Am. J. Trop. Med. Hyg., 59(5), 1998, pp. 837–842

RESPONSE TO CONJUGATE *HAEMOPHILUS INFLUENZAE* B VACCINE AMONG INFANTS IN NIAMEY, NIGER

GERARD CAMPAGNE, AMADOU GARBA, ANNE SCHUCHAT, DENIS BOULANGER, BRIAN D. PLIKAYTIS, MARIAMA OUSSEINI, AND JEAN-PHILIPPE CHIPPAUX

Centre de Recherche sur les Meningites et les Schistosomoses, Niamey, Niger; Respiratory Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; Centre de Sante Maternelle et Infantile de Yantala, Niamey, Niger

Abstract. Despite near elimination of *Haemophilus influenzae* b (Hib) meningitis from several industrialized countries following introduction of conjugate Hib vaccines into infant immunization schedules, Hib remains a major cause of meningitis and pneumonia in resource-poor countries. In Niger, Hib causes nearly 200 cases of meningitis per 100,000 children < one year of age, and > 40% of cases are fatal. We evaluated the immunogenicity of Hib polysaccharide–tetanus toxoid conjugate vaccine (PRP-T) administered in the same syringe as diphtheria-tetanus-pertussis (DTP) vaccine among infants in Niger. Infants were randomized into group 1 (PRP-T at six, 10, and 14 weeks), group 2 (PRP-T at 10 and 14 weeks), or a control group (meningococcal A/C polysaccharide vaccine). By 14 weeks of age, all subjects in groups 1and 2 had ≥ 0.15 μg/ml of anti-PRP antibody, and 82% versus 76% had ≥ 1.0 μg/ml of antibody (P = not significant). By nine months of age the proportion of infants with ≥ 0.15 and ≥ 1.0 μg/ml was group 1 = 97% and 76%; group 2 = 93% and 67%; controls = 10% and 2.6%. Four weeks after the first, second, and third doses of PRP-T, infants in group 1 showed geometric mean titers (GMTs) of 0.19, 3.97, and 6.09 μg/ml while infants in group 2 had GMTs of 2.40 and 4.41 μg/ml four weeks after the delayed first and second doses. Both PRP-T groups had significantly higher GMTs at 18 weeks and nine months of age than infants in the control group. The Hib PRP-T vaccine was immunogenic in infants in Niger. The strong response after PRP-T was initiated one month after the first DTP vaccination may reflect carrier priming. Two dose schedules of PRP-T should be given serious consideration, particularly if their reduced cost permits vaccine introduction that would be otherwise unaffordable.

Routine use of *Haemophilus influenzae* b (Hib) conjugate vaccines in infant immunization programs has led to near elimination of Hib meningitis from several industrialized countries.[1,2] Although *H. influenzae* is a major cause of meningitis in developing countries and is associated with high case fatality and substantial neurologic sequelae, Hib vaccines are not routinely used in countries with limited resources.[3–6] In countries within the meningitis belt of sub-Saharan Africa, the contribution of *H. influenzae* b to childhood meningitis may be unrecognized since microbiologic confirmation of meningitis cases is typically limited to a few reference hospitals, and concern about epidemics of meningococcal meningitis may overshadow appreciation for the importance of specific causes of endemic meningitis.

Review of data regarding bacterial meningitis in Niamey, Niger from 1981 to 1996 identified a minimal estimate for the incidence of meningitis due to *H. influenzae* of 195/100,000 children less than one year of age and 52/100,000 children less than five years of age. More than 80% of Hib meningitis cases among children less than five years of age occurred during the first year of life (Figure 1), with 36% evident by six months of age. The early peak of disease evident in Niamey is typical of other developing countries, and has important implications for design of suitable vaccination programs for these areas.[3–5]

Although numerous studies in industrialized countries have demonstrated the immunogenicity, efficacy, and effectiveness of Hib conjugate vaccines against invasive Hib disease,[7] relatively little information is available regarding the response to these vaccines in children in sub-Saharan Africa, where the presence of nutritional deficiencies, concurrent illnesses such as malaria, or socioeconomic conditions might lead to differences in vaccine-induced protection.[8–11] Recent demonstration that Hib conjugate vaccines protected infants in the Gambia from severe pneumonia as well as Hib meningitis suggest the enormous impact on childhood morbidity that these vaccines may have in Africa, where acute respiratory infections are a leading cause of childhood deaths.[11]

We determined the response among Nigerien infants to an Hib conjugate (tetanus toxoid) vaccine, PRP-T (Pasteur Mérieux Sérums and Vaccins, Lyon, France) administered in two different schedules in conjunction with routine vaccinations of the Expanded Program on Immunization (EPI). We compared a standard three-dose primary series with a delayed schedule to assess whether two doses of PRP-T administered with the second and third dose of diphtheria-tetanus-pertussis (DTP) vaccine provided comparable immunogenicity to the three-dose regimen, and could therefore offer a more economical way to protect young children in Africa. Since vaccine cost has been a limiting factor in adoption of Hib vaccines worldwide, these data have implications for other countries considering adoption of Hib vaccines where cost is currently a barrier.

METHODS

Site. The study was conducted between January and November 1995 at the Yantala Center for Maternal and Child Health (CSMI) in Niamey, Niger. Niamey (1995 estimated population = 550,000) is located in southern Niger, which lies within the meningitis belt of sub-Saharan Africa. The Yantala CSMI serves a population of approximately 100,000 residents of west Niamey and offers family planning, prenatal care, well-child visits, consultations for sick children, and provides vaccinations through the EPI. All phases of this study were integrated into routine EPI activities at the Center.

Subjects. Children between the ages of four and 12 weeks were recruited for the study upon presentation for routine

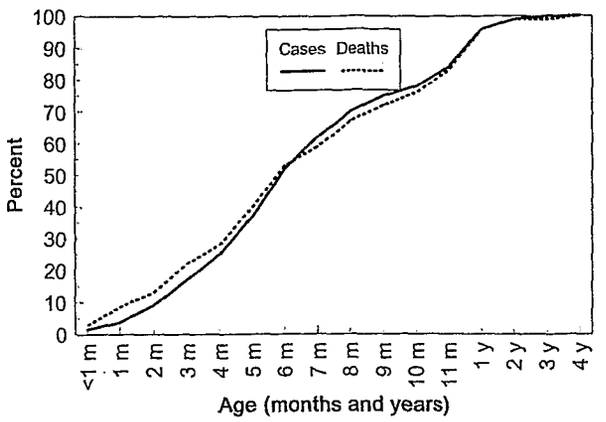


FIGURE 1. Cumulative age-specific incidence of *Haemophilus influenzae* type b meningitis cases (n = 611, 1981–1986) and deaths (n = 106, 1989–1996) in children < five years of age in Niamey, Niger.

EPI administration of the first diphtheria-tetanus-whole cell DTP vaccination. Parents gave informed consent to permit their children to participate in the study.

Study vaccine. The study vaccine was a single lot of polyribosylribitol phosphate-tetanus toxoid (PRP-T) conjugate vaccine produced by Pasteur Mérieux Sérums and Vaccins prepared with 10 μg of polysaccharide capsular antigen conjugated to tetanus toxoid. Lyophilized PRP-T vaccine was reconstituted with DTP for administration in the same syringe. The study vaccine or DTP alone was administered subcutaneously in the right subscapular fossa. Infants in the control group received serogroup A/C meningococcal polysaccharide vaccine produced by Pasteur Mérieux Sérums and Vaccins.

Expanded Program on Immunization vaccines. The EPI vaccines were provided to all infants enrolled in the study according to Niger's immunization schedule. Infants recruited into the study had already received BCG and oral polio vaccine (Pasteur Mérieux Sérums and Vaccins) at birth. At ages six, 10, and 14 weeks, they received a dose of DTP vaccine (Pasteur Mérieux Sérums and Vaccins) subcutaneously in the right subscapular region as well as a dose of oral polio vaccine (Pasteur Mérieux Sérums and Vaccins). At nine months of age, measles and yellow fever (Pasteur Mérieux Sérums and Vaccins) vaccines were administered.

Study design. Children enrolled in the study were randomized into one of three groups (Table 1). Group 1 received three doses of PRP-T at the same time as DTP injections, scheduled for six, 10, and 14 weeks of age. Group 2 also received three doses of PRP-T, but administered with the second and third DTP injections at 10 and 14 weeks of age, and the last dose at nine months of age, at the visit when measles and yellow fever vaccines were administered. Children in group 3 served as controls. These infants received two doses of meningococcal A/C polysaccharide vaccine at six and 14 weeks of age. Infants in the control group were given three doses of PRP-T vaccine at the end of the study (age nine months), with one month intervals between vaccinations.

Blood was collected by heel, toe, or fingerstick before each vaccination for groups 1 and 2 at six, 10, 14, and 18 weeks, as well as at nine months of age. Subjects in the control group had blood collected at six weeks, 18 weeks, and nine months of age (Table 1).

The study sample size was designed to permit follow-up of a minimum of 38 children per group.

Reactogenicity. Children in the study were observed in the clinic for 15 min after each vaccination. Local and systemic reactions were evaluated in a sample of children from groups 1 and 3 one day after the first vaccination. Evaluation was conducted by a physician working at Yantala CSMI who was blinded to the study group of the infant.

Laboratory methods. Antibody directed against the polysaccharide PRP was measured by the Farr-type radioimmunoassay (RIA) method and expressed in terms of micrograms per milliliter. The reference serum for these assays used human anti-Hib (CBER/FDA lot 1983; Office of Biological Reagents and References, Food and Drug Administration, Bethesda, MD) containing 70 μg/ml of antibody. Assays were performed at the Clinical Seroimmunology Laboratory of Pasteur Mérieux Sérums and Vaccins on coded specimens. The lower limit of antibody detectable by the assay was 0.07 μg/ml. The PRP-antibody concentrations of ≥ 0.15 μg/ml and ≥ 1.0 μg/ml are considered to reflect short-term and long-term protection, respectively.

Statistical analysis. The SAS software version 6.12 (SAS Institute, Cary, NC) was used for the analyses. Proportions of subjects in each study group that reached antibody concentrations ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at each age were

TABLE 1
Schedule of vaccination and blood collection by study group*

Group	Age at each contact				
	6 weeks (4–12 weeks)	10 weeks (8–16 weeks)	14 weeks (12–20 weeks)	18 weeks (16–24 weeks)	9 months (8–9 months)
1: Hib	1 dose Blood sample	1 dose Blood sample	1 dose Blood sample	– Blood sample	– Blood sample
2: Hib	– Blood sample	1 dose Blood sample	1 dose Blood sample	– Blood sample	1 dose Blood sample
3: Mnc A + C (control)	1 dose Blood sample	– –	1 dose –	– Blood sample	– Blood sample
All groups EPI vaccines	OPV/DTP	OPV/DTP	OPV/DTP		Yellow fever Measles

* Parenthesis refers to authorized range of age, in weeks or months, at each contact; Hib = *Haemophilus influenzae* type b; – = no vaccination or blood sample; Mnc A + C = *Neisseria meningitidis* serogroup A and C polysaccharide vaccine; OPV = oral polio vaccine; DTP = diphtheria-tetanus-pertussis vaccine; EPI = Expanded Program on Immunization.

TABLE 2

Anti-PRP levels expressed as geometric mean titers (GMT) in Nigerien children who received different schedules of PRP-T (groups 1 and 2) or control vaccine*

	Age (weeks or months)				
	6 weeks (prevaccination)	10 weeks	14 weeks	18 weeks	9 months
Group	GMT (95% CI)	GMT (95% CI)	GMT (95% CI)	GMT (95% CI)	GMT (95% CI)
1	0.06 (0.04, 0.09)	0.19 (0.11, 0.34)	3.97 (2.74, 5.74)	6.09 (3.67, 10.09)	2.14 (1.35, 3.40)
2	0.06 (0.04, 0.10)	0.03 (0.02, 0.05)	2.40 (1.70, 3.39)	4.41 (3.03, 6.42)	1.67 (1.07, 2.61)
3 (control)	0.06 (0.04, 0.11)	–	–	0.04 (0.02, 0.08)	0.03 (0.02, 0.05)

* GMT in micrograms per milliliter; CI = confidence interval; – = no data.

compared using the chi-square test or, where appropriate, Fisher's exact test. A mixed model analysis of variance (ANOVA) was used to examine differences in geometric mean serologic antibody concentrations among the vaccine and control groups. The vaccine and control groups and the serum bleed number accounted for the fixed effects; the random assignment of individuals to one of the three study groups constituted the random effect. The mixed ANOVA was performed taking into account the repeated measures design of this study and the inherent collinearity among bleeds within an individual. A resultant longitudinal data analysis was performed using the MIXED procedure in SAS. For a number of specimens, the lower and upper limit of antibody concentrations were not titrated precisely (i.e., the lower limit was < 0.07 μg/ml and the upper limit ranged from > 2.2 to > 22.0 μg/ml). An accelerated failure time model was used to calculate geometric mean concentrations and 95% confidence intervals using the LIFEREG procedure in SAS. This method adjusted for the left and right censoring of these data.

Ethical approval. This study was approved by the Ethics Committee of the government of Niger as well as by the Institutional Review Boards of the Centers for Disease Control and Prevention (Atlanta, GA) and the World Health Organization (Geneva, Switzerland).

RESULTS

A total of 180 children were recruited for the study (Group 1= 59, Group 2 = 62, Group 3 = 59), 87 (48.3%) of whom were female. The mean age at each visit was recruitment = eight weeks (range = 4–12); second contact = 13 weeks (range = 9–22); third contact = 18 weeks (range = 13–29); fourth contact = 24 weeks (range = 18–38 weeks), and final contact = nine months. Of these, 151 (83.9%) children completed the study. Sufficient sera for RIA analysis were available for 165 subjects at enrollment, 134 (74%) at the fourth contact, and 119 (66%) at the nine-month visit.

Adverse reactions. No serious adverse reaction was identified in any study subject. There was no difference between the frequency of fever, vomiting, diarrhea, excessive crying, or refusal to feed between groups 1 and 3. Swelling at the point of injection was more common among subjects who received the Hib vaccine (4 of 19) than among those who received the meningococcal polysaccharide vaccine (1 of 30) ($P = 0.07$, by Fisher's exact test, two-tailed).

Immune response. Table 2 shows the geometric mean titers (GMTs) of anti-PRP antibody at baseline and subsequent visits for each study group. There was no difference between baseline anti-PRP antibody titers between children in the three groups. Children in groups 1 and 2 had significantly higher antibody levels than the control group at the 18-week and nine-month visit.

Infants in group 1 showed increased antibody levels after each dose, with a peak GMT at 18 weeks of 6.09 μg/ml (95% confidence interval [CI] = 3.67, 10.09). Subjects in group 2 responded strongly to the first PRP-T dose administered at the 10-week visit, reaching a GMT of 2.40 (95% CI = 1.70, 3.39) four weeks later. Antibody titers at 18 weeks did not differ significantly between groups 1 and 2. By nine months of age, GMTs decreased to 2.14 in children who had received three doses of PRP-T and 1.67 in children who had received two doses. These results did not differ significantly.

The proportion of children in each study group with antibody levels ≥ 0.15 and ≥ 1.0 μg/ml is shown in Table 3. Prior to vaccination, children in the three groups did not differ in the proportion with antibody levels ≥ 0.15 or ≥ 1.0 μg/ml. As expected, a higher proportion of children in group 1 than group 2 had antibody levels ≥ 0.15 or ≥ 1.0 μg/ml at the 10-week visit, when only group 1 infants had previously received PRP-T vaccine. Four weeks after the second PRP-T injection in group 1 and after the first PRP-T injection in group 2, all subjects had antibody levels > 0.15 μg/ml, and a similar proportion (82% and 76%) had levels ≥ 1.0 μg/ml. The proportion of children in group 1 and group 2 with levels ≥ 0.15 or ≥ 1.0 μg/ml at subsequent visits did not differ significantly.

Additional analyses revealed no effect of the precise age at vaccination or the interval (in weeks) between vaccinations on subsequent PRP antibody levels. There were too few subjects with high baseline PRP antibody to assess whether maternally acquired antibody to PRP affected response to subsequent PRP-T vaccine.

DISCUSSION

The PRP-T Hib conjugate vaccine administered in the same syringe as the DTP vaccine in conjunction with the routine childhood immunization schedule was immunogenic in infants in Niger. Overall, 86% of the children achieved antibody levels ≥ 1.0 μg/ml four weeks after a primary series of either two or three doses of PRP-T, and 71% maintained this level by nine months of age; 99% of the infants had antibody levels ≥ 0.15 μg/ml four weeks after the second or third PRP-T immunization and 95% maintained this level at nine months of age. These data suggest that Hib

TABLE 3
Proportion of infants with antibody levels of ≥0.15 and ≥1.0 μg/ml by study group*

	Age (weeks or months)									
	6 weeks	10 weeks		14 weeks		18 weeks		9 months	
Group	≥0.15 (%)	≥1.0 (%)	≥0.15 (%)	≥1.0 (%)	≥0.15 (%)	≥1.0 (%)	≥0.15 (%)	≥1.0 (%)	≥0.15 (%)	≥1.0 (%)
1	17/56 (30.4)	1/56 (1.8)	26/47 (55.3)	8/47 (17.0)	50/50 (100)	41/50 (82.0)	43/44 (98.0)	39/44 (88.6)	36/37 (97.3)	28/37 (75.7)
2	15/54 (27.8)	4/54 (7.4)	9/48 (18.8)	0/48 (0.0)	37/37 (100)	27/37 (72.9)	54/54 (100.0)	45/54 (83.3)	40/43 (93.0)	29/43 (67.4)
3 (control)	20/55 (36.4)	4/55 (7.3)	–	–	–	–	8/36 (22.2)	1/36 (2.8)	4/39 (10.3)	1/39 (2.6)

* No significant difference between group 1 and group 2 in % ≥0.15 or ≥1.0 μg/ml at 14 weeks, 18 weeks, or nine months of age. $P < 0.001$ for difference between group 1 and group 3 at ages 18 weeks and nine months, and between group 2 and group 3 at ages 18 weeks and nine months. $P < 0.001$ for difference in % ≥0.15 μg/ml between group 1 and group 2 at 10 weeks and $P = 0.003$ for difference in % ≥1.0 μg/ml between group 1 and group 2 at age 10 weeks. – = no data.

PRP-T vaccine is likely to provide excellent protection against Hib disease when administered to young infants according to the EPI schedule. There was little difference in the antibody levels at 18 weeks and nine months of age between children who had received two versus three doses of PRP-T vaccine when the two-dose schedule was delayed to begin one month after the first dose of DTP vaccine. Antibody response to Hib vaccines has varied in different studies according to diverse factors, including age at immunization, type of conjugate vaccine, and whether the vaccine is administered in the same syringe as other antigens.[12–18] Response to certain Hib conjugate vaccines varied in different populations, suggesting that factors such as nutrition, pre-existing antibody to Hib or other antigens, or genetic influences could potentially affect immune response.[19,20] It is therefore reassuring to find that Hib antibody levels among infants in Niamey vaccinated with PRP-T were comparable with those identified in children in industrialized countries as well as those identified in recent studies of PRP-T vaccine in the Gambia,[10,11] since this suggests that efficacy against invasive Hib disease in Niger is likely to be comparable with the high levels observed elsewhere.

Given the large burden of Hib disease in sub-Saharan Africa, and the excellent immune response identified in this study as well as those conducted in the Gambia, the potential benefits of routine use of Hib conjugate vaccine among infants in this region are evident.[10,11] However, cost of vaccine has been a major barrier to adoption of Hib conjugate vaccines even among wealthier, transitional economies.[21] Although the price of vaccines supplied to the poorest countries is often negotiated through large volume purchases by international donors and may be provided below cost to certain regions, reducing the number of doses necessary for an immunization series could reduce cost, and consequent price, substantially. Our study addressed the question of whether infants immunized with a delayed two-dose schedule of vaccine during the first months of life would have similar protection to infants who received three doses.

By four weeks after the last vaccination and nine months of age, GMTs against PRP were similar for the three-dose and two-dose groups, with both groups achieving antibody levels significantly higher than those among control subjects who received meningococcal A/C polysaccharide vaccine. The GMT following the first dose of PRP-T in infants vaccinated at 10 weeks of age (2.40 μg/ml) was significantly higher than the GMT obtained after the first dose of PRP-T was administered at six weeks of age (0.19 μg/ml). Although some of this difference may reflect improved response in infants immunized at an older age, the strong response to PRP-T among infants in group 2 may also reflect the response to carrier priming, since infants in this group had received tetanus toxoid included in the DTP vaccine administered at six weeks of age, and may therefore have been primed to have enhanced response to the tetanus toxoid carrier included in the PRP-T vaccine given four weeks later.[22] These data suggest that omitting administration of a dose of PRP-T with the first DTP injection should not have adverse consequences on short- and longer-term immune response, at least measured up to nine months of age, or through much of the at-risk period among infants in Niger. Although there may be logistic difficulties associated with scheduling dif-

ferent vaccines for administration at the six- and 10-week visits, these data suggest that where this schedule is feasible, a reduced number of doses of PRP-T should offer comparable protection to infants at lower cost.

An additional concern in considering the optimal number of Hib doses is the magnitude of Hib disease that occurs before the delayed schedule would provide protection, since if this is large, the cost savings of eliminating a dose may be outweighed by the benefits of a three-dose schedule that protects against early disease. Surveillance for Hib meningitis in Niamey during the period 1981–1996 revealed that only 40 (6.5%) of 611 cases of Hib meningitis among children < five years of age occurred in infants < 12 weeks of age; 17 (2.8%) occurred among infants between eight and 12 weeks of age, cases that would potentially be prevented only by the traditional three-dose schedule (assuming two weeks are needed for antibody production following vaccination with the conjugate vaccine). Despite the official EPI vaccination schedule of six, 10, and 14 weeks of age, children in this trial received routine and study vaccines somewhat later (8, 13, and 18 weeks of age). One potential risk of delaying the first dose of PRP-T is that children in certain areas may not receive their second DTP injection until much later in infancy. Consideration of local circumstances, such as the coverage and timeliness of infant immunizations, can help EPI program managers and policymakers determine whether a delayed, two-dose schedule of PRP-T is appropriate for their setting. Other approaches, such as neonatal or maternal immunization programs, may address the earliest cases of infant Hib disease in areas where these cases constitute an important burden.[23]

We did not evaluate response to a booster dose of Hib conjugate vaccine. Data from the United Kingdom, where no booster dose is used, suggest that in an industrialized country, the primary series is adequate to dramatically reduce Hib meningitis.[22] The earlier onset of Hib disease in developing countries might make a booster dose even less important for these populations, since most disease, 84% in Niamey, occurs by one year of age. However, carriage of Hib is more common among children in developing countries.[24] The impact of the infant vaccination series on rates of carriage and disease in older children is not clear, although vaccinating older children had substantial impact on disease rates in younger infants in industrialized countries.[1,2] Because the EPI infrastructure for delivering vaccines in Africa focuses on children less than one year of age, proposing a vaccination schedule that requires a dose administered to older children is problematic. Since most Hib disease occurs before 12 months of age, and exposure to carriers is likely to boost vaccine-induced immunity among children as they age, the potential value of a booster dose of Hib vaccine in developing countries is unlikely to merit the cost and infrastructure development it would entail.

Infants included in this study were typical of those residing in Niger, and probably representative of children throughout sub-Saharan Africa, in terms of nutritional status and exposure to other endemic diseases such as malaria. Vaccine administered in this study, provided in the same syringe as DTP by EPI personnel administering routine immunizations, was well-tolerated and immunogenic. These data therefore extend the observations collected in the Gambia regarding the potential benefits of Hib vaccine in children in Africa[11] and are probably applicable to other tropical, resource poor areas where Hib disease rates are high.[25] These findings also suggest that two-dose schedules should be given serious consideration, particularly when the economic savings of limiting the series to two doses would be sufficient to prompt policymakers to introduce Hib vaccines into routine use.

The excellent immune response evident among Nigerien infants suggests that these vaccines could prevent substantial illness in this region. Although much attention is routinely given to meningococcal epidemics, surveillance in Niamey over a six-year period including a major epidemic (1994–1995) revealed that meningitis deaths due to Hib and pneumococcus exceeded those due to meningococcus. Until success is achieved with other approaches to reducing the cost of Hib vaccines, such as through research into economic production of entirely synthetic Hib vaccine or investigations of lower dose formulations,[26] reducing the number of injections may be the best means available to permit the largest number of the world's children the benefit of protection against *H. influenzae* b disease.

Acknowledgments: We thank the personnel at CSMI Yantala, especially Aissa Moumouni, for excellent assistance in conducting the study; and Dr. Soga Garba (Ministry of Public Health, Niger) and Dr. Bernard Ivanoff (World Health Organization) for assistance in development of the study. We are also grateful to Dr. Christine Blondeau (Pasteur Mérieux Sérums and Vaccins) for performing the laboratory assays, to Drs. Pierre Saliou, Michel Piollet, and Chantal Ethevenaux (Pasteur Mérieux Sérums and Vaccins) for providing vaccines and technical advice.

Financial support: This work was supported by the French Ministry of Cooperation and the World Health Organization Global Program on Vaccines.

Authors' addresses: Gerard Campagne and Amadou Garba, Centre de Recherche sur les Meningites et les Schistosomoses, BP 10887, Niamey, Niger. Anne Schuchat, Respiratory Diseases Branch, Centers for Disease Control and Prevention, Mailstop C-23, Atlanta, GA 30333. Denis Boulanger and Jean-Philippe Chippaux, Institut Français de Recherche Scientifique pour le Développement en Coopération/Centre de Recherche sur les Meningites et les Schistosomoses/ Organisation de Coopération et de Coordination pour la lutte Contre les Grandes Endémies, BP 10887, Niamey, Niger. Brian D. Plikaytis, Biostatistics and Information Management Branch, Centers for Disease Control and Prevention, Mailstop C-09, Atlanta, GA 30333. Mariama Ousseini, CSMI Yantala, Niamey, Niger.

Reprint requests: Anne Schuchat, Respiratory Diseases Branch, Centers for Disease Control and Prevention, Mailstop C-23, Atlanta, GA 30333.

REFERENCES

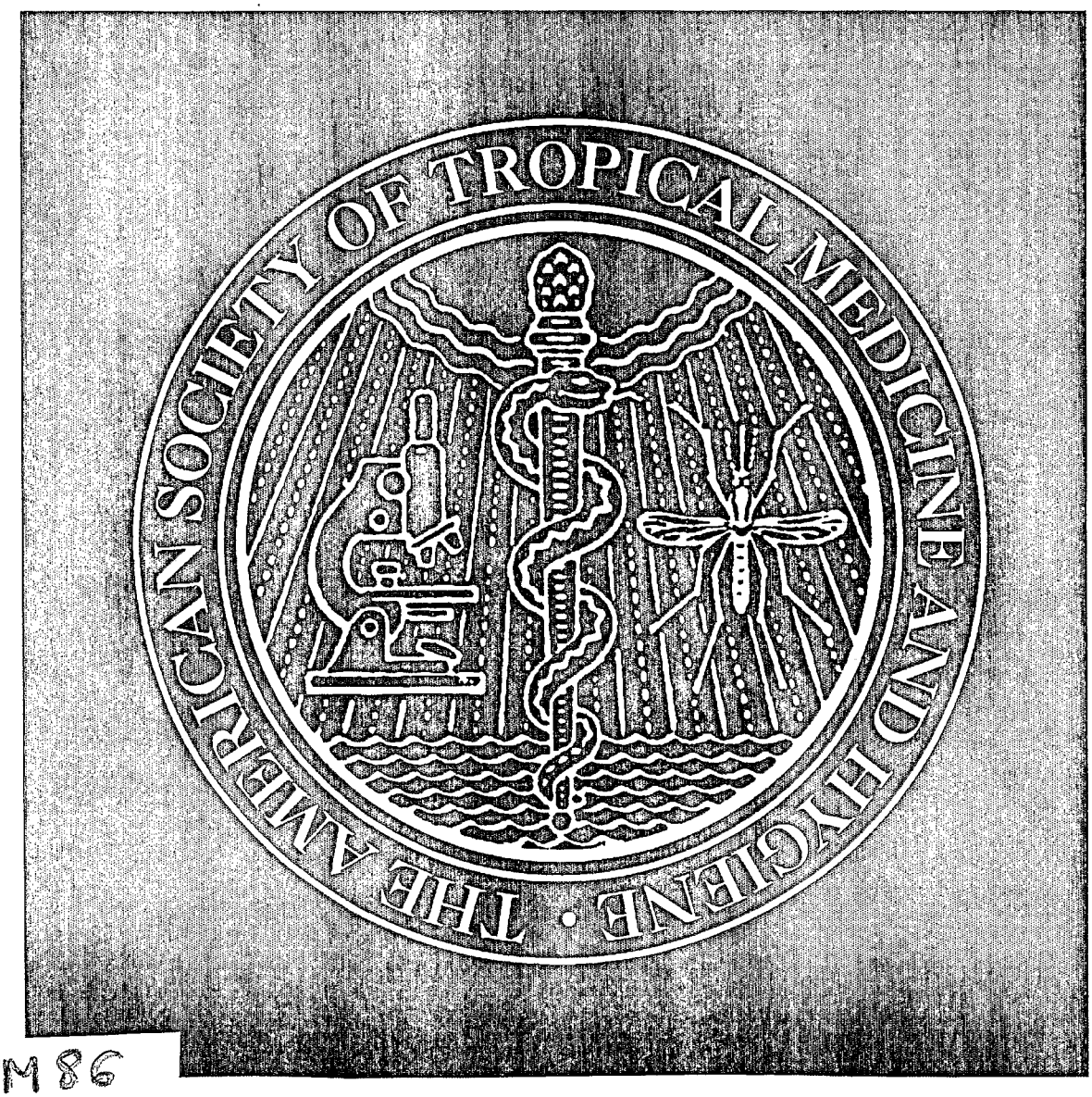
1. Adams WG, Deaver KA, Cochi SL, Plikaytis BD, Zell ER, Broome CV, Wenger JD, 1993. Decline of childhood *Haemophilus influenzae* type b (Hib) disease in the Hib vaccine era. *JAMA 269:* 221–226.
2. Peltola H, Kilpi T, Anttila M, 1992. Rapid disappearance of *Haemophilus influenzae* type b meningitis after routine childhood immunization with conjugate vaccines. *Lancet 340:* 592–594.
3. Funkhouser A, Steinhoff MC, Ward J, 1991. *Haemophilus influenzae* disease and immunization in developing countries. *Rev Infect Dis 13 (suppl 6):* S542–S554.
4. Bjilmer HA, van Alphen L, Greenwood BM, Brown J, Schnei-

der G, Hughes A, Menon A, Zanen HC, Valkenburg HA, 1990. The epidemiology of *Haemophilus influenzae* meningitis in children under five years of age in The Gambia, West Africa. *J Infect Dis 161:* 1210–1215.

5. Cadoz M, Denis F, Diop Mar I, 1981. Etude épidémiologique de cas de méningites purulentes hospitalisés à Dakar pendant la décennie 1970-1979. *Bull OMS 59:* 575–584.
6. Pécoul B, Varaine F, Keita M, Soga G, Djibo A, Soula G, Abdou A, Etienne J, Rey M, 1991. Long-acting chloramphenicol versus intravenous ampicillin for treatment of bacterial meningitis. *Lancet 338:* 862–866.
7. Ward JH, 1994. Influenzae vaccines. Plotkin SA, Mortimer EA Jr, eds. *Vaccines.* Second edition. Philadelphia: W.B. Saunders Co., 33–386.
8. Campbell H, Byass P, Ahonkai VI, Vella PP, Greenwood BM, 1990. Serologic response to an *Haemophilus influenzae* type b polysaccharide-*Neisseria meningitidis* outer membrane protein conjugate vaccine in very young Gambian infants. *Pediatrics 86:* 102–107.
9. Mulholland EK, Byass P, Campbell H, Fritzell B, Greenwood AM, Todd J, Greenwood BM, 1994. The immunogenicity and safety of *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine in Gambian infants. *Ann Trop Paediatr 14:* 183–188.
10. Mulholland EK, Hoestermann A, Ward JI, Maine N, Ethevenaux C, Greenwood BM, 1996. The use of *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine mixed with diphtheria-tetanus-pertussis vaccine in Gambian infants. *Vaccine 14:* 905–909.
11. Mulholland K, Hilton S, Adegbola R, Usen S, Oparaugo A, Omosigho C, Weber M, Palmer A, Schneider G, Jobe K, Lahai G, Jaffar S, Secka O, Lin K, Ethevenaux C, Greenwood B, 1997. Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate for prevention of pneumonia and meningitis in Gambian infants. *Lancet 349:* 1191–1197.
12. Decker MD, Edwards KM, Bradley R, Palmer P, 1992. Comparative trial in infants of four conjugate *Haemophilus influenzae* type b vaccines. *J Pediatr 120:* 184–189.
13. Granoff DM, Anderson EL, Osterholm MT, Holmes SJ, McHugh JE, Belshe RB, Medley F, Murphy TV, 1992. Differences in the immunogenicity of three *Haemophilus influenzae* type b conjugate vaccines in infants. *J Pediatr 121:* 187–194.
14. Kayhty H, Eskola J, Peltola H, Ronnberg P-R, Kela E, Karanko V, Saarinen L, 1991. Antibody responses to four *Haemophilus influenzae* type b conjugate vaccines. *Am J Dis Child 145:* 223–227.
15. Holmes SJ, Murphy TV, Anderson RS, Kaplan SL, Rothstein EP, Gan VN, Granoff DM, 1991. Immunogenicity of four *Haemophilus influenzae* type b conjugate vaccines in 17- to 19-month-old children. *J Pediatr 118:* 364–371.
16. Capeding MRZ, Nohynek H, Pascual LG, Kayhty H, Sombrero LT, Eskola J, Ruutu P, 1996. The immunogenicity of three *Haemophilus influenzae* type b conjugate vaccines after a primary vaccination series in Philippine infants. *Am J Trop Med Hyg 55:* 516–520.
17. Watemberg N, Dagan R, Arbelli Y, Belmaker I, Morag A, Hessel L, Fritzell B, Bajard A, Peyron L, 1991. Safety and immunogenicity of *Haemophilus* type b-tetanus protein conjugate vaccine, mixed in the same syringe with diphtheria-tetanus-pertussis vaccine in young infants. *Pediatr Infect Dis J 10:* 758–761.
18. Dagan R, Botujansky C, Watemberg N, Arbelli Y, Belmaker I, Ethevenaux C, Fritzell B, 1994. Safety and immunogenicity in young infants of *Haemophilus* b-tetanus protein conjugate vaccine, mixed in the same syringe with diphtheria-tetanus-pertussis-enhanced inactivated poliovirus vaccine. *Pediatr Infect Dis J 13:* 356–361.
19. Eskola J, Peltola H, Takala AK, Käyhty H, Hakulinen M, Karanko V, Kela E, Rekola P, Rönnberg PR, Samuelson JS, Gordon LK, Mäkelä PH, 1987. Efficacy of *Haemophilus influenzae* type b polysaccharide-diphtheria toxoid conjugate vaccine in infancy. *N Engl J Med 317:* 717–722.
20. Ward J, Brenneman G, Letson GW, Heyward WL, and the Alaska *H. influenzae* Vaccine Study Group, 1990. Limited efficacy of a *H. influenzae* type b conjugate vaccine in Alaska Native infants. *N Engl J Med 323:* 1381–1387.
21. Steinhoff MC, 1997. *Haemophilus influenzae* type b infections are preventable everywhere. *Lancet 349:* 1186–1187.
22. Booy R, Heath PT, Slack MPE, Begg N, Moxon ER, 1997. Vaccine failures after primary immunisation with *Haemophilus influenzae* type-b conjugate vaccine without booster. *Lancet 349:* 1197–1202.
23. Kurikka S, Olander, R, Eskola J, Kayhty H, 1996. Passively acquired anti-tetanus and anti-*Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine in infancy. *Pediatr Infect Dis J 15:* 530–535.
24. Bijlmer HA, Campbell H, Van Alphen LWA, Greenwood BM, Valkenburg HA, Zanen HC, 1989. Carriage of *Haemophilus influenzae* in healthy Gambian children. *Trans R Soc Trop Med Hyg 83:* 831–835.
25. Bryan JP, de Silva HR, Tavares A, Rocha H, Scheld WM, 1990. Etiology and mortality of bacterial meningitis in northeastern Brazil. *Rev Infect Dis 12:* 128–135.
26. Mendelman PM, Feeley L, Bird S, Staub T, Matthews H, Del Beccaro MA, Overturf G, Lee A, Ellis R, Staub J, Szymanski S, Donnelly J, Hennessey JP, Kniskern P, 1997. Immunogenicity and safety of *Haemophilus influenzae* type b polysaccharide-*Neisseria meningitidis* conjugate vaccine in 7.5 mg liquid formulation: a comparison of three lots with the 15.0 mg lyophilized formulation. *Vaccine 15:* 775–781.

VOLUME 59 NOVEMBER 1998 NUMBER 5

The American Journal of TROPICAL MEDICINE & HYGIENE



OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

ISSN 0002-9637