Hepatitis B virus lineages in mammalian hosts: Potential for bidirectional cross-species transmission

Cibele R Bonvicino, Miguel A Moreira, Marcelo A Soares

Abstract

The hepatitis B virus (HBV) is a cosmopolitan infectious agent currently affecting over 350 million people worldwide, presently accounting for more than two billion infections. In addition to man, other hepatitis virus strains infect species of several mammalian families of the Primates, Rodentia and Chiroptera orders, in addition to birds. The mounting evidence of HBV infection in African, Asian and Neotropical primates draws attention to potential cross-species transmission of these viruses to man. Moreover, recent evidence also suggests the humans may also be a source of viral infection to other mammals, particularly to domestic animals like poultry and swine. We list evidence of HBV and HBV-like infection of nonhuman mammals and discuss their potential roles as donors/recipients of these viruses to humans and to other closely-related species.

Key words: Hepatitis B; Hepatitis B virus nonhuman host; Cross-species transmission; Hepatitis B virus

Core tip: Hepatitis B virus (HBV) is an infectious agent affecting humans worldwide. Other HBV-related strains infect mammalian species of primates, rodents and bats, in addition to birds. Evidence of HBV infection in African, Asian and Neotropical primates draws attention to potential cross-species transmission of these viruses to man. Mounting evidence suggests humans may also be a source of viral infection to other mammals, particularly to domestic animals like poultry and swine. We list evidence of HBV and HBV-like infection of nonhuman mammals and discuss their potential roles as donors/recipients of these viruses to humans and to other closely-related species.

© 2014 Baishideng Publishing Group Inc. All rights reserved.
HBV prevalence varies worldwide, with countries showing high (>8%), intermediate (2%-8%) and low (<2%) estimates. In areas of high prevalence, approximately 70%-90% of the population has been infected by HBV before the age of 40 and 8% are chronic carriers. Approximately 45% of the world population live in areas of high endemicity, including Europe, Asia, the Pacific (excluding Japan, Australia and New Zealand), sub-Saharan Africa, Amazonia, Middle East regions, Central Asian republics, the Arctic and some east European countries. Low prevalence regions include North America, western and northeastern Europe, Australia and some parts of South America while the remaining world regions show an intermediate prevalence. Among indigenous populations of the United States, Canada, New Zealand and Australia, HBV prevalence has been found to be above 5%.

HBV classification followed a historical chronology since its initial identification in humans. The first criterion of classification was based on the viral surface antigen, hepatitis B surface antigen (HBsAg). The determinant “a” (HBsAg amino acid residues 124 to 147) is common to all human HBV isolates and does not provide discriminating information. On the other hand, residues 122 and 160 are used to classify the second and the third determinants, and their combination is used for determining HBV subtypes. The four major subtypes are further subdivided, adding up to a total of ten described subtypes: ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw3, adw4q-, adrq+, and adrq-.

Currently, HBV classification is based on viral genotypes and clades derived from phylogenetic analyses of partial or full-length nucleotide sequences. When whole genomes are compared, the established nucleotide divergence must be at least 7.5% for defining a genotype while a classification exclusively based on the S gene requires at least a 4% divergence. To present, eight different HBV genotypes have been described based on full-length sequences, named A to H. Genotype I has been proposed for HBV samples found in Laos and Vietnam but its formal recognition is still controversial. Another new genotype, named J, has also been recently described in a Japanese individual but it has not been consensually accepted.

Genotypes diverging between 4% and 7.5% are further subdivided in to sub-genotypes A1 to A5, B1 to B8, C1 to C7, D1 to D7 and F1 to F4. A sub-genotype D8 has been recently proposed, resulting from a recombination event between HBV/D and HBV/E genotype, and circulating in Niger. Two F sub-genotypes are further divided in two clades, F1 (a-d) and F2 (a and b).

HBV genotypes predominate in different geographic regions. HBV/A and HBV/D are worldwide distributed while HBV/B and HBV/C are prevalent in Asia, Oceania and North America, HBV/E in Africa, HBV/F in Latin America, HBV/G in Central America and Europe, and HBV/H in Central America.

Many HBV genotypes co-circulate in different regions where an increased risk of co-infections has been observed, particularly with HBV/B and C and with HBV/A and D. As viral recombination necessarily presumes co-infection with at least two different genotypes, areas of co-circulation show increased rates of HBV genomic recombination. Recombination often occurs in the pre-C/C genomic region and several recombinants have been described between HBV genotypes A and D, B and C, and A and C. In the case of B/C recombinants, two divergent viral strains with different geographic distribution have been identified and assigned to different B sub-genotypes.

**HBV infection in nonhuman hosts**

HBV belongs to the Hepadnaviridae family comprising two genera: Orthohepadnavirus and Avihepadnavirus, the former infecting mammals and the latter infecting birds. Orthohepadnaviruses have been identified in several mammals, including the woodchuck (Marmota monax), the ground squirrel (Spermophilus beecheyi), the Arctic ground squirrel (Spermophilus paradoxus), the pig (Sus scrofa), the neotropical woolly monkey (Lagothrix lagotricha), and Old World primate genera like Gorilla, Pongo, Hylobates, Nomascus and Pan (Table 1, Figure 1). Like most hepatnaviruses, HBV only replicates in specific hosts, although cross-species transmission between hosts of different species has been constantly occurred, representing a matter of concern in view of the ability of HBV to cross species barriers despite its genetic divergence. Evidence of recombination between human and ape HBV and different nonhuman primate variants suggested that these viruses are capable of sharing hosts in nature.

A short genome length with overlapping coding regions and genome replication with an intermediate RNA molecule that is retrotranscribed by a viral reverse transcriptase are singular characteristics of hepatnaviruses. It might be initially assumed that these characteristics might restrict HBV of evolving too drastically despite its large host diversity. A combination of two, non-exclusive models can be proposed for HBV evolution: host-viral co-evolution and cross-species transmission. The divergence observed in avian and mammalian hepatnaviruses and the exclusive characteristics of each group, like the (doubtful) presence of the X gene in avian hepatnavirus (Avihepadnavirus), suggested an early split between these viral groups without cross-species transmission events between mammals and birds. The same can be proposed for the HBV found in primate, rodent and bat hosts where the observed divergence did not suggest interspecific transmission between mammals of different orders. On the other hand, transmission between closely-related species has been proposed for primate HBV.
| Taxa | Pos/Tot | HBV strain | Locality | Ref. |
|------|---------|-------------|----------|------|
| Order primates | | | | |
| Family hominidae | | | | |
| Pan paniscus | 5/27 | chHBV | Captive | Heckeletal. | |
| Pan troglodytes | 11 | gibHBV | Wild caught and captive | Warren et al. | |
| Pan troglodytes | 7/57 | chHBV | Germany captive | Heckel et al. | |
| Pan troglodytes | 1/4 | chHBV | Captive | Hu et al. | |
| Pan troglodytes schweinfurthi | 6/62 | chHBV | Cameroon wild born | Starkman et al. | |
| Pan troglodytes troglodytes | 2/8 | chHBV | Southwest Cameroon | Starkman et al. | |
| Pan troglodytes troglodytes | 1/46 | chHBV | Wild Gabon | Makuwaa et al. | |
| Pan troglodytes vellerensis | 7 | chHBV | Congo, Cameroon, Gabon wild | Makuwaa et al. | |
| Pan troglodytes versus | 3 | chHBV | Cameroon wild born | MacDonald et al. | |
| Pan troglodytes versus | 1 | chHBV | Gabon captive | Heckel et al. | |
| Pongo pygmaeus | 8/38 | chHBV | South-eastern Nigeria | Starkman et al. | |
| Pongo pygmaeus | 7/28 | chHBV | Taiwan captive | Huang et al. | |
| Pongo pygmaeus | 40/53 | gibHBV | Thailand, prov. Ratchaburi, KhaoPratub Chang | Sa-nguanmoo et al. | |
| Gorilla gorilla | 2/11 | chHBV | Cameroon wild born | Lyons et al. | |
| Gorilla gorilla | 4/36 | chHBV | Captive | Heckel et al. | |
| Gorilla gorilla | 1 | chHBV | Cameroon wild born | Grethe et al. | |
| Family hyllobatidae | | | | |
| Hyllobates lar | 5/22 | chHBV | Paigrton Zoo-captive born | Starkman et al. | |
| Hyllobates agilis | | | | |
| Nomasus gabrielii | 9/19 | gibHBV | Taiwan | Starkman et al. | |
| Hyllobates agilis | 1 | gibV HBV | Taiwan captive | Huang et al. | |
| Hyllobates concolor | 2 | gibV HBV | Thailand, Duit | Grethe et al. | |
| Hyllobates concolor | 4/7 | gibHBV | North Vietnam and Central China | Noppornpanth et al. | |
| Hyllobates lar | 3 | gibIIHBV | Germany captive | Grethe et al. | |
| Hyllobates lar | 1 | gibIIHBV | Thailand, Patas | Grethe et al. | |
| Hyllobates lar | 3/10 | gibHBV | Taiwan captive | Huang et al. | |
| Hyllobates lar | 11/72 | gibHBV | Thailand wild and captive born | Noppornpanth et al. | |
| Hyllobates lar | 1/2 | gibHBV | Bangkok, Duit zoo | Sa-nguanmoo et al. | |
| Hyllobates leucogenys | 1 | gibVHBV | Thailand, Duit | Grethe et al. | |
| Hyllobates leucogenys | 1 | gibVHBV | Vietnam, Cuc Phuong, | Grethe et al. | |
| Hyllobates moloch | 1 | gibVHBV | Germany captive | Grethe et al. | |
| Hyllobates muelleri | 1/3 | gibIIHBV | Taiwan captive | Huang et al. | |
| Hyllobates pilatus | 1 | gibIIHBV | France captive | Grethe et al. | |
| Hyllobates pilatus | 12/20 | gibHBV | Thailand wild and captive born | Noppornpanth et al. | |
| Hyllobates pilatus | 2/6 | gibHBV | Bangkok, Duit zoo | Sa-nguanmoo et al. | |
| Hyllobates pilatus | At least 1 | gibHBV | Thailand (originally from Vietnam and China) | Huang et al. | |
| Nomasus concolor | 4/7 | gibHBV | Bangkok, Duit zoo | Noppornpanth et al. | |
| Nomasus gabrielii | 1/1 | gibHBV | Thailand (originally from Vietnam and China) | Sa-nguanmoo et al. | |
| Nomasus gabrielii | 1/2 | gibHBV | Taiwan captive | Huang et al. | |
| Nomasus leucogenys | 3/7 | gibHBV | Taiwan captive | Huang et al. | |
| Nomasus leucogenys | 5/6 | gibHBV | Bangkok, Duit zoo | Sa-nguanmoo et al. | |
| Family cercopithecidae | | | | |
| Cercopithecus aethiops | 1 | Captive | | Heckeletal. | |
| Lophocebus albigena | 1/5 | HBV genD | Cameroon wild born | Duspinay et al. | |
| Macaca fascicularis | 31/120 | HBV genD | Mauritius Island (introduced) | Lyons et al. | |
| Mandrillus sphinx | 2/9 | HBV genA2 | Cameroon wild born | Lyons et al. | |
| Papio ursinus orientalis | 15/69 | HBV genA2 | S Africa, W, E Cape and Limpopo prov. | Dickens et al. | |
| Family atelidae | | | | |
| Lagotricha lagotricha | 13/16 | WMHBV | United States, Louisville Zoo. Garden captive | Landford et al. | |
| Order chiroptera | | | | |
| Family vespertilionidae | | | | |
| Miniopterus fuliginosus | 22 | TBHBV | Kachin State, Myanmar | He et al. | |
| Family hipposideridae | | | | |
| Hipposideros cf. ruber | 4/51 | HBBHV | Gabon | Drexler et al. | |
| Family rhinolophidae | | | | |
| Rhinolophus alcyons | 1/16 | RBHBV | Gabon | Drexler et al. | |
| Family philantopidae | | | | |
| Subfam. sternocentrotinae | | | | |
Comprehensive phylogenetic analyses including avianhepadnaviruses and orthohepadnaviruses clearly showed a high divergence at the nucleotide level between these two groups \([24-26]\). These analyses also revealed three groups of mammalian HBV, each associated with a different mammalian order: Rodentia, Chiroptera and Primates.

**HBV infection in old world primates**

Active and resolved HBV infections have been found in several species belonging to the genera *Pan*, *Gorilla*, *Hylobates*, *Nomascus* and *Pongo* \([17,27-29]\). Prevalence of infection in these animals is comparable to those found among humans in endemic areas \([21]\). Specific HBV strains were found in gorillas \([30]\), chimpanzees \([31]\) and gibbons \([21,27,28]\). Recent findings showed occurrence of recombination between HBV strains of human and chimpanzee \([31]\), human and gibbon \([31]\), and gorilla and chimpanzee \([21]\), confirming the ability of HBV to cross species barriers. These findings suggested that transmission from humans to nonhuman primates or *vice-versa* were likely to occur wherever their habitats overlap.

Orangutans are apes of the Hominidea family with two extant species, *Pongo pygmaeus* and *Pongo abelii* (Figure 1). They are the only great apes found outside Africa, in the islands of Borneo and Sumatra \([31]\). Orangutans are highly endangered as a result of poaching and widespread destruction of their habitats resulting from human intrusions in their rainforest habitat. The accumulation of relatively solitary orangutans in reintroduction centers also increases the potential of transmission of viral pathogens, either of orangutan or human origin. Previous studies have shown the role of *Pongo pygmaeus* as an HBV host \([31,32]\), carrying a specific HBV strain \([34]\) and with individuals potentially becoming chronic HBV carriers \([33]\). In some places, prevalence of HBV in orangutans was as high as 59%, with 10% of them representing chronic carriers \([35]\).

Chimpanzees are apes of the Hominidea family comprising two extant species, the gracile chimpanzee or bonobo (*Pan paniscus*), and the robust or common chimpanzee *P. troglodytes* (Figure 1). With four subspecies: the western common chimpanzee (*P. troglodytes verus*), the central common chimpanzee (*P. troglodytes troglodytes*), the eastern common chimpanzee (*P. troglodytes schweinfurthii*), and *P. troglodytes vellerus* (Figure 1) \([37-39]\). Wild chimpanzees still dwell in several forested regions of the lowest latitudes of sub-Saharan Africa \([26]\). This species has been the primary experimental model of HBV infection and they host indigenous nonhuman primate HBV strains \([40]\). Viral infection is widespread throughout the entire range of chimpanzee habitats; all four subspecies being infected with HBV-like viruses, collectively termed chHBV \([17,20,28,29,31]\). Strong associations between chHBV strains and their host geographic distribution have been found \([20,41]\). Chronic HBV infections usually result from perinatal infection and the presence of chHBV sequences in wild newborn chimpanzees suggests that natural perinatal transmission is responsible for their infection \([40]\). The finding of HBV in fecal samples collected from wild *P. t. troglodytes* showed that HBV detected in captive apes were related to viruses circulating in the wild \([42]\). Contacts between human and chimpanzees via the bushmeat trade, as family pets and caretakers, together with the number of viruses harbored by chimpanzees, pointed that these animals constitute putative reservoirs of infectious agents \([37]\). High prevalence rates of chHBV, of up to 25% in some wild communities (Table 1), further enhances the risk of cross-species transmission events.

Gorillas are apes of the Hominidea family belonging to the genus *Gorilla* comprising two species: *Gorilla beringei* with two subspecies (*G. b. beringei* and *G. b. graueri*), and *Gorilla gorilla* with two subspecies (*G. g. gorilla* and *G. g. diehli*) \([37]\). Gorillas are ground dwelling, predominantly herbivorous apes inhabiting the tropical or subtropical forests of central Africa (Figure 1). Evidence of past HBV infection was found in 11% to 30% of tested gorillas \([43,44]\), none of which reported with current infection. Until now, only one western lowland gorilla (*Gorilla gorilla gorilla*) from Cameroon has been reported with an HBV-like infection \([45]\). Whether this gorilla HBV sequence differed from that of chimpanzee HBV has remained unknown although some studies showed their close relationship \([20,42,45]\). The last authors suggested that sympatry of these two primate taxa, in the forests of west Africa, makes the possibility of cross-species transmission likely \([42]\).

**Table 1**

| Family sciuridae | Order rodentia | THBV | HBV | THBV |
|------------------|----------------|------|-----|------|
| Marmota monax    | WHV            | United States captive | China, Beijing | United States, California |
| Otolemur albigaster | GSVH          | United States, Alaska  | China, Beijing | United States, Philadelphia |
| Spermophilus variegatus | ASHV          | United States, Philadelphia | Brazil | United States, California |
| Sciurus carolinensis pennsylvanicus | THBV       | China, Beijing | Brazil | United States, California |
| Domestic animals |                | China, Beijing | Brazil | United States, California |

HBV: Hepatitis B virus; Pos/Tot: Number of HBV positive animals/total number of animals analyzed for the presence of HBV infection; ASHV: Arctic ground squirrel HBV; GSHV: Californian ground squirrel HBV; gHBV: Gibbon HBV; HBV genA: Human HBV genotype A; HBHBV: Horseshoe bat HBV; RBHBV: Roundleaf bat HBV; TBHBV: Tent-making bat HBV; THBV: Tree squirrel HBV; WHV: Woodchuck HBV; WMHBV: Woolly monkey HBV.
Gibbons are lesser apes belonging to the Hylobatidae family, comprising four genera, *Hylobates*, *Nomascus*, *Hoolock*, and *Symphalangus*, and distributed in tropical and subtropical rainforests from northeastern India to Indonesia and northern to southern China, and the islands of Sulawesi, Borneo, and Java (Figure 1). Phylogenetic analysis of complete HBV surface (S) gene sequences revealed that gibbon viruses clustered separately from hepatavirus of other hosts. Several species of *Hylobates* and *Nomascus* were found to be infected by at least four different HBV strains. An HBV isolate from a *Nomascus leucogenys* found in Thailand was phylogenetically separate from those found in *Hylobates pileatus* and *Hylobates lar*, and was almost identical with an HBV isolate from *Hylobates concolor*, confirming the circulation of several HBV strains in gibbons. Evidence for horizontal and vertical transmission in captive gibbons was found, and HBV DNA has also been detected in the saliva of gibbon HBV carriers. Some gibbon species have been shown to become chronic HBV carriers. A previous study showed a high prevalence (ca. 41%) of infection by HBV in captive and possible horizontal transmission between infected gibbons in Taiwan. In this study, saliva samples of HBV carrier gibbons tested positive for HBV DNA, demonstrating a potential infection through contact with bodily fluids.

Phylogenies based on complete HBV genome sequences of different primate species suggest that interspecific transmissions might take place between man and closely-related genera (*Pan*, *Gorilla*, *Pongo*, and *Hylobates*). This can be deduced from the grouping of HBV genotypes found in the great apes with human specific HBV genotypes. However, analyses carried out with different HBV genomic regions showed a more complex picture, where recombination events between genotypes were demonstrated.

Recombination events between human HBV genotypes are frequently reported and some sub-genotypes clearly result from recombination events between different genotypes. These events were also hypothesized as part of the evolutionary history of HBV genotypes from *Homo sapiens*, *Pan*, *Gorilla*, *Pongo*, and *Hylobates*. In these cases, there is evidence that recombination has been a relevant process although it is not clear whether recombination events occurred before or after the initial infection in each species. An interesting case was reported by Tatematsu *et al.*, showing that the new human genotype J found in one patient resulted from a recombination event between human HBV/C and gibbon HBV. No other man or gibbon was found infected by this virus. Zhou and Homes, analyzing recombination events with different algorithms, suggested that recombination between HBV genotypes more frequently occurs between the positions 1627 and 3252. Lyons *et al.*, who analyzed recombination between human HBV genotypes and between great ape HBV genotypes, found similar results and showed evidence that recombination has recurrently taken place during evolution of HBV genotypes.

Baboons are social monkeys of the Cercopithecidae family, with five *Papio* species commonly recognized despite controversies on their *bona fide* status as valid species or subspecies. These comprise *Papio ursinus*, or the...
HBV infection in neotropical primates

Wooly monkeys belong to the *Lagothrix* genus, a neotropical primate taxon of the Atelidae family. *Lagothrix* comprises at least four species: *Lagothrix lagothricha*, *Lagothrix poeppigi*, *Lagothrix lagus*, and *Lagothrix cana* (Figure 1). They are the only neotropical monkeys found to host a specific HBV[56], 81% (13/16) of animals from the Louisville Zoo colony showed signs of ongoing or previous infections with wooly monkey HBV (WMHBV). Nine polymerase chain reaction (PCR)-positive animals showed consistent profiles with either acute or chronic infection. PCR analysis of archived sera showed that many infections were chronic and had been present in the colony for at least 9 years prior to the study[58]. Data of WMHBV infections in the Louisville colony were consistent with vertical transmission. At the time of that study, *Lagothrix* was considered to be a monotypic genus with a single species *L. lagothricha*. The current taxonomic arrangement splitting *L. lagothricha* in four species does not allow us to know which species was identified as the HBV reservoir.

WMHBV is the only HBV so far described in neotropical primates and was only detected in captive animals[57]. The WMHBV genome is capable of replicating and producing virions in human liver cell lines but experimental infection using the spider monkey (*Ateles geoffroyi*) as a model did not result in permanent infection, with viral clearance 16 wk after infection[57]. Phylogenetic analyses showed that WMHBV divergence occurred before the radiation of the remaining primate HBV genotypes, with a nucleotide sequence similarity ranging from 62% to 86% in different open reading frames between different genotypes. WMHBV was not detected in wild specimens, a reason why it is unclear whether this HBV might actually infect wild populations of wooly monkeys.

**HBV infection in domestic animals**

Research on HBV-like viruses in domestic animals has been carried out since 1985[58]. Recently, liver of captive swine and chickens were found to be naturally infected with HBV in China[58,59]. These findings, together with the known ability of HBV to cross species barriers[58], suggested that human and nonhuman HBV variants might share hosts in nature. Recently, serological data from several samples from swine from Brazil and partial genome sequencing (252-365 bp) of three of these samples confirmed HBV infection, with sequences sharing 93%-96% of identity with human HBV[60]. Although there is no evidence that human populations have been so far infected with HBV variants of animals used for food, animal source foods deserve a closer attention[58].

**HBV infection in bats**

Bats (order Chiroptera) are a source of a wide variety of emerging pathogens, including coronaviruses, filoviruses, Hendra and Nipah paramyxoviruses, lyssaviruses and HBV[61]. A recent study provided strong evidence of circulation of orthohepadnaviruses in *Miniopterus fuliginosus* bats from Myanmar[24]. *Miniopterus fuliginosus* was initially considered a junior synonymous of *M. schreibersii*, but molecular studies inferred from mitochondrial cytochrome *b* sequences showed that *M. fuliginosus* was a valid species[62]. The virus found in this bat species differed from currently known members of the genus Orthohepadnavirus, representing a new species. Prevalence of bat hepatitis viruses in *Miniopterus fuliginosus* from two localities was 2.2% and 4.7%, respectively, indicating that this species was likely a natural reservoir of BatHV[63]. These bats are widely spread and host other viruses, including coronaviruses and betaherpesviruses[63-65].

A screening of 3080 bat specimens belonging to 54 species and 11 families showed ten specimens (0.3%) from Panama and Gabon carrying unique hepadnaviruses in co-ancestral relation to HBV, putatively classified as orthohepadnavirus species[66]. Infected livers showed histopathologic alterations compatible with hepatitis. Phylogenetic analyses carried out with generated virus sequences suggested that bat HBV was more closely-related to primate HBV than to those of other mammalian orders.

**HBV infection in rodents**

Woodchuck (*Marmota monax*), a rodent of the Sciuridae
family, is distributed in Canada and United States, including Alaska (Figure 1)[70]. This species is common and territorial, with highly variable densities ranging from 0.1 to 3.3/hectare and with loosely structured populations in burrow systems without spatial clusters[66]. Viruses similar to HBV were found in a laboratory population of woodchucks and designated woodchuck hepatitis virus (WHV)[67]. Subsequently, WHV was found in a natural population of woodchucks from southeastern Pennsylvania, central New Jersey, and north central Maryland[40].

*Spermophilus beecheyi*, currently known as *Otospermophilus beecheyi* (ground squirrel), is a rodent of the Sciuridae family distributed in United States and Mexico (Figure 1)[65,70]. This species lives in rocky habitats and is widespread and locally abundant in most of its habitats, including agricultural areas, but can be rare in other places[71]. The ground squirrel hepatitis virus shared many of the unique characteristics of HBV, and has been found in Beechey ground squirrels of northern California[71].

*Spermophilus parryii kennicotti*, currently known as *Urocitellus parryii kennicotti* (arctic ground squirrel), is a rodent of the Sciuridae family. *Urocitellus parryii* is distributed in Canada, Alaska in United States, and Russia (Figure 1)[70]. This species lives in colonies with complex system of shallow burrows (up to 1 m) with several entrances and nests[69]. Testut et al[29] found that 14% of the 56 analyzed animals were positive for ASHB (arctic ground squirrel HBV).

The tree squirrel *Sciurus carolinensis*, a rodent of the Sciuridae family, occurs in United States and Canada (Figure 1), while *S. c. pennsylvanicus* occurs in the northeast of this distribution[70]. Based on histological evidence of hepatitis in 14 of 94 samples of tree squirrel livers, DNA polymerase and cross-reactive surface antigen activities in 3 of 14 livers, a virus similar to, but different from HBV was identified, named tree squirrel hepatitis B virus (THBV)[74].

**CONCLUSION**

Several hypotheses have been postulated to explain the origin and evolution of HBV. The manifold genotypes found in humans might have originated by multiple episodes of zoonotic transmissions from several nonhuman primate species[29]. This hypothesis is similar to the one proposed by the human immunodeficiency virus (HIV) type 1 from at least four separate cross-species transmission from different subspecies of chimpanzees or gorillas[75,76] while human infection with HIV type 2 in west Africa arose independently through contact with sooty mangabeyes[77]. Like for HIV, the constant and increasingly frequent exposure of humans to blood, meat and bodily fluids of infected wild and domestic animals during poaching and meat processing and preparation provides a recurrent source of cross-species transmission events of HBV-like viruses to humans. Such events might be even more frequent than perceived, since only a small fraction of cross-species transmitted viruses is thought to culminate with successful establishment of infection leading to virus replication and pathogenesis. The higher physical stability of HBV-like viruses (e.g., compared to HIV)[78] may enhance such scenario of successful establishments in the human host.

The dynamic interplay between the host and the virus depends on viral facts such as viral genetic variation and viral genotype[25]. The increase in reports on the circulation of HBV in different species of mammals and birds has stimulated interest in identifying new reservoirs and genotypes, indicating the need for additional studies to a greater understanding of the dynamics of transmission of HBV to humans and other species susceptible to the virus. Although transmission of human hepatitis B virus variants to nonhuman primates is well documented, it remains to be elucidated whether nonhuman primate HBV and those from other vertebrate species are transmissible to man.

**ACKNOWLEDGMENTS**

We would like to thank Dr. Héctor N. Seuánez for reviewing the draft version of the manuscript.

**REFERENCES**

1. Abdou Chekaraou M, Brichler S, Mansour W, Le Gal F, Garba A, Dény P, Gوردien E. A novel hepatitis B virus (HBV) subgenotype D (D8) strain, resulting from recombination between genotypes D and E, is circulating in Niger along with HBV/E strains. *J Gen Virol* 2010; 91: 1609-1620 [PMID: 20147517 DOI: 10.1099/vir.0.018127-0]
2. Heathcote EJ. Demography and presentation of chronic hepatitis B virus infection. *Am J Med* 2008; 121: S3-11 [PMID: 19185072 DOI: 10.1016/j.amjmed.2008.09.024]
3. Schiff ER. Optimizing management strategies in patients with chronic hepatitis B. Introduction. *Am J Med* 2008; 121: S1-S2 [PMID: 19185069 DOI: 10.1016/j.amjmed.2008.09.023]
4. Tan AT, Koh S, Goh V, Bertolletti A. Understanding the immunopathogenesis of chronic hepatitis B virus: an Asian perspective. *J Gastroenterol Hepatol* 2008; 23: 833-843 [PMID: 18565018 DOI: 10.1111/j.1440-1746.2008.05385.x]
5. Cougot D, Neuvet C, Buendia MA. HBV induced carcinogenesis. *J Clin Virol* 2005; 34 Suppl 1: S75-S78 [PMID: 16461228]
6. Custer B, Sullivan SD, Hazlet TK, Iloeje U, Veenstra DL, Kowdley KV. Global epidemiology of hepatitis B virus. *J Clin Gastroenterol* 2004; 38: S158-S168 [PMID: 15602165]
7. Gish RG. Diagnosis of chronic hepatitis B and the implications of viral variants and mutations. *Am J Med* 2008; 121: S12-S21 [PMID: 19185070 DOI: 10.1016/j.amjmed.2008.09.025]
8. Carey WD. The prevalence and natural history of hepatitis B in the 21st century. *Cleve Clin J Med* 2009; 76 Suppl 3: S2-S5 [PMID: 19465705 DOI: 10.3949/ccjm.09676.s.001]
9. World Health Organization. Hepatitis B. Accessed on 10/23/2013. Available from: http://www.who.int/emc/2002
10. Kay A, Zoulim F. Hepatitis B virus genetic variability and evolution. *Virus Res* 2007; 127: 164-176 [PMID: 17383765]
11. Kurbanov F, Tanaka Y, Mizokami M. Geographical and genetic diversity of the human hepatitis B virus. *Hepatol Res* 2010; 40: 14-30 [PMID: 20156297 DOI: 10.1111/j.1872-054X.2009.00601.x]
12. Olinger CM, Jutavijittum P, Hübschen JM, Yousukh A, Samountry B, Thanmayong T, Toriyama K, Muller CP. Possible new hepatitis B virus genotype, southeast Asia. *Energ...
Hepatitis B virus: significance of genotypes.

De Castro L, Niel C, Gomes SA. Low frequency of mutations in the core promoter and precore regions of hepatitis B virus in anti-HBe positive Brazilian carriers. BMC Microbiol 2001; 1: 10.

Mello FC, Souto FJ, Nabuco LC, Villela-Nogueira CA, Coelho HS, Franz HC, Saraiva JC, Virgolinho HA, Motta-Castro AR, Melo MM, Martins RM, Gomes SA. Hepatitis B virus genotypes circulating in Brazil: molecular characterization of genotype F isolates. BMC Microbiol 2007; 7: 103 [PMID: 18036224 DOI: 10.1186/1471-2180-7-103]

De Castro L, Niel C, Gomes SA. Low frequency of mutations in the core promoter and precore regions of hepatitis B virus in anti-HBe positive Brazilian carriers. BMC Microbiol 2001; 1: 10.

Vartanian JP, Pineau P, Henry M, Hamilton WD, Muller MN, Wrangham RW, Wain-Hobson S. Identification of a hepatitis B virus genome in wild chimpanzees (Pan troglodytes schweinfurthii) from East Africa indicates a wide geographical dispersion among equatorial African primates. J Virol 2002; 76: 11155-11158 [PMID: 12368630]

Li W, She R, Liu L, You H, Yin J. Prevalence of a virus similar to human hepatitis B virus in swine. Viral J 2010; 7: 60 [PMID: 20233455 DOI: 10.1186/1743-422X-7-60]

Simmonds P, Midgley S. Recombination in the genesis and evolution of hepatitis B virus genotypes. J Virol 2005; 79: 15467-15476 [PMID: 16306618 DOI: 10.1128/JVI.79.24.15467-15476.2005]

Hu X, Javadan A, Gagneux P, Robertson BH. Paired chimpanzee hepatitis B virus (ChHBV) and mtDNA sequences suggest different ChHBV genetic variants are found in geographically distinct chimpanzee subspecies. Virus Res 2001; 79: 103-108 [PMID: 11551650]

Lyons S, Sharp C, LeBreton M, Djojo CF, Kiyang JA, Lankester F, Bibila TG, Tamoufè U, Fair J, Wolfe ND, Simmonds P. Species association of hepatitis B virus (HBV) in non-human apes; evidence for recombination between gorilla and chimpanzee variants. PLoS One 2012; 7: e33430 [PMID: 22432021 DOI: 10.1371/journal.pone.0033430]

Murakami S, Hawaii HBx-like protein from a hidden open reading frame. Acta Virol 2001; 45: 342-346 [PMID: 11551650]

Chang SF, Netter HJ, Hildt E, Schuster R, Schaefer S, Hsu YC, Rang A, Will H. Duck hepatitis B virus expresses a regulatory HBx-like protein from a hidden open reading frame. J Virol 2001; 75: 161-170 [PMID: 11119585]

He B, Fan Q, Yang F, Hu T, Qiu W, Feng Y, Li Z, Li Y, Zhang F, Guo H, Zou X, Tu C. Hepatitis virus in long-fingered bats, Myanmar. Emerg Infect Dis 2013; 19: 638-640 [PMID: 23631923 DOI: 10.3202/j19.121655]

Locarnini S, Littlejohn M, Azin MN, Yuen L. Possible origins and evolution of the hepatitis B virus (HBV). Semin Cancer Biol 2013; 23: 561-575 [PMID: 24013024 DOI: 10.1016/j.semcancer.2013.08.006]

Drexler JF, Geipel A, König A, Corman VM, van Riel D, Leijten LM, Bremer CM, Rasche A, Cottontail VM, Maganga GD, Schlegel M, Müller MA, Adam IS, Klose SM, Carneiro AJ, Stöcker A, Franke CR, Gloza-Rausch F, Geyer J, Annan A, Adu-Sarkodie Y, Oppong S, Binger T, Vallo P, Tschapka M, Ulrich RG, Gerlich WH, Leroy E, Kuiken T, Grebe D, Drosten C. Bats carry pathogenic hepadnaviruses antigenically related to hepatitis B virus and capable of infecting human hepatocytes. Proc Natl Acad Sci USA 2013; 110: 16151-16156 [PMID: 24043818 DOI: 10.1073/pnas.1308094110]

Norder H, Ebert JW, Fieb Canvas, Hepatitis B virus infection in non-human primates. J Virol 2000; 74: 5377-5381 [PMID: 10796168 DOI: 10.1128/JVI.74.11.5377-5381.2000]

MacDonald DM, Holmes EC, Lewis JC, 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 2000; 74: 4253-4257 [PMID: 10756109 DOI: 10.1128/JVI.74.9.4253-4257.2000]

Njouom R, Mba SA, Nerrienet E, Foupouapoougyni Y, Rouset D. Detection and characterization of hepatitis B virus strains from wild-caught gorillas and chimpanzees in Cameroon, Central Africa. Infect Genet Evol 2010; 10: 790-796 [PMID: 20471498 DOI: 10.1016/j.meegid.2010.05.002]

Takahashi K, Brozman B, Usuda S, Mishiro S, Prince AM. Full-genome sequence analyses of hepatitis B virus (HBV) strains recovered from chimpanzees infected in the wild: implications for an origin of HBV. Virolology 2000; 267: 58-64 [PMID: 10641813]

Magiorkinis EN, Magiorkinis GN, Paraskevis DN, Hatzakis AE. 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 2005; 349: 165-171 [PMID: 15777724]

Sa-Ngumnool P, Rianthavorn P, Amornsaowadattana S, Poovorawan Y. Hepatitis B virus infection in non-human primates. Acta Virol 2009; 53: 82-89 [PMID: 19537907]

Husson S, Wich SA, Marshall AJ, Dennis RD, Ancrenaz M, Brasse Y, Gunal M, Heeney J, Meier J, Simorangkir T, Singleton I. Orangutan distribution, density, abundance and impacts of disturbance. In: Wich SA, Utami Atmoko SS, Mitra Setia T, van Schaik CP, editors. Orangutans: Geographical variation in behavioral ecology and conservation. Oxford: Oxford University Press, 2009: 77-96

Warren KS, Niphuis H, Heriyanto EJ, Swan RA, Heeney J. Seroprevalence of specific viral infections in confiscated orangutans (Pongo pygmaeus). J Med Primatol 1998; 27: 33-37 [PMID: 9606041]

Warren KS, Heeney JL, Swan RA, Heriyanto EJ. A new group of hepadnaviruses naturally infecting orangutans (Pongo pygmaeus). J Virol 2001; 75: 7860-7865 [PMID: 11038880]

Groves CP. Primates. In: Wilson DE, Reeder DM, editors. Mammal Species of the World. 3rd ed. Baltimore: Johns Hopkins University Press, 2005: 181-182

Hey J. The divergence of chimpanzee species and subspecies as revealed in multipopulation isolation-with-migration analyses. Mol Biol Evol 2010; 27: 921-933 [PMID: 19955478 DOI: 10.1093/molbev/msp298]

Butynski TM, The Robust Chimpanzee Pan troglodytes: Taxonomy, Distribution, Abundance, and Conservation Status. In: Kormos R, Boesch C, Bakarr MI, Butynski TM, editors. West African primates. 1st ed. United Kingdom: IUCN The World Conservation Union, 2003: 5-12

Hu X, Margolis HS, Purcell RH, Ebert J, Robertson BH. Identification of hepatitis B virus indigenous to chimpanzees. Proc Natl Acad Sci USA 2000; 97: 1661-1664 [PMID: 10677515]

Makau M, Muller F, Hufert FT. Molecular epidemiology of hepatitis B virus variants in nonhuman primates. J Virol 2000; 74: 5377-5381 [PMID: 10796168 DOI: 10.1128/JVI.74.11.5377-5381.2000]

Makau M, Souquière S, Bourry O, Rouquet P, Telfer P, Maeloué P, Kazanji M, Roques P, Simon F. Complete-genome analysis of hepatitis B virus from wild-born chimpanzees in central Africa demonstrates a strain-specific geographical cluster. J Gen Virol 2007; 88: 2679-2685 [PMID: 17872519]

Makau M, Souquière S, Clifford SL, Mouning-Ondeme A, Bawo-Johnson M, Wikings EJ, Latour S, Simon F, Roques P. Identification of hepatitis B virus genome in faecal sample
from wild living chimpanzee (Pan troglodytes troglodytes) in Gabon. J Clin Virol 2005; 34 Suppl 1: S83-S88 [PMID: 16461230]

43 Heckel JD, Riettschel W, Hufert FT. Prevalence of hepatitis B virus infections in nonhuman primates. J Med Primatol 2001; 30: 14-19 [PMID: 11396859]

44 Makuwa M, Souquiere S, Telfer P, Leroy E, Bourry O, Rouquet P, Clifford S, Wikings EJ, Roques P, Simon F. Occurrence of hepatitis viruses in wild-born non-human primates: a 3 year (1998-2001) epidemiological survey in Gabon. J Med Primatol 2003; 32: 307-314 [PMID: 14617185]

45 Starkman SE, MacDonald DM, Lewis JC, Holmes EC, Simmons P. Geographic and species association of hepatitis B virus genotypes in non-human primates. Virology 2003; 314: 381-393 [PMID: 14517090]

46 Ma S, Wang Y, Pirier FE. 1988. Taxonompy, distribution and status of Gibbons (Hylobates) in southern China and adjacent areas. Primates 1988; 29: 277-286 [DOI: 10.1007/BF02381129]

47 Huang CC, Chiang YC, Chang CD, Wu YH. Prevalence and phylogenetic analysis of hepatitis B virus among nonhuman primates in Taiwan. J Zoo Wild Med 2009; 40: 519-528 [PMID: 19768688]

48 Noppornpanth S, Haagmans BL, Bhattarakosol P, Ratana-korn P, Niesters GH, Osterhaus AD, Poovorawan Y. Molecular epidemiology of gibbon hepatitis B virus transmission. J Gen Virol 2003; 84: 147-155 [PMID: 12533711]

49 Shi W, Zhang Z, Ling C, Zheng W, Zhu C, Carr MJ, Higgins DC. Hepatitis B virus subgenotyping: history, effects of recombination, misclassifications, and corrections. Infect Genet Evol 2013; 16: 355-361 [PMID: 23538336 DOI: 10.1016/j.meegid.2013.03.021]

50 Zhou Y, Holmes EC. Bayesian estimates of the evolutionary rate and age of hepatitis B virus. J Mol Evol 2007; 65: 197-205 [PMID: 17684696]

51 Dickens C, Kew MC, Purcell RH, Kramvis A. Occult hepatitis B virus infection in chacma baboons, South Africa. Emerg Infect Dis 2013; 19: 598-605 [PMID: 23631817 DOI: 10.3201/eid1904.121107]

52 Deinhardt F. Hepatitis in primates. Adv Virus Res 1976; 20: 113-157 [PMID: 818890]

53 Kedda MA, Kramvis A, Kew MC, Locatas G, Paterson AC, Aspillan S, Stark JH, De Klark WA, Griddle B. Susceptibility of chacma baboons (Papio ursinus) to infection by hepatitis B virus. Transplantation 2000; 69: 1429-1434 [PMID: 11079676]

54 Dupinay T, Gheit T, Roques P, Cova L, Chevallier-Queyron P, Tashatsu SI, Le Grand R, Simon F, Cordier G, Wakrim L, Benjelloun S, Trépo C, Chémin I. Discovery of naturally occurring hepatitis B virus of humans. J Virol 2013; 86: 7351-7356 [PMID: 24223210 DOI: 10.1128/JVI.00497-13]

55 Perelman P, Johnson WE, Roos C, Seuñane HN, Harvath JE, Moreira MA, Kassing B, Pontius J, Roelke M, Rampeter Y, Schneider MP, Silva A, O'Brien SJ, Pecon-Slattery J. A molecular phylogeny of living primates. PLoS Genet 2011; 7: e1001342 [PMID: 21436896 DOI: 10.1371/journal.pgen.1001342]

56 Lanford RE, Chavez D, Brasky KM, Burns RB, Hahn BH. Isolation of a hepadnavirus from the woolly monkey, a New World primate. Proc Natl Acad Sci USA 1998; 95: 5759-5761 [PMID: 9576957]

57 Lanford RE, Chavez D, Barrera A, Brasky KM. An infectious clone of woolly monkey hepatitis B virus. J Virol 2003; 77: 7814-7819 [PMID: 12929821]

58 Qifeng X. Experimental infection on chickens with hepatitis B virus. Chines J Nat 1985; 9: 238-239

59 Tian J, Xia K, She R, Li W, Ding Y, Wang J, Chen M, Yin J. Detection of hepatitis B virus in serum and liver of chickens. Vet Res 2012; 42: 2 [PMID: 22217003 DOI: 10.1186/1740-422X-9-2]

60 Vieira YR, Vieira AA, Ciacci-Zanella JR, Barquero G, Lago BV, Gomes SA, Silva, MFM, Santos DRL, Pinto MA, da Paula VS. Serological and molecular evidence of hepatitis B virus infection in swine from Brazil. Vet Virol 2012; 17 Suppl 1: 402-403

61 Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev 2006; 19: 531-545 [PMID: 16847084]

62 Gao F, Yue L, White AT, Pappas PG, Barchue J, Hanson AP,...
Greene BM, Sharp PM, Shaw GM, Hahn BH. Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature* 1992; 358: 495-499 [PMID: 1641038]

Sa-nguanmoo P, Thongmee C, Ratanakorn P, Pattanaranngsan R, Boonyaritichaiikij R, Chodapisitkul S, Theamboolers A, Tangkijvanich P, Poovorawan Y. Prevalence, whole genome characterization and phylogenetic analysis of hepatitis B virus in captive orangutan and gibbon. *J Med Primatol* 2008; 37: 277-289 [PMID: 18466280 DOI: 10.1111/j.1600-0684.2008.00290.x]

P-Reviewers: Guo JS, Wilhelm B  S-Editor: Zhai HH  L-Editor: A  E-Editor: Wang CH
