Universal immunization of infants with low doses of a low-cost, plasma-derived hepatitis B vaccine in South Africa

B.D. Schoub,[1] U. Matai,[2] B. Singh,[3] N.K. Blackburn,[4] & J.B. Levin[5]

Objective To evaluate the effectiveness of universal vaccination against viral hepatitis B in South Africa among 18-month-old rural children.

Methods Children were immunized with a course of low-dose (1.5 μg), plasma-derived hepatitis B vaccine at 6, 10 and 14 weeks of age, and blood samples from the children were tested for three hepatitis B markers: hepatitis B surface antigen (HBsAg), anti-HBs and anti-HBc.

Findings One year after vaccination, a protective anti-HBs antibody titre of at least 10 IU/l was present in 669/769 (87.0%) of blood serum samples tested. Only 3/756 children (0.4%) were HBsAg positive and a fourth child was anti-HBc positive (HBsAg negative). This is a marked decrease compared to the hepatitis B prevalences reported in previous studies. Among rural migrant mine-workers, for example, HBsAg prevalence was 9.9%, and was 10.1% among children 0–6 years of age in the Eastern Cape Province.

Conclusion The low-dose, plasma-derived hepatitis B vaccine, which is affordable to most developing countries, was very successful in controlling endemic hepatitis B infection, where the virus is predominantly spread by horizontal transmission among infants and young children.

Keywords Hepatitis B vaccines/administration and dosage/economics; Hepatitis B surface antigens/blood; Child; Endemic diseases/immunology; Immunization programs; Evaluation studies; Cross-sectional studies; South Africa (*source: MeSH, NLM*).

Mots clés Vaccin anti-hépatite B/administration et posologie/économie; Antigène HBS/sang; Enfant; Maladie endémique/immunologie; Programmes de vaccination; Etude évaluation; Etude section efficace; Afrique du Sud (*source: MeSH, INSERM*).

Palabras clave Vacunas contra la hepatitis B/administración y dosificación/economía; Antígenos de superficie de la hepatitis B/sangre; Niño; Enfermedades endémicas/inmunología;Programas de inmunización; Estudios de evaluación; Estudios transversales; Sudáfrica (*fuente: DeCS, BIREME*).

Bulletin of the World Health Organization 2002;80:277-281.

Voir page 280 le résumé en français. En la página 280 figura un resumen en español.

Introduction

The World Health Assembly called for all countries with moderate or high hepatitis B virus (HBV) endemicity to institute routine immunization of all infants by 1995, and by 1997 over 80 countries had introduced HBV vaccine into their national programmes (*1*). Nevertheless, even though the effectiveness of immunizing infants has been demonstrated (*2–5*), many countries cannot afford HBV vaccine, the most expensive of the six vaccines in the Expanded Programme on Immunization (EPI). As a result, extensive reservoirs of HBV still exist, with some 95% of the 350 million carriers of the virus residing in poorer developing countries (*6*).

To reduce costs, countries have turned to plasma-derived HBV vaccines, rather than the more expensive recombinant vaccines. For example, the lowest tender price for recombinant vaccine in 1999 was US$ 5.50 (R 42.89) per 10-dose vial, compared to a tender price of US$ 3.72 (R 29.03) in 2000 for plasma derived vaccine. Although fears about the safety of plasma-derived vaccines still persist, it is generally accepted that they are both safe and effective (*7*). The efficacy of reduced doses of HBV vaccine has been investigated in an attempt to further decrease costs. Efficacy studies in young adults have shown highly satisfactory seroresponses using only half the recommended dose (*8, 9*), and adequate immunogenicity has been demonstrated using one-fifth of the recommended dosage in neonates (*10*), infants, and young children (*11, 12*). On the other hand, suboptimal seroresponses were observed when half the recommended dose was used to immunize neonates born to non-carrier mothers in Hong Kong (*13*) and to HBV-carrier mothers in China (Taiwan) (*14*). WHO has cautioned against using arbitrary dose reductions to save costs without approval from the national controlling authority and without first obtaining evidence that adequate immunogenicity would still be retained (*15*). In other studies, reducing the number of doses also resulted in unsatisfactory seroresponses (*11, 16*).

A low-cost plasma-derived HBV vaccine (Hepaccine B, Cheil Foods and Chemicals Company, Seoul, Republic of Korea) has been developed by inactivating the plasma-derived vaccine through flash heating — a procedure that is claimed to enhance HBV immunogenicity (*17*). In some adult studies this vaccine has been found to be highly immunogenic, with 94–

[1] National Institute for Virology and Department of Virology, University of the Witwatersrand, South Africa. Correspondence should be addressed to this author at the following address: National Institute for Virology, Private Bag X4, Sandringham 2131, South Africa (email: schoub@niv.ac.za).

[2] Expanded Programme on Immunization, Department of Health, Pretoria, South Africa.

[3] National Institute for Virology and Department of Virology, University of the Witwatersrand, South Africa.

[4] National Institute for Virology and Department of Virology, University of the Witwatersrand, South Africa.

[5] Medical Research Council, Pretoria, South Africa.

Ref. No. 00-0920

97% seroconversion using only a 3-μg dose (*18, 19*). However, the seroconversion rate has been reported to be as low as 52% in one study (*20*), while in another the seroresponse was slower with low-dose than with conventional doses of the vaccine (*21*). Similarly, in infants and children, both satisfactory (94% seroconversion) (*22*) and highly unsatisfactory seroresponses (58% seroconversion) (*23*) have been reported. Nevertheless, an efficacy study in a rural South African infant population immunized with Hepaccine B at the EPI scheduled ages of 6, 10 and 14 weeks demonstrated that 93% of the infants developed protective titres of antibodies to anti-hepatitis B surface (anti-HBs) markers (*24*), and in April 1995 the South African government introduced the Hepaccine B vaccine into the routine EPI programme (*25*).

To date, there has been no study of the effectiveness of this low-dose plasma-derived vaccine under field conditions, even though it could be an affordable solution for universal immunization programmes in developing countries. South Africa is a good location to carry out such a study, since HBV endemicity is high — estimates of the prevalence of hepatitis B surface antigen (HBsAg) range from 9.9% among adult migrant mineworkers from rural areas (*26*) to 10.1% among rural African children aged 0–6 years in the Eastern Cape Province (*27*). In this paper, we describe the effectiveness of the South African universal EPI-based immunization programme, which uses the Hepaccine B vaccine.

Methods

Study design

The study was a cross-sectional investigation of the prevalence of HBsAg, anti-HBs, and anti-hepatitis B core (anti-HBc) markers among 18-month-olds one year after they had been immunized for viral hepatitis B according to the EPI schedule at 6, 10, and 14 weeks of age. Plasma-derived Hepaccine B vaccine (Cheil Foods and Chemicals Company, Seoul, Republic of Korea) is used for universal immunization in South Africa, and was administered intramuscularly at the manufacturer's recommended dose of 1.5 μg in 0.5ml. In 1999, the vaccine cost ca US$ 0.40 (R 2.90) per dose in a 10-dose vial.

Study population

The study was carried out in rural districts of all nine provinces of South Africa. Rural populations were examined because HBV endemicity was significantly higher among them than urban populations. Between February and November 1999, we recruited healthy 18-month-olds attending clinics for routine second measles immunizations and for follow-up poliomyelitis and diphtheria–pertussis–tetanus immunizations. The first 20 such children who attended each clinic and who had received the full three-dose schedule of HBV vaccine at 6, 10 and 14 weeks (as documented in their road-to-health card) were enrolled in the study, after obtaining informed consent from their mothers or caregivers. Blood samples were then taken from the children and tested for three hepatitis markers.

The provincial EPI manager selected the clinics that would participate in the study (one rural clinic per region), choosing those that regularly submitted immunization statistics and had good cold chain practices (determined by inspection and questionnaire). The distribution of blood samples taken from the study children is shown by province in Table 1.

Table 1. Distribution of blood samples tested for markers of hepatitis B virus, South Africa, by province

Province	% of total population	No. of regions in province	No. of samples submitted
Eastern Cape	15.53	5	73 (9.9)[a]
Free State	6.49	6	77 (10.0)
Gauteng	18.11	5	90 (11.7)
KwaZulu Natal	20.74	7	80 (10.4)
Mpumalanga	6.90	3	50 (6.5)
Northern Cape	2.07	6	77 (10.0)
Northern Province	12.14	7	152 (19.8)
Western Cape	8.27	5	92 (12.0)
North West	9.75	4	78 (10.1)
Total	–	–	769 (100)

[a] Figures in parentheses are percentages of the total.

The purpose of the study was explained in the vernacular to the mothers or caregivers of children attending the clinics. In addition, it was pointed out to the community that the study was valuable because it would help to establish the effectiveness of the vaccination programme, and that it would also benefit the children by determining their immunity to HBV. Children who tested negative for HBV antibodies were offered reimmunization and follow-up testing. No remuneration or reward was offered to any study participants. A signature or other identification mark on the consent form indicated both an understanding of the programme and its implications, as well as a willingness to participate in the study. Study results were communicated to provincial EPI coordinators and clinics, to relevant provincial health departments, and to the Department of Health national EPI programme. The study protocol was reviewed and approved by the Committee for Research on Human Subjects and Ethics, University of the Witwatersrand, as well as by the ethics review bodies of each of the nine provincial governments.

Serology

A 5-ml sample of venous blood was taken from each child and sent under cold chain conditions to the National Institute for Virology in Johannesburg, where they were tested for HBsAg, anti-HBs and anti-HBc markers, using commercial enzyme immunoassay kits (Sanofi Bio-Rad Laboratories, Sanofi Diagnostics, 92430 Marnes-la-Coquette, France) performed according to the manufacturer's recommendations. The blood sample immunoassay responses were converted to titres (IU/l) using standard response–titre curves generated with serum samples provided by the manufacturer. A titre of at least 10 IU/l was considered to be protective (*28*).

Statistical analysis

The survey was regarded as a stratified, two-stage sample, with stratification by province and the two stages being clinics and children within clinics. The data were analysed using Stata (version 7) software, and estimates of the proportion of children with a protective titre took into account the survey design (*29*). To test whether the proportion of children with protective titres differed between provinces, we used the Rao–Scott correction to the Pearson χ^2 statistic for association in a two-way table (*30*).

Results

A total of 782 blood samples were collected, 12 of which were rejected because their volume was too low to perform any tests. Of the remaining blood samples, 770 were tested for anti-HBc, 769 for anti-HBs, and 756 for HBsAg. There are different totals for the three markers because 14 blood samples had sufficient volume to test for anti-HBc and anti-HBs antibodies, but not for HBsAg; and one sample had sufficient volume only for the anti-HBc test. Protective titres of anti-HBs antibodies ($\geqslant$10 IU/l) were found in 669 of 769 (87.0%) blood samples tested (95% confidence interval (CI) = 84.6–89.4%). This proportion did not differ significantly between provinces (Rao–Scott design-based F test (6.11, 232.10) = 0.9522, P = 0.4595; uncorrected χ^2 test = 7.73 (8 degrees of freedom (Table 2)). More than half (55.7%) of all sera had titres >100 IU/l (Fig. 1).

Only three children (from North-West Province) had evidence that they carried HBV (i.e. they were positive for both HBsAg and anti-HBc). One was born to a mother known to be positive for human immunodeficiency virus (HIV) (the other children's HIV status was not known) and who had been seen several times at the clinic for failure to thrive. A fourth child, also from North-West Province, was anti-HBc (and anti-HBs) positive, but HBsAg negative. Thus, the positivity rate was 3 in 756 (0.4%) for HBsAg, and 4 in 770 (0.5%) for anti-HBc.

Table 2. Prevalence of serological markers for hepatitis B virus among 18-month-olds, in South Africa, by province

Province	HBsAg		Anti-HBc		Anti-HBs	
	n	No. positive	*n*	No. positive	*n*	No. positive[a]
Eastern Cape	72	0 (0)[b]	73	0 (0)	73	61 (84)[c]
Free State	74	0 (0)	77	0 (0)	77	66 (86)[c]
Gauteng	87	0 (0)	90	0 (0)	90	77 (86)[c]
KwaZulu/Natal	78	0 (0)	80	0 (0)	80	69 (86)[c]
Mpumalanga	50	0 (0)	50	0 (0)	50	48 (96)[c]
Northern Cape	77	0 (0)	77	0 (0)	77	66 (86)[c]
Northern Province	149	2 (1.3)	153	2 (1.3)	152	138 (91)[c]
Northwest	92	1 (1)	92	2 (2)	92	79 (86)[c]
Western Cape	77	0 (0)	78	0 (0)	78	65 (83)[c]
Total	756	3 (0.13)	770	4 (0.5)	769	669 (87.0)[c]

[a] A blood sample was considered positive if the anti-HBs titre was $\geqslant$ 10IU/l.
[b] Figures in parentheses are percentages.
[c] Rao-Scott design-based F test (6.11, 232.10) = 0.9522; P = 0.4595; uncorrected χ^2 test = 7.73 on 8 degrees of freedom.

Discussion

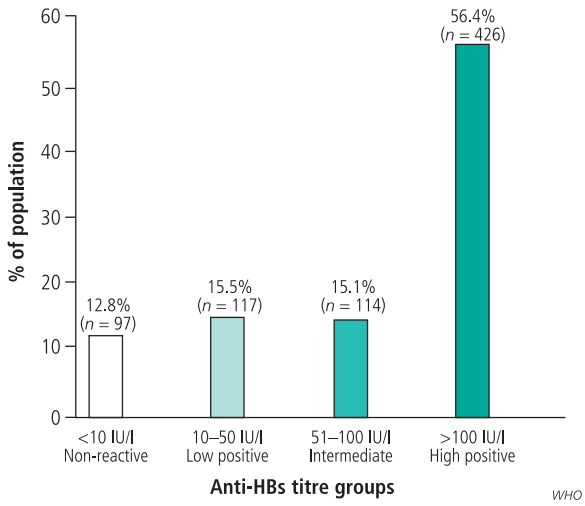
The effectiveness of universal HBV immunization programmes has been demonstrated in many countries. China (Taiwan) was one of the first areas to introduce mass HBV immunization, in July 1984, and over the following 10 years the prevalence of HBsAg antigenaemia dropped from 9.8% to 1.3%, anti-HBc prevalence fell from 26% to 4%, and 79% of the population had developed protective anti-HBs antibodies (*1*). Similarly, in the rest of China the prevalence of HBsAg dropped from 8.2% to 0.3% over a 15-year period (*2*); and in Saudi Arabia it dropped from 6.7% to 0.3% over the period 1989–97 (*4*). In Africa, routine HBV immunization was first introduced to newborn infants in the Gambia, in 1990: nine years later the HBV carrier rate among vaccinated children was 0.6%, compared to 10% among unvaccinated children, a vaccine efficacy of 83% against infection and 95% against chronic carriage (*3*).

HBV is highly endemic in South Africa, as it is in other sub-Saharan countries. HBsAg carriage rates among migrant mine workers from rural areas in South Africa varied from 5.5% to 14%, with an overall prevalence of 9.9% (*26*). In Ovamboland, in neighbouring Namibia, HBsAg prevalence was 17% and 11%, respectively, among adult males and females. Among infants younger than 6 months of age, the prevalence was only 1%, but rose to 13% among children aged over 1 year. Thus in south-west Africa, horizontal spread of HBV in infants and young children was a far more important route of viral transmission than perinatal spread (*31*). Similarly, in Kangwane, in the rural eastern part of South Africa, the HBsAg carriage rate rose from 1% among infants aged less than 6 months to 2.3% among 1–2-year-olds, 9.7% among 2–3-year-olds, and 11.8% among 3–5-year-olds (*32*). In the KwaZulu/Natal Province, HBsAg antigenaemia in rural children aged 6–14 years was 18.5%, compared to 10% among urban children (*33*); in urban African children in Soweto, the prevalence was even lower, at 0.97% (*34*).

In April 1995, the South African Department of Health introduced universal immunization of infants for HBV and added the vaccine to the existing EPI schedule at 6, 10, and 14 weeks (*25*). Hepaccine B was chosen for the programme because of cost constraints, even though low seroconversion rates had been reported in Melanesia (*23*). In contrast, a local study in Shoshanguve, north of Pretoria, reported an anti-HBs seroconversion rate of 93% (*24*).

We investigated the effectiveness of Hepaccine B in a cross-sectional study of sera from rural African children, one year after completing their full course of HBV immunization. In the Eastern Cape Province, immediately prior to the introduction of universal HBV vaccination, the overall HBsAg prevalence was 10.1% in unvaccinated rural children aged 0–6 years, but varied from 8.1% among infants aged 0–6 months to 15% among 5–6-year-olds (*27*). In contrast, none of 75 children from the same population tested positive for HBsAg, and none of 78 children were positive for anti-HBc, one year after receiving the full course of three doses of

Fig. 1. **Distribution of anti-HBs titres in South Africa**



Hepaccine B vaccine. In the country as a whole, only 3 of 756 children examined for HBsAg were positive, one of whom was possibly immunosuppressed (born to an HIV-positive mother and failing to thrive). A fourth child, who was positive for both anti-HBc and anti-HBs antibodies, also had evidence of infection with HBV.

The results demonstrated that the universal HBV vaccination programme has produced a dramatic change in the epidemiology of viral hepatitis B. The prevalence of protective anti-HBs antibodies (titres ($\geqslant$10 IU/l) in immunized children was 85%, and over half of all the sera tested had titres >100 IU/l one year after receiving three doses of Hepaccine B. Although the sustainability of the anti-HBs seroresponse will need to be assessed in the future, the results of other studies are encouraging. Several long-term investigations found that anti-HBs antibodies do persist (*1–4*), and in the Gambia the HBV transmission rate has been steadily declining since the introduction of immunization (*35*).

Because of operational restrictions, our investigation was subject to some sampling deficiencies and potential biases. First, the sample was biased towards children who had ready access to health care facilities. The sampling for each province was selectively drawn from that portion of the population that returned for the second measles vaccination at 18 months of age, and it may not be representative of the entire population. Second, the eligibility for enrolment into the study was relatively uncontrolled, since all overtly healthy children (evaluated by a clinic nurse) were eligible. Third, it was not possible to randomize the selection process; instead children coming to the clinic were enrolled in succession. However, these sampling constraints were identical to those of earlier pre-immunization studies (e.g. *27*). The fact that neither clinics nor children were randomly selected was a weakness of the study design and arose for logistic reasons, but this should be reviewed in any future studies. For example, it would be better to sample every sixth child to visit a clinic. Nevertheless, there were no significant differences between provinces in terms of the proportions of children with protective anti-HBs, nor evidence of clustering within clinics (Table 2), suggesting that the results are unlikely to be seriously biased.

The effectiveness of the South African universal hepatitis B vaccination programme is important for developing countries because it demonstrates that endemic HBV infection can be controlled by routine immunization with a low-cost vaccine. Even more important was the demonstration that the secondary effects of immunization can be extensive at the population level, when the organism depends on chronically infected individuals to maintain endemicity. Immunization reduced the overall reservoir of HBV in the population and decreased the transmission rate, thus providing a significant degree of protection to non-immunized individuals. Similarly, group immunity benefits have been demonstrated in infants using *Haemophilus influenzae* type B protein conjugate vaccines, which have virtually eliminated the infection from the USA with only modest vaccine coverage (*36*). ■

Acknowledgements

We thank the EPI coordinators in all nine provinces of South Africa for organizing the collection and transport of specimens; the nursing sisters for taking specimens and interviewing the participants; and the mothers and infant caregivers. We also thank Miriam Mashele for excellent technical assistance in the laboratory. This study was funded and supported by the Department of Health, South Africa.

Conflicts of interest: none declared.

Résumé

Vaccination universelle des nourrissons par de faibles doses de vaccin anti-hépatite B de coût modique dérivé de plasma

Objectif Evaluer l'efficacité de la vaccination universelle contre l'hépatite virale B chez les enfants de 18 mois dans des zones rurales d'Afrique du Sud.

Méthodes Les enfants ont été vaccinés par une série de faibles doses (1,5 µg) de vaccin anti-hépatite B dérivé de plasma administrées à l'âge de 6, 10 et 14 semaines, et trois marqueurs de l'hépatite B ont été ultérieurement recherchés dans des prélèvements de sang : l'antigène de surface de l'hépatite B (HbsAg), les anticorps anti-HBs et les anticorps anti-HBc.

Résultats Un an après la vaccination, un titre d'anticorps protecteurs anti-HBs d'au moins 10 UI/l était présent dans 669 échantillons de sérum testés sur 769 (87,0 %). Seuls 3 enfants sur 756 (0,4 %) étaient positifs pour l'HbsAg et un quatrième était positif pour les anticorps anti-HBc (et négatif pour l'HbsAg). Ces valeurs représentent une baisse marquée par rapport à la prévalence de l'hépatite B rapportée dans des études antérieures. Par exemple, la prévalence de l'HbsAg était de 9,9 % parmi les mineurs migrants d'origine rurale et elle était de 10,1 % chez les enfants de 0 à 6 ans dans la province du Cap oriental.

Conclusion Le vaccin anti-hépatite B dérivé de plasma administré à faible dose, à la portée financière de la plupart des pays en développement, était donc très efficace contre l'infection endémique par le virus de l'hépatite B là où ce dernier se propage principalement par transmission horizontale chez les nourrissons et les jeunes enfants.

Resumen

Inmunización universal de lactantes contra la hepatitis B con dosis bajas de una vacuna de bajo costo derivada del plasma

Objetivo Evaluar la eficacia de la vacunación universal contra la hepatitis viral B en Sudáfrica entre niños de 18 meses de zonas rurales.

Métodos Se inmunizó a los niños mediante la administración de dosis bajas (1,5 µg) de vacuna anti-hepatitis B derivada del plasma a las 6, 10 y 14 semanas de edad, extrayéndose muestras de sangre para determinar la presencia de tres marcadores de la enfermedad: antígeno de superficie de la hepatitis B (HBsAg), anticuerpos anti-HBs, y anticuerpos anti-HBc.

Resultados Un año después de la vacunación, el 87,0% (669/769) de las muestras de suero sanguíneo analizadas presentaban niveles protectores de anticuerpos anti-HBs, de 10 UI/l como mínimo. Sólo el 0,4% (3/756) de los niños eran HbsAg-positivos, y un cuarto niño era anti-HBc-positivo (HBsAg-negativo). Esto supone una marcada disminución en comparación con las prevalencias de hepatitis B notificadas en estudios anteriores. Entre los mineros migrantes rurales, por ejemplo, la prevalencia de HBsAg fue del 9,9%, y entre los niños de 0 a 6 años de la Provincia de El Cabo Oriental, del 10,1%.

Conclusión La administración de dosis bajas de vacuna anti-hepatitis B derivada del plasma, asequible para la mayoría de los países en desarrollo, permitió controlar muy eficazmente la infección endémica por hepatitis B, situación en la que el virus se propaga predominantemente por transmisión horizontal entre lactantes y niños de corta edad.

References

1. Kane MA. Global status of hepatitis B immunisation. *Lancet* 1996;348:696.
2. Chen HL, Chang MH, Ni YH, Hsu HY, Lee PI, Lee CY, et al. Seroepidemiology of hepatitis B virus infection in children: ten years of mass vaccination in Taiwan. *Journal of the American Medical Association* 1996;276:906-8.
3. Liao SS, Li RC, Li H, Yang JY, Zeng XJ, Gong J, et al. Long-term efficacy of plasma-derived hepatitis B vaccine: a 15-year follow-up study among Chinese children. *Vaccine* 1999;17:2661-6.
4. Viviani S, Jack A, Hall AJ, Maine N, Mendy M, Montesano R, et al. Hepatitis B vaccination in infancy in The Gambia: protection against carriage at 9 years of age. *Vaccine* 1999;17:2946-50.
5. Al-Faleh FZ, Al-Jeffri M, Ramia S, Al-Rashed R, Arif M, Rezeig M, et al. Seroepidemiology of hepatitis B virus infection in Saudi children 8 years after a mass hepatitis B vaccination programme. *Journal of Infection* 1999;38: 167-70.
6. Ayoola EA. Viral hepatitis in Africa. In: Zuckerman AJ, editor *Proceedings of the International Symposium on Viral Hepatitis and Liver Disease.* New York: Alan R Liss Inc;1988. p.161-9.
7. The Children's Vaccine Initiative and the Global Programme for Vaccines and Immunization. Recommendations from the Special Advisory Group of Experts, Part I. *Weekly Epidemiological Record* 1996;71(35):261-6.
8. Goh KT, Oon CJ, Heng BH, Lim GK. Long-term immunogenicity and efficacy of a reduced dose of plasma-based hepatitis B vaccine in young adults. *Bulletin of the World Health Organization* 1995;73:523-7.
9. Bryan JP, Craig PG, Reyes L, Hakre S, Jaramillo R, Harlan H, et al. Randomized comparison of 5 and 10 μg doses of two recombinant hepatitis B vaccines. *Vaccine* 1995;13:978-82.
10. Moyes CD, Milne A, Dimitrakakis M, Goldwater PN, Pearce N. Very-low-dose hepatitis B vaccine in newborn infants: an economic option for control in endemic areas. *Lancet* 1987;1:29-30.
11. Yvonnet B, Coursaget P, Leboulleux D, Barres JL, Fritzell B, Sarr G, et al. Low-dose hepatitis B vaccine immunisation in children. *Lancet* 1987;1:169.
12. Milne A, Moyes C. Response to hepatitis B vaccine in New Zealand children: using low doses in a two-month versus six-month schedule. In: Zuckerman AJ, editor. *Proceedings of the International Symposium on Viral Hepatitis and Liver Disease.* New York: Alan R Liss Inc;1988. p. 977-9.
13. Lee SS. Hepatitis B vaccination strategy for newborn babies. *Lancet* 1995; 346: 900-1.
14. Lee C-Y, Hwang L-Y, Palmer Beasley R. Low-dose hepatitis B vaccine. *Lancet* 1989;2:860-1.
15. Kane M. Reduced doses of hepatitis B vaccines: is it a good idea? *Bulletin of the World Health Organization* 1995;73:529-30.
16. Manyike PT, Aspinall S, Summers RS. Immunogenicity of recombinant hepatitis B vaccine in urban black children from Ga-Rankuwa, Bophuthatswana, South Africa. *Pediatric Infectious Diseases Journal* 1992;11:726-30.
17. Prince AM, Vnek J, Brotman B. An affordable multideterminant plasma-derived hepatitis B virus vaccine. In: Olufemi WA, O'Conor GT, de-The GB, Johnson CA, editors. *Virus associated cancers in Africa.* Oxford: Oxford University Press; 1984. p. 355-72. IARC Publication No. 63.
18. Chung WK, Sun HS, Chung KW, Kim BS, Min BK, Prince AM. Safety and immunogenicity of a new heat-inactivated hepatitis B virus vaccine in adult recipients. *Vaccine* 1987;5:175-8.
19. Pennie RA, O'Connor AM, Dulberg CS, Bottiglia A, Manga P, Kang CY.Low-cost hepatitis B vaccine improves uptake among self-paying health-care students. *Journal of Medical Virology* 1992;37:48-53.
20. Chadha MS, Arankalle VA, Banerjee K. Comparative immunogenicity of different hepatitis B vaccines among certain high risk groups in India. *Journal of the Association of Physicians of India* 1992;40:584-8.
21. Phanuphak P, Phanpanich T, Wongurai S, Sirivichayakul S, Sriwanthana B, Panmuong W, et al. Comparative immunogenicity study of four plasma-derived hepatitis B vaccines in Thai young adults. *Vaccine* 1989;7:253-6.
22. Kim SH et al. Studies on the safety and immunogenicity of a heat-inactivated hepatitis B vaccine in children. *Korean Journal of Infectious Diseases* 1985;18:39-44.
23. Milne A, Rodgers E, Hopkirk N. Hepatitis B vaccination of babies in Melanesia. *Lancet* 1995;346:318.
24. Aspinall S, Kocks DJ. Immunogenicity of a low-cost hepatitis B vaccine in the South African Expanded Programme on Immunisation. *South African Medical Journal* 1998;88:36-9.
25. Department of Health. New childhood immunisation schedule. *South African Medical Journal* 1995;85:298.
26. Dusheiko GM, Conradie JD, Brink BA, Marimuthu T, Sher R. Regional prevalence of hepatitis B, Delta, and human immunodeficiency virus infection in southern Africa: a large population survey. *American Journal of Epidemiology* 1989; 129:138-45.
27. Vardas E, Mathai M, Blaauw D, McAnerney J, Coppin A, Sim J. Preimmunization epidemiology of hepatitis B virus infection in South African children. *Journal of Medical Virology* 1999;58:111-5.
28. Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. What level of hepatitis B antibody is protective? *Journal of Infectious Diseases* 1999;179:489-92.
29. *Stata Statistical Software Release 7.0.* College Station (TX); Stata Corporation: 2001.
30. Rao JNK, Scott AJ. The analysis of categorical data from complex sample surveys: chi-squared tests for goodness of fit and independence in two-way tables. *Journal of the American Statistical Association* 1981;76:221-30.
31. Botha JF, Dusheiko GM, Ritchie MJJ. Hepatitis B virus carrier state in black children in Ovamboland: role of perinatal and horizontal infection. *Lancet* 1984;1:1210-2.
32. Prozesky OW, Szmuness W, Stevens CE, Kew MC, Harley EJ, Hoyland JA, et al. Baseline epidemiological studies for a hepatitis B vaccine trial in Kangwane. *South African Medical Journal* 1983;64:891-3.
33. Abdool Karim SS, Coovadia HM, Windsor IM, Thejpal R, van den Ende J, Fouche A. The prevalence and transmission of hepatitis B virus infection in urban, rural and institutionalized black children of Natal/KwaZulu, South Africa. *International Journal of Epidemiology* 1988;17:168-73.
34. Dibisceglie AM, Kew MC, Dusheiko GM, Berger EL, Song E, Patersonm AC, et al. Prevalence of hepatitis B virus infection among black children in Soweto. *British Medical Journal* 1986;292:1440-2.
35. Whittle HC, Maine N, Pilkington J, Mendy M, Fortuin M, Bunn J, et al. Long-term efficacy of continuing hepatitis B vaccination in infancy in two Gambian villages. *Lancet* 1995;345:1089-92.
36. Centers for Disease Control and Prevention. Progress towards elimination of *Haemophilus influenzae* type b disease among infants and children — United States, 1987–1993. *Morbidity and Mortality Weekly Report* 1994; 43(8):144-8.