IMMUNIZATION OF SIX-MONTH-OLD INFANTS WITH DIFFERENT DOSES OF EDMONSTON–ZAGREB AND SCHWARZ MEASLES VACCINES

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Abstract Because measles causes an estimated 2 million deaths per year among children in developing countries, including a substantial proportion of infants less than nine months old — the age at which vaccination is recommended — there has been interest in using different strains of vaccine and higher doses to achieve immunization of younger infants.

We conducted a randomized trial of three different doses of Edmonston–Zagreb and of Schwarz measles vaccines in infants to evaluate the effect of the strain and dose of vaccine on the serologic response and acute adverse reactions to vaccination. Six-month-old infants received a standard, medium, or high dose of one of the vaccines, and nine-month-old infants received a standard dose. Antibody levels were measured before and after vaccination, by means of a plaque-reduction neutralization assay, in 1061 six-month-olds and 299 nine-month-olds.

Edmonston–Zagreb vaccine produced higher rates of seroconversion and seropositivity than comparable doses of Schwarz vaccine. Among the six-month-old infants, the seroconversion rate 18 weeks after vaccination with the standard dose of Edmonston–Zagreb vaccine was 92 percent, that with the medium dose was 96 to 97 percent, and that with the high dose was 98 percent; the rates for the corresponding doses of Schwarz vaccine were 66 percent, 76 percent, and 91 percent, respectively. Higher seroconversion rates were observed with an increase in the dose of either Edmonston–Zagreb ($P<0.01$) or Schwarz ($P<0.001$) vaccine. The seroconversion rates produced by high and medium doses of Edmonston–Zagreb vaccine in six-month-olds were equal to or significantly higher than the rate produced by a standard dose of Schwarz vaccine in nine-month-olds (87 percent). Clinical adverse reactions were not associated with the strain or dose of a vaccine.

We conclude that Edmonston–Zagreb vaccine is more immunogenic than Schwarz vaccine in infants and can induce effective immunization against measles at six months of age. (N Engl J Med 1990; 322:580-7.)

MEASLES remains a major cause of childhood morbidity and mortality in developing countries. It is estimated that more than 2 million children die of measles each year.[1] Measles also causes blindness, diarrhea, and malnutrition in many children who survive the acute illness. Live attenuated measles vaccines, available since the 1960s, are an effective means of reducing the incidence of measles in many countries. The administration of these vaccines results in seroconversion rates of over 90 percent in older infants; however, transplacentally acquired maternal antibody interferes with the successful immunization of young infants.[2-4] Therefore, the World Health Organization has recommended that measles vaccine be given at nine months of age to infants in developing countries,[5] an age at which most infants have lost maternal antibody and at which seroconversion rates can be acceptably high. However, infants in areas endemic for measles who lose maternal antibody before they are nine months old remain at high risk for measles and account for 20 to 30 percent of all patients with measles in some large urban areas.[6-8]

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Because of the high morbidity and mortality due to measles in infants younger than the recommended age for vaccination, there has been considerable interest in developing alternative strategies for immunizing young infants, including administering vaccine by different routes[9-12] or in higher doses[13] and using different strains.[13-17] Recent data suggest that the Edmonston–Zagreb measles vaccine, which was developed from the Edmonston-B vaccine strain and further attenuated by passage in human diploid cells,[18] may be more immunogenic in young infants than the widely used Schwarz strain, which was attenuated in chick-embryo fibroblasts.[9-17] In some trials, seroconversion rates of more than 90 percent have been observed among infants four to six months old who received Edmonston–Zagreb vaccine. However, doses of vaccine that were higher than conventional doses were used in several of these studies,[11,13,14] raising the question of whether the strain or dose of the vaccine was responsible for the greater-than-expected immunogenicity of Edmonston–Zagreb vaccine.

To answer questions about the effect of the vaccine dose and strain on seroconversion rates and acute adverse reactions, we conducted a randomized, double-blind, comparative trial of Edmonston–Zagreb and Schwarz vaccines administered subcutaneously to six-month-old infants at three different doses and to nine-month-old infants in a standard dose.

METHODS

Vaccines

Schwarz vaccine was provided by Dr. M. Roumiantzeff (Institut Mérieux, Lyon, France). Lot 1050 contained 3.8 $\log_{10}$ plaque-forming units (pfu) (standard dose), lot 1261 contained 4.5 $\log_{10}$ pfu

(medium dose), and lot 0980 contained 5.3 $\log_{10}$ pfu (high dose) per dose (Table 1). An Edmonston–Zagreb vaccine produced in Yugoslavia was provided by Drs. M. Beck and S. Smerdel (Institute of Immunology, Zagreb); lot 90/8 contained 4.5 $\log_{10}$ pfu (medium dose), and lot 81 contained 5.6 $\log_{10}$ pfu (high dose) per dose. An Edmonston–Zagreb vaccine produced in Mexico was provided by Dr. J. De Mucha (National Institute of Virology, Mexico City)[19]; lot 33/11 contained 3.7 $\log_{10}$ pfu (standard dose), and lot 1/33 contained 4.6 $\log_{10}$ pfu (medium dose) per dose.

Study Population and Design

The study was performed in three districts of Mexico City: Iztapalapa, Gustavo A. Madero, and Venustiano Carranza. Approximately 80 percent of the children one to four years of age in Mexico City have received measles vaccine.

We studied nine vaccination groups — seven groups of six-month-old infants and two groups of nine-month-old infants (Table 1). Two groups of six-month-olds received medium doses of Edmonston–Zagreb vaccine to allow the Edmonston–Zagreb vaccines from the two manufacturers to be compared.

Infants were enrolled in the study and vaccinated from June 22 through July 24 and September 7 through October 23, 1987. The names of infants of the appropriate age were obtained from the civil registry of births in Mexico City. Workers visited the infants' homes, explained the study to their parents or guardians, and requested informed consent for participation. Infants were not eligible for the study if they had a history of measles, had received measles vaccine or oral poliomyelitis vaccine in the previous two weeks, or were immunocompromised or severely malnourished. During the first period of enrollment and vaccination, six-month-old infants were randomly assigned to one of the nine groups by a computerized random-number generator. During the second period, both six-month-old and nine-month-old infants were enrolled; the six-month-olds were assigned to one of seven groups, and the nine-month-olds to one of two groups, according to a random-number table.

Vaccination and collection of blood specimens were conducted in the infants' homes. The vaccines were reconstituted in distilled water, and 0.5 ml was administered subcutaneously in the arm. Blood specimens were obtained immediately before vaccination and 8 and 18 weeks afterward. Two prevaccination specimens were obtained at six and nine months of age from infants assigned to receive vaccine at nine months of age. Infants who did not seroconvert by 18 weeks after vaccination were revaccinated.

Blood samples obtained by finger puncture were collected in capillary tubes (Microvette), transported on ice to the laboratory, and centrifuged. Serum samples were diluted 1:3 in phosphate-buffered saline and frozen at −20°C until assayed. Specimens were labeled with a study number to keep the laboratory evaluation unbiased.

The parents of infants were asked to report any adverse reactions immediately to study physicians or their local health clinic. Information on reactions occurring during the three weeks after vaccination was obtained for all infants at the visit made eight weeks after vaccination. In addition, all infants vaccinated during the last three weeks of the second period of enrollment and vaccination were also enrolled in a study of adverse reactions. Home visits were made every third day, from day 3 to day 24 after vaccination, by nurses who were unaware of the infants' vaccination group. Unvaccinated controls six and nine months old were selected from the neighborhoods of the vaccinated infants and were also followed up through home visits every third day for 24 days. The infants were examined, their rectal temperature was measured, and their mothers were asked whether symptoms had occurred during the previous two days.

The trial was approved by the ethics committee of the Ministry of Health (Mexico), the institutional review board at the Centers for Disease Control, and the National Institutes of Health.

Vaccine Potency

The infectivity titer of each vaccine was measured by plaque assay of Vero-cell cultures. The vaccines were diluted in fourfold steps, and 0.2 ml of each dilution was inoculated onto each of three dishes 35 mm in diameter and cultured for 1.5 hours. The inoculum was replaced with 4.0 ml of 0.5 percent agarose (SeaKem Agarose Me, FMC Corp., Rockland, Me.) in Eagle's minimal essential medium containing 5 percent inactivated fetal-calf serum, 100 U of penicillin per milliliter, 100 U of streptomycin per milliliter, 50 μg of gentamicin per milliliter, and 0.75 μg of amphotericin B per milliliter (Fungizone). The glutamine content was 0.03 percent (wt/vol). On days 4 and 6, 1.5 ml of 0.5 percent agarose was added. On day 6 the agarose contained an additional 150 μg per milliliter (wt/vol) of neutral red. The overlay was stripped on day 7, the dishes were dried, and the plaques were counted. When the titers were calculated, only dilutions yielding an average between 10 and 100 pfu per petri dish were used. The first International Reference Reagent Measles Vaccine (Live), code no. 82/638, which has an assigned titer of 3.7 $\log_{10}$ pfu per vial, had a mean titer of 4.01 $\log_{10}$ pfu per vial in our laboratory. The vaccine potencies reported below were determined in our laboratory and were not adjusted relatively to the International Reference preparation. Testing, performed before the study and after each vaccination period, revealed no decrease in vaccine potency during the study.

Plaque-Reduction–Neutralization Assay

The plaque-reduction–neutralization assay was performed as previously described.[20] Plaque-reduction–neutralization antibodies were measured at a starting dilution of 1:24, which included the 1:3 predilution in phosphate-buffered saline. All specimens from each infant were assayed simultaneously. The plaque-reduction–neutralization titer was considered to be the serum dilution that reduced the number of plaques by 50 percent.

The international standard preparation of anti-measles serum, which has an assigned value of 10 IU of antibody, was obtained from the Statens Seruminstitut (Copenhagen, Denmark) and reconstituted to a volume of 1.0 ml. The mean reciprocal plaque-reduction–neutralization titer of the reference preparation was 6000. Therefore, the minimal measurable antibody titer in this study (1:24) corresponded to 40 mIU. The titer of an aliquot of the international standard preparation was determined in parallel with the titer of each assay specimen. Only tests in which the titer of the international standard preparation varied by no more than 20 percent from the mean were considered valid.

Definitions of Vaccine Response

We defined seroconversion as a change from seronegative to seropositive status in infants in whom antibody was not detected before vaccination (<40 mIU). If the prevaccination antibody level was ≥40 mIU, we required a fourfold rise in the level above the calculated titer expected at 8 or 18 weeks after vaccination on the basis of a 6-week half-life of maternal antibody (≥1.58 times the prevaccination titer at 8 weeks, and ≥0.5 times the prevaccination titer at 18 weeks).[21] We also calculated seropositivity rates ≥200 mIU 18 weeks after vaccination, since data have suggested that levels of vaccine-induced antibody above 200 mIU may protect against disease[22] and since we wanted to allow our results to be compared with those from other studies in which antibody was detected only above this level.

Statistical Analysis

Because of differences in the distribution of prevaccination antibody titers among the vaccination groups containing six-month-olds (Table 1), we standardized the rates of seroconversion and seropositivity in each group to the overall distribution of prevaccination antibody levels among six-month-olds in four subgroups (<40 mIU, 40 to 99 mIU, 100 to 199 mIU, and ≥200 mIU), using the direct method for standardization.[23] Univariate 95 percent confidence intervals were calculated on the basis of variance from standardized data. To compare seroconversion rates, seropositivity rates, and adverse reactions among the groups, we used a procedure for analyzing categorical data in which linear models were fitted to functions of categorical data by the weighted least-squares estimation. Hypothesis testing was performed with the use of the generalized Wald statistic.[24,25] Linear trends were evaluated with the Mantel–Haenszel chi-square test.

Geometric mean titers were compared by analysis of variance

and the Tukey–Kramer studentized range test for pairwise comparisons (two-tailed).[26] To determine the effect of the vaccine dose on the postvaccination titer, we fitted a linear regression model to the $\log_2$ of titers determined 8 and 18 weeks after vaccination.

The levels of significance reported were obtained directly from the tests and were not adjusted for multiple comparisons.

Results

Study Population

Two adequate postvaccination serum specimens were obtained from each of 1399 of the 1969 infants (71.1 percent) who were initially enrolled in the study. Of these 1399, 13 were excluded from analysis because they were vaccinated at an age inappropriate for the study, received another measles vaccine after enrollment in the trial, or may have had measles. We obtained prevaccination samples at six and nine months of age from 92 infants vaccinated at nine months of age. Among these, 6 (7 percent) had an increase in titer between six and nine months of age. These 6 were also excluded from the analysis.

Thus, a total of 1380 six-month-old and nine-month-old infants were included in the analysis; their median ages were 26 and 37 weeks, respectively (Table 1). Among the six-month-olds, 39 percent had prevaccination antibody levels ≥40 mIU, including 5 percent whose prevaccination levels were ≥200 mIU.

Seroconversion and Seropositivity Rates

Crude and standardized rates of seroconversion and seropositivity differed by 1 to 5 percentage points. Only standardized rates for the six-month-old infants are presented in this report.

Effect of Strain

Among the six-month-old infants, the Edmonston–Zagreb vaccines produced significantly higher seroconversion rates than similar doses of the Schwarz vaccine both 8 and 18 weeks after vaccination (Table 2). The differences in seroconversion rates were greatest between the Edmonston–Zagreb vaccine produced in Mexico and the Schwarz vaccine when given in standard doses, both 8 weeks (82 vs. 57 percent; $P<0.0001$) and 18 weeks (92 vs. 66 percent; $P<0.0001$) after vaccination. The differences were smallest between the Edmonston–Zagreb vaccine produced in Yugoslavia and the Schwarz vaccine when given in high doses, both 8 weeks (94 vs. 86 percent; $P<0.05$) and 18 weeks (98 vs. 91 percent; $P<0.01$) after vaccination. There was no difference in the seroconversion rates when the Mexican Edmonston–Zagreb vaccine was compared with the Yugoslavian vaccine given in medium doses.

When seropositivity at ≥200 mIU was used as a measure of serologic response, the Edmonston–Zagreb vaccines were found to be more immunogenic than the Schwarz vaccine ($P<0.01$ for comparisons at each dose level). There was no difference in the seropositivity rates between the Mexican and Yugoslavian Edmonston–Zagreb vaccines in medium doses.

Among the nine-month-old infants, the Mexican Edmonston–Zagreb vaccine produced higher seroconversion rates than the Schwarz vaccine when given in standard doses, 8 weeks (97 vs. 85 percent; $P<0.0001$) and 18 weeks (96 vs. 87 percent; $P<0.01$) after vaccination. However, there was no difference between these two vaccination groups in seropositivity at ≥200 mIU.

Effect of Dose

According to any of the criteria for serologic response to vaccination, rates of response to the Schwarz vaccine were higher with increasing dose ($P<0.001$ by the Mantel–Haenszel chi-square test). Significant differences in rates were found between each of the dose levels. A dose effect on seroconversion and seropositivity rates was also observed for the Edmonston–Zagreb vaccines ($P<0.01$ by the Mantel–Haenszel chi-square test). However, although the differences in rates between the standard and high doses of Edmonston–Zagreb vaccines were statistically significant ($P<0.001$), those between the medium and high doses of Yugoslavian Edmonston–Zagreb vaccine were not.

Table 1. Vaccination Groups and Characteristics of the 1380 Study Infants.

Vaccination Group	Vaccine Dose*	No. of Infants	Infants with Prevaccination Antibody ≥40 mIU	Age	
				Median	Range
	$\log_{10}$ pfu		*%*	*wk*	
Infants 6 mo old (n = 1081)					
Edmonston–Zagreb-vaccine dose†					
Standard — M	3.7	151	47	26	23–29
Medium — M	4.6	160	38	26	21–29
Medium — Y	4.5	168	32	26	23–29
High — Y	5.6	136	45	26	22–29
Schwarz-vaccine dose‡					
Standard	3.8	146	42	26	21–29
Medium	4.5	158	37	26	21–29
High	5.3	162	35	26	23–29
Infants 9 mo old (n = 299)					
Edmonston–Zagreb-vaccine dose†					
Standard — M	3.7	171	8	37	33–41
Schwarz-vaccine dose‡					
Standard	3.8	128	9	37	33–41

*Vaccine potency was determined in our laboratory, and values were not adjusted in relation to the International Reference Measles Vaccine.

†M denotes the Edmonston–Zagreb vaccine produced by the National Institute of Virology, Mexico, and Y the Edmonston–Zagreb vaccine produced by the Institute of Immunology, Zagreb, Yugoslavia.

‡The Schwarz vaccine was produced by Institut Mérieux, Lyon, France.

Effect of Prevaccination Antibody Level

Seroconversion rates decreased with increasing levels of antibody present before vaccination in all

Table 2. Seroconversion and Seropositivity Rates after Vaccination.

Vaccination Group (No.)	Seroconversion at 8 Wk*		Seroconversion at 18 Wk*		Seropositivity ≥200 mIU at 18 Wk*	
	% (95% CI)	P value†	% (95% CI)	P value†	% (95% CI)	P value†
Infants 6 mo old						
Edmonston–Zagreb-vaccine dose‡						
Standard — M (151)	82 (76–88)	NS	92 (88–97)	NS	66 (58–74)	NS
Medium — M (160)	89 (85–94)	NS	97 (95–100)	<0.01	85 (79–90)	NS
Medium — Y (168)	90 (86–95)	NS	96 (93–99)	<0.01	91 (86–95)	NS
High — Y (136)	94 (90–98)	>0.05	98 (96–100)	<0.001	94 (90–98)	<0.01
P value§	*<0.001*		*<0.01*		*<0.001*	
Schwarz-vaccine dose¶						
Standard (146)	57 (49–65)	<0.001	66 (58–73)	<0.001	49 (40–57)	<0.001
Medium (158)	74 (68–81)	>0.05	76 (69–83)	>0.05	62 (55–70)	<0.001
High (162)	86 (81–91)	NS	91 (87–95)	NS	79 (73–85)	NS
P value§	*<0.001*		*<0.001*		*<0.001*	
Infants 9 mo old						
Edmonston–Zagreb-vaccine dose‡						
Standard — M (171)	97 (95–100)	<0.001	96 (93–99)	<0.01	79 (73–85)	NS
Schwarz-vaccine dose¶						
Standard (128)	85 (79–91)	Reference	87 (81–93)	Reference	82 (75–89)	Reference

*Rates are standardized according to the antibody levels present in six-month-old infants before vaccination. Univariate 95 percent confidence intervals (CI) for six-month-old groups are based on variance from standardized data.

†P values show the significance of differences between each group and the group of nine-month-old infants given the standard dose of Schwarz vaccine; NS denotes not significant.

‡M denotes the Edmonston–Zagreb vaccine produced by the National Institute of Virology, Mexico, and Y the Edmonston–Zagreb vaccine produced by the Institute of Immunology, Zagreb, Yugoslavia.

§By Mantel–Haenszel chi-square test for trend in serologic response to different doses of each vaccine strain.

¶The Schwarz vaccine was produced by Institut Mérieux, Lyon, France.

vaccination groups of six-month-old infants (Fig. 1). Among the infants given the Schwarz vaccine, a dose effect was apparent at each prevaccination antibody level, even in infants who had not had detectable antibody (<40 mIU). A dose effect of Edmonston–Zagreb vaccine was seen among infants with higher prevaccination antibody levels. However, infants without detectable prevaccination antibody responded equally well (>98 percent) to all doses.

Comparison with the Standard Dose of Schwarz Vaccine at Nine Months of Age

Because Schwarz vaccine is one of the most widely used measles vaccines in infants nine months of age, we compared the seroconversion and seropositivity rates for each vaccine administered at six months with the rates for the Schwarz vaccine administered in a standard dose at nine months. According to any of the criteria for response to vaccination, the high dose of the Yugoslavian Edmonston–Zagreb vaccine given at six months of age produced significantly higher response rates than the standard dose of Schwarz vaccine given at nine months (Table 2). A medium dose of either Edmonston–Zagreb vaccine administered at six months of age also produced significantly higher seroconversion rates as observed 18 weeks after vaccination than the standard dose of Schwarz vaccine administered at nine months.

Geometric Mean Titers

Infants who seroconverted eight weeks after vaccination were included in the analysis of geometric mean titers (Table 3). Within each vaccination group the titers were higher in the infants who had no antibody before vaccination. The differences were statistically significant in the groups given the medium and high doses of Schwarz vaccine and in those given the medium and high doses of Yugoslavian Edmonston–Zagreb vaccine ($P<0.01$). Between 8 and 18 weeks after vaccination, there was no significant decline in titers among infants vaccinated at six months of age. However, significant declines were observed among infants who received vaccine at nine months.

Effect of Strain

Among the six-month-old infants without detectable antibody before vaccination, the standard and medium doses of Mexican Edmonston–Zagreb vaccine produced lower geometric mean titers than did the standard and medium doses of Schwarz vaccine 8 and 18 weeks after vaccination ($P<0.01$). Among the nine-month-old infants, the standard dose of the Mexican Edmonston–Zagreb vaccine also produced lower titers than did the standard dose of the Schwarz vaccine. There were no significant differences between the titers produced by the Yugoslavian Edmonston–Zagreb and Schwarz vaccines.

Effect of Dose

Among the infants without detectable antibody before vaccination, there appeared to be a trend for an increasing geometric mean titer with increasing doses of the Schwarz vaccine; however, this trend was not statistically significant. The Schwarz vaccine had no dose effect on geometric mean titers in infants with detectable antibody before vaccination, nor did the

Edmonston–Zagreb vaccines from the same manufacturer in infants with or without detectable antibody before vaccination.

Comparison with the Standard Dose of Schwarz Vaccine at Nine Months of Age

The geometric mean titer eight weeks after vaccination in the nine-month-old group given the standard dose of Schwarz vaccine was higher than that in all groups except the groups of six-month-olds given the high dose of Schwarz vaccine and the medium dose of Yugoslavian Edmonston–Zagreb vaccine. By 18 weeks after vaccination, the titers in this group had decreased so that the geometric mean titer was significantly higher only in relation to those in the groups of six-month-olds given standard and medium doses of Mexican Edmonston–Zagreb vaccine and the group of nine-month-olds given the standard dose of this vaccine.

Acute Clinical Reactions

No severe adverse clinical reactions were detected during the follow-up study or reported during the visit for the collection of blood eight weeks after vaccination.

The clinical follow-up study of adverse reactions included 750 vaccinated infants and 63 controls (Table 4). The rates of adverse reactions attributable to vaccination were calculated by subtracting the reaction rate for the controls from the rate for each vaccination group. The rate of fever (rectal temperature, ≥38.5°C) ranged from 1 to 14 percent (rate of attributable occurrences, 0 to 3 percent). There were no differences between the maximal temperature (39.5 to 40.0°C) or the median duration of fever (1.0 to 2.5 days). The rate of occurrence of rash ranged from 8 to 21 percent. The median duration (2 to 4 days) and mean first day the rash appeared (7 to 10 days after vaccination) did not differ according to the strain or dose of vaccine. Similar results were obtained when the analysis was restricted to infants who had seroconverted. As compared with the control group, all vaccination groups except the group of six-month-olds given the standard dose of Mexican Edmonston–Zagreb vaccine had a higher rate of rashes, all groups had a higher rate of diarrhea, and the groups given the standard and medium doses of Schwarz vaccine had a higher rate of conjunctivitis.

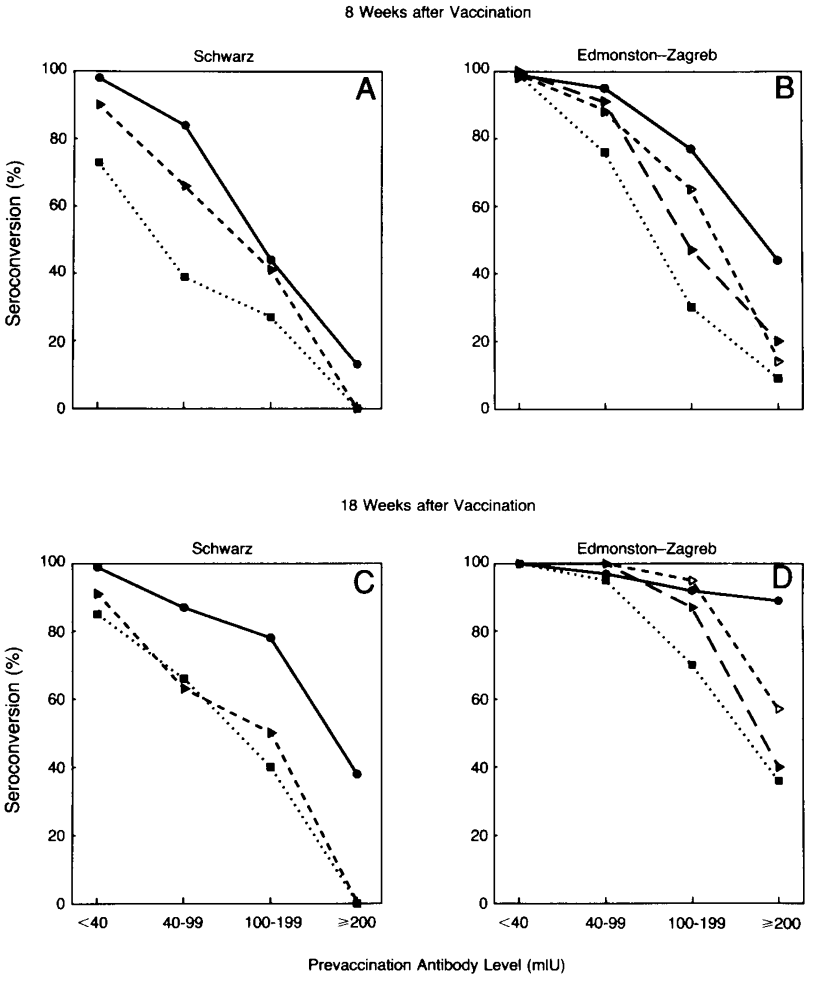

Figure 1. Seroconversion Rates 8 and 18 Weeks after Vaccination with Schwarz (A and C) and Edmonston–Zagreb (B and D) Measles Vaccines, According to the Prevaccination Antibody Titer Determined by the Plaque-Reduction–Neutralization Assay.

Seroconversion rates measured after administration of the standard dose of the Schwarz vaccine are denoted by ■, after the medium dose by ▲, and after the high dose by ●. Rates measured after administration of the standard dose of Mexican Edmonston–Zagreb vaccine are denoted by ■, after the medium dose of the Mexican vaccine by ▲, after the medium dose of the Yugoslavian Edmonston–Zagreb vaccine by △, and after the high dose of the Yugoslavian vaccine by ●.

Discussion

Our study was a large, randomized trial comparing the Edmonston–Zagreb and Schwarz strains of measles vaccine in different doses in infants. Our finding — that Edmonston–Zagreb vaccines induced significantly higher rates of seroconversion and seropositivity than the Schwarz vaccine at all three dose levels in six-month-olds — confirms earlier reports that suggested the Edmonston–Zagreb strain of measles vaccine had superior immunogenicity.[9-17] Furthermore, we found that the rates of seroconversion induced by all doses of Edmonston–Zagreb vaccine in six-month-

Table 3. Geometric Mean Titers after Vaccination, According to Antibody Titer before Vaccination.

Vaccination Group	Prevaccination Titer	No. Studied*	Postvaccination Geometric Titer† at 8 wk	at 18 wk
			mIU	
Infants 6 mo old				
Edmonston–Zagreb-vaccine dose				
Standard — M	<40	80	436	457
	≥40	36	371	383
Medium — M	<40	97	577	691
	≥40	46	429	540
Medium — Y	<40	114	1826	1731
	≥40	42	735	739
High — Y	<40	74	1083	1350
	≥40	52	722	782
Schwarz-vaccine dose				
Standard	<40	62	1384	1293
	≥40	21	997	1059
Medium	<40	89	1675	1504
	≥40	30	516	324
High	<40	103	1813	1530
	≥40	37	736	657
Infants 9 mo old				
Edmonston–Zagreb-vaccine dose				
Standard — M	<40	152	690	539
	≥40	13	675	296
Schwarz-vaccine dose				
Standard	<40	101	3013	1921
	≥40	8	2632	1260

*Number who seroconverted eight weeks after vaccination.

†Brackets denote a significant difference ($P<0.01$ by Tukey–Kramer studentized range test for pairwise comparisons [two-tailed]) between the groups indicated.

old infants were comparable to or significantly greater than the rates of seroconversion induced by the standard dose of Schwarz vaccine in nine-month-olds. When we used a more conservative definition of response to vaccination (a titer of at least 200 mIU 18 weeks after vaccination), we had the same findings, although the response rates were lower in all groups.

The reason for the greater immunogenicity of the Edmonston–Zagreb vaccine strain in infants is not known. Edmonston–Zagreb vaccine was derived from the Edmonston-B vaccine by passages in primary dog-kidney cells followed by 19 passages in WI-38 human diploid cells; large plaques were selected during several passages.[18] The Schwarz vaccine was derived from the Edmonston-A vaccine and attenuated by passages in chick-embryo fibroblasts.[27] It has been suggested that attenuation in human diploid cells may be responsible for a greater affinity of the Edmonston–Zagreb vaccine virus for human cells[28] and for its greater immunogenicity. Although some differences in the growth, morphology of plaque types,[18,29] and content of defective viral particles[30] between Edmonston–Zagreb, Schwarz, and other vaccine strains have been described, the molecular virologic mechanisms and properties of Edmonston–Zagreb vaccine that may be responsible for its superior immunogenicity have not been identified.

Our data demonstrate that the strain of a vaccine has an effect on seroconversion and seropositivity rates, even though the high dose of the Edmonston–Zagreb vaccine used in this study contained 0.3 $\log_{10}$ more plaque-forming units than the high dose of Schwarz vaccine. There may also have been non-equivalent amounts of viral particles in the vaccines found to be of similar potency, because of different sensitivities of the Vero-cell test of the potency for the two vaccine strains. Nevertheless, the effect of the strain is evident, since the standard dose of Edmonston–Zagreb vaccine and the high dose of Schwarz vaccine, which had 100-fold more plaque-forming units per dose, produced similar seroconversion rates.

Since viral replication after vaccination greatly increases the amount of antigen available for processing, it has been assumed that increasing the dose of live measles vaccine would not affect the response to the vaccine. However, our study demonstrates a dose effect on seroconversion and seropositivity for both vaccine strains. Earlier studies did not find that the dose of live-measles vaccine affected seroconversion rates[31-39]; however, many of these trials were carried out in older infants or in infants who were seronegative at the time of vaccination. Although one trial in infants three to seven months old found no dose effect with Schwarz vaccine,[40] other studies have suggested that the dose may be important when vaccine is administered while maternal antibody is present.[41-43] The current requirement of the World Health Organi-

Table 4. Rates of Acute Clinical Reactions.

Group	No. Studied	Reaction* Fever (≥38°C)	Rash	Conjunctivitis	Diarrhea
		% of infants with reaction			
Control infants	63	11	2	10	10
Infants 6 mo old					
Edmonston–Zagreb-vaccine dose					
Standard — M	62	5	8	18	42†
Medium — M	71	4	20†	20	45†
Medium — Y	70	14	21†	17	47†
High — Y	62	2	18†	15	39†
Schwarz-vaccine dose					
Standard	75	11	19†	24†	44†
Medium	76	7	20†	30†	49†
High	80	6	18†	15	40†
Reactions attributable to vaccination		*0–3*	*6–19*	*5–20*	*29–39*
Infants 9 mo old					
Edmonston–Zagreb-vaccine dose					
Standard — M	145	1	11	17	38†
Schwarz-vaccine dose					
Standard	109	8	15	18	34†
Reactions attributable to vaccination		*0*	*9–13*	*7–8*	*24–28*

*Bracket denotes a significant difference ($P<0.01$) between the groups of six-month-old infants indicated.

†$P<0.01$ as compared with controls.

zation for measles vaccine specifies that it contain no less than 3.0 $\log_{10}$ pfu per dose. Since our data suggest that higher doses can produce higher seroconversion rates in infants with maternal antibody, this requirement may need to be changed to achieve optimal responses to vaccination when maternal antibody may still be present.

Only 7 percent of the 92 infants from whom blood was obtained twice before vaccination (at six and nine months of age) had a rise in antibody. This indicates that almost all of the occurrences of seroconversion we observed were due to vaccination rather than to exposure to wild virus. In addition, the increase in seroconversion rates between 8 and 18 weeks after vaccination was greater than the increase expected because of exposure to circulating measles. This "delayed immune response" was also observed by Sabin and colleagues after administration of measles vaccine by aerosol and subcutaneous injection.[9,10] The reasons for this delay are not known and require further investigation.

Schwarz vaccine produced higher geometric mean titers than either the Yugoslavian or Mexican Edmonston–Zagreb vaccines; however, the differences were significant only between the Schwarz and Mexican Edmonston–Zagreb vaccines. Although producing similar seroconversion rates, the Yugoslavian vaccine also produced significantly higher titers than the Mexican vaccine. Traditionally, the magnitude of the antibody titer observed after measles vaccination has not been considered important[44]; however, recent data suggest that it might be a predictor of long-term immunity.[45] Determining the reasons for the differences in geometric mean titers will be important, particularly if the postvaccination antibody titer correlates with the duration of protection.

There were no severe adverse reactions in any of the vaccinated infants. Although minor differences in the reaction rates were observed between the vaccine strains, none were statistically significant. Furthermore, there were no trends of increased adverse reactions in relation to the dose of vaccine or age at vaccination. However, the small number of infants in the study of adverse reactions precluded the detection of less common adverse reactions or of small differences between the vaccination groups. In our sample population, the power to detect differences between rates of 5 and 15 percent was approximately 40 percent.

Our data suggest that Edmonston–Zagreb vaccine can be administered safely to infants six months of age and can produce seroconversion rates at least comparable to those achieved at nine months of age with the standard dose of Schwarz vaccine. If the medium and high doses of the Edmonston–Zagreb vaccines used in this trial are administered, higher seroconversion rates can be reached than those achieved with the standard dose of Schwarz vaccine given at nine months. We also found that increasing the dose of Schwarz vaccine can result in acceptably high rates of seroconversion in younger infants. However, increasing by 10-fold or 100-fold the current minimal required dose of any measles vaccine may pose technical difficulties in the manufacture of the vaccine and increase its cost. The optimal formulation of measles vaccine depends on the maternal-antibody profile of the population to be vaccinated.[46] In our study, there was no difference between the responses to the medium and high doses of Edmonston–Zagreb vaccines. However, since the effect of the dose of Edmonston–Zagreb vaccine appears to be greatest among infants with high titers of maternal antibody at the time of vaccination, differences between the effects of these doses may be observed in infants with titers higher than those of the infants we studied.

Questions remain concerning the persistence of antibody in young infants vaccinated against measles[47] and the long-term efficacy of the vaccine. Therefore, study populations receiving Edmonston–Zagreb vaccine at an early age should continue to be monitored. Our data suggest, however, that in populations in which protection at an earlier age is desirable, administering Edmonston–Zagreb vaccine at six months can provide effective immunization against measles.

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