Khao Pratub Chang Wildlife Breeding Center, Ratchaburi and the Krabok Koo Wildlife Breeding Center, Cha Choeng Sao. All steps of sample collection were done during the routine check up under permission of each zoo. This research project had been approved by the Ethics Committee of Chulalongkorn University, Faculty of Medicine and the Faculty of Veterinary Science, Animal Care and Use Committee (FVS-ACUC), Mahidol University.

LIMITATION

To avoid unnecessary restraint of non-human primates by anesthetic drug, sera stored during previous studies and collected from routine health check were used. The volume of each sample was limited.

OPERATIONAL DEFINITION

Hepatitis B carrier is a term used to describe those non-human primates that have hepatitis B surface antigen (HBsAg) in the blood and HBV DNA is detected in the infectious particles circulating in blood circulation. *In vivo* HBV infection of severe combined immunodeficiency mice (SCID) with human hepatocytes will be accepted when HBV DNA is detected in infected mice sera.

EXPECTED BENEFIT

- Measuring prevalence of non-human primate HBV infection in Thailand.
- Generating the knowledge of the evolution of non-human primate HBV and the probability of cross-transmission from non-human primate HBV to human by using SCID mice model.

RESEARCH METHODOLOGY

1. Sample collection

Year 2001:

Krabok Koo Wildlife Breeding Center, Cha Choeng Sao (n = 11)

7 Pileated gibbons (*Hylobates Pileatus*)

4 White-handed gibbons (*Hylobates lar*)

Year 2004:

Dusit zoo, Bangkok (n = 40)

10 Long-tail macaques (*Macaca fascicularis*)

4 Southern pigtail macaques (*Macaca nemestrina*)

2 Stump-tailed macaques (*Macaca arctoides*)

1 Rhesus macaque (*Macaca mulatta*)

4 Silvered langurs (*Semnopithecus cristatus*)

1 Phayre's langur (*Semnopithecus phayrei*)

3 Dusky langur (*Semnopithecus obscurus*)

6 White-cheeked gibbons (*Nomascus leucogenys*)

1 Yellow-cheeked gibbon (*Nomascus gabriellae*)

6 Pileated gibbons (*Hylobates pileatus*)

2 White-handed gibbons (*Hylobates lar*)

Year 2006:

Khao Pratub Chang Wildlife Breeding Center, Ratchaburi (n = 53)

53 orangutans (*Pongo pygmaeus*)

Year 2008:

Re-collected sera (n = 13)

Dusit zoo (n =7)

1 Silvered langurs (*Semnopithecus cristatus*)

1 Pileated gibbon (*Hylobates pileatus*)

4 White-cheeked gibbons (*Nomascus leucogenys*)

1 Yellow-cheeked gibbon (*Nomascus gabriellae*)

Krabok Koo Wildlife Breeding Center, Cha Choeng Sao (n = 6)

2 Pileated gibbons (*Hylobates Pileatus*)

3 White-handed gibbons (*Hylobates lar*)

1 Black crested gibbons (*Nomascus concolor*)

New sera (n = 11)

Dusit zoo (n = 6)

5 White-cheeked gibbons (*Nomascus leucogenys*)

1 Orangutan (*Pongo pygmaeus*)

Chiangmai Zoo, Chiangmai (n = 5)

3 White-cheeked gibbon (*Nomascus leucogenys*)

2 Hybrid gibbons (*Nomascus leucogenys+Hylobates lar*)

2. Process of study

- Serum collection

- Screening methods: HBsAg test (ELISA), HBV DNA detection (real-time PCR)

- Molecular characterization and evolution study of non-human primate HBVs (Bioinformatic tools)

- Cross-species transmission study (SCID mice model)

3. Data analysis

- Percentage of non-human primate HBV carriers

- Nucleotide and amino acid analysis

Chromas LITE ver. 2.01

ClustalX multiple alignment program ver. 2

SEQUENCHER® DNA sequence assembly software

ExPaSy translation tool

- Phylogenetic analysis

Molecular Evoultionary Genetics Analysis software (MEGA) ver. 4

BEAST package ver. 1.5.4

Tracer program

FigTree program

CHAPTER II

REVIEW AND RELATED LITERATURES

1. HEPATITIS B VIRUS

1.1 Discovery Dr. Baruch S. Blumberg who discovered the hepatitis B virus was interested in the genetics of disease susceptibility (Fig. 1A). In early 1950's, he noted that inherited traits could make different groups of people more or less susceptible to the same disease. He and his colleagues decided to travel aroud the world to collect blood samples from the native populations in remote areas. He planned to study the genetic differences and the association with a disease. He focused on hemophiliac patients who had received multiple blood transfusions. He found that these patients from New York City would have been exposed to blood serum protein that they themselves had not inherited, but had been inherited by their donors. Dr. Blumberg (1965) decided to use antibodies from hemophilic patients to test all blood samples that he and his colleagues collected around the world. The match was found between antibody from a New York hemophiliac patient and antigen found in the blood sample of an Australian aborigine (Fig. 1B). This antigen was called the "Australia antigen" or Au [36, 37]. Prince et al. (1968) also found the identical antigen, term "serum-hepatitis-related antigen" or SH [38]. Further analysis revealed that Au and SH is the same [39] and the antigen later represented the hepatitis B surface antigen (HBsAg) [40, 41].

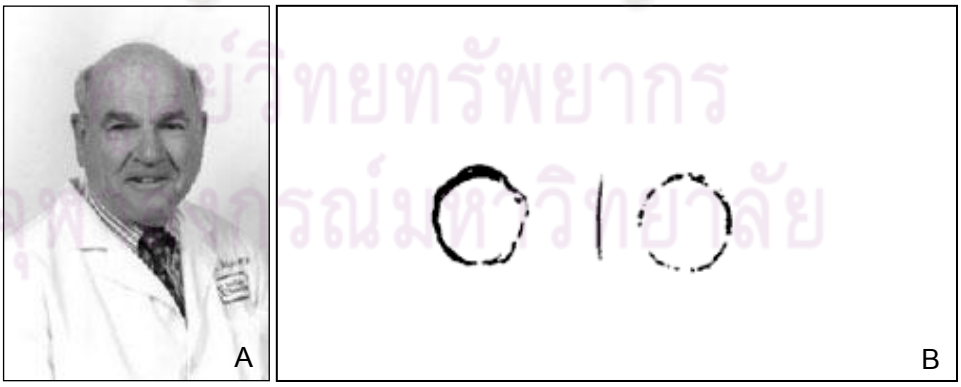


Fig. 1 Dr. Baruch Blumberg, M.D., Ph.D. Nobel laureate (A) [37] and precipitation between Australia antigen (HBsAg) of Australian aborigine serum and hemophiliac patient serum containing antibody against antigen (anti-HBs) (B) [36].

1.2 Taxonomy and host range of HBVs HBV is a partially double-stranded DNA virus with the smallest genome of approximately 3 kb [42, 43] that belongs to the family *Hepadnaviridae* [44]. HBV occurs in 3 forms: Dane particle [45], spherical form and filamentous form [46] (Fig. 2A). The Dane particles contain viral genome, while the other forms comprise only glycoproteins and host-derived lipid envelopes [41]. The Dane particle has a diameter of 42 nm and contains 3 proteins, the large (L), middle (M), and small (S) surface proteins in its outer layer. The inner layer has an icosahedral nucleocapsid of 22 nm in diameter contains viral genome [47] (Fig. 2B).

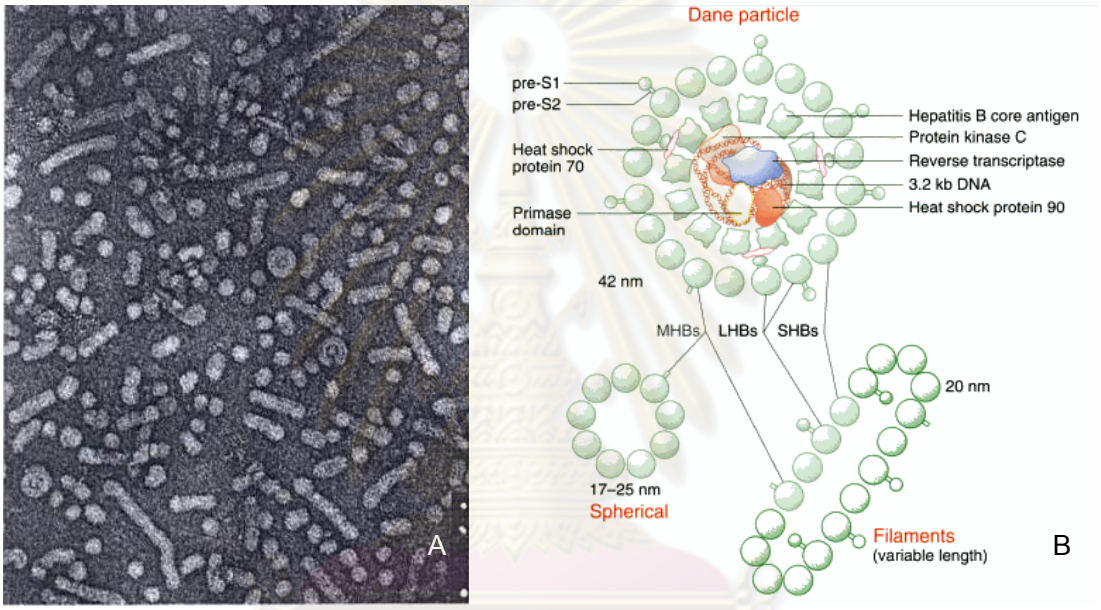


Fig. 2 Electron microscopic picture of HBV particles (magnification approximately 300,000x) (A) [46] and schematic diagram of Dane particle, spherical form and filamentous form (B) [48].

Hepadnaviruses are divided into two genera *Orthohepadnavirus* and *Avihepadnavirus* based on their specific host, mammals and birds, respectively [24] (Table 1). Orthohepadnaviruses can infect humans, apes, and rodents. Non-human primate HBVs infect gorillas (*Gorilla gorilla*) [4], chimpanzees (*Pan troglodytes*) [21, 23, 49-52], orangutans (*Pongo pygmaeus*) [2, 53], gibbons (*Hylobates* sp. and *Nomascus* sp.) [3, 4, 9, 54] and woolly monkeys (*Lagothrix lagothricha*) [5]. According to the phylogenetic analysis, the non-human primate HBVs, in particular Gibbon hepatitis B virus (GiHBV), Orangutan hepatitis B virus (OuHBV), Chimpanzee hepatitis B virus

(ChHBV), and Gorilla hepatitis B virus (GoHBV) are closely related to human HBVs (Fig. 3).

The geographic distribution of the non-human primate HBVs is shown in Fig.4 [1-5]. HBVs can also be found in New World rodents such as woodchucks (*Marmota monax*), ground squirrels (*Spermaphilus beecheyi*), and arctic ground squirrels (*Spermaophilus parryi*) infected by Woodchuck hepatitis virus (WHV), Ground squirrel hepatitis virus (GSHV), and Arctic ground squirrel hepatitis virus (ASHV), respectively [55-57]. The nucleotide identity for Woolly monkey hepatitis B virus (WMHBV), non-human primate HBVs, and rodent HBVs is 78%, 90%, and 54-70%, respectively, in comparison with the human HBV genome [24]. Avihepadnaviruses exclusively infect birds. HBV infection is very common among ducks and geese [58-63]. Other possible hosts are herons, storks, and cranes. The avihepadnaviruses share only 40% identity with the human HBV genome [24].

1.3 Subtype, genotype and sub-genotype of HBV in human In order to classify HBV isolates, the first classification was done by serotyping based on reactivity of HBsAg and the standard anti-sera [64]. For subtyping, the subtype specificities are located in the external loops of HBsAg (amino acids 110-180). The “a” determinant is a major immunogenic region (amino acids 124-147). Two major subtype epitopes are the *d/y* and *r/w* specificities. These specificities were depending on the amino acid residues 122 and 160 of HBsAg, respectively [65]. If amino acid residue 122 is Lys (K), the specificity is *d* and if this residue is Arg (R), then the specificity is *y* [66]. Similarly amino acid residue 122, if amino acid residue 160 is Lys (K), the specificity is *w* and if this residue Arg (R), then the specificity is *r* [67], The *w* specificity is not homogenous. Using the amino acid residues 127, the *w* specificity can be dividing into 4 groups: *w1*, *w2*, *w3*, and *w4* [68]. The *q* specificity was found in *adw*4 [69] and later found in *adr* subtype [70]. It is classified by amino acid residue 158 and 178 for *adw*4. In *adr* subtype, the classification is belonging to amino acid residue 159 and 177. Thus, subtypes of human HBVs were distinguised into nine types: *ayw*1, *ayw*2, *ayw*3, *ayw*4, *ayr*, *adw2*, *adw4q-*, *adrq+*, and *adrq-* [65]. Based on antigenic determinants of surface antigen, HBV subtypes have been classified in Table 2

Table 1 Host and their Hepadnaviruses.

	Hepadnavirues	
	Name	Host
Orthohepadnaviruses	Chimpanzee HBV (ChHBV)	Chimpanzees (*Pan troglodytes*) [1]
	Gorilla HBV (GoHBV)	Gorillas (*Gorilla gorilla*) [4]
	Gibbon HBV (GiHBV)	Gibbons(*Hylobates*sp., *Nomascus* sp.) [3, 54]
	Orangutan HBV (OuHBV)	Orangutans (*Pongo pygmaeus*) [2]
	Woolly monkey HBV(WMHBV)	Woolly monkeys (*Lagotrix lagotricha*) [5]
	Human HBV (HBV)	Humans (*Homo sapiens*) [45]
	Woodchuck HBV (WHV)	Woodchucks (*Marmota monax*) [55]
	Arctic ground squirrel HBV (ASHV)	Arctic ground squirrels (*Spermophilus parryi*) [56]
	California ground squirrel HBV (ASHV)	California ground squirrels (*Spermophilus beecheyi*) [57]
Avihepadnaviruses	Duck HBV (DHBV)	Ducks (*Anas domesticus*) [58]
	Ross's goose HBV (RSGV)	Ross's goose (*Anser rossi*)
	Snow goose HBV (SGHBV)	Snow goose (*Anser caerulescens*) [59]
	Grey teal HBV (GTHBV)	Grey teals (*Anas gibberifrons gracilis*) [60]
	Maned duck HBV (MDHBV)	Maned Ducks (Chenonetta jubata) [60]
	Heron HBV (HHBV)	Herons (*Adrea cinerea*) [61]
	Stork HBV (STHBV)	Storks (*Ciconia ciconia*) [62]

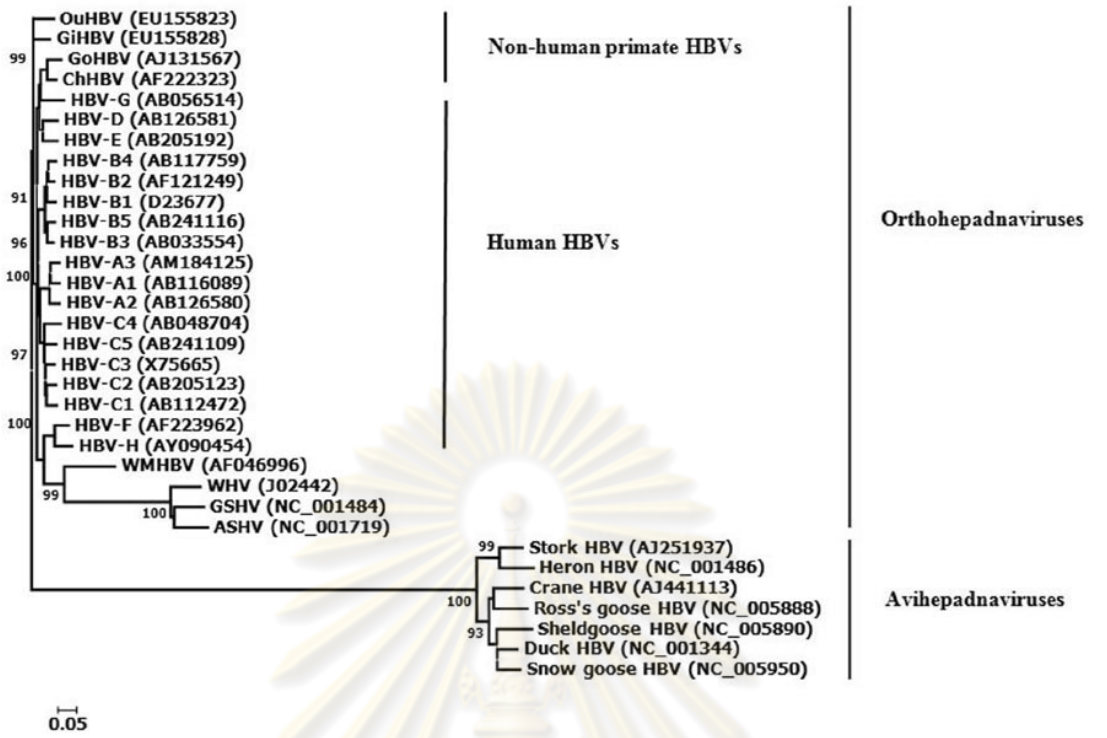


Fig. 3 Phylogenetic tree of various hepadnaviruses based on entire genome. Percentage bootstrap values (>75%) are shown at the respective nodes. The scale bar at the bottom indicate the genetic distance.

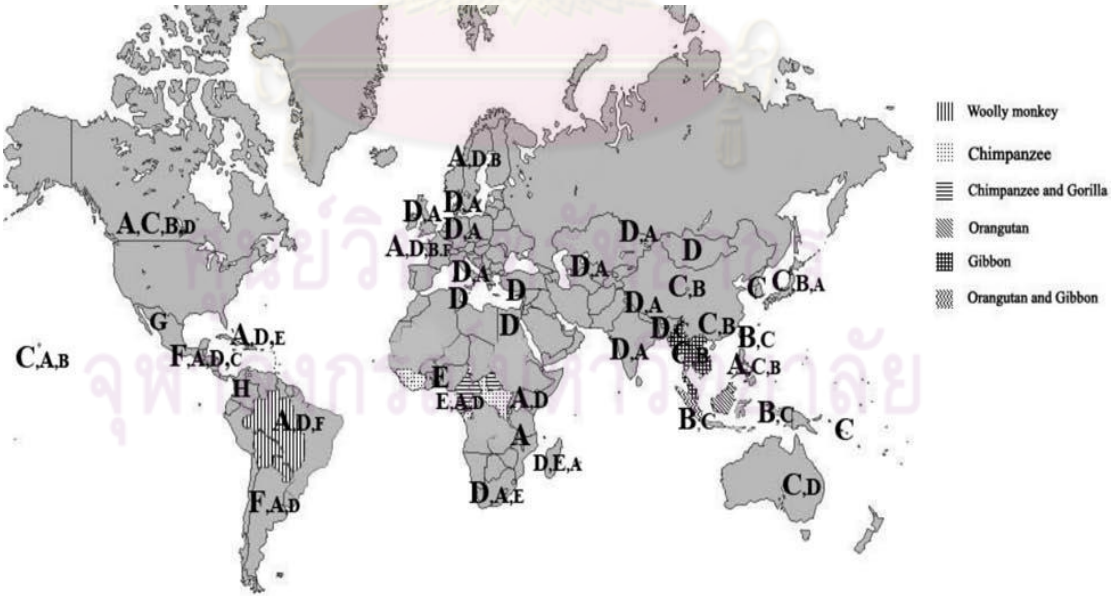


Fig. 4 Geographic distribution of non-human HBVs and their hosts. The size of letters represents the extent of prevalence of specific genotype of human HBV in the area.

Table 2 Subtype classification of HBV isolates [68. 70]

Position		Specificity	Position		Specificity	Position		Specificity	Position		Specificity
122	Lys (K)	*d*	160	Lys (K)	*w*						
						127	Pro (P)	*w1*/w2*			
							Thr (T)	*w3*			
							Leu/Ile (L/I)	*w4*			
									158/178	Leu (L)/Gln (Q)	*q-*
	Arg (R)	*y*		Arg (R)	*r*						
									159/177	Val (V)/Ala (A)	*q-*
										Ala (A)/Val (V)	*q+*

***** The expression of *w*1 specificity also required Arg122, Phe134, and/or Ala 159.

Human HBVs are divided into 8 genotypes (HBV-A to HBV-H) based on at least 8% divergence within the viral genome [71]. Each genotype can be further sub-divided based on at least 4% divergence of the viral genome into the following sub-genotypes: HBV-A1 to HBV-A5, HBV-B1 to HBV-B5, HBV-C1 to HBV-C5, HBV-D1 to HBV-D5 and HBV-F1 to HBV-F4 [24, 72]. Recently, a new genotype of HBV has been proposed (HBV-I) [17, 18]. However, some researchers regard this HBV-I genotype as a new recombinant virus rather than a novel genotype [19]. Interestingly, genotype J was discovered from a Japanese patient with hepatocellular carcinoma. Human HBV genotype J was closer to GiHBV and OuHBV than human strains. Moreover, this genotype has a deletion of 33 nucleotides at the start of *preS1* gene as same as discovered from ape strains [20]. The distribution of human HBV, subtype, genotype, sub-genotype and genome structure are shown in Table 3.

Table 3 Differences between HBV genotype, sub-genotype and geographic origin (adapted from Schaefer, 2007 [24], Norder et al., 1992 [68], and Norder et al., 2004 [70]).

Genotype	Subgenotype	Synonyms	Subtype	Genome length (bp)	ORF-differences	Geographic origin
A	A1	Aa, A'	*adw2, ayw1*	3221	Insertion of aa 153 and 154 in HBc	Africa, (Asia, South America)
	A2	Ae, A-A'	*adw2, ayw1*			Europe
	A3	Ac	N/A			Gabon, Cameroon
	(A4)		N/A			Mali
	(A5)		N/A			Nigeria
B	B1	Bj	*adw2*	3215		Japan

Table 3 (continued)

Genotype	Subgenotype	Synonyms	Subtype	Genome length (bp)	ORF-differences	Geographic origin
	B2	Ba	*adw2, adw3*			Asia without Japan
	B3		*adw2*			Indonesia, Philippines
	B4		*ayw1*			Vietnam
	B5		N/A			Philippines
C	C1	Cs	*adw2,adw3,adrq+,ayr*	3215		Southeast Asia
	C2	Ce	*adrq+,ayr*			Far East (Korea, Japan, Northern China)
	C3		*adrq,adw2, adrq+*			Micronesia
	C4		*ayw3*			Australia
	C5		N/A			Philippines, Vietnam
D	D1		*ayw2*	3182	Deletion of aa 1-11 in preS1	Mongolia, Belarus, Europe?
	D2		*adw3,ayw3, ayw4*			India?
	D3		*ayw3,ayw2*			South Africa, East India, Serbia
	D4		*ayw2*			Australia
	D5		N/A			East India

Table 3 (continued)

Genotype	Subgenotype	Synonyms	Subtype	Genome length (bp)	ORF-differences	Geographic origin
E			*ayw4*	3212	Deletion of aa 1-11 in preS1	West Africa
F	F1		*adw4, ayw4*	3215		South and central America
	F2		*adw,adw4, ayw4*			South America
	F3		N/A			Bolivia
	F4		N/A			Argentina
G			*adw2*	3248	Insertion of 12 aa in HBc	USA, Germany, Belgium
H			*adw4*	3215		Nicaragua
I			N/A	3215		Vietnam
J			N/A	3182	Deletion of aa 1-11 in preS1	Japan

N/A = data not available

1.4 Viral genes and their products The entire genome of HBV is a partially double-stranded DNA which complete genome is approximately 3.2 kb length and the shorter strand is approximately 1.7 kb [48]. The construction of HBV genome is an open circular structure which overlaps (approximately 240 nt) between the two DNA

strands to maintain the circular configuration of the DNA [73]. One of the DNA strands is incomplete [74]. The complete strand is named as minus strand defining 5' and 3' ends which are linked to the viral DNA polymerase [75]. In uncompleted strand, it is named as plus strand and defined 5' end but variable 3' end [76]. The genome contains two directly repeated sequences of DR1 and DR2 [77]. HBV genome comprises of 4 ORFs (ORF S, ORF P, ORF C, and ORF X) (Fig.5). ORF S is located in the ORF P, ORF C and X overlap partially with ORF P [78]. Some ORFs of virus encode more than one protein from one ORF by using the internal AUG start sites. ORF S encodes 3 protein: large S protein (*preS1/preS2/S*), middle S protein (*preS2/S*) and small S protein (S) [79]. ORF C encodes HBe and HBc protein [80]. ORF X encodes HBx protein [81] and may encode more than one protein [82]. Totally, 4 ORFs can encode at least 7 proteins.

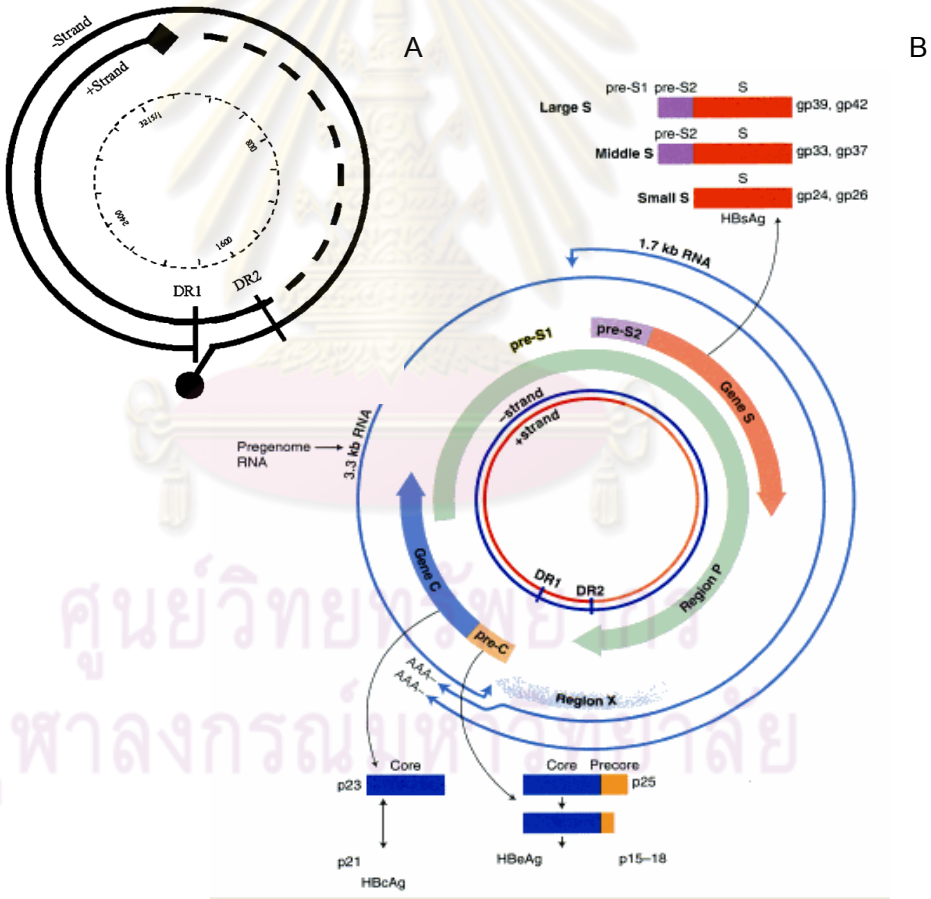


Fig. 5 Genome (A) [76] and protein (B) structure of HBV [48]. Solid circle, primaes; diamond, DNA polymerase.

The names of HBV proteins are listed below:

Small S protein (SHBs): It is the most abundant protein which known as HBsAg [41]. Sequence of SHBs starts at the third conserved AUG of ORF S and ends at the stop codon of ORF S. Mostly amino acids of SHBs are hydrophobic types (Fig. 6A). However, the region between aa 100 and 160 is the major hydrophilic region of MHR [83]. This region contains the subtype-specific region that reacts with antibodies (Fig. 6B). The variation at aa 144 and 145 is the most presistent in the vaccine escape patients [78].

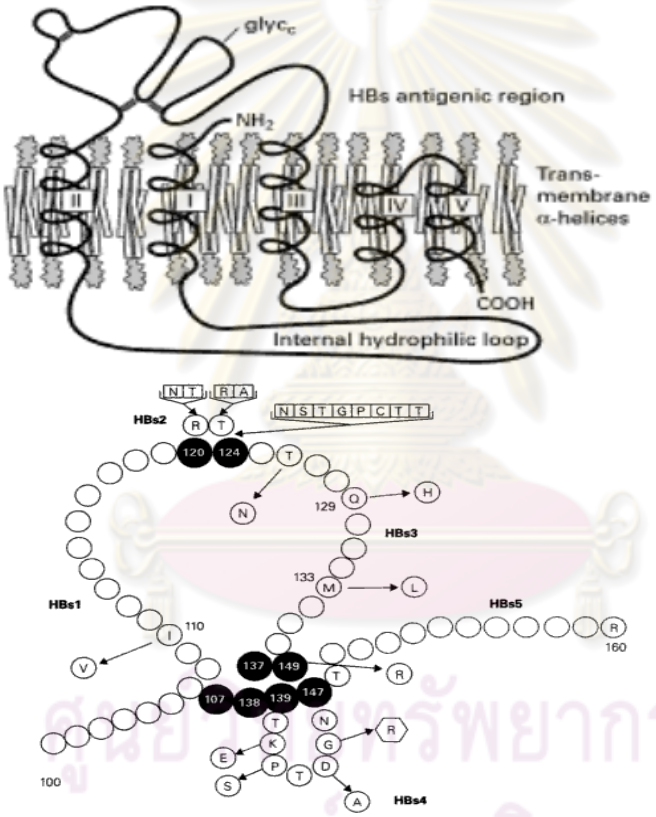


Fig. 6 Topological models of small HBs protein. The epitopes are generated by the complex folding of the external hydrophilic loop between ∝-helices II and III (A) [78]. The region between aa 100 and 160 shows the two major loops (aa 107/137 and aa 139/147) and one minor loop (aa 121/124) in MHR (B). HBs1-5, antigenic region; Circle, vaccination-associated variants; Hexagon, variant which affect the ability of specific monoclonal antibody to bind; Square, the insertions seen in diagnostic failures [83].

Middle S protein (MHBs): It is the minor component of surface antigen. The sequences of amino acids are the combination between SHBs and 55 aa of preS2 domain [84]. MHBs is very sensitive to protease. The preS2 domain of MHBs partially covers the S domain (Fig. 7). The middle part of the preS2 domain can bind with a modified form of serum albumin. Actually, approximately 1 in 10,000 serum albumin molecules can bind to preS2 domain. However, serum albumin of non-human primates can not bind this region [85].

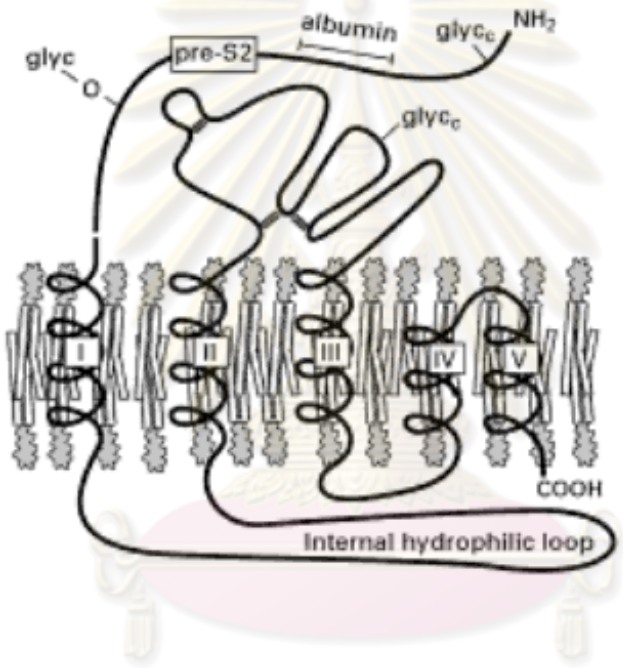


Fig. 7 Topological model of middle S protein [78].

Large S protein (LHBs): The extra domain of L is preS1 domain. From the topological model, the S and some parts of preS2 domain are covered by preS1 domain (Fig. 8). The preS1 is known as highly variable region. Some regions of preS1 overlap with some parts of the polymerase. This part is not necessary for replication. Moreover, surface structure has the highest chance to be selected by immune presure. So, the preS1 is known as highly variable region from these reasons.

SHBs, MHBs and LHBs are all the components of Dane particle, while LHBs and MHBs are found equally about 30% of the envelope protein content [86].

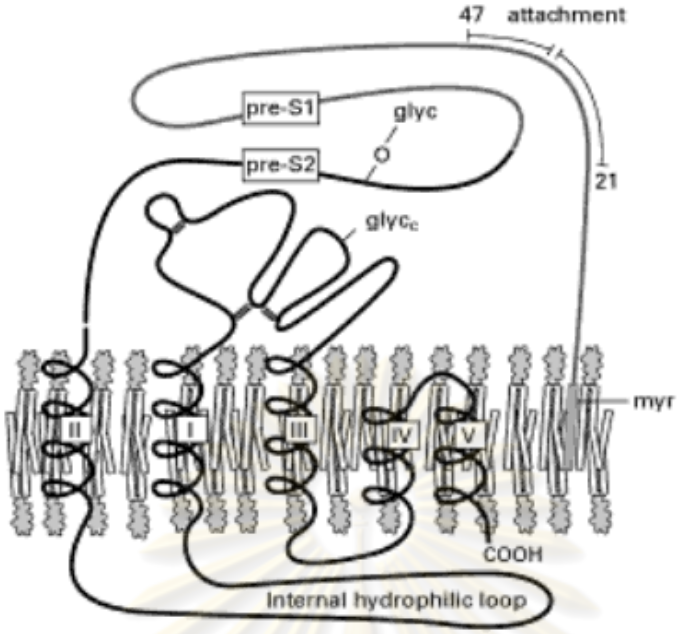


Fig. 8 Topological model of large S protein [78].

HBc protein: HBc contains 183 or 185 amino acids, depending on the genotype. The protein does not contain lipid or glycan but mostly contains hydrophilic and charged amino acid types [87]. HBc protein is essential for replication step by packaging its mRNA, the viral polymerase and a protein kinase into the core particle [88]. After that, this particle is enveloped by ER membrane that contains all three HBs forms. However, non-enveloped core particle are often found in the nucleus [89].

HBe protein: Up to now, the function of HBe has not been known, However, all hepadnaviruses have produced HBe protein. There is a high level of HBe protein in highly viraemic virus carriers. From previous studies, WHV with HBe-detected strain was infectious for newborn woodchuck while it failed to induce persistent infection [90], whereas the normal strain was successful to establish the persistent infection. These data suggest that HBe protein may be served as suppressor from the immune elimination of HBV-producing hepatocytes.

Polymerase protein: P protein comprises of four distinguishable domains (Fig. 9) [91, 92].

Domain 1 – The terminal protein domain. This domain is linked to 5' end of the minus stranded of viral DNA and necessary for priming of minus stranded synthesis

Domain 2 – It has no specific function and is also termed "spacer".

Domain 3 – This domain encodes the RNA- or DNA- dependent polymerase (reverse transcriptase).

Domain 4 – This carboxy terminal domain encodes Rnase H;-the enzyme that is used to cleave the RNA and DNA hybrids.

P protein is combined together with the pregenomic RNA inside core particles [93].

Fig. 9 Schematic model of polymerase protein [92].

HBx protein: HBx protein has 154 amino acids. It is present in the orthohepadnaviruses but absent in the avihepadnaviruses. This observation suggests that HBx protein may not play an important role in the mechanism of genome replication or virion assembly. There is no clear evidence that HBx protein serves as a structural component of virion. The amount of HBx protein expression is also unknown. The most significant effect of HBx protein expression is also unknown. The most significant effect of HBx protein is tumorigenic activity [94, 95].

1.5 Gene mutation Reverse transcriptase of HBV lack a proofreading activity; as a result, HBV population distributed in the host is inherently error prone. The HBV mutation rate has been estimated at approximately 1.4 – 3.2 x 10^{-5} substitutions per site per year [96].

Basal core promoter, precore and core gene: The major mutation in this region results in reducing or blocking HBeAg expression. First, the mutation affects the

translation of stop codon in the precore gene [97]. At nt 1896, a G to A substitution provides a premature termination of the precore/core protein which is the precursor of HBeAg [98]. Second, the mutation at the basal core promoter effects a transcriptional reduction mRNA. At nt 1762 (A to T) and nt 1764 (G to A). The double mutation of A1762T plus G1764A results in a decrease but not a disappearance of HBeAg production. Basal core promoter mutation results in reduced binding of liver specific transcription factors. This evidence effects transcribing fewer mRNA and proteins.

X gene: X region overlaps with the basal core promoter and enhancer II. The mutation of A1762T plus G1764A also causes changes in the *X* gene at aa130 (xK130M) and aa131 (xV131I). Moreover, the deletion of the basal core promoter leads to production of the truncated X protein [99].

Envelope gene: Point mutations, deletions and genetic recombinations within *preS* genes have been identified. Major HBsAg protein induces an immune response to the region located from residue 99 to 170. Mutations in this epitope have been selected during vaccination [100]. The sG145R mutation has been associated with vaccine failure [100] (Fig. 6B).

Polymerase gene: The treatment of nucleoside and nucleotide analogues has resulted in the mutation in polymerase gene (Fig. 10) [101]. Well-known lamivudine-resistant mutation has been located in the C domain of HBV polymerase at the aa204 or YMDD locus [102]. This mutation decreases sensitivity of drug from $\geq$ 20 fold to $>$ 100 – fold [92]. The substitution of amino acid N to T at codon 236, located in the D domain, and amino acid A to T/V at codon 181, located in the B domain, are responsible for adefovir resistance [103]. Resistance to entecavir was observed in the patient who was resistant to lamivudine. The mutation were mapped to domain B (rtS184G), domain C (rtS202I) and domain D (rtM205V) [92] (Fig. 10). Because of the identify between HBV and HIV infection. HIV therapy can provide a guideline for developing combination therapy of HBV infection [104]. No single drug is able to permanently use, control or eliminate chronic HBV infection.

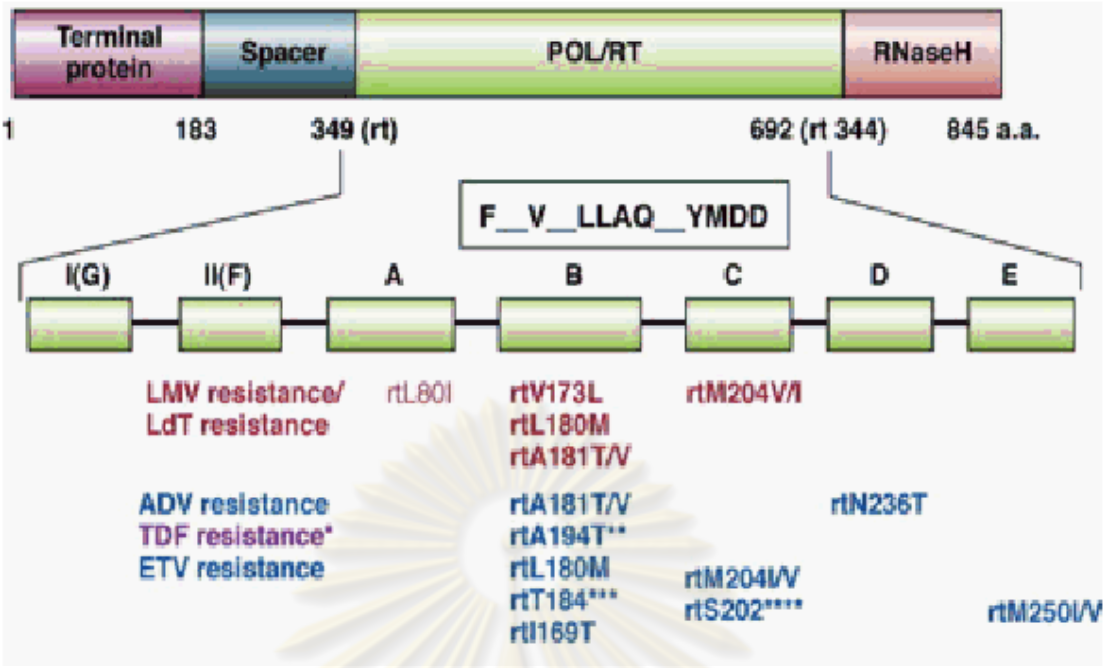


Fig. 10 The location of the major antiviral drug-resistant mutation associated with lamivudine (LMV), telbivudine (LdT), adefovir (ADV), tenofovir (TDF), and entecavir (ETV) resistance. *rtA181T/V and /or rtN236T cause reduced sensitivity; **ALT association with rtL180M+rtM204V; ***S/A/I/L/G/C/M; ****C/G/I [103].

1.6 HBV replication The HBV replication is the replication of the DNA genome by reverse transcription of an RNA intermediated [105]. The HBV life cycle begins with the virus attachment to the host cell membrane by the envelope proteins and fusing their membrane with the host cell membrane and releasing viral genome into the cell (Fig. 11). The early step of HBV infection consists of attachment, fusion an entry stage [106]. LHBs is essential for the attachment step. The antibody against the peptide preS1 domain (aa 21-47) was virus-neutralizing and protective antibody (Fig. 8). Moreover, the antibody against this site has neutralized the HBV infection in chimpanzee [107]. Paran et al. used the mutagenesis study and found that the QLDPAF sequence within preS1 region may play an important role in cell attachment and viral infection [108]. In case of the cell receptor for HBV attachment, Kuroki et al. found that a glycoprotein in duck hepatocyte (duck carboxypeptidase D, DCPD) could be co-precipitate with DHBV [109]. The carboxypeptidase D is a protease which is found in both the avian hepatocytes and the mammalian hepatocytes. The host cell receptor may have the protease function and the HBV infection may require proteolysis [106].

Moreover, Treichel et al. have found that HBV-particles from carriers could bind to the human asialogylcoprotein receptor (ASGPR). The inhibition of HBV-ASGPR interaction could be inhibited the HBV uptake into HepG2 and Huh7 cells. These data suggest that the ASGPR may be a site of HBV particle binding [110].

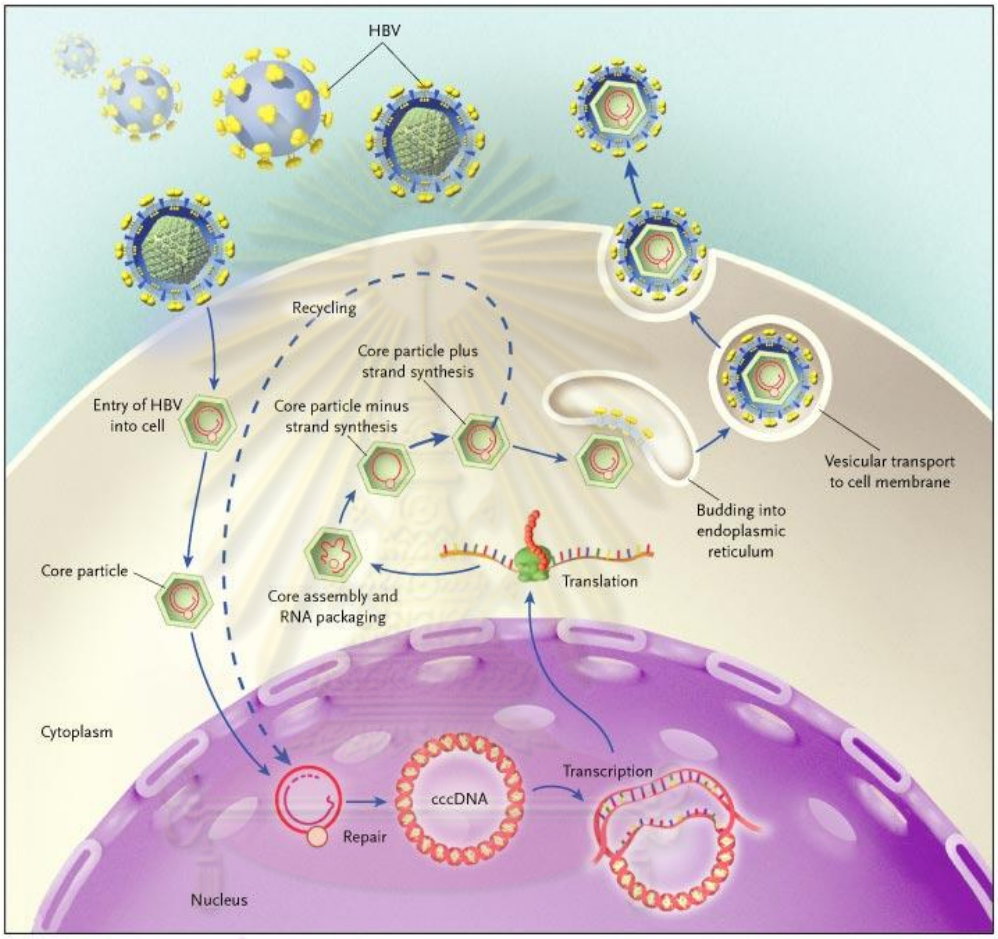


Fig. 11 Replication cycle of the hepadnaviral genome [41].

The next step is the viral fusion and proteolysis. This step is dependent on a “fusion motif”-a hydrophobic region within the viral surface protein [111]. Pre-digestion of HBV with V8 protease allowed the virus to enter into the unsusceptible cell line, V8 protease cleaves at “PEST fusion motif” (FLG-LL-AG). This evidence shows that unsusceptible cell lines such as HepG2 and Huh7 cell may lack of protease that is necessary for exposing the HBV fusion domain [112]. After the viral fusion and proteolysis steps, HBV requires the internalization step and transport the core particle

with viral genome to the nucleus. The information of the internalization of HBV is still unclear. After a putative fusion peptide of HBV was found in 1994, Stoeck et al. presented a new original entry mechanism involving an internalization motif (PLSSIFSRIGDP) in the preS2 domain of envelope protein. According to this study, the internalization motif was exposed on the surface of viral particles following a conformational change of envelope after virus binding at the cell surface and into the endosomal compartment. This evidence allows the translocation of viruses through the endosomal membrane into the cytosol [113].

After they penetrate into the hepatocyte, cores are presented to the cytosol and transport to the nucleus [114]. There, the partial double-stranded DNA undergoes repair and converts to a stable form called covalently closed circular DNA, or cccDNA form [114]. The cccDNA becomes the template for host RNA polymerase II to transcription of genomic and subgenomic products [75]. All viral RNA is transported to the cytoplasm where its translation to viral envelope protein, core protein and a polymerase protein as well as the X protein and precore polypeptide. In the cytoplasm, nucleocapsids are assembled, and during this process a single molecule of genomic RNA is incorporated into the viral core particle [115]. Next, the HBV polymerase enzyme synthesizes two viral DNA strands [115]. First DNA strand is synthesized from the RNA template, after the synthesis of DNA strand, the RNA template is degraded and the second DNA synthesis is preceded from using the first DNA strand as template [75, 105, 116]. Some core particles with the mature genome are transported back to the nucleus [114]. Most of nucleocapsid particles are routed to the membranes of the compartment between the endoplasmic reticulum (ER) and the golgi apparatus, which contains the viral surface proteins necessary for envelopment process [117]. After envelopment, these particles are migrated to the cell surface where they are released into the circulation.

1.7 Transmission routes Hepatitis B virus is transmitted by the exchange of body fluids. The routes of HBV transmission are divided into 2 ways. First is vertical or perinatal transmission and second is horizontal transmision. Perinatal transmission is the mother-to-chlid transmission. The mechanism of perinatal transmission remains unclear.

The infection may be occurs *in utero* or intrapartum [118, 119]. The children who are infected during the perinatal period will develop chronic infection approximately 90% [120].

Horizontal transmission is the transmission between person to person, not from parent to child. The people who most at risk from horizontal transmission include anybody who has unprotected sexual intercourse, intra-venous (IV) administrator and people who receive blood transfusion.

Several studies document the unusual cases of hepatitis B virus transmission. For example, the dentists were infected HBV from their patients [121], the teacher got HBV by receiving the saliva and nasal secretions from her students [122], the HBV outbreak was caused by patients sharing the inadequately sterilized needles [123], a butcher was infected because he shared the knife with his co-workers [124], and human bite is considered a possible route of transmission [125].

1.8 Prevalence of HBV in humans There is a wide range of hepatitis B surface antigen (HBsAg) prevalence in different countries such as 0.2-0.5% in USA, 2.4-4.7% in India, and 1.4-8.0% in Russia. The chronic HBV infection and its related hepatic complications are important particularly in Southeast Asian countries where the prevalence of the infection is relatively high, varying from 3-6% in Indonesia, Philippines, Myanmar, Laos, Cambodia and Vietnam [126-141]. In Thailand, the prevalence of HBV infection has declined upon implementation of the national HBV vaccination program, with present prevalence of approximately 4% [142-145]. The predominant HBV genotypes in this region are genotypes C and B (Fig. 12]

Fig. 12 The prevalence of HBV infection in Southeast Asian countries.

1.9 Pathogenicity and clinical outcome of HBV infection The pathogenicity and clinical outcome of hepatitis B are the interaction between virus and the host immune system. The host lymphocytes recognize various HBV-derived peptides located on the surface of the hepatocytes. This lymphocytes attack the HBV and cause liver injury. The final stage of patient with HBV infection is cirrhosis and most patients are likely to develop hepatocellular carcinoma (HCC) [146-149]. The stage of HBV infection comprises of 4 different stages as describe below (Fig. 13A):

Stage 1 – Immune tolerance: This stage is approximately 2-4 weeks for healthy adults, while the newborn is often decades. Infected patient has little viral replication, no elevation of the aminotransferase levels and no symptoms.

Stage 2 – Immune response: This stage is approximately 3-4 weeks for acute infection and 10 years or more for chronic infection. In this stage, the immune system attacks the hepatitis B-infected cell to clear the infection. There is HBeAg in patient sera and a

decline of the HBV DNA level. Many people develop symptoms because the immune system attacks HBV infected liver cells.

Stage 3 – Viral clearance: This step is also called “seroconversion”. HBV stops its viral replication. HBV DNA is also undetectable or lower level. The host antibodies are produced to target against HBeAg (anti-HBe). Aminotransferase level is still within normal range. HBsAg is still detected.

Stage 4 – Immunity to hepatitis B: HBV DNA is undetectable. Various antibodies to viral antigens have been produced to clear the HBV.

Newborn babies who infected HBV from their mothers are mostly become chronic carriers (Fig. 13B), while most infected adults can recover and develop life-long immunity.

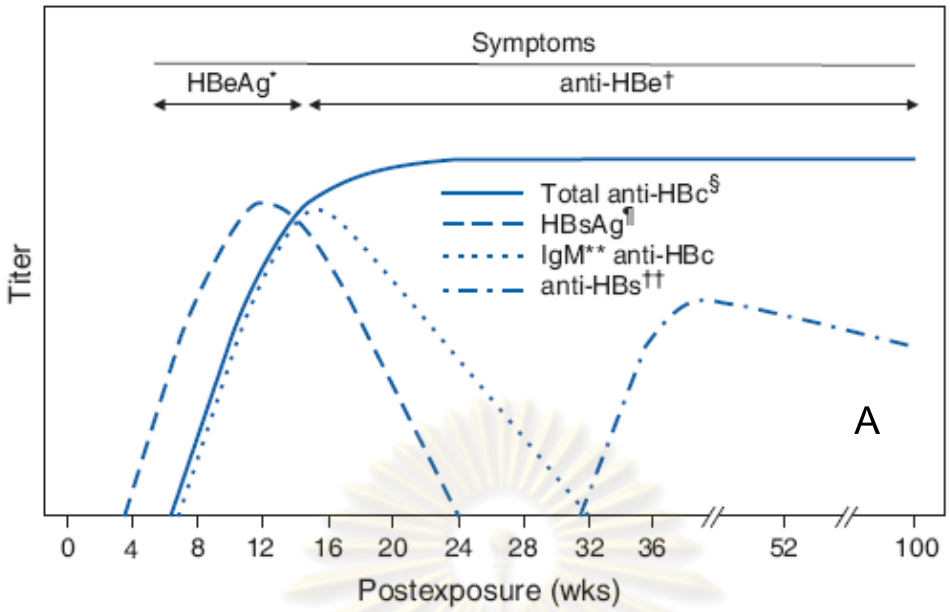
1.10 HBV markers The diagnosis of HBV infection can be made by the detection of HBsAg, anti-HBc and anti-HBs marker. However, there are several markers of HBV that can be detected in patients.

HBsAg (hepatitis B surface antigen): This is a marker of infectivity. If HBsAg is present, it indicates the acute or chronic HBV infection.

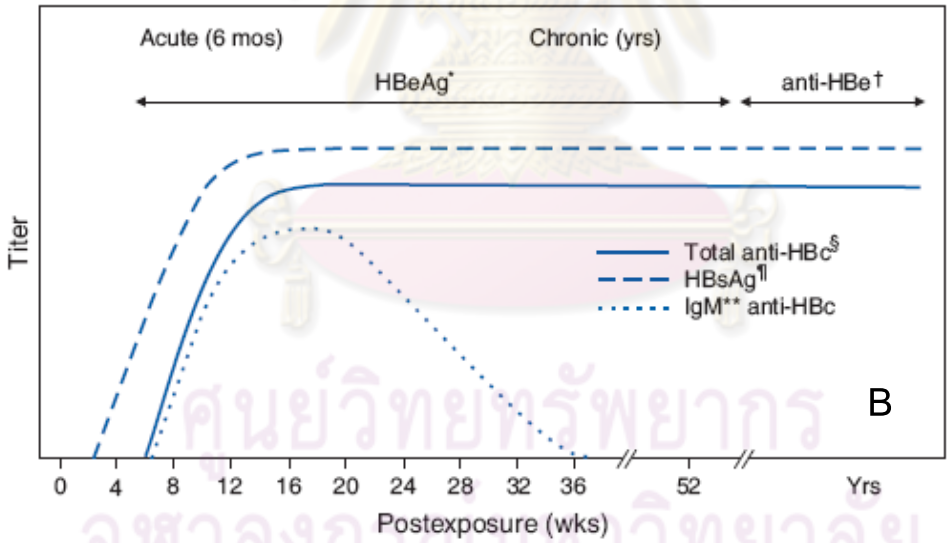
Anti-HBs (antibody to hepatitis B surface antigen or HbsAb): This is a marker of the immunity. If anti-HBs is present, it indicates an immune response to HBV infection, an immune response to vaccination.

HbcAg (hepatitis B core antigen): The antigen found in the surface of the nucleocapsid or core. This antigen can found in the hepatocyte and do not circulate in the blood circulation.

Anti-HBc (antibody to hepatitis B core antigen or HbcAb): It is non-specific marker of acute, chronic, resolved infection or vaccination. It may be used to identify previous exposure to HBV infection.



* Hepatitis B e antigen.
† Antibody to HBeAg.
§ Antibody to hepatitis B core antigen.
¶ Hepatitis B surface antigen.
** Immunoglobulin M.
†† Antibody to HBsAg.



* Hepatitis B e antigen.
† Antibody to HBeAg.
§ Antibody to hepatitis B core antigen.
¶ Hepatitis B surface antigen.
** Immunoglobulin M.

Fig. 13 Serological pattern of hepatitis B virus infection. Acute infection with recovery (A) and acute infection with progression to chronic infection (B) [150, 151].

HBeAg (hepatitis B “e” antigen): It is a marker of replication and infectivity. High concentration of HBeAg indicates high infectivity.

Anti-HBe (antibody to hepatitis B “e” antigen): This antigen indicates a low viral titer and low degree of infectivity.

HBV DNA (HBV deoxyribonucleic acid): It is a marker of viral replication and used to monitor the treatment of chronic HBV-infected patients.

Typical serological patterns of acute and chronic HBV infection are shown in Table 4. The people who are classified as having immune through vaccination, natural infection, acutely infected and chronically infected is not necessary to vaccination.

Table 4 Serological patterns of acute and chronic HBV infection [150-151].

Classification	HBsAg	anti-HBs	anti-HBc	HBeAg	anti-HBe
Susceptible (never exposed)	-	-	-	-	-
High infectivity chronic carrier	+	-	+	+	-
High infectivity chronic carrier	+	-	+	-	+
Low infectivity chronic carrier	+	-	+	-	+
Current acute infection	+	-	+	+/-	+/-
Vaccine immunity*	-	+	-	-	-
Past exposure	-	+	+	-	+/-

* anti-HBs positive with $\geq$ 10 mIU/ml; -, negative; +, positive

2. HBVs IN NON-HUMAN PRIMATES: HISTORY AND FACTS

The first association between human and non-human primate HBV infection occurred in the 1960s among the chimpanzee handlers in the USA [25]. Three years later, imported chimpanzees presented with human viral hepatitis like symptoms [26]. In 1970, 16 persons who had contacted with 2 imported chimpanzees from Sierra

Leone suffered from viral hepatitis infection [27]. Based on these facts, several researchers have successfully used chimpanzees as a model for human viral hepatitis by inoculating the animals with infected sera, saliva or semen collected from the HBV-infected people [28, 32]. Several researchers could reproduce similar experiments in other non-human primates such as gibbons [33, 34]. These data confirmed that human HBV can infect other non-human primates.

At the same time, the survey of HBV in other non-human primates began by using radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). HBV infection was documented in chimpanzees, orangutans, gorillas, woolly monkeys and gibbons. In 1978, 5 chimpanzees in London zoo were infected with HBV. Three of those had one of their parents being chronic carriers; hence, this suggested that the infection may have transmitted vertically [152]. It took a decade for the first ChHBV genome to be revealed in 1988. ChHBV genome has 3,182 bp with 4 open reading frames of surface, core, polymerase and *X* gene [1] without precore stop codon [23]. There is 33-nt deletion in the *preS1* region making the 3' end of the core region more suitable for phylogenetic analysis than the preS1 region, the 3' end of the core region is conserved among *Orthohepadnaviridae* [49].

For gibbon, 2 species (*Hylobates agilis* and *Hylobates moloch*) are susceptible to HBV infection. However, the infection does not appear to be as severe as in human [22]. The *preS* gene of gibbon HBV was first sequenced in 1993 [35], and the whole genome was constructed 3 years later [54].

In 1996, 4 baboons were induced HBV infection with serum from the endstage liver disease patients. However, the baboons did not show any abnormal clinical biochemical or pathological finding [153]. Subsequently, Kedda et al. (2000) found HBV viral particles by electron microscopic examination in baboon hepatocytes. This aborted the possibility of using baboons as a source of liver xenograft [154].

In 1998, Lanford et al. determined the complete genome of WMHBV (3,179 bp) for the first time by polymerase chain reaction (PCR), hybridization and sequence analysis. They inoculated WMHBV in the black-handed spider monkeys (*Ateles geoffroyi*) because woolly monkey has been an endangered species. It was

found that the spider monkeys were susceptible to WMHBV but the infection was subclinical. When they repeated the same procedure in chimpanzees, they found that chimpanzees were minimally susceptible to WMHBV. The authors concluded that the black-handed spider monkey was a suitable model for WMHBV. Subsequently, they used WMHBV particles from the medium of transfected culture of Huh7 cell line to infect a spider monkey. A week after inoculation, the spider monkey became viremic with 2.2 x 10^5 ge/ml in serum whereas baboons, rhesus monkeys, and tamarins were not infected [5]. For orangutans, complete *S* gene of HBV was described in 1999 [2].

Grethe et al. (2000) studied 21 HBsAg positive sera from 19 gibbons, a chimpanzee and a gorilla kept in Germany, France, Thailand and Vietnam. They found that *preS1* gene and the whole genome of ape clustered into 6 genomic groups - 5 groups of gibbons and 1 group of chimpanzee and gorilla. They observed that animals from the same location shared HBV from the same cluster and vice versa. Moreover, the genome of the gorilla HBV was in the same cluster as the genome of chimpanzee HBV because they shared the same habitat [4]. Hu et al. (2000) used the mitochondrial DNA to differentiate the sequences of ChHBV into three distinct clusters. They suggested that 3 clusters of complete ChHBV sequences came from 3 different subspecies of chimpanzees: *Pan troglodytes troglodytes*, *Pan troglodytes verus* and *Pan troglodytes vellerosus* [21]. Also, this held true for orangutans in Borneo [155]. These findings suggested that geography or sub-populations of the hosts had influence on virus variants.

Thornton et al. (2001) tried to vaccinate the mates of two HBsAg positive white-cheeked gibbons (*Hylobates leucogenys leucogenys* and *Hylobates leucogenys siki*) and a western lowland (*Gorilla gorilla gorilla*). The vaccinated animals showed the anti-HBs titre > 100 mIU/litre [156].

In 2002, Gheit et al. inoculated a replication-competent head-to-tail HBV DNA plasmid dimer to Barbary macaue (*Macaca sylvanus*) by intrahepatic route. Two days after inoculation, monkeys had increased ALT levels. Three weeks after inoculation, virus-like particles were in serum. Liver pathology was different to controls.

These results indicated that HBV can replicate in *M.sylvanus* and this monkey may be a suitable model for HBV replication [157].

In 2003, Starkman et al. tested 137 primates sera from Africa and South east Asia (gibbons and orangutans). *Cercopithecidae* showed negative results for PCR for HBV despite using the primers for DNA conserved in all human, non-human primates and rodents. Seven apes had positive PCR for HBV. The phylogenetic analysis reveals that GiHBV and OuHBV sequence can be divided into 3 groups: firstly, orangutan and gibbon strains with overlapping habitat in the Southern part of Southeast Asia; secondly, the gibbon strains (*Nomascus sp*. and *H. lar*) of Central Thailand and Laos, and the third group contain gibbon strain (*H. pileatus*) that is predominant in Cambodia and Central Thailand [8].

In the same year, Aiba et al. (2003) amplified full length HBV genome from 2 pileated gibbons. Three forms of HBV particles were observed in the sera under the immunoelectron microscopy. HBsAg and HBeAg were detected in the cytoplasm and nuclei of hepatocytes, respectively. Gibbon HBV genome shared 90.3% homology with HBV chimpanzee strain. Moreover, a study reported that gibbon HBV could also infect chimpanzees [158]

In order to analyze prevalence and possible route of gibbon HBV transmission, Noppornpanth et al. (2003) screened 101 captive gibbons from Central Thailand. Forty animals showed at least one marker for HBV infection. Twenty animals recovered from the infection and 19 animals were HBV carrier. Offsprings of two carrier gibbon mothers were HBsAg and HBV DNA positive. *PreS1* sequences showed small divergence of only 2 and 4 bases. Thus, vertical transmission must have occurred. Additionally, animals in different cages shared the same branches of phylogenetic tree indicating horizontal transmission. Four carriers had mutation at nt 1762 and 1764. All mutated samples had HBeAg positive [3].

In 2005, Sall et al. investigated the frequency and genetic relationships of GiHBV in Cambodia by PCR and the entire genome sequences were compared with the available published HBV sequences. The phylogenetic tree showed the difference in

HBV genotype distribution between gibbons from Cambodia and gibbons from Thailand comparing to the previous study [3]. This study showed several forms of HBV circulating in geographically separated gibbon populations in Southeast Asia [9]. The first evidence of potential recombination between ChHBV and human HBV sequences was documented [159]. Recombinations between human and non-human primate strain, between different gibbon genera and between different bird subfamilies were further confirmed [160].

All experiments above provide the information that helps us to better understanding the non-human primate HBV regarding background of the virus, hosts of virus and zoonotic transmission [32].

3. DIFFERENCES BETWEEN HUMAN AND NON-HUMAN PRIMATE HBVs

3.1 Molecular characterization The physical genome organization of human and non-human primate HBVs is similar. The HBV genome contains four ORFs: *preC/C*, *P*, *preS/S*, and *X* [161]. Orthohepadnaviruses produce 3 domains of hepadnaviral surface proteins encoded by an ORF with 3 alternative start codons. These three proteins are L protein encoded by *preS1/S* genes, M protein encoded by *preS2/S* genes, and *S* protein encoded only by *S*. Interestingly, *preS1* gene of non-human primate HBVs has 33 nt or 11 amino acid deletions at the 5' terminus after the start codon (Fig.14), which is not found in human HBVs except for HBV-D. In the process of protein modification, L protein of human HBVs is myristoylated at Gly2 at the N-terminus of preS1 region, what is not observed with non-human primate HBVs due to the deletion in this position [162]. Moreover, human HBVs show O-glycosylation at Thr37, whereas ChHBV and GiHBV display in the same position Asn37 and GoHBV Asp37.

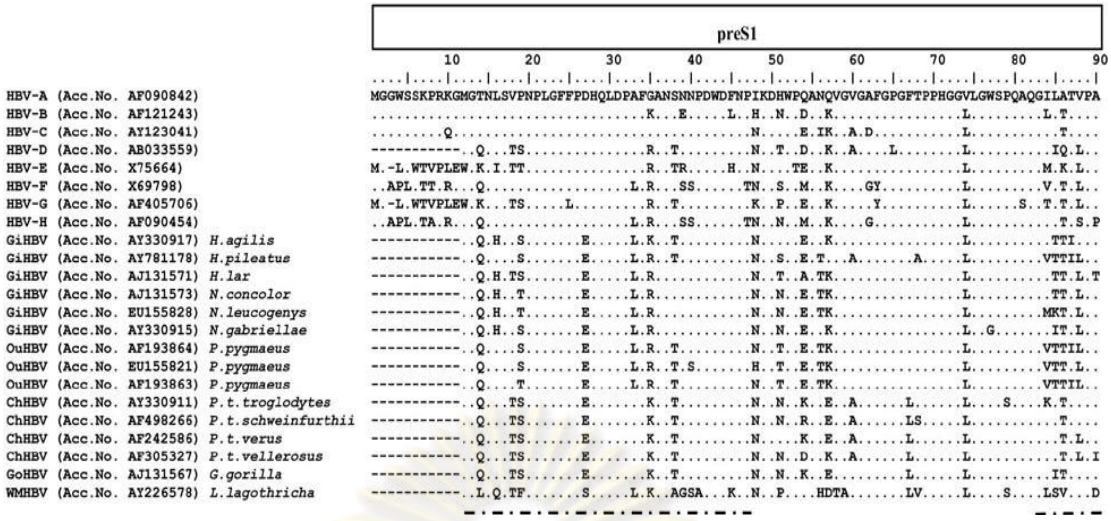


Fig. 14 Multiple alignment of sequences of preS protein of human and non-human primate HBVs. Human HBV-A (AF090842) served as a reference. (.) = identical amino acid, (-) = deletion, (_._._) = viral ligands.

The amino acids 12-20, 21-47, and 82-90 of the preS1 domain (Fig.14) are virus binding ligands [163,164]. The amino acid 27 is in the preS1 region. All genotypes of human HBVs contain Asp27 residue; whereas all non-human primate HBVs display Glu27 in this position. The M protein is not essential for infectivity [165]. Among human and non-human primate HBVs, the percentage of identity of nucleotide sequences ranges from 74% to 98%.

The “a” determinant in the S region of HBsAg is essential for induction of a protective immunity. Based on two pairs of amino acids in the position 122 (*d/y*) and 160 (*w/r*) of the “”a” determinant, human HBVs are divided into four serotypes *adw*, *ayw*, *adr* and *ayr* [66]. All human and non-human primate HBVs contain Gly145 in the “a” determinant region (Fig.15). This finding suggests that the recombinant HBV vaccine should prevent the infection with non-human primate HBVs as well.

As for hepadnaviral RNA ε signal, non-human HBVs show nucleotide U; whereas human HBVs display nucleotide G (G1896U) [166]. This variation affects the structure of bulge conformation (Fig.16). This conformation resembles HBV-A, which contains a G1896A mutation without a C1858T mutation resulting in the instability of the

stem-loop structure in this mutant virus. Instability of HBeAg contributes to the low levels of HBe antibodies in the HBV-infected patients [167,168].

```
                                                    122                 145                 160
                                                     |                   ⇩                  |
HBV-A (Acc.No. AF090842)                            PCKTCTTPAQGNSMFPSCCCTKPTDGNCTCIPIPSSWAFAK
HBV-B (Acc.No. AF121243)                            ..........T.............................
HBV-C (Acc.No. AY123041)                            ......I....T..........S..............R
HBV-D (Acc.No. AB033559)                            ..R........T..Y........S..............G.
HBV-E (Acc.No. X75664)                              ..R..M.L...T........S..S..............G.
HBV-F (Acc.No. X69798)                              .......L...T........S..S.............LG.
HBV-G (Acc.No. AF405706)                            ..............Y........S..............
HBV-H (Acc.No. AF090454)                            .......L...T..........S..............G.
GiHBV (Acc.No. AY330917) H.agilis                   ..R...I....T.L.........S..............R
GiHBV (Acc.No. AY781178) H.pileatus                 ..R...I....T.LY........S..............
GiHBV (Acc.No. AJ131571) H.lar                      ..R...IT...T.LY........S..............
GiHBV (Acc.No. AJ131573) N.concolor                 ......I....T.L.........................
GiHBV (Acc.No. EU155828) N.leucogenys               ......I....T.L.........................
GiHBV (Acc.No. AY330915) N.gabriellae               .........P.T.L.........S..............
OuHBV (Acc.No. AF193864) P.pygmaeus                 T.R...IS.P.T.L.........S..............R
OuHBV (Acc.No. EU155821) P.pygmaeus                 T.R...IS.P.T.L.........S..............R
OuHBV (Acc.No. AF193863) P.pygmaeus                 ..R...IS.P.T.L.........S..........P.....
ChHBV (Acc.No. AY330911) P.t.troglodytes            ..........T.LL........S..............V.
ChHBV (Acc.No. AF498266) P.t.schweinfurthii         .........H.T..........S..............R
ChHBV (Acc.No. AF242586) P.t.verus                  ..........T.LI........S..............
ChHBV (Acc.No. AF305327) P.t.vellerosus             ..........T.L.........S..............
GoHBV (Acc.No. AJ131567) G.gorilla                  .......T...T.LY........S..............
WMHBV (Acc.No. AY226578) L.lagothricha              ..R...PIVP.I.SY........................
```

Fig. 15 Multiple alignment of sequences of S protein of human and non-human primate HBVs. The underlining indicates “a” determinant region. Arrow represents specific glycine.

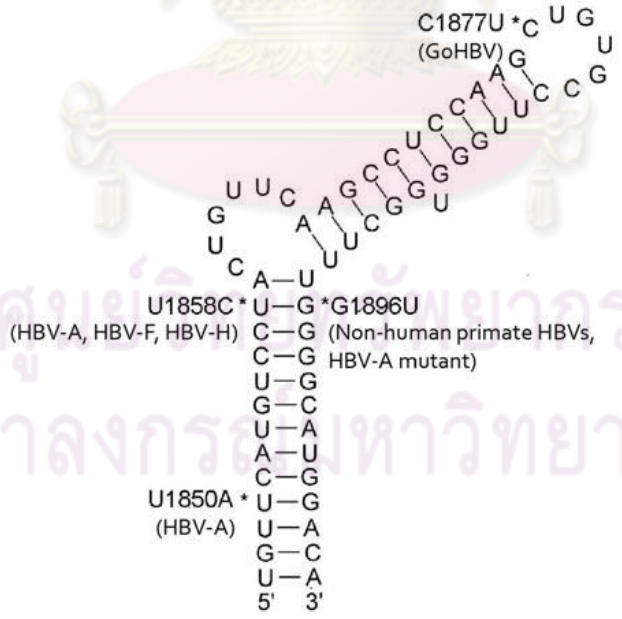


Fig. 16 Secondary structure of human and non-human primate HBV RNA ε signals (adapted from Kidd-Ljunggren *et al.*, 2002) [166].

3.2 Origin and evolution of HBVs in non-human primates Since the initial discovery of the human HBV in 1967 [46], several research studies have been conducted to elucidate the origin and evolution of this virus. Various theories have been proposed. Based on the substitution rate of the entire genome, ancestor of HBVs must have emerged between 2,300 and 3,100 years ago [169,170]. The first theory speculated that HBV originated in America and later it was introduced to Europe during the colonial period. Subsequently, the virus diversified to the different genotypes specific to Africa, central Asia, and China [171]. However, this hypothesis was contradicted by molecular clock calculations arriving at the conclusion that genotype development required a much longer period than 400 years [172]. Then, some researchers supposed that each HBV genotype may have emerged in America, before it spread to Europe. Genotype F was indigenous to South America [170]. Whether HBV evolution is host-dependent has remained elusive [23,172,173]. Like the human HBVs, the origin of non-human primates is widely dispersed but only humans can travel around the world. A theory suggested that human may acquire HBV infection from the non-human primates or vice versa [1,49]. This idea is contradicted by the nucleotide identities of non-human primate HBV genomes, by the significant difference between human and non-human primate HBV genomes and a lack of evidence of the cross-species infection. Thus, both the origin and evolution of HBVs have remained inconclusive [169].

3.3 Prevalence, pathogenesis, and clinical significance

3.3.1 Prevalence The prevalence of HBV infection in the nature primate habitat is unknown. Most data resulted from the studies performed on samples obtained from captive animals, wild-born or captive-born. HBV infection has not been documented in the family *Cercopithecidae*. The prevalence of HBV infection is very high in the families *Atelidae* and *Hylobatidae*. Among species in the family *Hominidae*, the orangutan has the highest incidence of HBV infection (Table 5).

3.3.2 Pathogenesis and clinical significance In humans, HBVs constitute one of the most important risk factors for development of cirrhosis and hepatocellular carcinoma (HCC). Knowledge about the natural history of HBV infection

in non-human primates is inadequate. HBV infection in non-human primates can cause biochemical and histological abnormalities, but not cirrhosis or HCC. HBsAg-positive gibbons displayed elevated alanine aminotransferase (ALT) levels compared with control animals 68.8 ± 48.1 vs. 33.0 ± 15.9 IU/l, respectively [3]. The autopsy of HBV-infected woolly monkeys showed hepatitis and liver necrosis without cirrhosis of HCC [5]. This could be attributable to the life span of these non-human primates being too short to develop cirrhosis or HCC.

4. POTENTIAL OF CROSS-TRANSMISSION

Despite an advancement of the knowledge in this field, cross-species transmission has not been proven yet. If the cross-species transmission does occur, the chance to eradicate HBV infection by immunization will be diminished due to the difficulty in controlling of natural virus reservoir.

4.1 Transmission of human HBVs to non-human primates In order to elucidate both the infectivity and biology of human HBVs, the experimental animals close to humans were used such as chimpanzees, gibbons, and baboons. So far, chimpanzees have proved to be the best model. After infection with HBV, the chimpanzees developed hepatic pathology similar to the acute HBV infection in humans, but they did not develop a chronic liver disease [174]. It has shown that the serum, saliva, and semen from HBsAg-positive humans can transmit HBV infection to other non-human primates as well. Chimpanzees and gorillas were HBsAg-positive and/or had elevated serum ALT [8, 27, 31, 33, 34]. Baboon sera were HBV DNA-positive [154]. Barbary macaques showed the presence of HBsAg and HBV DNA in serum. Moreover, Dane particles were found in the serum of Barbary macaques 3 weeks animal model to study the HBV replication [157].

4.2 Transmission of human HBVs to mice Mice are not a natural host for the hepadnaviruses. Several researchers developed transgenic mice in order to study the expression of coding regions for the surface antigens, products of *preS*, *S*, and *X* gene of HBVs. They found HBV DNA, transcribed RNA, and HBsAg in HBV-infected transgenic mice. However, none of them showed clinical symptoms or developed signs

Table 5 Prevalence of HBV infection in non-human primates as reported until 2008 (mostly adapted from Starkman et al., 2003) [8].

Family	Common Name	Systemic name	No. of animals			
			Total	HBsAg +	HBV DNA +	Carrier state (%)
Hominidae	Gorilla	*Gorilla gorilla spp.*	85	3/65	8/53	8/85 (9.41)
	Chimpanzee	*Pan troglodytes spp.*	734	47/702	40/205	63/734 (8.58)
	Bonobo	*Pan paniscus*	27	1/27	1/5	1/27 (3.70)
	Orangutan	*Pongo pygmaeus spp.*	531	80/375	45/165	80/531 (15.07)
Hylobatidae	Gibbon	*Hylobates spp.*	347	66/325	93/247	82/347 (23.3)
		Nomascus spp.	9	3/9	3/9	3/9 (33.33)
Cercopithecidae	Mandrill	*Mandrillus leucophaeus*	78	0/78	0/78	0
		Mandrillus sphinx	174	0/174	-	0
	Long-tail macaque	*Macaca fascicularis*	82	0/82	0/10	0
	Rhesus macaque	*Macaca mulatta*	32	0/32	0/1	0
	Stump-tailed macaque	*Macaca arctoides*	2	0/2	0/2	0
	Southern pigtail macaque	*Macaca nemestrina*	4	0/4	0/4	0
	Vervet monkey	*Cercopithecus aethiops*	58	0/48	-	0
	Cherry-capped mangabey	*Ceropithecus torquatus*	24	0/24	-	0
	Moustached monkey	*Cercopithecus cephus*	28	0/28	-	0
	Sun-tailed monkey	*Cercopithecus solatus*	16	0/16	-	0
	De Brazza's monkey	*Cercopithecus neglectus*	2	0/2	-	0
	Crowned guenon	*Cercopithecus pagonias*	2	0/2	-	0
	White-nosed guenon	*Cercopithecus nictitans*	24	0/24	0/2	0

Table 5 (continued)

Family	Common Name	Systemic name	No. of animals			
			Total	HBsAg +	HBV DNA +	Carrier state (%)
	Red-eared monkey	*Cercopithecus erythrotis*	3	0/3	0/3	0
	Silvered langur	*Semnopithecus cristatus*	4	0/4	0/4	0
	Phayre's langur	*Semnopithecus phayrei*	1	0/1	0/1	0
	Dusky langur	*Semnopithecus obscurus*	3	0/3	0/3	0
	Baboon	*Papio spp*	168	0/168	0/4	0
	Mangabey	*Cerocebus albigena*	6	0/6	-	0
	White-fronted capuchin	*Cebus albifrons*	10	0/10	-	0
	Talapoin	*Miopithecus talapoin*	5	0/5	-	0
	N/A	N/A	386	0/386	-	0
Atelidae	Woolly monkey	*Lagothrix lagotricha*	16	7/13	9/15	7/16 (43.75)
Cebidae	Common squirrel monkey	*Saimiri sciureus*	20	0/20	-	0
	Common marmoset	*Callithrix jacchus*	6	0/6	-	0
	Cotton-top tamarin	*Saguinus oedipus*	12	0/12	-	0
	N/A	N/A	49	0/49	-	0
Lemuridae	N/A	N/A	13	0/13	-	0
Callimiconidae	N/A	N/A	6	0/6	-	0

N/A = data not available. (-) = not done. Carrier state = HBsAg and HBV DNA were concurrently found.

of pathological liver [175,176]. Since the transgenic mice were not susceptible to HBVs, mice with severe combined immunodeficiency (SCID) were used for infection with HBVs. Actually, HBV-infected SCID mice developed chronic liver disease. In addition, they permitted HBV to replicate in human hepatocytes that were able to proliferate in these mice [174].

4.3 Transmission of non-human primate HBVs to non-human primates The inoculation of GiHBV to a chimpanzee resulted in the acute hapatitis infection. The virus isolated from the infected chimpanzee was GiHBV too [35]. Black-handed spider monkey was chosen as a suitable small primate model to study HBVs, because it belongs to the same family and subfamily as the woolly monkey. The results showed that the black-handed spider monkey was susceptible to WMHBV, but developed only a subclinical infection. The susceptibility of chimpanzees to WMHBV was only limited [5].

4.4 Computer-based analysis of HBV recombinants Unusual sequence within the core region of a strain of ChHBV was reported [50]. The core antigen of tested ChHBV shows only 78-82% amino acid identity to the known ChHBV strains. Moreover, the infectied animal was HBc antibody-negative. The *preS2* did not show the deletion at its 5'-end common to other non-human primate HBVs. The rest of the gene was identical to the ChHBV gene described in the previous studies [50]. Later, several reports have mentioned the recombinants between ChHBV, GiHBV, and HBV-C [35,159,160].

5. ESSENTIAL VERTICAL ROUTE OF HBV TRANSMISSION

A vertical transmission causes chronic HBV infection and is the major avenue of the HBV infection in endemic areas. By contrast, a horizontal transmission is a major route of HBV infection in the western world. The vertical transmission is essential for the transmission of HBV in the non-human primates. It was demonstrated that *S* gene of HBV isolated from gibbons and their offsprings displayed 99.5% identity [3]. Lanford et al. (1998) reported that 80% the offsprings coming from HBV-infected woolly monkey mothers tested positive for HBV infection [5].

However, there is also a possibility of horizontal transmission comfirmed by HBV DNA detection in gibbon saliva [3]. If the gibbons were housed in the same habitat, they would likely transmit HBV to the other gibbons. In order to study the possible routes of HBV transmission, Noppornpanth et al. studied 40 of 101 HBV-infected gibbons. Twenty animals recovered from the infection and 19 became the carriers [3]. The offsprings of two gibbon carrier mothers were HBsAg-and HBV DNA-positive. Sequences of *preS2* gene showed little divergence of only 2 and 4 bases. Thus, vertical transmission must have occurred. Interestingly, the gibbons in different cages were infected by HBV sharing the same phylogenetic tree branches; therefore, this study confirmed horizontal transmission.

6. PREVENTION AND TREATMENT

In humans, 2 groups of antiviral agents are currently used for treatment of the hepatitis, e.g. interferon (IFN) and nucleotide/nucleoside analogues (NA). These agents have been proven to prevent a disease progression in humans [177]. Due to the uncertainty of the natural history of HBV infection in non-human primates, the importance of HBV treatment is not known, but there is evidence suggesting that NA may be useful. The efficacy of lamivudine treatment in the HBV-infected chimpanzees was studied [178]. The HBV DNA level underwent a significant decrease over an 8-week period. Upon cessation of the lamivudine administration, the viral load rebound to the original level within 6 days [178]. Hence, lamivudine may be effective in treatment of the HBV-infected non-human primates. However, a larger control-trial study would be required to confirm this conclusion. For IFN, there is no data regarding its efficacy of HBV infection treatment in non-human primates.

As described previously, the amino acids in the “a” determinant region of the S region are highly conserved. Hence, the vaccine was speculated to exert a cross-protective effect in non-human primates (Fig.15). Several studies have shown that the vaccine to be beneficial in pre- and post- exposure prophylaxis in the non-human primates [179,180]. Ogata et al. evaluated the protective efficacy of a licensed HBV vaccine against HBV infection with an “a” determinant mutant. Four vaccinated

chimpanzees positive for HBsAg antibody were injected with HBV. These animals did not develop symptoms of the disease during a follow-up period of 2 years [179]. As to post exposure prophylaxis, Iwarson et al. (1988) conducted a study by administering HBV vaccine to 4 chimpanzees at 4, 8, 48, 72 hrs after HBV inoculation. The second and third doses of vaccine were given at 2 and 6 weeks after exposure. None of the animals developed HBsAg antibody or elevated serum ALT levels. However, the animals that received HBV vaccine 72 hours after exposure were HBc antibody-positive [180].

Knowledge of the role of HBV immune globulin (HBIG) for HBV post-exposure prophylaxis is limited. HBIG was administered to 5 HBV inoculated chimpanzees. Three animals that received HBIG after HBV inoculation developed anti-HBsAg antibodies and elevated serum ALT levels. In contrast, the other 2 animals that received HBIG and HBV simultaneously did not. One of these 2 chimpanzees also received HBV vaccine at the time of HBV inoculation. It was concluded that HBIg was effective only when administering simultaneously with HBV inoculation or with HBV vaccine [181].

7. NON-HUMAN PRIMATE

7.1 Overview of non-human primate Order primates comprise of prosimians, monkeys, apes and humans (Fig. 17). The members of this order have nails, hair or fur, self-regulate body temperature, nurse their young with mammary glands, vision-orientated and flexible behavior. Order primates are divided into three suborders which comprise of suborder Prosimii, suborder Tarsiiformes, and suborder Anthropoidea. Prosimii or prosimians members are lemurs of Madacascar, lorises, potto, angwantibo, and galagos. They inhabit in Africa, Asia, Sri Lanka, Madagascar and the East Indies. Tarsiiformes contain only one member, tarsiers. Tarsiers share characteristics of both prosimians and anthropoids. They are found in Indonesia and Philippines. Anthropoidea consist of new world monkeys, old world monkeys, ape and human. New world monkey comprises of 5 families: *Callitrichidae* (marmoset), *Cebidae* (capuchin monkeys and squirrel monkeys), *Aotidae* (owl monkeys), *Pitheciidae* (saki, ukari, and titi), and *Atelidae* (howler, spider and woolly monkeys).They are found in

Central and South America. Old world monkey or Cercopithecidae are found in Africa and Asia. The examples of the old world monkeys are Allen's swamp monkey, talapoin, macaque, mangabey, baboon, gelada, drill, colobus, langur, lutung, surili and douc. Ape is divided into 2 types: lesser ape (gibbon) and great ape (chimpanzees, bonobos, gorillas, and orangutans). The distribution of chimpanzee and gorila is Africa, while orangutan, bonobos and gibbon is Southeast Asia. To date, non-human primates are located in the limited natural habitats in tropical and subtropical area [Fig. 18].

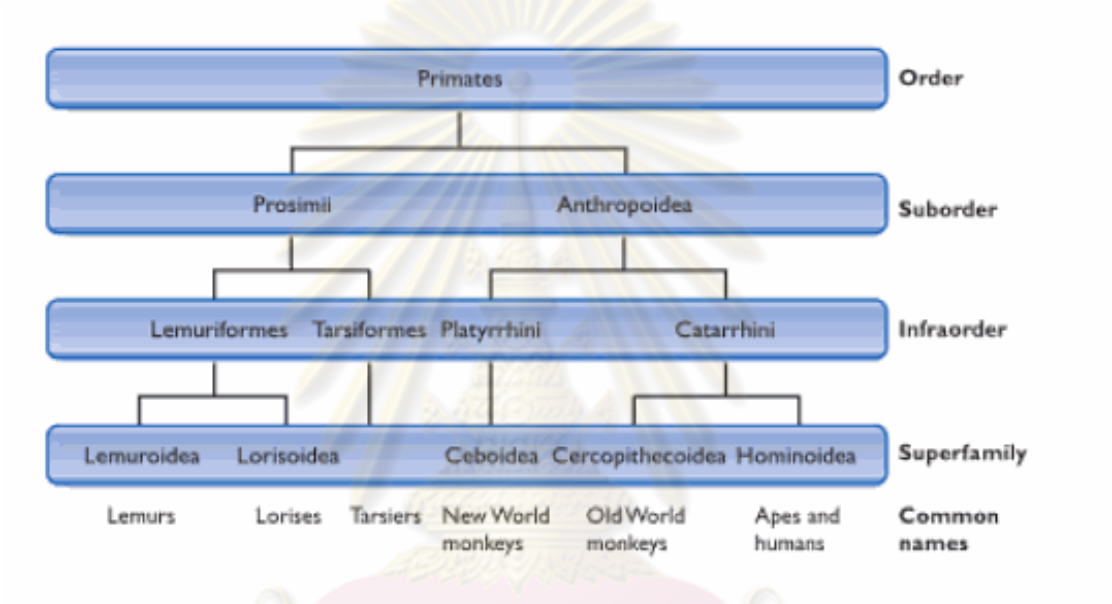


Fig. 17 The classification of primate [182].

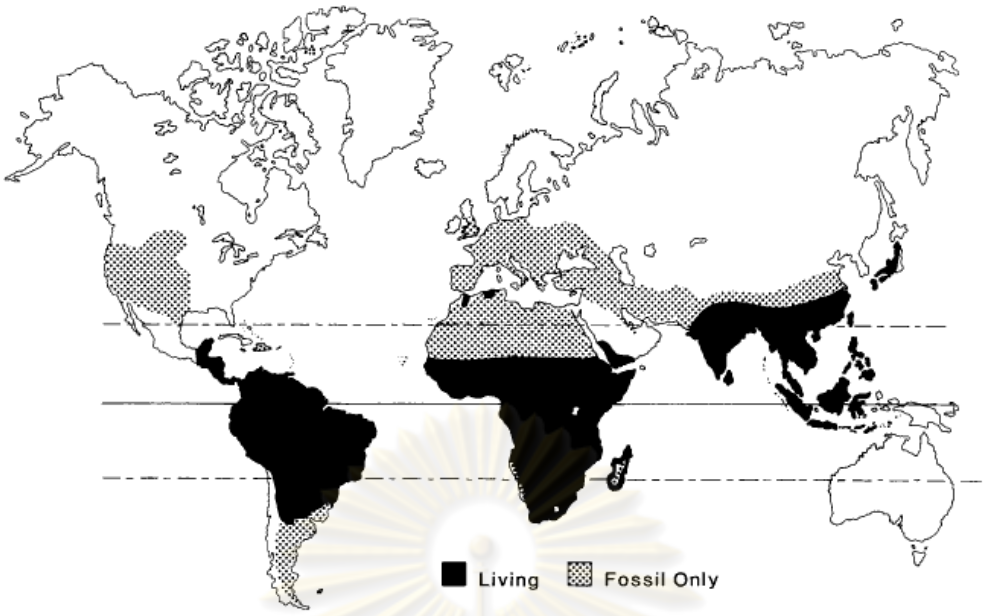


Fig. 18 The geographical distribution of extant non-human primates and extinct primate species [183].

7.2 Non-human primate in this study

7.2.1 Gibbons Gibbons are lesser apes. It belongs to the family *Hylobatidae*. This family comprises of 4 genera which are distinguished by diploid number of chromosomes (Table 6).

Table 6 Main divisions of the family *Hylobatidae* and their distribution [184].

Genus	Chromosome no. (2n)	Common name	Species	Distribution
Hylobates	44	Agile gibbon	*H. agilis*	Sumatra (Indonesia), Malaysia
		White-bearded gibbon	*H. albibarbis*	Southwest Borneo (Indonesia)
		Kloss's gibbon	*H. klossii*	Mentawai island (Indonesia)

Table 6 (continued)

Genus	Chromosome no. (2n)	Common name	Species	Distribution
		White-handed gibbon	*H. lar*	Malay peninsula, Thailand, Myanmar, Laos, Indonesia, Yunnan (China)
		Silvery gibbon	*H. moloch*	Java (Indonesia)
		Gray gibbon	*H. muelleri*	Borneo(Indonesia)
		Pileated gibbon	*H. pileatus*	East Thailand, West Cambodia
Hoolock	38	Western hoolock gibbon	*H. hoolock*	Assam, Bangladesh, Myanmar
		Eastern hoolock gibbon	*H. leuconedys*	Myanmar, west Yunnan
Nomascus	52	Black crested gibbon	*N. concolor*	Vietnam, Yunnan (China), Laos
		Hainan crested gibbon	*N. hainanus*	Hainan Island (China)
		Cao-vit crested gibbon	*N. nasutus*	Norteast Vietnam
		Yellow-cheeked gibbon	*N. gabriellae*	South Laos, South Vietnam, East Cambodia
		Southern whtie-cheeked gibbon	*N. siki*	South Laos, Central Vietnam
		Northern white-cheeked gibbon	*N. leucogenys*	North Laos, Northwest Vietnam, South Yunnan (China)
Symphalangus	50	Siamamg	*S. syndactylus*	Sumatra (Indonesia), Malay peninsula

Gibbon species used in this study are reviewed as below [184-190]:

Pileated gibbon (*Hylobates pileatus*)

Other names:	Capped, Crowned or Indo-Chineses lar gibbon
Distribution:	Cambodia, Laos and Thailand
IUCN status:	Vulnerable
Weight:	About 8 kg
Length:	Head to body 44 – 64 cm.
Habitat:	Rainforest, evergreen and mixed decidous – evergreen forest. Maximum age: 34 years
Description:	Pileated gibbon has long arms, tailless. Males are black with white hands and feet while, females have blondish fur with a black cap and chest.

White-handed gibbon (*Hylobates lar*)

Other names:	Lar gibbon
Distribution:	Sumatra, Malyasia, Myanmar, Thailand, China
IUCN status:	Endangered
Weight:	About 5 -6 kg
Length:	Head to body 45-53 cm
Habitat:	Forest and subtropical/tropical dry forest
Maximum age:	57 years
Description:	Tail-less and long-limbed like all other gibbons, this small ape has long and tapered hands. The coat is long and dense, varying in color from black to pale brown and yellowish. The face is black, surrounding by white hair. The tops of the hands and feet are white.

White-cheeked gibbon (*Nomascus leucogenys*)

Other names:	Chinese white-cheeked gibbon, northern white-cheeked gibbon, white-cheeked crested gibbon
Distribution:	China, Laos, Vietnam

IUCN status: Critically endangered

Weight: 6 kg

Length: 46 – 64 cm.

Habitat: Lowland forest

Maximum age: 44 years

Description: Males have black fur and black skin with white fur on their cheeks while, females are golden or reddish buff-colored with black faces and dark brown or black fur on top of their heads. Females do not have white cheek fur but have white fur around their faces.

Yellow-cheeked gibbon (*Nomascus gabriellae*)

Other names: Buff-cheeked crested gibbon, red-cheeked crested gibbon

Distribution: Vietnam, Laos, Cambodia

IUCN status: Endangered

Weight: 7.0 – 7.4 kg

Length: Head to body about 60 – 80 cm

Habitat: Tropical evergreen forest, lowland forest

Maximum age: Up to 50 years

Description: Adult male is black with small pale yellow or pale orange cheeks. Adult female has a bright yellow or pale orange with a black patch on the top of its head.

7.2.2 Orangutan (*Pongo pygmaeus*)

Distribution: Northern Sumatra, Indonesia, low-lying swamps in Borneo

IUCN status: Critically endangered

Weight: Female 50 kg, male 200 kg

Length: Standing hight 137 cm

Habitat: Tropical rain forest

Maximum age: Up to 50 years

Description: It is tail-less, with small ears and a small nose. The coat is long and soft, and reddish brown in color. The arched eyebrows are not very conspicuous, and the jaws are prominent. The head is pear-shaped, the eyes are small, and the lips are mobile. The arms are very long and strong, and its prehensile feet give it a 4-handed appearance. On the head of adult male there is a crest to which the powerful temporal muscles are attached.

7.2.3 Macaques

Long-tailed macaques (*Macaca fascicularis*)

Other names: Crab-eating macaque, cynomologus monkey, longtail macaque

Distribution: Philippines, Malaysia, Indonesia, Myanmar, India, Vietnam, Cambodia, Laos and Thailand

IUCN status: Last concern

Weight: Male: 4.7 to 8.3 kg , Female: 2.5 to 5.7 kg

Length: Male: 41.2 to 64.8 cm, Female: 38.5 to 50.3 cm

Habitat: Primary, secondary, coastal, mangrove, swamp and riverine forests from sea

Maximum age: 31 years

Description: There are various colors from light brown or grayish to brown fur covering backs, legs and arms. They have pinkish-brown faces and fur on their heads, often creating a crest of hair on the top of their heads. Both male and female have white coloring eyelids.

Southern pig-tailed macaques (*Macaca nemestrina*)

Other names:	Pig-tailed macaque, Sunda pig-tailed macaque, berok (Malay). Ling kaang (Thai)
Distribution:	Indonesia, Malaysia, Thailand, Bangladesh, India, China, Myanmar, Laos, Cambodia
IUCN status:	Vulnerable
Weight:	Male: 6.2 to 14.5 kg, Female: 4.7 to 10.9 kg
Length:	Male: 49.5 to 56.4 cm, Female: 46.7 to 56.4 cm
Habitat:	Lowland and hilly primary rainforests, swamp and secondary forest
Maximum age:	26 years
Description:	The fur on the top of their heads is dark brown or black and grown in the center of the top heads. They have olive brown fur over entire bodies, except for the underside which is white. Their tails are less than the length of the body from head to rump.

Stump-tailed macaques (*Macaca arctoides*)

Other names:	Bear macaque
Distribution:	China, India, Myanmar, Bangladesh, Malaysia, Thailand
IUCN status:	Vulnerable
Weight:	Male: 9.9 to 10.2 kg, Female: 7.5 to 9.1 kg
Length:	Male: 5.17 to 6.50 cm
Habitat:	Subtropical and tropical broadleaf evergreen forest
Maximum age:	30 years
Description:	They have thick, long, dark brown fur covering their bodies and short tails. They have bright pink or red faces. Males are much larger than females.

Rhesus macaques (*Macaca mulatta*)

Distribution:	Northern India, Afghanistan, Assam, Myanmar, China, Bhutan, Nepal, Thailand, Laos, Pakistan
IUCN status:	Least concern
Weight:	Male: 7.7 kg, Female: 5.34 kg
Length:	Male: 53 cm, Female: 47 cm
Habitat:	Forest, woodlands, rocky terrain, adapted to human environment, living near dwelling
Maximum age:	25 years
Description:	They has long, light brown coat, which is paler on the underside. The head is round, eyes are oval and the ears are small.

7.2.4 Langurs

Silvered langur (*Semnopithecus cristatus*)

Other names:	Silvered leaf monkey
Distribution:	Malay Peninsula, Sumatra, Borneo, Myanmar, Thailand, Cambodia, Vietnam
IUCN status:	Near threatened
Weight:	6.8 kg
Length:	49.3 to 57cm
Habitat:	Thick forest and tropical rain forest
Maximum age:	28 years
Description:	They have silvered fur due to the grey tips to their dark brown-black fur. The underside of tail is yellowish.

Phayre's langur (*Semnopithecus phayrei*)

Distribution:	Bangladesh, India, Myanmar, China, Thailand, Laos and Vietnam
IUCN status:	Endangered
Weight:	Male: 7.3 kg, Female: 6.2 kg
Length:	44 to 61 cm

Habitat: Evergreen forest

Maximum age: 28 years

Description: They have dark ashy-bluish brown on the dorsal side and whitish on the ventral side. The head and tail ends are darker than the rest of the body. The upper arms, legs, and tail are silvery gray. The lips and the area around eyes are whitish.

Dusky langur (*Semnopithecus obscurus*)

Distribution: Myanmar, India, Laos, Malaysia and Thailand

IUCN status: Near threatened

Weight: 7.08 kg

Length: 42 to 61 cm

Habitat: Closed primary forest, old-growth secondary forests, plantation forests and urban forest

Maximum age: 34 years

Description: The face is dark gray with white around the eyes and the center of the mouth. The back and limbs are gray, with dark gray hands and feet.

8. SEVERE COMBINED IMMUNODEFICIECY TRANSGENIC WITH UROKINASE-TYPE PLASMINOGEN ACTIVATOR MOUSE WITH HUMAN HEPATOCYTE

Up to now, the SCID-Alb-uPA mice with human hepatocytes are the best model for studies on human liver-specific pathogens such as HCV and HBV, human hepatic metabolism of pharmaceutical agent, and human hepatic toxicity of candidate antiproliferative agents [191]. The mice present evidence that more fully characterizes the repopulation of the mouse liver with human hepatocytes [192]. Histological study reveals that chimeric mice show the evidence of the integration component between mouse liver and human hepatocytes. Moreover, human albumin and 21 other human specific proteins can be detected in mice sera [193,194]. Discovery of the small powerful model was emerged by Brinster and his colleague [195]. In the experiment,

their mouse model of neonatal bleeding disorder carrying a urokinase-type plasminogen activator transgene controlled by an albumin promoter expressed urokinase overproduction leading to a bleeding phenotype and most of mice died from bleeding complication. Not all mice died at that time, they investigated the survival mice and found that bleeding phenotype had been lost [196]. From this evidence, the mice began to proliferate and replace their diseased parenchyma cell with normal hepatocytes. After that those mice were used to generate the woodchuck and human hepatocytes and they were able to support for infection with WHV and HBV [192,197]. The method that was used to construct the SCID-Alb-uPA mice with human hepatocyte was shown in Fig. 19.

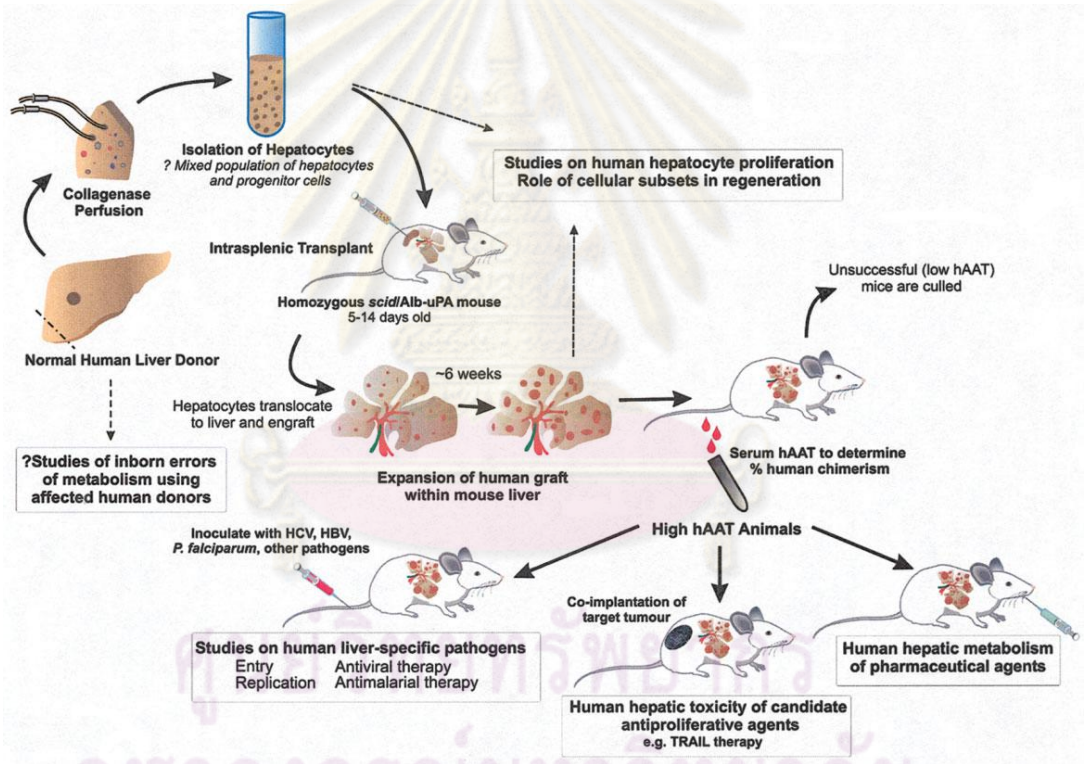


Fig. 19 Construction of the SCID-Alb-uPA mice [191].

CHAPTER III

MATERIALS AND METHODS

STUDY POPULATION

To investigate the potential reservoirs of HBV infection among captive non-human primates: 17 macaques (10 long-tailed macaques, *Macaca fascicularis*, 4 southern pig-tailed macaques, *Macaca nemestrina*, 2 stump-tailed macaques, *Macaca arctoides* and 1 rhesus macaque, *Macaca mulatta*), 20 gibbons (11 white-cheeked gibbons, *Nomascus leucogenys*, 1 yellow-cheeked gibbon, *Nomascus gabriellae*, 6 pileated gibbons, *Hylobates pileatus* and 2 white-handed gibbons, *Hylobates lar*), 8 langurs (4 silvered langurs, *Semnopithecus cristatus*, 1 Phayre's langur, *Semnopithecus phayrei*, and 3 dusky langurs, *Semnopithecus obscurus*) and 1 orangutan (*Pongo pygmaeus*) kept at Dusit zoo, Bangkok, 5 gibbons (3 white-cheeked gibbons, *Nomascus leucogenys,* 2 hybrid gibbon between white-cheeked gibbon and white-handed gibbon, *Nomascus leucogenys and Hylobates lar)* kept at Chiangmai zoo, Chiangmai and 53 orangutans (*Pongo pygmaeus*) kept at Khao Pratub Chang Wildlife Breeding Center, Ratchaburi, Thailand.

Additionally, eleven gibbons with HBsAg positive (7 pileated gibbons, *Hylobates Pileatus*, and 4 white-handed gibbons, *Hylobates lar*) from previous study (Year 2001) kept at the Krabok Koo Wildlife Breeding Center, Cha Choeng Sao were also used as subjects in this research project, which had been approved by the Faculty of Veterinary Science, Animal Care and Use Committee (FVS – ACUC), Mahidol University and the ethical committee of the Faculty of Medicine, Chulalongkorn University.

MATERIALS

1. Aluminum foil (Rainbow metal company, USA)
2. Barrier Tip: 20 – 200 (BioScience, USA)
3. Beaker: 5 ml, 50 ml, 100 ml, 200 ml, 500 ml, 1000 ml (Pyrex, England)
4. Combs (Bio-RAD, USA)

5. Cylinder: 25 ml (Pyrex, England)
6. Microcentrifuge tube: 0.2 ml, 1.5 ml (BioScience, USA)
7. Parafilm (Penchiney plastic packaging, USA)
8. Pipette rack (Eppendorf, Germany)
9. Pipette Tips: 10 µl, 200 µl, 1000 µl (BioScience, USA)
10. Polypropylene conical tube: 15 ml, 20 ml (Elkay, USA)
11. Reagent bottle: 250 ml, 500 ml, 1000 ml (Duran, Germany)
12. Stirring magnetic bar (V&P scientific, USA)

EQUIPMENTS

1. ABIPRISM™ 310 Genetic (Perkin-Elmer, USA)
2. Autoclave (Hydroclave MC10 Harvey, USA)
3. Balance (PB1520 Mettler Toledo, Switzerland)
4. Centrifuge (Beckman GS-6R, USA)
5. Chemical safety carbinet (Toxicap, France)
6. Class II microbiological safety carbinet (Envair, England)
7. Spectrophotometer (Eppendorf, Germany)
8. Cuvett (Eppendorf, Germany)
9. Deep Freezer -20°C (Sanyo, Japan)
10. Deep Freezer -80°C (Forma Scientific, USA)
11. Gel Doc 1000 (Bio-RAD, USA)
12. Incubator (Memmert, Germany)
13. Microcentrifuge 0.2 ml (Butterfly, Taiwan)
14. Microcentrifuge 1.5 ml (Denver, USA)
15. Multi-block heater (Lab-Line Instrument, USA)
16. PCR HEPA+ carbinet (LIO LAB, Thailand)
17. PCR safety carbinet (LIO LAB, Thailand)
18. Refrigerate microcentrifuge (Universal 16R Hettich, USA)
19. Refrigerator 4 °C (Mitsubishi, Japan)
20. Speed-Vac (Thomas Scientific, USA)
21. Stirring hot plate (Banstead/Thermolene, USA)

22. Thermal cycler (Eppendorf, MasterCycler, Germany)
23. LightCyCler™ Real-time PCR (Roach, Basel, Switzerland)
24. ABI 750 Fast Real-time PCR (Applied Biosystems, USA)
25. UV transilluminator (Fotyodyne, USA)
26. Water purification equipment (Yamato Scientific, Japan)

REAGENTS

1. Agarose molecular grade (Promega, USA)
2. Ethidium bromide (Sigma, Singapore)
3. Sucrose (USB, Hong Kong)
4. Bromphenal blue (Pharmacia, Hong Kong)
5. Boric acid (USB, Hong Kong)
6. Tris (USB, Hong Kong)
7. 100 base pair DNA ladder (Biolabs, USA)
8. Abosolute ethanol (Sigma, Singapore)
9. Chloroform (Sigma, Singapore)
10. Disodium ethylenediamine tetraacetic acid: EDTA (USB, Hong Kong)
11. Isoamyl alcohol (Sigma, Singapore)
12. Magnesium chloride (Sigma, Singapore)
13. Phenol (Singma, Singapore)
14. Proteinase K (Sigma, Singapore)
15. Sodium acetate (USB, Singapore)
16. Sodium chloride (USB, Hong Kong)
17. Tris-HCl (UDB, Hong Kong)
18. Eppendorf MasterMix (2.5X) (Eppendorf, Germany): Taq DNA polymerase (62.5 U/ml), 125 mM KCl, 75 mM Tris-HCl ph 8.3, 3.75 mM $Mg(Oac)_2$, 0.25% Igepal® - CA630, 500 µM of each dNTO and sterbilizer
19. 5 PRIME Mastermix (5 PRIME GmbH, Hamburg, Germany)
20. TaqMan® Universal PCR Master Mix (Applied Biosystems, USA)
21. Oligonucleotide primers (Proligo, Singapore)

22. SYBR Green (QIAGEN, Hilden, Germany)
23. Ampicillin (Phamacia, Hong Kong)
24. Isopropyl-1-thio-β-D-galactopyranoside: IPTG (Bio Basic, Germany)
25. Magnesium sulfate (Sigma, Singapore)
26. pGEM-T Easy Vector System (Promega, USA)
27. Tryptone (Giboco BRL, USA)
28. Yeast extract (Giboco BRL, USA)
29. 5-bromo-4-chloro-3-inodlyl- β-D-galactopyranoside: X-gal (Bio Basic, Germany)
30. ABI PRISM™ BigDye™ Terminator CyCle Sequencing Ready Reaction kit (Perkin-Elmer Applied Biosystems Division, Foster City, CA)
31. Gel Extraction kit (Eppendorf, Germany)

SOFTWARE AND BIOINFORMATIC PROGRAM

1. Chromas LITE ver. 2.01
2. ClustalX multiple alignment program ver. 2
3. SEQUENCHER® DNA sequence assembly software
4. ExPaSy translation tool
5. Molecular Evoultionary Genetics Analysis software (MEGA) ver. 4
6. BEAST package ver. 1.5.4
7. Tracer
6. FigTree

METHODS

1. Sample collection During the routine health check, all primates were anaesthetized. Animal blood samples were collected by venipuncture and transferred to EDTA anticoagulant coated test tubes. Plasma was separated by centrifugation at 3,000 rpm for 10 minutes and kept at -70°C until tested. The demographic data of the primates have been obtained from the records of the zoo and wildlife breeding center.

2. Serological method Plasma samples were subjected to a biochemical analyzer (Hitachi 912, Roche Diagnostic, Mannheim, Germany) for biochemical analysis of alanine amino-transferase (ALT) and aspartate transminase (AST) at Central Laboratory, King Chulalongkorn Memorial Hospital. Plasma was assayed for HBsAg, antibodies to HBsAg (anti-HBs), and antibodies to the HBV core antigen (anit-HBc) by enzyme linked immunosorbent assay (ELISA) using the Murex HBsAg Version 3, Murex anti-HBs and Murex anti-HBc kit, respectively (Murex, Biotech Limited, Dartford, Kent, England).

3. HBV DNA extraction HBV DNA was extracted from 100-µl plasma samples using proteinase K in lysis buffer (10 mM Tris-HCl ph 8.0, 0.1 M EDTA pH 8.0, 0.5% SDS and 20 mg/ml proteinase K) followed by phenol/chloroform extraction and ethanol precipitaion [144]. The DNA pellets were dissolved in 30 µl sterile distilled water.

4. HBV DNA detection Real-time PCR was performed to determine the quantitative HBV DNA levels as previously described [198]. Briefly, the HBV DNA standard plasmid was constructed by inserting the *preS* region (nt 2814-475) into pGem-T® Easy Vector through T-A cloning strategy. The concentration of the inserted plasmid was determined by measuring at OD_{260}. The serial dilution of the inserted plasmid from 10^2 to 10^{10} copies/µl was detected by real-time PCR method and used to prepare the standard curve for quantitative HBV DNA from the specimens. Primer which was used in this analysis allowed the amplification of a 280-bp product in *preS1* region. Sense primer (PreS1F+) and the antisense (PreS1R2) primer were shown in Table 7. The reaction mixture comprised with 1.0 µl of DNA sample, 5.0 µl of 2.5X MasterMix Solution, 0.5 µl of 25 mM Magnesium solution (5 PRIME Mastermix, 5 PRIME GmbH, Hamburg, Germany), 0.5 µl of 25 µM PreS1F+, 0.5 µl of PreS1R2, 0.2 µl of 10X SYBR Green (QIAGEN, Hilden, Germany) and distilled water was used in a final volume of 12.7 µl. Real-time PCR amplification was carried out in a LightCyCler™ (Roach, Basel, Switzerland). After a pre-incubation step at 95°C for 15 min in order to activate the Taq DNA polymerase, amplification was performed during 40 cycles including denaturation (94°C, 15s), annealing (60°C, 20s) and extension (72°C, 25s). A single fluorescent signal was obtained once per cycle at 78°C after the extension step. A standard curve

was created automatically in each run by plotting the threshold cycle number against the copy numbers of each standard, and HBV DNA quantitation of unknown samples was deduced from the regression line.

5. Whole genome amplification and sequencing HBsAg positive samples of non-human primates were subjected to complete HBV genome amplification by PCR using four primer sets selected from conserved regions so that the resulting amplicons overlapped contiguous fragments. The primer sequences of set one were PreS1F+ and R5; of set 2, F6 and X102; of set 3, X101 and CORE2 and of set 4; CORE1 and R1 (Table 7). The total 25- µl reaction mixture comprised 2 µl of a resuspended HBV viral DNA solution, 10 µl of 2.5X Eppendorf® MasterMix (Eppendorf, Hamburg, Germany), 0.5 µl of 25 µM primer, and sterile water. PCR amplification was performed under the following conditions: initial denaturation at 94°C for 30 s (denaturation), 55°C for 30 s (primer annealing), 72°C for 1.30 minutes (extension) and a final extension step at 72°C for7 minutes. PCR-amplified products were examined by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light. Subsequently, the bands of interest were purified applying the PerFectprep®Gel Cleanup kit (Eppendorf, Hamburg, Germany). Cycle sequencing was performed using the AmpliTaq™ DNA Polymerase FS dye terminator cycle sequencing chemistry of the ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer Applied Biosystems Division, Foster City, CA). The reaction was performed according to the manufacturer's specification. Nucleotide sequences were edited and assembled using SEQMAN (LASERGENE program package, DNASTAR) and submitted to the GenBank database.

Table 7 Primer sequences for HBV DNA detection and whole genome sequencing.

Name	Direction	Primer sequences (5' to 3')	Position
PreS1 F+	F	5'-GGG TCA CCA TAT TCT TGG GAA C-3'	2814-2835
PreS1 R2	R	5'-CCT GAG CCT GAG GGC TCC AC-3'	3094-3075
F3	F	5'-CTC GTG TTA CAG GCG GGG T-3'	191-209
F6	F	5'-ATA TGG ATG ATG TGG TAT TGG G-3'	737-758
FR5	F	5'-GAA TTG TGG GTC TTT TGG GCT-3'	995-1015
R3	R	5'-ACA AAC GGG CAA CAT ACC TTG-3'	475-455
R5	R	5'-AGC CCA AAA GAC CCA CAA TTC-3'	1015-995
RF1	R	5'-TGG CCC GAA TGC TCC CGC TCC T-3'	3042-3021
S2-1	F	5'-CAA GGT ATG TTG CCC GTT TG-3'	455-474
Xi1	F	5'-AGC TTG TTT TGC TCG CAG C-3'	1287-1305
Xi2	R	5'-CAG ATG AGA AGG CAC AGA C-3'	1569-1551
X101	F	5'-TCT GTG CCT TCT CAT CTG-3'	1552-1569
FMD	F	5'-GCA TGG AGA CCA CCG TGA AC-3'	1606-1625
FPC1	F	5'-GCC TTC TGA CTT CTT TC-3'	1957-1973
PC1	R	5'-GGA AAG AAG TCA GAA GGC-3'	1973-1957
CO2	R	5'-GTG AGG TGA ACA ATG TTC CG-3'	2053-2034
CORE1	F	5'-GAG TGT GGA TTC GCA CTC CTC C-3'	2268-2289
RCORE1	R	5'-GGA GGA GTG CGA ATC CAC ACT C-3'	2289-2268
CORE2	R	5'-CCC ACC TTA TGA GTC CAA GG-3'	2576-2457
FstX	F	5'-CAT GGC TGC TAG GYT GTG CT-3'	1373-1392
FCore2	F	5'-CCT TGG ACT CAY AAG GTG GG-3'	2457-2576

6. Phylogenetic analysis The two sequences of captive gibbon HBV (G25: accession no.AY077735 and G26: accession no. AY077736) determined in the course of our previous study [3] in the Krabok Koo Wildlife Breeding Center, and all HBV sequences obtained in the course of this study were aligned with each human genotype. Sequences were also compared with available complete genome sequences from chimpanzee, gibbon, orangutan, woolly monkey and gorilla. Phylogenetic and genetic comparisons of HBV isolates were performed applying the Clustal X version

2.0.10 multiple alignment program. Subsequent analysis was performed by Molecular Evolutionary Genetics Analysis (MEGA) software version 3.1. Amino acid translations were accomplished using the ExPASy translation tool (available from: http://www.expasy.ch/tools/dna.html).

7. Evolution study In this study, attempts were made to determine the evolutionary rate and clarify the origin of non-human primate HBV using molecular analysis.

7.1 Gibbon sequences On year 2008, four HBsAg positive gibbons (2 white-handed gibbons; *Hylobates lar* and 2 pileated gibbons; *Hylobates pileatus*) from the Krabok Koo Wildlife Breeding Center, Cha Cheng Sao and three HBsAg positive gibbons (one yellow-cheeked gibbon; *Nomascus gabriellae* and 2 white-cheeked gibbons; *Nomascus leucogenys*) from Dusit zoo, Bangkok were re-collected the sera. Complete HBV genome was amplified by PCR.

7.2 Estimating evolutionary rates For evolution rate study, seven pairs of gibbons HBV sequences on two time points were subjected to reconstruct the tree. The tree was built on the entire genome by Neighbor-Joining method on Tamura3 - parameter topology. Each pair of gibbon HBV genome was calibrated the molecular clock by MEGA software version 4.

7.3 Dating the origin of non-human primate HBV A reconstructed tree was constructed using the completed genome. To confirm the reliability of the phylogenetic tree, bootstrap resampling tests were also carried out 1,000 times. The origin of non-human primate HBV was estimated using the Bayesian Markov chain Monte Carlo (MCMC) approach available in the BEAST program (http://beast.bio.ed.ac.uk/Main_Page) [199]. Strict molecular clock was used in this calculation [200].

8. Cross-species transmission study in SCID/uPA mouse with human hepatocyte Chimeric mice were purchased from PhoenixBio Co, Ltd (Hiroshima, Japan). All methods were performed by using the company service. The highest DNA levels of HBV found in gibbon and orangutan samples were subjected to cross-transmission

study. The protocol of cross-species transmission study and detection was presented in Fig. 20. To quantitate HBV DNA level from SCID mice sera, HBV DNA was extracted from 10 µl mice sera by using QIAamp® DNA Mini kit (QIAGEN, Germany) as manufacturer's recommendation. The pellet was resuspended in the total volume 50 µl. Five microliters of DNA were subjected to quantitative HBV DNA by ABI 7500 Fast Real-time PCR (Applied Biosystems, USA). The reaction mixture was comprised of 12.5 µl TaqMan® Universal PCR MasterMix (Applied Biosystems, USA), 0.5 µl of 10µM forward primer (HBSF2: 5'-CTTCATCCTGCTGCTATGCCT-3'), 0.5 µl of 10µM reverse primer (HBSR2: 5'-AAAGCCCAGGATGATGGGAT-3'), 0.5 µl of 10µM probe (HBSP2: FAM-ATGTTGCC CGTTTGTCCTCTAATTCCAG-TAMRA) and 6 µl distilled water. The condition of Real-time PCR was performed in 95°C for 10 minutes (pre-denaturation), followed by 45 cycles of 95°C for 15 sec (denaturation) and 60°C for 30 sec (extension), and 4°C for holding step. A standard curve was created and HBV DNA quantitation of unknown samples was calculated by compared with standard curve.

Mice sera which positive for HBsAg were further study by amplifying the entire genome sequences. Briefly, one µl of DNA was used as template for first PCR. The entire genomes were distinguished into two segments: fragment A and fragment B. Fragment A was amplified by 10µM HBV17F-SARU forward primer (5'-CAAACTCTGCAAGATCCCAGAG-3') and 10µM HBV1799R-SARU reverse primer (5'-GACCAATTTATGCCTACAGCCTC-3'). Fragment B was amplified by 10µM HBV1595F-SARU forward primer (5'-CTTCACCTCTGCACGTTGCATGG-3') and 10µM HBV262R-SARU reverse primer (5'-CCACCACGAGTCTAGACTCTGTGG-3'). Both fragment A and fragment B used the same reaction mixture as follow: 5 µl of 10X buffer, 5 µl 25mM $MgCl_2$, 5 µl 2.5 mM dNTP, 0.33 µl LA-Taq (TaKaRa BIO INC, Japan) and 29.67 µl distilled water. The amplification was performed on GeneAmp® PCR System 9700 (Applied Biosystems, USA). The amplification program was continued as follow: 95°C for 2 min (pre-denaturation) and followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 2 min, and 72°C for 15 min (final extention).

For second PCR, two µl of first PCR was used as template. First PCR product of fragment A was nested by HBV47F-SARU forward primer (5'-CTGTATTTTCCTGCTGGTGGCTCCAG-3') and HBV1760R-SARU reverse primer (5'-TAACCTCGTCTCCGCCCCAAACTC-3'). First PCR product of fragment B was nested by HBV1608F-SARU (5'-GCATGGAGACCACCGTGAACG-3') and HBV201R-SARU (5'-TGTAACACGAGCAGGGGTCCTAGG-3'). Both fragment A and fragment B used all reaction mixtures as same as the first PCR except increasing the first PCR template to 2 µl and adjusting distilled water to 28.67 µl. The amplification program was performed as follow: 95°C for 2 min (pre-denaturation) and followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 2 min, and 72°C for 20 min (final extention).

Second PCR products were segregated by electrophoresis on 1% agarose gel stained with ethidium bromide. The bands of PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN, Germany). Purified products were further analysed by sequencing (ABI PRISM3100 Genetic Analyzer, Applied Biosystems, USA). Cycle sequencing was performed using BigDye Terminator 3.1V cycle sequencing kit (Applied Biosystems, USA) as manufacturer's suggestion. The condition of sequencing PCR was set on GeneAmp® PCR System 9700 (Applied Biosystems, USA) as follow: 95°C for 2 minutes, followed by 25 cycles of 95°C for 10 sec (denaturation), 50°C for 5 sec (annealing) and 60°C for 4 sec (extension), and 4°C for holding step.

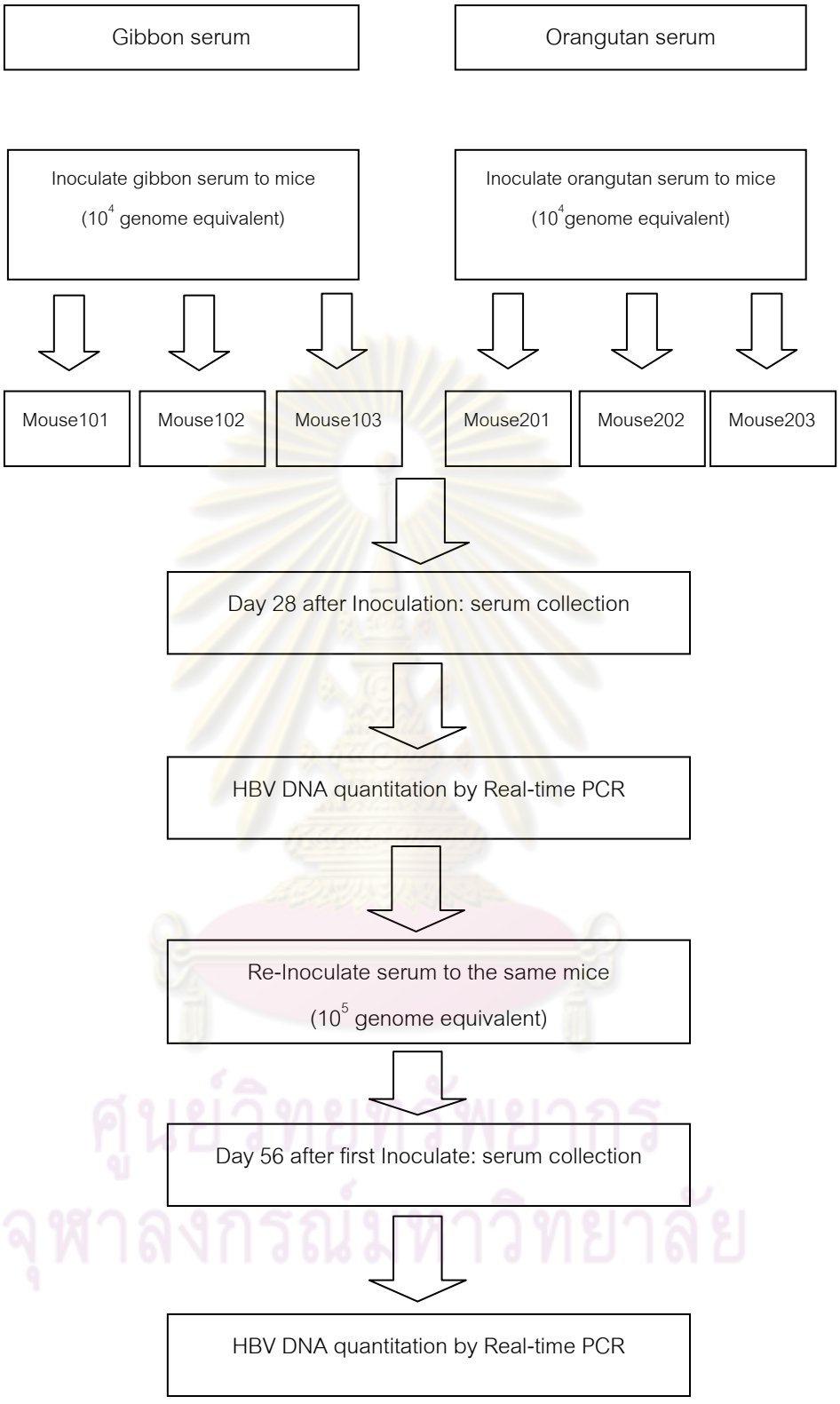


Fig. 20 Diagram of cross-species transmission study in SCID mice with human hepatocytes

CHAPTER IV

RESULTS

SEROPREVALENCE OF HBV IN NON-HUMAN PRIMATES

One hundred and four plasma samples taken from 40 various non-human primates (year 2004), five gibbons and one orangutan (year 2008) from Dusit, zoo, 53 orangutans from Khao Pratub Chang Wildlife Breeding Center, Ratchaburi (year 2006) and five gibbons from Chiangmai zoo, Chiangmai (year 2008) were tested for the presence of HBsAg, anti-HBs and anti-HBc antibodies. Sera positive for at least one marker of HBV infection were found in gibbons (10/25; 40%) and orangutans (40/54; 74.07%). The results were shown in Table 8. Moreover, five gibbons and seven orangutans were identified as chronic carriers. To determine the liver pathology associated with this infection, ALT and AST levels were determined in the plasma of four HBsAg positive gibbons and 36 HBsAg negative animals. With one exception (GD14), the ALT and AST levels upon comparison with HBsAg negative animals were evaluated (Table 9). The mean ± SD ALT and AST values in HBsAg negative animals were 33.64 ± 17.56 and 34.82 ± 15.15, respectively. Approximately 50% (5/10) of gibbons and 82.5% (33/40) of orangutans were non-carrier animals, as indicated by the presence of anti-HBs and anti-HBc antibodies.

DETECTION OF HBV DNA IN NON-HUMAN PRIMATES

To quantify HBV DNA in non-human primates, available plasma samples collected from various species were screened by real-time PCR according to the previously described method [198]. Eleven HBsAg positive gibbons from Krabok Koo Wildlife Breeding Center were also detected by real time PCR. The limitation of this method is 100 copies per microliter. The results from real-time PCR perfectly correlated with the results obtained by HBsAg screening. The levels of HBV DNA were shown in Table 9.

Table 8 Seroprevalence of HBV and HBV DNA among captive non-human primates.

Primate (n)	n	Positive HBV marker			
		HBsAg and anti-HBc n (%)	anti-HBs and anti-HBc n (%)	anti-HBc only n (%)	HBV DNA n (%)
1.Macaque (17)					
Macaca fascicularis	10	0 (0)	0 (0)	0 (0)	0 (0)
Macaca nemestrina	4	0 (0)	0 (0)	0 (0)	0 (0)
Macaca arctoides	2	0 (0)	0 (0)	0 (0)	0 (0)
Macaca mulatta	1	0 (0)	0 (0)	0 (0)	0 (0)
2. Langur (8)					
Semnopithecus cristatus	4	0 (0)	0 (0)	0 (0)	0 (0)
Semnopithecus phayrei	1	0 (0)	0 (0)	0 (0)	0 (0)
Semnopithecus obscurus	3	0 (0)	0 (0)	0 (0)	0 (0)
3. Gibbon (25)					
Nomascus leucogenys	14	3 (21.4)	3 (21.4)	0 (0)	3 (27.3)
Nomascus gabriellae	1	1 (100)	0 (0)	0 (0)	1 (100)
Hylobates pileatus	6	1 (16.7)	1 (16.7)	0 (0)	1 (16.7)
Hylonates lar	2	0 (0)	1 (50)	0 (0)	0 (0)
Hybrid*	2	0 (0)	0 (0)	0 (0)	0 (0)
4. Orangutan (54)					
Pongo pygmaeus	54	7 (13.0)	25 (46.3)	8 (14.8)	7 (13.0)
Total	104				

* *Nomascus leucogenys* and *Hylobates lar*

Table 9 Demographic data, ALT, AST, HBV serological markers and HBV viral load of captive non-human primates

No	Species	Sex	Age	Cage	Code	GenBank accession no.	ALT (U/l)	AST (U/l)	HBsAg	Anti-HBs	Anti-HBc	HBV DNA	HBV DNA (copies/µl)
1	*N. leucogenys*	F	ND	-	GD4	-	ND	ND	+	-	+	+	-
2	*N. gabriellae*	M	8	-	GD13	-	17	14	+	-	+	+	2.830×10^6
3	*N. leucogenys*	F	21	-	GD14	EU155828	79	75	+	-	+	+	2.002×10^7
4	*N. leucogenys*	M	11	-	GD21	EU155829	18	14	+	-	+	+	3.085×10^7
5	*H. pileatus*	F	ND	-	GD22	-	16	9	+	-	+	+	5.260×10^6
6	*P. pygmaeus*	M	6-8	3/10	OS6	EU155821	ND	ND	+	-	+	+	3.660×10^6
7	*P. pygmaeus*	F	3-4	3/10	OS9	EU155822	ND	ND	+	-	+	+	1.875×10^6
8	*P. pygmaeus*	M	5	3/6	OS23	EU155823	ND	ND	+	-	+	+	4.680×10^5
9	*P. pygmaeus*	M	5	3/5	OS25	EU155824	ND	ND	+	-	+	+	7.070×10^6
10	*P. pygmaeus*	M	6-8	3/5	OS27	EU155825	ND	ND	+	-	+	+	1.210×10^7
11	*P. pygmaeus*	F	6-8	3/5	OS28	EU155826	ND	ND	+	-	+	+	5.440×10^6
12	*P. pygmaeus*	M	5	3/1	OS39	EU155827	ND	ND	+	-	+	+	8.250×10^5

ALT and AST normal range: 0 -40 U/l. ALT and AST (Mean $\pm$ SD) values in HBsAg negative animals were 33.64 $\pm$ 17.56 and 34.82 $\pm$ 15.15. ND, no data.

GIBBON AND ORANGUTAN HBV NUCLEOTIDE SEQUENCES

To complete the HBV genomes of twelve non-human primates expressing HBsAg and positive for HBV DNA by real-time PCR, DNA from five gibbons *(*3 white-cheeked gibbons; *Nomascus lecogenys*: 1 yellow-cheeked gibbon; *Nomascus gabriellae*: 1 pileated gibbon; *Hylobated pileatus)* and from seven orangutans (*Pongo pygmaeus*) were amplified by conventional PCR. Moreover, DNA samples obtained from 11 HBsAg positive gibbons (7 pileated gibbons; *Hylobated pileatus*: 4 white-handed gibbons; *Hylobates lar*) from Krabok Koo Wildlife Breeding Center (year 2001) were also amplified. The details of HBsAg positive gibbons from Krabok Koo Wildlife Breeding Center are shown in Table 10. All gibbon and orangutan HBV comprised 3,182 nucleotides and showed genetic organization compatible with the human virus. The nucleotide sequences determined in this research study have been submitted to the GenBank database and assigned accession numbers EU155821 - EU155827 (Oragutan), EU155828 - EU155829 (Gibbon). All gibbon and orangutan HBV sequences were compared with the representative sequences in GenBank including the human HBV genotype B, C, D and J, orangutan, gibbon, chimpanzee, gorilla and woolly monkey sequences.

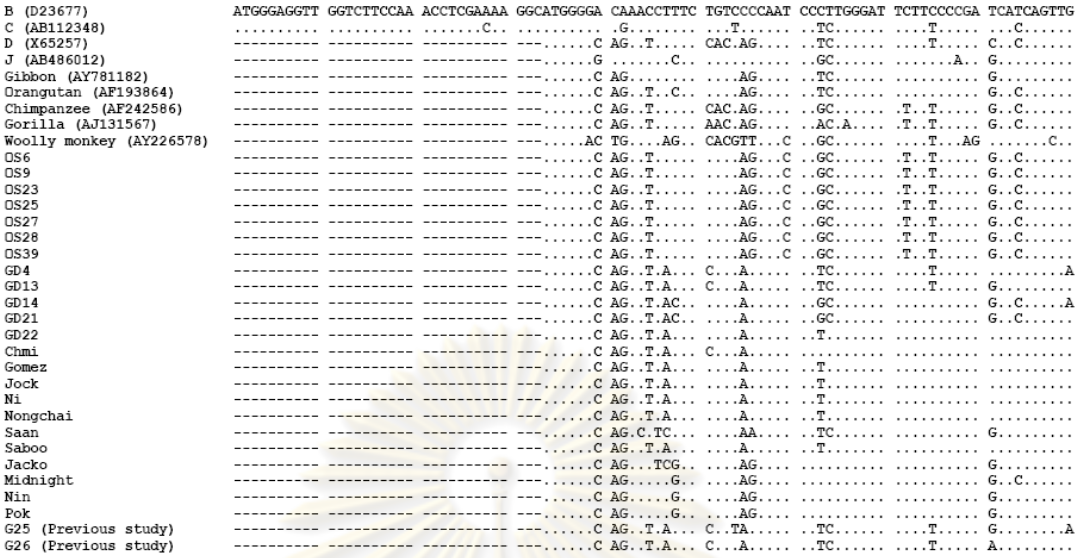
PreS/S gene: The nucleotides of the *PreS/S* region were aligned in order to establish nucleotide and amino acid differences between HBV isolates from gibbons, orangutans and human HBV genotypes B, C, D and J. Moreover, the GiHBV and OuHBV sequences were compared with other non-human primate HBV strains (Fig. 21A). HBV isolated from gibbons and orangutans in this study had a deletion of 33 nucleotides at the 5' end of the *preS1* region as had been established by previous studies. Based on the percentage of similarity, the highest percentage of similarity fell within each respective group, GiHBV and OuHBV displayed a higher percentage of similarity than to the human HBV (data not shown).

Table 10 Demographic data and HBV viral load of HBsAg positive gibbons from Krabok Koo Wildlife Breeding Center (Year 2001)

No	Species	Sex	Age	Cage	Code	ALT (U/l)	HBsAg	Anti-HBs	Anti-HBc	HBV DNA	HBV DNA (copies/µl)
1	*H. pileatus*	M	16	C20	Jock	46	+	-	+	+	4.05×10^7
2	*H. pileatus*	M	12	C16	Nong Chai	50	+	-	+	+	2.68×10^7
3	*H. pileatus*	M	11	C15	Saboo	207	+	-	+	+	4.24×10^7
4	*H. pileatus*	F	10	C15	Ni	70	+	-	+	+	3.71×10^7
5	*H. pileatus*	M	11	C14	Gomez	93	+	-	+	+	1.41×10^7
6	*H. pileatus*	F	11	C14	Chmi	87	+	-	+	+	6.92×10^6
7	*H. pileatus*	M	18	C13	Saan	46	+	-	+	+	2.99×10^7
8	*H. lar*	M	10	C2	Pok	39	+	-	+	+	4.33×10^7
9	*H. lar*	M	ND	R4	Jacko	50	+	-	+	+	7.27×10^7
10	*H. lar*	M	12	R27	Midnight	ND	+	-	+	+	6.51×10^7
11	*H. lar*	M	5	L14	Nin	ND	+	-	+	+	2.56×10^8

ALT and AST normal range: 0 -40 U/l. ND, no data.

(A) *PreS1* region



(B) *C* region

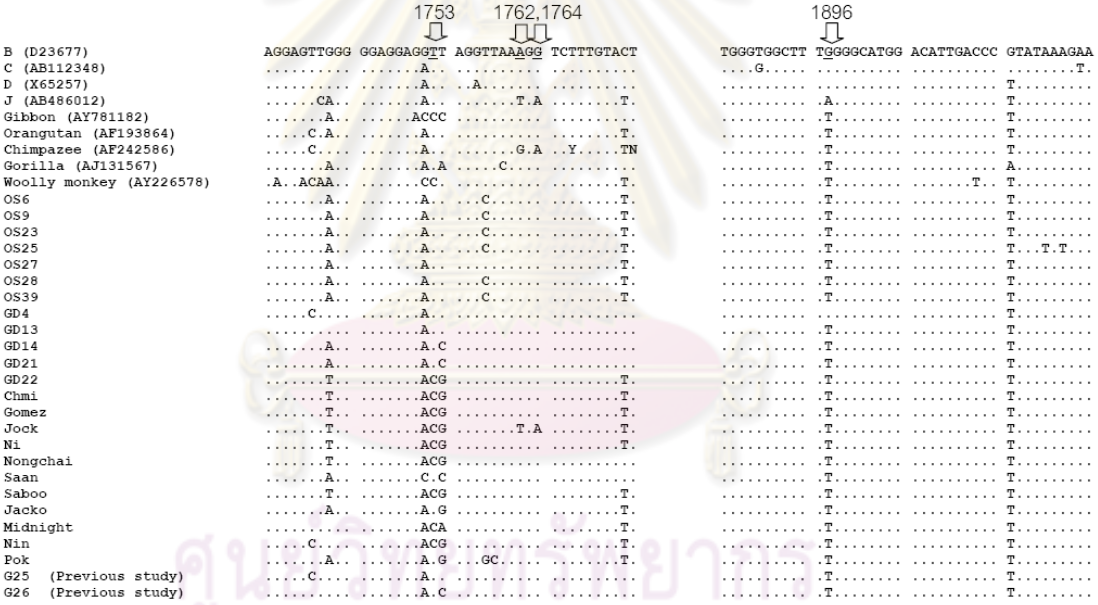


Fig. 21 *PreS* (A) and Core (B) alignment. All sequences were compared to human HBV genotype B, C, D, J and non-human primate HBV. Dot (.) indicates identical nucleotide. Dashes (-) indicate deletion nucleotide. GenBank accession numbers are given follow HBV strains.

The nucleotide sequences of 16 gibbons from this study, two gibbons from the previous study (G25 and G26) [3], seven orangutans and human HBV genotypes B, C, D and J were translated into amino acid sequences (Fig. 22A). GD4 and G25 harbored an insertion of Gln (Q) between Gly^{83} (G) and Ile^{84} (I). In addition, several mutations were found in the PreS2 and S regions, the deletion or insertion in these regions were not found in any strains. On closer investigation, the only altered amino acids found in the PreS1 region of orangutan and gibbon were Leu^{33} (L) and Thr^{56} (T), and in the S region, was Ile^{213} (I). The "a" determinant in the S region of HBsAg that has proven essential for induction of a protective immune response contains Gly145 in all non-human primate HBVs. This finding suggests that the recombinant HBV vaccine may prevent infection by non-human primate HBVs (Fig. 23)

PreC/C gene: The *PreC/C* gene sequences were less divergent than the *PreS/S* gene. In the core promoter region, a T1753A mutation was found in GD22, Chmi, Gomez, Ni, Nongchai, Saboo, Midnight and Nin, while T1753A/A1762T/G1764A was found in Jock. In the *PreC* region, there was no mutation at nucleotide positions 1896 and 1899 (G1896A and G1899A) (Fig. 21B). Yet, all gibbon and orangutan sequences except for GD4 showed a G to T mutation at position 1896. This mutation induced an amino acid change from Trp (W) to Leu (L) at amino acid residue 28 of the PreC region. The nucleotides at positions 2174 to 2413 of the core region were highly conserved (data not shown); the resulting amino acid sequences were similar for all isolates. Alignment of the core protein amino acids showed that the amino acids in this region are highly conserved among OuHBV, GiHBV and human HBV. The only non-human primate (orangutan and gibbon) amino acids are Leu28 in the PreC region and Asn51 (N), Ser70 (S), Pro179 (P) and Ala180 (A) in the C region (Fig.22B)

Complete HBV genome: All sequences were analyzed by comparison with each of the human HBV genotypes A-H. The results showed that all isolates were 98-99% identical within the orangutan group and 93-98% within the gibbon group. Comparison between gibbon and orangutan sequences showed 90-91% identity (data not shown).

Fig. 22A PreS/S protein

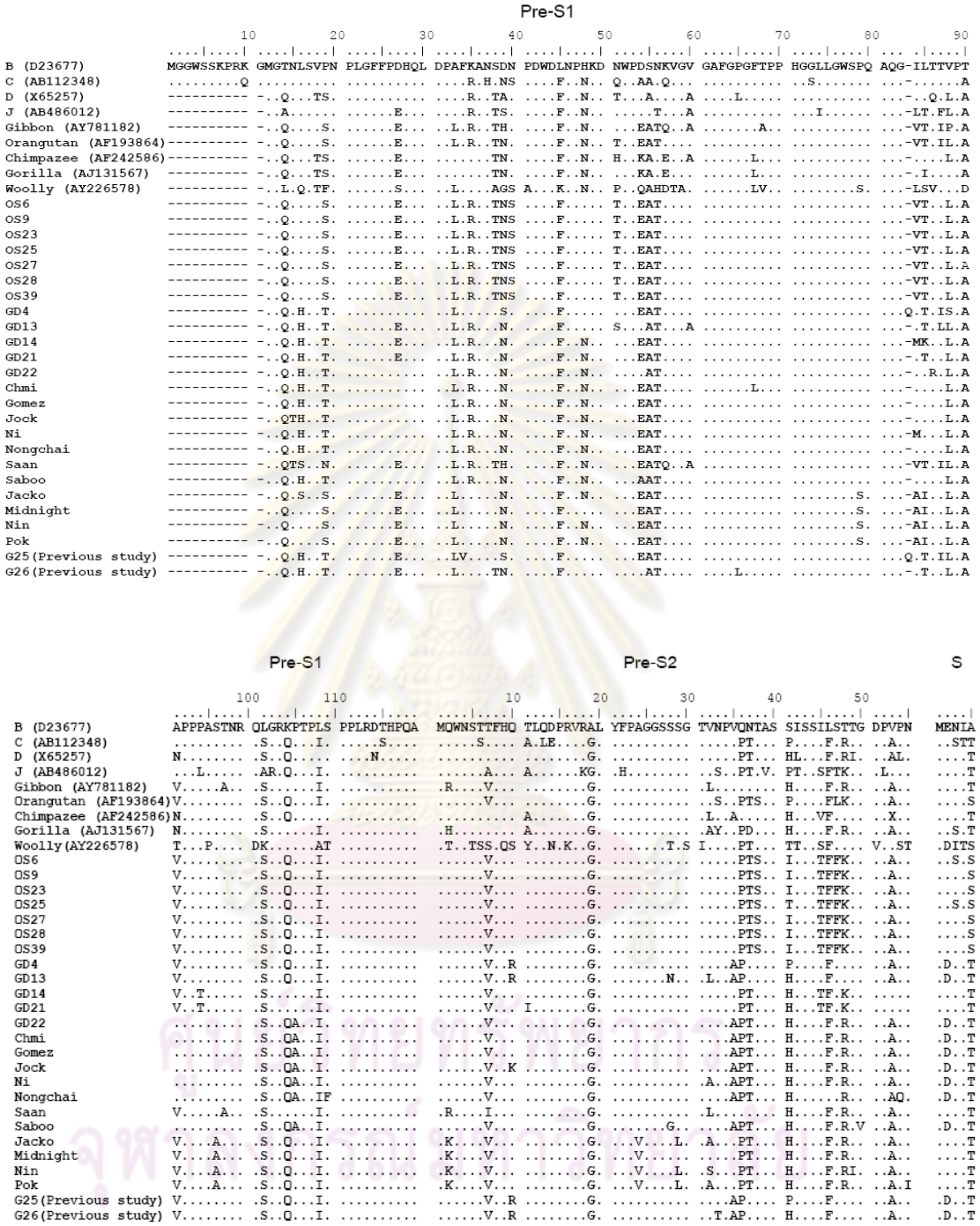


Fig. 22A (continued)

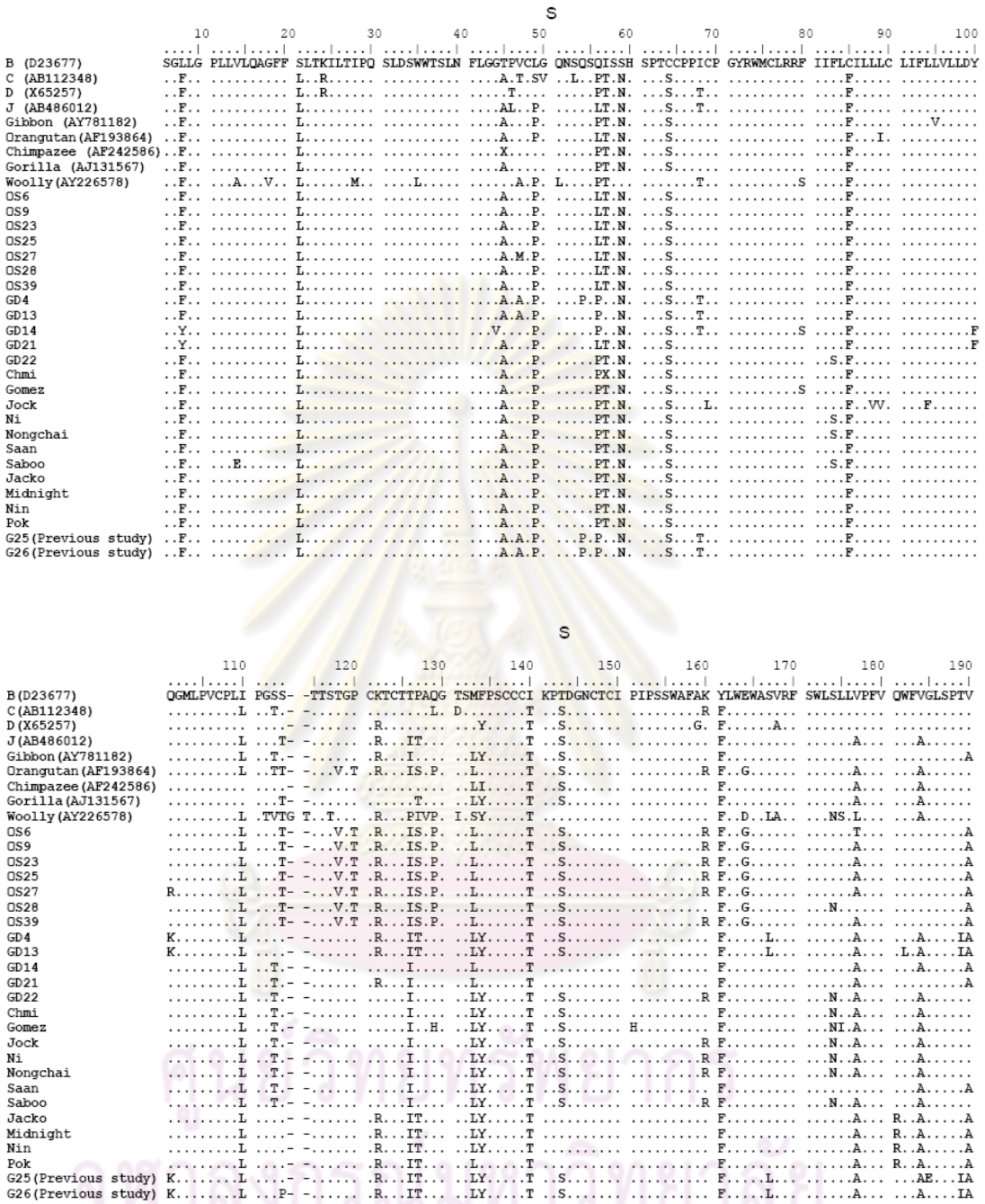


Fig. 22A (continued)

S

```
                          200        210        220
                    ....|....| ....|....| ....|....| ....|.
B (D23677)          WLSVIWMMWF WGPSLYNILS PFMPLLPIFF CLWAYI
C (AB112348)        .........Y .........N ..L....... ...V..
D (X65257)          .........Y ......S... ..L....... ...V..
J (AB486012)        .......I.Y .........N ..I....... ...V..
Gibbon(AY781182)    .......T.X ...N.....N ..I....... ......
Orangutan(AF193864) ..L....I.Y ...N.F.... ..I....... ......
Chimpazee(AF242586) .......T.. .......... ..I....... ...V..
Gorilla(AJ131567)   .........Y ...N...... ..I....... ...V..
Woolly(AY226578)    ..L....... .....FS... ..L....L.. W.....
OS6                 ..L....I.Y .......... ..I....... ......
OS9                 ..L....I.Y .......... ..I....... ......
OS23                ..L....I.Y .......... ..I....... ......
OS25                ..L......Y .......... ..I....... ......
OS27                ..L....I.Y .......... ..I....... ......
OS28                ..L....I.Y .......... ..I....... ......
OS39                ..L....I.Y .......... ..I....... ......
GD4                 .PLA.....Y .........N ..I....... ...V..
GD13                .........Y .........N ..I....... ...V..
GD14                ..LA...I.Y .........K ..I....... ...V..
GD21                ..LA...I.Y .........K ..I....... ...V..
GD22                ..L......Y .........N ..I....... ...V..
Chmi                ..L......Y .........N ..I....... ...V..
Gomez               ..L......Y .........N ..I....... ...V..
Jock                ..L......Y .........N ..I....... ...V..
Ni                  ..L......Y .........N ..I....... ...V..
Nongchai            ..L......Y .........N ..I....F.. ...V..
Saan                ..L....T.Y ...N.....N ..I....... ...V..
Saboo               ..L......Y .........N ..I.....P. SGRV..
Jacko               ..L....T.Y ...N.....N ..I....... ...V..
Midnight            ..L....I.Y ...N.....N ..I....... ...V..
Nin                 ..L....I.Y ...N.....N ..I....... ...V..
Pok                 ..L....I.Y ...N.....N ..I....... ...V..
G25 (Previous study).PL......Y .........N ..I....... ...V..
G26 (Previous study).PL......Y .........N ..I....... ...V..
```

Fig. 22B PreC/C protein

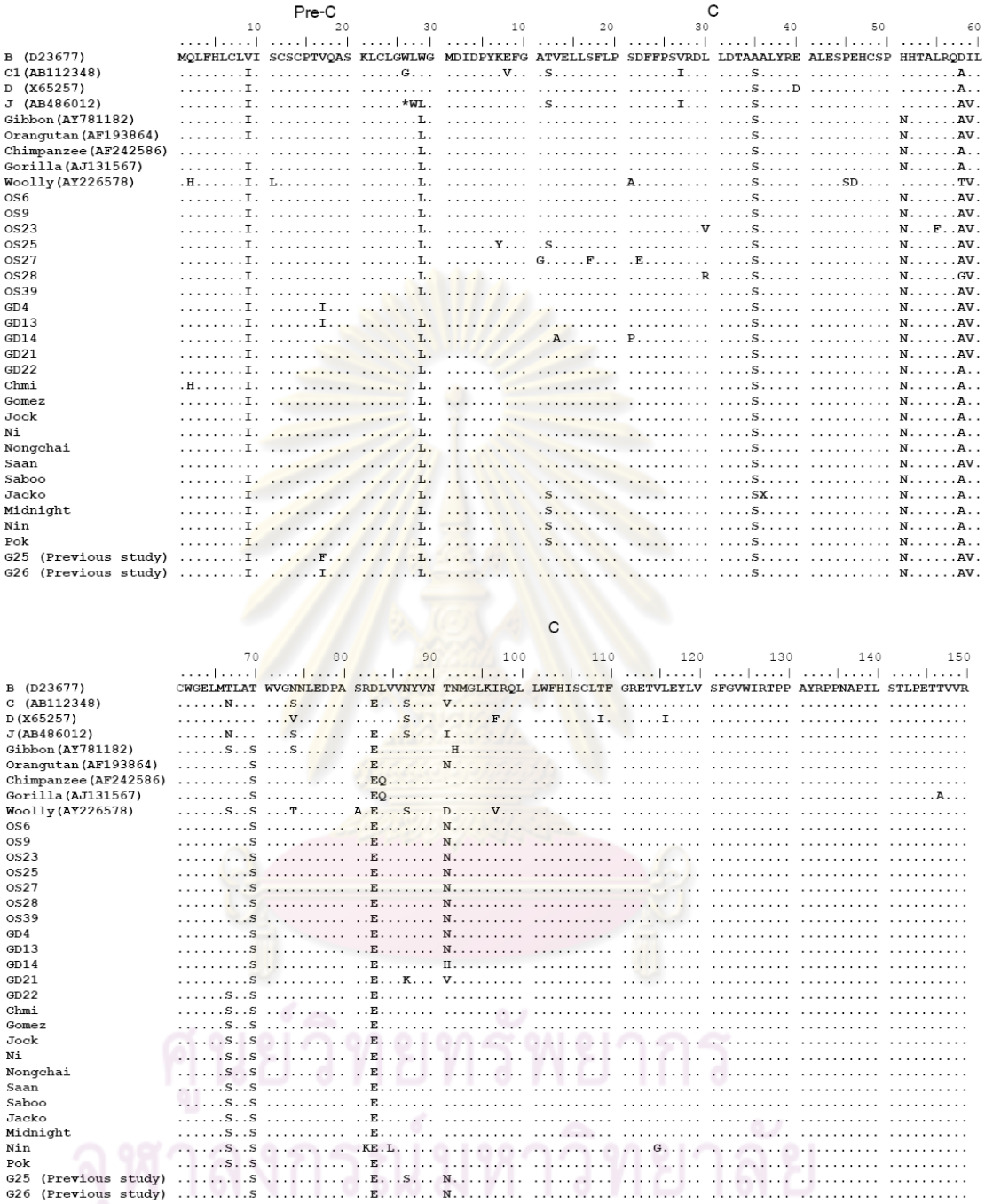


Fig. 22B (continued)

C

```
                          160         170         180
                     ....|....| ....|....| ....|....| ...
B (D23677)           RRGRSPRRRT PSPRRRRSQS PRRRRSQSRE SQC
C (AB112348)         .......... .......... .......... ...
D (X65257)           .......... .......... .......... ...
J (AB486012)         .......... .......... ........PS ...
Gibbon(AY781182)     .......... .......... ........PA P..
Orangutan(AF193864)  .......... .......... ........PA ...
Chimpanzee(AF242586) .......... .......... ........PA ...
Gorilla(AJ131567)    .......... .......... ........PA ...
Woolly(AY226578)     ..-.PSG... .......... ........PA .S.
OS6                  .......... .......... ........PA ...
OS9                  .......... .......... ........PA ...
OS23                 .......... .......... ........PA ...
OS25                 .......... .......... ........PA ...
OS27                 .......... .......... ........PA ...
OS28                 .......... .......... ........PA ...
OS39                 .......... .......... ........PA ...
GD4                  .......... .......... ........PA ...
GD13                 .......... .......... ........PA ...
GD14                 .......... .......... ........PA ...
GD21                 .......... .......... ........PA ...
GD22                 .......... .......... ........PA ...
Chmi                 .......... .......... ........PA ...
Gomez                .......... .......... ........PA ...
Jock                 .......... .......... ........PA P..
Ni                   .......... .......... ........PA ...
Nongchai             .......... .......... ........PA ...
Saan                 .......... .......... ........PA P..
Saboo                .......... .......... ........PA ...
Jacko                .......... .......... ........PA ...
Midnight             .......... .......... ........PA ...
Nin                  .......... .......... ........PA ...
Pok                  .......... .......... ........PA ...
G25 (Previous study) .......... .......... ........PA ...
G26 (Previous study) .......... .......... ........PA ...
```

Fig. 22 Alignment of amino acid sequences of the complete *S* gene (PreS1, PreS2 and S domain) (A) and complete *C* gene (PreC and Core domain) (B) from 18 gibbons (GD4, GD13, GD14, GD21, GD22, Chmi, Gomez, Jock, Ni, Nongchai, Saan, Saboo, Jacko, Midnight, Nin, Pok, G25 and G26) and seven orangutans (OS6, OS9, OS23, OS25, OS27 and OS39) with human HBV genotypes B, C, D and J. Dots indicated conserved amino acids. Dashes indicate deletion amino acids. Changing amino acids were indicated in letters. A master sequence based on the comparison was shown in the upper line. The percentages of similarity of the compete *S* genes were 99% within the orangutan group, 94% within the gibbon group, 89-91% between the orangutan and human HBV, 89-92% between the gibbon and human HBV and 91-92% between the orangutan and human HBV, 90-93% between the gibbon and human HBV and 92-94% similarity between the orangutan and gibbon.

```
B(D23677)                PCKTCTTPAQ GTSMFPSCCC IKPTDGNCTC IPIPSSWAFA K
C(AB112348)              .........L .D........ T..S...... .......... R
D(X65257)                ..R..... ....Y..... T..S...... .........G .
J(AB486012)              ..R...IT.. .......... T..S...... .......... .
Gibbon(AY781182)         ..R...I... ...LY..... T..S...... .......... .
Orangutan(AF193864)      T.R...IS.P ...L...... T..S...... .......... R
Chimpazee(AF242586)      .......... ...LI..... T..S...... .......... .
Gorilla(AJ131567)        .......T.. ...LY..... T..S...... .......... .
Woolly(AY226578)         ..R...PIVP .I.SY..... T......... .......... .
OS6                      T.R...IS.P ...L...... T..S...... .......... R
OS9                      T.R...IS.P ...L...... T..S...... .......... R
OS23                     T.R...IS.P ...L...... T..S...... .......... R
OS25                     T.R...IS.P ...L...... T..S...... .......... R
OS27                     T.R...IS.P ...L...... T..S...... .......... R
OS28                     T.R...IS.P ...L...... T..S...... .......... .
OS39                     T.R...IS.P ...L...... T..S...... .......... R
GD4                      ..R...IT.. ...LY..... T..S...... .......... .
GD13                     ..R...IT.. ...LY..... T..S...... .......... .
GD14                     ......I... ...L...... T......... .......... .
GD21                     ..R...I... ...L...... T......... .......... .
GD22                     ......I... ...LY..... T..S...... .......... R
Chmi                     ......I... ...LY..... T..S...... .......... .
Gomez                    ......I..H ...LY..... T..S...... .H........ .
Jock                     ......I... ...LY..... T..S...... .......... R
Ni                       ......I... ...LY..... T..S...... .......... R
Nongchai                 ......I... ...LY..... T..S...... .......... R
Saan                     ......I... ...LY..... T..S...... .......... .
Saboo                    ......I... ...LY..... T..S...... .......... R
Jacko                    ..R...IT.. ...LY..... T......... .......... .
Midnight                 ..R...IT.. ...LY..... T......... .......... .
Nin                      ..R...IT.. ...LY..... T......... .......... .
Pok                      ..R...IT.. ...L...... T......... .......... .
G25 (Previous study)     ..R...IT.. ...LY..... T..S...... .......... .
G26 (Previous study)     ..R...IT.. ...LY..... T..S...... .......... .
```

Fig. 23 S protein alignment of human HBV genotypes and non-human primate HBVs. The underlined nucleotides indicate the “a” determinant. The arrow indicates specific glycine.

PHYLOGENETIC ANALYSES OF GIBBON AND ORANGUTAN HBV

To determine the phylogenetic relationships, phylogenetic trees of the *PreS/S*, *PreC/C*, *P*, *X* region and the complete nucleotide sequences were constructed.

Complete HBV genome: The complete HBV sequences of non-human primates were compared with sequences representative for each group of human HBV genotype, orangutan, gibbon, gorilla, chimpanzee, and woolly monkey HBV in the GenBank database (Fig. 24A). The woolly monkey sequences were used as an out group. The data support that each of the human genotypes clustered separately from

non-human primates whereas all sequences obtained from gibbons and orangutans in this study can be grouped with previously published GiHBV and OuHBV sequences. The novel gibbon sequences clustered as a subgroup with the gibbon sequences previously obtained.

PreS/S gene: This phylogenetic tree comprises the *PreS/S* nucleotide sequences of OuHBV and GiHBV isolates from the present project, representative of non–human primates and of each human HBV genotype from GenBank. All *PreS/S* sequences including gibbon sequences from our previous study were examined by neighbor joining analysis. The results were shown in Fig. 24B. Furthermore, the HBV isolates from the gibbons (GD4, GD13, GD14, GD21, GD22, Chmi, Gomez, Jock, Ni, Nongchai, Saan, Saboo, Jacko, Midnight, Nin, Pok, G25 and G26) were found distantly (91-93%) related to the OuHBV sequences (OS6, OS9, OS23, OS25, OS27, OS28, and OS39). OuHBV in this study clustered with orangutan from Indonesia (AF193864) by 100% bootstrap value.

PreC/C gene: The results of phylogenetic analysis of the *PreC/C* gene were similar to the *PreS/S* gene. The *PreC/C* gene was on branches separate from each human genotype (Fig. 24C). The GiHBV sequence determined by previous research branched most closely with GD4 and G25. The bootstrap values were 93%. In contrast, GD14 and G21 were different from the GiHBVs of the preceding study. GiHBVs from Krabok Koo Wildlife Breeding Center could be divided into 3 branches. The first branch includes GiHBV strains from Nongchai, Ni, Jock, Gomez, Saboo, Chmi and GD22 strain from Dusit zoo, the second one GiHBV strains from Saan, and the third one GiHBV strains from Jacko, Pok, Nin, and Midnight. All orangutan sequences are related to the orangutan virus from Indonesia (AF193864).

P gene and *X* gene: The results of phylogenetic analysis of the *Pre-C/C* gene were similar to the *PreS/S* and *PreC/C* gene. The results are shown in Fig. 24D and 24E.

Fig. 24A Complete HBV genome

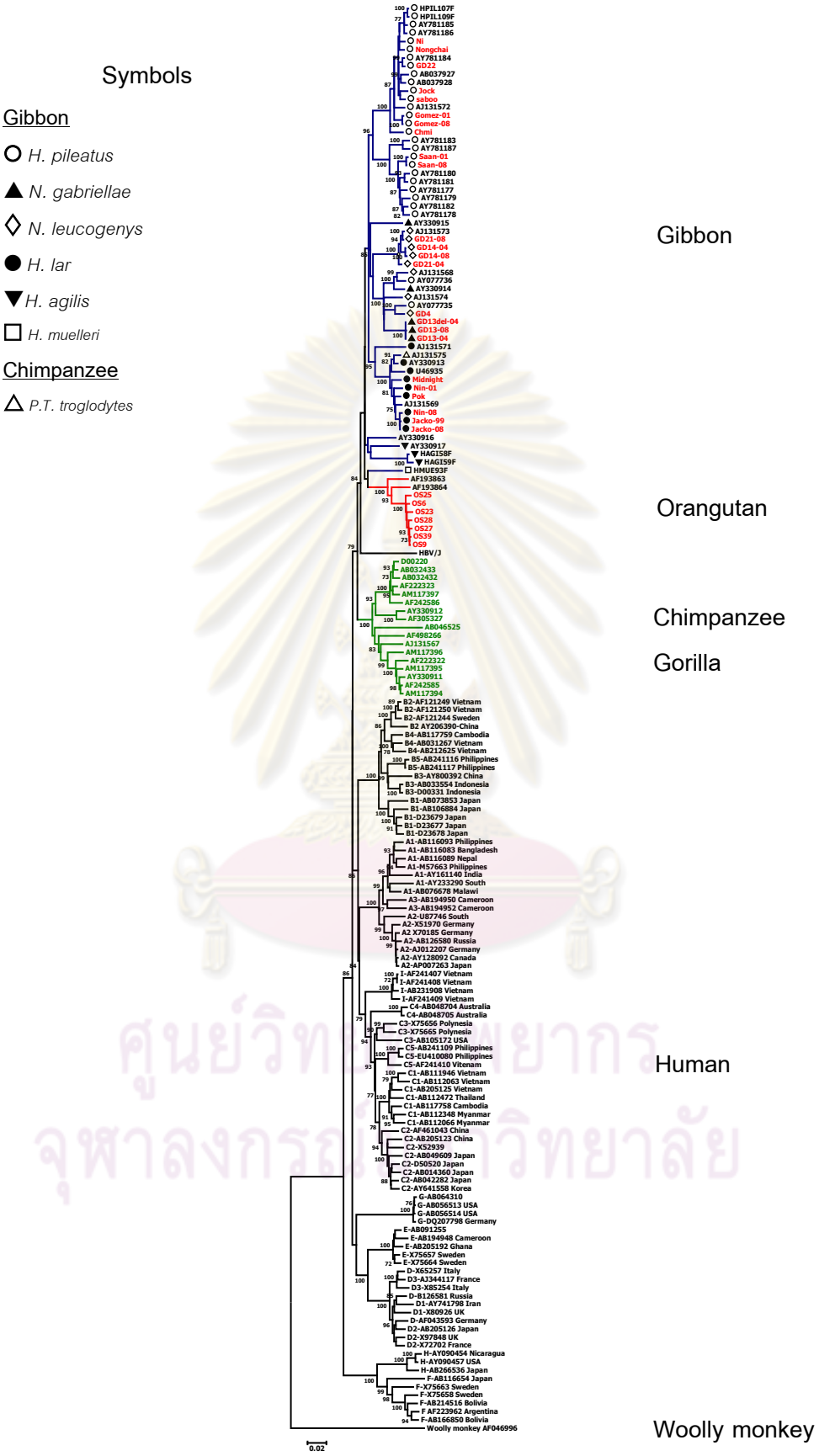


Fig. 24B Complete *S* gene

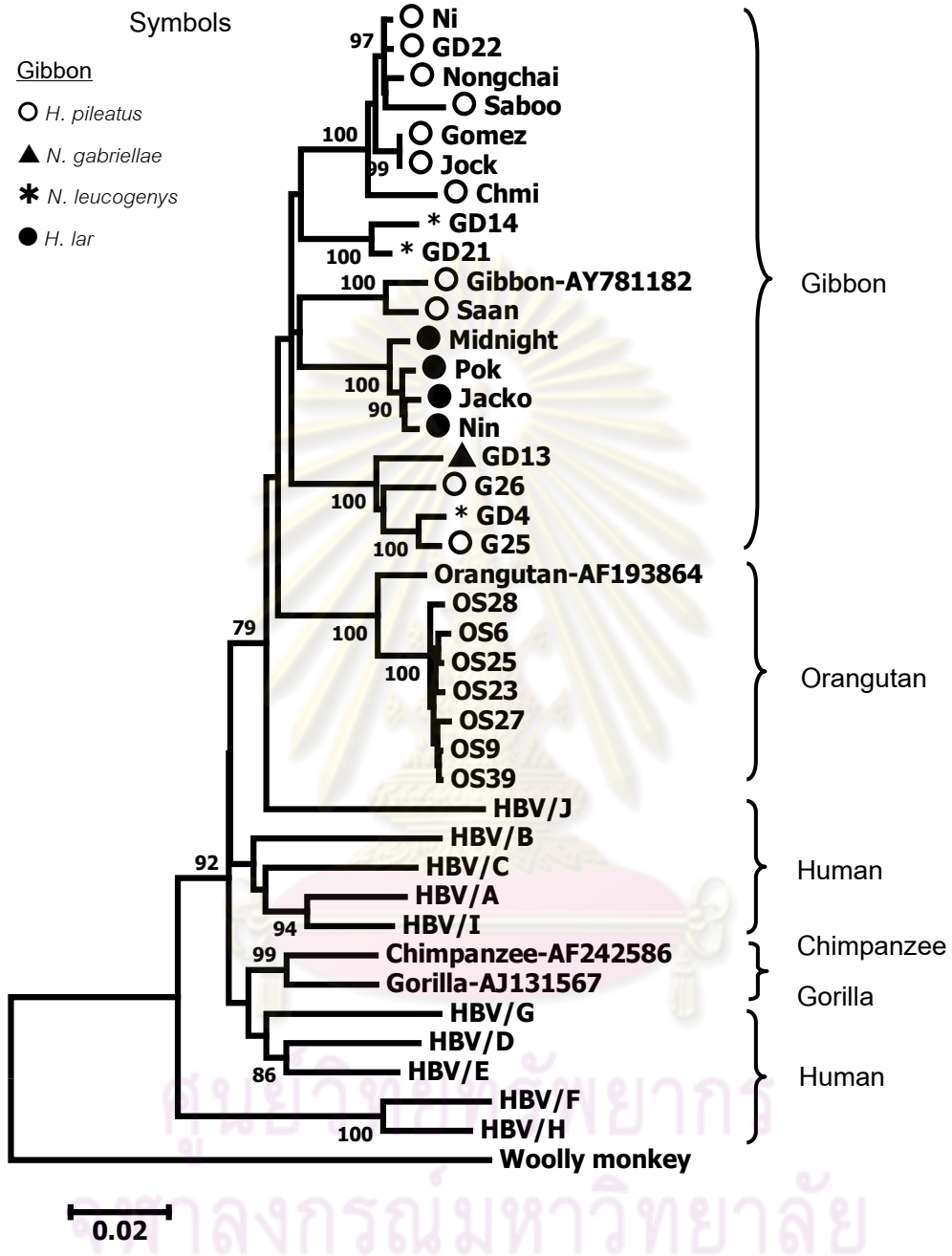


Fig. 24C *PreC/C* gene

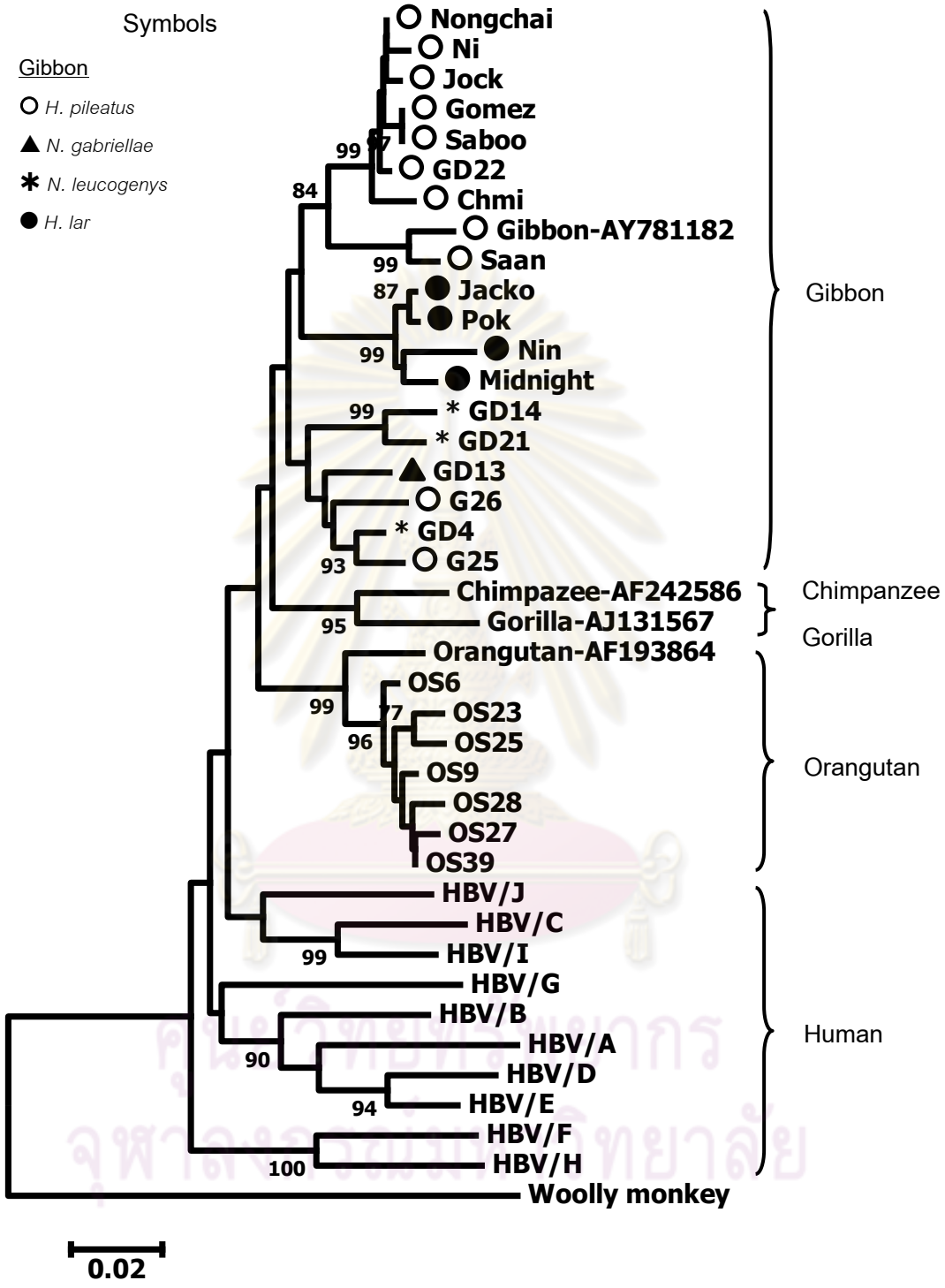


Fig. 24D *P* gene

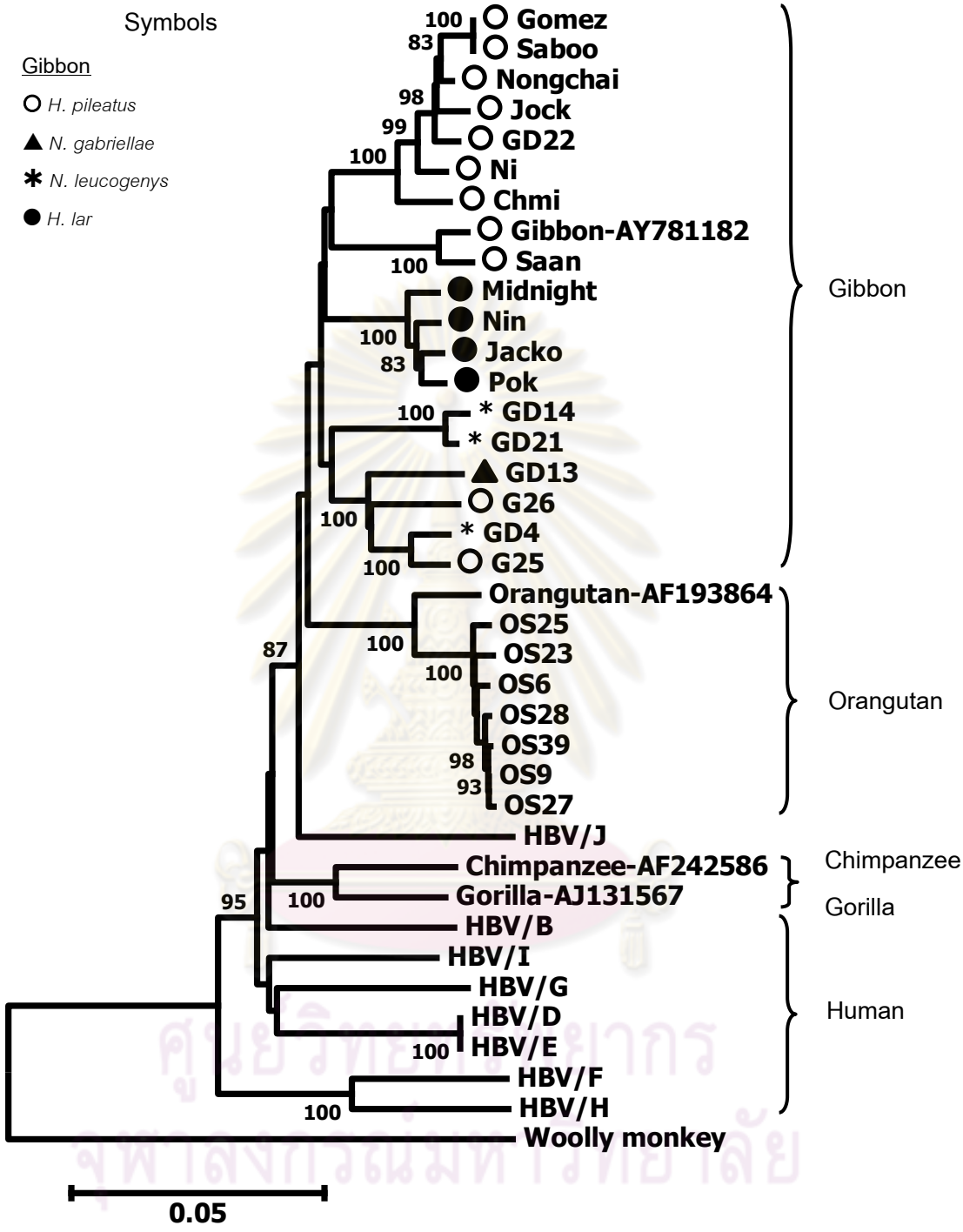


Fig. 24E *X* gene

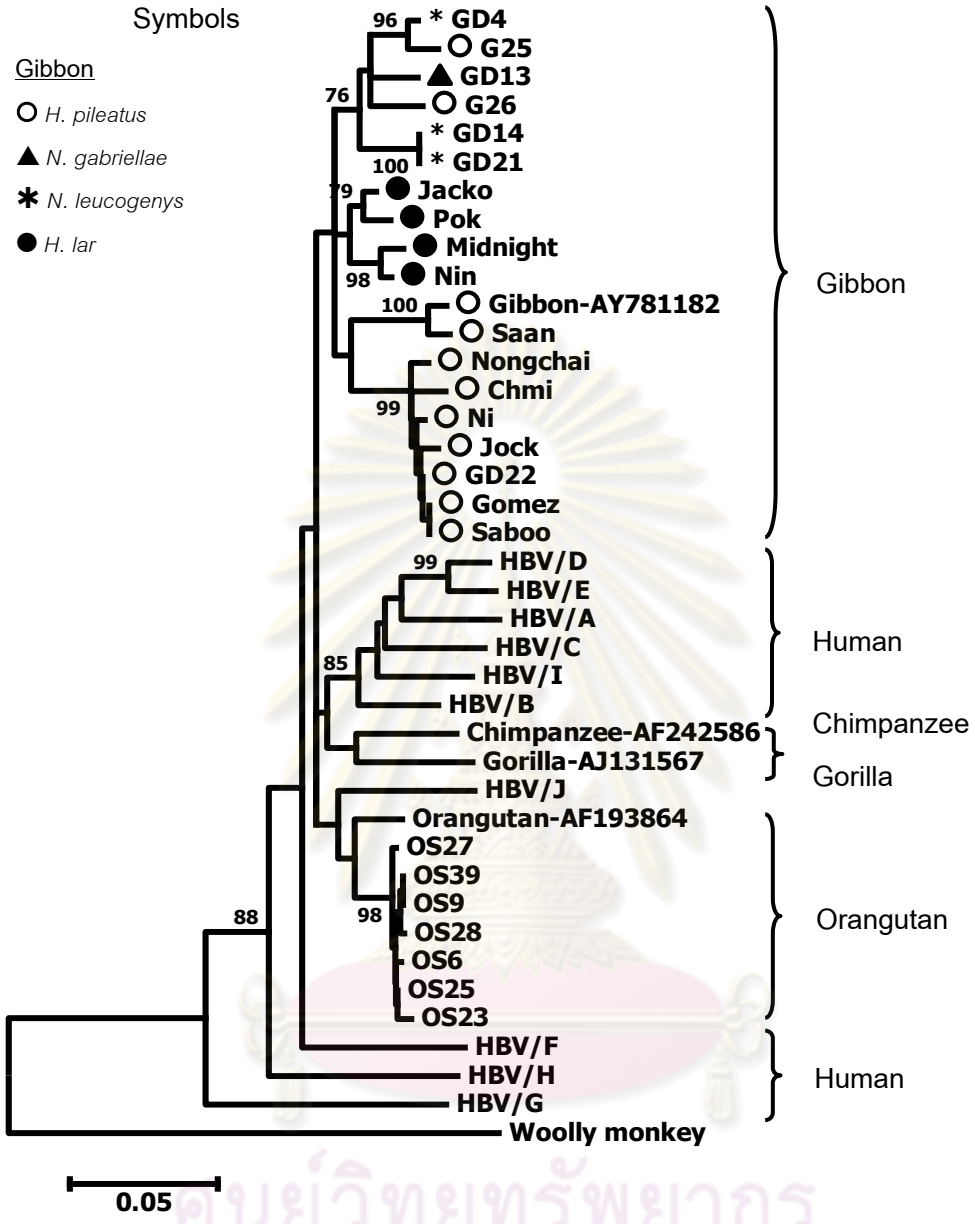


Fig. 24 Phylogram depicts the phylogenetic relationship between the sequence obtained from the present study and representative sequences of non-human HBV strains from GenBank. Regions include in the comparison were: (A) Complete HBV genome (B) the large *S* gene including *PreS1*, *PreS2* and HBsAg gene; (C) the *C* gene, including PreC and Core region; (D) the *P* gene; (E) the *X* gene. Percentage bootstrap values (>75%) were shown at the respective nodes. The scale bar at the bottom indicated the genetic distance. The species origin of sequences obtained in this study and in previous studies was indicated by the symbol.

EVOLUTION STUDY

In this study, attempts were made to determine the evolutionary rate and clarify the origin of non-human primate HBV using molecular analysis.

Phylogenetic analysis: The phylogenetic tree for 7 pairs of gibbon HBV at two time points is shown in Fig. 25. Each genome sequence of gibbon strains was significant clustered with its pairwised strains except 2 pairs of Nin and GD21.

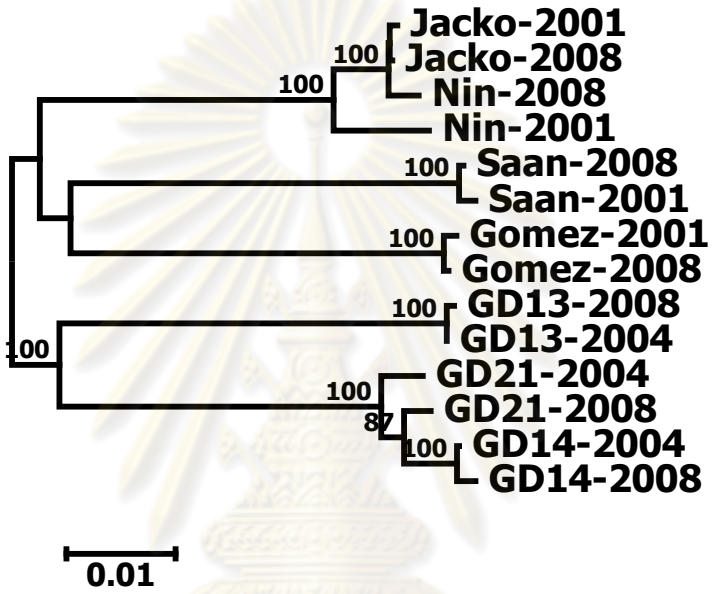


Fig. 25 Phylogenetic tree of gibbon HBV (7 pairs). Bootstrap support values (>75%) are shown for key nodes. The year of sampling is given as part of the isolate name.

Evolutionary rates in GiHBV: The molecular evolutionary rates of GiHBV are shown in Table 11. Mostly, the evolutionary rates ranged from 0.6 – 2.4 x 10^{-4} substitutions/site/year while Nin and Jacko showed high evolutionary rates with 10.8 and 13.8 x 10^{-4} substitutions/site/year, respectively.

Table 11 Substitution rates for gibbon HBV sequences.

Data set	Common name	Scientific name	Zoo	Substitution rate, x 10^{-4} subs/site/year
Jacko	White-handed gibbon	*H. lar*	Krabok Koo	0.6
Nin	White-handed gibbon	*H. lar*	Krabok Koo	10.8
Saan	Pileated gibbon	*H. pileatus*	Krabok Koo	1.4
Gomez	Pileated gibbon	*H. pileatus*	Krabok Koo	1.1
GD13	Yellow-cheeked gibbon	*N. gabriellae*	Dusit zoo	0.8
GD14	White-cheeked gibbon	*N. leucogenys*	Dusit zoo	2.4
GD21	White-cheeked gibbon	*N. leucogenys*	Dusit zoo	13.8

Population growth rate: To pinpoint the time when non-human primate HBVs originated and their population growth rate, all sequences of GiHBV and some sequences of OuHBV from this study were subjected to phylogenetic analysis in comparison with gibbon and orangutan HBV strains from a previous study. Because HBV genotype J was closely related to gibbon and orangutan strains it was used as the out-group. Phylogenetic analysis was applied to calculate population growth rate and determine the origin of non-human primate HBV (Fig. 26). The Bayesian skyline plots revealed that the effective number of infections remained constant from the time of the root in the phylogenetic tree until about 260 – 360 years ago (1750 – 1850), when the infections rapidly became epidemics that led to an increase in the effective population size (Fig. 27).

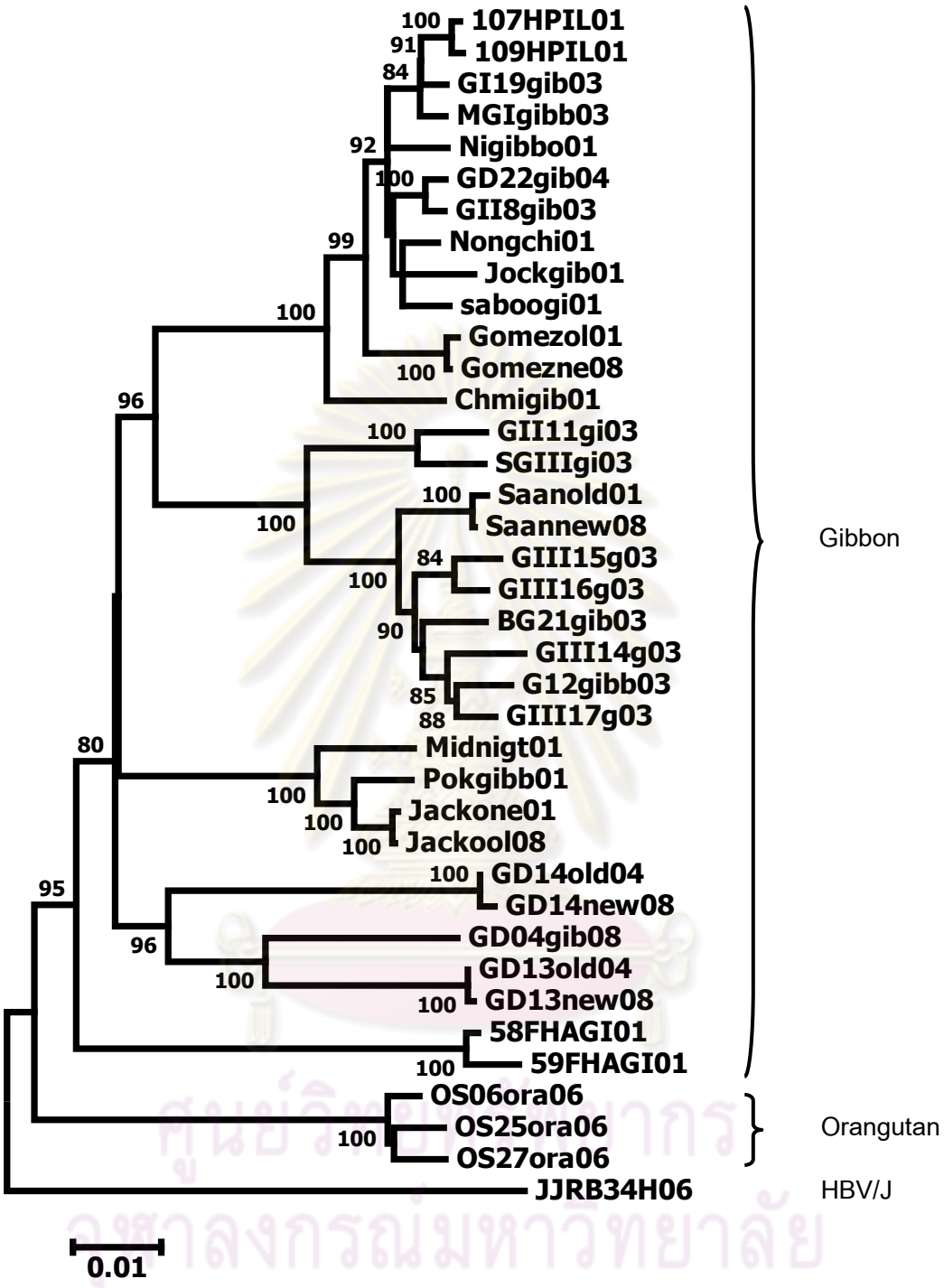


Fig. 26 Phylogenetic tree of GiHBV and OuHBV was used to calculate population growth rate and determine origin of non-human primate HBV.

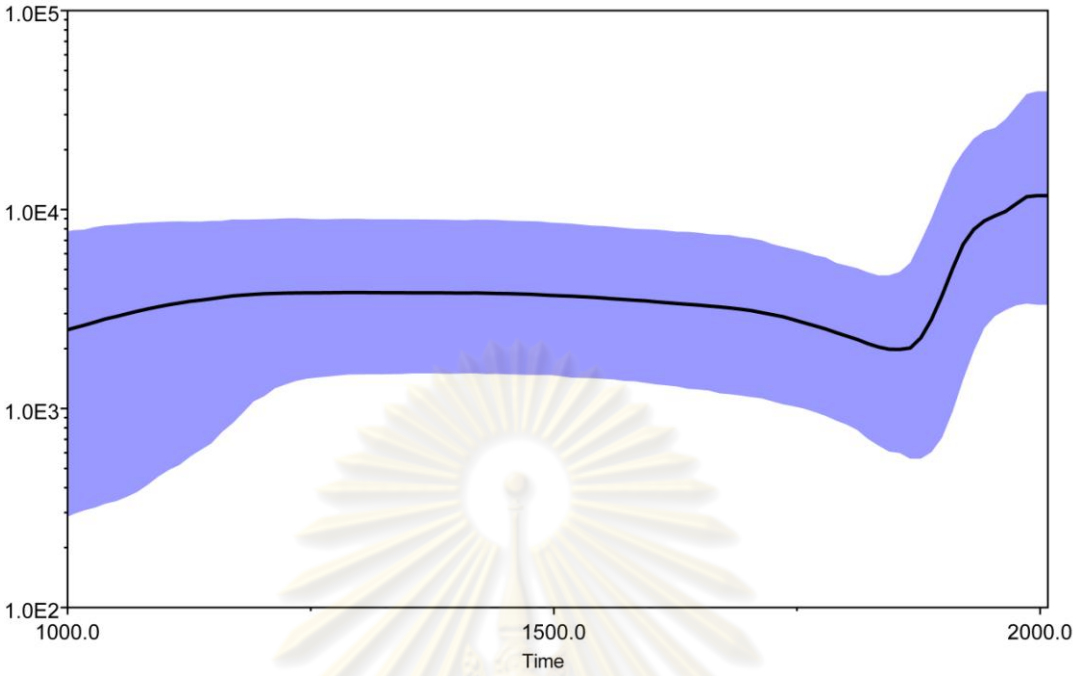
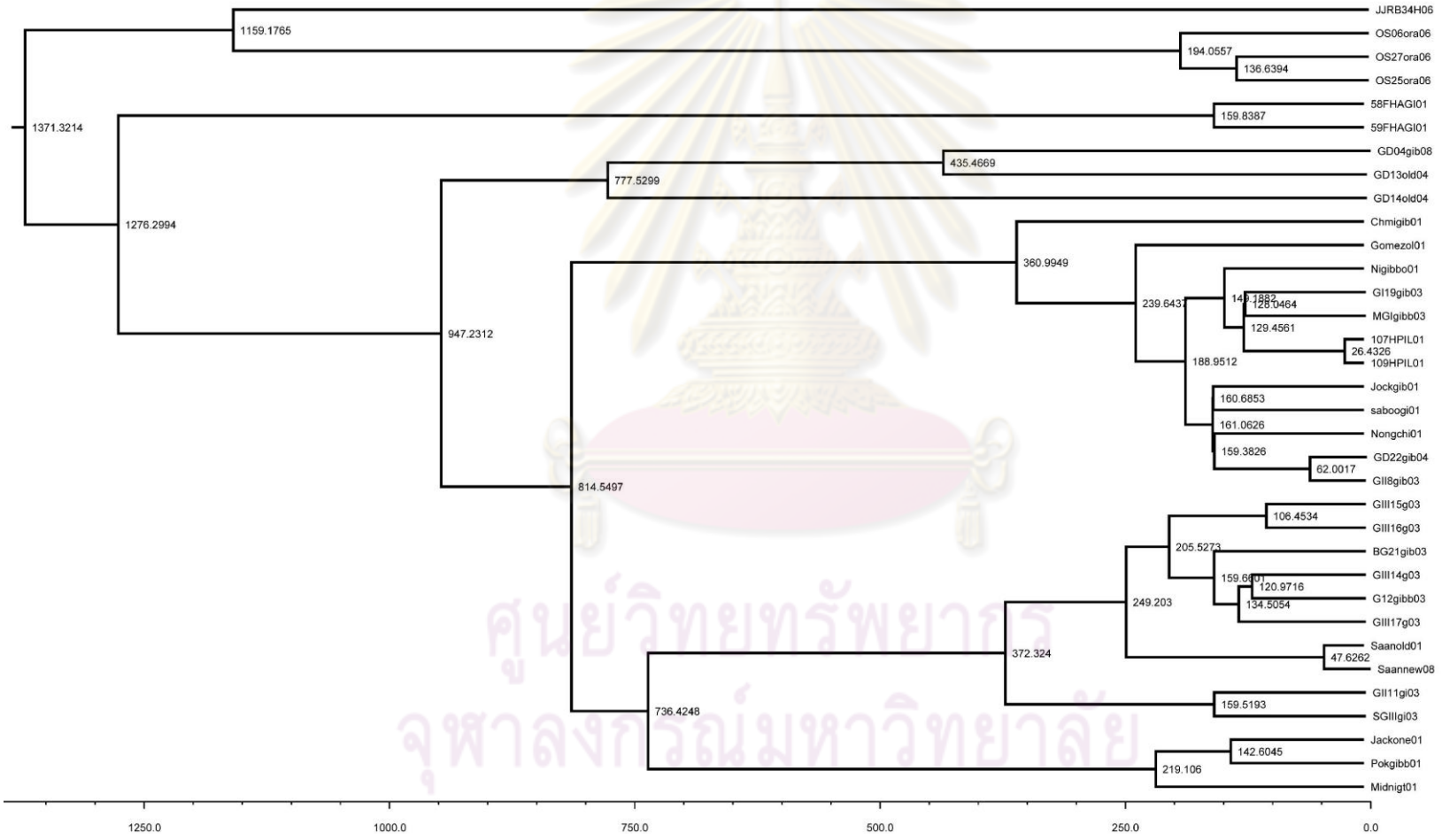


Fig. 27 Bayesian skyline plots of non-human primate HBV in this population, inferred from the entire genome. The plot depicts change in the effective number of infections (Y-axis) through time (X-axis), indicative of changing epidemiology dynamics (population growth rates).The thick solid line is the median estimate, and the blue area overlay shows the 95% highest posterior density (HPD) limits. Genetic distances have been depicted as number of years using estimates of the molecular clock.

The origin of non-human primate HBVs: To find the origin of non-human primate HBVs, the calculation was further tested. GiHBV was diverged from orangutan and genotype J ancestor at aprroximately 1,370 years ago. Human HBV genotype J were distinguished from OuHBv at about 1,600 years ago. However, time scale in this experiment was conflicted. The time which calculated from the evolutionary program and the true time scale was not matched in the same host. For example, HBV strains from Saan in year 2001 and year 2008 showed the common ancestor at about 48 years ago. Actually, these gibbon HBVs have a divergence time only 7 years (Fig. 28).

Fig. 28 Phylogenetic analysis of 34 full-length genome sequences with mean node ages. The last two digits of the strain names indicate the year of the isolate. Scale bar represents time in year.



CROSS-SPECIES TRANSMISSION STUDY

In this study, serum containing 10^4 copies of genome equivalent to gibbon and orangutan HBV was inoculated into SCID mice. Twenty-eight days after inoculation, mice sera were collected and HBV DNA was extracted. The samples were subjected to quantitative HBV DNA determination by real-time PCR. Unfortunately, HBV DNA could not be detected in all samples. Thus, all mice were re-inoculated with 10^5 copies of genome equivalent. Fifty-six days after the initial inoculation, mice sera were collected and subjected to quantitative HBV DNA analysis again. At this point, HBV DNA could be detected in the samples from 2 mice of the gibbon group and 2 mice of the orangutan group (Table 12).

Table 12 HBV DNA level in chimeric mice.

			Sample					
	Day	Genome inoculation	Gibbon serum			Orangutan serum		
Code			101	102	103	201	202	203
DNA conc. (copies/ml)	28	10^4	-	-	-	-	-	-
	56	10^5	$1.4x10^6$	-	$3.8x10^4$	$1.2x10^8$	$1.6x10^5$	-

-, negative

Two mice from gibbon (code 101 and 103) and orangutan (code 201 and 202) groups, each showed high levels of HBV DNA while all others remained negative. HBV DNA from all four mice was amplified and subjected to sequencing of the entire genome. The sequences from mice sera were identical to hepatitis B virus from gibbon and orangutan sera determined prior to inoculation. The comparison between the entire genome sequences from mice sera and gibbon or oruangutan sera were shown in table 13.

Table 13 Comparison between HBV derived from mice sera and gibbon or orangutan sera. Data are expressed as nucleotide number and percent identity.

Host	Gibbon	Orangutan	101	103	201	202
101	3172/3182 (99%)	-	-	3175/3182 (99%)	-	-
103	3165/3182 (99%)	-	3175/3182 (99%)	-	-	
201	-	3167/3182 (99%)	-	-	-	3176/3182 (99%)
202	-	3165/3182 (99%)	-	-	3176/3182 (99%)	-

HBV derived from mice sera inoculated with gibbon or orangutan HBV showed 99% identity with HBV derived from gibbon or orangutan sera, respectively.

CHAPTER V

DISCUSSION AND CONCLUSION

PREVALENCE, MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS

Upon screening various non-human primate species for HBV infection, approximately 40% of gibbons and 74% of orangutans showed at least one marker of HBV. This rate in gibbons is equal that found in our previous study [3]. In addition, the rates of active infection defined by detectable HBV DNA in gibbons (20%) and orangutans (13%) are similar to those of non-human primates in Central Africa and Southeast Asia [8]. However, the results in this study were obtained from wild-born primates kept in captivity, and the prevalence of HBV infection in wild gibbons and orangutans is unknown. In contrast, this study could neither detect any HBV marker nor HBV DNA by real-time PCR in either macaques or langurs. The finding that HBV can and does infect only the members of the families *Pongidae* (orangutan) and *Hylobatidae* (gibbon) strongly supports previous evidence of their rather narrow host range [6-8].

The results regarding HBV serological markers showed that the majority of orangutans and gibbons had been infected with the virus but managed to resolve the infection, which became apparent by positive results for anti-HBs and negative results for HBsAg and HBV DNA. In contrast, some orangutans and gibbons became chronic carriers and displayed positive results for serum HBsAg and HBV DNA. Interestingly, approximately 15% of orangutans displayed only anti-HBc without either HBsAg or anti-HBs usually accompanying this marker. Such atypical serology is likely attributable to resolved HBV infection since HBV DNA could not be detected in any of the orangutans' plasma. In humans, anti-HBc-positive individuals lacking HBsAg are usually considered to have been previously exposed to HBV infection, but a proportion of these patients may have subclinical or occult HBV infection as HBV DNA can be detected in the liver. Occult HBV status is in some cases associated with mutant virus undetectable by commercial HBsAg assays, but more frequently results from a strong suppression of virus replication and gene expression [201]. Although the significance of isolated anti-

HBc in non-human primates is unclear, the serological markers of HBV infection in gibbons and orangutans may be similar to those described in humans.

In humans, several researchers have reported that patients with chronic HBV infection often present mutations in the basic core promoter region [201]. Accordingly, in this study the core regions were aligned with analyzed nucleotide positions 1753, 1762 and 1764 including the *PreC* variant's nucleotide positions 1858, 1896 and 1899. We could not detect any mutations at those positions except for a G to T substitution at nucleotide position 1896. This substitution had occurred in all gibbon and orangutan HBV sequences described here, and was commonly found in non-human primate sequences. It has been proposed that the difference in RNA secondary structure between infected humans and non-human primates may be responsible for this discrepancy [168, 202].

Upon phylogenetic analysis, all seven complete genome sequences of HBV-infected orangutans obtained in this study grouped with those previously published. Indeed, they showed genetic relatedness to the HBV isolates from gibbons, particularly the *Hylobates* species that shared geographical habitat ranges. The branches occupied by all orangutans analyzed clustered together, and displayed very close phylogenetic relatedness to an HBV isolate obtained from Indonesia [2]. This isolate showed a very high percentage of sequence similarity (approximately 98-99% identity in the *PreS* gene) to all isolates described here, suggesting that they had originated from a common source. Although the geographic origin of the orangutans described in this study was unknown, prior to their capture, they probably inhabited Borneo and Sumatra, as wild living orangutans are generally restricted to these islands. Thus, the primary source of this HBV strain found in captive orangutans may have originated from the wild.

HBV isolates from gibbons in this study (*N. leucogenys*) were phylogenetically separate from gibbons (*H. pileatus* and *H. lar*) described in preceding research, but were almost identical to a gibbon isolate (*H. concolor*) that was reported to have originated from a Thai zoo [4], suggesting several strains of HBV circulated in

gibbons in Thailand. Previous data have shown that HBV gibbon strains from Thailand and Vietnam could be classified into four phylogenetically distinct genomic groups [4]. Likewise, there appears to be a substantial difference in HBV strain distribution between gibbons from Thailand and those from Cambodia [9]. These observations can be explained by the different geographical location as well as different species (and sub-species) of non-human primates, which in turn may have determined the particular HBV strains infecting those animals in this geographic region.

In humans, a high percentage of individuals who become infected by horizontal transmission during adolescence or adulthood have a short duration of infectivity and clear the virus. In contrast, mother-to-child perinatal transmission generally leads to life long chronic HBV infection due to a prolonged stage of immunological tolerance and it is considered to be an essential mechanism for the persistence of HBV infection in human populations. Similar to HBV infection in humans, a previous study has documented that HBV in captive gibbons can be transmitted by vertical and horizontal routes [3]. Frequent vertical transmission in captive gibbons would support the assumption that this may be a main mechanism for the continuation of HBV infection in gibbons and potentially other ape species in the wild [8]. In addition, horizontal transmission also represents an important route for HBV distribution in the wild as well as in captivity. For instance, orangutans in the wild are solitary apes with restricted contact to other individuals and thus, the possibility of horizontally acquired infection is limited. When captured and housed together, the probability of orangutans to be exposed to HBV appears to be increased. Indeed, the pronounced sequence similarity of HBV among infected orangutans described here strongly suggests that the transmission of HBV among these apes might have been relatively recent, possibly due to horizontal spread from an animal infected in the wild prior to its capture.

Despite the previous hypothesis of species-specific HBV infection, a geographical basis rather than species association accounting for the distribution of HBV variants has been increasingly recognized. For instance, HBV in orangutans consistently grouped within the gibbon clade from Southeast Asia and similarly, a gorilla sequence (AJ131576) clustered with chimpanzee sequences from Central Africa. Lack

of strict species-specificity of HBV variants was also reported for a chimpanzee sequence (AJ131575) grouped with a gibbon cluster, and another chimpanzee sequence (AB032431) grouped with human HBV genotype E. These observations support probable interspecies transmission which could be explained by sharing a common habitat in geographic regions with high prevalence of HBV infection, such as Southeast Asia and Central Africa. Thus, the more the regions both species inhabit overlap, the higher the probability of cross-species transmission [8,9]. This probability has currently been investigated by several researchers in order to elucidate the ultimate origin and evolution of HBV in humans and non-human primates.

The rate of nucleotide substitution in non-human primate HBV is still unknown. In this study, the evolutionary rate of gibbon HBV was approximately 10^{-4} subs/site/year. This rate was higher than that of human HBV with an evolutionary rate of 10^{-5} subs/site/year [169,203] The reason for this difference is not known. Further research performed on a much larger sample size would be required. In contrast, Nin and GD21 showed a high evolutionary rate and the sequences did not cluster indicating that gibbon HBV having infected Nin and GD21 is a quasispecies.

According to the Bayesian skyline plot, the exponential growth of non-human primate HBV took place in the 1750s, and reached a plateau in year 2000s. Since information on non-human primates in Thailand is scarce, this observation could not be confirmed. Maybe non-human primates were imported or migrated into Southeast Asia which may be reflected in their population growth rate. However, knowledge of the history of non-human primates in this region is necessary to confirm this hypothesis.

The origin of non-human primate HBV in this study revealed that the gibbon HBV had originated prior to orangutan HBV. Moreover, human HBV genotype J did not appear to have originated as the result of a cross-species transmission from orangutan or gibbon but shared the same ancestor with orangutan HBV.

Experimental transmission of human HBV to non-human primates by exposure to human saliva containing HBV has been reported [31,33]. Hu et al. constructed a phylogenetic tree and found that the *S* gene sequence from two chimpanzees clustered with human HBV genotypes A and C which could suggest

possible virus transmission from human to chimpanzee [21]. Currently, there is no evidence indicating natural infection of humans with non-human primate HBV [3]. Yet, non-human primate virus could probably be transmitted to humans as the respective HBV genomes are largely similar. Due to this similarity, HBV vaccine can be used to prevent cross-transmission between species. In fact, HBV isolated from gibbons and orangutans contain glycine at position 145 of the 'a' determinant indicating that HBV vaccines should be effective. However, the route of HBV transmission from non-human primates to humans ought to be elucidated. In this study, cross-species transmission was performed in chimeric mice containing human hepatocytes. The results showed that HBV DNA can be detected in sera of mice inoculated with HBsAg from orangutan or gibbon carriers. The entire genomes of HBV derived from mice sera confirmed that chimeric mice containing human hepatocytes can be infected with non-human primate HBV. This evidence may imply that humans can also be infected and suffer from non-human primate HBV such as GiHBV and OuHBV.

Non-human primates are the reservoir of many viruses that cause several diseases such as CeHV-1, lymphocryptoviruses, SIV, SFV, and rabies. Most viruses in non-human primates are not zoonotic. However, there are many viruses in non-human primates causing diseases in animal handlers, hunters, or in any person who is in close contact, bitten or scratched by these animals. Apes and woolly monkeys are susceptible to HBVs. According to phylogenetic analysis, non-human primate HBV genomes are very close to one another and very similar to the human HBV genome, but significantly different from avian HBVs. Despite advances in the field of HBVs during the past 4 decades, the origin an evolution of HBVs has remained inconclusive. Thus, the evolution of HBVs may or may not be independent from the evolution of its host, but non-human primate HBVs are quite host-specific. However, cross-species transmission has been reported. Several research projects have demonstrated that human HBVs can be tranmitted to non-human primates. Evidence of the non-human primate HBV transmission to humans has been suggested but not yet confirmed. The research presented here has confirmed the possibility of cross-species transmission of non-human primate HBVs to humans. This may imply that cross-species transmission from

non-human primates to humans does occur, diminishing the chance of HBV eradication due to the difficulty in controlling the natural virus reservoir. Thus, HBV infection will probably continue to be a global public health problem. Due to the fact that the amino acids in the “a” determinant region of all HBVs are highly conserved the cross-protective effect of the HBV vaccine and anti-viral medications in non-human primates has been proposed. Applying these agents may prevent infection and treat animals or persons infected with non-human primate HBVs. However, further studies will be required to develop a protocol for prevention or treatment of non-human primate HBV infection using vaccine or drugs.

Some parts of this dissertation were published in 3 journals as below:

1. Sa-nguanmoo, P., Thongmee, C., Ratanakorn, P., Pattanarangsan, R., Boonyarittichaikij, R, Chodapisitkul, S., et al. Prevalence, whole genome characterization and phylogenetic analysis of hepatitis B virus in captive orangutan and gibbon. J Med Primatol 37 (2008): 277-289.
2. Sa-nguanmoo, P., Rianthavorn, P., Amornsawadwattana, S., and Poovorawan, Y. Hepatitis B virus infection in non-human primates. Acta Virol 53 (2009): 72-82.
3. Sa-nguanmoo, P. and Yoovorawan, Y. Virus transmission from nonhuman primates to humans. Asian Biomed 2 (2008): 83-84.

REFERENCES

[1] Vaudin, M., Wolstenholme, A.J., Tsiquaye, K.N., Zuckerman, A.J., and Harrison, T.J. The complete nucleotide sequence of the genome of a hepatitis B virus isolated from a naturally infected chimpanzee. J Gen Virol 69 (1988): 1383-1389.

[2] Warren, K.S., Heeney, J.L., Swan, R.A., Heriyanto, and Verschoor, E.J. A new group of hepadnaviruses naturally infecting orangutans (Pongo pygmaeus). J Virol 73 (1999): 7860-7865.

[3] Noppornpanth, S., Haagmans, B.L., Bhattarakosol, P., Ratanakorn, P., Niesters, H.G., Osterhaus, A.D., et al. Molecular epidemiology of gibbon hepatitis B virus transmission. J Gen Virol 84 (2003): 147-155.

[4] Grethe, S., Heckel, J.O., Rietschel, W., and Hufert, F.T. Molecular epidemiology of hepatitis B virus variants in nonhuman primates. J Virol 74 (2000): 5377-81.

[5] Lanford, R.E., Chavez, D., Brasky, K.M., Burns, R.D. 3rd., and Rico-Hesse, R. Isolation of a hepadnavirus from the woolly monkey, a New World primate. Proc Natl Acad Sci U S A 95 (1998): 5757-5761.

[6] Deinhardt, F. Hepatitis in primates. Adv Virus Res 20 (1976): 113-157.

[7] Makuwa, M., Souquière, S., Telfer, P., Bourry, O., Rouquet, P., Kazanji, M., et al. Hepatitis viruses in non-human primates. J Med Primatol 35 (2006): 384-387.

[8] Starkman, S.E., MacDonald, D.M., Lewis, J.C.M., Holmes, E.C., and Simmond, P. Geographic and species association of hepatitis B virus genoytpes in non-human primates. Virology 314 (2003): 381-393.

[9] Sall, A.A., Starkman, S., Reynes, J.M., Lay, S., Nhim, T., Hunt, M, et al. Frequent infection of *Hylobates pileatus* (pileated gibbon) with species-associated variants of hepatitis B virus in Cambodia. J Gen Virol 86 (2005): 333-337.

[10] Okamoto, H., Tsuda, F., Sakugawa, H., Sastrosoewignjo, R.I., Imai, M., Miyakawa, Y., et al. Typing hepatitis B virus B by homology in nucleotide sequence: comparison of surface antigen subtypes. J Gen Virol 69 (1988): 2575-2583.

[11] Norder, H., Hammas, B., Löfdahl, S., Couroucé, A.M., and Magnius, L.O. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surfaces antigen and genomic classification of the corresponding hepatitis B virus strains. J Gen Virol 73 (1992): 1201-1208.

[12] Norder, H., Hammas, B., Lee, S.D., Bile, K., Couroucé, A.M., Mushahwar, I.K., et al. Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. J Gen Virol 74 (1993): 1341-1348.

[13] Norder, H., Couroucé, A.M., and Magnius, L.O. Complete genomes, phylogenetic relatedness, and structural protein of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 198 (1994): 489-503.

[14] Naumann, H., Schaefer, S., Yoshida, C.F., Gaspar, A.M., Repp, R., and Gerlich, W.H. Identification of a new hepatitis B virus (HBV) genotype from Brazil that expressed HBV surface antigen subtype adw4. J Gen Virol 74 (1993): 1627-1632.

[15] Stuyver, L., De Gendt, S., Van Geyt, C., Zoulim, F., Fried, M., Schinazi, R.F., et al. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol 81 (2000): 67-74.

[16] Arauz-Ruiz, P., Norder, H., Robertson, B.H., and Magnius, L.O. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central American. J Gen Virol 83 (2002): 2059-2073.

[17] Tran, T.T., Trinh, T.N., and Abe, K. New complex recombinant genotype of hepatitis B virus identified in Vietnam. J Virol 82 (2008): 5657-5663.

[18] Olinger, C.M., Jutavijittum, P., Hübschen, J.M., Yousukh, A., Samountry, B., Thammavong, T., et al. Possible new hepatitis B virus genotype, southeast Asia. Emerg Infect Dis 14 (2008):1777-1780.

[19] Kurbanov, F., Tanaka, Y., Kramvis, A., Simmonds, P., and Mizokami, M. When should 'I' consider a new hepatitis B virus genotype? J Virol 82 (2008): 8241-8242.

[20] Tatematsu, K., Tanaka, Y., Kurbanov, F., Sugauchi, F., Mano, S., Maeshiro, T., et al. A genetic variant of hepatitis B virus divergent from known human and ape

genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. J Virol 83 (2009): 10538-10547.

[21] Hu, X., Margolis, H.S., Purcell, R.H., Ebert, J., and Robertson, B.H. Identification of hepatitis B virus indigenous to chimpanzees. Proc Natl Acad Sci U S A 97 (2000): 1661-1664.

[22] Lanford, R.E., Chavez, D., Rico-Hesse, R., and Mootnick, A. Hepadnavirus infection in captive gibbons. J Virol 74 (2000): 2955-2999.

[23] MacDonald, D.M., Holmes, E.C., Lewis, J.C., and Simmonds, P. Detection of hepatitis B virus infection in wild-born chimpanzees (*Pan troglodytes* verus): phylogenetic relationships with human and other primate genotypes. J Virol 74 (2000): 4253-4257.

[24] Schaefer, S. Hepatitis B virus taxonomy and hepatitis B virus genoytpes. World J Gastroenterol 13 (2007): 14-21.

[25] Hillis, W.D. An outbreak of infectious hepatitis among chimpanzee handlers at a United States Air Force Base. Am J Hygl 73 (1961): 316-328.

[26] Hillis, W.D. Viral hepatitis associated with sub-human primates. Transfusion 3 (1963): 445-454.

[27] Maynard, J.E., Berquist, K.R., Hartwell, W.V., and Krushak, D.H. Viral hepatitis and studies of hepatitis associated antigen in chimpanzees. Can Med Assoc J 106 (1972): 473-479.

[28] Holmes, A.W., and Capps, R.B. The use of primates for transmission and study of human viral hepatitis. Medicine (Baltimore) 45 (1966): 553-555.

[29] Deinhardt, F., Holmes, A.W., Wolfe, L., and Junge, U. Transmission of viral hepatitis to nonhuman primates. Vox Sang 19 (1970): 261-269.

[30] Maynard, J.E., Krushak, D.H., Bradley, D.W., and Berquist, K.R. Infectivity studies of hepatitis A and B in non-human primates. Dev Biol Stand 30 (1975): 229-235.

[31] Alter, H.J., Purcell, R.H., Gerin, J.L., London, W.T., Kaplan, P.M., McAuliffe, V.J., et al. Transmission of hepatitis B to chimpanzees by hepatitis B surface antigen-positive saliva and semen. Infect Immun 16 (1977): 928-933.

[32] Robertson, B.H. Viral hepatitis and primates: historical and molecular analysis of

human and nonhuman primate hepatitis A, B, and the GB-related viruses. J Viral Hepat 8 (2001): 233-242.

[33] Bancroft, W.H., Snitbhan, R., Scott, R.M., Tingpalapong, M., Watson, W.T., Tanticharoenyos, P., et al. Transmission of hepatitis B virus to gibbons by exposure to human saliva containing hepatitis B surface antigen. J Infect Dis 135 (1977): 79-85.

[34] Scott, R.M., Snitbhan, R. Bancroft, W.H., Alter, H.J., and Tingpalapong, M. Experimental transmission of hepatitis B virus by semen and saliva. J Infect Dis 142 (1980): 67-71.

[35] Mimms, L.T., Solomon, L.R., Ebert, J.W., and Fields, H. Unique preS sequence in a gibbon–derived hepatitis B virus variant. Biochem Biophys Res Commun 195 (1993): 186-191.

[36] Blumberg, B.S., Alter, H.J., and Visnich, S. A “new” antigen in leukemia sera. JAMA 191 (1965): 541-546.

[37] Hepatitis B Fountation. Meet Dr. Blumberg. [online]. 2009. Available from : http://www.hepb.org/about/blumberg.htm [2009, Oct 23].

[38] Prince, A.M. An antigen detected in the blood during the incubation period of serum hepatitis. Proc Natl Acad Sci U S A 60 (1968): 814-821.

[39] Prince, A.M. Relation of Australia and SH antigens. Lancet 2 (1968): 462-463.

[40] Prince, A.M., Hargrove, R.L., Szmuness, W., Cherubin, C.E., Fontana, V.J., and Jeffries, G.H. Immunologic distinction between infectious and serum hepatitis. N Engl J Med 282 (1970): 987-991.

[41] Ganem, D., and Prince, A.M. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 350 (2004): 1118-1129.

[42] Qadri, I., Ferrari, M.E., and Siddiqui, A. The hepatitis B virus transactivator protein, HBx, interacts with single-stranded DNA (ssDNA). Biochemical characterizations of the HBx-ssDNA interactions. J Biol Chem 271 (1996): 15443-15450.

[43] Nassal, M., and Schaller, H. Hepatitis B virus replication. Trends Microbiol 1 (1993): 221-228.

[44] Seeger, C., and Mason, W.S. Hepatitis B virus biology. Microbiol Mol Biol Rev 64

(2000): 51-68.

[45] Dane, D.S., Cameron, C.H., and Briggs, M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet 1 (1970): 695-698.

[46] Blumberg, B.S. The Australia antigen story. In I. Millman, T.K. Eisenstein, B.S. Blumberg (eds.), Hepatitis B: the virus, the disease, and the vaccine. pp. 5-32. Pennsylvania : Springer, 1984.

[47] Bruss, V. Hepatitis B virus morphogenesis. World J Gastroenterol 13 (2007): 65-73.

[48] Shors, T. Hepatitis virus life cycles. In T. Shors (ed.), Understanding viruses, pp. 486-96. Massachusetts : Jones and Bartlett Publishers, 2008.

[49] Takahashi, K., Brotman, B., Usuda, S., Mishiro, S., and Prince, A.M. Full-genome sequence analyses of hepatitis B virus (HBV) strains recovered from chimpanzees infected in the wild: implications for an origin of HBV. Virology 267 (2000): 58-64.

[50] Takahashi, K., Mishiro, S., and Prince, A.M. Novel hepatitis B virus strain from a chimpanzee of Central Africa (*Pan troglodytes troglodytes*) with an unusual antigenicity of the core protein. Intervirology 44 (2001): 321-326.

[51] Hu, X., Javadian, A., Gagneux, P., and Robertson, B.H. Paired chimpanzee hepatitis B virus (ChHBV) and mtDNA sequences suggest different ChHBV genetic variants are found in geographically distinct chimpanzee subspecies. Virus Res 79 (2001): 103-108.

[52] Makuwa, M., Souquière, S., Bourry, O., Rouquet, P., Telfer, P., Mauclère, P., et al. Complete-genome analysis of hepatitis B virus from wild-born chimpanzees in central Africa demonstrates a strain-specific geographical cluster. J Gen Virol 88 (2007): 2679-2685.

[53] Warren, K.S., Niphuis, H., Heriyanto, Verschoor, E.J., Swan, R.A., and Heeney, J.L. Seroprevalence of specific viral infections in confiscated orangutans (*Pongo pygmaeus*). J Med Primatol 27 (1998): 33-37.

[54] Norder, H., Ebert, J.W., Fields, H.A., Mushahwar, I.K., and Magnius, L.O. Complete sequencing of a gibbon hepatitis B virus genome reveals a unique genoytpe distantly related to the chimpanzee hepatitis B virus. Virology 218 (1996): 214-223.

[55] Summers, J., Smolec, J.M., and Snyder, R. A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc Natl Acad Sci U S A 75 (1978): 4533-4537.

[56] Testut, P., Renard, C.A., Terradillos, O., Vitvitski-Trépo, L., Tekaia, F., Degott, C., et al. A new hepadnavirus endemic in arctic ground squirrels in Alaska. J Virol 70 (1996): 4210-4219.

[57] Marion, P.L., Oshiro, L.S., Regnery, D.C., Scullard, G.H., and Robinson, W.S. A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. Proc Natl Acad Sci U S A 77 (1980): 2941-2945.

[58] Mason, W.S., Seal, G., and Summers, J. Virus of Pekin ducks with structural and biological relatedness to human hepatitis B virus. J Virol 36 (1980): 829-836.

[59] Chang, S.F., Netter, H.J., Bruns, M., Schneider, R., Fröhlich, K., and Will, H. A new avian hepadnavirus infecting snow geese (*Anser caerulescens*) produces a significant fraction of virions containing single-stranded DNA. Virology 262 (1999): 39-54.

[60] Li, L., Dixon, R.J., Gu, X., and Newbold, J.E. Comparison of the sequences of the Grey Teal, Maned Duck and Duck Hepatitis B Viruses. In The molecular biology of hepatitis B virus, pp. 13. California : University of California San Diego, 1998.

[61] Sprengel, R., Kaleta, E.F., and Will, H. Isolation and characterization of a hepatitis B virus endemic in herons. J Virol 62 (1988): 3832-3839.

[62] Pult, I., Netter, H.J., Fröhlich, K., Kaleta, E.F., and Will, H. Identification, structural and functional analysis of a new avian Hepadnavirus from storks (STHBV). In The molecular biology of hepatitis B virus, pp. 2. California : University of California San Diego, 1998.

[63] Prassolov, A., Hohenberg, H., Kalinina, T., Scheneider, C., Cova, L., Krone, O., et al. New hepatitis B virus of cranes that has an unexpected broad host range. J Virol 77 (2003): 1964-1976.

[64] Couroucé, A.M., Lee, H., Drouet, J., Canavaggio, M., and Soulier, J.P. Monoclonal antibodies to HBsAg: a study of their specificities for eight different HBsAg subtypes. Dev Bio Stand 54 (1983): 527-534.

[65] Mohebbi, S.R., Amini-Bavil-Olyaee, S., Zali, N., Derakhshan, F., Sabahi, F., and Zali, M.R. An extremely aberrant subtype of hepatitis B virus genotype D in Iran. Hep Mon 9 (2009): 73-75.

[66] Le Bouvier, G.L. The heterogeneity of Australia antigen. J Infect Dis 123 (1971): 671-675.

[67] Bancroft, W.H., Mundon, F.K., and Russell, P.K. Detection of additional antigenic determinants of hepatitis B antigen. J Immunol 109 (1972): 842-848.

[68] Norder, H., Couroucé, A.M., and Magnius, L.O. Molecular basis of hepatitis B virus serotype variations within the four major subtypes. J Gen Virol 73 (1992): 3141-3145.

[69] Magnius, L., Kaplan, L., Vyas, G.N., and Perkins, H.A. A new virus specified determinant of hepatitis B surface antigen. Acta Pathol Microbiol Scand B 83 (1975): 295-297.

[70] Norder, H., Couroucé, A.M., Coursaget, P., Echevarria, J.M., Lee, S.D., Mushahwar, I.K., et al. Genetic diversity of hepatitis B virus strains derived worldwide: genetypes, subgenotypes, and HB_sAg subtypes. Intervirology 47 (2004): 289-309.

[71] Magnius, L.O., and Norder, H. Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. Intervirology 38 (1995): 24-34.

[72] Kramvis, A., and Kew, M.C. Relationship of genotypes of hepatitis B virus to mutations, disease progression and response to antiviral therapy. J Virol Hepat 12 (2005): 456-464.

[73] Sattler, F., and Robinson, W.S. Hepatitis B viral DNA molecules have cohesive ends. J Virol 32 (1979): 226-233.

[74] Landers, T.A., Greenberg, H.B., and Robinson, W.S. Structure of hepatitis B Dane particle DNA and nature of the endogenous DNA polymerase reaction. J Virol 23 (1977): 368-376.

[75] Will, H., Reiser, W., Weimer, T., Pfaff, E., Büscher, M., Sprengel, R., et al. Replication strategy of human hepatitis B virus. J Virol 61 (1987): 904-911.

[76] He, M.L., Wu, J., Chen, Y., Lin, M.C., Lau, G.K., and Kung, H.F. A new and sensitive

method for the quantification of HBV cccDNA by real-time PCR. Biochem Biophys Res Commun 295 (2002): 1102-1107.

[77] Dejean, A., Sonigo, P., Wain-Hobson, S., and Tiollais, P. Specific hepatitis B virus integration in hepatocellular carcinoma DNA through a viral 11-base-pair direct repeat. Proc Natl Acad Sci U S A 81 (1984): 5350-5354.

[78] Kann, M., and Gerlich, W.H. Hepatitis B virus and other *Hepadnaviridae*: structure and molecular virology. In H.C. Thomas, S. Lemon, A.J. Zuckerman, (eds.), Viral Hepatitis, pp. 149-180. Massachusetts : Blackwell Publishing, 2005.

[79] Heermann, K.H., Goldmann, U., Schwartz, W., Seyffarth, T., Baumgarten, H., and Gerlich, W.H. Large surface proteins of hepatitis B virus containing the pre-s surface. J Virol 52 (1984): 396-402.

[80] Ou, J.H., Laub, O, and Rutter, W.J. Hepatitis B virus gene function: the precore region targets the core antigen to cellular membranes and causes the secretion of the e antigen. Proc Natl Acad Sci U S A 83 (1986): 1578-1582.

[81] Tiollais, P., Pourcel, C., and Dejean, A. The hepatitis B virus. Nature 317 (1985): 489-495.

[82] Kwee, L., Lucito, R., Aufiero, B., and Schneider, R.J. Alternate translation initiation on hepatitis B virus X mRNA produces multiple polypeptides that differentially transactivate class II and III promoters. J Virol 66 (1992): 4382-4389.

[83] Carman, W.F., Jazayeri, M., Basuni, A., Thomas, H.C., and Karayiannis, P. Hepatitis B surface antigen (HBsAg) variants. In H.C. Thomas, S. Lemon, A.J. Zuckerman (eds.), Viral Hepatitis, pp. 225-241. Massachusetts : Blackwell Publishing, 2005.

[84] Stibbe, W., and Gerlich, W.H. Structural relationships between minor and major proteins of hepatitis B surface antigen. J Virol 46 (1983): 626-628.

[85] Machida, A., Kishimoto, S., Ohnuma, H., Baba, K., Ito, Y., Miyamoto, H., et al. A polypeptide containing 55 amino acid residues coded by the pre-S region of hepatitis B virus deoxyribonucleic acid bears the receptor for polymerized human as well as chimpanzee albumins. Gastroenterology 86 (1984): 910-918.

[86] Heermann, K.H., Kruse, F., Seifer, M., and Gerlich, W.H. Immunogenicity of the gene S and Pre-S domains in hepatitis B virions and HBsAg filaments. Intervirology 28 (1987): 14-25.

[87] Roossinck, M.J., and Siddiqui, A. In vivo phosphorylation and protein analysis of hepatitis B virus core antigen. J Virol 61 (1987): 955-961.

[88] Albin, C., and Robinson, W.S. Protein kinase activity in hepatitis B virus. J Virol 34 (1980): 297-302.

[89] Gudat, F., Bianchi, L., Sonnabend, W., Thiel, G., Aenishaenslin, W., and Stalder, G.A. Pattern of core and surface expression in liver tissue reflects state of specific immune response in hepatitis B. Lab Invest 32 (1975): 1-9.

[90] Chen, H.S., Kew, M.C., Hombuckle, W.E., Tennant, B.C., Cote, P.J., Gerin, J.L., et al. The precore gene of the woodchuck hepatitis virus genome is not essential for viral replication in the natural host. J Virol 66 (1992): 5682-5684.

[91] Schlicht, H.J., Bartenschlager, R., and Schaller, H. Biosynthesis and enzymatic functions of the hepadnaviral reverse transcriptase. In A. McLachhlan (ed.), Molecular biology of the hepatitis B virus, pp. 171-180. Boca Raton : CRC Press, 1991.

[92] Locarnini, S. Molecular virology of hepatitis B virus. Semin Liver Dis 24 (2004): 3-10.

[93] Bartenschlager, R., and Schaller, H. Hepadnaviral assembly is initiated by polymerase binding to the encapsidation signal in the viral RNA genome. EMBO L 11 (1992): 3413-3420.

[94] Seifer, M., Höhne, M., Schaefer, S., and Gerlich, W.H. In vitro tumorigenicity of hepatitis B virus DNA and HBx protein. J Hepatol 13 (1991): S61-65.

[95] Kim, C.M., Koike, K., Saito, I., Miyamura, T., and Jay, G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 351 (1991): 317-320.

[96] Okamota, H., Imai, M., Kametani, M., Nakamura, T., and Mayumi, M. Genomic heterogeneity of hepatitis B virus in a 54-year-old woman who contracted the infection through materno-fetal transmission. Jpn J Exp Med 57 (1987): 231-236.

[97] Lok, A.S., Akarca, U., and Greene, S. Mutations in the pre-core region of hepatitis B

virus serve to enhance the stability of the secondary structure of the pre-genome encapsidation signal. Proc Natl Acad Sci U S A 91 (1994): 4077-4081.

[98] Lee, W.M. Hepatitis B virus infection. N Engl J Med 337 (1997): 1733-1745.

[99] Günther, S., Fischer, L., Pult, I., Stemeck, M., and Will, H. Naturally occurring variants of hepatitis B virus. Adv Virus Res 52 (1999): 25-137.

[100] Carman, W.F., Zanetti, A.R., Karayiannis, P., Waters, J., Manzillo, G., Tanzi, E., et al. Vaccine-induced escape mutant of hepatitis B virus. Lancet 336 (1990): 325-9.

[101] National AIDS treatment advocacy project. Hepatitis B virus [Online]. 2009. Available from : http://www.natap.org/2009/images/111009/1111091/image008.gif.

[102] Stuyer, L.J., Locarnini, S.A., Lok, A., Richman, D.D., Carman, W.F., Dienstang, J.L., et al. Nomenclature for antiviral-resistant human hapatitis B virus mutations in the polymerase region. Hepatology 33 (2001): 751-757.

[103] Asgus, P., Vaughan, R., Xiong, S., Yang, H., Delaney, W., Gibbs, C., et al. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 125 (2003): 292-297.

[104] Locarnini, S., and Birch, C. Antiviral chemotherapy for chronic hepatitis B infection: lesson learned from treating HIV-infected patients. J Hepatol 30 (1999): 536-550.

[105] Summers, J., and Mason, W.S. Replication of the genome of a hepatitis B--like virus by reverse transcription of an RNA intermediate. Cell 29 (1982): 403-15.

[106] Lu, X., and Block, T. Study of the early steps of the Hepatitis B Virus life cycle. Int J Med Sci 1 (2004): 21-33.

[107] Neurath, A.R., Seto, B., and Strick, N. Antibodies to synthetic peptides from the preS1 region of the hepatitis B virus (HBV) envelope (env) protein are virus-neutralizing and protective. Vaccine 7 (1989): 234-236.

[108] Paran, N., Geiger, B., and Shaul, Y. HBV infection of cell culture: evidence for

multivalent and cooperative attachment. EMBO J 20 (2001): 4443-4453.

[109] Kuroki, K., Cheung, P., Marion, P.L., and Ganem, D. A cell surface protein that binds avian hepatitis B virus particles. J Virol 68 (1994): 2091-2096.

[110] Treichel, U., Meyer zum Büschenfelde, K.H., Diense, H.P., and Gerken, G. Receptor-mediated entry of hepatitis B virus particles into liver cells. Arch Virol 142 (1997): 493-498.

[111] Hungson, F.M. Structural characterization of viral fusion proteins. Curr Biol 5 (1995): 265-274.

[112] Lu, X., Block, T., and Gerlich, W.H. Protease-induced infectivity of hepatitis B virus for a human hepatoblastoma cell line. J Virol 70 (1996): 2277-2285.

[113] Stoeckl, L., Funk, A., Kopitzki, A., Brandenburg, B., Oess, S., Will, H., et al. Identification of a structural motif crucial for infectivity of hepatitis B viruses. Proc Natl Acad Sci U S A 103 (2006): 6730-6734.

[114] Tuttleman, J.S., Pourcel, C., and Summers, J. Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. Cell 47 (1986): 451-460.

[115] Pollack, J.R., and Ganem D. Site-specific RNA binding by a hepatitis B virus reverse transcriptase initiates two distinct reactions: RNA packaging and DNA synthesis. J Virol 68 (1994): 5579-5587.

[116] Wang, G.H., and Seeger, C. Novel mechanism for reverse transcription in hepatitis B viruses. J Virol 67 (1993): 6507-6512.

[117] Huovila, A.P., Eder, A.M., and Fuller, S.D. Hepatitis B surface antigen assembles in a post-ER, pre-Golgi compartment. J Cell Biol 118 (1992): 1305-1320.

[118] Beasley, R.P., Hwang, L.Y., Lee, G.C., Lan, C.C., Roan, C.H., Huang, F.Y., et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. Lancet 2 (1983): 1099-1102.

[119] Xu, D.Z., Yan, Y.P., Choi, B.C., Xu, J.Q., Men, K., Zhang, J.X., et al. Risk factors and mechanism of transplacental transmission of hepatitis B virus: a case-control study. J Med Virol 67 (2002): 20-26.

[120] Boxall, E.H., Sira, J., Standish, R.A., Davies, P., Sleight, E., Dhillon, A.P., et al. Natural history of hepatitis B in perinatally infected carriers. Arch Dis Child

Fetal Neonatal Ed 89 (2004): F456-460.

[121] Shaw, F.E. Jr., Barrett, C.L., Hamm, R., Peare, R.B., Coleman, P.J., Hadler, S.C. et al. Lethal outbreak of hepatitis B in a dental practice. JAMA 255 (1986): 3260-3264.

[122] Williams, I., Smith, M.G., Sinha, D., Kerman, D., Minor-Babin, G., Garcia, E., et al. Hepatitis B virus transmission in an elementary school setting. JAMA 278 (1997): 2167-2169.

[123] Kent, G.P., Brondum, J., Keenlyside, R.A., LaFazia, L.M., and Scott, H.D. A large outbreak of acupuncture-associated hepatitis B. Am J Epidemiol 127 (1988): 591-598.

[124] Mevorach, D., Eliakim, R., and Brezis, M. Hepatitis B--an occupational risk for butchers? Ann Intern Med 116 (1992): 428.

[125] Hui, A.Y., Hung, L.C., Tse, P.C., Leung, W.K., Chan, P.K., Chan, H.L. Transmission of hepatitis B by human bite-confirmation by detection of virus in saliva and full genome sequencing. J Clin Virol 33 (2005): 254-256.

[126] James, L., Fong, C.W., Foong, B.H., Wee, M.K., Chow, A., Shum, E., et al. Hepatitis B seroprevalence study 1999. Singapore Med J 42 (2001): 420-424.

[127] Merican, I., Guan, R., Amarapuka, D., Alexander, M.J., Chutaputti, A., Chien, R.N., et al. Chronic hepatitis B virus infection in Asian countries. J Gastroenterol Hepatol 15 (2000): 1356-1361.

[128] Srey, C.T., Ijaz, S., Teddar, R.S., and Monchy, D. Characterization of hepatitis B surface antigen strains circulation in the Kingdom of Cambodia. J Viral Hepat 13 (2006): 62-66.

[129] Sebastian, V.J., Bhattacharya, S., Ray, S., and Daud, J.H. Prevalence of hepatitis B-surface antigen in the pregnant women of Brunei Darussalam. Southeast Asian J Trop Med Public Health 21 (1990): 123-127.

[130] Alexander, M.J., Sinnatamby, A.S., Rohaimah, M.J., Harun, A.H., and Ng, J.S. Incidence of hepatitis B infection in Brunei Darussalam—analysis of racial distribution. Ann Acad Med Singapore 19 (1990): 344-346.

[131] Budihusodo, U., Sulaiman, H.A., Akbar, H.N., Lesmana, L.A., Waspodo, A.S.,

Noer, H.M., et al. Seroepidemiology of HBV and HCV infection in Jakarta, Indonesia. Gastroenterol Jpn 26 (1991): 196-201.

[132] Amirudin, R., Akil, H., Akahane, Y., and Suzuki, H. Hepatitis B and C virus infection in Ujung Pandang, Indonesia. Gastroenterol Jpn 26 (1991): 184-188.

[133] Utama, A., Octavia, T.I., Dhenni, R., Miskad, U.A., Yusuf, I., and Tai, S. Hepatitis B virus genotypes/subgenotypes in voluntary blood donors in Makassa, South Sulawesi, Indonesia. Virol J 6 (2009): 128.

[134] Lingao, A.L., Torres, N.T., Muñoz, N., Lansang, M.A., West, S.K., Bosch, F.X., et al. Mother to child transmission of hepatitis B virus in the Philippines. Infection 17 (1989): 257-259.

[135] Langsan, M.A. Epidemiology and control of hepatitis B infection: a perspective from the Philippines, Asia. Gut 38 (1996): S43-47.

[136] Nakai, K., Win, K.M., Oo, S.S., Arakawa, Y., and Abe, K. Molecular characteristic-based epidemiology of hepatitis B, C, and E viruses and GB virus C/hepatitis G virus in Myanmar. J Clin Microbiol 39 (2001): 1536-1539.

[137] Caruana, S.R., Kelly, H.A., De Silva, S.L., Chea, L., Nuon, S., Saykao, P., et al. Knowledge about hepatitis and previous exposure to hepatitis viruses in immigrants and refugees from the Mekong region. Aust N Z J Public Health 29 (2005): 64-68.

[138] Jutavijittum, P., Yousukh, A., Samountry, B., Samountry, k., Ounavong, A., Thammavong, T., et al. Seroprevalence of hepatitis B and C virus infections among Lao blood donors. Southeast Asian J Trop Med Public Health 38 (2007): 647-649.

[139] Thüring, E.G., Joller-Jemelka, H.I., Sareth, H., Sokhan, U., Reth, C., and Grob, P. Prevalence of markers of hepatitis viruses A, B, C and of HIV in healthy individuals and patients of a Cambodian province. Southeast Asian J Trop Med Public Health 24 (1993): 239-249.

[140] Duong, T.H., Nguyen, P.H., Henley, K., and Peters, M. Risk factors for hepatitis B infection in rural Vietnam. Asian Pac J Cancer Prev 10 (2009): 97-102.

[141] Thuy le, T.T., Ryo, H., Van Phung, L., Furitsu, K., and Nomura, T. Distribution of genotype/subtype and mutational spectra of the surface gene of hepatitis B

virus circulating in Hanoi, Vietnam. J Med Virol 76 (2005): 161-169.

[142] Luksamijarulkul, P., Thammata, N., and Tiloklurs, M. Seroprevalence of hepatitis B, hepatitis C and human immunodeficiency virus among blood donors, Phitsanulok Regional Blood Center, Thailand. Southeast Asian J Trop Med 33 (2002): 272-279.

[143] Suwannakarn, K., Tangkijvanich, P., Thawornsuk, N., Theamboonlers, A., Tharmaphornpilas P., Yoocharoen, P., et al. Molecular epidemiological study of hepatitis B virus in Thailand based on the analysis of *pre-S* and *S* genes. Hepatol Res 38 (2008): 244-251.

[144] Theamboonlers, A., Jantaradsamee, P., Kaew-In, N., Tangkijvanich, P., Hirsch, P., and Poovorawan, Y. The predominant genotypes of hepatitis B virus in Thailand. Ann Trop Med Parasitol 93 (1999): 737-743.

[145] Chongsrisawat, V., Yoocharoen, P., Theamboonlers, A., Tharmaphornpilas, P., Warinsathien, P., Sinlaparatsamee, S., et al. Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. Trop Med Int Health 11 (2006): 1496-1502.

[146] Fattovich, G., Giustina, G., Schalm, S.W., Hadziyannis, S., Sanchez-Tapias, J., Almasio, P., et al. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. The EUROHEP study group on hepatitis B virus and cirrhosis. Hepatology 21 (1995): 77-82.

[147] Chang, M.H., Chen, C.J., Lai, M.S., Hsu, H.M., Wu, T.C., Kong, M.S., et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan childhood hepatoma study group. N Engl J Med 336 (1997): 1855-1859.

[148] Yu, M.C., Yuan, J.M., Ross, R.K., and Govindarajan, S. Presence of antibodies to the hepatitis B surface antigen is associated with an excess risk for hepatocellular carcinoma among non-Asians in Los Angeles County, California. Hepatology 25 (1997): 226-228.

[149] Weinbaum, C.M., Williams, I., Mast, E.E., Wang, S.A., Finelli, L., Wasley, A., et al.

Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. MMWR Recomm Rep 57 (2008): 1-20.

[150] Dienstag, J.L., and Isselbacher, K.J. Chronic hepatitis. In E. Braunwald (ed.), Harrisson's Principles of Internal Medicine, pp. 1742-1752. New York : McGraw-Hill Companies Inc, 2001.

[151] Dienstag, J.L., and Isselbacher, K.J. Acute viral hepatitis. In E. Braunwald (ed.), Harrisson's Principles of Internal Medicine, pp. 1742-1752. New York : McGraw-Hill Companies Inc, 2001.

[152] Zuckerman, A.J., Thornton, A., Howard, C.R., Tsiquaye, K.N., Jones, D.M. and Brambell, M.R. Hepatitis B outbreak among chimpanzees at the London zoo. Lancet 2 (1978): 652-654.

[153] Michaels, M.G., Lanford, R., Demetris, A.J., Chavez, D., Brasky, K., Fung, J., et al. Lack of susceptibility of baboons to infection with hepatitis B virus. Transplantation 61 (1996): 350-351.

[154] Kedda, M.A., Kramvis, A., Kew, M.C., Lecatsas, G., Paterson, A.C., Aspinall, S., et al. Susceptibility of chacma baboons (*Papio ursinus orientalis*) to infection by hepatitis B virus. Transplantation 69 (2000): 1429-1434.

[155] Verschoor, E.J., Warren, K.S., Langenhuijzen, S., Heriyanto, Swan, R.A., and Heeney, J.L. Analysis of two genomic variants of orang-utan hepadnavirus and their relationship to other primate hepatitis B–like viruses. J Gen Virol 82 (2001): 893-897.

[156] Thornton, S.M., Walker, S., and Zuckerman, J.N. Management of hepatitis B virus infections in two gibbons and a western lowland gorilla in a zoological collection. Vet Rec 149 (2001): 113-115.

[157] Gheit, T., Sekkat, S., Cova, L., Chevallier, M., Petit, M.A., Hantz, O., et al. Experimental transfection of Macaca sylvanus with cloned human hepatitis B virus. J Gen Virol 83 (2002): 1645-1659.

[158] Aiba, N., Nishimura, H., Arakawa, Y., and Abe, K. Complete nucleotide sequence and phylogenetic analyses of hepatitis B virus isolated from two pileated gibbons. Virus Genes 27 (2003): 219-226.

[159] Magiorkinis, E.N., Magiorkinis, G.N., Paraskevis, D.N., and Hatzakis, A.E. Re-analysis of a human hepatitis B virus (HBV) isolate from an East African wild born *Pan troglodytes schweinfurthii*: evidence for interspecies recombination between HBV infecting chimpanzee and human. Gene 349 (2005): 165-171.

[160] Yang, J., Xi, Q., Deng, R., Wang, J., Hou, J., and Wang, X. Identification of interspecies recombination among hepadnaviruses infecting cross-species hosts. J Med Virol 79 (2007): 1741-1750.

[161] Fauquet, C.M., and Mayo, M.A. Virus Taxonomy. In C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, L.A. Ball (eds.), Eighth Report of the International Committee on Taxonomy of Viruses, pp. 371-440. Amsterdam : Elsevier-Academic Press, 2005.

[162] Glebe, D., and Urban, S. Viral and cellular determinants involved in hepadnaviral entry. World J Gastroenterol 13 (2007): 22-38.

[163] Neurath, A.R., Kent, S.B., Strick, N., and Parker, K. Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. Cell 46 (1986): 429-436.

[164] Ryu, C.J., Cho, D.Y., Gripon, P., Kim, H.S., Guguen-Guillouzo, C., and Hong, H.J. An 80-kilodalton protein that binds to the pre-S1 domain of hepatitis B virus. J Virol 74 (2000): 110-116.

[165] Fernholz, D., Galle, P.R., Stemler, M., Brunetto, M., Bonino, F., and Will, H. Infectious hepatitis B virus variant defective in pre-S2 protein expression in a chronic carrier. Virology 194 (1993): 137-148.

[166] Kidd-Ljunggren, K., Miyakawa, Y., and Kidd, A.H. Genetic variability in hepatitis B viruses. J Gen Virol 83 (2002): 1267-1280.

[167] Li, J.S., Tong, S.P., Wen, Y.M., Vitvitski, L., Zhang, Q., and Trépo C. Hepatitis B virus genotype A rarely circulates as an HBe-minus mutant: possible contribution of a single nucleotide in the precore region. J Virol 67 (1993): 5402-5410.

[168] Ingman, M., Lindqvist, B., and Kidd-Ljunggren, K. Novel mutation in Hepatitis B virus preventing HBeAg production and resembling primate strains. J Gen

Virol 87 (2006): 307-310.

[169] Hannoun, C., Horal, P., and Lindh, M. Long-term mutation rates in the hepatitis B virus genome. J Gen Virol 81 (2000): 75-83.

[170] Simmonds, S. Reconstructing the origins of human hepatitis viruses. Phil Trans R Soc Lond B Biol Sci 356 (2001): 1013-1026.

[171] Bollyky, P., Rambaut, A., Grassley, N., Carman, W., and Holmes, E. Hepatitis B virus has a recent new world evolutionary origin. J Hepatol 28 (1998): 96-96(1).

[172] Orito, E., Mizokami, M., Ina, Y., Moriyama, E.N., Kameshima, N., Yamamoto, M., et al. Host-independent evolution and a genetic classification of the hepadnavirus family based on nucleotide sequences. Proc Natl Acad Sci U S A 86 (1989): 7059-7062.

[173] Robertson, B.H., and Margolis, H.S. Primate hepatitis B viruses-genetic diversity, geography and evolution. Rev Med Virol 12 (2002): 133-141.

[174] Guha, C., Mohan, S., Roy-Chowdhury, N., and Roy-Chowdhury, J. Cell culture and animal models of viral hepatitis. Part I: hepatitis B. Lab Amim (NY) 33 (2004): 37-46.

[175] Burk, R.D., DeLoia, J.A., elAwady, M.K., and Gearhart, J.D. Tissue preferential expression of the hepatitis B virus (HBV) surface antigen gene in two lines of HBV transgenic mice. J Virol 62 (1988): 649-54.

[176] Yamamura, K., Araki, K., Hino, O., Tomita, N., Miyazaki, J., and Matsubara, K. HBV production in transgenic mice. Gastroenterol Jpn 25 (1990): 49-52.

[177] Leemans, W.F., Ter Borg, M.J., and de Man, R.A. Review article: Success and failure of nucleoside and nucleotide analogues in chronic hepatitis B. Aliment Pharmacol Ther 26 (2007): 171-182.

[178] Shata, M.T., Pfahler, W., Brotman, B., Lee, D.H., Tricoche, N., Murthy, K., et al. Attempted therapeutic immunization in a chimpanzee chronic HBV carrier with a high viral load. J Med Primatol 35 (2006): 165-171.

[179] Ogata, N., Cote, P.J., Zanetti, A.R., Miller, R.H., Shapiro, M., Gerin, J., et al. Licensed recombinant hepatitis B vaccines protect chimpanzees against infection with the prototype surface gene mutant of hepatitis B virus.

Hepatology 30 (1999): 779-786.

[180] Iwarson, S., Wahl, M., Ruttimann, E., Snoy, P., Seto, B., and Gerety, R.J. Successful postexposure vaccination against hepatitis B in chimpanzees. J Med Virol 25 (1988): 433-439.

[181] Wahl, M, Iwarson, S., Snoy, P., and Gerety, R.J. Failure of hepatitis B immune globulin to protect against exp infection in chimpanzees. J Hepatol 9 (1989): 198-203.

[182] Lewin, R. Part two: background to human evolution, unit 10 Primate Heritage. In R. Lewin (ed.), Human Evolution: An illustrated introduction, pp. 5-32. Oxford : Blackwell Publishing, 2005.

[183] Fleagle, J.G. Adaptation, evolution, and systematics. In J.G. Fleagle, (ed.), Primate adaptation and evolution, pp. 1-9. San Diego Academic Press, 1999.

[184] Geissmann, T. Gibbon systematics and species identification [online]. 2009. Available from : http://www.gibbons.de/main/system/system.html.

[185] National Primate Research Center, University of Wisconsin – Madison. Wisconsin primate research center library [online]. 2010. Available from : http://pin.primate.wisc.edu/factsheets/.

[186] Animal Info. Enhancement endangered animals of the world [online]. 2005. Available from : http://www.animalinfo.org.

[187] International Union for Conservation of Nature and Natural Resources. The ICUN red list of threatened species [online]. 2010. Available from : http://www.iucnredlist.org/apps/redlist/search.

[188] The website of everything.com. Primates [online]. 2010. Available from : http://thewebsiteofeverything.com/animals/mammals/Primates.

[189] The University of Michigan Museum of Zoology. Animal Diversity. [online]. Available from : http://animaldiversity.ummz.umich.edu/site.

[190] Pleasance, S. Primates, xenarthrans, pangolins, aardvark. In S. Anderson (ed.), Macdonald encyclopedia of mammals. pp.83-130. London : Macdonald & Co (Publishers) Ltd, 1986.

[191] Kneteman, N.M., and Mercer, D.F. Mice with chimeric human livers: Who says supermodels have to be tall? Hepatology 41 (2005): 703-706.

[192] Meuleman, P., Libbrecht, L., De Vos, R., de Hemptinne, B.. Gevaert, K., Vandekerckhove, J., et al. Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera. Hepatology 41 (2005): 847-856.

[193] Dandri, M., Burda, M.R., Török, E., Pollok, J.M., Iwanska, A., Sommer, G., et al. Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. Hepatology 33 (2001): 981-988.

[194] Mercer, D.F., Schiller, D.E., Elliott, J.F., Douglas, D.N., Hao, C., Rinfret, A., et al. Hepatitis C virus replication in mice with chimeric human livers. Nat Med 7 (2001): 927-933.

[195] Heckel, J.L., Sandgren, E.P., Degen, J.L., Palmiter, R.D., and Brinster, R.L. Neonatal bleeding in transgenic mice expressing urokinase-type plasminogen activator. Cell 62 (1990): 447-456.

[196] Sandgen, E.P., Palmiter, R.D., Heckel, J.L, Daugherty, C.C., Brinster, R.L., and Degen, J.L. Complete hepatic regeneration after somatic deletion of an albumin-plasminogen activator transgene. Cell 66 (1991): 245-256.

[197] Petersen, J., Dandri, M., Gupta, S., and Rogler, C.E. Liver repopulation with xenogenic hepatocytes in B and T cell-deficient mice leads to chronic hepadnavirus infection and clonal growth of hepatocellular carcinoma. Proc Natl Acad Sci U S A 95 (1998): 310-315.

[198] Payungporn, S., Tangkijvanich, P., Jantaradsamee, P., Theamboonlers, A., and Poovorawan Y. Simultaneous quantitation and genotyping of hepatitis B virus by real-time PCR and melting curve analysis. J Virol Methods 120 (2004): 131-140.

[199] Drummond, A.J., and Rambaut, A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7 (2007): 214.

[200] Michitaka, K., Tanaka, Y., Horike, N., Duong, T.N., Chen, Y., Matsuura, K., et al. Tracing the history of hepatitis B virus genotype D in Western Japan. J Med Virol 78 (2006): 44-52.

[201] Raimondo, G., Pollicino, T., Cacciola, I., and Squadrito, G. Occult hepatitis B virus infection. J Hepatol 46 (2007): 160-170.

[202] Jardi, R., Rodriguez, F., Buti, M., Costa, X., Valdes, A., Allende, H., et al. Mutations in the basic core promoter region of hepatitis B virus. Relationship with precore variants and HBV genotypes in a Spanish population of HBV carriers. J Hepatol 40 (2004): 507-514.

[203] Fares, M.A., and Holmes, E.C. A revised evolutionary history of hepatitis B virus (HBV). J Med Virol 54 (2002): 807-814.

APPENDICES

APPENDIX A

REAGENT AND BUFFER

1. 5X Tris borate buffer (5X TBE)

Tris-base	54	g
Boric acid	27.5	g
EDTA (pH 8.0)	20	ml
Distilled water to	1000	ml

2. 1X Tris borate buffer (1XTBE)

5X TBE	200	ml
Distilled water	800	ml

3. 2% (w/v) agarose gel

Agarose gel	4	g
1X TBE	200	ml

4. 10% Ethidium bromide

Ethidium bromide	30	µl
Distilled water	300	ml

5. Loading dye

0.25% Bromphenol blue

40% (w/v) sucrose

Distilled water to	50	ml

6. Proteinase K (20 mg/ml)

Proteinse K	100	mg
Distilled water	5	ml

7. Lysis buffer

Tris-HCl	0.105	g
EDTA	0.0125	g
SDS	0,335	g
Distilled water	50	ml

8. 25:24:1 (v/v) Phenol/chloroform/isoamyl alcohol

Phenol	25	volume
Chloroform	24	volume
Isoamyl alcohol	1	volume

9. 7.5 M Ammonium acetate

Ammonium acetate	57.81	g
Distilled water to	100	ml
Autoclave		

10. 20 µg/ml glycogen

Glycogen	4	g
Distilled water	1	ml

11. SOB medium

Tryptone	20	g
Yeast extract	5	g
NaCl	0,5	g
250 mM KCl	10	ml
Distilled water to	1000	ml
Autoclave		

12. SOC medium

SOB with 2 M $MgCl_2$ and 1.0 M glucolin

13. LB agar

NaCl	10	g
Tryptone	10	g
Yeast extract	5	g
Agar	15	g
Distilled water to	1000	ml
Autoclave		

14. LB broth

NaCl	10	g
Tryptone	10	g
Yeast extract	5	g
Distilled water to	1000	ml

15. Ampicillin stock solution (100 mg/ml)

Ampicillin sodium salt	5	g
Deionized water	50	ml
Filter-sterilize, keep at -20 °C		

APPENDIX B

GENERAL DESCRIPTION OF NON-HUMAN PRIMATES

Dusit zoo (Year 2004: n = 40)

Collection Date: 4/8/2004

No	Scientific name	Common name	Microchip	Sex	SGOT	SGPT
1	*M. fascicularis*	Long-tailed macaque	000-805-080	M	29	17
2	*M. fascicularis*	Long-tailed macaque	000-890-886	M	36	35
3	*M. fascicularis*	Long-tailed macaque	001-039-329	M	32	35
4	*M. fascicularis*	Long-tailed macaque	000-106-357	F	57	37
5	*M. fascicularis*	Long-tailed macaque	001-118-111	M	-	-

Collection Date: 5/8/2004

No	Scientific name	Common name	Microchip	Sex	SGOT	SGPT
6	*M. nemestrina*	Pig-tailed macaque	000-853-060	M	33	65
7	*M. nemestrina*	Pig-tailed macaque	000-070-043	F	35	60
8	*M. nemestrina*	Pig-tailed macaque	000-070-003	F	43	17
9	*M. nemestrina*	Pig-tailed macaque	000-892-274	F	27	17

Collection Date: 10/8/2004

No	Scientific name	Common name	Microchip	Sex	SGOT	SGPT
10	*M. arctoides*	Stump-tailed macaque	001-066-523	F	25	109
11	*M. arctoides*	Stump-tailed macaque	000-867-812	F	24	47
12	*M. mulatta*	Rhesus macaque	000-064-863	M	37	53

Collection Date: 17/8/2004

No	Scientific name	Common name	Microchip	Sex	SGOT	SGPT
13	*N. gabriellae*	Yellow-cheeked gibbon	001-051-271	M	14	17
14	*N. leucogenys*	White-cheeked gibbon	001-066-819	F	75	79
15	*N. leucogenys*	White-cheeked gibbon	001-117-267	F	14	15
16	*N. leucogenys*	White-cheeked gibbon	000-064-071	M	21	23
17	*N. leucogenys*	White-cheeked gibbon	000-870-256	M	26	20

Collection Date: 19/8/2004

No	Scientific name	Common name	Microchip	Sex	SGOT	SGPT
18	*H. pileatus*	Pileated gibbon	000-070-783	F	24	29
19	*H. pileatus*	Pileated gibbon	001-070-259	F	15	52
20	*H. pileatus*	Pileated gibbon	000-849-280	F	15	25
21	*N. leucogenys*	White-cheeked gibbon	001-100-014	M	14	18
22	*H. pileatus*	Pileated gibbon	000-066-079	F	9	6
23	*H. pileatus*	Pileated gibbon	001-084-791	M	20	49
24	*H. pileatus*	Pileated gibbon	001-054-283	M	25	17
25	*N. leucogenys*	White-cheeked gibbon	000-050-355	F	20	17

Collection Date: 24/8/2004

No	Scientific name	Common name	Microchip	Sex	SGOT	SGPT
26	*M. fascicularis*	Long-tailed Macaque	001-052-879	M	32	26
27	*M. fascicularis*	Long-tailed Macaque	000-022-805	M	30	15
28	*M. fascicularis*	Long-tailed Macaque	001-065-819	M	37	30
29	*M. fascicularis*	Long-tailed Macaque	001-071-323	M	39	45
30	*M. fascicularis*	Long-tailed Macaque	000-848-272	M	47	74

Collection Date: 26/8/2004

No	Scientific name	Common name	Microchip	Sex	SGOT	SGPT
31	*H. lar*	White-handed Gibbon	000-067-359	M	61	31
32	*H. lar*	White-handed Gibbon	000-561-812	F	14	18

Collection Date: 31/8/2004

No	Science name	Common name	Microchip	Sex	SGOT	SGPT
33	*S. cristatus*	Silvered Langur	001-052-359	F	55	43
34	*S. phayrei*	Phayre's Langur	001-071-267	F	38	27
35	*S. obscurus*	Dusky Langur	001-069-815	F	46	20
36	*S. obscurus*	Dusky Langur	000-890-074	M	43	12
37	*S. obscurus*	Dusky Langur	000-066-059	F	-	-

Collection Date: 2/9/2004

No	Science name	Common name	Microchip	Sex	SGOT	SGPT
38	*S.cristatus*	Silvered Langur	001-065-571	M	48	28
39	*S.cristatus*	Silvered Langur	001-067-835	F	44	44
40	*S.cristatus*	Silvered Langur	000-893-326	F	82	73

Dusit zoo (Year 2008: n = 13)

Collection Date: 22/6/2008

No	Scientific name	Common name	Microchip	Sex	Code	Note
1	*S.cristatus*	Silvered Langur	001-065-571	F	-	-

Collection Date: 6/5/2008

No	Scientific name	Common name	Microchip	Sex	Code	Note
2	*N. leucogenys*	White-cheeked gibbon	001-065-843	M	-	New
3	*N. leucogenys*	White-cheeked gibbon	000-371-294	F	-	New
4	*N. leucogenys*	White-cheeked gibbon	000-854-780	M	-	New
5	*N. leucogenys*	White-cheeked gibbon	000-888-536	F	GD4	New
6	*H. pileatus*	Pileated gibbon	001-054-283	M	-	-
7	*N. leucogenys*	White-cheeked gibbon	001-064-071	M	-	-
8	*N. leucogenys*	White-cheeked gibbon	001-117-267	F	-	-
9	*N. leucogenys*	White-cheeked gibbon	5752	M	-	New

Collection Date: 12/5/2008

No	Scientific name	Common name	Microchip	Sex	Code	Note
10	*N. leucogenys*	White-cheeked gibbon	000-066-819	F	GD14	-
11	*N. gabriellae*	Yellow-cheeked gibbon	001-051-271	M	GD13	-
12	*N. leucogenys*	White-cheeked gibbon	001-100-014	M	GD21	-

Collection Date: 2008

No	Scientific name	Common name	Sex	Code	Born
13	*P.pygmaeus*	Orangutan	M	Job	1985

Krabok Koo Wildlife Breeding Center (Year 2001: n = 11)

No	Name	Scientific name	Common name	Microchip	Sex	Born
1	Jock	*H. pileatus*	Pileated gibbon	116752443A	M	1985
2	Nongchai	*H. pileatus*	Pileated gibbon	116444145A	M	1989
3	Saboo	*H. pileatus*	Pileated gibbon	TN000770817	M	1990
4	Ni	*H. pileatus*	Pileated gibbon	116411213A	F	1991
5	Gomez	*H. pileatus*	Pileated gibbon	116869185A	M	1990
6	Chmi	*H. pileatus*	Pileated gibbon	116464221A	F	1990
7	Saan	*H. pileatus*	Pileated gibbon	122752495A	M	1983
8	Pok	*H. lar*	White-handed gibbon	122758331A	M	1991
9	Jacko	*H. lar*	White-handed gibbon	TN001097325	M	-
10	Midnight	*H. lar*	White-handed gibbon	122677730A	M	1989
11	Nin	*H. lar*	White-handed gibbon	116376534A	M	1996

Krabok Koo Wildlife Breeding Center (Year 2008: n = 6)

Collection Date: 14/10/2008

No	Name	Scientific name	Common name	Microchip	Sex	Born
1	Gomez	*H. pileatus*	Pileated gibbon	116869185A	M	1990
2	Saan	*H. pileatus*	Pileated gibbon	122752495A	M	1983
3	Pok	*H. lar*	White-handed gibbon	122758331A	M	1991
4	Jacko	*H. lar*	White-handed gibbon	TN001097325	M	-
5	Charlie	*N. leucogenys*	White-cheeked gibbon	-	M	-
6	Nin	*H. lar*	White-handed gibbon	116376534A	M	1996

Chiangmai zoo (Year 2008: n = 5)

Collection Date: 28/6/2008

No	Name	Scientific name	Common name	Microchip	Sex	NOTE
1	โอ๊ค	*N. leucogenys*	White-cheeked gibbon	000-050-355	M	-
2	อุ๋ง	*N. leucogenys*	White-cheeked gibbon	000-870-256	M	-
3	เนะ	*N. leucogenys*	White-cheeked gibbon	116-913-343A	F	Hybrid
4	เม้า	*N. leucogenys*	White-cheeked gibbon	115-231-521A	F	-
5	นิ้ง	*N. leucogenys*	White-cheeked gibbon	116-869-753A	F	Hybrid

Khao Pratub Chang Wildlife Breeding Center (Year 2006: n = 7)

Collection Date: 2006

No	Code	Scientific name	Common name	Microchip	Sex	Age	Nick name
1	OS6	*P. pygmaeus*	Orangutan	000669A3B5 AVID*007*557*537	M	6-8	Oven
2	OS9	*P. pygmaeus*	Orangutan	000669999B 977 200 000 723 666	F	3-4	-
3	OS23	*P. pygmaeus*	Orangutan	AVID*007*376*292 000 6698E10	M	5	-
4	OS25	*P. pygmaeus*	Orangutan	006 782 324 000 66ABAE3	M	5	-
5	OS27	*P. pygmaeus*	Orangutan	AVID*006*346*544 00066AD71F	M	6-8	-
6	OS28	*P. pygmaeus*	Orangutan	AVID*007*060*123 00066C155E	F	6-8	-
7	OS39	*P. pygmaeus*	Orangutan	977 200 000 726 028 00 6676854	M	3	-

APPENDIX C

SUBMITTED GiHBV AND OuHBV SEQUENCES

```
LOCUS     EU155821               3182 bp    DNA     circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain OS6, complete genome.
ACCESSION   EU155821
VERSION     EU155821.1  GI:162957044
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="OS6"
                     /host="orangutan (Pongo pygmaeus)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25920.1"
                     /db_xref="GI:162957046"
                  /translation="MPLSCQHFRKLLLLDEEAGPLEEELPRLADEGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTAPVFNPNWQTPSFPDIHLHQDIIDKCQQFVGPLTVN
                  EKRRLKLIMPARFYPNSTKYFPPDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESFCQQPAGIFSRAPVGPSVQSQHKQSRLGLQSPQ
                  GHLARGHQGRSGSIWARVHSTSRRSFGVEPTGSGRHHNIASSSSSCLHQSAVGKAAYS
                  HLSTAERHSSSGHAVELHSVPPNSAGSQSKGSVFPCWWLQFRNSEPCSDFCLHHIVNL
                  LQDWGPCTEHGEHLIRIPRTPARVTGGVFLVDKNPHNSSESRLVVDFSQFSRGSSSVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRINYHQRGNMQNLHDFCSRNLFVSLMLLYKTFGRKLHLYSHPIIMGFRKIPMGV
                  GLSPFLLAQFTNAICSVVRRAFPHCLAFSYMDDMVLGAKSVQHLESLYTAVTNFLLSL
                  GIHLNPSKTKRWGYSLHFMGYVIGSWGTLPQDHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYKCIQNRQAFTFSPTYKAFLRTQYLNLYPVARQRQG
                  VCQVFADATPTGWGLALGSLRMRGTFVAPLPIHTAELLAACFARSRSGANIIGTDNSV
                  VLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPT
```

```
                TGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP"
gene            join(2848..3182,1..835)
                /gene="S"
CDS             join(2848..3182,1..835)
                /gene="S"
                /note="surface protein"
                /codon_start=1
                /product="large S protein"
                /protein_id="ABY25919.1"
                /db_xref="GI:162957045"
                /translation="MGQNLSVSNPLGFFPEHQLDPLFRANTNSPDWDFNPHKDTWPEA
                TKVGVGAFGPGFTPPHGGLLGWSPQAQGVTTTLPAVPPPASTNRQSGRQPTPISPPLR
                DTHPQAMQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISSTFFKTGDP
                APNMESISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPVCPGQNSQS
                LTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGS
                TTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLWGWASVRFS
                WLSLLTPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFCLWAYI"
CDS             join(3172..3182,1..835)
                /gene="S"
                /codon_start=1
                /product="middle S protein"
                /protein_id="ABY25924.1"
                /db_xref="GI:162957050"
                /translation="MQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISS
                TFFKTGDPAPNMESISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPV
                CPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLP
                VCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLW
                GWASVRFSWLSLLTPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFC
                LWAYI"
CDS             155..835
                /gene="S"
                /codon_start=1
                /product="S protein"
                /protein_id="ABY25925.1"
                /db_xref="GI:162957051"
                /translation="MESISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGG
                APVCPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQG
                MLPVCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAR
                FLWGWASVRFSWLSLLTPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPI
                FFCLWAYI"
gene            1374..1838
                /gene="X"
CDS             1374..1838
                /gene="X"
                /codon_start=1
                /product="X protein"
                /protein_id="ABY25921.1"
                /db_xref="GI:162957047"
                /translation="MAARLCCQLDPARDVLCLRPVGAESRGRPFSGSVGALPPSSPSA
                VPPDHGAHLSLRGLPVCAFSSAGPCALRFTSARCMETTVNAPRNLPKVLHKRTLGLST
                MSTTGIETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene            1814..2452
                /gene="C"
CDS             1814..2452
                /gene="C"
                /codon_start=1
                /product="precore/core protein"
                /protein_id="ABY25922.1"
                /db_xref="GI:162957048"
                /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYKEFGATVEL
                LSFLPSDFFPSVRDLLDTASALYREALESPEHCSPNHTALRQAVLCWGELMTLASWVG
                NNLEDPASRELVVNYVNNNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS             1901..2452
                /gene="C"
```

```
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25923.1"
                     /db_xref="GI:162957049"
                   /translation="MDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYREALES
                   PEHCSPNHTALRQAVLCWGELMTLASWVGNNLEDPASRELVVNYVNNNMGLKIRQLLW
                   FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                   PSPRRRRSQSPRRRRSQSPASQC"
ORIGIN
        1 ctccacagtg ttccaccaaa ctctgcagga tcccagagta aggggtctgt atttccctgc
       61 tggtggctcc agttcaggaa cagtgaaccc tgttccgact tctgcctcca tcatatcgtc
      121 aaccttcttc aagactgggg accctgcacc gaacatggag agcatctcat caggattcct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaattcc
      241 tcagagtcta gactcgtggt ggacttctct caattttcta gggggagctc cagtgtgtcc
      301 tggccaaaat tcgcagtccc taacctccaa tcactcacca acctcttgtc ctccaatctg
      361 tcctggttat cgctggatgt gtctgcggcg ttttatcata ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga ctaccaaggt atgttgcccg tttgtcctct
      481 acttccagga tcaactacca ccagcgtggg aacatgcaga acctgcacga tttctgctcc
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct tcggacggaa attgcacctg
      601 tattcccatc ccatcatcat gggctttcgc aagattccta tgggggtggg cctcagtccg
      661 tttctcctgg ctcagtttac taacgccatt tgttcagtgg ttcgtagggc tttcccccac
      721 tgcttggctt ttagttatat ggatgatatg gtattggggg ccaagtctgt acaacatctt
      781 gagtcccttt ataccgctgt taccaatttt cttttgtctt tgggcataca tttaaaccct
      841 agcaaaacaa aaagatgggg ctattccctt catttcatgg ggtatgtgat tggaagttgg
      901 ggaaccttgc cacaagacca tattgtacag aaaatcaaac aatgttttcg caaacttcct
      961 gtaaatcgcc ctattgattg gaaagtatgt caaagaattg tgggactctt aggctttgcc
     1021 gctccttca ctcaatgtgg ctatcctgct ttaatgcctt tatataaatg catacagaat
     1081 cgtcaggctt ttactttctc gccaacttac aaggcctttc tgcgtacaca atatctgaac
     1141 ctttaccccg ttgcccggca acgacagggt gtgtgccaag tgtttgctga cgcaacccca
     1201 actggttggg gcttggcact gggttctctg cgcatgcgtg gaacctttgt ggctcctctg
     1261 ccgatccata ctgcggaact cctggccgct tgttttgctc gcagcaggtc tggagcaaac
     1321 attatcggta ccgacaattc tgttgtgttg tcgcggaaat atacatcttt cccatggctg
     1381 ctaggttgtg ctgccaactg gatcctgcgc gggacgtcct ttgtctacgt cccgtcggcg
     1441 ctgaatcccg cggacgaccc ttctcggggt cggttggggc tctaccgccc tcttctccgt
     1501 ctgccgttcc gcccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcatctgc cggtccgtgt gcacttcgct tcacctctgc acgttgcatg gagaccaccg
     1621 tgaacgcccc ccggaacttg ccaaaagtct tgcataagcg gactcttgga ctgtcaacaa
     1681 tgtcaacgac cggaattgag acatacttca aagactgtgt atttaaagac tgggaggagt
     1741 taggggagga gattaggcta aaggtctttg tattaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttataa
     1921 agaatttgga gctactgtgg agttactctc ttttttgcct tctgacttct ttccgtctgt
     1981 cagagatctc ctcgacaccg catcagccct gtatcgggag gccttagagt ctccagaaca
     2041 ttgttcacct aaccacacag cactcaggca agctgttctg tgctggggtg agttaatgac
     2101 tctggcttcc tgggtgggta ataatttgga agacccagca tctagggaac tagtagttaa
     2161 ctatgtcaat aataatatgg gcctaaaaat tagacaattg ctgtggtttc acatttcctg
     2221 tctcaccttt ggaagagaaa ccgttttaga atatttggtg tcttttggag tgtggattcg
     2281 cactcctcct gcgtacagac caccaaatgc ccctatcttg tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgaag
     2401 gtctcaatca ccgcgtcgca gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcacaa ggtgggaaac tttacggggc tttattcttc tactgcacct gtctttaacc
     2521 ctaactggca aactccctct tttcctgaca ttcatttgca tcaggacatt attgataagt
     2581 gtcagcaatt tgttggtcca cttacagtaa atgaaaaacg aagattgaaa ttaatcatgc
     2641 ctgctagatt ctatcctaac tctaccaaat attttccccc agataaaggt attaaaccct
     2701 attatccaga gcatgtggtt aatcattatt tccaaaccag acactattta cacactttgt
     2761 ggaaggcggg tatcttatat aagagagaaa ctacacgtag cgcttcattt tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc tttctgtcag caacccgctg
     2881 ggatttttttc ccgagcacca gttggaccct ctgttcagag ccaacacaaa cagtccagat
     2941 tgggacttca atccccacaa ggacacctgg cccgaggcca ccaaggtagg agtgggagca
     3001 tttgggccag ggttcactcc acctcacgga ggtcttttgg ggtggagccc acaggctcag
     3061 ggcgtcacca caacattgcc agcagttcct cctcctgcct ccaccaatcg gcagtcggga
     3121 aggcagccta ctcccatctc tccaccgctg agagacactc atcctcaggc catgcagtgg
     3181 aa
```

```
LOCUS       EU155822               3182 bp    DNA  circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain OS9, complete genome.
ACCESSION   EU155822
VERSION     EU155822.1  GI:162957052
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="OS9"
                     /host="orangutan (Pongo pygmaeus)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25926.1"
                     /db_xref="GI:162957053"
                  /translation="MPLSCQHFRKLLLLDEEAGPLEEELPRLADEGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTAPVFNPNWQTPSFPDIHLHQDIIDKCQQFVGPLTVN
                  EKRRLKLIMPARFYPNSTKYFPPDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESFCQQPAGIFSRAPVGPSVQSQHKQSRLGLQSPQ
                  GHLARGHQGRSGSIRARVHSTSWRSFGVEPTGSGRHHNIASSSSSCLHQSAVGKAAYS
                  HLSTAERHSSSGHAVELHSVPPNSAGSQSKGSVFPCWWLQFRNSEPCSDFCLHHIVNL
                  LQDWGPCTEHGEHLIRIPRTPARVTGGVFLVDKNPHNSSESRLVVDFSQFSRGSSSVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRINYHQRGNMQNLHDFCSRNLFVSLMLLYKTFGRKLHLYSHPIIMGFRKIPMGV
                  GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDMVLGAKSVQHLESLYTAVTNFLLSL
                  GIHLNPSKTKRWGYSLHFMGYVIGSWGTLPQDHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYNCIQNRQAFTFSPTYKAFLRTQYLTLYPVARQRQG
                  VCQVFADATPTGWGLALGSLRMRGTFVAPLPIHTAELLAACFARSRSGANIIGTDNSV
                  VLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPT
                      TGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP"
     gene            join(2848..3182,1..835)
                     /gene="S"
     CDS             join(2848..3182,1..835)
                     /gene="S"
                     /note="surface protein"
                     /codon_start=1
                     /product="large S protein"
```

```
                     /protein_id="ABY25927.1"
                     /db_xref="GI:162957054"
                     /translation="MGQNLSVSNPLGFFPEHQLDPLFRANTNSPDWDFNPHKDTWPEA
                     TKVGVGAFGPGFTPPHGGLLGWSPQAQGVTTTLPAVPPPASTNRQSGRQPTPISPPLR
                     DTHPQAMQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISSTFFKTGDP
                     APNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPVCPGQNSQS
                     LTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGS
                     TTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLWGWASVRFS
                     WLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFCLWAYI"
CDS                  join(3172..3182,1..835)
                     /gene="S"
                     /codon_start=1
                     /product="middle S protein"
                     /protein_id="ABY25930.1"
                     /db_xref="GI:162957057"
                     /translation="MQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISS
                     TFFKTGDPAPNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPV
                     CPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLP
                     VCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLW
                     GWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFC
                     LWAYI"
CDS                  155..835
                     /gene="S"
                     /codon_start=1
                     /product="S protein"
                     /protein_id="ABY25931.1"
                     /db_xref="GI:162957058"
                     /translation="MENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGG
                     APVCPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQG
                     MLPVCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAR
                     FLWGWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPI
                     FFCLWAYI"
gene                 1374..1838
                     /gene="X"
CDS                  1374..1838
                     /gene="X"
                     /codon_start=1
                     /product="X protein"
                     /protein_id="ABY25928.1"
                     /db_xref="GI:162957055"
                     /translation="MAARLCCQLDPARDVLCLRPVGAESRGRPFSGSAGALPPSSPSA
                     VPPDHGAHLSLRGLPVCAFSSAGPCALRFTSARCMETTVNAPRNLPKVLHKRTLGLST
                     MSTTGIETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene                 1814..2452
                     /gene="C"
CDS                  1814..2452
                     /gene="C"
                     /codon_start=1
                     /product="precore/core protein"
                     /protein_id="ABY25929.1"
                     /db_xref="GI:162957056"
                     /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYKEFGATVEL
                     LSFLPSDFFPSVRDLLDTASALYREALESPEHCSPNHTALRQAVLCWGELMTLASWVG
                     NNLEDPASRELVVNYVNNNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                     YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS                  1901..2452
                     /gene="C"
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25932.1"
                     /db_xref="GI:162957059"
                     /translation="MDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYREALES
                     PEHCSPNHTALRQAVLCWGELMTLASWVGNNLEDPASRELVVNYVNNNMGLKIRQLLW
                     FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                     PSPRRRRSQSPRRRRSQSPASQC"
```

ORIGIN

```
        1 ctccacagtg ttccaccaaa ctctgcagga tcccagagta aggggtctgt atttccctgc
       61 tggtggctcc agttcaggaa cagtgaaccc tgttccgact tctgcctcca tcatatcgtc
      121 aaccttcttc aagactgggg accctgcacc gaacatggag aacatctcat caggattcct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaattcc
      241 tcagagtcta gactcgtggt ggacttctct caattttcta gggggagctc cagtgtgtcc
      301 tggccaaaat tcgcagtccc taacctccaa tcactcacca acctcttgtc ctccaatctg
      361 tcctggttat cgctggatgt gtctgcggcg ttttatcata ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga ctaccaaggt atgttgcccg tttgtcctct
      481 acttccagga tcaactacca ccagcgtggg aacatgcaga acctgcacga tttctgctcc
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct tcggacggaa attgcacctg
      601 tattcccatc ccatcatcat gggctttcgc aagattccta tgggggtggg cctcagtccg
      661 tttctcctgg ctcagtttac tagcgccatt tgttcagtgg ttcgtagggc tttcccccac
      721 tgcttggctt ttagttatat ggatgatatg gtattggggg ccaagtctgt acaacatctt
      781 gagtcccttt ataccgctgt taccaatttt cttttgtctt tgggcataca tttaaaccct
      841 agcaaaacaa aaagatgggg ctattccctt catttcatgg ggtatgtgat tggaagttgg
      901 ggaaccttgc cacaagacca tattgtacag aaaatcaaac aatgttttcg caaacttcct
      961 gtaaatcgcc ctattgattg gaaagtatgt caaagaattg tgggactctt aggctttgcc
     1021 gctcctttca ctcaatgtgg ctatcctgct ttaatgcctt tatataattg catacagaat
     1081 cgtcaggctt ttactttctc gccaacttac aaggcctttc tacgtacaca atatctgacc
     1141 ctttaccccg ttgctcggca acgacagggt gtgtgccaag tgtttgctga cgcaacccca
     1201 actggctggg gcttggcact gggatctctg cgcatgcgtg gaacctttgt ggctcctctg
     1261 ccgatccata ctgcggaact cctggccgct tgttttgctc gcagcaggtc tggagcaaac
     1321 attatcggta ccgacaattc tgttgtgttg tcgcggaaat atacatcttt cccatggctg
     1381 ctaggttgtg ctgccaactg gatcctgcgc gggacgtcct ttgtctacgt cccgtcggcg
     1441 ctgaatcccg cggacgaccc ttctcggggt cggctggggc tctaccgccc tcttctccgt
     1501 ctgccgttcc gcccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcatctgc cggtccgtgt gcacttcgct tcacctctgc acgttgcatg gagaccaccg
     1621 tgaacgcccc ccggaacttg ccaaaggtct tgcataagcg gactcttgga ctgtcaacaa
     1681 tgtcaacgac cggaattgag acatacttca aagactgtgt atttaaagac tgggaggagt
     1741 taggggagga gattaggcta aaggtctttg tattaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttataa
     1921 agaatttgga gctactgtgg agttactctc ttttttgcct tctgacttct ttccgtctgt
     1981 ccgagatcta ctcgacaccg catcagccct gtatcgggag gccttagagt ctccagaaca
     2041 ttgttcacct aaccacacag cactcaggca agctgttctg tgctggggtg agttaatgac
     2101 tctggcttcc tgggtgggta ataatttgga agatccagca tctagggaac tggtagttaa
     2161 ctatgtcaat aataatatgg gcctaaaaat tagacaattg ctgtggtttc acatttcctg
     2221 tctcaccttt ggaagagaaa ccgttttaga atatttggtg tcttttggag tgtggattcg
     2281 cactcctcct gcgtacagac caccaaatgc ccctatcttg tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgaag
     2401 gtctcaatca ccgcgtcgca gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcacaa ggtgggaaac tttacggggc tttactcttc tactgcacct gtctttaacc
     2521 ctaactggca aactccctct tttcctgaca ttcatttgca tcaagacatt attgataagt
     2581 gtcagcaatt tgttggtcca cttacagtaa atgaaaaacg aagattgaaa ttaatcatgc
     2641 ctgcgagatt ctatcctaac tctaccaaat attttccccc agataaaggt atcaaaccct
     2701 attatccaga gcatgtggtt aatcattatt tccaaaccag acactattta cacactttgt
     2761 ggaaggcggg tatcttatat aagagagaaa ctacacgtag cgcttcattt tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc tttctgtcag caacccgctg
     2881 ggatttttc ccgagcacca gttggaccct ctgttcagag ccaacacaaa cagtccagat
     2941 tgggacttca atccccacaa ggacacctgg cccgaggcca ccaaggtagg agtgggagca
     3001 ttcgggccag ggttcactcc acctcatgga ggtcttttgg ggtggagccc acaggctcag
     3061 ggcgtcacca caacattgcc agcagttcct cctcctgcct ccaccaatcg gcagtcggga
     3121 aggcagccta ctcccatctc tccaccgctg agagacactc atcctcaggc catgcagtgg
     3181 aa
```

```
LOCUS       EU155823               3182 bp    DNA  circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain OS23, complete genome.
ACCESSION   EU155823
VERSION     EU155823.1  GI:162957060
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="OS23"
                     /host="orangutan (Pongo pygmaeus)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25933.1"
                     /db_xref="GI:162957061"
                  /translation="MPLSCQHFRKLLLLDEEAGPLEEELPRLADEGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTAPVFNPNWQTPSFPDIHLHQDIIDKCQQFVGPLTVN
                  EKRRLKLIMPARFYPNSTKYFPPDKGIKPYYPEHGVNHYFQTRHYLHTLWKAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESFCQQPAGIFSRAPVGPSVQSQHKQSRLGLQSPQ
                  GHLARSHQGRSGSIWARVHSTSRRSFGVEPTGSGSHHNIASSSSSCLHQSAVGKAAYS
                  HLSTAERHSSSGHAVELHSVPPNSAGSQSKGSVFPCWWLQFRNSEPCSDFCLHHIVNL
                  LQDWGPCTEHGEHLIRIPRTPARVTGGVFLVDKNPHNSSESRLVVDFSQFSRGSSSVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRINYHQRGNMQNLHDFCSRNLFVSLMLLYKTFGRKLHLYSHPIIMGFRKIPMGV
                  GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDMVLGAKSVQHLESLYTAVTNFLLSL
                  GIHLNPNKTKRWGYSLHFMGYVIGSWGTLPQDHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYNCIQNRQAFTFSPTYKAFLRTQYLTLYPVARQRQG
                  VCQVFADATPTGWGLALGSLRMRGTFVAPLPIHTAELLAACFARSRSGANIIGTDNSV
                  VLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPT
                      TGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP"
     gene            join(2848..3182,1..835)
                     /gene="S"
     CDS             join(2848..3182,1..835)
                     /gene="S"
                     /note="surface protein"
                     /codon_start=1
                     /product="large S protein"
```

```
                     /protein_id="ABY25934.1"
                     /db_xref="GI:162957062"
                     /translation="MGQNLSVSNPLGFFPEHQLDPLFRANTNSPDWDFNPHKDTWPEA
                     TKVGVGAFGPGFTPPHGGLLGWSPQAQGVTTTLPAVPPPASTNRQSGRQPTPISPPLR
                     DTHPQAMQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISSTFFKTGDP
                     APNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPVCPGQNSQS
                     LTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGS
                     TTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLWGWASVRFS
                     WLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFCLWAYI"
CDS                  join(3172..3182,1..835)
                     /gene="S"
                     /codon_start=1
                     /product="middle S protein"
                     /protein_id="ABY25937.1"
                     /db_xref="GI:162957065"
                     /translation="MQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISS
                     TFFKTGDPAPNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPV
                     CPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLP
                     VCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLW
                     GWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFC
                     LWAYI"
CDS                  155..835
                     /gene="S"
                     /codon_start=1
                     /product="S protein"
                     /protein_id="ABY25938.1"
                     /db_xref="GI:162957066"
                     /translation="MENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGG
                     APVCPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQG
                     MLPVCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAR
                     FLWGWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPI
                     FFCLWAYI"
gene                 1374..1838
                     /gene="X"
CDS                  1374..1838
                     /gene="X"
                     /codon_start=1
                     /product="X protein"
                     /protein_id="ABY25935.1"
                     /db_xref="GI:162957063"
                     /translation="MAARLCCQLDPARDVLCLRPVGAESRGRPFSGSVRALPPSSPSA
                     VPPDHGAHLSLRGLPVCAFSSAGPCALRFTSARCMATTVNAPRNLPKVLHKRTLGLST
                     MSTTGIETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene                 1814..2452
                     /gene="C"
CDS                  1814..2452
                     /gene="C"
                     /codon_start=1
                     /product="precore/core protein"
                     /protein_id="ABY25936.1"
                     /db_xref="GI:162957064"
                     /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYKEFGATVEL
                     LSFLPSDFFPSVRDVLDTASALYREALESPEHCSPNHTAFRQAVLCWGELMTLASWVG
                     NNLEDPASRELVVNYVNNNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                     YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS                  1901..2452
                     /gene="C"
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25939.1"
                     /db_xref="GI:162957067"
                     /translation="MDIDPYKEFGATVELLSFLPSDFFPSVRDVLDTASALYREALES
                     PEHCSPNHTAFRQAVLCWGELMTLASWVGNNLEDPASRELVVNYVNNNMGLKIRQLLW
                     FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                     PSPRRRRSQSPRRRRSQSPASQC"
```

ORIGIN

```
        1 ctccacagtg ttccaccaaa ctctgcagga tcccagagta aggggtctgt atttccctgc
       61 tggtggctcc agttcaggaa cagtgaaccc tgttccgact tctgcctcca tcatatcgtc
      121 aaccttcttc aagactgggg accctgcacc gaacatggag aacatctcat caggattcct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaattcc
      241 tcagagtcta gactcgtggt ggacttctct caattttcta gggggagctc cagtgtgtcc
      301 tggccaaaat tcgcagtccc taacctccaa tcactcacca acctcttgtc ctccaatctg
      361 tcctggttat cgctggatgt gtctgcggcg ttttatcata ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga ctaccaaggt atgttgcccg tttgtcctct
      481 acttccagga tcaactacca ccagcgtggg aacatgcaga acctgcacga tttctgctcc
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct tcggacggaa attgcacctg
      601 tattcccatc ccatcatcat gggctttcgc aagattccta tgggggtggg cctcagtccg
      661 tttctcctgg ctcagtttac tagcgccatt tgttcagtgg ttcgtagggc tttcccccac
      721 tgcttggctt ttagttatat ggatgatatg gtattggggg ccaagtctgt acaacatctt
      781 gagtcccttt ataccgctgt taccaatttt cttttgtctt tgggcataca tttaaaccct
      841 aacaaaacaa aaagatgggg ctattccctt catttcatgg ggtatgtgat tggaagttgg
      901 ggaaccttgc cacaagacca tattgtacag aaaatcaaac aatgttttcg caaacttccc
      961 gtgaatcgtc ctattgattg gaaagtatgt caaagaattg tgggactctt aggctttgcc
     1021 gctcctttca ctcaatgtgg ctatcctgca ttaatgcctt tatataattg catacagaat
     1081 cgtcaggctt ttactttctc gccaacttac aaggcctttc tacgtacaca atacctgacc
     1141 ctttaccccg ttgctcggca acgacagggt gtgtgccaag tgtttgctga cgcaacccca
     1201 actggctggg gcttggcact gggttctctg cgcatgcgtg gaacctttgt ggctcctctg
     1261 ccgatccata ctgcggaact cctggccgct tgttttgctc gcagcaggtc tggagcaaac
     1321 atcatcggta ccgacaattc tgttgtgttg tcgcggaaat atacatcttt cccatggctg
     1381 ctaggttgtg ctgccaactg gatcctgcgc gggacgtcct ttgtctacgt cccgtcggcg
     1441 ctgaatcccg cggacgaccc ttctcggggt cggttagggc tctaccgccc tcttctccgt
     1501 ctgccgttcc gcccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcatctgc cggtccgtgt gcacttcgct tcacctctgc acgttgcatg gcgaccaccg
     1621 tgaacgcccc ccggaacttg ccaaaggtct tgcataagcg gactcttgga ctgtcaacaa
     1681 tgtcaacgac cggaattgag acatacttca aagactgtgt atttaaagac tgggaggagt
     1741 taggggagga gattaggcta aaggtctttg tattaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttataa
     1921 agaatttgga gctactgtgg agttactctc ttttttgcct tcggatttct ttccgtctgt
     1981 cagagatgta cttgacaccg catcagccct gtatcgggag gccttagagt ctccagaaca
     2041 ttgttcacct aaccacacag cattcaggca agctgttttg tgttggggtg agttaatgac
     2101 tctggcttcc tgggtgggta ataatttgga agatccagca tctagggaac tagtagttaa
     2161 ctatgtcaat aataatatgg gcctaaaaat tagacaattg ctgtggtttc acatttcctg
     2221 tctcaccttt ggaagagaaa cagttttaga atatttggtg tcttttggag tgtggattcg
     2281 cactcctcct gcttacagac caccaaatgc ccctatcttg tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgaag
     2401 gtctcaatca ccgcgtcgca gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcacaa ggtgggaaac tttacggggc tttactcttc tactgcacct gtctttaacc
     2521 ctaactggca aactccctct tttcctgaca ttcatttgca tcaggacatt attgataagt
     2581 gtcagcaatt tgttggtcca cttacagtaa atgaaaaacg aagattgaaa ttaatcatgc
     2641 ctgctagatt ctatcctaac tctaccaaat atttcccccc cgataaaggt attaaaccct
     2701 attatcctga gcatggggtt aatcattatt tccaaaccag acactattta cacactttgt
     2761 ggaaggcggg tatcttatat aagagagaaa ctacacgtag cgcttcattt tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc tttctgtcag caacccgctg
     2881 ggatttttc ccgagcacca gttggaccct ctgttcagag ccaacacaaa cagtccagat
     2941 tgggacttca atccccacaa ggacacctgg cccgaagcca ccaaggtagg agtgggagca
     3001 tttgggccag ggttcactcc acctcacgga ggtcttttgg ggtggagccc acaggctcag
     3061 ggagtcacca caacattgcc agcagttcct cctcctgcct ccaccaatcg gcagtcggga
     3121 aggcagccta ctcccatctc tccaccgctg agagacactc atcctcaggc catgcagtgg
     3181 aa
```

```
LOCUS       EU155824               3182 bp    DNA  circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain OS25, complete genome.
ACCESSION   EU155824
VERSION     EU155824.1  GI:162957068
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="OS25"
                     /host="orangutan (Pongo pygmaeus)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25940.1"
                     /db_xref="GI:162957069"
                  /translation="MPLSCQHFRKLLLLDEEAGPLEEELPRLADEGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTAPVFNPNWQTPSFPDIHLHQDIIDKCQQFVGPLTVN
                  EKRRLKLIMPARFYPNSTKYFPPDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESFCQQPAGIFSRAPVGPSVQSQHKQSRLGLQSPQ
                  GHLARGHQGRSGSIWARVHSTSRRSFGVEPTGSGRHHNIASSSSSCLHQSAVRKAAYS
                  HLSTAERHSSSGHAVELHSVPPNSAGSQSKGSVFPCWWLQFRNSEPCSDFCLHHIVNL
                  LQDWGPCTEHGEHLIRIPRTPARVTGGVFLVDKNPHNSSESRLVVDFSQFSRGSSSVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRINYHQRGNMQNLHDFCSRNLFVSLMLLYKTFGRKLHLYSHPIIMGFRKIPMGV
                  GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKSVQHLESLYTAVTNFLLSL
                  GIHLNPSKTKRWGYSLHFMGYVIGSWGTLPQDHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYNCIQNRQAFTFSPTYKAFLRTQYLTLYPVARQRQG
                  VCQVFADATPTGWGLALGSLRMRGTFVAPLPIHTAELLAACFARSRSGANIIGTDNSV
                  VLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPT
                      TGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP"
     gene            join(2848..3182,1..835)
                     /gene="S"
     CDS             join(2848..3182,1..835)
                     /gene="S"
                     /note="surface protein"
                     /codon_start=1
                     /product="large S protein"
```

```
                     /protein_id="ABY25941.1"
                     /db_xref="GI:162957070"
                     /translation="MGQNLSVSNPLGFFPEHQLDPLFRANTNSPDWDFNPHKDTWPEA
                     TKVGVGAFGPGFTPPHGGLLGWSPQAQGVTTTLPAVPPPASTNRQSGRQPTPISPPLR
                     DTHPQAMQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASTISSTFFKTGDP
                     APNMESISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPVCPGQNSQS
                     LTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGS
                     TTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLWGWASVRFS
                     WLSLLAPFVQWFVGLSPTAWLLVIWMMWYWGPSLYNILSPFIPLLPIFFCLWAYI"
CDS                  join(3172..3182,1..835)
                     /gene="S"
                     /codon_start=1
                     /product="middle S protein"
                     /protein_id="ABY25944.1"
                     /db_xref="GI:162957073"
                     /translation="MQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASTISS
                     TFFKTGDPAPNMESISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPV
                     CPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLP
                     VCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLW
                     GWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMMWYWGPSLYNILSPFIPLLPIFFC
                     LWAYI"
CDS                  155..835
                     /gene="S"
                     /codon_start=1
                     /product="S protein"
                     /protein_id="ABY25945.1"
                     /db_xref="GI:162957074"
                     /translation="MESISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGG
                     APVCPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQG
                     MLPVCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAR
                     FLWGWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMMWYWGPSLYNILSPFIPLLPI
                     FFCLWAYI"
gene                 1374..1838
                     /gene="X"
CDS                  1374..1838
                     /gene="X"
                     /codon_start=1
                     /product="X protein"
                     /protein_id="ABY25942.1"
                     /db_xref="GI:162957071"
                     /translation="MAARLCCQLDPARDVLCLRPVGAESRGRPFSGSVGALPPSSPSA
                     VPPDHGAHLSLRGLPVCAFSSAGPCALRFTSARCMETTVNAPRNLPKVLHKRTLGLST
                     MSTTGIETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene                 1814..2452
                     /gene="C"
CDS                  1814..2452
                     /gene="C"
                     /codon_start=1
                     /product="precore/core protein"
                     /protein_id="ABY25943.1"
                     /db_xref="GI:162957072"
                     /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYYEFGASVEL
                     LSFLPSDFFPSVRDLLDTASALYREALESPEHCSPNHTALRQAVLCWGELMTLASWVG
                     NNLEDPASRELVVNYVNNNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                     YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS                  1901..2452
                     /gene="C"
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25946.1"
                     /db_xref="GI:162957075"
                     /translation="MDIDPYYEFGASVELLSFLPSDFFPSVRDLLDTASALYREALES
                     PEHCSPNHTALRQAVLCWGELMTLASWVGNNLEDPASRELVVNYVNNNMGLKIRQLLW
                     FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                     PSPRRRRSQSPRRRRSQSPASQC"
```

ORIGIN

```
        1 ctccacagtg ttccaccaaa ctctgcagga tcccagagta aggggtctgt atttccctgc
       61 tggtggctcc agttcaggaa cagtgaaccc tgttccgact tctgcctcca ccatatcgtc
      121 aaccttcttc aagactgggg accctgcacc gaacatggag agcatctcat caggattcct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaattcc
      241 tcagagtcta gactcgtggt ggacttctct caattttcta gggggagctc cagtgtgtcc
      301 tggccaaaat tcgcagtccc taacctccaa tcactcacca acctcttgtc ctccaatctg
      361 tcctggttat cgctggatgt gtctgcggcg ttttatcata ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga ctaccaaggt atgttgcccg tttgtcctct
      481 acttccagga tcaactacca ccagcgtggg aacatgcaga acctgcacga tttctgctcc
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct tcggacggaa attgcacctg
      601 tattcccatc ccatcatcat gggctttcgc aagattccta tgggggtggg cctcagtccg
      661 tttctcctgg ctcagtttac tagcgccatt tgttcagtgg ttcgtagggc tttcccccac
      721 tgcttggctt ttagttatat ggatgatgtg gtattggggg ccaagtctgt acaacatctt
      781 gagtcccttt ataccgctgt taccaatttt cttttgtctt tgggcataca tttaaaccct
      841 agcaaaacaa aacgatgggg ctattccctt catttcatgg ggtatgtgat tggaagttgg
      901 ggaaccttgc cacaagacca tattgtacag aaaatcaaac aatgttttcg caaacttcct
      961 gtaaatcgcc ccattgattg gaaagtatgt caaagaattg taggactctt aggctttgcc
     1021 gctcctttca cgcaatgtgg ctatcctgct ttaatgcctt tatataattg catacagaat
     1081 cgtcaggctt ttactttctc gccaacttac aaggcctttc tacgtacaca atatctgacc
     1141 ctttaccccg ttgctcggca acgacagggt gtgtgccaag tgtttgctga cgcaacccca
     1201 actggctggg gcttggcact gggttctctg cgcatgcgtg gaacctttgt ggctcctctg
     1261 ccgatccata ctgcggaact cctagccgct tgttttgctc gcagcaggtc tggagcaaac
     1321 attatcggta ccgacaattc tgttgtgttg tcgcggaaat atacatcttt cccatggctg
     1381 ctaggttgtg ctgccaactg gatcctgcgc gggacgtcct ttgtctacgt cccgtcggcg
     1441 ctgaatcccg cggacgaccc ttctcggggt cggttggggc tctaccgccc tcttctccgt
     1501 ctgccgttcc gcccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcatctgc cggtccgtgt gcacttcgct tcacctctgc acgttgcatg gagaccaccg
     1621 tgaacgcccc ccggaacttg ccaaaggtct tgcataagcg gactcttgga ctgtcaacaa
     1681 tgtcaacgac cggaattgag acatacttca aagactgtgt atttaaagac tgggaggagt
     1741 taggggagga gattaggcta aaggtctttg tattaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttatta
     1921 tgaatttgga gcttctgtgg agttactctc ttttttgcct tcggacttct ttccgtctgt
     1981 cagagatcta cttgacaccg catcagccct gtatcgggag gccttagagt ctccagaaca
     2041 ttgttcacct aaccacacag cactcaggca agctgttttg tgttggggtg agttaatgac
     2101 tctggcttcc tgggtgggta ataatttgga agatccagca tctagggaac tagtagttaa
     2161 ctatgtcaat aataatatgg gcctaaaaat tagacaattg ctctggtttc acatttcctg
     2221 tctcaccttt ggaagagaaa ctgttttaga atatttggtg tcttttggag tgtggattcg
     2281 cactcctcct gcttacagac caccaaatgc ccctatcttg tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgaag
     2401 gtctcaatca ccgcgtcgca gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcacaa ggtgggaaac tttacggggc tttactcttc tactgcacct gtctttaacc
     2521 ctaactggca aactccctct tttcctgaca ttcatttgca tcaggacatt attgataagt
     2581 gtcagcaatt tgttggtcca cttacagtaa atgaaaaacg aagattgaaa ttaattatgc
     2641 ctgcaagatt ctatcctaac tctaccaaat atttcccccc agataagggt atcaaaccct
     2701 attatccaga gcatgtggtt aatcattatt tccaaaccag acactattta catactttgt
     2761 ggaaggcggg tatcttatat aagagagaaa ctacacgtag cgcttcattt tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc tttctgtcag caacccgctg
     2881 ggatttttc ccgagcacca gttggaccct ctgttcagag ccaacacaaa cagtccagat
     2941 tgggacttca atccccacaa ggacacctgg cccgaggcca ccaaggtagg agtgggagca
     3001 tttgggccag ggttcactcc acctcacgga ggtcttttgg ggtggagccc acaggctcag
     3061 ggcgtcacca caacattgcc agcagttcct cctcctgcct ccaccaatcg gcagtcagga
     3121 aggcagccta ctcccatctc tccaccgctg agagacactc atcctcaggc catgcagtgg
     3181 aa
```

```
LOCUS       EU155825               3182 bp    DNA  circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain OS27, complete genome.
ACCESSION   EU155825
VERSION     EU155825.1  GI:162957076
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="OS27"
                     /host="orangutan (Pongo pygmaeus)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25947.1"
                     /db_xref="GI:162957077"
                  /translation="MPLSCQHFRKLLLLDEEAGPLEEELPRLADEGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTAPVFNPNWQTPSFPDIHLHQDIIDKCQQFVGPLTVN
                  EKRRLKLIMPARFYPNSTKYFPPDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESFCQQPAGIFSRAPVGPSVQSQHKQSRLGLQSPQ
                  GHLARGHQGRSGSIRARVHSTSWRSFGVEPTGSGRHHNIASSSSSCLHQSAVGKAAYS
                  HLSTAERHSSSGHAVELHSVPPNSAGSQSKGSVFPCWWLQFRNSEPCSDFCLHHIVNL
                  LQDWGPCTEHGEHLIRIPRTPARVTGGVFLVDKNPHNSSESRLVVDFSQFSRGSSNVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRINNHQRGNMQNLHDFCSRNLFVSLMLLYKTFGRKLHLYSHPIIMGFRKIPMGV
                  GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDMVLGAKSVQHLESLYTAVTNFLLSL
                  GIHLNPSKTKRWGYSLHFMGYVIGSWGTLPQDHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYNCIQNRQAFTFSPTYKAFLRTQYLTLYPVARQRQG
                  VCQVFADATPTGWGLALGSLRMRGTFVAPLPIHTAELLAACFARSRSGANIIGTDNSV
                  VLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPT
                  TGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP"
     gene            join(2848..3182,1..835)
                     /gene="S"
     CDS             join(2848..3182,1..835)
                     /gene="S"
                     /note="surface protein"
                     /codon_start=1
                     /product="large S protein"
```

```
                     /protein_id="ABY25948.1"
                     /db_xref="GI:162957078"
                     /translation="MGQNLSVSNPLGFFPEHQLDPLFRANTNSPDWDFNPHKDTWPEA
                     TKVGVGAFGPGFTPPHGGLLGWSPQAQGVTTTLPAVPPPASTNRQSGRQPTPISPPLR
                     DTHPQAMQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISSTFFKTGDP
                     APNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPMCPGQNSQS
                     LTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYRGMLPVCPLLPGS
                     TTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLWGWASVRFS
                     WLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFCLWAYI"
CDS                  join(3172..3182,1..835)
                     /gene="S"
                     /codon_start=1
                     /product="middle S protein"
                     /protein_id="ABY25951.1"
                     /db_xref="GI:162957081"
                     /translation="MQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISS
                     TFFKTGDPAPNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPM
                     CPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYRGMLP
                     VCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLW
                     GWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFC
                     LWAYI"
CDS                  155..835
                     /gene="S"
                     /codon_start=1
                     /product="S protein"
                     /protein_id="ABY25952.1"
                     /db_xref="GI:162957082"
                     /translation="MENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGG
                     APMCPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYRG
                     MLPVCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAR
                     FLWGWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPI
                     FFCLWAYI"
gene                 1374..1838
                     /gene="X"
CDS                  1374..1838
                     /gene="X"
                     /codon_start=1
                     /product="X protein"
                     /protein_id="ABY25949.1"
                     /db_xref="GI:162957079"
                     /translation="MAARLCCQLDPARDVLCLRPVGAESRGRPFSGSAGALPPSSPSA
                     VPPDHGAHLSLRGLPVCAFSSAGPCALRFTSARCMETTVNAPRNLPKVLHKRTLGLST
                     MSTTGIETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene                 1814..2452
                     /gene="C"
CDS                  1814..2452
                     /gene="C"
                     /codon_start=1
                     /product="precore/core protein"
                     /protein_id="ABY25950.1"
                     /db_xref="GI:162957080"
                     /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYKEFGGTVEL
                     LFFLPSEFFPSVRDLLDTASALYREALESPEHCSPNHTALRQAVLCWGELMTLASWVG
                     NNLEDPASRELVVNYVNNNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                     YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS                  1901..2452
                     /gene="C"
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25953.1"
                     /db_xref="GI:162957083"
                     /translation="MDIDPYKEFGGTVELLFFLPSEFFPSVRDLLDTASALYREALES
                     PEHCSPNHTALRQAVLCWGELMTLASWVGNNLEDPASRELVVNYVNNNMGLKIRQLLW
                     FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                     PSPRRRRSQSPRRRRSQSPASQC"
```

ORIGIN

```
        1 ctccacagtg ttccaccaaa ctctgcagga tcccagagta aggggtctgt atttccctgc
       61 tggtggctcc agttcaggaa cagtgaaccc tgttccgact tctgcctcca tcatatcgtc
      121 aaccttcttc aagactgggg accctgcacc gaacatggag aacatctcat caggattcct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaattcc
      241 tcagagtcta gactcgtggt ggacttctct caattttcta gggggagctc caatgtgtcc
      301 tggccaaaat tcgcagtccc taacctccaa tcactcacca acctcttgtc ctccaatctg
      361 tcctggttat cgctggatgt gtctgcggcg ttttatcata ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga ttaccgaggt atgttgcccg tttgtcctct
      481 acttccagga tcaacaacca ccagcgtggg aacatgcaga acctgcacga tttctgctcc
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct tcggacggaa attgcacctg
      601 tattcccatc ccatcatcat gggctttcgc aagattccta tgggggtggg cctcagtccg
      661 tttctcctgg ctcagtttac tagcgccatt tgttcagtgg ttcgtagggc tttcccccac
      721 tgcttggctt ttagttatat ggatgatatg gtattggggg ccaagtctgt acaacatctt
      781 gagtcccttt ataccgctgt taccaatttt cttttgtctt tgggcataca tttaaaccct
      841 agcaaaacaa aaagatgggg ctattccctt catttcatgg ggtatgtgat tggaagttgg
      901 ggaaccttgc cacaagacca tattgtacag aaaatcaaac aatgttttcg caaacttcct
      961 gtaaatcgcc ctattgattg gaaagtatgt caaagaattg tgggactctt aggctttgcc
     1021 gctcctttca ctcaatgtgg ctatcctgct ttaatgcctt tatataattg catacagaat
     1081 cgtcaggctt ttactttctc gccaacttac aaggcctttc tacgtacaca atatctgacc
     1141 ctttaccccg ttgctcggca acgacagggt gtgtgccaag tgtttgctga cgcaacccca
     1201 actggctggg gcttggcact gggatctctg cgcatgcgtg gaacctttgt ggctcctctg
     1261 ccgatccata ctgcggaact cctggccgct tgttttgctc gcagcaggtc tggagcaaac
     1321 attatcggta ccgacaattc tgttgtgttg tcgcggaaat atacatcttt cccatggctg
     1381 ctaggttgtg ctgccaactg gatcctgcgc gggacgtcct ttgtctacgt cccgtcggcg
     1441 ctgaatcccg cggacgaccc ttctcggggt cggctggggc tctaccgccc tcttctccgt
     1501 ctgccgttcc gcccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcatctgc cggtccgtgt gcacttcgct tcacctctgc acgttgcatg gagaccaccg
     1621 tgaacgcccc ccggaacttg ccaaaggtct tgcataagcg gactcttgga ctgtcaacaa
     1681 tgtcaacgac cggaattgag acatacttca aagactgtgt atttaaagac tgggaggagt
     1741 taggggagga gattaggtta aaggtctttg tattaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttataa
     1921 agaatttgga ggtactgtgg agttactctt ttttttgcct tcggaattct ttccgtctgt
     1981 cagagatcta ctcgacaccg catcagccct gtatcgggag gccttagagt ctccagaaca
     2041 ttgttcacct aaccacacag cactcaggca agctgttctg tgctggggtg agttaatgac
     2101 tctggcttcc tgggtgggta ataatttgga agatccagca tctagggaac tggtagttaa
     2161 ctatgtcaat aataatatgg gcctaaaaat tagacaattg ctgtggtttc acatttcctg
     2221 tctcaccttt ggaagagaaa ccgttttaga atatttggtg tcttttggag tgtggattcg
     2281 cactcctcct gcttacagac caccaaatgc ccctatcttg tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgaag
     2401 gtctcaatca ccgcgtcgca gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcacaa ggtgggaaac tttacggggc tttactcttc tactgcacct gtctttaacc
     2521 ctaactggca aactccctct tttcctgaca ttcatttgca tcaagacatt attgataagt
     2581 gtcagcaatt tgttggtcca cttacagtaa atgaaaaacg aagattgaaa ttaatcatgc
     2641 ctgcgagatt ctatcctaac tctaccaaat attttccccc agataaaggt atcaaaccct
     2701 attatccaga gcatgtggtt aatcattatt tccaaaccag acactattta cacactttgt
     2761 ggaaggcggg tatcttatat aagagagaaa ctacacgtag cgcttcattt tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc tttctgtcag caacccgctg
     2881 ggatttttc ccgagcacca gttggaccct ctgttcagag ccaacacaaa cagtccagat
     2941 tgggacttca atccccacaa ggacacctgg cccgaggcca ccaaggtagg agtgggagca
     3001 ttcgggccag ggttcactcc acctcatgga ggtcttttgg ggtggagccc acaggctcag
     3061 ggcgtcacca caacattgcc agcagttcct cctcctgcct ccaccaatcg gcagtcggga
     3121 aggcagccta ctcccatctc tccaccgctg agagacactc atcctcaggc catgcagtgg
     3181 aa
```

```
LOCUS       EU155826               3182 bp    DNA  circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain OS28, complete genome.
ACCESSION   EU155826
VERSION     EU155826.1  GI:162957084
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="OS28"
                     /host="orangutan (Pongo pygmaeus)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25954.1"
                     /db_xref="GI:162957085"
                  /translation="MPLSCQHFRKLLLLDEEAGPLEEELPRLADEGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTAPVFNPNWQTPSFPDIHLHQDIIDKCQQFVGPLTVN
                  EKRRLKLIMPARFYPNSTKYFPPDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESFCQQPAGIFSRAPVGPSVQSQHKQSRLGLQSPQ
                  GHLARSNQGRSGSIRARVHSTSRRSFGVEPTGSGRHHNIASSSSSCLHQSAVGKAAYS
                  HLSTAERHSSSGHAVELHSVPPNSAGSQSKGSVFPCWWLQFRNSEPCSDFCLHHIVNL
                  LQDWGPCTEHGEHLIRIPRTPARVTGGVFLVDKNPHNSSESRLVVDFSQFSRGSSSVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRINYHQRGNMQNLHDFCSRNLFVSLMLLYKTFGRKLHLYSHPIIMGFRKIPMGV
                  GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDMVLGAKSVQHLESLYTAVTNFLLSL
                  GIHLNPSKTKRWGYSLHFMGYVIGSWGTLPQDHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYNCIQNRQAFTFSPTYKAFLRTQYLTLYPVARQRQG
                  VCQVFADATPTGWGLALGSLRMRGTFVAPLPIHTAELLAACFARSRSGANIIGTDNSV
                  VLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPT
                  TGRTSLYAVSPSVPSHLPDRVHFASPLHVAWRPP"
     gene            join(2848..3182,1..835)
                     /gene="S"
     CDS             join(2848..3182,1..835)
                     /gene="S"
                     /note="surface protein"
                     /codon_start=1
                     /product="large S protein"
```

```
                     /protein_id="ABY25955.1"
                     /db_xref="GI:162957086"
                     /translation="MGQNLSVSNPLGFFPEHQLDPLFRANTNSPDWDFNPHKDTWPEA
                     TKVGVGAFGPGFTPPHGGLLGWSPQAQGVTTTLPAVPPPASTNRQSGRQPTPISPPLR
                     DTHPQAMQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISSTFFKTGDP
                     APNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPVCPGQNSQS
                     LTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGS
                     TTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAKFLWGWASVRFS
                     WLNLLVPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFCLWAYI"
CDS                  join(3172..3182,1..835)
                     /gene="S"
                     /codon_start=1
                     /product="middle S protein"
                     /protein_id="ABY25958.1"
                     /db_xref="GI:162957089"
                     /translation="MQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISS
                     TFFKTGDPAPNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPV
                     CPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLP
                     VCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAKFLW
                     GWASVRFSWLNLLVPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFC
                     LWAYI"
CDS                  155..835
                     /gene="S"
                     /codon_start=1
                     /product="S protein"
                     /protein_id="ABY25959.1"
                     /db_xref="GI:162957090"
                     /translation="MENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGG
                     APVCPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQG
                     MLPVCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAK
                     FLWGWASVRFSWLNLLVPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPI
                     FFCLWAYI"
gene                 1374..1838
                     /gene="X"
CDS                  1374..1838
                     /gene="X"
                     /codon_start=1
                     /product="X protein"
                     /protein_id="ABY25956.1"
                     /db_xref="GI:162957087"
                     /translation="MAARLCCQLDPARDVLCLRPVGAESRGRPFSGSAGALPPSSPSA
                     VPPDHGAHLSLRGLPVCAFSSAGPCALRFTSARCMETTVNAPRNLPKVLHKRTLGLST
                     MSTTGIETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene                 1814..2452
                     /gene="C"
CDS                  1814..2452
                     /gene="C"
                     /codon_start=1
                     /product="precore/core protein"
                     /protein_id="ABY25957.1"
                     /db_xref="GI:162957088"
                     /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYKEFGATVEL
                     LSFLPSDFFPSVRDRLDTASALYREALESPEHCSPNHTALRQGVLCWGELMTLASWVG
                     NNLEDPASRELVVNYVNNNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                     YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS                  1901..2452
                     /gene="C"
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25960.1"
                     /db_xref="GI:162957091"
                     /translation="MDIDPYKEFGATVELLSFLPSDFFPSVRDRLDTASALYREALES
                     PEHCSPNHTALRQGVLCWGELMTLASWVGNNLEDPASRELVVNYVNNNMGLKIRQLLW
                     FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                     PSPRRRRSQSPRRRRSQSPASQC"
```

```
ORIGIN
        1 ctccacagtg ttccaccaaa ctctgcagga tcccagagta aggggtctgt atttccctgc
       61 tggtggctcc agttcaggaa cagtgaaccc tgttccgact tctgcctcca tcatatcgtc
      121 aaccttcttc aagactgggg accctgcacc gaacatggag aacatctcat caggattcct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaattcc
      241 tcagagtcta gactcgtggt ggacttctct caattttcta gggggagctc cagtgtgtcc
      301 tggccaaaat tcgcagtccc taacctccaa tcactcacca acctcttgtc ctccaatctg
      361 tcctggttat cgctggatgt gtctgcggcg ttttatcata ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga ctaccaaggt atgttgcccg tttgtcctct
      481 acttccagga tcaactacca ccagcgtggg aacatgcaga acctgcacga tttctgctcc
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct tcggacggaa attgcacctg
      601 tattcccatc ccatcatcat gggctttcgc aaaattccta tgggggtggg cctcagtccg
      661 tttctcctgg ctcaatttac tagtgccatt tgttcagtgg ttcgtagggc tttcccccac
      721 tgcttggctt ttagttatat ggatgatatg gtattggggg ccaagtctgt acaacatctt
      781 gagtcccttt ataccgctgt taccaatttt cttttgtctt tgggcataca tttaaaccct
      841 agcaaaacaa aaagatgggg ctattccctt catttcatgg ggtatgtgat tggaagttgg
      901 ggaaccttgc cacaagacca tattgtacag aaaatcaaac aatgttttcg caaacttcct
      961 gtaaatcgcc ctattgattg gaaagtatgt caaagaattg tgggactctt aggctttgcc
     1021 gctcctttca ctcaatgtgg ctatcctgct ttaatgcctt tatataattg catacagaat
     1081 cgtcaggctt ttactttctc gccaacttac aaggcctttc tacgtacaca atatctgacc
     1141 ctttaccccg ttgctcggca acgacagggt gtgtgccaag tgtttgctga cgcaacccca
     1201 actggctggg gcttggcact gggatctctg cgcatgcgtg gaacctttgt ggctcctctg
     1261 ccgatccata ctgcggaact cctggccgct tgttttgctc gcagcaggtc tggagcaaac
     1321 attatcggta ccgacaattc tgttgtgttg tcgcggaaat atacatcttt cccatggctg
     1381 ctaggttgtg ctgccaactg gatcctgcgc gggacgtcct ttgtctacgt cccgtcggcg
     1441 ctgaatcccg cggacgaccc ttctcggggt cggctggggc tctaccgccc tcttctccgt
     1501 ctgccgttcc gcccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcatctgc cggaccgtgt gcacttcgct tcacctctgc acgttgcatg gagaccaccg
     1621 tgaacgcccc ccggaacttg ccaaaggtct tgcataagcg gactcttgga ctgtcaacaa
     1681 tgtcaacgac cggaattgag acatacttca aagactgtgt atttaaagac tgggaggagt
     1741 taggggagga gattaggcta aaggtctttg tattaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttataa
     1921 agaatttgga gctactgtgg agttactctc ttttttgcct tcggatttct ttccttctgt
     1981 cagagatcga ctcgacaccg catcagccct gtatcgggag gccttagagt ctccagaaca
     2041 ttgttcacct aaccacacag cactcaggca aggtgttttg tgctggggtg agttaatgac
     2101 tctggcttcc tgggtgggta ataatttgga agatccagca tctagggaac tggtagttaa
     2161 ctatgtcaat aataatatgg gcctaaaaat tagacaattg ctgtggtttc acatttcctg
     2221 tctcaccttt ggaagagaaa ccgttttaga atatttggtg tcttttggag tgtggattcg
     2281 cactcctcct gcttacagac caccaaatgc ccctatcttg tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgaag
     2401 gtctcaatca ccgcgtcgca gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcacaa ggtgggaaac tttacggggc tttactcttc tactgcacct gtctttaacc
     2521 ctaactggca aactccctct tttcctgaca ttcatttgca tcaagacatt attgataagt
     2581 gtcagcaatt tgttggtcca cttacagtaa atgaaaaacg aagattgaaa ttaatcatgc
     2641 ctgcgagatt ctatcctaac tctaccaaat attttccccc agataaaggt atcaaaccct
     2701 attatccaga gcatgtggtt aatcattatt tccaaaccag acactattta cacactttgt
     2761 ggaaggcggg tatcttatat aagagagaaa ctacacgtag cgcttcattt tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc tttctgtcag caacccgctg
     2881 ggatttttc ccgagcacca gttggaccct ctgttcagag ccaacacaaa cagtccagat
     2941 tgggacttca atccccacaa ggacacctgg cccgaagcaa ccaaggtagg agtgggagca
     3001 ttcgggccag ggttcactcc acctcacgga ggtcttttgg ggtggagccc acaggctcag
     3061 ggcgtcacca caacattgcc agcagttcct cctcctgcct ccaccaatcg gcagtcggga
     3121 aggcagccta ctcccatctc tccaccgctg agagacactc atcctcaggc catgcagtgg
     3181 aa
```

```
LOCUS       EU155827               3182 bp    DNA  circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain OS39, complete genome.
ACCESSION   EU155827
VERSION     EU155827.1  GI:162957092
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="OS39"
                     /host="orangutan (Pongo pygmaeus)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25961.1"
                     /db_xref="GI:162957093"
                  /translation="MPLSCQHFRKLLLLDEEAGPLEEELPRLADEGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTAPVFNPNWQTPSFPDIHLHQNIIDKCQQFVGPLTVN
                  EKRRLKLIMPARFYPNSTKYFPPDKGIKPYYPEHVVNHYFQTRHYLHTLWKAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESFCQQPAGIFSRAPVGPSVQSQHKQSRLGLQSPQ
                  GHLARGHQGRSGSIRARVHSTSWRSFGVEPAGSGRHHNIASSSSSCLHQSAVGKAAYS
                  HLSTAERHSSSGHAVELHSVPPNSAGSQSKGSVFPCWWLQFRNSEPCSDFCLHHIVNL
                  LQDWGPCTEHGEHLIRIPRTPARVTGGVFLVDKNPHNSSESRLVVDFSQFSRGSSSVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRINYHQRGNMQNLHDFCSRNLFVSLMLLYKTFGRKLHLYSHPIIMGFRKIPMGV
                  GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDMVLGAKSVQHLESLYTAVTNFLLSL
                  GIHLNPSKTKRWGYSLHFMGYVIGSWGTLPQDHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYNCIQNRQAFTFSPTYKAFLRTQYLTLYPVARQRQG
                  VCQVFADATPTGWGLALGSLRMRGTFVAPLPIHTAELLAACFARSRSGANIIGTDNSV
                  VLSRKYTSFPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFRPT
                  TGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP"
     gene            join(2848..3182,1..835)
                     /gene="S"
     CDS             join(2848..3182,1..835)
                     /gene="S"
                     /note="surface protein"
                     /codon_start=1
                     /product="large S protein"
```

```
                     /protein_id="ABY25962.1"
                     /db_xref="GI:162957094"
                     /translation="MGQNLSVSNPLGFFPEHQLDPLFRANTNSPDWDFNPHKDTWPEA
                     TKVGVGAFGPGFTPPHGGLLGWSPQAQGVTTTLPAVPPPASTNRQSGRQPTPISPPLR
                     DTHPQAMQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISSTFFKTGDP
                     APNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPVCPGQNSQS
                     LTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGS
                     TTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLWGWASVRFS
                     WLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFCLWAYI"
CDS                  join(3172..3182,1..835)
                     /gene="S"
                     /codon_start=1
                     /product="middle S protein"
                     /protein_id="ABY25965.1"
                     /db_xref="GI:162957097"
                     /translation="MQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTSASIISS
                     TFFKTGDPAPNMENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPV
                     CPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLP
                     VCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFARFLW
                     GWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPIFFC
                     LWAYI"
CDS                  155..835
                     /gene="S"
                     /codon_start=1
                     /product="S protein"
                     /protein_id="ABY25966.1"
                     /db_xref="GI:162957098"
                     /translation="MENISSGFLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGG
                     APVCPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQG
                     MLPVCPLLPGSTTTSVGTCRTCTISAPGTSLFPSCCCTKPSDGNCTCIPIPSSWAFAR
                     FLWGWASVRFSWLSLLAPFVQWFVGLSPTAWLLVIWMIWYWGPSLYNILSPFIPLLPI
                     FFCLWAYI"
gene                 1374..1838
                     /gene="X"
CDS                  1374..1838
                     /gene="X"
                     /codon_start=1
                     /product="X protein"
                     /protein_id="ABY25963.1"
                     /db_xref="GI:162957095"
                     /translation="MAARLCCQLDPARDVLCLRPVGAESRGRPFSGSAGALPPSSPSA
                     VPPDHGAHLSLRGLPVCAFSSAGPCALRFTSARCMETTVNAPRNLPKVLHKRTLGLST
                     MSTTGIETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene                 1814..2452
                     /gene="C"
CDS                  1814..2452
                     /gene="C"
                     /codon_start=1
                     /product="precore/core protein"
                     /protein_id="ABY25964.1"
                     /db_xref="GI:162957096"
                     /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYKEFGATVEL
                     LSFLPSDFFPSVRDLLDTASALYREALESPEHCSPNHTALRQAVLCWGELMTLASWVG
                     NNLEDPASRELVVNYVNNNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                     YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS                  1901..2452
                     /gene="C"
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25967.1"
                     /db_xref="GI:162957099"
                     /translation="MDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYREALES
                     PEHCSPNHTALRQAVLCWGELMTLASWVGNNLEDPASRELVVNYVNNNMGLKIRQLLW
                     FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                     PSPRRRRSQSPRRRRSQSPASQC"
```

ORIGIN

```
        1 ctccacagtg ttccaccaaa ctctgcagga tcccagagta aggggtctgt atttccctgc
       61 tggtggctcc agttcaggaa cagtgaaccc tgttccgact tctgcctcca tcatatcgtc
      121 aaccttcttc aagactgggg accctgcacc gaacatggag aacatctcat caggattcct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaattcc
      241 tcagagtcta gactcgtggt ggacttctct caattttcta gggggagctc cagtgtgtcc
      301 tggccaaaat tcgcagtccc taacctccaa tcactcacca acctcttgtc ctccaatctg
      361 tcctggttat cgctggatgt gtctgcggcg ttttatcata ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga ctaccaaggt atgttgcccg tttgtcctct
      481 acttccagga tcaactacca ccagcgtggg aacatgcaga acctgcacga tttctgctcc
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct tcggacggaa attgcacctg
      601 tattcccatc ccatcatcat gggctttcgc aagattccta tgggggtggg cctcagtccg
      661 tttctcctgg ctcagtttac tagcgccatt tgttcagtgg ttcgtagggc tttcccccac
      721 tgcttggctt ttagttatat ggatgatatg gtattggggg ccaagtctgt acaacatctt
      781 gagtcccttt ataccgctgt taccaatttt cttttgtctt tgggcataca tttaaaccct
      841 agcaaaacaa aaagatgggg ctattccctt catttcatgg ggtatgtgat tggaagttgg
      901 ggaaccttgc cacaagacca tattgtacag aaaatcaaac aatgttttcg caaacttcct
      961 gtaaatcgcc ctattgattg gaaagtatgt caaagaattg tgggactctt aggctttgcc
     1021 gctcctttca ctcaatgtgg ctatcctgct ttaatgcctt tatataattg catacagaat
     1081 cgtcaggctt ttactttctc gccaacttac aaggcctttc tacgtacaca atatctgacc
     1141 ctttaccccg ttgctcggca acgacagggt gtgtgccaag tgtttgctga cgcaacccca
     1201 actggctggg gcttggcact gggatctctg cgcatgcgtg gaacctttgt ggctcctctg
     1261 ccgatccata ctgcggaact cctggccgct tgttttgctc gcagcaggtc tggagcaaac
     1321 attatcggta ccgacaattc tgttgtgttg tcgcggaaat atacatcttt cccatggctg
     1381 ctaggttgtg ctgccaactg gatcctgcgc gggacgtcct ttgtctacgt cccgtcggcg
     1441 ctgaatcccg cggacgaccc ttctcggggt cggctggggc tctaccgccc tcttctccgt
     1501 ctgccgttcc gcccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcatctgc cggtccgtgt gcacttcgct tcacctctgc acgttgcatg gagaccaccg
     1621 tgaacgcccc ccggaacttg ccaaaggtct tgcataagcg gactcttgga ctgtcaacaa
     1681 tgtcaacgac cggaattgag acatacttca aagactgtgt atttaaagac tgggaggagt
     1741 taggggagga gattaggcta aaggtctttg tattaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttataa
     1921 agaatttgga gctactgtgg agttactctc ttttttgcct tcggacttct ttccgtctgt
     1981 cagagatcta ctcgacaccg catcagccct gtatcgggag gccttagagt ctccagaaca
     2041 ttgttcacct aaccacacag cactcaggca agctgttctg tgctggggtg agttaatgac
     2101 tctggcttcc tgggtgggta ataatttgga agatccagca tctagggaac tggtagttaa
     2161 ctatgtcaat aataatatgg gcctaaaaat tagacaattg ctgtggtttc acatttcctg
     2221 tctcaccttt ggaagagaaa ccgttttaga atatttggtg tcttttggag tgtggattcg
     2281 cactcctcct gcttacagac caccaaatgc ccctatcttg tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgaag
     2401 gtctcaatca ccgcgtcgca gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcacaa ggtgggaaac tttacggggc tttactcttc tactgcacct gtctttaacc
     2521 ctaactggca aactccctct tttcctgaca ttcatttgca tcaaaacatt attgataagt
     2581 gtcagcaatt tgttggtcca cttacagtaa atgaaaaacg aagattgaaa ttaatcatgc
     2641 ctgcgagatt ctatcctaac tctaccaaat attttccccc agataaaggt atcaaaccct
     2701 attatccaga gcatgtggtt aatcattatt tccaaaccag acactattta cacactttgt
     2761 ggaaggcggg tatcttatat aagagagaaa ctacacgtag cgcttcattt tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc tttctgtcag caacccgctg
     2881 ggatttttc ccgagcacca gttggaccct ctgttcagag ccaacacaaa cagtccagat
     2941 tgggacttca atccccacaa ggacacctgg cccgaggcca ccaaggtagg agtgggagca
     3001 ttcgggccag ggttcactcc acctcatgga ggtcttttgg ggtggagccc gcaggctcag
     3061 ggcgtcacca caacattgcc agcagttcct cctcctgcct ccaccaatcg gcagtcggga
     3121 aggcagccta ctcccatctc tccaccgctg agagacactc atcctcaggc catgcagtgg
     3181 aa
```

```
LOCUS       EU155828               3182 bp    DNA  circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain GD14, complete genome.
ACCESSION   EU155828
VERSION     EU155828.1  GI:162957100
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="GD14"
                     /host="gibbon (Nomascus leucogenys)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25968.1"
                     /db_xref="GI:162957101"
                  /translation="MPLSYQHFRKLLLLDEEAGPLEEELPRLADDGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTVPVFNPEWQTPSFPDIHLHQDIIDRCQQFVGPLTVN
                  EKRRLKLIMPARFYPNATKYLPLEKGIKPYYPEHVVNHYFQTRHYLHTLWEAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESLCHQSAGILPRAPVRPSVQSQLKQSRLGLQPQQ
                  GQLARSHQGRSGCIRARVHPTTRRPFGVEPSGSGNENNLASSSSYCLHQSAVRKETYA
                  NLSTFERHPSAGHAVELHSIPPDSTRSQSKGPVFSCWWLQFRDSEPCSDYCLSHLVNL
                  LEDWGPCTEHGKHHIRIPRTPARVTGGVFLVDKNPHNSAESRLVVDFSQFSRGNTRVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRNIDHQHGTMQNLHDSCSRNLFVSLMLLYKTYGRKLHLYSHPIVMGFRKIPMGV
                  GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDLVLGAKSVQHLEALYTTVTNFLLSL
                  GIHLNPDKTKRWGYSLHFMGYVIGSWGTLPQEHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYACIHAKQAFTFSHTYKAFLRDQYLNLYPVARQRPG
                  LCQVFADATPTGWGLAIGHQRLRGTFVAPLPIHTAELLAACFARSRSGAKLIGTDNSV
                  VLSRKYTSFPWLLGCAVNWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFQPT
                      TGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP"
     gene            join(2848..3182,1..835)
                     /gene="S"
     CDS             join(2848..3182,1..835)
                     /gene="S"
                     /note="surface protein"
                     /codon_start=1
                     /product="large S protein"
```

```
                     /protein_id="ABY25969.1"
                     /db_xref="GI:162957102"
                     /translation="MGQNHSVTNPLGFFPEHQLDPLFRANSNNPDWDFNPNKDNWPEA
                     TKVGVGAFGPGFTPPHGGLLGWSPQAQGMKTTLPAVPPTASTNRQSGRKPTPISPPLR
                     DTHPQAMQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTTASHISSTFSKTGDP
                     VPNMENITSGYLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGVTPVCPGQNSQS
                     PISNHSPTSCPPTCPGYRWMCLRRSIIFLFILLLCLIFLLVLLDFQGMLPVCPLLPGT
                     STTSTGPCKTCTIPAQGTSLFPSCCCTKPTDGNCTCIPIPSSWAFAKFLWEWASVRFS
                     WLSLLAPFVQWFAGLSPTAWLLAIWMIWYWGPSLYNILKPFIPLLPIFFCLWVYI"
CDS                  join(3172..3182,1..835)
                     /gene="S"
                     /codon_start=1
                     /product="middle S protein"
                     /protein_id="ABY25972.1"
                     /db_xref="GI:162957105"
                     /translation="MQWNSTVFHQTLQDPRVRGLYFPAGGSSSGTVNPVPTTASHISS
                     TFSKTGDPVPNMENITSGYLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGVTPV
                     CPGQNSQSPISNHSPTSCPPTCPGYRWMCLRRSIIFLFILLLCLIFLLVLLDFQGMLP
                     VCPLLPGTSTTSTGPCKTCTIPAQGTSLFPSCCCTKPTDGNCTCIPIPSSWAFAKFLW
                     EWASVRFSWLSLLAPFVQWFAGLSPTAWLLAIWMIWYWGPSLYNILKPFIPLLPIFFC
                     LWVYI"
CDS                  155..835
                     /gene="S"
                     /codon_start=1
                     /product="S protein"
                     /protein_id="ABY25973.1"
                     /db_xref="GI:162957106"
                     /translation="MENITSGYLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGV
                     TPVCPGQNSQSPISNHSPTSCPPTCPGYRWMCLRRSIIFLFILLLCLIFLLVLLDFQG
                     MLPVCPLLPGTSTTSTGPCKTCTIPAQGTSLFPSCCCTKPTDGNCTCIPIPSSWAFAK
                     FLWEWASVRFSWLSLLAPFVQWFAGLSPTAWLLAIWMIWYWGPSLYNILKPFIPLLPI
                     FFCLWVYI"
gene                 1374..1838
                     /gene="X"
CDS                  1374..1838
                     /gene="X"
                     /codon_start=1
                     /product="X protein"
                     /protein_id="ABY25970.1"
                     /db_xref="GI:162957103"
                     /translation="MAARLCCQLDPTRDVLCLRPLGAESRGRPLSGPLGALPPPSPSA
                     VPADHGAHLSLRGLPVCAFSPAGPCALRFTSARCMETTVNAPRSLPRTLHKRTLGLSA
                     MSTTEVETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene                 1814..2452
                     /gene="C"
CDS                  1814..2452
                     /gene="C"
                     /codon_start=1
                     /product="precore/core protein"
                     /protein_id="ABY25971.1"
                     /db_xref="GI:162957104"
                     /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYKEFGATAEL
                     LSFLPPDFFPSVRDLLDTASALYREALESPEHCSPNHTALRQAVLCWGELMTLASWVG
                     NNLEDPASRELVVNYVNHNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                     YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS                  1901..2452
                     /gene="C"
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25974.1"
                     /db_xref="GI:162957107"
                     /translation="MDIDPYKEFGATAELLSFLPPDFFPSVRDLLDTASALYREALES
                     PEHCSPNHTALRQAVLCWGELMTLASWVGNNLEDPASRELVVNYVNHNMGLKIRQLLW
                     FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                     PSPRRRRSQSPRRRRSQSPASQC"
```

```
ORIGIN
        1 ctccacagta ttccaccaga ctctacaaga tcccagagta aggggcctgt attttcctgc
       61 tggtggctcc agttcaggga cagtgaaccc tgttccgact actgcctctc acatctcgtc
      121 aaccttctcg aagactgggg accctgtacc gaacatggaa aacatcacat caggatacct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaattcc
      241 gcagagtcta gactcgtggt ggacttctct caattttcta ggggtaacac ccgtgtgtcc
      301 tggccaaaat tcgcagtccc caatctccaa tcactcacca acctcttgtc ctccaacttg
      361 tcctggctat cgctggatgt gtctgcggcg ttctatcatc ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga cttccaaggt atgttgcccg tttgtcctct
      481 acttccagga acatcgacca ccagcacggg accatgcaaa acctgcacga ttcctgctca
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct acggacggaa attgcacctg
      601 tattcccatc ccatcgtcat gggctttcgc aaaattccta tgggagtggg cctcagtccg
      661 tttctcctgg ctcagtttac tagcgccatt tgttcagtgg ttcgcagggc tttcccccac
      721 tgcttggctt ttagctatat ggatgatctg gtattggggg ccaagtctgt acaacatctt
      781 gaagcccttt ataccactgt taccaatttt cttttgtctc tgggtataca tttgaatcct
      841 gacaaaacca aaagatgggg gtattccctt catttcatgg gttacgtgat cggaagttgg
      901 ggtaccttac cacaggaaca tattgtacaa aaaatcaaac agtgtttcag aaagctccct
      961 gtaaacaggc ctattgattg gaaagtgtgc caacgaattg tgggtctttt aggcttcgct
     1021 gccccttta cacaatgtgg atatcctgct ttaatgcctt tatatgcatg tatacacgct
     1081 aagcaggctt ttacttttc ccacacttac aaggcctttc tacgcgatca atatctgaac
     1141 ctttaccccg ttgcccggca acggccaggc ctatgccaag tgtttgctga cgcaaccccc
     1201 actggttggg gcttggccat cggccatcag cgcttgcgcg gaacctttgt ggctcctctg
     1261 ccgatccata cagcggaact cctagccgct tgttttgctc gcagccggtc tggggcaaag
     1321 ctcatcggga ctgacaactc tgttgttctc tcccgcaaat atacatcgtt tccatggctg
     1381 ctaggctgtg ctgtcaactg gatcctacgc gggacgtcct ttgtttacgt cccctcggcg
     1441 ctgaatcccg cggacgaccc ctctcggggc cgcttggggc tctaccgccc ccttctccgt
     1501 ctgccgttcc agccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcacctgc cggtccgtgt gcacttcgct tcacctctgc acgttgcatg gagaccaccg
     1621 tgaacgcccc ccggagcttg ccaaggacat tgcacaagag gactcttgga ctttcagcaa
     1681 tgtcaacgac cgaagttgaa acatacttca aagactgtgt gtttaaggac tgggaggagt
     1741 taggggagga gatcaggtta aaggtctttg tactaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttataa
     1921 agaatttgga gctactgcgg agttactctc tttcttgcct cctgacttct ttccgtcggt
     1981 cagagatctc ctcgacaccg cctcagctct gtatcgagaa gccttagagt ctccagagca
     2041 ttgttcacct aaccatacag cactcaggca agctgttctg tgttggggtg agttgatgac
     2101 tctggcttcc tgggtgggca ataatttgga agacccagca tctagagaac tagtagtcaa
     2161 ttatgttaat cacaacatgg gtttgaaaat caggcaactt ttgtggtttc acatttcctg
     2221 tctcactttt ggaagagaaa ctgtccttga gtatttggtg tcttttggag tgtggattcg
     2281 cactcctccc gcttacagac caccaaatgc ccctatctta tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgacg
     2401 gtctcaatca ccgcgtcgcc gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcataa ggtgggaaac tttactgggc tttattcttc tactgtacct gtctttaatc
     2521 ctgagtggca aactccttcc tttcctgata tacatttaca tcaagatatt attgatagat
     2581 gtcaacaatt tgtgggccct ctcacagtaa atgaaaagcg aagattaaaa ttaattatgc
     2641 ctgctagatt ctatcctaat gctaccaaat atttgcctct agagaaaggt attaaacctt
     2701 attatccaga gcatgtagtt aatcattact tccaaaccag acactatttg cacactctgt
     2761 gggaggcggg catcttatat aagagagaga cgacacgtag cgcctccttc tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc actctgtcac caatccgctg
     2881 ggattcttcc ccgagcacca gttagaccct ctgttcagag ccaactcaaa caatccagat
     2941 tgggacttca accccaacaa ggacaactgg ccagaagcca ccaaggtagg agtgggtgca
     3001 ttcgggccag ggttcacccc accacacgga ggccttttgg ggtggagccc tcaggctcag
     3061 ggaatgaaaa caaccttgcc agcagttcct cctactgcct ccaccaatcg gcagtcagga
     3121 aggaaaccta cgccaatctc tccacctttg agagacaccc atccgcaggc catgcagtgg
     3181 aa
```

```
LOCUS       EU155829               3182 bp    DNA  circular VRL 15-JAN-2009
DEFINITION  Hepatitis B virus strain GD21, complete genome.
ACCESSION   EU155829
VERSION     EU155829.1  GI:162957108
KEYWORDS    .
SOURCE      Hepatitis B virus
  ORGANISM  Hepatitis B virus
            Viruses; Retro-transcribing viruses; Hepadnaviridae;
            Orthohepadnavirus.
REFERENCE   1  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Ratanakorn,P., Pattanarangsan,R.,
            Boonyarittichaikij,R., Chodapisitkul,S., Theamboonlers,A.,
            Tangkijvanich,P. and Poovorawan,Y.
  TITLE     Prevalence, whole genome characterization and phylogenetic
analysis
            of hepatitis B virus in captive orangutan and gibbon
  JOURNAL   J. Med. Primatol. 37 (6), 277-289 (2008)
   PUBMED   18466280
REFERENCE   2  (bases 1 to 3182)
  AUTHORS   Sa-nguanmoo,P., Thongmee,C., Praianantathavorn,K.,
Thawornsuk,N.,
            Ratanakorn,P., Pattanarangsan,R., Boonyarittachaikij,R.,
            Theamboonlers,A. and Poovorawan,Y.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-SEP-2007) Pediatrics, Faculty of Medicine,
            Chulalongkorn University, Center of Excellence in Clinical
            Virology, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..3182
                     /organism="Hepatitis B virus"
                     /mol_type="genomic DNA"
                     /strain="GD21"
                     /host="gibbon (Nomascus leucogenys)"
                     /db_xref="taxon:10407"
                     /country="Thailand"
     gene            join(2307..3182,1..1623)
                     /gene="P"
     CDS             join(2307..3182,1..1623)
                     /gene="P"
                     /codon_start=1
                     /product="polymerase"
                     /protein_id="ABY25975.1"
                     /db_xref="GI:162957109"
                  /translation="MPLSYQHFRKLLLLDEEAGPLEEELPRLADDGLNHRVAEDLNLQ
                  LPNVSIPWTHKVGNFTGLYSSTVPVFNPEWQTPSFPDIHLHQDIIDRCQQFVGPLTVN
                  EKRRLKLIMPARFYPNSTKYLPLEKGIKPYYPEHVVNHYFQTRHYLHTLWEAGILYKR
                  ETTRSASFCGSPYSWEQELQHGAESLCHQSAGILPRAPVGPSVQSQLKQSRLGLQPQQ
                  GQLARSHQGRSGCIRARVHPTTRRPFGVEPSGSGNNNNLASSSSYCLHQSAVRKETYS
                  NLSTFERHSSSGHAVELHSIPPDSTRSQSKGPVFSCWWLQFRNSEPCSDYCLSHLVNL
                  LEDWGPCTEHGKHHIRIPRTPARVTGGVFLVDKNPHNTSESRLVVDFSQFSRGSTRVS
                  WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLPRYVARL
                  SSTSRNIDHQHGTMQNLHDSCSRNLFVSLMLLYKTYGRKLHLYSHPIVMGFRKIPMGV
                  GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDLVLGAKSVQHLEALYTTVTNFLLSL
                  GIHLNPDKTKRWGYSLNFMGYVIGSWGTLPQEHIVQKIKQCFRKLPVNRPIDWKVCQR
                  IVGLLGFAAPFTQCGYPALMPLYACIHAKQAFTFSHTYKAFLRDQYLNLYPVARQRPG
                  LCQVFADATPTGWGLAIGHQRLRGTFVAPLPIHTAELLAACFARSRSGAKLIGTDNSV
                  VLSRKYTSFPWLLGCAVNWILRGTSFVYVPSALNPADDPSRGRLGLYRPLLRLPFQPT
                  TGRTSLYAVSPSVPSHLPVRVHFASPLHVAWRPP"
     gene            join(2848..3182,1..835)
                     /gene="S"
     CDS             join(2848..3182,1..835)
                     /gene="S"
                     /note="surface protein"
                     /codon_start=1
                     /product="large S protein"
```

```
                     /protein_id="ABY25976.1"
                     /db_xref="GI:162957110"
                     /translation="MGQNHSVTNPLGFFPEHQLDPLFRANSNNPDWDFNPNKDNWPEA
                     TKVGVGAFGPGFTPPHGGLLGWSPQAQGITTTLPAVPPTASTNRQSGRKPTPISPPLR
                     DTHPQAMQWNSTVFHQILQDPRVRGLYFPAGGSSSGTVNPVPTTASHISSTFSKTGDP
                     VPNMENITSGYLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPVCPGQNSQS
                     LTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDFQGMLPVCPLLPGT
                     STTSTGPCRTCTIPAQGTSLFPSCCCTKPTDGNCTCIPIPSSWAFAKFLWEWASVRFS
                     WLSLLAPFVQWFVGLSPTAWLLAIWMIWYWGPSLYNILKPFIPLLPIFFCLWVYI"
CDS                  join(3172..3182,1..835)
                     /gene="S"
                     /codon_start=1
                     /product="middle S protein"
                     /protein_id="ABY25979.1"
                     /db_xref="GI:162957113"
                     /translation="MQWNSTVFHQILQDPRVRGLYFPAGGSSSGTVNPVPTTASHISS
                     TFSKTGDPVPNMENITSGYLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGGAPV
                     CPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDFQGMLP
                     VCPLLPGTSTTSTGPCRTCTIPAQGTSLFPSCCCTKPTDGNCTCIPIPSSWAFAKFLW
                     EWASVRFSWLSLLAPFVQWFVGLSPTAWLLAIWMIWYWGPSLYNILKPFIPLLPIFFC
                     LWVYI"
CDS                  155..835
                     /gene="S"
                     /codon_start=1
                     /product="S protein"
                     /protein_id="ABY25980.1"
                     /db_xref="GI:162957114"
                     /translation="MENITSGYLGPLLVLQAGFFLLTKILTIPQSLDSWWTSLNFLGG
                     APVCPGQNSQSLTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDFQG
                     MLPVCPLLPGTSTTSTGPCRTCTIPAQGTSLFPSCCCTKPTDGNCTCIPIPSSWAFAK
                     FLWEWASVRFSWLSLLAPFVQWFVGLSPTAWLLAIWMIWYWGPSLYNILKPFIPLLPI
                     FFCLWVYI"
gene                 1374..1838
                     /gene="X"
CDS                  1374..1838
                     /gene="X"
                     /codon_start=1
                     /product="X protein"
                     /protein_id="ABY25977.1"
                     /db_xref="GI:162957111"
                     /translation="MAARLCCQLDPTRDVLCLRPLGAESRGRPLSGPLGALPPPSPSA
                     VPADHGAHLSLRGLPVCAFSPAGPCALRFTSARCMETTVNAPRSLPRTLHKRTLGLSA
                     MSTTEVETYFKDCVFKDWEELGEEIRLKVFVLGGCRHKLVCSPAPCNFFTSA"
gene                 1814..2452
                     /gene="C"
CDS                  1814..2452
                     /gene="C"
                     /codon_start=1
                     /product="precore/core protein"
                     /protein_id="ABY25978.1"
                     /db_xref="GI:162957112"
                     /translation="MQLFHLCLIISCSCPTVQASKLCLGWLLGMDIDPYKEFGATVEL
                     LSFLPSDFFPSVRDLLDTASALYREALESPEHCSPNHTALRQAVLCWGELMTLASWVG
                     NNLEDPASRELVVKYVNVNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPA
                     YRPPNAPILSTLPETTVVRRRGRSPRRRTPSPRRRRSQSPRRRRSQSPASQC"
CDS                  1901..2452
                     /gene="C"
                     /codon_start=1
                     /product="core protein"
                     /protein_id="ABY25981.1"
                     /db_xref="GI:162957115"
                     /translation="MDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYREALES
                     PEHCSPNHTALRQAVLCWGELMTLASWVGNNLEDPASRELVVKYVNVNMGLKIRQLLW
                     FHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRGRSPRRRT
                     PSPRRRRSQSPRRRRSQSPASQC"
```

ORIGIN

```
        1 ctccacagta ttccaccaga ttctacaaga tcccagagta aggggcctgt attttcctgc
       61 tggtggctcc agttcaggaa cagtgaaccc tgttccgact actgcctctc acatctcgtc
      121 aaccttctcg aagactgggg accctgtacc gaacatggaa aacatcacat caggatacct
      181 aggacccctg ctcgtgttac aggcggggtt tttcttgttg acaaaaatcc tcacaatacc
      241 tcagagtcta gactcgtggt ggacttctct caattttcta gggggagcac ccgtgtgtcc
      301 tggccaaaat tcgcagtccc taacctccaa tcactcacca acctcttgtc ctccaatttg
      361 tcctggttat cgctggatgt gtctgcggcg ttttatcatc ttcctcttca tcctgctgct
      421 atgcctcatc ttcttgttgg ttcttctgga cttccaaggt atgttgcccg tttgtcctct
      481 acttccagga acatcgacca ccagcacggg accatgcaga acctgcacga ttcctgctca
      541 aggaacctct ttgtttccct catgttgctg tacaaaacct acggacggaa attgcacctg
      601 tattcccatc ccatcgtcat gggctttcgc aaaattccta tgggagtggg cctcagtccg
      661 tttctcctgg ctcagtttac tagcgccatt tgttcagtgg ttcgtagggc tttcccccac
      721 tgcttggctt ttagctatat ggatgatctg gtattggggg ccaagtctgt acaacatctt
      781 gaagcccttt ataccactgt taccaatttt cttttgtctc tgggtataca tttaaatcct
      841 gataaaacga aaagatgggg gtattccctt aatttcatgg gttacgtgat cggaagttgg
      901 ggtaccttac cacaggaaca tattgtacaa aaaatcaaac agtgtttcag aaagctccct
      961 gtaaacaggc ctattgattg gaaagtgtgc caacgaattg tgggtctttt aggcttcgct
     1021 gccccttta cacaatgtgg atatcctgct ttaatgcctt tatatgcatg tatacacgct
     1081 aagcaggctt ttacttttc ccacacttac aaggcctttc tccgcgatca atatctgaac
     1141 ctttaccccg ttgcccggca acggccaggc ctgtgccaag tgtttgctga cgcaaccccc
     1201 actggttggg gcttggccat cggccatcag cgcttgcgcg gaacctttgt ggctcctctg
     1261 ccgatccata cagcggaact cctagccgct tgttttgctc gcagccggtc tggggcaaag
     1321 ctcatcggga ctgacaactc tgttgttctc tcccgcaaat atacatcgtt tccatggctg
     1381 ctaggctgtg ctgtcaactg gatcctacgc gggacgtcct ttgtttacgt cccctcggcg
     1441 ctgaatcccg cggacgaccc ctctcggggc cgcttggggc tctaccgccc ccttctccgt
     1501 ctgccgttcc agccgaccac ggggcgcacc tctctttacg cggtctcccc gtctgtgcct
     1561 tctcacctgc cggtccgtgt gcacttcgct tcacctctgc acgttgcatg gagaccaccg
     1621 tgaacgcccc ccggagcttg ccaaggacat tgcacaagag gactcttgga ctttcagcaa
     1681 tgtcaacgac cgaagttgaa acatacttca aagactgtgt gtttaaggac tgggaggagt
     1741 taggggagga gatcaggtta aaggtctttg tactaggagg ctgtaggcat aaattggtct
     1801 gttcaccagc accatgcaac tttttcacct ctgcctaatc atctcatgtt catgtcctac
     1861 tgttcaagcc tccaagctgt gccttgggtg gcttttgggc atggacattg acccttataa
     1921 agaatttgga gctactgtgg agttactctc tttcttgcct tctgacttct ttccgtcggt
     1981 cagagatctc ctcgacaccg cctcagctct gtatcgagaa gccttagagt ctccagagca
     2041 ttgttcacct aaccatacag cactcaggca agctgttctg tgttggggtg agttgatgac
     2101 tctggcttcc tgggtgggaa ataatttgga agacccagca tctagggaac tagtagtcaa
     2161 atatgttaat gttaatatgg ggttgaaaat caggcaacta ttgtggtttc acatttcctg
     2221 tcttactttt ggaagagaaa ctgtccttga gtatttggtg tcttttggag tgtggattcg
     2281 cactcctccc gcttacagac caccaaatgc ccctatctta tcaacacttc cggaaactac
     2341 tgttgttaga cgaagaggca ggtcccctag aagaagaact ccctcgcctc gcagacgacg
     2401 gtctcaatca ccgcgtcgcc gaagatctca atctccagct tcccaatgtt agtattcctt
     2461 ggactcataa ggtgggaaac tttactgggc tttattcttc tactgtacct gtctttaatc
     2521 ctgagtggca aactccttcc tttcctgata tacatttaca tcaagatatt attgatagat
     2581 gtcaacaatt tgtgggccct ctcacagtaa atgaaaagcg aagattaaaa ttaattatgc
     2641 ccgctagatt ctatcctaat tctaccaaat atttgccccc agagaaaggt attaaacctt
     2701 attatccaga gcatgtagtt aatcattact tccaaaccag acactatttg cacactctgt
     2761 gggaggcggg catcttatat aagagagaga cgacacgtag cgcctccttc tgtgggtcac
     2821 catattcttg ggaacaagag ctacagcatg gggcagaatc actctgtcac caatccgctg
     2881 ggattcttcc ccgagcacca gttggaccct ctgttcagag ccaactcaaa caatccagat
     2941 tgggacttca accccaacaa ggacaactgg ccagaagcca ccaaggtagg agtgggtgca
     3001 ttcgggccag ggttcacccc accacacgga ggccttttgg ggtggagccc tcaggctcag
     3061 ggaataacaa caaccttgcc agcagttcct cctactgcct ccaccaatcg gcagtcagga
     3121 aggaaaccta ctccaatctc tccacctttg agagacactc atcctcaggc catgcagtgg
     3181 aa
```

BIOGRAPHY

Name: Ms.Pattaratida Sa-nguanmoo

Birth: 17 May 1981, Bangkok, Thailand

Education:

1999 – 2002 B.Sc. (Genetics), Faculty of Science, Chulalongkorn University

2000 – 2005 B.A. (Thai), Faculty of Humanities, Ramkhamhaeng University

2003 – 2005 M.Sc. (Molecular biology and genetics), Faculty of Medicine, Chulalongkorn University

2006 – 2009 Ph.D. (Biomedical Sciences), Faculty of Graduate School, Chulalongkorn University

Scholarships: The Chulalongkorn University Graduate Scholarship to Commemorate the 72nd Aniversary of His Majesty King Bhumibol Adulyadej and the exchange program fellowship under the program "Strategic Scholarships for Frontier Research Network" of Thailand's Commission on Higher Education

Award: "Travel Award" - Molecular epidemiology of hepatitis B virus in migrant workers in Thailand, The 8th JSH Single Topic Conference "HBV Now in Asia", 21st-22nd November, 2009, Tokyo, Japan [Oral presentation]

Training: Department of Virology, Nagoya City University Graduate School of Medical Science, Japan, 21st July-25th November, 2009

Publications: (This study)

1. Sa-nguanmoo, P., Thongmee, C., Ratanakorn, P., Pattanarangsan, R., Boonyarittichaikij, R, Chodapisitkul, S., et al. Prevalence, whole genome characterization and phylogenetic analysis of hepatitis B virus in captive orangutan and gibbon. J Med Primatol 37 (2008): 277-289.
2. Sa-nguanmoo, P., Rianthavorn, P., Amornsawadwattana, S., and Poovorawan, Y. Hepatitis B virus infection in non-human primates. Acta Virol 53 (2009): 72-82.