Natural and experimental infection of wild mallard ducks with duck hepatitis B virus

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Wild duck populations were investigated over a 4 year period for duck hepatitis B virus (DHBV) infection and liver disease. It appeared that DHBV is endemic in wild migratory mallards from France and the U.S.A., although neither hepatocellular carcinoma nor viral DNA integration could be detected in liver samples examined. The follow up of natural infection indicated that wild mallards developed significantly higher serum titres to DHBV DNA than Pekin ducks. The results of experimental transmission demonstrated that such differences in viraemia were not related to the breed of ducks but to the virus isolate, since the wild mallard-isolated DHBV (DHBVwM) induced significantly higher viraemia in both mallard and Pekin ducklings compared to the domestic Pekin DHBV (DHBVP) isolate. The naturally infected mallard and Pekin ducks had only minor histological lesions of the liver compared with experimentally infected birds. There was no correlation between the intensity of viraemia and the severity of liver lesions, suggesting that as for mammalian hepadnaviruses the hepatic injury in DHBV-infected ducks is probably immunologically mediated.

Biological study of human hepatitis B virus (HBV), the prototype member of the hepadnavirus family, is limited by the narrow host range of this virus and failure to infect cell lines in culture. The closely related duck hepatitis B virus (DHBV), commonly found in Pekin ducks (Anas domesticus), has played a major role in the characterization of hepadnaviruses’ replication and biology (reviewed by Schödel et al., 1989). In contrast to mammalian hepadnaviruses, which are known to cause hepatocellular carcinoma (HCC) after a long period of chronic infection (Popper et al., 1987; Marion et al., 1986), the oncogenicity of DHBV is unclear. To date, HCC has been reported only in domestic Pekin and brown ducks from a single region of China (Zhou, 1980; Yokosuka et al., 1985), whereas DHBV carrier Pekin ducks in other parts of the world do not develop HCC (Marion et al., 1984). These geographical variations in the prevalence of HCC in ducks may reflect the differences in duck breed, age, DHBV strain, and environmental factors such as dietary aflatoxins (Cova et al., 1990).

We have previously demonstrated the presence of DHBV in wild mallard (Anas platyrhynchos) ducks (Cova et al., 1986). With our interest directed towards defining the oncogenic potential of DHBV, we investigated wild duck populations over a 4 year period for signs of HCC and liver disease with relation to DHBV infection. In addition, as little is known of the biological properties of hepadnavirus isolates, it was of interest to characterize further DHBV isolated from wild mallard ducks (DHBVwM). In this study, we report that DHBVwM induces unusually high viraemia titres in its natural host and compare its pathogenic potential with that of other DHBV isolates.

To investigate the wild duck populations for DHBV infection and liver disease, sera and liver samples originating from wildlife reserves and from ducks shot by hunters were collected in France and U.S.A. Out of the 531 wild ducks’ serum samples obtained from French wildlife reserves collected between 1984 and 1990, 54 (10-2%) were positive for DHBV DNA (Table 1). The frequency of DHBV infection varied in different years, from 1 to 20%. DHBV was also detected in two out of 130 necropsy liver samples from ducks shot by hunters and in 1.8% of wild mallards originating from north-east U.S.A. Altogether, these results demonstrate that DHBV is endemic in the wild duck populations. A closely related virus, the heron hepatitis B virus has been isolated recently from grey heron living in the wild (Sprengel et al., 1988). In addition, DHBV had been also detected in wild maned ducks (Chemonetta jubata) in Australia (R. J. Dixon, personal communication). Thus, wild birds may represent a natural reservoir of avian hepadnaviruses.

Our investigation of liver samples from naturally
infected wild mallard ducks revealed neither HCC nor viral DNA integration (Table 1). However, the limited number of samples available, of which the majority originated from young birds, does not allow us to rule out the occurrence of HCC in wild mallards. HCC could develop in wild ducks, although this is difficult to test because surveying liver disease in waterfowl is hampered by the lack of systematic autopsy studies of birds which die either on their flyway or on reserve territory.

The initial screening of ducks for DHBV DNA suggested that wild mallards exhibited higher serum levels of viral DNA than observed in Pekin ducks (data not shown). It was therefore of interest to compare further the course of viraemia in wild and domestic birds. Wild mallard eggs were collected in the reserve of Dombes, France and hatched ducklings were screened for DHBV infection. Pekin ducklings congenitally infected with DHBV were a progeny of a flock of DHBV carrier ducks maintained at our institute (Cova et al., 1985). The two groups of 1-week-old DHBV carrier mallard (14 birds) and Pekin (12 birds) ducklings were individually monitored for DHBV DNA in serum over a 1 year period. To quantify DHBV DNA, 50 µl of duck sera and cloned DHBV DNA ranging from 100 ng to 0.1 pg were spotted in duplicates onto nitrocellulose filters (Cova et al., 1990) and probed with cloned, genome length, DHBV DNA labelled by nick translation (Rigby et al., 1977) to a specific activity of 0-8 to 1.2 × 10⁶ c.p.m./µg. The filters were hybridized overnight with radiolabelled DHBV probe in 50% formamide at 42 °C, as previously described (Cova et al., 1985), washed under stringent conditions, dried, and liquid scintillation counting of each individual spot was done. From the curve derived from DHBV DNA blots, the counts obtained for each spot were converted into pg DHBV DNA and then to virus genome equivalents (v.g.e.), assuming each DHBV genome to be 3.3 × 10⁻⁶ pg (Jilbert et al., 1988). Fig. 1 indicates that at the peak of viraemia (1 to 5 weeks post-hatch), wild mallards scored significantly higher DHBV titres (P < 0.001), reaching 2 × 10¹¹ v.g.e./ml for some animals compared to Pekin ducks for which titres did not exceed 3 × 10¹⁰ v.g.e./ml by the same age. Decreased and fluctuating DHBV DNA titres were observed thereafter. All naturally infected ducks were chronic virus carriers 1 year post-hatch and no DHBV DNA integration was observed in the liver samples analysed (data not shown).

To assess whether the high viraemia titres observed in wild mallards were related to the greater susceptibility of wild birds to DHBV infection or to the higher infectivity of some viral isolates, an experimental transmission of three DHBV isolates (Lambert et al., 1990) originating from a highly viraemic wild mallard (DHBVwM), a Chinese brown duck (DHBVc) and a domestic Pekin duck (DHBVP) was therefore undertaken. Ten-day-old Pekin and mallard ducklings (12 to 15 birds for each DHBV isolate) were inoculated intravenously with DHBV-positive serum diluted to contain similar amounts of virus corresponding to 10⁹ v.g.e./ml by the same age. Decreased and fluctuating DHBV DNA titres were observed thereafter. All naturally infected ducks were chronic virus carriers 1 year post-hatch and no DHBV DNA integration was observed in the liver samples analysed (data not shown).

![Table 1. Detection of DHBV in American and French wild duck populations](image)

**Table 1. Detection of DHBV in American and French wild duck populations**

<table>
<thead>
<tr>
<th>Year</th>
<th>Origin</th>
<th>Number of birds</th>
<th>Number DHBV-positive</th>
<th>%</th>
<th>Number of livers examined</th>
<th>HCC</th>
<th>DHBV status in liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1979</td>
<td>Michigan*</td>
<td>2 juvenile</td>
<td>1</td>
<td>50</td>
<td>NA†</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 adults</td>
<td>1</td>
<td>10</td>
<td>NA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1981</td>
<td>Michigan*</td>
<td>89 age ND‡</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Illinois*</td>
<td>26 juvenile</td>
<td>1</td>
<td>1</td>
<td>19</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27 adults</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>North Dakota*</td>
<td>17 age ND</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1984</td>
<td>Dombes§</td>
<td>20 adults</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>0/2</td>
<td>Free</td>
</tr>
<tr>
<td></td>
<td>Somme¶</td>
<td>20 adults</td>
<td>1</td>
<td>5</td>
<td>NA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1985</td>
<td>Dombes§</td>
<td>20 adults</td>
<td>3</td>
<td>15</td>
<td>2</td>
<td>0/2</td>
<td>Free</td>
</tr>
<tr>
<td></td>
<td>Dombes shot¶</td>
<td>174 juvenile</td>
<td>36</td>
<td>20-7</td>
<td>6</td>
<td>0/6</td>
<td>Free</td>
</tr>
<tr>
<td></td>
<td></td>
<td>105 adults</td>
<td>1</td>
<td>0</td>
<td>95</td>
<td>1</td>
<td>0/1</td>
</tr>
<tr>
<td>1986</td>
<td>Dombes§</td>
<td>38 adults</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84 juvenile</td>
<td>6</td>
<td>7-15</td>
<td>NA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1989</td>
<td>Dombes§</td>
<td>45 adults</td>
<td>4</td>
<td>8-9</td>
<td>NA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Alsace shot¶</td>
<td>25 adults</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>0/1</td>
<td>Free</td>
</tr>
</tbody>
</table>

* Ducks from north-east U.S.A. as part of a study of wild waterfowl undertaken by the American National Wildlife Health Laboratory.
† NA, Not available.
‡ ND, Not determined.
§ National wildlife reserve of Dombes.
¶ National wildlife reserve of Somme.
* National wildlife reserve of Alsace.
¶ Ducks shot by hunters in the Dombes lake district or in the Alsace district.

![Fig. 1](image)
Fig. 1. Comparative viraemia follow-up in mallard and Pekin ducks naturally infected with DHBV. Two groups of 1-week-old DHBV carrier mallard and Pekin ducklings were individually monitored for DHBV DNA in serum over a 1 year period. To quantify viral DNA 50 μl of duck serum was spotted onto nitrocellulose filters and probed with radiolabelled DHBV DNA probe followed by liquid scintillation counting of each individual spot as described in the text. Each column corresponds to the mean value ± S.E.M. of serum virus genome equivalents/ml from (■) mallard (14 birds) and (□) Pekin (12 birds) ducks.

those which received DHBVP. In mallard ducklings DHBVwm also peaked (day 4) to significantly higher viral DNA titres than DHBVP (day 8) (Fig. 2b). The viraemia in mallard ducklings was remarkably similar for DHBVc and DHBVwm, reaching its highest titres 4 days post-infection (Fig. 2b).

Table 2 shows that the histological examination of liver samples from highly viraemic mallard ducks naturally infected with DHBV (group 1) did not reveal significant liver lesions. This is consistent with findings in humans and woodchucks which indicate that the severity of liver disease is not related to the level of virus replication, but to the host immune response (Alberti et al., 1983; Frommel et al., 1984). The absence of liver disease in ducklings congenitally infected with DHBV is similar to chronic asymptomatic perinatal HBV infection of babies who usually do not develop liver pathology. By contrast, the significant portal inflammatory reaction and necrosis which we observed in Pekin as well as in mallard ducklings experimentally infected with DHBVP, DHBVc or DHBVwm at 10 days (Table 2, groups 3 to 8) seem to correlate with the development of the immune response of birds which begins to function 3 to 5 days post-hatch (Solomon, 1966; Fukuda et al., 1987).

The main finding of this study was the characterization of the DHBV isolate from naturally infected wild mallards, which induced unusually high viraemia titres

Table 2. Histological study of naturally and experimentally DHBV-infected Mallard and Pekin ducks

<table>
<thead>
<tr>
<th>Degree of hepatitis*</th>
<th>Natural infection</th>
<th>Experimental infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mallard</td>
<td>Pekin</td>
</tr>
<tr>
<td>0</td>
<td>11/13</td>
<td>6/9</td>
</tr>
<tr>
<td>+ to ++</td>
<td>2/13</td>
<td>3/9</td>
</tr>
<tr>
<td>++ +</td>
<td>0/13</td>
<td>0/9</td>
</tr>
</tbody>
</table>

* Degree of hepatitis according to Popper et al. (1980): 0, normal liver; + to ++, minor to moderate portal and parenchymal lesions; ++ +, portal infiltration and parenchymal lesions associated with focal necrosis of hepatitis. Livers scored as 0 were either normal or characterized by fatty changes.
in its natural host. Our results of experimental transmission clearly demonstrated that such differences in viraemia were not related to the breed of duck but indeed to virus isolate, since DHBVWM induced significantly higher viraemia in both mallard and Pekin ducks as compared to DHBVP. Until recently, our understanding of the genetic basis for biological characteristics such as host range or virulence of hepadnaviruses has been limited. HBV variants have been discovered with an inactive pre-core gene, due to stop codon or frameshift mutations, which lead to clinically significant changes in virus pathogenicity (Carman et al., 1989; Brunetto et al., 1989; Tong et al., 1990). This finding stimulated interest in other naturally occurring hepadnavirus variants (Alberti, 1990). In this regard the isolation of DHBVWM which induces unusually high viraemia in both mallard and Pekin ducks appears of great interest. The sequence determination of molecularly cloned DHBVWM in association with in vitro transfection of hepatoma cell lines (Galle et al., 1988; Fourel et al., 1989), will allow the functional analysis of viral genes responsible for the high viraemia induced by this isolate. Whether this reflects increased viral replication or more efficient virus packaging and export is not clear at present.

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References


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