Comparison of the immune response of four different dosages of a yeast-recombinant hepatitis B vaccine in Singapore children: a four-year follow-up study

K.T. Goh,[1] K.L. Tan,[2] K.H. Kong,[3] C.J. Oon,[4] & S.H. Chan[5]

The immunogenicity of four different dosages of yeast-derived hepatitis B vaccine (Merck, Sharp & Dohme: 0.6 μg, 1.25 μg, 2.5 μg and 5.0 μg), administered at 0, 1 and 6 months (0–1–6 schedule) intramuscularly, was evaluated in 122 seronegative healthy children 1–12 years of age. Three months after the first dose, 83.9–100% of the vaccinees seroconverted. Peak geometric mean titres (GMT) of between 1088 mIU/ml and 1699 mIU/ml were attained 3 months after completion of the vaccination schedule. After 24 months, anti-HBs (antibody to hepatitis B surface antigen) was detected in 93.1–100% of the vaccinees, but the GMT dropped to between 214.3 mIU/ml and 303.5 mIU/ml. After 48 months, 88.8–100% of the vaccinees continued to possess anti-HBs and 70.3–87% had titres above 10 mIU/ml. As expected, the GMT declined further to between 72.6 mIU/ml and 118.8 mIU/ml. There were no significant differences in seroconversion rates and GMT among the different dosage groups. All the vaccinees remained asymptomatic and free from hepatitis B virus infection.

The study showed that reduced dosages of the vaccine (0.6 μg, 1.25 μg and 2.5 μg) were as immunogenic as the standard dose (5 μg); the 2.5-μg dose was recommended for the national childhood immunization programme in Singapore. No booster is necessary for at least four years after vaccination.

Introduction

The scarcity of suitable plasma for the manufacture of hepatitis B vaccine and the possible transmission of human immunodeficiency virus (*1*) led to the development of genetic-engineered vaccines. One of the recombinant DNA hepatitis B vaccines is produced in yeast (*Saccharomyces cerevisiae*) that has been inactivated by formalin, absorbed in aluminium hydroxide and purified by hydrophobic chromatography (*2*). The safety and immunogenicity of the yeast-derived vaccines in children and adults have been demonstrated (*3–5*). The vaccine is well tolerated, with side-effects similar to those of plasma-based vaccine (*4*). There was no increase in antibody titre to *Candida albicans* (*3*) or elevations of IgE-specific antibodies to *S. cerevisiae* (*6*) among the vaccinees. The vaccine is as efficacious as the plasma-based vaccine in the prevention of perinatal transmission of hepatitis B virus (HBV) infection (*7*).

Owing to the high cost of the DNA-recombinant yeast-derived vaccine (as high as the plasma-based vaccine when it was first marketed), clinical trials were conducted in Singapore to evaluate the immunogenicity and efficacy of reduced dosages of the recombinant vaccine in infants, children, teenagers (*8*) and adults (*9*). If a lower dosage is compatible with both immunogenicity and efficacy, the cost of the vaccination programme could be reduced considerably. This is of particular importance in developing countries where HBV infection is endemic. We report the findings of our study on the immune response of healthy children to four different dosages of the vaccine, with follow-up for 4 years.

Materials and methods

The study population were 1–12-year-olds who were on regular follow-up in a postnatal clinic at Kandang Kerbau Hospital. The purpose of the trial was carefully explained to the parent or guardian and informed consent was obtained before venous blood (5 ml) was collected from each child using disposable needles and syringes. At the time of blood collection,

[1] Head, Quarantine & Epidemiology Department, Ministry of the Environment, 40, Scotts Road, Singapore 0922. Requests for reprints should be sent to this author.

[2] Professor, Department of Paediatrics, National University of Singapore, Singapore.

[3] Registrar, Quarantine & Epidemiology Department, Ministry of the Environment, Singapore.

[4] Principal Investigator, Department of Clinical Research, Singapore General Hospital, Singapore.

[5] Professor, Department of Microbiology, National University of Singapore, Singapore.

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relevant demographic data such as date of birth, weight, height, sex and ethnic group of each child were recorded.

At the end of each collection, the samples were immediately dispatched to the Ransome Research Laboratory, Department of Clinical Research, Singapore General Hospital, where the sera were separated and stored at –70 °C before analysis in batches. Hepatitis B surface antigen (HBsAg) and antibody to hepatitis B surface antigen (anti-HBs) were tested by radio-immunoassay (AUSRIA II and AUSAB, respectively, Abbott Laboratories, Chicago) and antibody to hepatitis B core antigen (anti-HBc) by enzyme immunoassay (CORZYME, Abbott Laboratories, Chicago). Serum alanine aminotransferase (ALT) was determined by the Greiner Analyser G-400 (Greiner Electronics Diagnostica, Switzerland).

Children found to be negative for HBsAg, anti-HBs and anti-HBc, with normal ALT levels (9–36 IU/ml) and with no previous history of hepatitis B vaccination were randomized into 4 groups (about 30 in each group) to receive 3 doses of 0.6 μg, 1.25 μg, 2.5 μg or 5.0 μg of Merck, Sharp and Dohme yeast-derived hepatitis B vaccine. The vaccine was mixed well and then given intramuscularly into the upper deltoid muscle using a 0.5 ml insulin syringe type U100 at 0, 1 month and 6 months (0–1–6 schedule). Prior to the administration of the vaccine, the parent or guardian was questioned regarding allergies and children with known allergies to yeast were excluded. The parent or guardian was asked to record any local or systemic complaints which occurred after each vaccination. Blood samples were obtained at 3 months, 6 months (before the last dose was given), 9 months, 12 months, 24 months and 48 months after the first dose, and tested for HBsAg, anti-HBs, anti-HBc and ALT levels. The reciprocal anti-HBs titres were expressed in milli-international units (mIU)/ml, based on a WHO Reference Standard (supplied by the International Laboratory for Biological Standards, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service). Seroconversion is defined as a rise in anti-HBs titre to ≥2.1 mIU/ml. Significant differences between seroconversion rates and between mean values were tested by Fisher's exact test and Student's *t*-test, respectively. A P value of <0.05 is considered statistically significant.

Results

Of the 146 children tested, 125 (85.6%) were negative for all the three HBV markers. One with elevated serum ALT was excluded, while two other children left the country. There was no significant difference in the mean age, sex, ethnic group, mean weight, mean height, and mean ALT levels among the 122 children randomized to the 4 different dosage groups (Table 1).

Three months after the first dose, 83.9% of the vaccinees in the 0.6-μg group, 93.3% in the 1.25-μg group, 93.3% in the 2.5-μg group and 100% in the 5-μg group seroconverted. The seroconversion rate increased to 90%, 93.4%, 93.3% and 100%, respectively, at 6 months, just before the third and last dose was administered. Three months after completion of the third dose, anti-HBs titres ≥10 mIU/ml were detected in between 93% and 100% of the vaccinees, with 58.1–61.3% having titres ≥1000 mIU/ml (Fig. 1). The GMT increased from 50 mIU/ml at 3 months to 1088.2 mIU/ml at 9 months for vaccinees in the 0.6-μg dose group, 85.5 mIU/ml to 1135.1 mIU/ml in the 1.25-μg group, 122.7 mIU/ml to 1689.1 mIU/ml in the 2.5-μg group, and 145.3 mIU/ml to 1699.4 mIU/ml in the 5-μg group (Fig. 2).

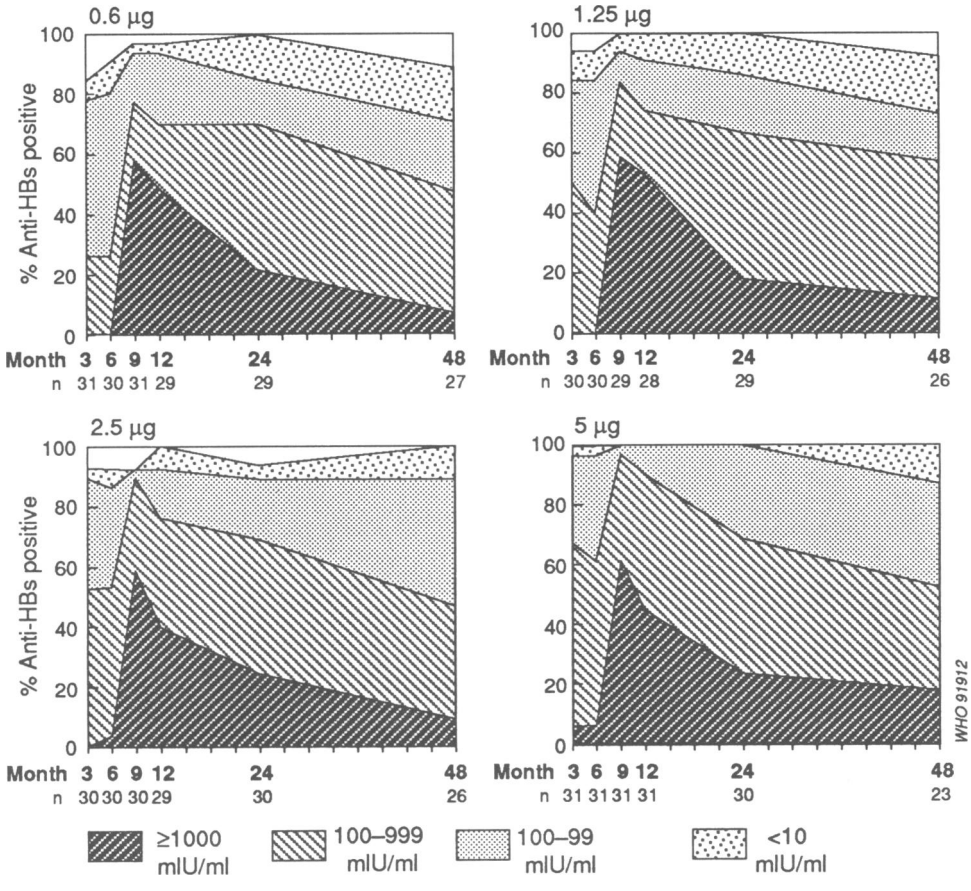
The anti-HBs positivity rate at 12 months and 24 months remained high at between 93.1% and 100%, but dropped slightly at 48 months to 88.8% in the 0.6 μg dose group, 92.2% in the 1.25 μg dose group and 92.3% in the 2.5-μg dose group. All the vacci-

Table 1: **Demographic characteristics of 122 seronegative children who were vaccinated with four different dosages of Merck, Sharp and Dohme yeast-derived hepatitis B vaccine**

	0.6 μg	1.25 μg	2.5 μg	5.0 μg
No. of children	31	30	30	31
Age (years)	4.7 ± 2.5[a]	4.5 ± 2.6	4.7 ± 2.8	5.5 ± 2.4
Weight (kg)	17.4 ± 6.7[a]	17.9 ± 5.9	18.6 ± 6.9	19.7 ± 6.6
Height (cm)	107.0 ± 13.8[a]	106.4 ± 16.7	109.6 ± 15.8	113.6 ± 15.8
ALT (IU/l)	11.5 ± 2.9[a]	11.6 ± 3.0	10.9 ± 3.8	10.9 ± 3.0
Sex (% male)	58.1	43.3	56.7	54.8
Ethnic group (% Chinese)	100	90	100	100

[a] Values are the mean ± SD.

Fig. 1. **Antibody responses of 122 seronegative healthy children aged 1–12 years, immunized with four different dosages of yeast-derived hepatitis B vaccine at 0, 1 and 6 months, and followed up for 48 months.**



nees in the 5.0-µg group continued to possess anti-HBs. However, anti-HBs titres ≥10 mIU/ml declined to between 70.3% and 73% in the 0.6-µg and 1.25-µg groups and to between 80.8% and 87% in the 2.5-µg and 5.0-µg groups. There was a significant decline in the GMT from the peak value at 9 months to between 214.3 mIU/ml and 271.2 mIU/ml at 24 months and between 72.6 mIU/ml and 118.8 mIU/ml at 48 months.

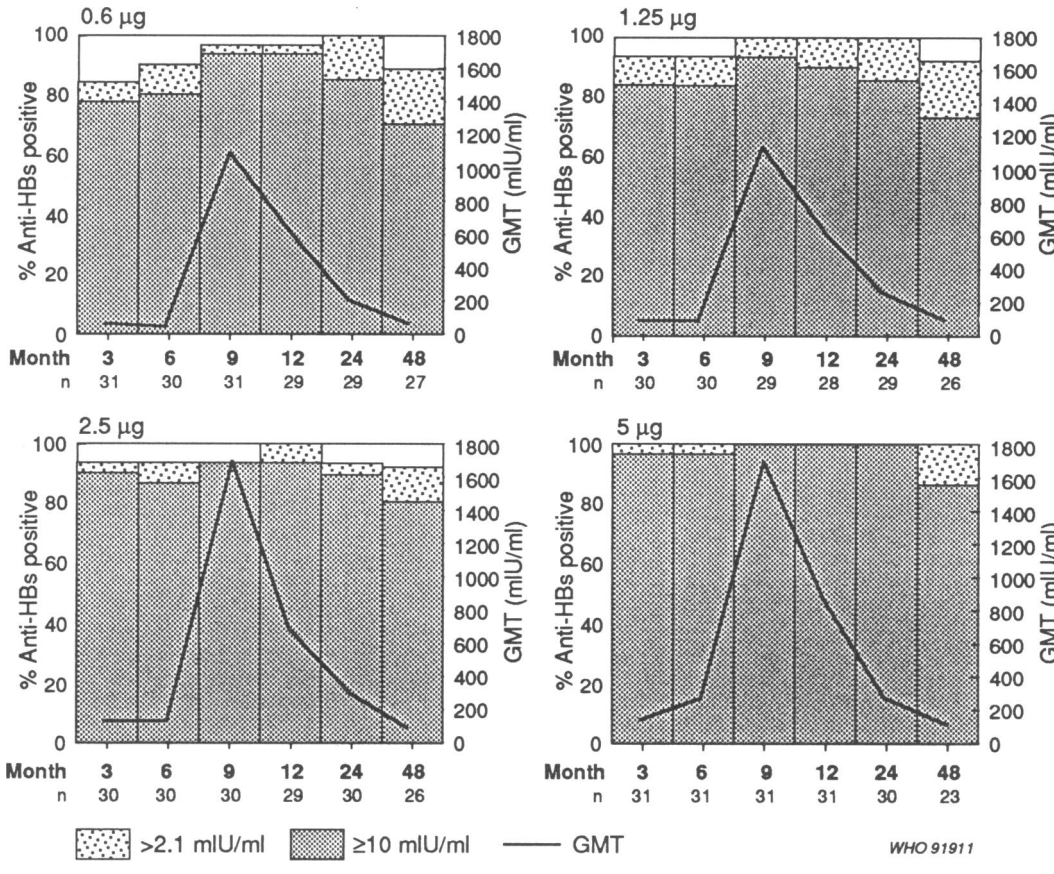
The persistence of anti-HBs is related to the peak level achieved at 9 months. For vaccinees with peak anti-HBs titres between 10 and 99 mIU/ml, 46.2% continued to have a level ≥10 mIU/ml at 24 months and 15.4% at 48 months. For those in the 100–999 mIU/ml range, anti-HBs persisted at 24 months but dropped to 55.6% at 48 months. Anti-

Table 2: **Relationship between peak anti-HBs level attained 9 months after vaccination and persistence of anti-HBs in healthy seronegative children vaccinated with yeast-derived hepatitis B vaccine at 0, 1 month and 6 months**

Peak anti-HBs level at 9 months (mIU/ml)	% with anti-HBs ≥10 mIU/ml at:		
	12 months	24 months	48 months
10–99 (*n* = 14)	100 (14/14)[a]	46.2 (6/13)	15.4 (2/13)
100–999 (*n* = 25)	100 (25/25)	100 (24/24)	55.6 (10/18)
≥1000 (*n* = 76)	100 (72/72)	100 (76/76)	100 (67/67)

[a] Figures in parentheses are the proportions and numbers of children involved.

Fig. 2. **Seroconversion rates and geometric mean titres (GMT) of 122 seronegative healthy children aged 1–12 years, immunized with four different dosages of yeast-derived hepatitis B vaccine at 0, 1 and 6 months, and followed up for 48 months.**



HBs was maintained at ≥10 mIU/ml at 48 months in all the vaccinees who developed a peak titre ≥1000 mIU/ml (Table 2).

The GMT at 3 months and at 6 months for the 0.6-µg group was significantly lower than that of the 2.5-µg dose (P=0.022 and P=0.0036, respectively) and that of the 5.0-µg dose (P=0.0086 and P=$<10^{-6}$, respectively). The GMT of the 1.25-µg dose was also significantly lower than that of the 5.0-µg dose at 6 months (P=0.0026). However, from 9 months onwards, there was no significant difference in GMT in the 4 different dosage groups. As for seroconversion rates, a significant difference was only noted between the 0.6-µg and 5.0-µg groups at 3 months (P=0.0263).

There was only one vaccinee, a six-year-old child in the 0.6-µg dose group, who did not seroconvert. When he was revaccinated with three doses of 5 µg vaccine at 2 years, there was an excellent anti-HBs response with a titre of 1785.6 mIU/ml one month after completion of the vaccination. (This child was subsequently excluded in the data analysis). None of the vaccinees had elevated ALT throughout the duration of follow-up. Transient anti-HBc was detected in 5 children before completion of the vaccination schedule; 2 in the 0.6-µg dose group and 2 in the 1.25-µg dose group at 3 months, and one in the 2.5-µg group at 6 months. The mothers of these children were HBsAg-positive but hepatitis B *e* antigen negative. Infection could have been acquired

from HBsAg carriers within or outside the family setting. The anti-HBs levels were between 6.0 mIU/ml and 1031.2 mIU/ml when anti-HBc was detected. After the third dose was administered, there was a further increase in anti-HBs concentration and anti-HBc disappeared completely. HBsAg was not detected in any of the vaccinees at any time point and none developed clinical hepatitis. The vaccine was well tolerated with no reports of adverse side-effects, such as anaphylactoid or other allergic reactions.

Discussion

The immunogenicity of reduced dosages of the Merck, Sharp & Dohme yeast-derived hepatitis B vaccine has been demonstrated in children (*10*), teenagers (*8*, *11*) and adults (*4*, *9*, *12*) when administered according to the 0–1–6 schedule. The excellent anti-HBs response of reduced dosages in Singapore children was confirmed in this clinical trial.

The anti-HBs response and GMT appeared to be dose-related before the third dose was administered, with significantly lower levels in vaccinees given the 0.6-μg dose, and to a certain extent, the 1.25-μg dose, but such differences were not discernible after completion of the vaccination schedule. Seroconversion rate was excellent and the GMT increased by 12 to 22 times in all the 4 dosage groups three months after the last dose was given. Although the number of subjects recruited in each group was relatively small, the pattern of anti-HBs response was so similar that even with an increased number of vaccinees, no significant difference is likely to be observed.

The national childhood hepatitis B vaccination programme in Singapore was implemented in two phases beginning with the immunization of babies born to carrier mothers in October 1985 (*13*). A reduced dosage of Merck, Sharp & Dohme plasma-derived hepatitis B vaccine was recommended as a local vaccine trial showed that the 5-μg dose was as immunogenic and efficacious as the standard dose (10 μg) in the prevention of perinatal transmission (*14*). In the second phase which began in September 1987, the programme was extended to cover all the newborns and the plasma-based vaccine was replaced by the Merck, Sharp & Dohme yeast-derived hepatitis B vaccine.

The 9-month follow-up results of this clinical trial provided the basis for the formulation of the schedule and dosage of hepatitis B vaccination in children using the yeast-derived vaccine. In recommending the dosages to be used in the childhood immunization programme in Singapore, the Expert Committee on the Immunization Programme took into consideration the somewhat poorer response in infants who get the vaccine at birth or within the first two months of life, and the particularly good response in children 1–10 years of age (*10*), the difficulty of administering a vaccine dose which is one-eighth to one-quarter of the standard dose, and the variations in immune response of vaccinees and possible loss of vaccine potency during storage under suboptimal field conditions. The 2.5-μg dose was therefore recommended for healthy infants and children. It is interesting to note that with modifications to the original production process and a second yeast master seed, there have been further improvements in the purity and immunogenicity of the newer batch of the vaccine (*10*). A 2.5-μg dose instead of the 5.0-μg dose is currently recommended by the manufacturer for infants and children.

However, a reduced dosage cannot be recommended at present for high-risk children born to carrier mothers as the preliminary results of an ongoing local clinical trial comparing the immunogenicity and efficacy of the 2.5-μg and 5.0-μg doses of yeast-derived hepatitis B vaccine are still equivocal. For babies born to hepatitis B *e* antigen (HB*e*Ag) negative carrier mothers, the 2.5-μg dose was as immunogenic and efficacious as the 5.0-μg dose in the prevention of perinatal transmission when three doses were given at birth, one month and 6 months without hepatitis B immunoglobulin (HBIG). None of them developed HBsAg at the end of one year's follow-up. In the case of babies born to HB*e*Ag carrier mothers, three of 27 babies vaccinated with three doses of 2.5-μg dose vaccine (together with HBIG at birth) were found to have HBsAg since birth and were probably infected *in utero*. However, HBsAg was not detected in any of the 28 babies vaccinated with the 5.0-μg dose. In many developing countries where HBeAg screening of pregnant women is not done, it is important not to administer a reduced dosage when the efficacy may be compromised. In Burma, a 92% efficacy was reported in infants born to HB*e*Ag carrier mothers when the 5-μg dose was administered according to the 0–1–6 schedule without HBIG (*10*), which was comparable to the rate of 94% when HBIG was also given at birth (*7*).

The rate of decline of anti-HBs is closely related to the peak level attained after completion of the 3-dose schedule, as in the case of adult vaccinees (*15*); 80% of the vaccinees given the 2.5-μg dose still possessed anti-HBs above the level considered to be protective (>10 mIU/ml) (*16*) 4 years after vaccination. The relationship between persistence of anti-HBs and duration of protection is still uncertain. Low or undetectable levels of circulating anti-HBs

may not necessarily indicate loss of protection as it has been shown that protection persists even when humoral antibody is no longer detectable (*17*). In our study, all the vaccinees in the 4 dosage groups with no detectable anti-HBs or with levels <10 mIU/ml continued to be protected against HBV infection. In teenager and adult vaccinees, when a booster dose was given to those with anti-HBs <10 mIU/ml at 12 months, an anamnestic response was elicited (*8*, *9*). The same phenomenon was also observed in healthy adults whose anti-HBs became undetectable 5–7 years after vaccination (*12*). This shows that once an immune response has been induced, it can be re-stimulated by exposure to the wild virus with active increase in anti-HBs during the early phase of the incubation period of the disease, thereby protecting against clinical illness or development of the carrier state. In an endemic area, it is possible that continued exposures to carriers could sustain or even stimulate the anti-HBs response without any serological evidence of an infection (*18*). Because of persistence of immunological memory, a booster dose may not be indicated in vaccinees who have responded to the full schedule of immunization.

Résumé

Comparaison de la réponse immunitaire suscitée par quatre doses différentes de vaccin antihépatite B recombinant produit sur levures chez des enfants de Singapour: une étude de suivi sur quatre ans

Compte tenu du coût élevé du vaccin antihépatite B produit sur levures lorsqu'il a été commercialisé pour la première fois, on a effectué un essai clinique afin de déterminer si des doses plus faibles de ce vaccin seraient aussi immunogènes que la dose standard. On a effectué une étude randomisée sur 122 enfants en bonne santé, séronégatifs, âgés de 1 à 12 ans, à qui l'on a administré 0,6 µg, 1,25 µg, 2,5 µg ou 5,0 µg de vaccin Merck, Sharp & Dohme par voie intramusculaire à 0, 1 et 6 mois. On a contrôlé l'apparition d'anticorps anti-antigène de surface du virus de l'hépatite B (anti-HBs) au bout de 3, 6, 9, 12, 24 et 48 mois. On a observé une séroconversion chez 83,9 à 100% des vaccinés à 3 mois et chez 90 à 100% à 6 mois. Le titre moyen géométrique (TMG) d'anticorps a atteint un pic situé entre 1088 mUI/ml et 1699 mUI/ml au bout de 9 mois. Ce TMG semblait lié à la dose avant que la vaccination ne soit terminée, avec des concentrations sensiblement inférieures chez les vaccinés ayant reçu la dose de 0,6 µg par rapport à ceux ayant reçu 2,5 µg et 5,0 µg. Toutefois, au-delà de 9 mois, aucune différence significative n'a été observée dans les taux de séroconversion ni dans le TMG, quelle que soit la dose administrée. A 24 mois, on a retrouvé l'anti-HBs chez 93,1 à 100% des vaccinés, mais le TMG avait chuté et se situait entre 214,3 mUI/ml et 303,5 mUI/ml. A 48 mois, 88,8 à 100% des vaccinés possédaient encore des anti-HBs, 70,3 à 87% étant au-dessus de 10 mUI/ml et le TMG avait encore chuté, se situant entre 72,6 mUI/ml et 118,8 mUI/ml. Tous les vaccinés sont restés asymptomatiques et indemnes de toute infection par le virus de l'hépatite B une fois la vaccination achevée, comme l'a montré l'absence d'antigène de surface de l'hépatite B et d'anticorps dirigés contre l'antigène central de l'hépatite B. L'étude a confirmé l'immunogénicité des doses réduites de vaccin, un résultat particulièrement important pour les pays en développement où l'infection par le virus de l'hépatite B est endémique. A Singapour, on a recommandé une dose de 2,5 µg dans le cadre du programme national de vaccination des enfants contre l'hépatite B. Rien n'indique qu'il faille par la suite administrer un rappel, du moins pendant les 4 ans qui suivent la vaccination.

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