Transmission of Hepatitis B Virus to Gibbons by Exposure to Human Saliva Containing Hepatitis B Surface Antigen

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A pool of whole-mouth saliva collected from five human carriers of hepatitis B surface antigen, subtype adr, was found to contain antigen particles with mean diameters of 23.3 and 41.8 nm as seen by immune electron microscopy. Two gibbons received subcutaneous injections of the pooled saliva and developed serological and, in at least one animal, biochemical evidence of hepatitis B virus infection at 12 and 22 weeks, respectively. Although none of eight other gibbons that were exposed by the nasal or oral routes were infected, the experiment demonstrated that human saliva can serve as a vehicle for the transmission of hepatitis B virus.

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The presence of hepatitis B surface antigen (HBsAg) in the whole-mouth saliva of antigenemic people has been reported [1-7]. The high prevalence of the antigen and of antibody to HBsAg in members of the same family [8-13], children in institutions [14, 15], and sexual partners [13, 16, 17], and the high incidence of hepatitis in dental personnel [18, 19] have raised the possibility that saliva may be a vehicle for the transmission of hepatitis B virus (HBV) [20]. A human bite has been implicated in the transmission of HBV in one case [21]. Since no reports have demonstrated the infectivity of saliva, we attempted to transmit HBV by exposing captive gibbons to a pool of human saliva containing HBsAg.

Materials and Methods

Saliva collection. Seven male and four female Thai adults, who were known to have HBsAg-positive sera, were asked to provide fresh saliva specimens. Since HBsAg is inconstantly present in saliva [6, 7], samples were collected from the donors on more than one day. Collections were made at least 2 hr after eating any food or brushing the teeth.

Saliva was collected by two methods. The first involved spitting into sterile containers. These samples were considered to be whole-mouth saliva, since they included all oral secretions. No sialagogues were used to obtain these samples. The second type of collection utilized a circular, hard plastic cup, 2 cm in diameter, with a peripheral suction ring and a central drainage port attached to a flexible polyethylene drainage tube. The cup was positioned to cover completely the opening of one parotid duct and was attached to the buccal mucosa by gentle suction. This type of collection was considered to consist of unilateral parotid secretions. During the collection of parotid secretions, the donor was sometimes permitted to suck on a hard candy to promote secretory activity.

During collection, the saliva samples were kept chilled in an ice bath. After removal of a 1.0-ml aliquot for testing, the remainder of each sample was promptly frozen at -70°C until pooled. Samples were selected for inclusion in a saliva pool on the basis of the HBsAg test results. All saliva samples were tested for occult blood (Labstix; Ames Company, Elkhart, Ind.). Multiple serum samples were drawn from each donor.
Table 1. Frequency of hepatitis B surface antigen (HBs Ag) in two types of human saliva preparations.

<table>
<thead>
<tr>
<th>Donor no.</th>
<th>Whole-mouth saliva</th>
<th>Parotid secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of specimens</td>
<td>No. occult blood-positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. HBs Ag-positive*</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>16</td>
</tr>
</tbody>
</table>

*Specimens positive for HBs Ag as tested by radioimmunoassay.

to determine the geometric mean titer of antigen during the weeks of saliva collection.

Preparation of the saliva pool. Five people provided samples of whole-mouth saliva and parotid secretion; HBs Ag was present in 16 (61%) of 23 of the former samples and only one (12.5%) of eight of the latter specimens (table 1). The single antigen-positive sample of parotid secretion was of an unusually large volume, a fact which suggested that the collection cup may have dislodged allowing HBs Ag to enter the sample from the oral cavity. Because of the low yield of antigen, parotid secretions were not included in the final saliva pool.

Six of the 11 saliva donors had HBs Ag in one or more samples. Five of the six carried HBs Ag/adr; the other had the adw subtype. To maintain the uniformity of the saliva pool, we used 18 samples from the adr carriers only (table 2). The samples were thawed quickly, mixed together thoroughly, and centrifuged at 12,100 g for 30 min at 4 C; the supernate was used as the saliva pool. The pool appeared clear to the eye, was positive for occult blood, and gave a reaction for HBs Ag by radioimmunoassay only.

The pool was divided into aliquots for convenient use and cultured for bacterial growth. Portions reserved for sc injection were treated

Table 2. Samples of whole-mouth saliva from carriers of hepatitis B surface antigen (HBs Ag)/adr used in the human saliva pool.

<table>
<thead>
<tr>
<th>Donor no. (sex, age)</th>
<th>HBs Ag titer*</th>
<th>e antigen</th>
<th>Occult blood reaction†</th>
<th>No. tested</th>
<th>No. HBs Ag-positive‡</th>
<th>Volume added to saliva pool (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (M, 36)</td>
<td>1:128</td>
<td>+</td>
<td>4+</td>
<td>2</td>
<td>2</td>
<td>62.6</td>
</tr>
<tr>
<td>2 (M, 36)</td>
<td>1:32</td>
<td>−</td>
<td>4+</td>
<td>2</td>
<td>2</td>
<td>75.0</td>
</tr>
<tr>
<td>3 (F, 35)</td>
<td>1:28</td>
<td>−</td>
<td>4+</td>
<td>1</td>
<td>1</td>
<td>20.0</td>
</tr>
<tr>
<td>4 (M, 33)</td>
<td>1:11</td>
<td>NT</td>
<td>4+</td>
<td>1</td>
<td>1</td>
<td>12.2</td>
</tr>
<tr>
<td>5 (F, 28)</td>
<td>1:11</td>
<td>+</td>
<td>Trace</td>
<td>1</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>171.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Age is given in years, M = male; F = female; (+) = positive result; (−) = negative result; NT = not tested.
*Geometric mean titer of HBs Ag by CF.
†Graded according to the Labstix test (Ames Company, Elkhart, Ind.) on a scale of trace (minimal reaction) to 4+ (maximal reaction).
‡Specimens positive for HBs Ag as tested by radioimmunoassay.
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with penicillin (1,000 units/ml) and streptomycin (1,000 µg/ml). Bacterial cultures of the untreated saliva yielded a few colonies of α-hemolytic streptococci; the saliva containing antibiotics was bacteriologically sterile. All portions were kept frozen at −70°C until used.

Management and selection of gibbons. White-handed gibbons (Hylobates lar) were housed in animal rooms designed to permit free circulation of air with the outside area. The rooms were double-screened to prevent the entry of insect vectors. The gibbons selected for exposure to the saliva were isolated in one room. Each gibbon was kept in an individual cage, and cages were spaced apart to prevent direct contact between animals. Animals were fed a commercial primate diet supplemented with fruits and vegetables. Each gibbon had an individual supply of food and water. Only veterinary personnel who had no HBsAg were permitted to care for the gibbons selected for study.

Ten gibbons with no detectable HBsAg or antibody to HBsAg (anti-HBs) were selected for exposure to the saliva pool. They included five males and five females and ranged in age from one to 11 years. One other gibbon (B-66S) had anti-HBs and was followed as a control for the methods of antibody detection. Studies of the transmission of viral hepatitis had never been done before in the gibbon colony.

Exposure to the saliva pool. Two gibbons (Pc-13 and Pc-14) received sc injections of 1.7 ml of saliva every other day for three days (table 3). Eight other gibbons were divided into pairs and exposed to 1.0 ml of saliva on each of five successive days by aerosol spray in the nose, aerosol spray in the mouth, brushing of the teeth with the saliva, or ingestion of saliva injected into a banana. Each animal received a total of 5.0 or 5.1 ml of the pooled saliva over the same five days. It may be assumed that a large portion of the saliva administered by the oral and nasal routes was swallowed. The antibody-positive control gibbon (B-66S) was not exposed to the saliva.

Follow-up study. The first day of exposure to the saliva pool was designated day 0. On every subsequent day each animal was observed for altered behavior, and the rectal temperature was recorded. Each week, the gibbons were weighed and examined, and a blood sample was drawn after sedation with a rapidly acting anesthetic (phencyclidine hydrochloride or ketamine hydrochloride).

Detection of HBsAg, anti-HBs, and e antigen. All samples of serum and saliva were tested for HBsAg by solid-phase radioimmunoassay (Austria II, Abbott Laboratories, North Chicago, Ill.) without preliminary preparation [22]. Positive reactions were confirmed by ≥50% reduction of test serum reactivity after incubation with a human serum containing anti-HBs. Titers of antigen in serum were determined by CF tests [23], HBsAg subtypes were detected by immunodiffusion [24], and e antigen [25] was detected in serum by immunodiffusion with serum from a Thai blood donor as antibody.

Anti-HBs was detected by a solid-phase radioimmunoassay (Ausab, Abbott Laboratories) and passive HA [26] with use of erythrocytes coated with HBsAg/ad (Electronucleonics Laboratory, Bethesda, Md.).

*The sc doses were given on days 0, 2, and 4. All other methods of exposure were used on days 0–4 inclusive.
†F = female; M = male.
manner. Second, 5.0 ml of each supernate was centrifuged in a Beckman L-550 ultracentrifuge (Beckman Instruments, Fullerton, Calif.) with an SW 39L rotor at ~100,000 g for 16 hr at 4 C. The top 4.85 ml of supernate was removed with a pipette. The sediment was suspended in the remaining 0.15 ml of supernate to give a 33-fold concentration of antigen.

As a third step, each concentrate of saliva was divided in two, and 0.07 ml was mixed with either 0.1 ml of a 1:50 dilution of rabbit anti-HBs/adr or an equal dilution of serum from the same rabbit obtained before it was immunized. The rabbit antiserum was absorbed to excess with normal human serum. In addition, separate preparations were made of a partially purified, HBs Ag/adr in CsCl; these preparations were known to contain small particles, filaments, and Dane particles [27]. Each sample was incubated at 25 C for 1 hr and then overnight at 4 C.

The fourth step involved centrifugation of each sample at 94,800 g in the Sorvall RC2-B centri­fuge for 90 min [28]. Each sediment was resus­pended in three drops of distilled water, placed on a collodion-carbon-coated copper 200-mesh grid, stained with 2% uranyl acetate, and examined under a Hitachi HU-11C electron microscope (Hitachi, Ltd., Tokyo, Japan).

Results

HBV infections in exposed gibbons. Twelve weeks after the first inoculation of the pooled saliva, gibbon Pc-13 gave positive results in tests for HBs Ag (figure 1). This gibbon had detectable antigenemia for the next two weeks accompanied by a distinct rise in levels of alanine aminotransferase (SGPT). Anti-HBs was detected in all subsequent samples of sera. During the period of antigenemia, Pc-13 had a normal temperature, normal eating behavior, and no change in weight. Jaundice, hepatomegaly, lymphadenopathy, skin rash, and other abnormal physical findings were not found.

Figure 1. Response of gibbon Pc-13 to the sc injection of a pool of human saliva containing hepatitis B surface antigen (HBs Ag). Antigenemia (HBs Ag) was followed sequentially by elevations in the level of alanine aminotransferase (SGPT) and the appearance of antibody detected by passive HA (PHA) and radioimmunoassay (RIA). Anti-HBs = antibody to HBs Ag.

Figure 2. Response of gibbon Pc-14 to sc inoculation of a pool of human saliva containing hepatitis B surface antigen (HBs Ag). Elevation in the level of alanine aminotransferase (SGPT) occurred at 11 weeks after exposure; this elevation immedi­ately followed surgery on one hand of the gibbon. Antibody to HBs Ag (anti-HBs) was first detected by radioimmunoassay (RIA) at 22 weeks. HBs Ag was not detected. PHA = passive HA.
Gibbon Pp-14 remained well until 10 weeks and four days after exposure, when he escaped from his cage and was bitten severely on the hand by gibbon P-16. After the amputation of one finger, the SGPT value rose to 61 units and the aspartate aminotransferase (SGOT) to 45 units (figure 2). Both values were normal one week later. At 22 weeks, the serum first contained anti-HB$_s$, as seen by radioimmunoassay. The next week the passive HA titer of anti-HB$_s$ was 1:32. HB$_s$ Ag was not detected at any time. The incubation period is assumed to be 22 weeks, although

![Figure 3](https://academic.oup.com/jid/article-abstract/135/1/79/836210/192000)

**Figure 3.** Electron micrographs of clumps of hepatitis B surface antigen (HB$_s$ Ag) particles derived from human plasma and saliva. *A,* partially purified HB$_s$ Ag/adr (EH017) with normal rabbit serum; *C,* EH017 with rabbit antibody to HB$_s$ Ag (anti-HB$_s$); *B* and *D,* similar small and large particles from the human saliva pool precipitated with rabbit anti-HB$_s$. Filamentous forms were not found in the saliva. The bar represents 100 nm (X 192,000).
it is not possible to be certain that the rise in SGPT and SGOT at 11 weeks was entirely due to the traumatic injury. None of the gibbons exposed to the saliva by the oral or nasal routes developed HB$_b$ Ag or anti-HB$_b$ at any time.

**HBV infections in unexposed gibbons.** Tests of 55 animals were performed with blood samples collected from gibbons living in the colony between March 1973 and December 1975. Although some animals left the colony during this period, others were born into it. Two gibbons were found to be chronic carriers of HB$_b$ Ag in March 1973; only one of these was still present during the experiment. Throughout the 34-month observation period, none of the unexposed gibbons developed HB$_b$ Ag or anti-HB$_b$.

**Immune electron microscopy.** The partially purified HB$_b$ Ag/adr (EH017) was found to contain some clusters of particles even without the addition of rabbit anti-HB$_b$. In the presence of antiserum, clusters of particles were more abundant. The mean dimensions of the principal morphological forms were: small spheres, 21.6 $\pm$ 3.6 nm in diameter; filaments, up to 177 nm long; and large spheres, 36.1 nm in diameter (figure 3). Small particles similar to those in EH017 were seen in the control saliva that was mixed with HB$_b$ Ag/adr serum only after rabbit antiserum was added.

The saliva pool contained no clusters of particles in the absence of antiserum. However, the addition of specific antibody precipitated many clusters, which included small particles with a mean diameter of 23.3 $\pm$ 2.7 nm and large spheres averaging 41.8 nm in diameter (figure 3). Filamentous forms were not found in the saliva pool.

**Discussion**

It is certain that gibbons Pc-13 and Pc-14 were infected with hepatitis B virus after exposure to the pool of human saliva. Because of the precautions taken to avoid other exposures, it is highly unlikely that they were infected in any other way. During the study, one other gibbon and four technicians were known to carry HB$_b$ Ag; all were kept away from the gibbons being studied. The detection of large particles in the saliva pool, similar in size to Dane particles, gives added weight to the probability that the saliva was the source of the infection.

Why didn't the eight gibbons exposed by the oral and nasal routes become infected? It is probable that the concentration of infectious material was too low. It can be assumed that each animal swallowed some of the saliva. Krugman [14] demonstrated that MS-2 serum was highly infectious by mouth. The MS-2 serum contained a concentration of HB$_b$ Ag detectable by immunodiffusion [29]. The saliva pool used in this study had antigen detectable only by radioimmunoassay. The relative insensitivity of immunodiffusion compared with the sensitivity of the radioimmunoassay [30] suggests that the MS-2 serum contained a much higher titer of HB$_b$ Ag than did the saliva and probably contained more infectious particles as well.

The incubation periods of 12 and 22 weeks observed for the gibbons were similar to the ranges reported for chimpanzees (two to 15 weeks) [31–34], rhesus monkeys (12 to 15 weeks) [35], and human children (four to 15 weeks) [14, 36].

HB$_b$ Ag was most frequently found in saliva when there was a relatively high concentration of occult blood and a high titer of antigen in serum. However, exceptions to this generalization were observed. The presence of e antigen in the donor's blood may be another important factor related to the infectivity of saliva. Further investigation is needed to confirm the infectivity of human saliva and to define the characteristics of infectious donors.

**References**

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