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Epidemiology of Hepatitis B Infection in Liberian Infants

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To provide background for a hepatitis B vaccine efficacy trial, sera were collected from 0- to 4-year-old Liberian infants and their mothers, on two occasions an average of 14.75 months apart, and tested for serological markers of hepatitis B virus infection. The prevalence of the hepatitis B surface antigen (HBsAg) was 2.9% in the 0- to 6-month age group and 23% in infants 3 to 4 years of age. HBsAg persisted for the 14.75-month average follow-up period in 80.8% of the infants tested. The annual incidence of development of HBsAg was 18.9% for infants less than 1 year of age and 13.6% in infants 3 to 4 years of age. Infants born to HBsAg carrier mothers had significantly higher age-specific prevalence and incidence of hepatitis B virus infection. However, it was estimated that only a minor proportion of hepatitis B infections in Liberia are derived by vertical transmission from carrier mothers.

Numerous studies have documented a high prevalence of hepatitis B virus (HBV) infections in many parts of the world, particularly subsaharan Africa, Southeast Asia, Taiwan, and the Western Pacific islands (1, 8). These areas are known also to have a high prevalence of chronic liver disease, including hepatocellular carcinoma (12). Many lines of evidence have suggested that the HBV chronic carrier state may play an important role in the etiology of chronic liver disease and hepatocellular carcinoma. Prevention of the HBV carrier state by active immunization with an HBV vaccine in high-prevalence regions of the world may thus result in prevention of a high proportion of cases of chronic liver disease and hepatocellular cancer.

Previous studies have shown that the majority of HBV infections in high-prevalence regions occur in childhood (14, 15). There is, however, little information available on the relative rates of infection in the first few years of life. Vertical transmission by HBV carrier mothers has been shown to be important in some parts of the world (6, 11) but not in others (7, 9, 10).

This study was designed to provide background for an HBV vaccine efficacy trial in West Africa. In particular, we wished to determine age-specific prevalence and incidence of HBV infection during the first 4 years of life and to assess the role of HBV carrier mothers in transmission of HBV infections.

MATERIALS AND METHODS

Populations sampled. The survey was carried out in 22 villages in Grand Cape Mount County, Liberia. In Garwula district, these included: Jondu, Tienimani, Vonzuahn, Sinje, Joni, Fandoh, Manivalo, Sanjanamalo, Madina, Bendu, Kongja, Mani, Bamboja, and Gohn; in Tewor district, the villages studied were Jenewonde, Mambo, Maiyehn, Wuilo, Jenne-Liberia, Fahnjo, Gbesse, and Bouma. These villages contained between 7 and 78 sleeping houses. The population of these villages was predominantly from the Vai tribe. The study region is coastal secondary rain forest devoted largely to shifting subsistence agriculture.

During the first survey (April through August 1978), blood specimens were obtained from infants up to the age of 4 and their mothers. In the second survey (June through November 1979), we attempted to obtain follow-up samples from all of the initially tested infants as well as from some additional infants not tested in the first survey. Only mothers who requested that a second sample be tested were rebled during the second survey. The average interval between paired samples was 14.75 months. We estimate that about 40% of the eligible infants in the villages studied were included in the first survey.

Procedure for obtaining informed consent. In each village, a town meeting was held during the evening with town and clan chiefs, village elders, and the majority of inhabitants present. At this time the purpose of the study, its procedures, and its risks were carefully explained through a Vai interpreter. Questions concerning the study were elicited and carefully answered. It was emphasized that the study would help determine the age at which infants should be immunized when the new vaccine for hepatitis becomes available, that it would not provide any immediate benefit for the participants, and that participation was entirely voluntary.

Procedure for identification of subjects. Subjects were identified by name, age, sex, tribal origin, birthplace, name of mother, head of household, location of household on a map prepared by the survey team, and the number of the infant's clinic card. In addition, a handprint of each infant was placed on the

back of the data form.

Blood specimens. Finger or heel prick blood specimens were obtained with disposable lancets. Approximately 100 μl of blood was collected on each of one to four filter papers (Schleicher & Schuell no. 903 specimen collection paper, 1-inch circles). These were air dried on pins before being placed in glassine envelopes for storage in a desiccator over $CaCl_2$ at 4°C while in the field and subsequently at −70°C. Specimens were identified by serial accession numbers. Each filter paper was rehydrated for testing by holding overnight at 4°C in 1.0 ml of 0.15 M NaCl–0.01 M phosphate buffer, pH 7.4, containing 0.1% sodium azide and 50% fetal or newborn calf serum. This resulted in a 1:20 dilution, i.e., 50 μl of serum in 1 ml of eluent.

Serological methods. Specimens were first tested for hepatitis B surface antigen (HBsAg) by Ausria II (Abbott Laboratories). Specimens negative for HBsAg were then tested for anti-HBs by the Ausab test (Abbott Laboratories). Those negative by both assays were tested for anti-HBc by the Corab assay (Abbott Laboratories). All positive assay results were repeated. Only repeatable positives were accepted.

RESULTS

Between 18 April and 24 August 1978, 544 samples from infants and 407 maternal blood samples were collected. During the second survey (22 June to 8 November, 1979), 513 samples were collected from infants; 395 of these were from infants bled in the first survey. Sixty-three samples were obtained during the second survey from mothers. Twenty-nine of these were from mothers not previously bled, and 34 were from mothers who had been tested during the previous year. The average interval between successive bleedings was 14.75 months.

To obtain follow-up samples from 72% of infants bled in the first survey, it was necessary to visit each village three times. Of the follow-up specimens, 244 were obtained on the first of these visits, 105 on the second, and 46 on the third. Of the 157 infants lost to follow-up, 80 had moved out of the study region, 33 had died, and 44 were still living in the study region but could not be located.

Age-specific prevalence of HBV markers. Table 1 and Fig. 1 summarize the age-specific prevalence of serological markers of hepatitis infection. These data include all infants and mothers bled for the first time in 1978 or 1979. The prevalence of HBsAg was 2.9% in the 0- to 6-month age group; higher prevalences were found in the older age groups. Of the 3- to 4-year-old children, 23% had HBsAg. The prevalence of HBsAg in all infants born to HBsAg carrier mothers (25%) was significantly higher ($\chi^2 = 5.7$; $P < 0.025$) than that seen in infants born to noncarrier mothers (11.9%).

TABLE 1. *Age-specific prevalence of hepatitis B markers*[a]

Age (mo)	No. tested	HBsAg		Anti-HBs[b]		Anti-HBc only[c]		Any marker	
		No. positive	%	No. positive	%	No. positive	%	No. positive	%
Infants born to HBsAg-negative mothers									
0–6	128	4	3.1	2	1.6	12	9.4	18	14.0
7–12	127	10	7.9	1	0.8	5	3.9	16	12.6
13–24	118	17	14.4	2	1.7	3	2.5	22	18.6
25–36	110	18	16.4	5	4.5	13	11.8	36	32.7
37–48	94	20	21.3	5	5.3	10	10.6	35	37.2
Total	577	69	11.9[d]	15	2.6	43	7.4	127	22.0[e]
Infants born to HBsAg-positive mothers									
0–6	7	0	0	0	(0)	5	71.4	5	71.4
7–12	9	4	44.4	0	(0)	0	(0)	4	44.4
13–24	11	1	9.0	0	(0)	0	(0)	1	9.0
25–36	7	2	28.6	0	(0)	1	14.3	3	42.8
37–48	6	3	50.0	0	(0)	1	16.6	4	66.6
Total	40	10	25.0[d]	0	(0)	6	15.0	16	40.0[e]
Mothers									
15–45 yr	436	33	7.6	75	17.2	159	36.5	267	61.2

[a] Includes infants tested in 1978 and those tested for the first time in 1979, except for 45 infants whose mothers were not tested.

[b] Tested for in all HBsAg-negative sera.

[c] Tested for only in HBsAg-negative and anti-HBs-negative sera.

[d] $\chi^2 = 5.7$; $P < 0.025$.

[e] $\chi^2 = 6.8$; $P < 0.01$.

The prevalence of anti-HBs and anti-HBc was higher in the 0- to 6-month age group than in the 7- to 12-month group in infants born to both HBsAg-positive and -negative mothers. This probably reflects detection of maternal antibody in the 0- to 6-month age group. There was no significant difference in the prevalence of HBV serological markers between male and female infants. Among the mothers, 7.6% were found to have detectable HBsAg, 17.2% had anti-HBs, and 36.5% had anti-HBc only.

Persistence of HBsAg in infants. Forty-seven of the 99 initially HBsAg-positive infants were retested in 1979. HBsAg was again detected in 38 (80.8%) (Table 2). Eight of the nine infants who were found to have terminated the infection were females.

Age-specific incidence of hepatitis B markers. Table 3 and Fig. 2 summarize the age-specific incidence of HBV serological markers over the 14.75-month interval between bleedings in those infants who were seronegative when first tested. The incidence of development of HBsAg, anti-HBs, or anti-HBc remained relatively stable during the first 3 years of life and then fell slightly during the 4th year. The incidence of development of HBsAg in offspring of HBsAg-positive mothers (40%) was significantly higher than for infants born to HBsAg-negative mothers (17.7%) ($\chi^2 = 4.59$; $P < 0.05$). The average incidence of development of HBsAg of 19.4% for all infants for the 14.75-month period between bleedings corresponds to an annual incidence of 15.8%.

DISCUSSION

The high prevalence and incidence of markers of HBV infection found among infants in this study are in accord with previous reports indicating that in high-prevalence regions infection with this virus occurs predominantly in the early years of life (14, 15).

Analysis for age-specific prevalence and incidence revealed both to be higher in offspring of chronic carrier mothers, indicating that vertical transmission may play a role in the spread of this infection in Liberia. The possible significance of vertical transmission in infants is illus-

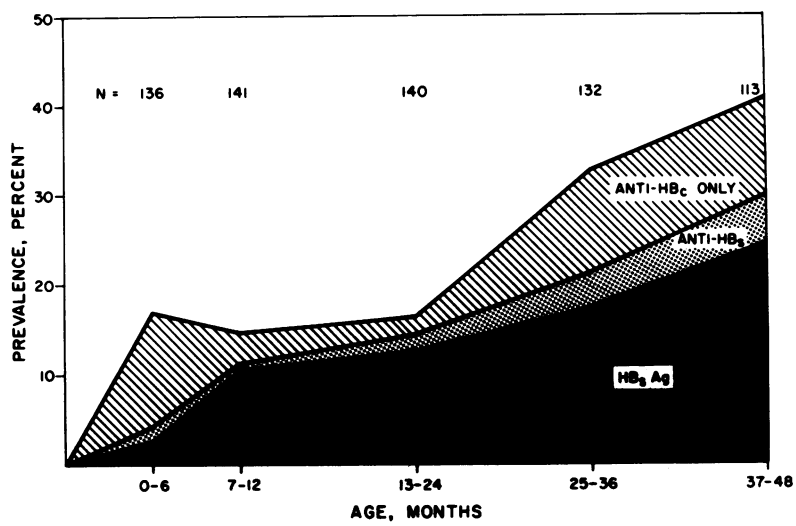

FIG. 1. *Age-specific prevalence of markers of HBV infection in infants in Liberia. In addition to the infants described in Table 1, this analysis includes results on 45 additional infants whose mothers were not tested.*

TABLE 2. *Results of retesting HBsAg-positive infants after an average of 14.75 months*[a]

Age when first tested (mo)	No. tested	HBsAg		Anti-HBc only		Seronegative	
		No. positive	%	No. positive	%	No. positive	%
0–6	1	1	100.0	0	0	0	0
7–12	7	6	85.7	0	0	1	14.3
13–24	12	11	91.7	1	8.3	0	0
25–36	12	9	75.0	3	25.0	0	0
37–48	15	11	73.3	1	6.7	3	20.0
Total	47	38	80.8	5	10.6	4	8.5

[a] No infants were anti-HBs positive.

TABLE 3. *Age-specific incidence of hepatitis B markers*[a]

Age when first tested (mo)	No. tested	HBsAg		Anti-HBs[b]		Anti-HBc[c]		Any marker	
		No. positive	%	No. positive	%	No. positive	%	No. positive	%
Infants born to HBsAg-negative mothers									
0–6	49	10	20.4	0	0	3	6.1	13	26.5
7–12	62	13	21.0	2	3.2	3	4.8	18	29.0
13–24	65	11	16.9	4	6.1	3	4.6	18	27.7
25–36	49	8	16.3	2	4.1	4	8.2	14	28.6
37–48	24	2	8.3	0	0	1	4.2	3	12.5
Total	249	44	17.7[d]	8	3.2	14	5.6	66	26.5
Infants born to HBsAg-positive mothers									
0–6	1	1	100.0	0	0	0	0	1	100.0
7–12	2	2	100.0	0	0	0	0	2	100.0
13–24	9	2	22.2	0	0	0	0	2	22.2
25–36	1	0	0	0	0	1	100.0	1	100.0
37–48	2	1	50.0	0	0	0	0	1	50.0
Total	15	6	40.0[d]	0	0	1	6.7	7	46.6

[a] For 14.75-month interval between bleedings; does not include data on 10 infants whose mothers were not tested.

[b] Tested for in all HBsAg-negative sera.

[c] Tested for only in HBsAg-negative and anti-HBs-negative sera.

[d] $\chi^2 = 4.59$; $P < 0.05$.

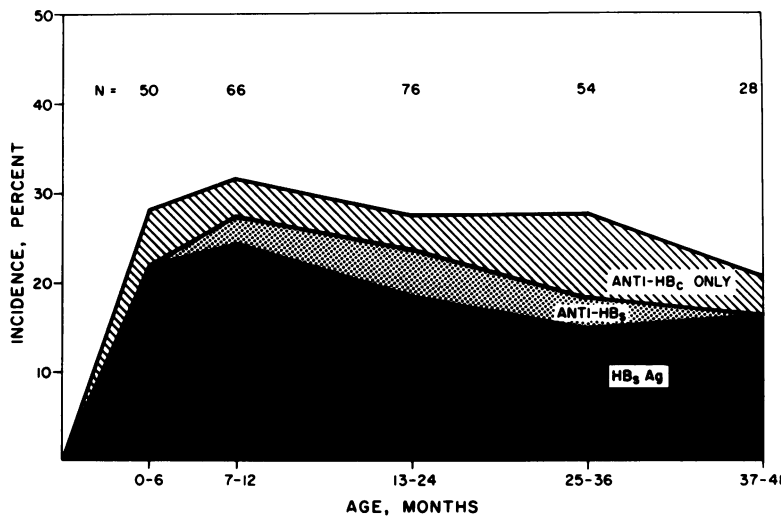

FIG. 2. *Age-specific incidence of markers of HBV infection in infants in Liberia. In addition to the infants described in Table 1, this analysis includes data from 10 infants whose mothers were not tested.*

trated by the higher proportion of antigenemic infants born to HBsAg-positive mothers (10 of 40 versus 69 of 577).

However, 12% of infants born to antigen-negative mothers also developed antigenemia; thus, a similar proportion of the infants born to HBV carrier mothers could have been infected by nonvertical routes. If 12% of the infants born to carrier mothers were infected via nonvertical means, then only 13% of antigenemic infants of carrier mothers and 6.6% [10 − (0.119 × 40) ÷ 79; see Table 1] of all antigenemic infants could attribute their infection directly to their mother. Maternal transmission thus appears to be a minor factor in the spread of hepatitis B infection in Liberia. It would appear that the infectivity of mothers in this region resembles more that of carrier mothers in Europe, the United States,

and Greece (7, 9, 10) than those in Taiwan and Japan (6, 11). It should be noted that interpretation of the above data is limited by the small number of antigenemic mothers and children of these mothers tested.

The HBV carrier rate of 7.6% of mothers in this study is less than that which we observed in another study of Liberian adult males, in which 14.4% were found to be carriers (A. M. Prince, T. White, N. Pollock, J. Riddle, B. Brotman, and L. Richardson, unpublished data). This is in accord with the generally lower carrier rate observed in females throughout the world. The difference between the HBsAg prevalence in adult males and females and the lack of such a difference among infants is in agreement with the fact that although both sexes are equally susceptible to infection with HBV, males more frequently become chronic carriers (13). Indeed, in the present study eight of the nine infants who terminated the infection were female. In a previous study from Liberia, Frentzel-Beyme et al. reported 11.7% of adult males and 11.1% of adult females to have HBsAg, using the less sensitive gel diffusion assay (4).

Our study showed 2.9% of children in the 0- to 6-month age group to have HBsAg. Infections at this age usually lead to development of the chronic carrier state (5). It will therefore be important to investigate the possibility of immunization with hepatitis B vaccines at birth. Only at this time is there potential for complete eradication of the chronic carrier state by active immunization. The higher prevalence of anti-HBs and anti-HBc in the 0- to 6-month versus 7- to 12-month age group probably reflects detection of maternal antibody in the younger age group. Although the average antibody titer appears to be quite low, the effect of maternal antibody on the immune response to HBV vaccine will need to be evaluated.

Twenty-three percent of the 3- to 4-year-old children had HBsAg, and this remained detectable in 73% of these children when they were retested. As the carrier rate in adults is about 14% in males and 8% in females, it is clear that not all of the children who had HBsAg in both samples will be lifelong carriers. Thus, transient infections in infancy and early childhood may last longer than the 6-month period traditionally used to separate self-limited from persisting HBV infections in adults.

The prevalence of anti-HBs was unexpectedly low in all groups. This resulted in a larger than expected percentage of samples which were anti-HBc positive but anti-HBs negative. This may be due to the 1:20 dilution necessitated by the elution of the filter paper samples. In another survey carried out by this laboratory, anti-HBs was detected in 77% of 111 venous blood samples from male employees and job applicants at The Liberian Institute for Biomedical Research. Additionally, we tested 79 serum specimens from venous blood obtained for routine diagnostic purposes from infants (admitted with diagnoses other than hepatitis) at the Elwa Hospital in Monrovia, Liberia. Sixty-two percent of the specimens were from infants less than 12 months of age, 30% were from infants aged 1 to 2 years, and the remaining 8% were from children 2 to 4 years of age. Six of the 79 sera (8%) contained HBsAg, 24 (30%) contained anti-HBs, and 9 (11%) contained anti-HBc alone. Eighteen of the 24 (75%) sera containing anti-HBs showed P/N ratio units in the Ausab assay of <10. Such low-titer sera would be negative in this test if diluted 1:20. These findings suggest that only 25% of anti-HBs-positive sera would be detected by the filter paper technique; thus, the true anti-HBs prevalence in 3- to 4-year-old children may be about 20% and not 5.3% as determined in this study, which would raise the estimate of the total infection rate in this age group to about 5%. This is compatible with previous findings in Senegal in which the peak prevalence of HBsAg was in the 7- to 8-year age group.

It is noteworthy that the observed prevalence for HBsAg in the 1- to 2-year age group was lower than that expected on the basis of the observed incidence. This difference could, however, be explained by an excess mortality in infants developing HBsAg during the 1st year of life. We analyzed case retrieval rates as a function of age and serological status, and the results, though they do not reach statistical significance, are of interest: 42% of HBsAg-positive infants 1 year and under in 1978 were found for retesting in 1979; however, 66% of the seronegative infants in this age group were available for retesting. An unusual frequency of fulminant hepatitis in HBV infections during the 1st year of life has been reported (3) but has not been observed in other studies (5, 6, 9, 11). Autopsy studies on infants who die during the 1st year of life in West Africa could determine whether fulminant HBV infections constitute a significant cause of death in this age group.

The data obtained in this study provide a quantitative basis for calculation of sample sizes required for hepatitis B vaccine efficacy trials in the perinatal period in Liberia. The extraordinarily high incidence of HBV infection in infants documented in this study underscores the need for such trials.

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LITERATURE CITED

1. **Blumberg, B. S., B. J. S. Gerstley, D. A. Hungerford, W. T. London, and A. I. Sutnick.** 1967. A serum antigen (Australia antigen) in Down's syndrome, leukemia, and hepatitis. Ann. Intern. Med. **66**:924-931.
2. **Blumberg, B. S., A. I. Sutnick, W. T. London, and L. Melartin.** 1972. Sex distribution of Australia antigen. Arch. Intern.Med. **130**:227-231.
3. **Dupuy, J. M., D. Fromel, and D. Alagille.** 1975. Severe viral hepatitis type B in infancy. Lancet **i**:191-194.
4. **Frentzel-Beyme, R. R., T. Traavik, J. Ulstrup, and A. M. Tønjum.** 1977. The prevalence of HB-Ag (Australia antigen) in the population of Liberia. Soc. Sci. Med. **11**:749-756.
5. **Gerety, R. J., J. H. Hoofnagle, J. A. Markenson, and L. F. Barker.** 1974. Exposure to hepatitis B virus and development of the chronic HBAg carrier state in children. J. Pediatr. **84**:661-665.
6. **Okada, K., T. Yamada, Y. Miyakawa, and M. Mayumi.** 1975. Hepatitis B surface antigen in the serum of infants after delivery from asymptomatic carrier mothers. J. Pediatr. **87**:360-363.
7. **Papaevangelou, G., and J. H. Hoofnagle.** 1979. Transmission of hepatitis B infection by asymptomatic chronic HBsAg carrier mothers. Pediatrics **63**:602-605.
8. **Prince, A. M.** 1970. Prevalence of serum hepatitis related antigen (SH) in different geographic regions. Am. J. Trop. Med. Hyg. **19**:872-879.
9. **Schweitzer, I. L.** 1975. Infection of neonates and infants with the hepatitis B virus. Prog. Med. Virol. **20**:27-48.
10. **Skinhoj, P., J. Cohn, and A. F. Bradburne.** 1976. Transmission of hepatitis type B from healthy HBsAg positive mothers. Br. Med. J. **1**:10-11.
11. **Stevens, C. E., R. P. Beasley, J. Tsui, and W. C. Lee.** 1975. Vertical transmission of hepatitis B antigen in Taiwan. N. Engl. J. Med. **292**:771-774.
12. **Szmuness, W.** 1978. Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. Prog. Med. Virol. **24**:40-69.
13. **Szmuness, W., E. J. Harley, H. Ikram, and C. E. Stevens.** 1978. Sociodemographic aspects of the epidemiology of hepatitis B, p. 297-320. *In* G. N. Vyas, S. N. Cohen, and R. Schmid (ed.), Viral hepatitis. Franklin Institute Press, Philadelphia.
14. **Szmuness, W., A. M. Prince, G. Diebolt, L. Leblanc, R. Baylet, R. Masseyeff, and J. Linhard.** 1973. The epidemiology of hepatitis B infections in Africa: results of a pilot survey in the republic of Senegal. Am. J. Epidemiol. **98**:104-110.
15. **Tabor, E., and R. J. Gerety.** 1979. Hepatitis B virus infection in infants and toddlers in Nigeria: the need for early intervention. J. Pediatr. **95**:647-650.