Vitamin A Supplementation at Birth Delays Pneumococcal Colonization in South Indian Infants[1]

Christian L. Coles,* Lakshmi Rahmathullah,† Reba Kanungo,** R. D. Thulasiraj,‡ Joanne Katz,*[2] Mathuram Santhosham* and James M. Tielsch*

*Department of International Health, John Hopkins School of Hygiene & Public Health, Baltimore 21205; †Aravind Center for Women, Children And Community Health, Madurai, India; **Department of Clinical Microbiology, Jawaharlal Institute of Post-graduate Medical Education and Research, Pondicherry, India and ‡Executive Secretariat, Lions-Aravind Institute for Community Ophthamology, Madurai, India

ABSTRACT Nasopharyngeal colonization is a risk factor for pneumococcal disease, a leading cause of complications and death in infants. We assessed the impact of vitamin A supplementation in reducing pneumococcal colonization in infants from an area with endemic vitamin A deficiency. We recruited 464 2-mo-old infants from a rural area in South India. Infants were randomly assigned to receive two 7000-μg retinol equivalent doses of vitamin A (n = 239) or placebo (n = 225) orally at birth, and nasopharyngeal specimens were collected at ages 2, 4 and 6 mo. We studied the effect of vitamin A on culture-confirmed pneumococcal colonization and on the distribution of pneumococcal serotypes. Analyses were conducted by intention-to-treat. The risk of colonization among infants aged 4 mo who were not colonized by age 2 mo was significantly reduced in the vitamin A group compared with the placebo group [odds ratio 0.51 (0.28, 0.92), P = 0.02). The odds of colonization were 27% lower in the treatment group than in the placebo group [odds ratio 0.73 (0.48, 1.1), P = 0.13]. No differences were detected in the prevalence of invasive serotypes. The risk of colonization with penicillin-resistant isolates was 74% lower in the vitamin A group than in the placebo group at 2 mo of age. However, the prevalence of penicillin-resistant isolates was only 4%. Neonatal vitamin A supplementation may play a role in lowering morbidity rates associated with pneumococcal disease by delaying the age at which colonization occurs. J. Nutr. 131: 255–261, 2001.

KEY WORDS: • *vitamin A supplementation* • *pneumococcal carriage* • *pneumococcal colonization* • *community trial* • *India*

Pneumococcal infections are a leading cause of complications and death in young children worldwide, resulting in ~650,000 infant deaths in the developing world each year (Garenne et al. 1992, World Health Organization 1997). *Streptococcus pneumoniae* is a leading cause of fatal bacterial pneumonia, the most common cause of otitis media and sepsis in children younger than 2 y and a leading cause of meningitis in infants (World Health Organization 1997). Case management of pneumonia, which relies on early diagnosis and prompt treatment with antibiotics, has been effective in reducing pneumonia-related deaths by 50% (Sazawal and Black 1992). However, the recent emergence and spread of drug-resistant strains may make this strategy less effective and more expensive (Butler et al. 1998, Hart and Kariuki 1998).

Preventive strategies have significant advantages over treatment in children with pneumonia. Vaccination is the optimal solution, but currently available vaccines contain antigens that are not immunogenic in those in whom the risk of pneumonia-related death is greatest: children younger than 2 y (Douglas et al. 1983, Sniadack et al. 1995). Although a seven-valent pneumococcal conjugate vaccine was recently licensed for use in the United States, several candidate pneumococcal conjugate vaccines containing 9–11 of the more invasive serotypes are currently being evaluated for use in developing countries (Eskola and Anttila 1999, Shinefield et al. 1999). Unfortunately, conjugate vaccines are expensive to produce and may not be affordable in developing countries for some time (Eskola and Anttila 1999, Shann and Steinhoff 1999, Sniadack et al. 1995). Moreover, temporal, age and geographic variations in serotype distributions present serious challenges to vaccine development. Research on alternative or adjuvant prevention strategies is therefore indicated.

Studies in several countries with endemic vitamin A deficiency and high rates of pneumococcal disease have reported rapid and abundant pneumococcal colonization of the nasopharynx in early infancy (Berman 1991, Gratten et al. 1986, Lloyd-Evans et al. 1996, Woolfson et al. 1997, World Health Organization 1995). Although the relationship between car-

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[2] To whom correspondence should be addressed at Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe St., Room W5515, Baltimore, MD 21205. E-mail: jkatz@jhsph.edu

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riage and infection is not well understood, there is evidence to suggest that pneumococcal strains that bind tightly to the respiratory tract cause local infections (i.e., otitis media and pneumonia), whereas those that bind loosely are responsible for systemic infections (i.e., bacteremia and meningitis) (Andersson et al. 1986, Stenfors and Raisanen 1992). Laboratory studies suggest that several components of mucosal immunity play important roles in inhibiting pneumococcal colonization (Stenfors and Raisanen 1993). Secretory antibody (sIgA)[3] interferes with bacterial adhesion to mucosal surfaces, and nonspecific barrier defenses lyse and clear aspirated bacteria from the respiratory tract (Kurono et al. 1991, Niederman et al. 1986, Virolainen et al. 1995). Low serum retinol concentrations are associated with impaired mucosal immunity and reflect alterations in tissue integrity, decreasing sIgA and diminishing the effectiveness of the mucociliary clearance of bacteria (Chandra 1988, Biesalski and Stofft 1992, Semba 1998, Sirisinha et al. 1980). These changes may result in greater bacterial adherence and an apparent increase in bacterial colonization.

This body of evidence suggests that reversal of vitamin A deficiency may reduce the rate of colonization and decrease associated morbidity rates. In addition to lowering the risk for pneumococcal infection in the individual, decreasing carriage could lower the pool of infection in the community. Alternatively, supplementation may delay colonization, in which case it would lower morbidity rates in the age group at highest risk. Under these conditions, vitamin A supplementation may present a cost-effective approach in the event that immunization is not effective or as an adjunct to vaccination for decreasing the risk of pneumococcal disease in infants in developing countries.

Results from a recent multicenter, hospital-based study in India reported that the most common invasive serotypes/groups in children younger than 5 y were 1, 4, 5, 6, 7, 12, 14,19 and 45 (Anonymous 1999). Serotypes 1 and 5 accounted for 29% of all disease. A candidate vaccine that includes nine serotypes (1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F) frequently associated with invasive pneumococcal disease in developing countries is undergoing phase III trials in South Africa (Mbelle et al. 1999). Should vitamin A narrow the distribution of invasive pneumococcal serotypes/groups that colonize the nasopharynx, it could alter vaccine formulation and reduce the cost of pneumococcal conjugate vaccines. Similarly, if vitamin A reduces the likelihood of colonization with resistant serotypes, it may help to maintain the efficacy of inexpensive antibiotics used to treat pneumococcal infections through reduction in the likelihood of resistance.

We conducted a randomized, double-blind, placebo-controlled vitamin A supplementation trial to evaluate the impact of vitamin A on pneumococcal nasopharyngeal colonization in young South Indian infants in an area of endemic vitamin A deficiency.

METHODS

Study population. The population of the Infant Pneumococcal Acquisition/Colonization in Tamil Nadu (InPACT) study was drawn from the ongoing Vitamin A Supplementation in Newborns (VASIN) trial. VASIN is a 3-y trial that was initiated on August 13, 1998, to evaluate the impact of vitamin A supplementation administered at birth on mortality, infectious disease morbidity and growth rates in newborns through the first 6 mo of life. The trial is being carried out in the two rural areas of Natham and Karriyapatty in the south Indian state of Tamil Nadu. These two areas are characterized by endemic vitamin A deficiency typical of rural areas in the state (Rahmathullah et al. 1990, Rahi et al. 1995, Ramakrishnan et al. 1995, Rahmathullah and Vennila 1994), with a high incidence of acute respiratory infections and demographic similarities typical of many rural communities in South Asia.

VASIN enrollment. Women >12 wk pregnant who live in or plan to deliver in the study area are eligible for enrollment in the VASIN trial. Participation in the trial is voluntary, and oral consent is mandatory for enrollment. Eligible women are identified through a variety of sources, including local nongovernment organizations, government- and nongovernment organization–run antenatal care clinics and traditional birth attendants. At the time of enrollment, demographic data are collected along with expected due date and information on plans for delivery. Women are randomized into groups to have their newborn receive either two doses of 7000 μg retinol equivalents (RE) of oil-soluble vitamin A (Hoffman-LaRoche, Nutley, NJ) or placebo within 48 h of birth. The decision to use two 7000-μg RE doses rather than one 14,000-μg RE dose was based on safety concerns. Enrolled women are visited once every 2 wk by field staff to update any changes in plans for delivery. Within 48 h of delivery, information on the newborn's vital status, sex and weight (Seca model 727 electronic infant weighing scale) is recorded. At this time, a vitamin A supplement or placebo is administered directly into the infant's mouth by study staff. Vital status and morbidity assessments are collected every 15 d for the first 6 mo of life, and at 6 mo of age, all infants enrolled in the study receive a 30,000-μg RE dose of vitamin A.

InPACT enrollment. Four hundred sixty-four VASIN study infants between the ages of 2 and 2.5 mo were recruited into the InPACT study between October 1998 and January 1999. Assuming a control rate of colonization of 50%, our initial sample size of 464 infants enabled us to detect a 17.5% reduction in pneumococcal NP colonization [odds ratio (OR) = 0.54] with 80% power (α = 0.05), after 20% loss to follow-up. Eligible infants were identified through VASIN trial records. Study staff explained the study to parents before seeking to enroll infants in the trial. Voluntary participation was emphasized. Infants were eligible for whom parental consent was received for the InPACT trial and who lived in one of eight selected supervisory areas within the Natham district. Natham was chosen as the study area because it had several logistical advantages over Karriyapatty, including a larger and denser population. The eight supervisory areas in Natham have the highest birth rates in the district. InPACT enrollment began in October 1998, with the last follow-up completed in June 1999.

Ethical review. Both the VASIN and InPACT studies were approved by the Ethical Committee of the Aravind Eye Hospital & Institute, the Lions Aravind Institute for Community Ophthalmology and the Committee on Human Research of the Johns Hopkins University School of Hygiene and Public Health. Oral informed consent was obtained and was noted on study forms. Due to the low level of literacy in this community, verbal consent was appropriate.

Data collection. Demographic and household data were collected as part of the VASIN study. Household data include family religion and caste, number of siblings under age 5, level of maternal education, source of cooking fuel, total number of cigarettes smoked in the household per day and type of transportation owned. Data collected by field staff at the time of delivery include weight and sex of the infant, maternal history of night-blindness during pregnancy and infant colostrum ingestion.

Nasopharyngeal specimen collection. Five trained field workers collected nasopharyngeal specimens from infants enrolled in the InPACT study. These specimens were collected from each infant at 2 mo (enrollment), 4 mo and 6 mo of age. Any sample that was not collected during the period 2 d before and 14 d after the appropriate collection date was classified as a "missed visit." The protocol for nasopharyngeal swab collection called for the insertion of a small, flexible rayon-tipped swab (DIFCO Culture Swab Transport System with Amies Medium) into the posterior nasopharynx for either 5 s or

[3] Abbreviations used: InPACT, Infant Pneumococcal Acquisition/Colonization in Tamil Nadu; NGO, nongoverment organization; OR, odds ratio; RE, retinol equivalent; sIgA, secretory antibody; VASIN, Vitamin A Supplementation in Newborns.

with 180-degree rotation before removal. The swab containing the sample was then placed in Amies transport medium and was carried to the microbiology laboratory of the Aravind Eye Hospital within 10 h of collection.

Laboratory procedures. We performed standard microbiological assays for the isolation and identification of pneumococci (Facklam and Washington 1991). Within 10 h of arrival at the laboratory, nasopharyngeal swabs were inoculated onto tryptic soy agar plates (Becton Dickinson, Sparks, MD) with 5% sheep blood and 5 mg gentamicin/L (Nathan Pirumal, Bombay, India). In cases when laboratory staff was not available to inoculate the specimens within 12 h, the swabs were transferred from the Amies media into 0.5 mL of skim milk media and were frozen at −20°C for up to 2 d before inoculation. Inoculated plates were incubated at 37°C with 5% CO_2 for 18–24 h. Over a 2-d period, plates were examined for growth of colonies that displayed typical *S. pneumoniae* morphology on a daily basis. In the plates with pneumococcal growth, three or four colonies were selected, transferred to new media and reincubated with a 6-mm Optochin disk (Taxo, Franklin Lakes, NJ) for 24 h. The zone of growth inhibition surrounding the Optochin disk was the basis for confirming the presence of pneumococci. A bile solubility test (HiMedia, Mumbai, India) was performed to confirm the presence of pneumococci in the event that the results of the Optochin test were indeterminate. Quality control was maintained through the use of pneumococcal reference strains (5603; American Type Culture Collection, Rockville, MD). PNEUMOTEST kits (Staten Serum Institute, Copenhagen, Denmark) were used to serogroup/type the culture-confirmed pneumococcal isolates (Lalitha et al. 1999). The serogroups/types contained in the licensed 23-valent pneumococcal polysaccharide vaccine can be identified using the pooled antisera in the kits.

Randomization. Women enrolled in the VASIN study were assigned a unique study number. Each participant was then randomized, stratified by cluster and blocked within geographic cluster area. The block size used was four. Each cluster was composed of four or five hamlets. The randomization scheme and coded packages of supplements were prepared by project management staff members in the city of Madurai. None of these staff members were involved in the recruitment of pregnant women and follow-up of infants. The treatment assignment codes were kept in a sealed enveloped in a locked cabinet at the Johns Hopkins School of Hygiene and Public Health.

The vitamin A and placebo capsules were identical. Each packet contained two capsules and was labeled with both the participant's name and study identification number to avoid errors during dosing.

Definition and measurement of outcomes. A number of outcomes were considered in the InPACT study. The primary outcome of interest was the prevalence of pneumococcal NP carriage. A specimen with an Optochin inhibition zone of >13 mm was classified as "culture positive" for pneumococci. Those with an inhibition zone of 9–13 mm were classified as "culture indeterminate." In cases of culture indeterminate specimens, a bile solubility test was used to confirm the presence of pneumococci (Facklam and Washington 1991). The secondary outcome of interest was the prevalence of "invasive" serotypes/groups of pneumococci. Invasive serotypes were identified using PNEUMOTEST kits and were analyzed using two different definitions. The first definition of invasive serotypes included those serotypes/groups identified in a recent multihospital survey in India as most likely to cause invasive pneumococcal disease in children under the age of 5 y (serotypes/groups 1, 4, 5, 6, 7, 12, 14 and 45) (Anonymous 1999). The second definition used those serotypes/groups included in the nine-valent pneumococcal conjugate vaccine currently under evaluation for use in developing countries that has shown promise in phase III trials (serogroups 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F) (Mbelle et al. 1999). An additional outcome of interest was the prevalence of susceptibility to antibiotics including penicillin, erythromycin and co-trimoxazole. Antibiotic susceptibility was determined using the Bauer and Kirby disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (Doern 1995) (National Committee for Clinical Laboratory Standards 1998). All susceptibility testing was carried out with Mueller-Hinton medium supplemented with 5% lysed sheep blood.

Data management and statistical analysis. Several methods were used to ensure high quality data collection and management. Participants were regularly reinterviewed at random by field supervisors to verify the reliability of data collected. In addition, field supervisors conducted meetings to review data collection forms on a weekly basis. The project block officer reviewed all corrected forms. All forms were forwarded to the data center in Madurai, where the forms were tallied and the data were entered and verified. The database was analyzed for missing and illogical data, which was forwarded back to the field for correction. After final corrections were made, the master file was updated.

Treatment assignment codes for women participating in the InPACT study were broken at the Johns Hopkins School of Hygiene and Public Health. The treatment assignments have not been revealed to the project staff in India because the main VASIN trial is still under way. The effects of vitamin A supplementation on pneumococcal NP colonization and serotype distribution were analyzed by intention-to-treat. The *t* test for continuous variables and the two-tailed χ^2 analysis or Fisher's exact test for contingency data were used, as appropriate, to assess treatment group differences at baseline (Stata 6.0; Stata Corporation, College Station, TX). Where appropriate, bivariate associations between treatment and colonization at 2, 4 and 6 mo of age were determined using the two-tailed χ^2 tests or Fisher's exact test. Bivariate associations between covariates and colonization were analyzed by the same methods mentioned here. Logistic regression models were developed to analyze colonization as a function of treatment assignment using covariates that were statistically significant at a level of $P \leq 0.10$ in the bivariate analysis for at least one of the time periods. Due to the dynamics of pneumococcal colonization and the collection of samples at 2-mo intervals, the observed prevalence of pneumococcal colonization most likely underestimates the true incidence of colonization in the population studied. Therefore, the OR rather than estimates of relative risk were used to assess associations between potential risk factors and colonization.

RESULTS

Enrollment. We approached 465 of the 539 infants eligible for the InPACT trial at age 2 mo; of the 539 infants, 11 (6 in the vitamin A group and 5 in the placebo group) had died and 63 (29 in the vitamin A group and 34 in the placebo group) had migrated before becoming eligible at 2 mo of age. Mothers of infants not approached were significantly more likely to report episodes of night-blindness during pregnancy than were mothers of enrolled infants (33.3% versus 8.8%, $P = 0.02$). Mean birth weight among migrants was comparable to that of InPACT enrollees, however, the mean birth weight among infants who died was significantly lower than that of enrollees (2199.8 versus 2672.4 g, $P < 0.001$). However, they did not differ by other socioeconomic measures.

Follow-up. Of the 465 infants approached in InPACT, there was one refusal in the placebo group. Of the 464 infants enrolled, 239 (51.5%) had been randomly assigned to the vitamin A group, and 225 (48.5%) had been randomly assigned to the placebo group (**Fig. 1**). Of the enrollees, 3 (2 in the treatment group and 1 in the placebo) had not been dosed because the infants were not reachable for dosing before their 1-mo birthday. At age 4 mo, specimens were collected from 87.4% (209) of the infants in the vitamin A group and 87.6% (197) of the infants in the placebo group. By 4 mo, there were 2 deaths, 3 refusals and 34 migrations out of the study area. In addition, 19 infants were missed. During follow-up at age 6 -mo, specimens were collected from 78.7% (188) infants in the vitamin A group and 76.0% (171) in the placebo group; these included specimens collected from 13 infants who were unavailable at the 4-mo follow-up visit (7 migrants, 5 missed infants and 1 infant who parents had initially refused). Three infant deaths and 62 migrations out of the study area occurred before the 6-mo specimen collection, and 38 infants were missed. Follow-up rates at ages 4 and 6 mo were 87.5% (406 of 464) and 77.3% (359 of 464), respectively. Migration (13.4%

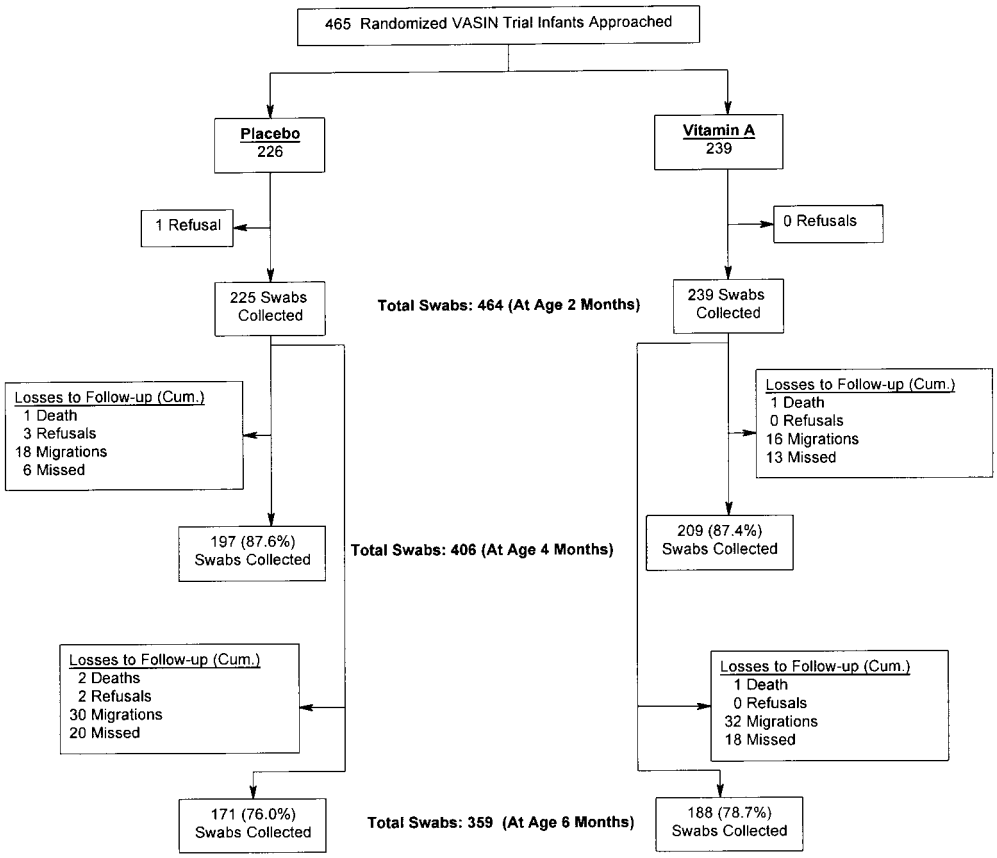

FIGURE 1 Infant Pneumococcal Acquisition/Colonization in Tamil Nadu Trial profile indicating numbers of infants enrolled, randomly assigned to placebo or vitamin A groups and followed throughout the trial. Cum., cumulative.

in the vitamin A group versus 13.3% in the placebo group) and missed infants (7.5% versus 8.9%) accounted for the majority of losses to follow-up and were similar in both treatment arms. Infants in both treatment groups who were lost to follow-up at age 4 mo were similar to infants retained in the trial. However, birth weights were significantly lower in both treatment arms among those infants lost to follow-up at 6 mo than among those followed (2580.4 versus 2693.7 g, $P = 0.025$).

Baseline comparability. Treatment groups were comparable at baseline with respect to known risk factors for acute respiratory infections. They were also similar for various socioeconomic status indicators with the exception of type of conveyance owned by the household (**Table 1**). A greater proportion of the vitamin A group reported bicycle ownership than the placebo group (40.2% versus 27.1%, $P = 0.002$).

Effect of vitamin A on pneumococcal NP colonization. At 2 mo of age, the prevalence of colonization was equivalent between vitamin A and placebo arms (**Table 2**). The odds of colonization tended to be lower (27%) in the vitamin A arm than in the placebo arm [OR 0.73 (0.48, 1.1) among infants aged 4 mo, $P = 0.13$], and by age 6 mo, the arms did not differ.

To investigate further the protective effects of vitamin A, we constructed multivariate logistic regression models that included independent predictors of colonization: infant's sex, number of siblings younger than 5 y, the number of cigarettes smoked in the household per day, socioeconomic status, history of night-blindness during pregnancy in the mother, cooking fuel, infant's colostrum intake and mother's level of education. The treatment effect was not statistically significant at ages 2, 4 and 6 mo. The adjusted OR for vitamin A protection were similar to the unadjusted OR.

Effect of vitamin A on delaying colonization. Vitamin A significantly reduced the risk of colonization among infants aged 4 mo who were not colonized at age 2 mo [OR 0.51 (0.28, 0.92), $P = 0.023$] but had no impact on the risk of colonization in those who were already colonized. There was no effect from age 2 to 6 mo or from age 4 to 6 mo (**Table 3**).

Effect of vitamin A on colonization with invasive serotypes. We evaluated the effect of vitamin A supplementation on colonization with nine invasive serotypes that are prevalent in India (**Table 4**). Colonization rates by these serotypes did not differ significantly between the two treatment groups at 2, 4 and 6 mo of age. Similarly, the distribution of serotypes included in the candidate $PncCRM_{197}$ pneumococcal conjugate vaccine was comparable between treatment groups at ages 2, 4 and 6 mo.

Effect of vitamin A on colonization with antibiotic-resistant pneumococci. We examined the impact of vitamin A on colonization with isolates resistant to four antibiotics commonly used for treating suspected bacterial pneumonia cases in India: penicillin, gentamicin, erythromycin and co-trimoxazole. Colonization with penicillin-resistant isolates was 74% lower in the vitamin A group than in the placebo group at 2 mo [OR = 0.26 (0.06, 1.16), $P = 0.08$] (**Table 5**). The power to detect differences was relatively low with penicillin, as the prevalence of penicillin resistance was 3.9%. We did not observe a similar effect in any other age group or among isolates resistant to the other three antibiotics. Approximately

TABLE 1

Comparison of distributions of infant characteristics by vitamin A treatment group, Tamil Nadu, India

Characteristic	Placebo group		Vitamin A group	
	n	%	*n*	%
Gender				
Male	124	55.1	139	58.2
Female	101	44.9	100	41.8
Birth weight, *g*[1]				
<2500	67	29.8	80	33.5
2500–2749	61	27.1	68	28.5
2750–2999	59	26.2	47	19.7
3000–3499	27	12.0	37	15.5
≥3000	11	4.9	7	2.9
Siblings <5 y, *n*				
0	92	40.9	84	35.1
1	102	45.3	121	50.6
≥2	31	13.8	34	14.2
Cigarettes smoked by household members, *n/d*				
0	103	45.8	115	48.1
1–9	52	23.1	50	20.9
10–19	44	19.6	48	20.1
≥20	26	11.6	26	10.9
Colostrum fed to newborn[2]				
Yes	181	80.4	199	83.3
No	44	19.6	40	16.7
History of night blindness during pregnancy				
Yes	20	8.9	21	8.8
No	205	91.1	218	91.2
Cooking fuel				
Wood	214	95.1	225	94.1
Bio gas	4	1.8	1	0.4
Kerosene	7	3.1	13	5.4
Land owner				
Yes	109	48.4	130	54.4
No	116	51.6	109	45.6
Mother's education, *y*				
0	106	47.1	103	43.1
≥1	119	52.9	136	56.9
Electricity				
Yes	111	49.3	117	49.2
No	114	50.7	121	50.8
Conveyance[3]				
None	143	63.6	134	56.1
Bicycle	61	27.1	96	40.2
Motorized vehicle	21	9.3	9	3.7
Roof				
Thatch	101	44.9	104	43.5
Tile	106	47.1	113	47.3
Concrete	18	8.0	20	8.4
Other	0	0.0	2	0.8

[1] Means ± SD birth weight was 2683.7 ± 414.7 and 2661.9 ± 395.2 g in the placebo and vitamin A group, respectively.
[2] All study infants were breastfed.
[3] *P*-values for comparison of distributions were >0.05 for all variables except conveyance ($P < 0.01$).

27% (92) of the isolates were resistant to three or more antibiotics.

DISCUSSION

We observed that two 7000-μg RE doses of vitamin A administered orally to neonates mostly within 48 h of birth produced a 27% reduction in the odds of pneumococcal carriage at age 4 mo but not at ages 2 and 6 mo. These findings are consistent with results from a neonatal vitamin A supplementation trial in Indonesia that showed a slight reduction in signs and symptoms that may be consistent with local pneumococcal disease (e.g., ear infections, cough with fever) among infants aged 4 mo in the vitamin A group (Humphrey et al. 1996). The results of our trial also provide some support for the finding of Chandra (1988) that vitamin A deficiency enhances bacterial colonization of the nasopharynx.

TABLE 2

Prevalence of pneumococcal nasopharyngeal colonization and odds ratios estimating vitamin A treatment effect by age at examination, Tamil Nadu, India

Age, *mo*	Placebo group		Vitamin A group		Odds ratio	95% confidence interval
	n	%	*n*	%		
2	225	54.2	237	53.6	0.97	0.68–1.41
4	196	67.9	208	60.6	0.73	0.48–1.10
6	169	69.8	183	70.5	1.03	0.65–2.62

There are little data on the impact of vitamin A supplementation on morbidity- and mortality-associated respiratory infections in vitamin A–deficient infants younger than 6 mo (Anonymous 1995). In contrast to the Indonesia trial cited here, a trial conducted in Nepal found that the relative risk of death from all causes was equivalent between the two treatment arms in infants younger than 6 mo (West et al. 1995). A potentially important difference between these two trials was the time of dosing. In the Indonesia trial, infants were dosed within 24 h of birth, whereas study infants in Nepal were dosed, on average, 2–3 wk after birth. It is possible that timing of the dose may be critical to the development of immunity to respiratory and other pathogens.

One of the limitations of this study is that we did not measure serum retinol levels at each interval. However, there is good evidence of vitamin A deficiency in the population from which the study infants are drawn, with 17–37% of preschool-age children having serum retinol levels below 0.7 μmol/L (Rahi et al. 1995, Rahmathullah et al. 1990, Rahmathullah and Vennila 1994, Ramakrishnan et al. 1995), as well as documented night-blindness in 9% of mothers of study infants. Evidence from three studies conducted in Nepal show that the prevalence of night-blindness is a sensitive indicator of endemic vitamin A deficiency and that these women are at greater risk for death, infection and anemia during pregnancy

TABLE 3

Proportion of infants colonized at the end of an age interval among those who were not colonized at beginning of that interval and odds ratios estimating the effect of vitamin A treatment on delay of colonization, Tamil Nadu, India

Age, *mo*	Placebo		Vitamin A		Odds ratio	95% confidence interval
	n	%	*n*	%		
2–4	93	66.7	99	50.5	0.51	0.28–0.91
4–6	57	59.6	66	68.2	1.45	0.69–3.04
2–6	82	64.6	86	60.4	0.84	0.44–1.57

(Christian et al. 1998a, 1998b and 2000). Hence, it is likely that the treatment effect seen here is what can be expected within a moderately deficient population. Rahman et al. (1997) examined the effect of vitamin A supplementation on cell-mediated immunity among infants younger than 6 mo in Bangladesh. Their results show cell-mediated immunity responses were improved among infants with adequate serum retinol concentrations (>0.7 μmol/L) after supplementation, but there was no improvement among children with low serum retinol levels after supplementation. Similarly, results from the trial in Indonesia showed a significant reduction in mortality rates only in infants with normal birth weight and who were not malnourished. It is possible that despite vitamin A supplementation, many of the children in our study never attained an adequate serum retinol concentration, which may explain the magnitude of the effect.

At age 4 mo, the vitamin A group tended to have a lower prevalence of colonization than the placebo group ($P = 0.13$); no effect was seen at ages 2 and 6 mo. The lack of effect at age 6 mo is consistent with data showing that the duration of the effect of a 30,000-μg RE dose of vitamin A is 2–4 mo (Sommer and West 1997). It is unclear why no effect was seen at age 2 mo. It is possible that there is an immunological maturation threshold level that is required for vitamin A to be protective. Data from laboratory studies indicate that vitamin A deficiency decreases the amount of secretory antibody in mucosal secretions (Biesalski and Stofft 1992, Beisel 1982, Semba 1998). There is evidence indicating that sIgA to pneumococcal capsular polysaccharide, which interferes with the adherence of pneumococci to mucosal epithelial cells, appears in the secretions of infants as early as 6 mo of age (Nieminen et al. 1996 and 1999). Yet, it is possible that sIgA to capsular polysaccharide may appear earlier in infants living in areas where the risk of infection is great. Alternatively, sIgA may be present in the secretions of young infants but at levels not detectable by current assessment techniques. The Indonesia trial reported a significant reduction in mortality rates between ages 1 and 4 mo.

Vitamin A significantly reduced the risk of colonization among infants aged 4 mo who were not colonized at age 2 mo but had no impact on the risk of colonization in those who were already colonized. This finding suggests that vitamin A may delay colonization and in turn lower the risk of disease in an age group at high risk for pneumococcal disease and death, although this delay is no more than 2 mo in duration. In addition, it suggests that vitamin A may have role in maintaining a barrier against colonization but has little effect once the barrier has been compromised.

TABLE 4

Prevalence of colonization with invasive serotypes and odds ratios estimating the impact of vitamin A treatment on colonization by age at examination, Tamil Nadu, India

Age, mo	Placebo group		Vitamin A group		Odds ratio	95% confidence interval
	n	%	*n*	%		
Carriage of invasive serotypes (India) by treatment group and age group						
2	89	31.5	94	28.7	0.88	0.47–1.65
4	130	28.5	123	35.8	1.40	0.82–2.38
6	117	29.9	129	28.7	0.94	0.54–1.63
Carriage of 9-valent $PncCRM_{197}$ vaccine serotypes by treatment group and age at examination						
2	89	55.1	94	46.8	0.72	0.40–1.29
4	151	32.5	144	30.6	0.92	0.56–1.50
6	138	35.5	145	30.3	0.79	0.48–1.30

TABLE 5

Prevalence of penicillin-resistant isolates and odds ratios estimating the impact of vitamin A treatment on colonization with these isolates by age at examination, Tamil Nadu, India

Age, mo	Placebo group		Vitamin A group		Odds ratio	95% confidence interval
	n	%	*n*	%		
2	88	8.0	91	2.2	0.26	0.06–1.17
4	127	5.5	122	2.5	0.43	0.11–1.66
6	115	3.5	128	2.3	0.67	0.15–3.02

The distributions of serotypes associated with invasive disease in developing countries and in India were comparable in the treatment arms in every age group. Based on these data, vitamin A supplementation is unlikely to alter the formulation or cost of producing a pneumococcal conjugate vaccine slated for use in developing countries.

The risk of colonization with penicillin-resistant pneumococci was much lower in the vitamin A group than in the placebo arm, but the prevalence of penicillin-resistance strains in this population was very low, ~4%, so the impact of this finding is likely to have minimal public health significance. Vitamin A supplementation may have a greater impact in settings where penicillin-resistant pneumococcal strains are prevalent.

Although the power to detect differences between treatment groups in this study was relatively low, the preliminary results are suggestive of a potential role for the use of vitamin A supplementation in reducing or delaying the risk of pneumococcal infections, which may reduce or delay complications and deaths from pneumococcal disease in this high-risk age group. The consequences of this intervention are likely to vary across different populations and settings because of the prevalence of pneumococcal diseases and the severity of infant malnutrition. In high exposure areas like South India, vitamin A supplementation must reduce the risk of acquisition of pneumococci soon after birth if it is to be an effective strategy for lowering the risk of pneumococcal infection in infants, but more research is warranted to explore this question. Given the growing problem of antibiotic resistance and the unrealized prospects for an affordable pneumococcal conjugate vaccine for young infants, further studies are needed to evaluate the impact of vitamin A on clinical outcomes and on lowering the pool of infection and to elucidate the conditions under which newborn supplementation may reduce the risk of pneumococcal disease in young infants in developing countries.

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