

THE BLACKFLY *SIMULIUM BUISSONI* AND INFECTION BY HEPATITIS B VIRUS ON A HOLOENDEMIC ISLAND OF THE MARQUESAS ARCHIPELAGO IN FRENCH POLYNESIA

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Abstract. The hematophagous blackfly *Simulium buissoni* causes skin lesions on an island in the Marquesas archipelago that is holoendemic for hepatitis B virus (HBV). To test the hypothesis of the possible role of this fly in the transmission of hepatitis B, 506 children (age range 2-11 years) were examined for the presence of skin lesions, and attempts were made to detect HBV DNA in and on blackflies using two polymerase chain reaction methods. The mean number of skin lesions showed a positive correlation with the age of these children ($r = 0.12$, $P < 0.05$). Furthermore, it was significantly higher in the rural zone than in the urban zone (mean \pm SD 41.02 ± 31.71 versus 17.73 ± 13.43 ; $P < 0.05$), and showed a correlation with a higher infection rate (73.9% versus 41.3%). 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 (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.

Hepatitis B virus (HBV) infection represents a major worldwide public health problem because of the ability of this virus to cause a chronic infection in infected individuals, of whom a significant number develop liver disease. Several epidemiologic surveys throughout the Pacific have demonstrated high overall prevalences of HBV infection.¹⁻⁴ 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.^{5,6} In the latter group of islands, the level and tendency for acquisition of infection were different, depending on the island considered; the infection rate among children was dramatically high on Nuku-Hiva Island (74% in the rural area) while it was moderate on Hiva-Oa Island (19% in rural area). These two islands are very similar in size (330 and 350 km², respectively), habitation type (one main population cluster and three or four small villages around the island), total population (2,099 inhabitants on Nuku-

approximately 1:2 on both islands. Although the socioeconomic and demographic factors that usually influence the incidence of HBV infection appear identical on the two islands, they cannot explain the differences observed between these two populations.

On Nuku-Hiva Island, the hematophagous blackfly *Simulium buissoni* (discovered by Roubaud in 1906) is a notorious pest of humans. The adult females feed on blood, resulting in painful and allergic reactions in the host. Although this species is not known to transmit disease in humans, its abundance causes considerable skin lesions among children. The swarming of this species, especially in the small, remote villages, poses the question of the association between the presence of this insect and the high seroprevalence of HBV on this island. The hypothesis of the possible role of *S. buissoni* in the transmission of HBV, either directly as a conveyor of the virus or indirectly through the numerous itching lesions produced on the skin, has been proposed because this fly is not present

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 Hatihieu) 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 overall

TABLE 1
Nuku Hiva Island, according to age and habitation zone*

Age	Children			Total
	7-23 months	2-4 years	5-10 years	
0-1)	0/22 (0)	2/14 (14.2)	5/19 (26.3)	7/63 (11.1)
1-2)	6/23 (26)	5/20 (25)	4/18 (22.2)	15/69 (21.7)
3-4)	6/23 (26)	2/13 (15.4)	12/19 (63.2)	26/63 (41.3)
5-6)	13/25 (52)	15/18 (83.3)	19/19 (100)	51/69 (73.9)
7-17)				

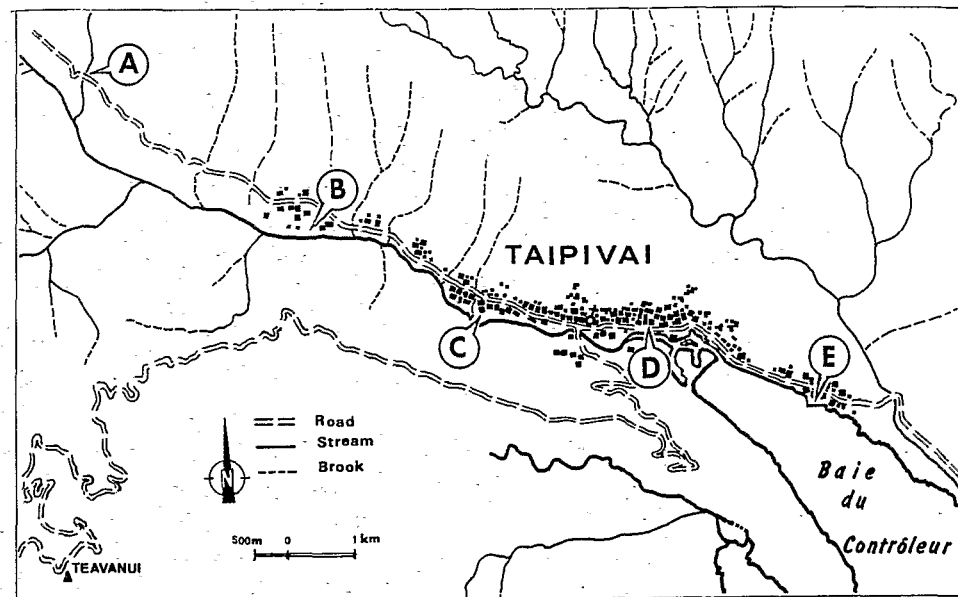


FIGURE 1. Map of Taipivai village on Nuku-Hiva Island in the Marquesas archipelago. The locations of *Simulium buissoni* sampling sites (A, B, C, D, and E) are shown.

ers) $\times 0.90$. This coefficient of 0.90 is the generally admitted risk for a one-year-old child of pour les Sciences et le Marketing, Delta Soft, Mévlan, France).

pH 8) plus 1% NP40. This supernatant should contain the HBV particles washed from the external surface of the blackflies (legs, wings, and mandibles). Subsequently, the insects were removed, blotted on filter paper, and transferred to a new microtube where they were homogenized in 200 μ l of TE buffer plus 1% NP40 with a glass rod. This homogenate should contain the HBV particles extracted from internal regions of the flies (intestinal contents, ovaries, and salivary glands). The DNA in the supernatants and homogenates was purified by incubating them for 1 hr at 70°C in 200 μ l of lysing solution (10 mM Tris HCl, 10 mM EDTA, 10 mM NaCl, pH 8, 5% sodium dodecyl sulfate, 50 μ g/ml of protein-

abo, Auber Villiers, France). After amplification, 10 μ l of the PCR product was subjected to electrophoresis on a horizontal 1.5% agarose gel in 1 \times Tris-borate-EDTA buffer (100 mM Tris-boric acid, 2 mM EDTA). The gel was stained with ethidium bromide and DNA bands were visualized with an ultraviolet transilluminator.

Southern blot analysis with digoxigenin-labeled DNA probes

For the Southern blots, the DNAs were transferred to nylon membranes (Hybond N; Amersham, Arlington Heights, IL) using 20 \times SSC (0.3 M sodium citrate, 3 M NaCl) and irradiated with

TABLE 2
Number of scars and lesions in 2-11-year-old children on Nuku Hiva Island

Zone	No. of children	Mean \pm SD no. of lesions (range)
Urban		
Taiohae	348	17.73 \pm 13.83* (1-91)
Rural		
Taipivai	76	46.12 \pm 38.53 (2-200)
Hakapa	36	41.31 \pm 22.72 (8-101)
Hatiheu	46	32.37 \pm 22.62 (8-94)
Total	156	41.02 \pm 31.71* (2-200)
Total	506	25.00 \pm 23.68 (1-200)

* $P < 0.05$ versus total urban and total rural children.

TABLE 3
*Results of the two polymerase chain reactions (PCR) for hepatitis B virus on supernatants of Simulium buissoni blackflies**

	Sampling sites					Negative control†
	A	B	C	D	E	
X gene PCR	0/9	1‡/9	1/9	1/9	0/9	0/5
S gene PCR	0/9	1‡/9	0/9	0/9	0/9	0/5

* Values are the no. of positive pools/total no. tested.

† Blackflies from an uninhabited area.

‡ Same pool positive using both PCR assays.

fold more sensitive than the S gene PCR, and both PCR systems were 10-fold more sensitive

1 2 3 4 5 6 7 8 M

detectable in the supernatants of one of nine pools of insects caught at each of three sites (B, C, and

cause 1) only three of 45 pools of insects tested were HBV positive, 2) the probability that a new infection rather than an intact skin

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