All 28 serum samples were then tested blindly for the presence of HCV RNA sequences by a standard HCV RNA reverse transcription–nested PCR protocol [2]. In accord with our previous experience with diagnosing human immunodeficiency virus by PCR [3], we tested samples with two sets of primers (two in- and two out-primers), both coding for sequences in the 5′ noncoding region of the HCV genome [1, 2]. Samples were considered reactive if they showed one positive result out of two amplifications. Positive samples were those that showed two positive results after amplification with the two primer sets (table 1).

If one considers that all samples showing at least one positive PCR result are viremic, a good correlation can be established between PCR and RIBA-2 results. Five samples were PCR- and RIBA-positive. Four were PCR-reactive and RIBA-positive. Although reactive samples were generally positive with one primer set, the use of a second set made it possible to detect reactivity in 1 more patient. This fact enforces our theory that multiple primer pairs should be routinely used for virus diagnostic purposes. In four samples we had completely different results from both assays (samples 3, 16, 18, and 28). The great divergence of results between the EIA tests from two manufacturers (Ortho and Abbott) with samples 3 and 16 (RIBA-negative, PCR-positive) is notable. Both samples showed high ratios of OD cutoff ($\geq$4.8) with the Ortho test; in contrast, ratios were $\leq$0.17 with the Abbott test.

In these cases, the 4 patients must be followed longer to establish the reason(s) for the divergent results: The RIBA-positive, PCR-negative results may be due to low virus production, genomic divergence of the isolate, or even false positivity of the RIBA; the RIBA-negative, PCR-positive results may be due to low antibody production by the patient or false positivity of the PCR assay.

In both cases in which the RIBA did not give clear results, PCR turned out to be very valuable. Sample 7 had to be classified as indeterminate according to the manufacturer's instructions. Sample 10 came from the same patient but, unfortunately, has not been tested by RIBA. However, PCR detected viremia in both samples. For sample 12 the reactivity by PCR gives support for the extreme weak positivity by RIBA.

Finally, the analysis of results suggests a division of patients into four groups: Group 1, patients with hepatitis due to HCV; group 2, patients with hepatitis of unknown etiology; group 3, probable false positivity of the ELISA; and group 4, patients to be followed because of divergent RIBA and PCR results.

We believe that the use of RIBA-2 and reverse transcriptase–nested PCR in combination is an efficient way to improve the quality of HCV diagnosis.

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Safety and Immunogenicity of *Salmonella typhi* Ty21a Liquid Formulation Vaccine in 4- to 6-Year-Old Thai Children

Colleagues—Typhoid fever remains a serious public health problem in many developing areas of the world [1, 2]. The attenuated *Salmonella typhi* Ty21a vaccine strain confers significant protection against typhoid fever without the attendant adverse reactions associated with the use of the parenteral vaccine [3–5]. A liquid Ty21a vaccine formulation has recently been found to provide greater protection than the currently available enteric-coated capsules in Chile and Indonesia [4, 5]. The vast majority of the study populations were schoolchildren (>6 years of age) and young adults. It is becoming increasingly apparent that there is a high incidence of *S. typhi* infection in children 2–5 years of age. Unfortunately, comparatively little is known about the safety and immunogenicity of the Ty21a vaccine in this high-risk age group [6].

Therefore, we conducted a randomized, double-blind, placebo-controlled trial to evaluate the safety and immunogenicity of the liquid Ty21a vaccine formulation in Thai children 4–6 years of age.

The study was conducted at 6 day care centers involving a total of 170 healthy children. The vaccine and placebo were packaged in identical-appearing aluminum foil sachets bearing a letter code. Each dose of vaccine contained 3×10^9 cfu of *S. typhi* Ty21a, while each placebo packet contained 2×10^9 heat-killed *Escherichia coli* K12. Each buffer pack was composed of 1.3 g of sodium bicarbonate and 0.8 g of ascorbic acid. The resuspended sachet was ingested within 10 min of preparation. Upon reconstitution, the Ty21a bacteria remain viable for at least 30 min. The children were observed by a physician for 1.5 h after vaccination for immediate reactions. Parents were given an adverse reaction report sheet to complete after each vaccination. Three doses of vaccine or placebo were given on alternate

Written informed consent was obtained from parents or guardians, and the trial design and consent procedure were approved by the Thai Ministry of Health.

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days. A venous blood sample was collected immediately before and 21 days after vaccination.

Anti-*S. typhi* lipopolysaccharide (LPS) antibody levels were measured by ELISA [7, 8]. A significant rise in antibody level (seroconversion) was defined as a net increase of 0.15 OD units above preimmunization values [7].

No adverse reactions that could be attributed to immunization were reported by the 88 participants who received the Ty21a vaccine. Of the 82 placebo recipients, 3 noted transient symptoms such as malaise, headache, rash, and low-grade fever.

Paired serum samples were obtained from 160 children. The serum anti-*S. typhi* LPS antibody response is shown in table 1. The seroconversion rate for vaccine recipients of all ages was 83% versus 14% for those who received the placebo ($P < .005$). There was no difference in the response rate for children aged 4, 5, or 6 (80%–86%; $P > .5$). The seroconversion rate among placebo recipients was higher than would have been predicted. We have not been able to pinpoint the reason for this observation.

Fully 37% of children (63/170) had elevated baseline antibody levels (OD $\geq$ 0.75, based on levels in North American volunteers who ingested *S. typhi* in vaccine challenge studies), suggesting prior exposure to *S. typhi* or another group D *Salmonella* species [7]. Of this subgroup, 89% of those who received vaccine seroconverted (31/35), while only 39% of placebo recipients did so (11/28; $P < .005$). This finding indicates that vaccine "take" is not inhibited by prior exposure to *S. typhi* and may boost background levels of immunity.

Prior attempts to immunize young children with Ty21a showed that ~40% of children aged 6–9 years seroconverted, while the response was meager (~20%) in 2- to 3.5-year-olds [9]. The superior immunogenicity noted herein may be attributed to one or more factors including vaccine formulation, higher level of previous exposure to *S. typhi* or group D *Salmonella* species among Thai children, or genetic differences.

Table 1. Anti-*Salmonella typhi* lipopolysaccharide antibody response following ingestion of *S. typhi* Ty21a vaccine or placebo.

Age (years)	Formulation	No. seroconverting/ total (%)	P*
4	Placebo	3/20 (15)	
	Vaccine	19/22 (86)	<.01
5	Placebo	6/36 (16)	
	Vaccine	30/37 (80)	<.01
6	Placebo	2/20 (10)	
	Vaccine	20/24 (83)	<.01
Total	Placebo	11/76 (14)	
	Vaccine	69/83 (83)	<.01

* Significance determined by χ^2 analysis.

A significant rise in serum IgG anti-*S. typhi* LPS antibody was found to correlate well with protection against disease in field trials with school-age children in Santiago, Chile [3]. Therefore, seroconversion may serve as an accurate marker of vaccine take and predictor of immunity.

The excellent safety and immunogenicity record of the Ty21a vaccine in 4- to 6-year-old Thai children paves the way for studies in younger children. At present, a study of similar design is planned for 2- to 4-year-old children. If similar results are obtained in this younger age group, it may prove possible to immunize before the age at which disease incidence peaks, thereby achieving the maximum public health benefit.

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