

## Long-term effectiveness of plasma-derived hepatitis B vaccine 22–28 years after immunization in a hepatitis B virus endemic rural area: is an adult booster dose needed?

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### SUMMARY

Longan County is considered a highly endemic area for hepatitis B virus (HBV). The plasma-derived vaccine has been used in newborns in this area since 1987. A cross-sectional survey was conducted to evaluate the long-term effectiveness of this vaccine. In total, 1634 participants born during 1987–1993 and who had received a series of plasma-derived HB vaccinations at ages 0, 1, and 6 months were enrolled. Serological HBV markers were detected and compared with previous survey data. Overall the prevalence of hepatitis B surface antigen (HBsAg) in all participants was 3·79%; 3·47% of subjects who had received the first dose within 24 h were HBsAg positive, and 8·41% of subjects who had received a delayed first dose were also HBsAg positive. There were 1527 subjects identified who had received the first dose within 24 h and whose HBsAg and anti-HBc prevalence increased yearly after immunization, while the anti-HBs-positive rate and vaccine effectiveness declined. The geometric mean concentration of antibody in the anti-HB-positive participants was 55·13 mIU/ml and this declined after immunization. Fewer than 2·0% of participants had anti-HB levels  $\geq 1000$  mIU/ml. The data show that the protective efficacy of the plasma-derived vaccinations declined and administration of HB vaccine within 24 h of birth was very important. To reduce the risk of HBV infection in this highly endemic area, a booster dose might be necessary if anti-HBs levels fall below 10 mIU/ml after age 18 years. Furthermore, studies on the immune memory induced by plasma-derived HB vaccine are needed.

**Key words:** Hepatitis B virus, long-term effectiveness, plasma-derived hepatitis B vaccine, primary immunization.

### INTRODUCTION

Hepatitis B virus (HBV) infection is a public health problem of major concern worldwide. It is estimated

that 240 million people are chronically infected with HBV, and about 780 000 people die of hepatitis B infection annually – 650 000 from cirrhosis and liver cancer and 130 000 from acute HB [1]. Immunization is the most effective method to control and prevent HBV infection and its serious long-term consequences. According to the World Health Organization, universal immunization has been

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implemented in 168 countries and has an outstanding record of safety and efficacy [2]. The effective implementation of HBV immunization has resulted in substantial decreases in disease burden, carriage rates, and HB-related morbidity and mortality [2–6]. After implementation of HBV immunization in China, the prevalence of hepatitis B surface antigen (HBsAg) fell from 10.3% in 1980 to 9.8% in 1992 and 7.2% in 2006 [7]. A recent study showed that the decline in HBsAg prevalence in all age groups in China was strongly associated with increased vaccine coverage [8]. Guangxi was one of the provinces that had a high HBV prevalence estimated in 1980 [9], but by 2006 the prevalence had decreased to 8.3% [10].

Longan County is a remote, undeveloped district in rural Guangxi province. In 1985, it was an HBV-endemic area with an HBsAg prevalence of 16.9% in those aged between 1 and 60 years [11]. After the completion of baseline surveys on the prevalence of HBV infection in 1985, large-scale HBV immunization was undertaken in five areas of China: Longan in Guangxi province, Zhengding in Hebei province, Xiangtan in Hunan province, Tongde in Qinhai province, and Shanghai city [12]. From 1987 to 1996, newborns were immunized with a primary series of three 10- $\mu$ g doses of plasma-derived HB vaccine regardless of the mother's HBV infection status. The long-term effectiveness of HB vaccine was evaluated in 2009 in Longan County. The efficacy of a plasma-derived HB vaccine use for 13–23 years and a yeast-derived recombinant HB vaccine for 12 years was analysed. The data showed that the efficacy of the vaccines was stable [12, 13]. As a follow-up, in order to analyse antibody persistence and protective effectiveness, we evaluated the plasma-derived HB vaccine after 22–28 years of use in this HBV-endemic rural area in 2015.

## MATERIALS AND METHODS

### Participants

This cross-sectional study was carried out in 10 towns in Longan County in February 2015. Eligible participants were born between 1987 and 1993 and had received three 10- $\mu$ g doses of plasma-derived HB vaccine at ages 0, 1, and 6 months. Those who had received the first dose of vaccine more than 72 h after birth were excluded. The vaccines were manufactured by the Beijing Institute of Biological Products (Beijing, China). Eligible participants were identified

from a database containing the records of all participants from previous cross-sectional surveys. The database included the participant's ID number, name, date of birth, dates of HBV immunization, and information identifying their parents. Individuals who had received a HB booster vaccination were excluded from the evaluation.

### Interviews and blood sample collection

Multi-stage random sampling was used to select participants. The primary sampling unit was the village, the secondary unit was the town, and the sample size calculation was based on the HBV infection rate in Longan County [11]. About 30% of the population was sampled; the non-response rate was less than 10%. Following rural community approval, trained field staff identified eligible subjects by verifying their name, date of birth, and village of residence as well as the name of the spouse, mother, or father from the study participant lists. Field workers checked the participants' immunization record booklets and recorded their demographic information, including sex, birth date, ethnicity, place of birth, and immunization history. Blood specimens (3.0–5.0 ml) were collected by venepuncture, transported in a cool box during field work, and transferred to a laboratory freezer within 5 h of collection for storage at  $-20^{\circ}\text{C}$ .

### HBV serology

Serological testing was carried out at the Guangxi Centers for Disease Control and Prevention. Serum samples were tested for three HBV markers: HBsAg, anti-HBsAg (anti-HBs), and anti-HB core antigen (anti-HBc) using micro-particle enzyme immunoassay (MEIA) kits (AXSYM, Abbott, USA). Positivity was defined as an HBsAg S/N  $\geq 2.0$ , an anti-HBc CO/S  $> 1.0$ , and an anti-HBs concentration of  $\geq 10$  mIU/ml.

### Statistical analysis

All participant information and laboratory data were entered into an SPSS v. 14.0 (SPSS Inc., USA) database and statistical analysis was carried out with SAS v. 8.0 software (SAS Institute Inc., USA). Statistical comparisons of the prevalence of HBV serological markers in men and women and across the various birth-year cohorts were performed using  $\chi^2$  tests. The trend of change in the prevalence of HBV markers with birth year was evaluated using the  $\chi^2$  trend test.

Table 1. HBsAg, anti-HBc, anti-HBs positive rates and GMCs of on-time birth-dose participants for birth years 1987–1993

Birth year	Year after vaccination	Sample size	HBsAg				Anti-HBc				Anti-HBs			
			No. of positive	Positive rate*	95% CI	No. of positive	Positive rate†	95% CI	No. of positive	Positive rate‡	95% CI	GMCs§	95% CI	
1987	28	235	11	4.68	2.63–8.19	54	22.98	18.06–28.77	45	19.15	14.63–24.66	43.7	31.42–55.97	
1988	27	243	12	4.94	2.71–8.04	56	23.05	18.19–28.73	51	20.99	16.34–26.54	47.7	36.05–59.35	
1989	26	240	10	4.17	2.28–7.50	51	21.25	16.55–26.86	50	20.83	16.17–26.41	52.59	40.60–64.58	
1990	25	253	7	2.77	1.35–5.60	55	21.74	17.10–27.22	60	23.72	18.89–29.33	58.08	46.60–69.57	
1991	24	242	6	2.48	1.14–5.30	46	19.01	14.56–24.42	70	28.93	23.58–34.93	55.71	45.33–66.09	
1992	23	168	4	2.38	0.93–5.96	25	14.88	10.29–21.04	55	32.74	26.10–40.15	61.57	50.09–70.04	
1993	22	146	3	2.05	0.70–5.87	22	15.07	10.17–21.76	50	34.25	27.04–42.26	64.22	50.41–78.04	
Total	–	1527	53	3.47	2.66–4.51	309	20.24	18.30–22.32	381	24.95	22.85–27.18	55.13	50.74–59.53	

GMC, Geometric mean concentration; CI, confidence interval.

\*  $\chi^2_{\text{trend}} = 4.80, P < 0.05$ , †  $\chi^2_{\text{trend}} = 7.10, P < 0.001$ , ‡  $\chi^2_{\text{trend}} = 19.85, P < 0.001$ , §  $\chi^2_{\text{trend}} = 7.74, P < 0.001$ .

As a measure of vaccine effectiveness, we assessed long-term protection by determining HBsAg prevalence. Vaccine effectiveness was calculated as:

$$1 - \frac{\text{HBsAg prevalence in vaccinated subjects in 2015}}{\text{HBsAg prevalence in unvaccinated subjects in 1985}} \times 100\%.$$

All *P* values were two-tailed and *P* < 0.05 was considered to be significant. The 95% confidence intervals (CIs) for the prevalence of HBV serological markers were estimated.

**Ethical approval**

The study was approved by the Ethics Committee of the Chinese Center for Disease Control and Prevention. The purpose of the study and the use of the information gathered were explained to the participants by research staff. Written informed consent was obtained from each participant before enrolment and blood collection.

**RESULTS**

**Participant demographics**

A total of 1774 subjects who were born between 1987 and 1993 and had been immunized with plasma-derived HB vaccine were included in the study; 140 subjects had received a booster dose and were therefore excluded from the analysis. The remaining 1634 participants were enrolled, of whom 1072 (65.61%) were men and 562 (34.39%) were women. Most of the participants (1623, 99.33%) were of Zhuang ethnicity. The first dose of HB vaccine had been given to 1527 of the participants (93.45%) within 24 h of birth and to 107 participants (6.55%) more than 24 h after birth but within 72 h.

**Anti-HBs positivity and geometric mean concentration (GMC)**

The overall anti-HBs positivity rate of the 1527 participants who received the first dose within 24 h of birth was 24.95% (95% CI 22.85–27.18). The positivity rate decreased significantly annually following immunization ( $\chi^2_{\text{trend}} = 19.85, P < 0.001$ ). Of the 1987 birth cohort, only 19.15% of participants were anti-HBs

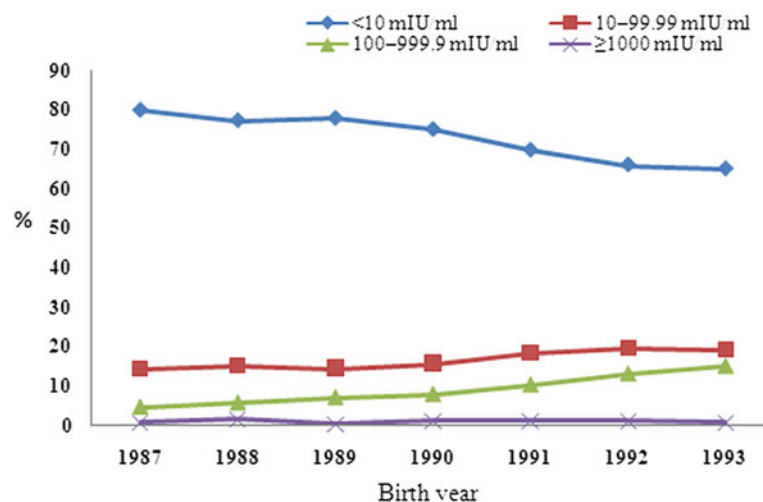


Fig. 1. Geometric mean concentration proportion of subjects for birth years 1987–1993 who received routine plasma-derived hepatitis B vaccine immunization with at ages 0, 1, and 6 months.

positive (Table 1). The GMC of all anti-HBs-positive participants was 55.13 mIU/ml, with the value highest in the 1993 birth group (64.22 mIU/ml) and the lowest value in the 1987 birth group (43.70 mIU/ml). The anti-HBs GMC decreased significantly after immunization ( $\chi^2_{\text{trend}} = 7.74$ ,  $P < 0.001$ ; Table 1). The anti-HBs-positive participants were stratified by anti-HBs concentration (Fig. 1). Most positive participants (14.47–16.96%) had anti-HBs concentrations of 10–99.99 mIU/ml, with 4.68–15.07% having concentrations between 100 and 999.9 mIU/ml. No more than 2% (0.42–1.65%, 1.05% in total) had concentrations  $\geq 1000$  mIU/ml. The size of each of these groups decreased gradually following immunization from 1987 to 1993. However, the proportion of participants with an anti-HBs concentration  $< 10$  mIU/ml increased over time.

#### HBsAg and anti-HBc positivity

The overall HBsAg prevalence of the participants was 3.79% (62/1634), which for participants who were administered an ‘on-time’ (i.e. within 24 h) birth dose was 3.47% (53/1527), while that of participants who had received a delayed first dose of vaccine was 8.41% (9/107) ( $\chi^2 = 5.40$ ,  $P < 0.05$ ). The HBsAg prevalence in men (3.73%, 40/1072) did not differ significantly from women (3.91%, 22/562,  $\chi^2 = 0.034$ ,  $P > 0.05$ ). The positivity rate of subjects who received the first vaccine dose within 24 h of birth increased by year following immunization ( $\chi^2_{\text{trend}} = 4.80$ ,  $P < 0.05$ , Table 1, Fig. 2). The overall anti-HBc positivity

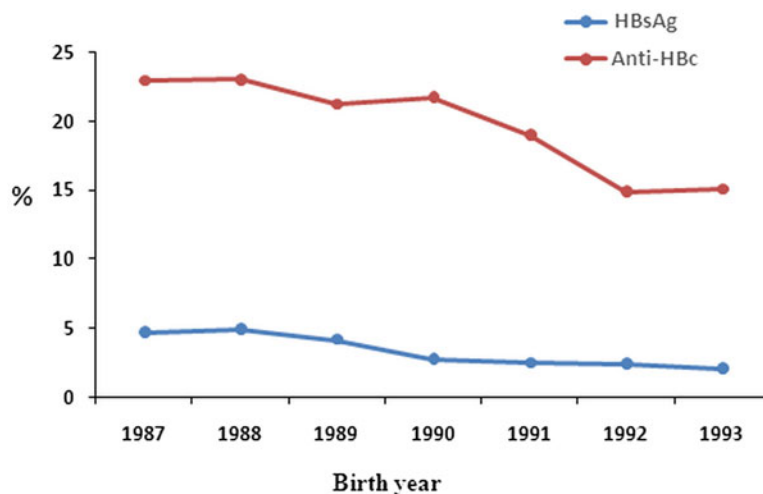
rate of the 1527 subjects was 20.24% (95% CI 18.30–22.32), and the rates increased by year following immunization ( $\chi^2_{\text{trend}} = 7.10$ ,  $P < 0.001$ , Table 1, Fig. 2). Correlation analysis of the HBsAg and Anti-HBs positivity rate was carried out. The data show that the positive value of HBsAg and anti-HBs were correlatively significant (correlation coefficient = 0.899,  $P < 0.05$ , Fig. 3).

#### Vaccine effectiveness of on-time birth-dose participants

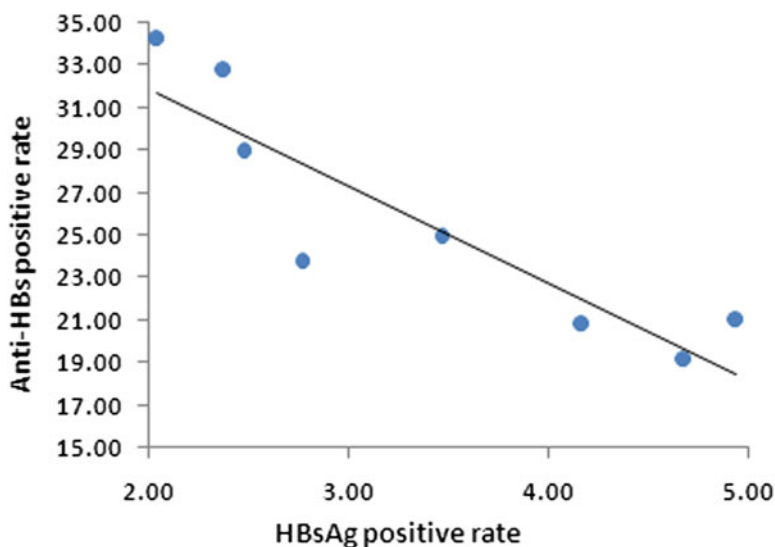
Compared to the 17.9% HBsAg-positive ratio reported in the 1985 survey of individuals aged  $> 20$  years [11], the overall protective effectiveness of the vaccine (i.e. in preventing HBsAg positivity) was found to be 73.91% in this study. Effectiveness declined with the number of years since immunization, being lowest in participants born in 1987 and highest in those born in 1993 (73.86, 72.40, 73.70, 84.52, 86.15, 87.70, and 88.55%, respectively).

#### HBsAg-positive rate in four previous cross-sectional surveys

Four cross-sectional surveys have been performed, based on the population analysed in the current study. The HBsAg-positive rates observed in each survey were significantly lower than the baseline data obtained in 1985 (Fig. 4). However, in the current survey, conducted in 2015, the HBsAg-positive rates for the 1987, 1988, and 1989 birth cohorts were higher than those for 1994 and 2005 [11, 14]. There may



**Fig. 2.** HBsAg and anti-HBc positive rates of subjects for birth years 1987–1993 who received routine plasma-derived hepatitis B vaccine immunization at ages 0, 1, and 6 months.



**Fig. 3.** Correlation analysis result of HBsAg and anti-HBs positive rate, 2015.

have been some bias in the results of the survey carried out in 2009 because of the small sample analysed, but the HBsAg-positive rates in the 1987 and 1989 birth cohorts were more than 6% [12, 15].

**DISCUSSION**

Before evaluating the long-term effectiveness of HB vaccine, confounding factors such as dosage, immunization procedure, type of vaccine, and age at immunization should be taken into consideration. To ensure the current survey results were comparable with historical data, we selected participants who had received three 10- $\mu$ g doses of plasma-derived HB vaccine

during infancy, using the same immunization schedule. The results demonstrate that the average HBsAg prevalence rate in the ‘on-time’ birth-dose group was significantly lower than that in the delayed-dose group. These findings confirm those of our previous study in Longan County [12, 13] and indicate that it is crucial to administer the first dose of HB vaccine within 24 h of birth in order to prevent and control HBV infection.

In this survey it was found that both the overall anti-HBs positivity rate (24.95%) and the GMCs (55.13 mIU/ml) of all participants decreased with time after immunization. The proportion of participants with anti-HBs GMCs from 10 to 99.99 mIU/ml

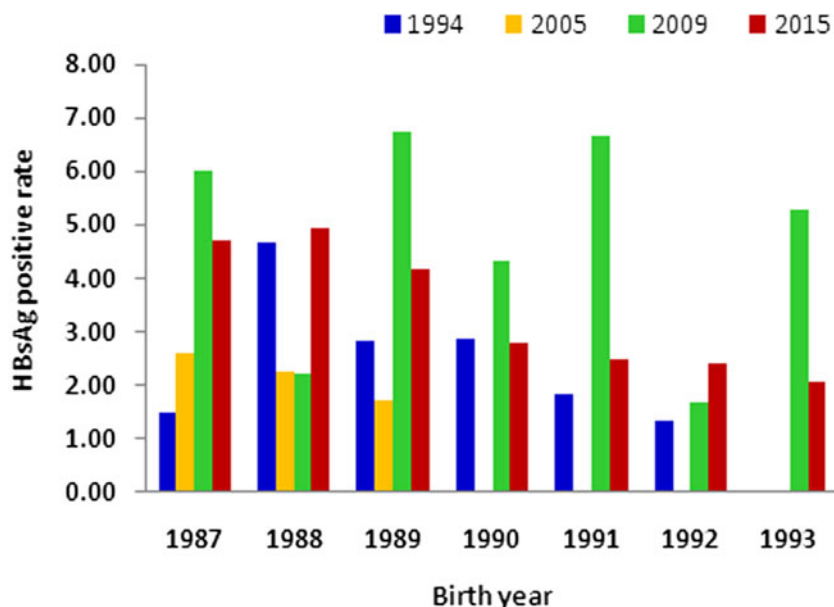


Fig. 4. Survey results of HBsAg-positive rate of 1987–1993 birth cohorts in 1994, 2005, 2009 and 2015, respectively.

and from 100 to 999.99 mIU/ml decreased in each age cohort from 1993 to 1987. These findings are consistent with those of our study carried out in 2009 [12, 13, 15]. Interestingly, 18 participants were found to be both anti-HBs and anti-HBc positive, and 16 of these participants had antibody concentrations  $\geq 1000$  mIU/ml. This may be explained by HBV natural infection (a potential natural booster), as a natural infection would have a similar effect as a vaccine booster. A study from Poland found that despite a low incidence of HB, natural boosters such as those related to dental or surgical treatments, may stimulate post-vaccination immunity. It was also reported that HBV infection and chronic HB may occur in previously vaccinated children [16].

It was found that the HBsAg-positive rates of this survey were decreased for different birth years. The main possible reason for this phenomenon was the protective effectiveness of anti-HBs decreased by age. The HBsAg positivity rates of the 1987–1993 birth cohorts were higher than those reported in previous studies carried out in 1994 and 2005 in the same population. For participants born in 1987, HBsAg positivity was 2.58% (5/194) in the 2005 survey (age 18 years) [14], and 6% (6/100) in 2009 (age 22 years) [13, 15]. In this survey, conducted in 2015 (age 28 years), the HBsAg positivity rate was 4.68% (11/235). It is suspected that the probability of HBV infection increases when the immunized population enters adulthood, i.e. aged  $>18$  years. Moreover, similar to

HBsAg, the anti-HBc positivity rates were also significantly higher than previous studies. There are three possible reasons for the high HBsAg and anti-HBc positivity rates in Longan County. First, the failure to prevent mother-to-child transmission (PMTCT) may be a major concern, especially for newborns whose mothers have a high titre of HBV DNA. The prevalence of HBsAg in women of child-bearing age was over 15% in Longan County before the introduction of HB immunization. Administration of hepatitis B immunoglobulin (HBIG) was a crucial procedure for PMTCT [17]. However, HBIG administration was not used for immunoprophylaxis in 1987–1993 in Longan County. Moreover, it was reported that serum HBV DNA can be detected in children born to HBsAg-positive mothers even after anti-HBs antibodies are induced by HB immunization [18, 19], which suggests that children born to HBsAg-positive mothers were at risk of becoming HBV carriers even when immunoprophylaxis was successfully administered. Second, some participants may have lost immune memory 22–28 years after receiving HB immunization. Many studies have shown that although anti-HBs positivity rates and GMC decline gradually after immunization, immune memory still functions when faced with HBV invasion [20–24]. However, a survey conducted in Longan County in 2006 reported that in a group of participants who had received plasma-derived vaccine 16–18 years previously and were anti-HBs negative, 20.18% remained



anti-HBs negative after a single booster dose [25]. Jan *et al.* reported that 24.4% of 127 college students aged 18–23 years in Taiwan who had received HB immunization were seronegative for HBsAg, anti-HBc and anti-HBs antibodies and remained negative 1 month post-booster vaccination [26]. These studies suggest that some people lose immune memory as well as serum antibody stimulated by the HB vaccine, and the loss of protection places them at risk of HBV infection. Third, the age of the survey participants ranged from 22 to 28 years. As adults (aged >18 years) they may encounter numerous risks of HBV exposure and infection. These include infected household contacts, sexual contact with HBV-infected partners, unsafe injections, haemodialysis, transfusion, organ transplantation, and dental and surgical procedures [27, 28]. When children become adults, the onset of sexual activity may thus challenge their HB immunity. Therefore, it is expected that those study participants without sufficient protection from anti-HBs antibodies would be at increased risk of HBV infection in Longan County, a high HBV prevalence area.

In conclusion, we showed that the HBsAg and anti-HBc positivity rates in Longan County, a high HBV endemic area, increased 22–28 years after immunization. Anti-HBs positivity and GMCs decreased significantly in the years after vaccination, and although sampling bias may have influenced the results, the long-term effectiveness of the plasma-derived HBV vaccine also declined during this period. A booster dose may be necessary for subjects with serum anti-HBs concentrations <10 mIU/ml who are not infected with HBV. In addition, it would be helpful to study T-cell immune memory in response to HB vaccine  $\geq$ 22 years after primary immunization. However, there are some limitations to this survey, such as some baseline information, including smoking, drinking, history of drug abuse being absent, the loss to follow-up rate of each stage and possible sampling bias. Further observations of the long-term protection of this plasma-derived HBV vaccine should be continued in future years.

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#### DECLARATION OF INTEREST

None.

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