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REVIEW

Hepatitis B virus infection in non-human primates

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Summary. – Hepatitis B viruses (HBVs) represent a serious public health problem affecting 350 to 400 million HBV carriers worldwide. The virus does not exclusively infect humans, but can also be found in non-human primates as in the families *Hominidae* (chimpanzee, gorilla, orangutan) and *Hylobatidae* (gibbon), which are distributed over Africa (chimpanzee and gorilla) and Southeast Asia (orangutan and gibbon), the endemic areas of human HBV. The prevalence of asymptomatic HBV carriers reaches in gibbons 23–33% and in orangutans 15%. 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 viruses causing severe diseases. Enhanced awareness of prevalence, genetic relatedness and evolution of non-human primate HBVs will help prevent further spread and cross-transmission of these viruses between humans and non-human primates.

Keywords: Hepatitis B virus; humans; non-human primates; prevalence; transmission; evolution

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Abbreviations: ALT= alanine aminotransferase; ASHV = Arctic ground squirrel hepatitis virus; CeHV-1 = Cercopithecine herpesvirus 1; ChHBV = Chimpanzee hepatitis B virus; GiHBV = Gibbon hepatitis B virus; GoHBV = Gorilla hepatitis B virus; GSHV = Ground squirrel hepatitis virus; HBIG = hepatitis B immune globulin; HBVs = Hepatitis B virus(es); HCC = hepatocellular carcinoma; IFN = interferon; NA = nucleotide/nucleoside analogues; OuHBV = Orangutan hepatitis B virus; SCID = severe combined immunodeficiency; SFV = Simian foamy virus; SIV = Simian immunodeficiency virus; WHV = Woodchuck hepatitis virus; WMHBV = Woolly monkey hepatitis B virus

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1. Taxonomy and host range of HBVs

HBV is a partially double-stranded DNA virus with the smallest genome of approximately 3 kb (Qadri *et al*., 1996; Nassal *et al*., 1993) that belongs to the family *Hepadnaviridae* (Seeger and Mason, 2000). HBV occurs in 3 forms, Dane particle (Dane *et al*., 1970), spherical form or filamentous form (Blumberg, 1984). The Dane particles contain viral genome, while the other forms comprise only glycoproteins and host-derived lipid envelopes (Ganem and Prince, 2004). The Dane particle has a diameter of 42 nm and contains 3 proteins L, M, and S in its outer layer. The icosahedral nucleocapsid of 27 nm in diameter contains viral genome (Bruss, 2007).

Hepadnaviruses are divided into two genera *Orthohepadnavirus* and *Avihepadnavirus* based on their specific host, mammals and birds, respectively (Schaefer, 2007). Orthohepadnaviruses can infect humans, apes, and rodents. Human HBVs are divided into 8 genotypes (HBV-A to HBV-H) based on at least 8% divergence within the viral genome (Magnius and Norder, 1995). 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 (Schaefer, 2007; Kramvis and Kew, 2005). Recently, a new genotype of HBV has been proposed (HBV-I) (Tran *et al.*, 2008, Olinger *et al*., 2008). However, some researchers regard this HBV-I genotype as a new recombinant virus rather than a novel genotype (Kurbanov *et al*., 2008).

Non-human primate HBVs infect gorillas (*Gorilla gorilla*) (Grethe *et al*., 2000), chimpanzees (*Pan troglodytes*) (Hu *et al*., 2000; MacDonald *et al*., 2000; Takahashi *et al*., 2000; Hu *et al*., 2001; Takahashi *et al*., 2001; Makuwa *et al*., 2007), orangutans (*Pongo pygmaeus*) (Warren *et al*., 1998; Warren *et al*., 1999; Sa-nguanmoo *et al*., 2008b), gibbons (*Hylobates* sp. and *Nomascus* sp.) (Grethe *et al*., 2000; Noppornpanth *et al*., 2003; Sall *et al*., 2005; Sa-nguanmoo *et al*., 2008b) and woolly monkeys (*Lagothrix lagothricha*) (Lanford *et al.*, 1998). 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. 1). The geographic distribution of the non-human primate HBVs is shown in Fig. 2. HBVs can also be found in New World rodents such as woodchucks (*Marmota monax)*, ground squirrels (*Spermophilus beecheyi*), and arctic squirrels (*Spermophilus parryi*) infected by Woodchuck hepatitis virus (WHV), Ground squirrel hepatitis virus (GSHV), and Arctic ground squirrel hepatitis virus (ASHV), respectively. 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 (Schaefer, 2007).

Avihepadnaviruses exclusively infect birds. HBV infection is very common in among ducks and geese (Prassolov *et al*., 2003). Other possible hosts are heron, stork, and crane. The avihepadnaviruses shares only 40% identity with the human HBVs genome (Schaefer, 2007).

2. HBVs in non-human primates: history and facts

The first outbreak of hepatitis infection occurred in the 1960s among chimpanzee handlers in USA (Hillis, 1961). Three years later, the captive chimpanzees showed symptoms related to the human viral hepatitis (Hillis, 1963). In 1970, sixteen people who had been in contact with 2 imported chimpanzees from Sierra Leone suffered from viral hepatitis infection (Maynard *et al.*, 1972). Based on these facts, several researchers successfully used chimpanzee as a model animal for research of human HBV by inoculating the chimpanzees with human sera, saliva, or semen collected from the HBV-infected people (Holmes and Capss, 1966; Deinhardt *et al*., 1970; Desmyter *et al*., 1973; Maynard *et al*., 1975; Alter *et al*., 1977;

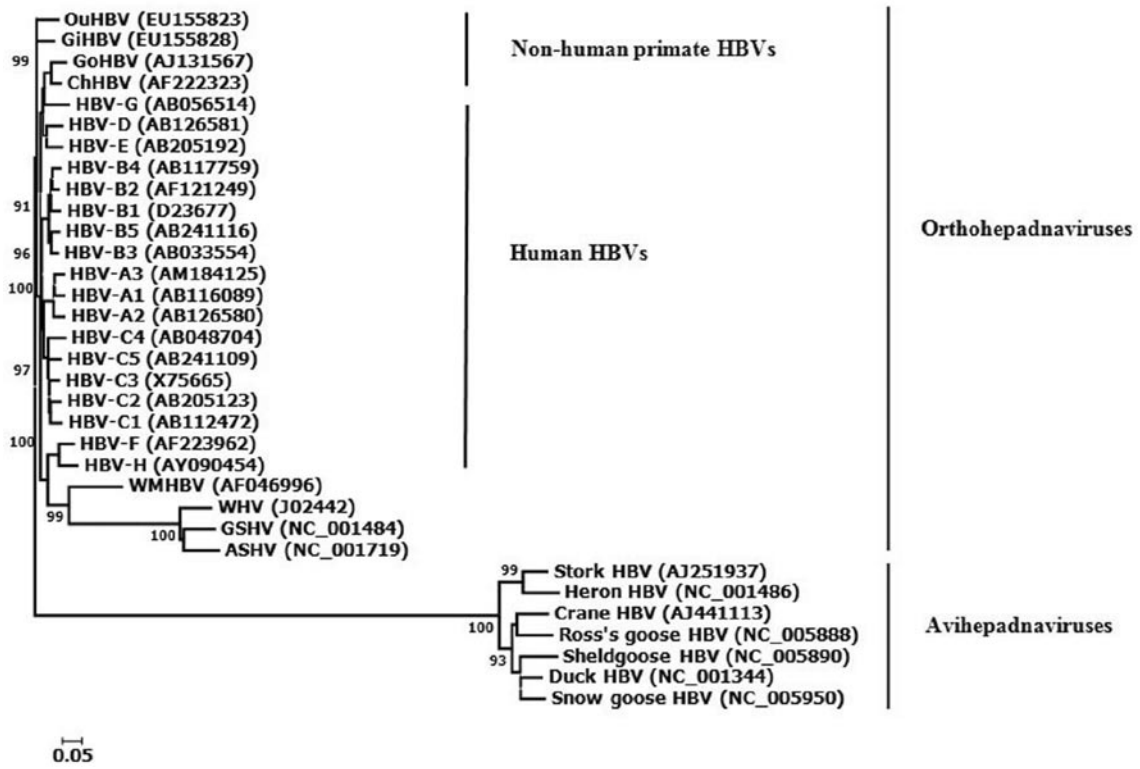

Fig. 1

Phylogenetic tree of various hepadnaviruses based on entire genome

Percentage bootstrap values (>75%) were shown at the respective nodes. The scale bar at the bottom indicated the genetic distance.

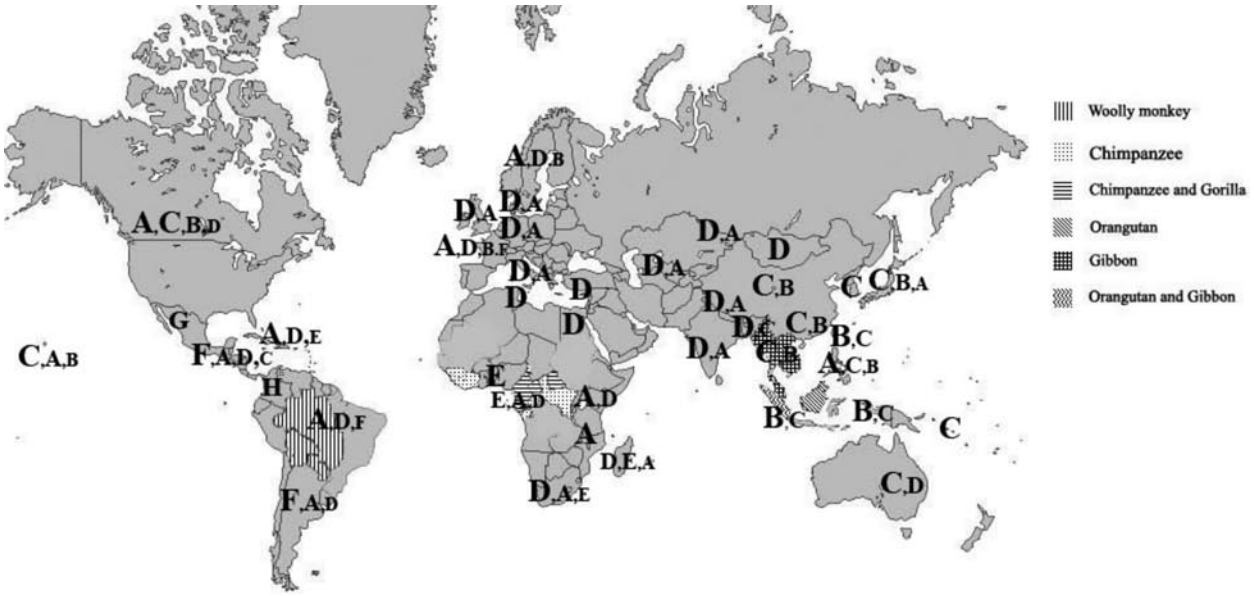

Fig. 2

Geographic distribution of non-human primate HBVs and their hosts

The size of letters represents the extent of prevalence of specific genotype of human HBV in the area.

Robertson, 2001). Similar experiments have been performed also in other non-human primates such as gibbons (Bancroft *et al*., 1977; Scott *et al*., 1980). These data confirmed the fact that human HBVs can infect several non-human primates.

The HBV infection was documented in chimpanzees, orangutans, gorillas, woolly monkeys, and gibbons. In 1978, five chimpanzees in the London zoo were infected with HBV. Three of them were chronic carriers similarly as one of their parents, what suggested that the infection may have been transmitted vertically (Zuckerman *et al.*, 1978). It took nearly a decade to sequence ChHBV genome that comprises 3,182 bp with 4 ORFs encoding the surface (*preS/S*), precore/core (*preC/C*), polymerase (*P*), and *X* gene (Vaudin *et al*., 1988) without pre-core stop codons (MacDonald *et al.,* 2000). The preS/S gene contains a 33 nt deletion in preS1 region that renders the 3'-end of the core region more suitable for the phylogenetic analysis of HBV evolution than the preS1 region. The 3'-end of the core region is conserved among orthohepadnaviruses (Takahashi *et al*., 2000).

The *preS/S* gene of GiHBV was first sequenced in 1993 and the whole genome was sequenced 3 years later. The GiHBV genome shares 90.3% identity with the ChHBV genome. The complete WMHBV genome (3,179 nts) was known in 1998 and *preS/S* gene of OuHBV was described in 1999 (Warren *et al*., 1999).

HBV infection was induced in 4 baboons by the inoculation of sera from the incurably ill liver disease patients. The baboons did not show any abnormal clinical, biochemical, or pathological findings (Michaels *et al*., 1996). By electron microscopic examination HBV viral particles were found in the baboon hepatocytes. This finding rendered the use of baboons as a source of liver xenograft impossible (Kedda *et al*., 2000).

Grethe *et al*. (2000) provided evidence that geographical distribution or host sub-populations have an effect on the formation of HBV variants. This effect was confirmed by the phylogenetic analysis (Starkman *et al*., 2003; Sall *et al*., 2005; Sa-nguanmoo *et al*., 2008b). The first evidence of potential recombination between ChHBV and human HBV genome was documented (Magiorkinis *et al*., 2005). Recombination between human and non-human primate HBV strains, between GiHBV strains from different genera of gibbons, and between bird HBV strains from different avian sub-families were confirmed (Yang *et al*., 2007). All those experiments provided data allowing us to better understand the background, virus hosts, and zoonotic transmission of the non-human primate HBVs (Robertson, 2001).

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* (Fauquet *et al*., 2005). 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 nts or 11 amino acid deletions at the 5' terminus after the start codon (Fig. 3), which is not found in human HBVs except for HBV-D. In the process of protein

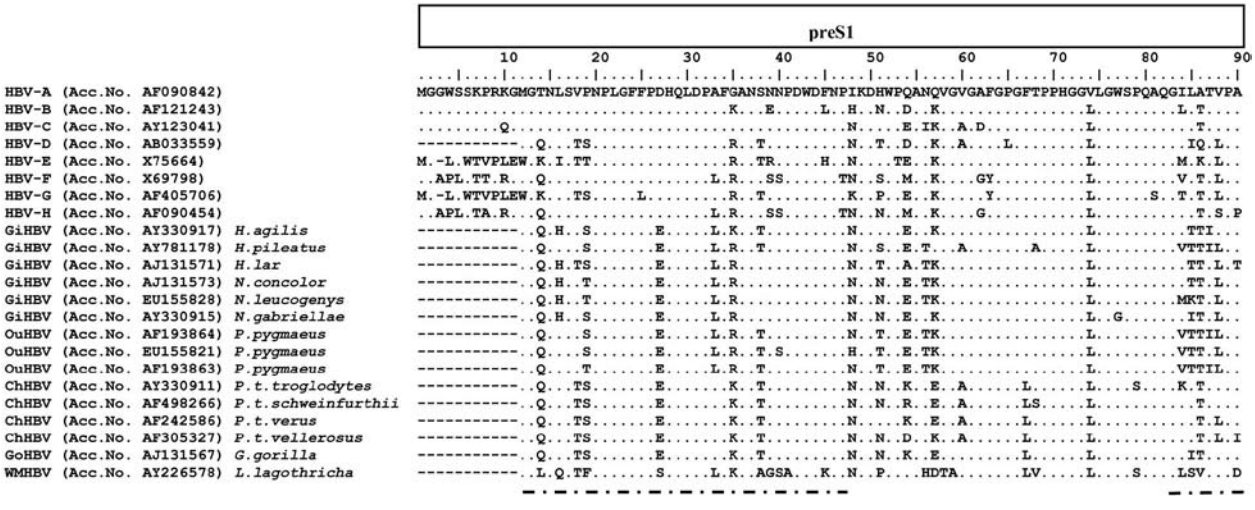

Fig. 3

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.

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                                                    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........................
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Fig. 4

Multiple alignment of sequences of S protein of human and non-human primate HBVs

The underlining indicates "a" determinant region. Arrow represents specific glycine.

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 (Glebe and Urban, 2007). Moreover, human HBVs show O-glycosylation at Thr37, whereas ChHBV and GiHBV display in the same position Asn37 and GoHBV Asp37.

The amino acids 12–20, 21–47, and 82–90 of the preS1 domain (Fig. 3) are virus binding ligands (Neurath *et al*., 1986; Ryu *et al*., 2000). The amino acids in these regions are not species-specific except for amino acid 27 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 (Fernholz *et al*., 1993). Among human and non-human primate HBVs, the % 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 positions 122 (d/y) and 160 (w/r) of the "a" determinant, human HBVs are divided into four serotypes adw, ayw, adr and ayr (Le Bouvier, 1971). All human and non-human primate HBVs contain Gly145 in the "a" determinant region (Fig. 4). 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

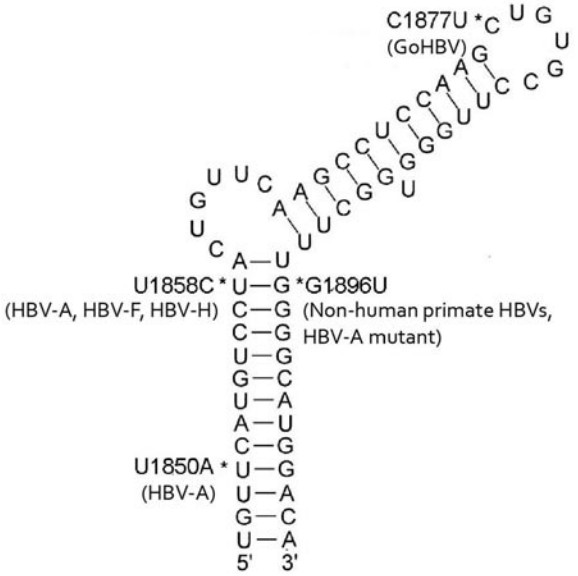

Fig. 5

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

G (G1896U) (Kidd-Ljunggren *et al.*, 2002). This variation affects the structure of bulge conformation (Fig. 5). This confor-

mation resembles HBV-A, which contains a G1896A mutation without a C1858T mutation to stabilize its structure. In human HBVs, lack of the C1858T mutation results 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 (Li *et al*., 1993; Ingman *et al*., 2006).

3.2 Origin and evolution of HBVs in non-human primates

Since the initial discovery of the human HBV in 1967 (Blumberg, 1984), 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 2300 and 3100 years ago (Hannoun *et al*., 2000; Simmonds, 2001). The first theory speculated that HBV originated in America and later was introduced to Europe during the colonial period. Subsequently, the virus diversified to the different genotypes specific to Africa, Central Asia, and China (Bollyky *et al*., 1998). However, this hypothesis was contradicted by molecular clock calculations arriving at the conclusion that genotype development required a much longer period than 400 years (Simmonds, 2001). Then, some researchers supposed that each HBV genotype may have emerged in America, before it spread to Europe. This theory was opposed by the fact that the only genotype F was indigenous to South America (Simmonds, 2001). Whether HBV evolution is host-dependent has remained elusive (Orito *et al*., 1989; MacDonald *et al*., 2000; Robertson *et al*., 2002). Like with the human HBVs, the origin of non-human primate HBVs is still unknown. The habitat of non-human primates is widely dispersed but only humans can travel around the world. A theory suggested that humans may acquire HBV infection from the non-human primates or vice versa (Zuckerman *et al*., 1978; Vaudin *et al*., 1988; Takahashi *et al*., 2000). This idea is contradicted by the nt 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 (Hannoun *et al*., 2000).

3.3 Prevalence, pathogenesis, and clinical significance

3.3.1 Prevalence

The prevalence of HBV infection in the natural 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 1).

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 (Noppornpanth *et al*., 2003). The autopsy of HBV-infected woolly monkeys showed hepatitis and liver necrosis without cirrhosis or HCC (Lanford *et al*., 1998). 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 (Guha *et al*., 2004). It has been shown that the serum, saliva, and semen from HBsAg-positive humans can transmit HBV infection also to other non-human primates. Chimpanzees and gorillas were HBsAg-positive and/or had elevated serum ALT (Maynard *et al*., 1972; Alter *et al*., 1977; Bancroft *et al*., 1977; Scott *et al*., 1980; Starkman *et al*., 2003). Baboon sera were HBV DNA-positive (Kedda *et al*., 2000). 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 after infection. Thus, Barbary macaques can serve as a good animal model to study the HBV replication (Gheit *et al*., 2002).

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 devel-

Table 1. Prevalence of HBV infection in non-human primates as reported until 2008 (mostly adapted from Starkman *et al.*, 2003)

Family	Common Name	Systemic name	No. of animals				References
			Total	HBsAg-positive	HBV DNA-positive	Carrier state (%)	
Hominidae	Gorilla	*Gorilla gorilla spp.*	85	3/65	8/53	8/85 (9.41)	Zuckerman *et al.*, 1978; Linnemann, Jr. *et al.*, 1984; Grethe *et al.*, 2000; Heckel *et al.*, 2001; Thornton *et al.*, 2001; Worley and Stalis, 2002; Makuwa *et al.*, 2003
	Chimpanzee	*Pan troglodytes spp.*	734	47/702	40/205	63/734 (8.58)	Deinhardt *et al.*, 1976; Zuckerman *et al.*, 1978; Eichberg and Kalter, 1980; Vaudin *et al.*, 1988; Ogata *et al.*, 1993; Grethe *et al.*, 2000; Hu *et al.*, 2000; MacDonald *et al.*, 2000; Takahashi *et al.*, 2000; Heckel *et al.*, 2001; Hu *et al.*, 2001; Takahashi *et al.*, 2001; Makuwa *et al.*, 2003; Starkman *et al.*, 2003
	Bonobo	*Pan paniscus*	27	1/27	1/5	1/27 (3.70)	Heckel *et al.*, 2001
	Orangutan	*Pongo pygmaeus spp.*	531	80/375	45/165	80/531 (15.07)	Deinhardt, 1976; Vaudin *et al.*, 1988; Warren *et al.*, 1998; Warren *et al.*, 1999; Davis *et al.*, 2000; Heckel *et al.*, 2001; Starkman *et al.* 2003; Sa-nguanmoo *et al.*, 2008b
Hylobatidae	Gibbon	*Hylobates spp.*	347	66/325	93/247	82/347 (23.3)	Deinhardt, 1976; Vaudin *et al.*, 1988; Norder *et al.*, 1996; Grethe *et al.*, 2000; Lanford *et al.*, 2000; Heckel *et al.*, 2001; Thornton *et al.*, 2001; Noppornpanth et al, 2003; Starkman *et al.*, 2003; Sall *et al.*, 2005; Sa-nguanmoo *et al.*, 2008b
		Nomascus spp.	9	3/9	3/9	3/9 (33.33)	Sall *et al.*, 2005, Sa-nguanmoo *et al.*, 2008b
Cercopithecidae	Mandrill	*Mandrillus leucophaeus*	78	0/78	0/78	0	Starkman *et al.*, 2003
		Mandrillus sphinx	174	0/174	–	0	Starkman *et al.*, 2003; Makuwa *et al.*, 2006
	Long-tail macaque	*Macaca fascicularis*	82	0/82	0/10	0	Makuwa *et al.*, 2006; Sa-nguanmoo *et al.*, 2008b
	Rhesus macaque	*Macaca mulatta*	32	0/32	0/1	0	Makuwa *et al.*, 2006; Sa-nguanmoo *et al.*, 2008b
	Stump-tailed macaques	*Macaca arctoides*	2	0/2	0/2	0	Sa-nguanmoo *et al.*, 2008b
	Southern pigtail macaque	*Macaca nemestrina*	4	0/4	0/4	0	Sa-nguanmoo *et al.*, 2008b
	Vervet monkey	*Cercopithecus aethiops*	58	0/48	–	0	Eichberg and Kalter, 1980; Makuwa *et al.*,2006
	Cherry-capped mangabey	*Ceropithecus torquatus*	24	0/24	–	0	Starkman *et al.*, 2003; Makuwa *et al.*, 2006
	Moustached monkey	*Cercopithecus cephus*	28	0/28	–	0	Makuwa *et al.*, 2006
	Sun-tailed monkey	*Cercopithecus solatus*	16	0/16	–	0	Makuwa *et al.*, 2006
	De Brazza's monkey	*Cercopithecus neglectus*	2	0/2	–	0	Makuwa *et al.*, 2006
	Crowned guenon	*Cercopithecus pagonias*	2	0/2	–	0	Makuwa *et al.*, 2006
	White-nosed guenon	*Cercopithecus nictitans*	24	0/24	0/2	0	Starkman *et al.*, 2003; Makuwa *et al.*, 2006
	Mona monkey	*Cercopithecus mona*	3	0/3	0/3	0	Starkman *et al.*, 2003
	Red-eared monkey	*Cercopithecus erythrotis*	3	0/3	0/3	0	Starkman *et al.*, 2003
	Silvered langur	*Semnopithecus cristatus*	4	0/4	0/4	0	Sa-nguanmoo *et al.*, 2008b
	Phayre's langur	*Semnopithecus phayrei*	1	0/1	0/1	0	Sa-nguanmoo *et al.*, 2008b
	Dusky langur	*Semnopithecus obscurus*	3	0/3	0/3	0	Sa-nguanmoo *et al.*, 2008b
	Baboon	*Papio spp.*	168	0/168	0/4	0	Deinhardt, 1976; Eichberg and Kalter, 1980; Michaels *et al.*, 1996
	Mangabey	*Cerocebus albigena*	6	0/6	–	0	Makuwa *et al.*, 2006
	White-fronted capuchin	*Cebus albifrons*	10	0/10	–	0	Eichberg and Kelter, 1980
	Talapoin	*Miopithecus talapoin*	5	0/5	–	0	Makuwa *et al.*, 2006
	N/A	*N/A*	386	0/386	–	0	Heckel *et al.*, 2001
Atelidae	Woolly monkey	*Lagothrix lagotricha*	16	7/13	9/15	7/16 (43.75)	Lanford *et al.*, 1998
Cebidae	Common squirrel monkey	*Saimiri sciureus*	20	0/20	–	0	Eichberg and Kelter, 1980
	Common marmoset	*Callithrix jacchus*	6	0/6	–	0	Eichberg and Kelter, 1980
	Cotton-top tamarin	*Saguinus oedipus*	12	0/12	–	0	Eichberg and Kelter, 1980
	N/A	*N/A*	49	0/49	–	0	Heckel *et al.*, 2001
Lemuridae	N/A	*N/A*	13	0/13	–	0	Heckel *et al.*, 2001
Callimiconidae	N/A	*N/A*	6	0/6	–	0	Heckel *et al.*, 2001

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

oped signs of pathological liver (Burk *et al*., 1988; Yamamura *et al*., 1990). 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 (Guha *et al*., 2004).

4.3 Transmission of non-human primate HBVs to non-human primates

The inoculation of GiHBV to a chimpanzee resulted in the acute hepatitis infection. The virus isolated from the infected chimpanzee was GiHBV too (Mimms *et al*., 1993). 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 WMBHV was only limited (Lanford *et al*., 1998).

4.4 Computer-based analysis of HBV recombinants

The unusual sequence within the core region of a strain of ChHBV was reported (Takahashi *et al*., 2001). The core antigen of tested ChHBV shows only 78–82% amino acid identity to the known ChHBV strains. Moreover, the infected animal was HBc antibody-negative. The preS1 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 (Takahashi *et al.,* 2001). Later, several reports have mentioned the recombinants between ChHBV, GiHBV, and HBV-C (Mimms *et al*., 1993; Magiorkinis *et al*., 2005; Yang *et al*., 2007).

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 in 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 (Noppornpanth *et al*., 2003). Lanford *et al.* (1998) reported that 80% of the offsprings coming from HBV-infected woolly monkey mothers tested positive for HBV infection.

However, there is also a possibility of horizontal transmission confirmed by HBV DNA detection in gibbon saliva (Noppornpanth *et al*., 2003). 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 (Noppornpanth *et al.,* 2003*)*. The offsprings of two gibbon carrier mothers were HBsAg- and HBV DNA-positive. Sequences of preS1 gene showed little divergence by 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 confirming 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 (Leemans *et al*., 2007). 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 (Shata *et al*., 2006). 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 (Shata *et al.*, 2006). 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 are 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. 4). Several studies have shown the vaccine to be beneficial in pre- and post-exposure prophylaxis in the non-human primates (Ogata *et al*., 1999; Iwarson *et al*., 1988). 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, strain AS. These animals did not develop symptoms of the disease during a follow-up period of 2 years (Ogata *et al*., 1999). 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 wks after exposure. None of the animals developed HBsAg antibody or elevated serum ALT levels. However, the animals that received HBV vaccine 72 hrs after exposure were HBc antibody-positive (Iwarson *et al*., 1988).

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 (Walh *et al*., 1989).

7. Conclusions

Non-human primates are the reservoir of many viruses that cause several diseases such as CeHV-1, lymphocryptoviruses, SIV, SFV (Sa-nguanmoo and Poovorawan, 2008a), 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. Old World monkeys and woolly monkeys are susceptible to HBVs. According to the 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 the advance in the field of HBVs during the past 4 decades, the origin and evolution of HBVs remains 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 transmitted to non-human primates. Evidence of the non-human primate HBV transmission to humans has been suggested but not yet confirmed. If the cross-species transmission from the non-human primates to humans does occur, the chance of HBV eradication will be diminished due to the difficulty in controlling the natural virus reservoir and HBV infection will 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 antiviral medications in non-human primates has been proposed. These agents may be able to prevent and treat animals or persons who are infected with non-human HBVs. However, further studies will be required to set up a practical protocol for prevention or treatment of non-human HBV infection using vaccines or drugs.

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