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## HEPATITIS B VIRUS VACCINATION OF SILVERY GIBBONS (*Hylobates moloch*) AT THE PERTH ZOO

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### Abstract

Hepatitis B virus (HBV) has been identified in a number of nonhuman primate species, including the Silvery gibbon (*Hylobates moloch*). The Perth Zoo has a successful Silvery gibbon breeding program and has developed an HBV vaccination regime to protect offspring of this species. Serologic testing has demonstrated that this vaccination regime has been successful in producing a serologic response consistent with vaccine-induced immunity.

### Introduction

Hepatitis B virus (HBV) infections involving strains that are distinct from human HBV genotypes, have been identified in gibbons (GiHBV), orangutans (OuHV), chimpanzees (ChHBV), gorillas (GoHBV) and woolly monkeys (WMHBV).<sup>4,6,7,13,14</sup> Recent studies have demonstrated wide-spread infection of wild primate populations with these species-specific HBV infections.<sup>3,5,8,12-14</sup> Recent studies provide evidence that these viruses are indigenous to these nonhuman primate species, suggesting that a transmission event in the Old World between humans and nonhuman primates may have occurred, involving a common ancestor virus from which the nonhuman primate HBV variants and the Old World human HBV genotypes (A to E) have evolved.<sup>4,5,8,13</sup> However, other researchers favour the theory that these nonhuman primate HBV variants are more likely the result of recent cross-species transmission of the virus between humans and nonhuman primates.<sup>6,7</sup> Fulminant hepatitis has been reported in a woolly monkey infected with WMHBV,<sup>6</sup> and elevated alanine aminotransferase (ALT) levels were reported in gibbons infected with GiHBV.<sup>10</sup> However, the pathogenicity of the HBV infections in other nonhuman primate species remains unknown.<sup>7,14</sup> Experimental transmission of human HBV to chimpanzees and gibbons has been documented,<sup>1,10,11</sup> and infectivity of gibbon HBV to a chimpanzee has also been demonstrated.<sup>9</sup> To date there is limited knowledge as to the zoonotic potential of these nonhuman primate viruses. Although it seems likely that these viruses are potentially zoonotic,<sup>9</sup> studies have not been able to confirm transmission of HBV from nonhuman primates to humans.<sup>10</sup>

The Perth Zoo currently houses one of the few successful breeding colonies of Silvery gibbons (*Hylobates moloch*) in the world and the only one in the Australasian region. Serologic testing of the two breeding adults at the Perth Zoo has shown cross-reactions with human HBV antigens and

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both are considered chronic carriers of an HBV-like virus (i.e., positive for HBV surface antigen [HBsAg+ve] and negative for HBV surface antibody [anti-HBs -ve]) (Tables 1 and 2). The breeding female is further classified as a high-infectivity chronic carrier, due to the cross-reaction of her sera with HBV e antigen (HBeAg). The likelihood that human babies born to high-infectivity chronic carrier mothers will develop HBV infection and become chronic carriers is greater than 90%, as compared to the 10-15% risk of infection associated with babies born to low-infectivity chronic carrier mothers.<sup>2,3</sup> Although transmission studies of the disease in nonhuman primates have been limited, previous studies have demonstrated a similar perinatal transmission pattern to that of HBV in humans.<sup>4,7</sup> DNA sequencing currently being undertaken by the author has demonstrated that the HBV-like virus isolated from the breeding female at the Perth Zoo is closely related to the previously identified GiHBV rather than human HBV.<sup>7,9,10</sup>

An unrelated adult male the Perth Zoo Silvery gibbon, who was also classified as a high-infectivity chronic carrier, died recently after a period of liver-related illness. This individual was found on postmortem to have evidence of liver cirrhosis consistent with pathology seen in humans with chronic hepatitis B infection, and had demonstrated elevated levels of ALT on multiple occasions (up to 613 U/L).

Based on this information, the Perth Zoo was concerned that there would be a high probability of offspring born to this Silvery gibbon breeding pair becoming infected with the virus, developing chronic carrier status, and possibly developing liver pathology associated with the infection in later life. Given the critically endangered status of the species however, and the obvious compatibility of the pair, it was decided to continue breeding these animals. A vaccination program to immunize newborn Silvery gibbons against HBV was developed with the help of human virologists, to reduce the risk of transmission of the disease to future offspring.

### **Development of an HBV Vaccination Regime**

The Silvery gibbon pair has given birth to four offspring since 1995, the most recent birth was in March 2003. All four offspring have been vaccinated against HBV. The initial recommended vaccination regime involved vaccinating newborn gibbons within the first 24-72 hr of birth, with 0.5 ml of recombinant Hepatitis B vaccine (Energix B®, SmithKline Beecham Biologicals, B-1330 Rixensart, Belgium) administered intramuscularly (i.m.) and 0.3 ml HBV Immunoglobulin (100 IU/ml) administered i.m. at a separate site. Booster vaccinations of 0.5 ml Energix B® vaccine i.m. were to be given at 4 mo and 12 mo of age. The immunoglobulin administered with the initial vaccination served to provide passive transfer of immunity against potential viral exposure during the birthing process. Recommended doses to be used were extrapolated from human doses and reduced according to body weight differences between the species.

The vaccination regime was used on the first offspring (OF1), however the second booster was delayed to 15 mo for management reasons. Blood testing at the time of the second booster demonstrated that this animal had developed a serologic response consistent with immunity to HBV. However, the presence of HBV core antibody (anti-HBc) as well as anti-HBs indicated that this

immunity was derived from past exposure rather than vaccination (Table 2). As the vaccine contains surface antigen only, the presence of core antibodies suggests that the animal has been exposed to actual virus rather than just vaccine. It is not possible from these results to determine if the anti-HBs has resulted from exposure to vaccine, virus or a combination of both. Repeat blood testing 5 yr after the initial vaccination showed that immunity had been maintained. Transfer of this animal to another institution shortly after this time meant that further testing could not be performed.

Due to the lack of serologic response to vaccination attained by the initial vaccination regime in OF1, the regime was amended for the second offspring (OF2) to involve a vaccination at 24-72 hr with 0.5 ml Energix B® vaccine i.m. and 0.1 ml HBV Immunoglobulin (100 IU/ml) i.m., followed by boosters of 0.5 ml Energix B® vaccine i.m. at 1 mo and 6 mo. Due to management and animal factors, the initial Energix B® vaccine and HBV Immunoglobulin injections were given at 72 hr, the first booster at 6 wk, and the second booster at 5 mo. Blood testing 1 yr after the second booster demonstrated that this animal had no evidence of exposure to the disease and had developed a serologic response consistent with vaccine-induced immunity (anti-HBc -ve, anti-HBs +ve). Serum from this individual was collected recently and has shown that this immunity has been maintained (4.5 yr after the initial vaccination).

The third offspring (OF3) was vaccinated with 0.5 ml Energix B® vaccine i.m. and 0.1 ml HBV Immunoglobulin (100 IU/ml) i.m. at 48 hr, with a single booster of 0.5 ml Energix B® vaccine i.m. given at 8 mo. This vaccination regime had been modified following discussions with human virologists, and the initial Energix B® vaccine booster (given previously at 1 mo) was eliminated due to problems encountered while darting the dam in order to vaccinate the previous offspring (OF2) at 1 mo of age. Blood collected from OF3 2 yr after initial vaccination demonstrated that this animal had also developed a serologic response consistent with vaccine-induced immunity and had no evidence of exposure to the disease (anti-HBc -ve, anti-HBs +ve).

Due to the success of the modified vaccination regime used for OF3, this regime was repeated for the fourth offspring (OF4), born in March 2003. This baby was vaccinated with 0.5 ml Energix B® vaccine i.m. and 0.1 ml HBV Immunoglobulin (100 IU/ml) i.m. at 48 hr. A single booster of 0.5 ml Energix B® vaccine i.m. will be given at 8 mo.

## Discussion

Based on serologic testing, the vaccination regimes used for OF2 and OF3 appear to have been successful in producing vaccine-induced immunity that has been maintained for at least 2 yr since the initial vaccination. The regime used for OF3 was preferred since the booster vaccination at 8 mo was able to be performed without the need for anesthesia of the dam. All previous vaccinations given prior to this age required general anesthesia of the dam to allow access to the baby and to ensure good acceptance of the baby when returned to the dam.

Although the initial regime used for OF1 did not produce a serologic response consistent with vaccine-induced immunity, serology from this animal is suggestive of immunity secondary to

exposure to infection, and therefore OF1 can now be safely involved in a breeding program without the risk of transmitting the disease to future offspring or other in-contact animals.

Although the number of animals in this study is low, it appears that the modified regime, involving vaccination at 24-72 hr with 0.5 ml Energix B® vaccine i.m. and 0.1 ml HBV Immunoglobulin (100IU/ml) i.m., followed by a booster vaccination of 0.5 ml Energix B® vaccine i.m. at 8 mo, is successful in providing a serologic response consistent with vaccine-induced immunity. It is recommended that this vaccination regime be used for any future offspring born to this pair of Silvery gibbons at the Perth Zoo. Virologists have also recommended that repeat boosters of Energix B® vaccine are given every 5 yr, on an opportunistic basis, in order to maintain immunity.

Despite the apparent success of the program, there are inherent risks to the dam, offspring and staff associated with the vaccination procedures, especially when darting and general anesthesia are involved. However, the benefits of the vaccination procedure in successfully establishing vaccine induced-immunity in Silvery gibbon offspring at the Perth Zoo was deemed to outweigh the risks involved in the zoo's situation. The suitability of such a vaccination regime for other institutions would depend on the number and status of the animals involved.

The effectiveness of vaccinations used in exotic species has often been questioned, as it is difficult to ascertain the extent of true protection afforded by vaccines that have been designed for other species (in this case, humans). This is certainly applicable in this situation, and whether these animals are truly "immune" to infection is likely to be debatable. In a critically endangered species such as the Silvery gibbon, we do not have the luxury of being able to run challenge tests to determine whether or not the immunity is truly protective. It would appear though, that in the cases at the Perth Zoo true protection has been achieved as both OF2 and OF3 developed immunity that has been maintained for several years while living in very close contact with two chronic HBV carriers. The fact that OF1 showed evidence of exposure-induced immunity demonstrates that the virus is transmissible in a captive situation, and as such there is a real risk of infection to all future offspring. Neither OF2 nor OF3 have shown evidence of exposure however, suggesting that their vaccinations provided true protective immunity. An alternative explanation could be that the dam had seroconverted to become a low-infectivity carrier during the time between the birth of OF1 and OF2. In this scenario, the risk of transmission of the virus to the later offspring would be reduced from > 90% to 10-15%. If this were the case, the serologic immunity demonstrated may not be indicative of true protective immunity. However, serum samples collected from the dam at the time of vaccinating OF4 have confirmed she is still a high-infectivity carrier, supporting the theory that the immunity attained in OF2 and OF3 is truly protective.

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**PROCEEDINGS**

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