Hepatitis B virus (HBV) infection represents a major worldwide public health problem because of the chronic hepatitis that HBV infection can cause in infected individuals, and of whom a significant number develop liver disease. Several epidemiologic surveys throughout the Pacific have demonstrated high overall prevalences of HBV infection. In French Polynesia, an exceptionally high HBV infection prevalence with a horizontal transmission pattern has been demonstrated in the populations of the Austral archipelago and the Marquesas archipelago. In the latter group of islands, the level and tendency of acquisition of infection were different, depending on the island considered; the infection rate among children was dramatically higher on Nuku-Hiva Island (74% in the rural area) than in the urban zone (mean 46.7%). Of the 500 children examined for the presence of skin lesions, and showed a correlation with a higher infection rate (73.9% versus 45.5%). Of the 45 pools of 10 insects tested, HBV DNA was not detectable on the inside of the insect, but was detectable on the flies in three pools (1–10 particles/insect in three positive pools). Infection by HBV conveyed by the flies is theoretically possible, but their indirect role via the numerous skin lesions caused on children is likely to explain such a high level of transmission.

In French Polynesia, the hematophagous blackfly Simulium buissoni (discovered by Roubaud in 1906) is a notorious pest of humans. The adult females feed on blood, resulting in the high seroprevalence of HBV on this island. The hematophagous blackfly is a conveyor of the virus or indirectly through the transmission of HBV to children. The hematophagous blackfly is a conveyor of the virus or indirectly through the transmission of HBV to children.
and used the polymerase chain reaction (PCR) to detect HBV DNA in and on female blackflies collected in Taipivai, a village that is holoendemic for this virus.

SUBJECTS AND METHODS

Study population

The total population of Nuku-Hiva Island was 2,099 in 1989. It was composed of an urban center (Taiohae) with 1,420 inhabitants and three small rural villages (Taipivai, Akapa, and Hatiheu) with 679 inhabitants. The 132 children included in the HBV seroprevalence study were randomly selected from the entire population of 602 children.

Epidemiology of HBV infection on Nuku-Hiva Island

Seroepidemiologic data regarding HBV infection in 1989 for the 132 children (<10 years old) and their 112 respective mothers (age range 13-63 years) is shown in Table 1. The infection rate of hepatitis B core antigen was significantly higher in the rural area than in the urban area among both mothers ($\chi^2 = 5.25, P < 0.05$) and children ($\chi^2 = 14.4, P < 0.001$). The same observation was made with regard to the sero-prevalence rate of hepatitis B surface antigen (HBSAg), but the difference was significant only for the mothers ($\chi^2 = 10.5, P < 0.01$). The HBSAg rate among children was significantly and surprisingly low (22.2%; $\chi^2 = 5.69, P < 0.02$) compared with that of the mothers (54.4%), when one considers that almost all the children are infected by age 10. There is no obvious reason that may explain this discrepancy, except to assume that the situation of the mothers was even worse than that of the children, since the earlier the infection occurs, the higher the risk of becoming a chronic HBsAg carrier. However, the health and hygiene conditions have improved dramatically in the Marquesas islands during the past 20 years. The current prominence of the horizontal transmission pattern supports this result.

The theoretical vertical risk of transmission is the expected proportion of HBsAg-positive children at age one. It is determined using the following formula: % HBsAg positive x % hepatitis B e antigen [HBe] positive among mothers) x 0.90.$^7$ This coefficient of 0.90 is the generally admitted risk for a one-year-old child of being a carrier when the mother is a double carrier (HBsAg positive and HBe positive). This calculated vertical risk was 1.7% in the urban area and 6.3% in the rural area. They were lower than expected with regard to the HBsAg seroprevalence because of the relatively low HBe seroprevalence among these mothers (7.7% in the urban area and 12.9% in the rural area). The observed vertical risks in these areas are 0% and 11.1%, respectively, which are not significantly different from the theoretical values.

Examination of children for scars and lesions

A total of 506 (98%) of 516 children (263 boys and 243 girls, age range 2-11 years) were examined. Three hundred forty-eight were from the urban center and 158 were from the rural area of the island. There was no difference in the age distribution of urban and rural children. For each child, the total number of scars and lesions on the arms and legs were scored. Statistical analysis was done using the PCSM statistical package (Programme Conversationnel de Statistiques pour les Sciences et le Marketing, Delta Soft, Meylan, France).

Collection of S. buissoni blackflies on Nuku-Hiva Island

Blood-seeking female blackflies were collected during the day by human bait catches. The catchers were free of HBV infection as determined by serologic and PCR tests. The flies were sampled in the village of Taipivai (380 inhabitants), where the highest infection rate has been observed. Blood-seeking female blackflies were collected in an uninhabited area (Terre Deserte). After collection, the insects were frozen in liquid nitrogen, sent by air to Tahiti, and stored at -80°C until tested.

Preparation of DNA samples from S. buissoni

For each sampling site, nine pools of 10 insects each were extracted (a total of 45 pools for the five sampling sites). Each pool of insects was soaked for 30 min in a microtube containing 200 µl of TE buffer (10 mM Tris HCl, 1 mM EDTA,
Southern blot analysis with digoxigenin-labeled DNA probes

For the Southern blots, the DNA samples were transferred to nitrocellulose membranes (Hybond N; Amersham, Arlington Heights, IL) using 20× SSC (0.3 M sodium citrate, 3 M NaCl) and irradiated with ultraviolet light. The DNA probes used (MD09 and MD24) were specific to the S and X amplified fractions and they do not overlap in sequence with the primers. These probes were 3'-end labeled with digoxigenin-dUTP (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions.10 The filters were prehybridized, then hybridized with the labeled probes under stringent conditions for 1 hr at 65°C.

Detection limits of the PCR assays

To test the sensitivity of the two PCR methods, negative control blackfly homogenates and supernatants were spiked with a range of 10-fold serial dilutions of HBV DNA standard (Abbott Laboratories, North Chicago, IL). The amount of HBV particles extracted from one fly may be assumed to contain 10 HBV particles (3-9 pg of DNA) in one PCR reaction using the calibrated positive controls (MD09 and MD24) directed the amplification of a 243-bp DNA fragment in the X gene that contained 112 specific nucleotides. The MD24/MD26 primers directed the amplification of a 128-basepair (bp) DNA fragment in the S gene that contained 112 specific nucleotides. The MD24/MD26 primers directed the amplification of a 243-bp DNA fragment in the X gene that contained 112 specific nucleotides.

HBV DNA was performed in a 50-μl reaction mixture containing 10 μl of the DNA template and 40 μl of a mixture containing two units of Taq polymerase (Perkin-Elmer-Cetus, Norwalk, CT), 1× PCR buffer (10 mM Tris HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatine) 0.2 μM of each primer, and 100 μM of each dNTP. 9 The reaction mixture was overlaid with 50 μl of mineral oil. The DNA was denatured at 94°C for 5 min, and 35 amplification cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min were performed, followed by an extension at 72°C for 5 min in an automatic thermo-cycler (Hybaid; Ceralab, Auber Villiers, France). After amplification, 10 μl of the PCR product was subjected to electrophoresis on a horizontal 1.5% agarose gel in 1× T-borate-EDTA buffer (100 mM Tris-borato-acid, 2 mM EDTA). The gel was stained with ethidium bromide and DNA bands were visualized with an ultraviolet transilluminator.

Polymerase chain reaction for HBV

Two PCR assays (S gene and X gene amplifications) were used to detect HBV DNA. The sequences of the primers were selected for the conserved regions of the viral genome determined from subtypes adw, adw, and ayw (C. Brechot, Institut Pasteur, Paris, France). The MD24/MD26 primers directed the amplification of a 128-basepair (bp) DNA fragment in the S gene that contained 112 specific nucleotides. The MD24/MD26 primers directed the amplification of a 243-bp DNA fragment in the X gene that contained 112 specific nucleotides.

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RESULTS

Scars and lesions among children

The results, according to the habitation zone, are shown in Table 2. The overall mean ± SD number of lesions was significantly higher (P < 0.05, Student's t-test) in the rural area (41.02 ± 31.71) than in the urban zone (17.73 ± 13.83). This was found to be positively correlated with age (all children analyzed; Pearson's coefficient r = 0.12 with the regression line Y = 16.2897 + 1.2595 X, P < 0.05. There was no significant difference in the number of lesions between sexes (26.63 ± 25.26 for boys and 23.24 ± 23.68 for girls).

Detection of HBV by PCR

Specificity. All supernatants and homogenates from the five pools of negative control flies caught in Terre Deserte, an uninhabited area of Nuku Hiva Island, were negative for HBV DNA in either of the two PCR systems.

Detection limits. In the homogenates, the detection limit was 100 HBV particles (3-9 × 10^4 pg of DNA) in one PCR reaction using the S gene PCR, while it was 1-3 HBV particles (3-9 × 10^4 pg of DNA) in one PCR reaction using the X gene PCR. In the homogenates, the detection limit was 100-300 HBV particles (3-9 × 10^4 pg of DNA) in one PCR reaction using the S gene PCR, while it was 10-30 HBV particles (3-9 × 10^4 pg of DNA) in one PCR reaction using the X gene PCR. The X gene PCR was 10-
FIGURE 2. Southern blot of amplified S gene products of hepatitis B virus (HBV) (128 basepairs [bp], primers MD03/MD06) with specific digoxigenin-labeled DNA probe MD09. Lane 1, positive control containing three HBV particles; lane 2, no DNA (control); lane 3, positive control containing 30 HBV particles; lanes 4-8, supernatants from sampling sites A, B, C, D, and E, respectively. M = DNA molecular weight markers.

FIGURE 3. Southern blot of amplified X gene products of hepatitis B virus (HBV) (243 basepairs [bp], primers MD24/MD26) with specific digoxigenin-labeled DNA probe MD29. Lane 1, positive control containing three HBV particles; lane 2, no DNA (control); lane 3, positive control containing 30 HBV particles; lanes 4-8, supernatants from sampling sites A, B, C, D, and E, respectively. M = DNA molecular weight markers.
Human rhinoviruses (HRV) include more than 100 different immunotypes, and type-specific immunity is associated with neutralizing antibody found in nasal secretions and in serum. These antibody titers remain relatively constant and can be detected serologically. Brown and Taylor-Robinson found neutralizing antibody and can be detected serologically. Brown and Taylor-Robinson found neutralizing antibody and can be detected serologically.

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