Evidence for the Presence of Duck Hepatitis B Virus in Wild Migrating Ducks

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(Accepted 11 November 1985)

SUMMARY

A virus closely related to duck hepatitis B virus (DHBV) was isolated from serum and liver samples of wild migratory ducks (mallards) caught in two separate wildlife reserve parks in France. In the first one (Dombes region) 12% of wild mallards were positive for DHBV, and in the second (River Somme) 3% of mallards were found positive. The DHBV isolated from the serum of wild mallards was also associated with an endogenous DNA polymerase activity capable in vitro of completing a partially double-stranded viral DNA into a fully double-stranded DNA of 3 kb. The various replicative DNA forms reported for DHBV were also detected in the liver of wild viraemic mallards. The DNA restriction enzyme pattern of the wild mallard strain differed from that of American and French strains of DHBV. The wild mallard strain DHBV was experimentally transmitted to mallard and Pekin ducklings and induced a chronic viraemia in both varieties of infected birds. This strain might be the common ancestor of all DHBV strains isolated from domestic ducks world-wide. The discovery of a DHBV-related virus in the natural wild population might be an important clue in the study of the different roles of environmental, host and viral factors in the pathogenesis of DHBV infection, and their possible oncogenic action in ducks.

INTRODUCTION

Duck hepatitis B virus (DHBV) is the fourth reported member of the rapidly expanding hepadnavirus family (Robinson et al., 1981). This new class of viruses includes human hepatitis B virus (HBV), woodchuck hepatitis virus (WHV) and ground squirrel hepatitis virus (GSHV). These hepatotropic viruses share an unusual genome structure consisting of a circular partially double-stranded DNA of 3000 to 3300 base pairs. The single-stranded gap may be filled by an endogenous virion-associated DNA polymerase reaction in vitro (Summers et al., 1975). The hepadnaviruses induce chronic liver disease in their hosts and, as documented for humans and woodchucks, hepatocellular carcinoma (Shafritz & Rogler, 1984).

In the past few years, molecular cloning and sequencing of DNA have increased our knowledge of the structure and replication of these viruses (Charnay et al., 1979; Cummings et al., 1980; Galibert et al., 1981; Mandart et al., 1984). However, the lack of an in vitro tissue culture system for hepadnavirus replication has hampered progress in the study of the biology of these viruses. Some difficulties have been overcome by the use of domestic Pekin ducks (Anas domesticus) infected by DHBV as an animal model. The DHBV is transmitted vertically through the egg (O’Connell et al., 1983) allowing the study of virus replication in congenitally infected
ducks (Mason et al., 1980, 1982; Halpern et al., 1983; Marion et al., 1984) or in experimentally infected ducklings and embryos (Mason et al., 1983; Halpern et al., 1983; Omata et al., 1984; Tsiquaye et al., 1985).

DHBV was initially discovered in the sera of domestic Pekin ducks from the People's Republic of China and was subsequently isolated from commercial flocks of Pekin ducks in the United States (Mason et al., 1980, 1981). Recently, the presence of DHBV has also been reported in Pekin ducks from Germany (Sprengel et al., 1984) and France (Cova et al., 1985). It has been reported that hepatocellular carcinoma occurs in Chinese domestic Pekin ducks, which are chronically infected by DHBV in China, while surprisingly no hepatocellular carcinoma has been observed in DHBV carrier ducks from the United States (Marion et al., 1984; Omata et al., 1983). Since all breeds of domestic duck such as Pekin, Khaki Campbell, Indian Runner and Aylesbury are derived from wild mallards (Delacour, 1973) it was of interest to search for the presence of DHBV in the wild bird population.

In this paper, we report the discovery of a virus similar to DHBV in the serum and liver of wild migrating mallards. This newly isolated virus may well be the common ancestor of all DHBV strains isolated from domestic ducks world-wide. We therefore studied in parallel the experimental transmission, DNA polymerase activity and DNA restriction fragment patterns of the mallard virus with these characteristics of previously described DHBV strains to further compare its properties with those of the American and French strains of DHBV.

**METHODS**

**Experimental animals.** Wild ducks were obtained from a 'gastronomic' farm where mallards (Anas platyrhynchos) caught in the wild were maintained and bred in captivity, or from national wildlife reserve parks. Two distant parks were investigated. The first is located along the estuary of the Somme river, a marshy coastal area in the north-west of France. The other is located in the lake district of Dombes, close to Lyons in south-east France. In these wildlife parks studies are conducted on duck migration, birds being captured at regular intervals, identified, banded and released. Many species of wild duck including mallards and shelduck (Tadorna tadorna) have their winter quarters in these regions. Previous studies have demonstrated the presence of avian influenza viruses among migratory mallards and shelduck passing through these reserves (Hannoun & Devaux, 1980). Serum samples were taken from birds caught during the winter period of 1984.

White Pekin ducks (A. domesticus), originally obtained from a commercial breeder, and among which congenital transmission of DHBV to 6% of progeny has been previously documented (Cova et al., 1985), were used. Ducklings were screened for DHBV at 1 day after hatching and viraemic birds were maintained in our animal facilities.

**Assay for the presence of DHBV in duck serum.** Viraemia was demonstrated by a DNA spot hybridization test. Briefly, 50 µl serum samples were directly applied to a nitrocellulose filter (0-45 µm, Schleicher & Schüll) using a HybiDot manifold apparatus (Bethesda Research Laboratories). The filter was floated for 20 min on 1 M-NaCl/0-2 M-NaOH, then soaked in 1 M-NaCl/0-5 M-Tris-HCl pH 7-4 and rinsed in 2 x SSC (Mason et al., 1982) (1 x SSC is 0-15 M-NaCl, 0-015 M-sodium citrate).

The filters were air-dried and baked for 3 h at 80 °C. The hybridization was carried out as described by Maniatis et al. (1982), overnight at 42 °C in a solution containing 50% formamide, 5 x SSC, 3 x Denhardt's solution, 100 µg/ml denatured salmon sperm DNA and DHBV DNA radiolabelled by nick translation as described below. DHBV DNA cloned in the plasmid pBR322 was a generous gift from Dr J. Summers.

Filters were initially washed at 68 °C in 2 x SSC, 0-1% SDS, then twice in 1 x SSC, 0-1% SDS and again twice in 0-5 x SSC, 0-1% SDS. The filters were air-dried and exposed at -80 °C against Kodak XRP5 film using a Dupont Cronex Plus intensifying screen. The sensitivity of this assay for the detection of specific viral sequences was of the order of 1 pg, which is graded +. Subsequent semi-quantitative estimation of DHBV DNA levels in serum was scored by crosses (+++, ---). Experimental transmission. Ten- to 15-day-old Pekin and wild mallard ducklings born in captivity and negative for DHBV according to the DNA spot hybridization test were inoculated intravenously (i.v.) with 200 µl of viraemic serum containing similar levels of the endogenous DNA polymerase activity, obtained from either mallard or Pekin ducks. All ducks were bled by an occipital sinus puncture 5 and 10 days after inoculation.
control group composed of uninfected, DHBV-seronegative Pekin and wild mallard ducklings was maintained in the same animal facilities.

**Electron microscopy.** Viral particles were concentrated from 200 μl of duck serum by ultracentrifugation for 1 h at 48,000 r.p.m. in a SW50.1 rotor (Beckman). Concentrated virus samples were resuspended in 15 μl phosphate buffer pH 7.0 and applied to 300-mesh Formvar carbon-coated grids. The samples were stained with 2% phosphotungstic acid (pH 6-0) or 2% uranyl acetate and examined by electron microscopy (Jeol 1800 S) at an original magnification of 60,000.

**Agarose gel analysis of DNA radiolabelled by the endogenous DNA polymerase reaction.** DHBV particles from duck sera were sedimented by ultracentrifugation on a 10 to 20% (w/v) sucrose gradient for 3 h at 48,000 r.p.m. in a SW50.1 rotor. The pellet was resuspended in TEN buffer (10 mM-Tris-HCl pH 7.4, 1 mM-EDTA, 100 mM-NaCl) and assayed for 3 h at 37 °C for incorporation of $[\alpha-^3P]dCTP$ into viral DNA by the endogenous DNA polymerase reaction as described previously (Hantz et al., 1984). The radiolabelled DNA was analysed by electrophoresis for 16 h on 1% (w/v) agarose gels (Cova et al., 1985).

**Analysis of DHBV DNA in the liver of infected ducks.** Total DNA was extracted from 0-1 to 0-2 g of liver as described by Mason et al. (1982). The DNA was subjected to electrophoresis in a horizontal slab gel of 1.2% agarose and blotted onto a nitrocellulose filter (0.45 μm, Schleicher & Schüll) by the method of Southern (1975) as modified by Wahl et al. (1979). Detection of DHBV-specific sequences was achieved by hybridization with a DHBV DNA probe radiolabelled by nick translation (Rigby et al., 1977) to a specific activity of 0-8 × 10⁸ to 1.2 × 10⁹ c.p.m./μg, using a nick translation kit (Amersham) in a reaction containing $[\alpha-^3P]dCTP$ (800 Ci/mmol) (Amersham). Filters were hybridized with the radiolabelled probe and washed as described above for the DNA spot hybridization test.

**Restriction enzyme analysis of DHBV DNA.** The single-stranded part of DHBV DNA was completed to fully double-stranded molecules in an endogenous DNA polymerase reaction. The concentrated viral particles were incubated for 5 h at 37 °C with unlabelled nucleotides, according to conditions previously described (Hantz et al., 1984). The reaction was stopped by incubation with proteinase K (Sigma, 100 μg/ml) for 1 h at 37 °C. Subsequent deproteinization was done by two phenol-chloroform and one chloroform extractions and DNA was precipitated by ethanol. The DNA was resuspended in 10 mM-Tris-HCl pH 7.5, 1 mM-EDTA and digested with various restriction endonucleases (EcoRI, BamHI, HindIII, Arai, XhoI) for 6 h at 37 °C using the buffers recommended by the supplier (Boehringer). The digested DNAs were separated by electrophoresis in a 1.2% (w/v) agarose gel, transferred to nitrocellulose filters and hybridized with the DHBV-specific radiolabelled probe as described for the liver DNA analysis.

**RESULTS**

**Detection of DHBV in wild duck populations**

Out of 180 mallards captured in the wild and maintained in captivity, seven (3.8%) were positive for DHBV by the spot hybridization assay (Table 1). Of the mallards originating from the Dombes, 12.5% and of those from the River Somme wildlife reserve park 3.1% gave a strong positive signal in the DNA hybridization test (Table 1). The frequency of DHBV infection was similar for males and females, but all sera from 60 shelducks were negative (Table 1).

**Experimental transmission of the wild strain of DHBV to mallard and Pekin ducklings**

In order to confirm that the positive signal observed in the DNA hybridization test was indeed due to the DNA of a transmissible agent, experimental infection of various ducks (Pekin ducklings and mallard ducklings born in captivity) were undertaken using the serum (200 μl for each duck injected i.v.) from a wild viraemic mallard (no. 369) as inoculum. The presence of DHBV in this inoculum was confirmed by electron microscopy, DNA polymerase and DNA hybridization tests.

Table 2 shows that all inoculated mallard ducklings developed acute viraemia within 5 days of inoculation demonstrable by both DHBV DNA hybridization and endogenous DNA polymerase assays. However, only young (10 to 20 days old) ducklings were susceptible to DHBV, whereas 4-month-old captured mallards (10 birds) infected and tested in the same inoculation experiment were found to be resistant (data not shown).

The same inoculum was used to infect Pekin ducklings (200 μl for each duck injected i.v.) (Table 2). All infected ducklings were susceptible to the wild mallard strain of DHBV. Ten days post-infection a significant decrease of viral DNA, down to undetectable levels for ducks 951
Table 1. Detection of DHBV in wild duck populations

<table>
<thead>
<tr>
<th>Duck species</th>
<th>Origin</th>
<th>No. of birds tested</th>
<th>No. of DHBV in dot hybridization test</th>
<th>Percentage positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. platyrhynchos</em> (mallard)</td>
<td>Wild-caught mallards maintained in captivity</td>
<td>180</td>
<td>+ + + (3)*</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Wild mallards from Dombes</td>
<td>32</td>
<td>+ (1)</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Wild mallards from River Somme estuary</td>
<td>32</td>
<td>+ + (1)</td>
<td>3.1</td>
</tr>
<tr>
<td><em>T. tadorna</em> (shelduck)</td>
<td>Wild shelducks from River Somme estuary</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* DHBV DNA levels in sera of infected ducks are indicated on the scale described in Methods.
† Numbers in parentheses represent number of birds.

Table 2. Experimental transmission of DHBV from wild mallards to captive mallard and Pekin ducklings

<table>
<thead>
<tr>
<th>Duck species</th>
<th>Inoculum*</th>
<th>Bird no.</th>
<th>Presence of viral DNA in duck serum (dot hybridization)†</th>
<th>DNA polymerase activity in duck sera (Δ c.p.m.) at 5 days p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild mallards</td>
<td>Serum DHBV + + + from wild mallard (no. 369)</td>
<td>951</td>
<td>+ + +</td>
<td>ND§</td>
</tr>
<tr>
<td></td>
<td></td>
<td>953</td>
<td>+ + +</td>
<td>5960</td>
</tr>
<tr>
<td></td>
<td></td>
<td>954</td>
<td>+ + +</td>
<td>3050</td>
</tr>
<tr>
<td></td>
<td></td>
<td>956</td>
<td>+ +</td>
<td>1323</td>
</tr>
<tr>
<td></td>
<td></td>
<td>957</td>
<td>+ + +</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>960</td>
<td>+ + +</td>
<td>ND</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>970 to 980</td>
<td>-</td>
<td>5 days p.i.</td>
</tr>
<tr>
<td>Pekin ducks</td>
<td>Serum DHBV + + + from wild mallard (no. 369)</td>
<td>172</td>
<td>+ +</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>193</td>
<td>+ + +</td>
<td>3442</td>
</tr>
<tr>
<td></td>
<td></td>
<td>209</td>
<td>+ + +</td>
<td>4001</td>
</tr>
<tr>
<td>Serum DHBV + + + from Pekin duck (no. 910)</td>
<td>101</td>
<td>+ + +</td>
<td>2262</td>
<td></td>
</tr>
<tr>
<td></td>
<td>102</td>
<td>+ + +</td>
<td>2118</td>
<td></td>
</tr>
<tr>
<td></td>
<td>103</td>
<td>+ + +</td>
<td>944</td>
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<td></td>
<td>104</td>
<td>+ + +</td>
<td>2562</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>120 to 130</td>
<td>-</td>
<td>5 days p.i.</td>
</tr>
</tbody>
</table>

* The inoculum from the wild mallard no. 369 had a similar endogenous DNA polymerase activity to that from the Pekin duck (no. 910).
† DHBV DNA levels in sera of infected ducks are indicated on the scale described in Methods.
‡ p.i., Post-infection.
§ ND, Not determined.

and 957, was observed in sera of both mallard and Pekin ducklings (Table 2). This was followed by further fluctuations of DNA polymerase activity and DNA levels in the serum of infected ducks. Out of six mallard ducklings inoculated with the wild mallard strain of DHBV, five developed a persistent viraemia lasting for more than 240 days.

Similar results were observed for a control group of four Pekin ducklings inoculated with the Pekin duck strain of DHBV (no. 910). However, it seems that the inoculum obtained from wild
mallard induced a more intense viraemia in Pekin ducklings, as suggested by the higher values for endogenous DNA polymerase activity, than the inoculum from Pekin ducks. No horizontal virus transmission was observed, since uninfected ducklings housed with the viraemic ones for 6 months remained seronegative for DHBV. Two naturally infected mallards which were followed for 200 days remained DHBV carriers. The transmission of the Pekin duck strain of DHBV to wild mallards is in progress.

Endogenous DNA polymerase activity

To confirm that the DNA polymerase activity detected in sera of wild mallards was virus-associated, concentrated viral particles were tested for endogenous DNA polymerase activity, using radiolabelled nucleotides, and the product of the reaction was analysed by agarose gel electrophoresis.

When submitted to 1% agarose gel electrophoresis the radiolabelled product of the endogenous DNA polymerase reaction migrated as a major band of about 3.3 kb representing the full-length double-stranded DNA in relaxed circular conformation, preceded by a smear consisting of faster migrating species of partially single-stranded DNA (Fig. 1b). Similar migration patterns were observed for radiolabelled DNAs obtained from the sera of Pekin ducks infected with the French (Fig. 1a) and American (Fig. 1c) strains of DHBV and run on the same gel as controls.

Electron microscopy

DHBV particles obtained from the serum of viraemic wild mallards and from Pekin ducks had a similar morphology under the electron microscope (Fig. 2). However, DHBV particles from wild mallards (Fig. 2b) had a slightly different appearance from the virus particles from Pekin ducks (Fig. 2a), which might be related to differences in the permeability of the particles to the stain.

Both wild mallard and Pekin duck virus preparations contained two types of roughly spherical particles 40 to 60 nm in diameter. Particles unpenetrated by stain had an opaque appearance.
and showed no clear internal structure. Particles penetrated by stain, with a partially disrupted envelope, revealed an outer shell surrounding an internal core.

The size and morphology of negatively stained viral particles were similar to those described previously for DHBV (Mason et al., 1980; Tsiquaye et al., 1985). As described by Tsiquaye et al. (1985), uranyl acetate stain allowed a better presentation of morphological details of DHBV (Fig. 2) than phosphotungstic acid (data not shown).

**Presence of DHBV-specific DNAs in the liver of infected wild mallards**

Analyses by Southern blot of the viral DNAs derived from sera and livers from naturally infected wild mallards and Pekin ducks are shown in Fig. 3. Virion DNA isolated from the serum of a mallard duck (Fig. 3a) migrated as two bands; the 4.5 kb band represented the relaxed circular (RC) conformation of viral DNA while the faster migrating band which had an identical mobility (3 kb) to that of a cloned, linear DHBV corresponded to the linear (L) form of viral DNA. The liver DNA from two wild mallard ducks (Fig. 3b, c) and from one French Pekin duck (Fig. 3d) exhibited additional, faster migrating DNA species which had the characteristic electrophoretic mobilities of the various replicating DHBV DNA forms previously described by Mason et al. (1982). The band marked CC probably corresponded to the supercoiled closed circular DNA of 3 kb, while the species marked SS represented the single-stranded viral DNA. Indeed, the electrophoretic mobility of the SS species was similar to that of heat-denatured (100 °C, 10 min) DHBV DNA and their migration was not affected by prior heating (data not shown). No high molecular weight, slow migrating bands suggestive of integration of viral DNA into cellular DNA was observed in any of the liver samples studied.

**Restriction endonuclease analysis**

The restriction enzyme patterns of DNAs from a wild mallard duck (no. 908) (lanes W) and a French Pekin duck (no. 92) (lanes F) obtained by Southern blot analysis (Fig. 4a) were compared with that of the American DHBV isolate predicted from its DNA sequence (kindly communicated by F. Galibert) (Fig. 4b). It allowed a rough evaluation of the similarity between the restriction maps of these viruses.
Duck HBV in wild mallards

Fig. 3. Southern blot analysis of DHBV DNAs in the virions and livers of naturally infected mallard and Pekin ducks. DNAs were subjected to electrophoresis on a 1.2% agarose gel, transferred to nitrocellulose, and detected by hybridization with a 32P-labelled DHBV probe as described in Methods. (a) Virion DNA from concentrated particles from serum of a wild mallard duck (no. 369). (b) Liver DNA (18 μg) from a wild mallard duck (no. 908). (c) Liver DNA (18 μg) from a wild mallard duck (no. 369). (d) Liver DNA (18 μg) from French Pekin duck (no. 57). RC, double-stranded 3 kb relaxed circular DNA; L, double-stranded 3 kb linear DNA; CC, double-stranded 3 kb supercoiled DNA; SS, single-stranded 3 kb DNA. Markers (numbers on the right, kb) are sizes of DNA fragments, HindIII-digested.

Wild mallard DHBV DNA can be differentiated from the French Pekin DHBV DNA by its AvaI and XhoI restriction endonuclease pattern (Fig. 4a). Wild mallard DHBV DNA had one XhoI cleavage site, at the same position (1212 bp) as the American DHBV isolate. This was deduced from the position, in addition to the major band of linear DNA, of two discrete bands (respectively 1750 and 1250 bp) deriving from the XhoI cleavage of the nicked, linear form of wild mallard DHBV DNA. The French Pekin DHBV DNA had no XhoI cleavage site (Fig. 4a, b). Furthermore, DNA from both the wild mallard and the French Pekin DHBV strains differed from the sequenced DNA of the American DHBV strain (Mandart et al., 1984) by the absence of the EcoRI cleavage site (Fig. 4a). The HindIII digestion pattern (two major bands of respectively 1700 and 1080 bp) and BamHI digestion pattern (one site at 1658 bp position linearizing RC DNA) of the DNAs were similar for all three strains of DHBV studied (American Pekin, French Pekin and wild mallards) (Fig. 4a, b). Restriction enzyme analysis of another wild mallard isolate (duck no. 919) showed a slightly different pattern (data not shown).

DISCUSSION

We report here the presence of a virus similar to DHBV in serum and liver from wild migrating ducks. This wild mallard strain is closely enough related to the original domestic Pekin duck DHBV to be detectable by DNA hybridization using cloned DHBV DNA as a probe. This new hepatotropic virus of wild ducks induced viraemia associated with an endogenous DNA polymerase which is capable of converting a partially double-stranded DNA into the fully double-stranded form in vitro. The various replicating DNA forms reported for DHBV, WHV and HBV were also demonstrated in the livers of wild mallards. All these properties are indeed characteristic of the hepadnavirus group (Robinson et al., 1981). The morphology of the two viruses is indistinguishable when viewed by electron microscopy. It may be concluded therefore that the virus isolated from wild mallard ducks is a new strain of DHBV.
Fig. 4. (a) Comparative restriction enzyme analysis of the DHBV DNA extracted from wild mallard duck serum and from French Pekin duck serum. DHV particles were sedimented from duck sera on a sucrose gradient and resuspended in TEN buffer. The single-stranded region of the viral DNA was completed to a fully double-stranded form in an endogenous DNA polymerase reaction using unlabelled nucleotides followed by DNA extraction and precipitation as described in Methods. Viral DNA was digested with various restriction endonucleases (EcoRI, BamHI, AvaI, HindIII, XhoI), separated on a 1.2% agarose gel, transferred to nitrocellulose and detected by hybridization with 32P-labelled DHBV probe as described in the text. Lanes F, virion DNA from a French Pekin duck (no. 92) naturally infected with DHBV. Lanes W, virion DNA from a wild mallard duck (no. 908) naturally infected with DHBV. RC, 3 kb, relaxed circular form of DHBV DNA. L, 3 kb, linear form of DHBV DNA. The position of this linear form is also indicated by arrows. Minor bands on the autoradiogram are derived from digestion of the nicked, linear form of DHBV DNA. Samples treated with HindIII were only partially digested. Markers (numbers on the right, kb) are sizes of λ DNA fragments, HindIII-digested. (b) Physical map of the American DHBV isolate predicted from its DNA sequence. Positions of the restriction enzyme cleavage sites (EcoRI, BamHI, AvaI, HindIII, XhoI) of the DNA from the American DHBV were deduced from its sequence (Mandart et al., 1984). The arrow indicates the position of the nick as determined by Mason et al. (1982). Distances are in kb. ▲, Restriction enzyme cleavage sites conserved in DHBV DNA from a French Pekin duck (no. 92) compared to the American isolate. ●, Restriction enzyme cleavage sites conserved in DHBV DNA from a wild mallard duck (no. 908) as compared to the American isolate.

For this study two wildlife reserve parks were chosen which are located on the two major migratory pathways of ducks across France. The Baie de la Somme ornithological park is on the flyway for ducks from the north European region (Iceland, Scandinavia, north-west U.S.S.R.), while the Dombes are one of the winter quarters for ducks of eastern origin (central north Europe, Germany, central Siberia). The detection of DHBV in two distant wildlife reserve parks located on the routes of two different duck flyways suggests that this virus circulates in wild duck populations.

It has been documented that wild ducks are a natural reservoir of influenza and parainfluenza viruses and are involved in the dissemination of these viruses all over the world (Webster et al., 1976; Hinshaw et al., 1980; Alexander, 1980; Kessler et al., 1979; Hannoun & Devaux, 1980).
Furthermore, based on the detection of antigenically related viruses in both domestic and feral ducks, the migratory birds may have introduced influenza and parainfluenza viruses into domestic flocks (Webster et al., 1976, 1978; Hinshaw et al., 1979; Nerome et al., 1983). Similarly it could be speculated that wild ducks play a role in the spread of the DHBV found in domestic ducks in Asia, north America and Europe. However, if the transmission of avian influenza and parainfluenza viruses occurs via the faecal–oral route through water contamination (Webster et al., 1976, 1978; Hinshaw et al., 1979), the transmission of DHBV is primarily vertical, via the egg (O'Connell et al., 1983; Urban et al., 1985). The occasional horizontal transmission of DHBV has been reported by Mason et al. (1983), but we did not observe horizontal transmission of DHBV from viraemic birds to the controls even though virus-free and infected Pekin and mallard ducks were kept together over a 6 month period. Moreover, close contact of wild ducks with domestic ones may not be a common occurrence.

The most likely hypothesis is that the DHBV strain circulating in wild mallard populations is the common ancestor of all the virus isolates from the various Chinese, American and European domestic ducks. Indeed, all breeds of domestic duck, such as Pekin, Khaki Campbell, Indian Runner etc., are derived from wild mallards (Delacour, 1973). The domestication of wild mallards is very ancient, and occurred as early as the 12th century in Europe and probably much earlier in China (Delacour, 1973). The vertical and horizontal modes of transmission are not mutually exclusive and probably both, together with the eventual mating between wild and domestic ducks, have contributed to the perpetuation and diffusion of the virus from wild to domestic ducks or vice versa at some point in time.

The results reported here indicate that the wild duck strain of DHBV differs in its DNA restriction enzyme pattern from the American and French strains of DHBV. This is not surprising since, as for HBV (Summers et al., 1975), DHB viruses vary from one another. The minor sequence heterogeneity easily explains the differences in the restriction enzyme pattern which has been previously reported not only between the German and American strains of DHBV (Sprengel et al., 1984), but also between different DHBV isolates of American origin (Mandart et al., 1984; F. Galibert, personal communication). Only the nucleotide sequence determination of the wild duck DHBV DNA, and its comparison to that of the Chinese and American DHBV strains will provide further information on phylogenetic relationships among these viruses.

The discovery of DHBV in the wild duck population might be crucial for the study of the possible association between DHBV infection and hepatocellular carcinoma in ducks. It has been reported that hepatocellular carcinoma does occur in Chinese domestic Pekin ducks chronically infected by DHBV in China, while no hepatocellular carcinoma has been observed in DHBV carrier Pekin ducks in the U.S.A. (Marion et al., 1984; Omata et al., 1983). This difference may be related to environmental factors such as aflatoxin, life span of the birds or to intrinsic differences in the oncogenic potential of the various DHBV strains. It is known for woodchucks that a high frequency of hepatocellular carcinoma occurs in animals which live out a natural life span (Snyder & Summers, 1980). In previous studies the DHBV-infected domestic ducks from commercial flocks were only 1 or 2 years old, whereas the expected life span of these birds is 10 years or more. In this regard, wild mallard ducks infected with DHBV may prove to be of great interest.

Up to now we have only analysed the state of DHBV in two liver samples from wild viraemic mallards and these had only unintegrated DHBV DNA. An extensive investigation of wild duck populations for liver disease and possible DHBV DNA integration in the liver would be most helpful in the elucidation of the roles of environmental, host and viral factors in the pathogenesis of DHBV infection and their possible oncogenic action in ducks.

We are indebted to Professor M. Aymard for helpful discussions. We thank J. Summers and W. Mason for generously providing us with the DHBV genome cloned in pBR322. We are grateful to F. Galibert for stimulating interest and advice and to A. Kay for communicating the restriction enzyme analysis of the American strain of DHBV. We thank Dr C. Hannoun for advice in the choice of wildlife reserve parks. We also thank for their kind cooperation Mr R. Malavieille and Mr P. Cordonnier in providing access to the birds from Dombes wildlife reserve park and Mr M. Jeanson who provided facilities for collecting samples from the ducks in the Marquenterre.


Duck HBV in wild mallards


(Received 7 August 1985)