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IMMUNOGENICITY OF A LOCALLY PRODUCED HEPATITIS B VACCINE WITH THE BIRTH DOSE STORED OUTSIDE THE COLD CHAIN IN RURAL VIETNAM

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Abstract. The heat stability of hepatitis B vaccine (HepB vaccine) should enable its storage outside the cold chain (OCC), increasing access to the birth dose in areas lacking refrigeration. We compared the immunogenicity of a locally produced vaccine among infants who received three doses stored within the cold chain (n = 358) or for whom the first dose was stored OCC for up to one month (n = 748). Serum was collected from these infants at age 9–18 months. The vaccine was protective in 80.3% of all infants. There were no differences in the prevalence of a protective level of antibody or antibody titer among groups of infants according to storage strategy. Differences in antibody titer between certain groups of infants could be explained by different vaccination schedules. Where birth dose coverage will be improved, HepB vaccine can be taken OCC for up to one month without affecting its immunogenicity.

INTRODUCTION

Chronic infection with the hepatitis B virus (HBV) is a major public health problem in Vietnam and usually results from exposure to the virus early in life. The World Health Organization (WHO) has for more than 12 years recommended that all infants be vaccinated against HBV.[1]

Our population-based, age-stratified serosurvey of HBV markers in Vietnam[2] indicated a very high seroprevalence of HBV infection commencing early in life. It confirmed the findings of other published[3–5] and unpublished surveys, and highlighted the need for a program of immunization beginning at birth. However, delivery of vaccines to newborns in Vietnam is not easy because the rate of home birth is high (average = 21.3%, and up to 56.1% in the northern highlands[6]) and access of health workers in rural areas can be very difficult. Furthermore, the scarcity of refrigeration at local health stations makes it difficult for these workers to provide heat-sensitive vaccines on a daily basis. Similar problems exist throughout the Mekong region.

After a preliminary report from China on the performance of hepatitis B vaccine (HepB vaccine) stored at ambient temperature,[7] studies in Indonesia have confirmed the feasibility[8] and success (in terms of immunogenicity)[9] of storing it outside the cold chain (OCC) for administration within a few days of birth to infants born at home. These field studies have made use of the thermostability of HepB vaccine, as demonstrated in controlled animal[10] and human studies,[11,12] and as verified by WHO.[13] However, published field studies[7,9] lack detail on the temperatures at which the vaccine was exposed, the average duration of OCC storage prior to use, and in one case[9] failed to allow for maternal antibody amongst the young infants assessed.

From 1998 to 2001, we implemented a project to evaluate three alternative strategies for the introduction of HepB vaccine in rural Vietnam. Two of these strategies included the administration of a birth dose after storage of the vaccine at ambient temperature for up to one month. We used a locally produced, low-dose vaccine, at that time the only option for the Expanded Program on Immunization (EPI) for Vietnam.

The project was evaluated by assessing the operational success of the different strategies used and their scientific outcome in terms of the immunogenicity and protective efficacy of the vaccine used. We add to the existing knowledge on field use of HepB vaccines after storage at ambient temperature by comparing the immunogenicity of three doses of the local vaccine after storage of the first dose OCC or refrigerated.

METHODS

Project timing and location. Field work was conducted from 1998 to 2000 and took place in three geographically, ethnically, and economically distinct districts of Thanh Hoa province (population approximately 3.4 million, population density = 305/km^2), which is located approximately 160 km south of Hanoi, the capital of Vietnam. This evaluation was conducted in two of these districts, Quang Xuong and Ngoc Lac, which are described elsewhere.[2]

Project activities began in nine geographically dispersed communes in each district in early 1998. The population of these communes was approximately 107,000 and the prevailing population growth rate was approximately 1.5%.

Vaccine. The HepB vaccine used was produced by the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi. It is a plasma-derived vaccine that has been assessed by the national licensing authority as safe, immunogenic, and heat stable. The vaccine is sold to the EPI at a dosage of 2.5 μg/0.5 mL of hepatitis B surface antigen (HBsAg) protein in two-dose vials. No information was available regarding the lot-to-lot variation of this vaccine or whether the vaccine used came from the same or different lots. Independent testing for the HBsAg content of the vaccine was not possible, but the same national licensing authority was involved in vaccine quality assurance during the project period.

Vaccination strategies. In Vietnam the EPI is conducted as a monthly activity and reliable refrigeration is not available beyond district level in most areas. In project areas, the first

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EPI contact of many infants was not until two or more months after birth. To improve this, alternate strategies of HepB vaccination were introduced. In the first strategy, birth doses were available using vials stored at ambient temperature (OCC) in commune health centers for up to one month, followed by two additional doses stored inside the cold chain (ICC) of the EPI and given at ages two and three months. In the second strategy, all doses of HepB vaccine were stored ICC, and were thus only available on the monthly EPI day.

The OCC strategy was introduced in six communes and the ICC strategy in three communes in each of the two districts. Since the capacity of certain communes to administer a birth dose of HepB vaccine was anticipated to be limited, allocation of these 18 communes to their respective strategies was geographically dispersed, but not random, to ensure that an adequate number of infants received a birth dose for our research purposes.

Vaccination commenced in these communes in November 1998. Doses were provided free of charge and at the time these were the only rural communes in the country to have introduced free, routine HepB vaccination.

Serologic and vaccination survey. The OCC and ICC groups were compared by means of a serologic survey conducted over an eight-day period in mid-October 2000. Venous blood was collected from 1,106 infants 9–18 months of age, the entire reported birth cohort. Data pertaining to the birth dates of infants and each dose of HepB vaccine were obtained from EPI registers held at the health center of each commune.

Informed oral consent for participation of their infants was given by mothers after a community-wide publicity campaign before this survey. All project activities including this survey were reviewed and approved by the NIHE, the seat of the Vietnam EPI, leaders of the participating communities, and the Vietnam Ministry of Health. Mothers and infants who participated were given a small, non-monetary gift in return for their cooperation.

Analysis of samples. After separation of the samples on the evening of collection, serum was frozen for later analysis at NIHE. The NIHE participates in the WHO quality assurance program and conducted serologic testing for this study according to standard laboratory procedures. All sera were coded with a unique identifier, but laboratory staff were not blind as to the origin of each sample. All sera were assessed by enzyme-linked immunosorbent assay (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) for HBsAg. The HBsAg-negative samples were tested for antibody to HBsAg (anti-HBs). Quality assurance testing was not done in this survey because laboratory capacity was previously deemed adequate.[2]

Data analysis. Data were entered by two different persons using Epi-Info version 6.04d (Centers for Disease Control and Prevention, Atlanta, GA) and analyzed using Stata release 6 (Stata Corporation, College Station, TX). Only infants who had received three doses of vaccine were included in analyses of anti-HBs titers. The geometric mean titer (GMT) was used to compare anti-HBs in infants grouped by district and first-dose strategy (OCC or ICC). Because of the skewed distribution of anti-HBs levels, for comparison among infants within the OCC group the titer of each infant was transformed by taking its natural logarithm. Comparisons between groups of infants were performed using the chi-square test for proportions and the *t*-test for means. Multiple linear regression was used to examine the associations between independent variables and anti-HBs titers, simultaneously adjusting for potential confounders. Variables were selected into the multivariate model based on prior knowledge, and included the quadratic terms of two continuous variables known to influence anti-HBs titer, the delay between doses 2 and 3[14–16] and that between dose 3 and sampling,[17] and the delay between birth and dose 1. It also included the two-way interaction between district and strategy.

To further assess the validity of the calculated GMTs, predicted anti-HBs levels were also assessed for each group of infants based on the mathematical model available at http://www2.stat.unibo.it/palareti/vaccine.htm.[17] This model enables the prediction of anti-HBs levels on any date in the future based on the titer at baseline and the delay until it is measured again. To make these estimates, the GMT for each group was compared with the actual level from the group with the shortest delay between dose 3 and sampling.

RESULTS

Survey sample characteristics and vaccination data. The sample of 1,106 infants was composed of 583 boys and 523 girls 9–18 months of age (mean = 13.7). Five hundred sixty-six were in Quang Xuong and 540 were in Ngoc Lac. Seventy hundred forty-eight received dose 1 of vaccine stored OCC and 358 received vaccine stored ICC.

Most infants (98.4% in Quang Xuong and 77% in Ngoc Lac, total = 973) had received all three doses of vaccine at the time of sampling. There were wide differences in the delay between doses 2 and 3. In Quang Xuong, in each case the mean delay in this interval for OCC, ICC, and all communes was 31 days, compared with 58, 83, and 60 days, respectively, in Ngoc Lac. In each case these differences were significantly longer in Ngoc Lac ($P < 10^{-4}$ for each comparison).

As expected, the mean delay between dose 3 and the date of sampling varied widely. For infants in Quang Xuong (324 days) it was much longer than those in Ngoc Lac (257 days) ($t = 11.4$, $P < 10^{-5}$). When assessed by strategy, the mean delay was also longer among infants in OCC (300 days) than ICC (283 days) communes ($t = 2.5$, $P = 0.01$). However, when broken down by district, there was no difference in this delay between infants in OCC (324 days) and ICC communes (323 days) in Quang Xuong, but a large difference between Ngoc Lac OCC (272 days) and ICC communes (205 days) ($t = 5.4$, $P < 10^{-5}$).

Prevalence of HBsAg. The overall proportion of infants who were HBsAg positive was 6.5% (n = 72), a decrease of 6% from the 12.5% in our published baseline survey.[2] The current proportion ranged from 5% in Quang Xuong (n = 28), a decrease of 3.3% from baseline, to 8.2% (n = 44) in Ngoc Lac, a decrease of 8.3% from baseline.

Prevalence of anti-HBs. Overall, anti-HBs was detected among 81.5% of 1,105 infants able to be tested (95% confidence interval [CI] = 79.1–83.8). A protective level of anti-HBs (≥ 10 mIU/mL) was present in 80.3% (95% CI = 77.8–82.7) of 1,068 infants for whom sufficient serum was available for quantitative testing. Among the 996 HBsAg-negative infants for whom quantitative anti-HBs levels were measured, 86.1% (95% CI = 83.8–88.2) were protected.

Table 1 shows the prevalence of a protective level of anti-

TABLE 1

Prevalence of a protective level of antibody to hepatitis B virus surface antigen (anti-HBs) after three doses of hepatitis B vaccine among infants by first dose strategy, and number of days of heat exposure for dose 1 in communes storing vaccine outside the cold chain (OCC)*

	ICC communes (95% CI)	OCC communes: days of storage outside the cold chain	
		1–14	15–31
All infants	77.9 (72.4–82.7) (n = 271)	83.6 (78.4–88.0) (n = 250)	82.7 (78.9–86.1) (n = 452)
Quang Xuong	78.2 (71.4–84.0) (n = 179)	83.7 (77.3–88.9) (n = 172)	83.5 (77.7–88.3) (n = 206)
Ngoc Lac	77.2 (67.2–85.3) (n = 92)	83.3 (73.2–90.8) (n = 78)	82.1 (76.7–86.7) (n = 246)

* A positive result was assumed for the small number of infants from whom there was insufficient blood to test quantitatively for anti-HBs; these infants were equally distributed across the groups. ICC = within the cold chain; CI = confidence interval.

HBs after three doses of vaccine by district and strategy and, in the relevant communes, by the number of days dose 1 was OCC. Prevalence did not differ between any groups of infants (by chi-square test).

Influences on immunogenicity of the vaccine. The GMT of anti-HBs among the 858 HBsAg-negative infants for whom sufficient serum was available was 121 mIU/mL (95% CI = 111–134). Table 2 shows the GMTs among infants who had received three doses of vaccine by district and strategy. The titer was slightly higher in ICC than OCC communes, but this difference was not significant on crude analysis ($t = 1.19$, $P = 0.23$). Infants in Ngoc Lac had significantly higher titers than those in Quang Xuong both overall ($t = 2.92$, $P < 0.01$) and in the OCC group ($t = 2.50$, $P = 0.01$), but not in the ICC group ($t = 1.34$, $P = 0.18$).

Table 2 also shows GMTs differentiated by duration of storage of dose 1 OCC. In Quang Xuong, where mean dose intervals did not vary across communes, there was no difference between the titers of infants in ICC communes (GMT = 114 mIU/mL) and those in communes storing dose 1 OCC for 15–31 days (94 mIU/mL) ($t = 0.84$, $P = 0.40$). A similar comparison was not attempted for infants in Ngoc Lac because of the widely differing intervals between doses 2 and 3, and thus delays between dose 3 and sampling.

Figure 1 shows a dot plot of the natural logarithms of anti-HBs titers of infants in the OCC communes according to the number of days the vial containing their first dose had been kept at ambient temperature. The line connects the medians of these logarithms for all infants receiving dose-1 on each day. The flat slope of this line suggests no relationship between anti-HBs titers and the duration for which dose 1 was stored OCC.

In multivariate analysis, we simultaneously controlled for strategy (OCC or ICC), district, the interaction between strategy and district, the age and sex of the infant, the age of the mother, and the intervals (in days) between birth and dose 1, dose 2, and dose 3, and between dose 3 and the survey. The result confirmed that infants in Ngoc Lac had a higher GMT than those in Quang Xuong ($P = 0.003$) and that there was no difference in anti-HBs levels between infants in ICC and OCC communes ($P = 0.80$). No other variable was found to be associated with anti-HBs titer.

Anti-HBs decay. Table 2 also shows the GMTs of anti-HBs predicted by extrapolating from the group with the shortest delay between dose 3 and sampling (Ngoc Lac ICC group) using a published mathematical model of anti-HBs decay.[17] Although there was overall support for the decay model, in each case in Quang Xuong the actual GMT was lower than predicted.

Temperature exposure. Table 3 shows the average maximum and minimum temperatures in the two project districts. The distinct seasonal changes noted are typical of lowland northern Vietnam and also of many other tropical countries north of the Tropic of Cancer. However, night-time temperatures during the summer months are higher than in many tropical areas and have an impact on the average temperature to which vaccine OCC was exposed.

DISCUSSION

These results provide more evidence for the immunogenicity of HepB vaccine after a period of storage at ambient temperature. Overall, the vaccine used was immunogenic, regardless of storage of the vials used for dose 1 for up to one month OCC in a tropical environment. There were no differences between the prevalence of a protective level of anti-HBs or the GMT of antibody measured in groups of infants who either received all three doses of this vaccine stored ICC, or OCC for the first and ICC for the remaining two doses. There was also no relationship between the duration of storage OCC and these two parameters.

It is possible that maternal antibody was still present in

TABLE 2

Geometric mean titer (GMT) of antibody to hepatitis B virus surface antigen and predicted GMT* by district and storage strategy, and GMT among infants in OCC communes according to number of days dose 1 was OCC

District and strategy		GMT (actual) (95% CI)	Predicted GMT*	GMT by days of storage OCC	
				1–14 days	15–31 days
District	Quang Xuong	100 (88–112)	120		
	Ngoc Lac	150 (128–175)	151		
Strategy	OCC	113 (101–126)	130	111 (91–135)	115 (100–131)
	ICC	135 (111–163)	137		
Quang Xuong	OCC	93 (81–108)	120	93 (73–118)	94 (78–113)
Ngoc Lac		141 (119–166)	143	160 (112–231)	136 (113–164)
Quang Xuong	ICC	114 (93–140)	121		
Ngoc Lac†		189 (127–283)	189		

* Allowing for decay in antibody between dose 3 and bleeding, in comparison to the Ngoc Lac ICC group which had the shortest such delay, based on http://www2.stat.unibo.it/palareti/vaccine.htm. OCC = outside the cold chain; ICC = within the cold chain.

† The titer of this group was used to adjust that of others in calculating the predicted GMT.

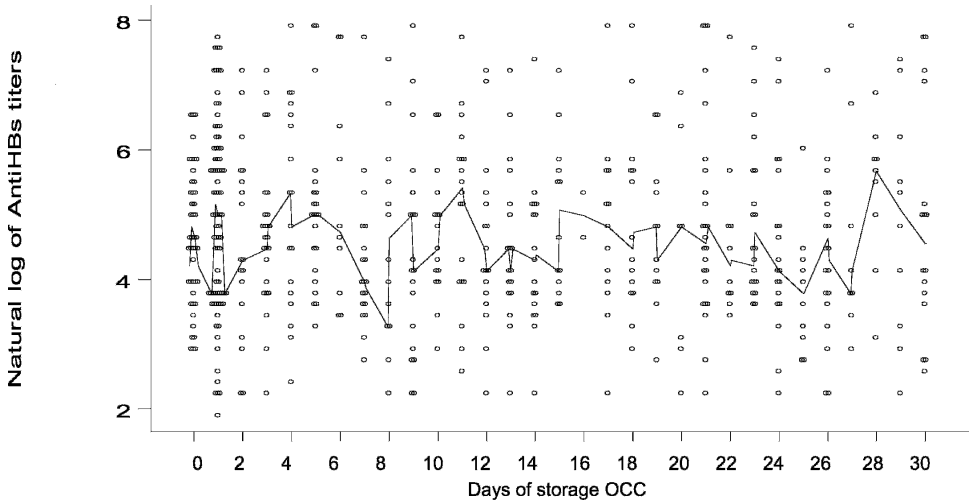

FIGURE 1. Dot plot of the natural logarithm of antibody to hepatitis B surface antigen (anti-HBs) among infants born in OCC communes according to duration of heat-exposure of dose 1 in days, showing median titer for each day. The line represents median anti-HBs titers of infants receiving vaccine on each day. OCC = outside the cold chain.

some of the youngest infants sampled, but not in more than a few percent[16] and presumably equally across the groups. It is also possible that one or even two of the doses in any individual infant or group was impotent, but that the anti-HBs response was still adequate to provide protection. However, if this was more common among the infants in OCC communes and due to heat spoilage, one might expect the GMT to be lower in this group, which it was not.

We were careful to allow for programmatic influences on the titers of antibody among infants in the different groups, and the analyses suggest that these variables did influence anti-HBs titer. In Ngoc Lac, there were longer intervals between doses 2 and 3 of vaccine, which has usually been shown to result in higher antibody titers.[14–16,18] This applied overall, as well as in ICC and OCC communes, when assessed separately. A shorter interval between the last dose of vaccine and the collection of blood allows less time for antibody decay.[17] This interval was shorter in Ngoc Lac, but it was also much shorter in Ngoc Lac ICC than OCC communes. This difference did not apply in Quang Xuong; thus, the overall shorter delay in ICC than OCC communes must have been due to the influence of Ngoc Lac. We conclude that the differences in the titers of anti-HBs observed between the different groups of infants may be explained by differences in the timing of doses between the two districts and between ICC and OCC communes in Ngoc Lac, and not by any other variable assessed.

Ngoc Lac ICC communes clearly performed relatively poorly in vaccinating infants on time. As described, the choice of vaccine storage for the nine communes in each district was not random. Of necessity for our research on the efficacy of the vaccine used, in some cases this choice included consideration of whether staff in those communes would likely be able to deliver a birth dose of vaccine to all infants, as required by the OCC strategy. Some Ngoc Lac ICC communes were more remote or had a weaker preventive health service than others. This obviously affected their capacity to deliver vaccines in a timely way.

The relatively low rate of a protective level of anti-HBs (80.3%) in this study warrants comment. The dosage of HBsAg protein in the vaccine assessed is lower than that used in many commercially available HepB vaccines. In another unpublished study, we evaluated the immunogenicity of the same locally produced, plasma-derived HepB vaccine in two different doses and compared it with that of two 10 μg/dose Korean recombinant HepB vaccines. In that trial, after vaccine storage in experimental conditions at 2–8°C and administration of all doses to each infant by the same nurse or doctor, the 2.5 μg/dose vaccine induced a protective level of antibody in 85.9% of 71 eight-month old infants whose mothers were seronegative for all HBV markers. This was not as high as the percentages among those given 5 μg doses of this vaccine or the Korean vaccines, but compared well to another field trial of a HepB vaccine of similar HBsAg content.[19]

In addition, the rate of 80.3% was achieved under field conditions, with the associated risks of vaccine freezing (which can render it impotent[10]), variable standard injection techniques, and a wide variation in scheduling. Infants in this

TABLE 3
Average monthly minimum and maximum temperatures (°C) in the two districts during the months of birth of infants surveyed

		1999									
District		Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Quang	Minimum	18.9	22.8	23.8	25.8	27.2	25.6	24.6	22.9	19.9	13.5
Xuong	Maximum	24.0	27.0	29.0	31.7	34.5	31.7	31.1	27.9	25.2	19.6
Ngoc Lac	Minimum	19.1	21.3	23.1	25.4	25.4	25.0	24.0	22.5	19.7	12.5
	Maximum	25.7	29.4	29.9	33.8	32.9	31.4	31.4	29.1	25.9	20.9

study were also older than those in the controlled study, which allowed time for antibody decay. Finally, overall rates of birth dosing were rather low in this cohort of infants, which may have influenced rates of perinatal infection and thus HBsAg prevalence. The background rate of HBsAg positivity among mothers in the two districts surveyed was 15% in our previous survey.[2] Thus, it seems likely that a number of infants were infected by their mothers very early in life, which affected the rate of response to the vaccine. The higher rate of protection among HBsAg-negative infants (86.1%) almost certainly includes a higher proportion of infants born to HBsAg-negative mothers, and is therefore reassuring with regard to the overall immunogenicity of the vaccine. Our studies suggest that higher antigen content in the local vaccine might yield a higher rate of protection, but in most infants the existing formulation, which will be phased out in 2006, is immunogenic and protective into the second year of life.

The GMTs calculated by the decay model were lower in Quang Xuong than predicted. Our use of the model assumed that infants were selected from the same population and represented by the group with the shortest delay between dose 3 and sampling. However, this was not the case because the infants in Quang Xuong were vaccinated with a shorter interval between doses 2 and 3, which is a key factor in the highest level of anti-HBs achieved before commencement of decay, than those in the reference group (Ngoc Lac ICC communes). Thus, although there was support for the model in the Ngoc Lac group, it was not appropriate to use it for infants who were vaccinated according to a different schedule. This is further indirect evidence for the influence of the interval between doses 2 and 3 on anti-HBs levels.

The prevalence rates of HBsAg among the infants surveyed were not as low as in most field trials of HepB vaccine.[18] A discussion of these findings is beyond the scope of this report, but they may relate to the low dose of vaccine used,[20] vaccination technique, the timing of the first dose (which was often late in Ngoc Lac), or possibly freezing of the vaccine before it was taken OCC.

This report adds to the existing literature on use of HepB vaccine OCC and expands upon certain parameters not previously discussed. In particular, as well as providing information on the range of ambient temperatures to which dose 1 of the vaccine was exposed, we have shown that the prevalence of anti-HBs or the GMT induced did not vary according to the duration of this exposure up to one month. This concurs with the existing scientific information on heat stability of HepB vaccine,[7,9,11–13] and provides further support for its storage OCC in rural areas of developing countries that lack refrigeration. Moreover, the recent application of high-temperature vaccine vial monitors on HepB vaccines of many manufacturers and the assertion of WHO that these may be used to monitor vaccines stored OCC[21] is also reassuring on the safety and effectiveness of HepB vaccines stored OCC.

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REFERENCES

1. World Health Organisation, 1992. Expanded program on immunization. Global Advisory Group–Part 1. *Wkly Epidemiol Rec 67:* 11–15.
2. Hipgrave DB, Nyugen TV, Vu NH, Hoang TL, Do TD, Tran NT, Jolley D, Maynard JE, Biggs BA, 2003. Hepatitis B infection in rural Vietnam and the implications for a national program of infant immunization. *Am J Trop Med Hyg 69:* 88–94.
3. Tran VB, Buu M, Nguyen TM, Morris GE, 1993. Hepatitis B in Ho Chi Minh City, Viet Nam. *Trans R Soc Trop Med Hyg 87:* 262.
4. Nakata S, Song P, Duc DD, Nguyen XQ, Murata K, Tsuda F, Okamoto H, 1994. Hepatitis C and B virus infections in populations at low or high risk in Ho Chi Minh and Hanoi, Vietnam. *J Gastroenterol Hepatol 9:* 416–419.
5. Katelaris PH, Robertson G, Bradbury R, Tippett G, Hoa DQ, Ngu MC, 1995. Seroprevalence of hepatitis viruses in children in rural Viet Nam. *Trans R Soc Trop Med Hyg 89:* 487.
6. *Vietnam Demographic and Health Survey, 2002*. Calverton, MD: Committee for Population, Family and Children (Vietnam) and ORC Macro., 2003.
7. Anonymous, 1991. Hepatitis B vaccine delivery outside the cold chain: the Long An County, China example. *Global Perspectives Hepatitis 2:* 3–4.
8. Sutanto A, Suarnawa IM, Nelson CM, Stewart T, Soewarso TI, 1999. Home delivery of heat-stable vaccines in Indonesia: outreach immunization with a prefilled, single-use injection device. *Bull World Health Organ 77:* 119–126.
9. Otto BF, Suarnawa IM, Stewart T, Nelson C, Ruff TA, Widjaya A, Maynard JE, 1999. At-birth immunisation against hepatitis B using a novel pre-filled immunisation device stored outside the cold chain. *Vaccine 18:* 498–502.
10. Diminsky D, Moav N, Gorecki M, Barenholz Y, 2000. Physical, chemical and immunological stability of CHO-derived hepatitis B surface antigen (HBsAg) particles. *Vaccine 18:* 3–17.
11. Just M, Berger R, 1988. Immunogenicity of a heat-treated recombinant DNA hepatitis B vaccine. *Vaccine 6:* 399–400.
12. van Damme P, Cramm M, Safary A, Vandepapeliere P, Meheus A, 1992. Heat stability of a recombinant DNA hepatitis B vaccine. *Vaccine 10:* 366–367.
13. Galazka A, Milstien J, Zaffram M, 1998. *Thermostability of Vaccines. Global Programme for Vaccines and Immunization*. Geneva: World Health Organization. WHO/GPV/98.07.
14. Hadler SC, de Monzon MA, Lugo DR, Perez M, 1989. Effect of timing of hepatitis B vaccine doses on response to Vaccine in Yucpa Indians. *Vaccine 7:* 106–110.
15. West DJ, 1993. Scope and design of hepatitis B vaccine clinical trials. Ellis RW, ed. *Hepatitis B Vaccines in Clinical Practice*. New York: Dekker Inc., 159–178.
16. Moulia-Pelat JP, Spiegel A, Martin PM, Cardines R, Boutin JP, Roux JF, Excler JL, Saliou P, 1994. A 5-year immunization field trial against hepatitis B using a Chinese hamster ovary cell recombinant vaccine in French Polynesian newborns: results at 3 years. *Vaccine 12:* 499–502.
17. Honorati MC, Palareti A, Donzani D, Busachi CA, Rizzoli R,

Facchini A, 1999. A mathematical model predicting anti-HBs decay after vaccination against hepatitis B. *Clin Exp Immunol 116:* 121–126.

18. Mast E, Mahoney F, Kane M, Margolis H, 2004. Hepatitis B vaccine. Plotkin S, Orenstein W, Offit P, eds. *Vaccines*. Fourth edition. Philadelphia: W. B. Saunders Co., 299–337.
19. Lo KJ, Tsai YT, Lee SD, Wu TC, Wang JY, Chen GH, Yeh CL, Chiang BN, Yeh SH, Goudeau A, Coursaget P, Tong MJ, 1985. Immunoprophylaxis of infection with hepatitis B virus in infants born to hepatitis B surface antigen-positive carrier mothers. *J Infect Dis 152:* 817–822.
20. Andre FE, Zuckerman AJ, 1994. Review: protective efficacy of hepatitis B vaccines in neonates. *J Med Virol 44:* 144–151.
21. Department of Vaccines and Biologicals, 2002. *Getting Started with Vaccine Vial Monitors*. Geneva: World Health Organization. WHO/V&B/02.35.