

ORIGINAL ARTICLE

A Randomized, Controlled Trial of an Aerosolized Vaccine against Measles

N. Low, A. Bavdekar, L. Jeyaseelan, S. Hirve, K. Ramanathan, N.J. Andrews, N. Shaikh, R.S. Jadi, A. Rajagopal, K.E. Brown, D. Brown, J.B. Fink, O. John, P. Scott, A.X. Riveros-Balta, M. Greco, R. Dhere, P.S. Kulkarni, and A.M. Henao Restrepo

ABSTRACT

BACKGROUND

Aerosolized vaccine can be used as a needle-free method of immunization against measles, a disease that remains a major cause of illness and death. Data on the immunogenicity of aerosolized vaccine against measles in children are inconsistent.

METHODS

We conducted an open-label noninferiority trial involving children 9.0 to 11.9 months of age in India who were eligible to receive a first dose of measles vaccine. Children were randomly assigned to receive a single dose of vaccine by means of either aerosol inhalation or a subcutaneous injection. The primary end points were seropositivity for antibodies against measles and adverse events 91 days after vaccination. The noninferiority margin was 5 percentage points.

RESULTS

A total of 1001 children were assigned to receive aerosolized vaccine, and 1003 children were assigned to receive subcutaneous vaccine; 1956 of all the children (97.6%) were followed to day 91, but outcome data were missing for 331 children because of thawed specimens. In the per-protocol population, data on 1560 of 2004 children (77.8%) could be evaluated. At day 91, a total of 662 of 775 children (85.4%; 95% confidence interval [CI], 82.5 to 88.0) in the aerosol group, as compared with 743 of 785 children (94.6%; 95% CI, 92.7 to 96.1) in the subcutaneous group, were seropositive, a difference of -9.2 percentage points (95% CI, -12.2 to -6.3). Findings were similar in the full-analysis set (673 of 788 children in the aerosol group [85.4%] and 754 of 796 children in the subcutaneous group [94.7%] were seropositive at day 91, a difference of -9.3 percentage points [95% CI, -12.3 to -6.4]) and after multiple imputation of missing results. No serious adverse events were attributable to measles vaccination. Adverse-event profiles were similar in the two groups.

CONCLUSIONS

Aerosolized vaccine against measles was immunogenic, but, at the prespecified margin, the aerosolized vaccine was inferior to the subcutaneous vaccine with respect to the rate of seropositivity. (Funded by the Bill and Melinda Gates Foundation; Measles Aerosol Vaccine Project Clinical Trials Registry-India number, CTRI/2009/091/000673.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Henao Restrepo at Implementation Research and Economic Evaluation, Department of Immunization, Vaccines and Biologicals, World Health Organization, 20 Ave. Appia, CH-1211, Geneva 27, Switzerland, or at henaorestrepo@who.int.

N Engl J Med 2015;372:1519-29.

DOI: 10.1056/NEJMoa1407417

Copyright © 2015 Massachusetts Medical Society.

THE GLOBAL VACCINE ACTION PLAN AIMS to eliminate measles from at least five World Health Organization (WHO) regions by 2020.¹ A safe and effective injectable measles vaccine has been widely available since 1963,² and intensified efforts between 2000 and 2010 reduced measles-related deaths by 74%.³ Nevertheless, major outbreaks continue, particularly in resource-poor countries that lack investment in health care systems and the health service infrastructure. In these countries, immunization coverage through routine services and mass campaigns remains low.⁴

New approaches to measles vaccination could contribute to reaching elimination goals, particularly if they increase coverage, can be administered by people without clinical training, and do not cause injection-associated infections.⁵⁻⁷ Aerosolized vaccine against measles was developed in Mexico and has been used in more than 4 million children there since 1980.⁸ Nebulization delivers vaccine to the site of natural infection and induces measles-specific antibodies and cell-mediated immunity.^{9,10}

Data about the efficacy of this aerosolized vaccine against measles in children are inconsistent, however. Two comprehensive reviews concluded that it was as good as or better than vaccine delivered subcutaneously in children 9 months of age or older.^{11,12} However, the two randomized, controlled trials comparing these routes of administration in children 8 to 13 months of age were small, associated with a risk of bias, and inconsistent.^{9,10} A subsequent systematic review pooled data about each route of administration from randomized, controlled trials and observational studies. Among children 10 to 35 months of age, the pooled seroconversion rate in five studies among those who received aerosolized vaccine against measles was 93.5% (95% confidence interval [CI], 89.4 to 97.7), and the pooled seroconversion rate in two studies among those who received the vaccine subcutaneously was 97.1% (95% CI, 92.4 to 100).¹³ After subcutaneous vaccination, approximately 92% (interquartile range, 84 to 96) of children 9 to 10 months of age and 99% (interquartile range, 93 to 100) of children 11 to 12 months of age underwent seroconversion.⁷

Given the established record of injectable measles vaccine, alternative delivery methods should show noninferiority. A phase 1 trial in India of aerosolized vaccine against measles

showed evidence of safety, immunogenicity, and feasibility in children older than 4 years of age and in adults.¹⁴ In this individually randomized, open-label noninferiority trial involving children in India, we aimed to compare the immunogenicity and safety of a primary dose of aerosolized vaccine against measles with that of a primary dose of vaccine delivered subcutaneously.

METHODS

STUDY DESIGN AND OVERSIGHT

The WHO Initiative for Vaccine Research coordinated the Measles Aerosol Vaccine Project, and the Centers for Disease Control and Prevention and the American Red Cross were partners in the project. An independent data and safety monitoring board had access to unblinded data to assess serious adverse events. A product development group (see the Supplementary Appendix, available with the full text of this article at NEJM.org) reviewed progress. Ethics committees of the WHO, Christian Medical College (in Vellore, India), the National Institute of Virology, and King Edward Memorial Hospital Research Centre (in Pune, India) approved the protocol. The trial was designed and conducted in accordance with Good Clinical Practice¹⁵ and Good Laboratory Practice¹⁶ guidelines.

The Serum Institute of India provided all vaccines free of charge, and Aerogen provided the delivery devices free of charge. Additional details about the trial design, conduct, and analysis are provided in the Supplementary Appendix and in the full protocol, including the statistical analysis plan, available at NEJM.org.

STUDY PARTICIPANTS AND CLINICAL SETTING

The trial was conducted in villages served by eight primary health centers in Pune. One study has shown that more than 90% of infants in Pune are breast-fed from birth, for a median of 4.7 months.¹⁷ Children between 9.0 and 11.9 months of age were eligible to participate in the study if they were due to receive primary measles vaccination. Children were excluded from participation if they were ineligible to receive measles vaccine according to WHO criteria.¹⁸

RANDOMIZATION AND VACCINATION

From December 20, 2009, through April 5, 2010, we randomly assigned children, in a 1:1 ratio, to receive measles vaccine by means of aerosol in-

halation or by means of a subcutaneous injection. A detailed description of the randomization process is provided in the Supplementary Appendix. After obtaining written informed consent from the parents or guardians of the children, the study nurses telephoned a centralized Web-based service and recorded the study assignments. At the Vadu site, which is part of a demographic surveillance system,¹⁹ two random subgroups of 100 children were selected to have blood drawn at either day 28 or day 364 for the monitoring of serologic responses.

We used a measles vaccine (Serum Institute of India), licensed by the WHO, that contained at least 1000 viral infective units of the live attenuated Edmonston–Zagreb strain of measles virus in each dose. The vaccine was delivered in 10-dose vials.

The study nurses reconstituted the 10-dose vials of measles vaccine and stored them until use at 2 to 8°C. They reconstituted the vaccine for aerosol delivery in 2-ml diluent and administered a single 0.2-ml dose, nebulized for 30 seconds through a single-use nonvented face mask, using a battery-operated Aeroneb vibrating mesh nebulizer (Aerogen). The nebulizer generated aerosol with a volume median diameter of 5.1 μm (geometric standard deviation, 2.1 μm) as determined by means of laser diffraction (Spraytec) (details are provided in the Supplementary Appendix).¹⁴ The study nurses reconstituted the vaccine for subcutaneous delivery in 5-ml diluent and administered a single 0.5-ml dose into the left upper arm. The rooms for delivery of aerosolized vaccine and subcutaneous vaccine were separate so that children receiving subcutaneous vaccine were not exposed unintentionally to aerosolized vaccine. Reconstituted vaccine was discarded after 6 hours.

END POINTS

Immunogenicity

The prespecified primary end point was seropositivity for serum antibodies against measles 91 days after vaccination. We defined seropositivity as 0.1 or more optical-density units on an enzyme-linked immunosorbent assay (ELISA) (Enzygnost Anti-Measles Virus/IgG, Siemens) or, in samples containing less than 0.1 optical-density units, a measles antibody concentration of 120 mIU per milliliter or more as measured with the use of a plaque-reduction neutralization test (PRNT). The testing algorithm was based on

a study that showed a positive predictive value of 99.4% for ELISA (cutoff value, 0.1 optical-density units) as compared with PRNT (cutoff value, 120 mIU per milliliter).²⁰

All samples at baseline and day 91 were tested by means of ELISA. Specimens with less than 0.1 optical-density units and all samples from the Vadu site were tested by means of PRNT. Paired prevaccination and post-vaccination samples were tested in the same run. All specimens were tested at the Virus Reference Department, Public Health England (formerly the United Kingdom Health Protection Agency), United Kingdom, in March 2012. The specimens had been tested in Pune, but the results of a random 10% sample of ELISA and PRNT analyses did not meet the prespecified quality-assurance criteria, so all samples were shipped to the United Kingdom.

Prespecified secondary end points were seroconversion and geometric mean concentrations of antibodies. Seroconversion was defined as a change from seronegative at day 0 to seropositive at day 91. Secondary outcomes were the difference in rates of seroconversion (among participants who were seronegative at baseline), the ratio of geometric mean concentrations, and geometric mean concentrations at days 0, 28, 91, and 364 in children in Vadu.

Safety

The prespecified primary safety outcome was the frequency of all solicited and unsolicited reports of adverse events up to 91 days after vaccination on day 0.²¹ Study nurses observed all children during and for 30 minutes after vaccination. Adverse events were then assessed according to the report of parents and guardians during home visits on days 3, 7, 10, 17, 21, 28, and 56 and by clinical examination on days 14 and 91 (Fig. S1 in the Supplementary Appendix). All children who were enrolled in Vadu were evaluated at day 364.

We collected information with the use of questionnaires that solicited information on 16 events (Table S1 in the Supplementary Appendix), by means of active surveillance both for events requiring treatment or hospitalization and for deaths, and through unsolicited reports of events from parents or guardians. We sent reports of all adverse events to the data and safety monitoring board, which had the authority to stop the trial if a single serious adverse event was judged to be attributable to the vaccine.

FOLLOW-UP AND BLINDING

We followed all participants until day 91 (from March through August 2010, depending on the date the patient underwent randomization). Case-based active surveillance with serologic confirmation of cases of fever and rash (Enzygnost Anti-Measles Virus IgM ELISA, Siemens) was in place in the entire trial area.²² Parents or guardians, children, and study staff were aware of the route of vaccine administration.

To reduce bias, all outcome assessments were blinded. Field workers used follow-up case-report forms that did not record the vaccination assignment, laboratory staff conducted analyses of coded specimens, and statisticians conducted data checks and preliminary analyses of blinded data.

STATISTICAL ANALYSIS

We aimed to show that seropositivity after receipt of aerosolized vaccine against measles was no more than 5 percentage points lower than seropositivity after subcutaneous vaccination. This estimate was based on the 4 percentage-point difference in a previous systematic review of studies involving children 10 to 35 months of age¹³; in addition, with a bigger margin, aerosolized vaccine would not provide levels of protection required for herd immunity.¹⁸

We assumed that exactly 90% of the children in each group would be seropositive at day 91, and we allowed for lower immunogenicity in children younger than 10 months of age.⁷ For noninferiority to be shown with the use of the confidence-interval approach, the lower limit of the two-sided 95% confidence interval for the difference (aerosol group minus subcutaneous group) in the proportion of seropositive children at day 91 had to be above -5 percentage points in the per-protocol population. If the upper confidence interval was below -5 percentage points, we would conclude inferiority, and if the lower interval was above zero, we would conclude superiority (Fig. S2 in the Supplementary Appendix). We estimated that with a sample of 800 children per group, the study would have 90% power to detect these differences. We planned to enroll 1000 children per group to allow for 20% who would not have results that could be evaluated at follow-up.

We calculated the difference between the

proportion of children who were seropositive after receiving the aerosolized vaccine and the proportion who were seropositive after receiving the subcutaneous vaccine in the per-protocol population (which consisted of children who received the assigned vaccine, did not have any major protocol deviations, and had specimen results at day 91) and the full-analysis population (which consisted of all children who underwent randomization, excluding children for whom outcome data were missing). The full-analysis population was equivalent to a modified intention-to-treat population. We used the Wilson score method to estimate 95% confidence intervals.²³ Multiple imputation was used to predict seropositivity when outcome data were missing, and we repeated the analysis with inclusion of all participants (see the Supplementary Appendix).²⁴

For the secondary end points, we used logistic regression to investigate the association of prespecified factors with lack of seroconversion after receipt of aerosolized vaccine. For safety outcomes, we calculated the percentages of children (with 95% confidence intervals) who had any solicited or unsolicited reports of an adverse event or serious adverse event up to day 91.

RESULTS**STUDY PARTICIPANTS**

In total, 2004 children underwent randomization and 1996 received their assigned vaccine (Fig. 1). The baseline characteristics of the two groups were balanced (Table 1, and Table S3 in the Supplementary Appendix). We followed 1956 of 2004 children (98%) until day 91. Specimens obtained from 331 participants (17%) thawed for a period during transport and were, therefore, excluded from the primary-outcome analyses; these specimens were distributed equally between the aerosol and subcutaneous groups (Fig. S3 and Tables S4 and S5 in the Supplementary Appendix).

The per-protocol population for the primary outcome included 77% of the children (775 of 1001 children) who were randomly assigned to aerosolized vaccine and 78% (785 of 1003 children) who were randomly assigned to subcutaneous vaccine (Fig. 1). The full-analysis set included 79% (788 of 1001) of the children who were

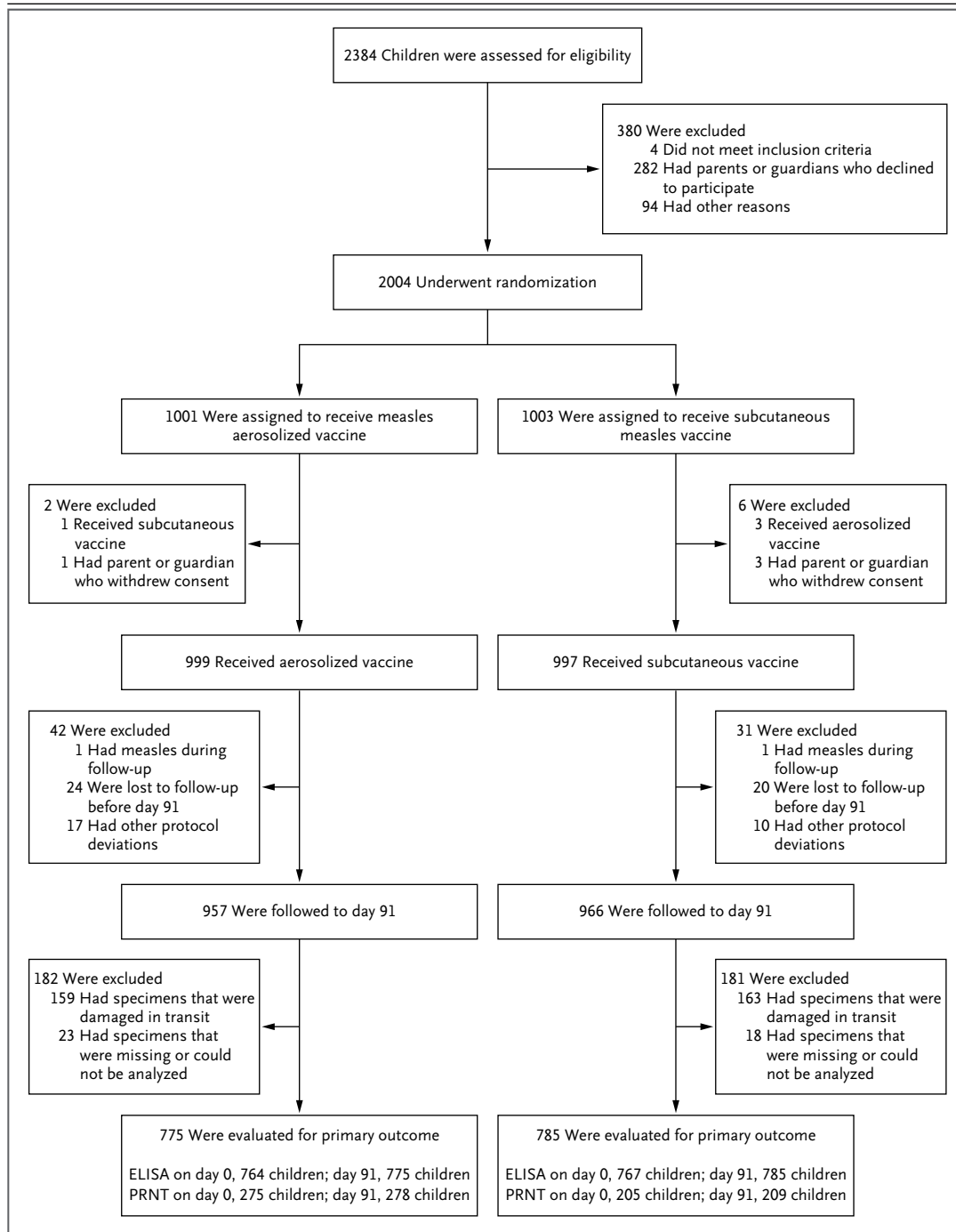


Figure 1. Eligibility, Randomization, and Follow-up of Children in the Per-Protocol Population.

Data on children in the full-analysis set (788 children in the aerosol group and 796 children in the subcutaneous group) are provided in Figure S3 in the Supplementary Appendix; in that analysis, 48 children were lost to follow-up before day 91, and data on 331 children with missing laboratory results because of damage to specimens during transport were excluded from the analysis of the primary end point. ELISA denotes enzyme-linked immunosorbent assay, and PRNT plaque-reduction neutralization test.

Table 1. Baseline Characteristics of the Participants.*

Characteristic	Aerosolized-Vaccine Group (N=1001)	Subcutaneous-Vaccine Group (N=1003)
Age — no. (%)		
9.0 to <10.0 mo	730 (72.9)	749 (74.7)
10.0 to <11.0 mo	198 (19.8)	165 (16.5)
11.0 to 11.9 mo	73 (7.3)	89 (8.9)
Female sex — no. (%)	488 (48.8)	489 (48.8)
Usual health care provider — no. (%)		
Government	184 (18.4)	191 (19.0)
Private	812 (81.1)	802 (80.0)
Other	5 (0.5)	10 (1.0)
Health history — no. (%)†		
Preexisting medical condition	2 (0.2)	7 (0.7)
Clinically significant illness in past 3 mo	29 (2.9)	24 (2.4)
Measurements outside normal range for age — no./total no. (%)‡		
Height	474/997 (47.5)	480/996 (48.2)
Weight	187/1000 (18.7)	185/1001 (18.5)
Location of primary health care center — no. (%)		
Alandi	64 (6.4)	64 (6.4)
Chakan	134 (13.4)	135 (13.5)
Nhavara	155 (15.5)	155 (15.5)
Pabal	130 (13.0)	122 (12.2)
Shel Pimpalgaon	83 (8.3)	84 (8.4)
Talegaon Dhamdhere	63 (6.3)	76 (7.6)
Vadu	191 (19.1)	181 (18.0)
Wagholi	181 (18.1)	186 (18.5)
Measles antibodies at baseline — no. (%)§		
Yes	23 (2.3)	16 (1.6)
No	762 (76.1)	768 (76.6)
Could not be evaluated	216 (21.6)	219 (21.8)

* There were no significant differences in characteristics between the study groups.

† Preexisting conditions were reported by the parents or guardians of the children and recorded on the case-report form (see Table S3 in the Supplementary Appendix).

‡ Recorded values that were greater than 6 SD from the mean were considered by the software package used to calculate anthropometric measurements (WHO Anthro, version 3.1.0) to be outliers and were excluded from the analysis.

§ Seropositivity for serum antibodies against measles was defined as 0.1 or more optical-density units on ELISA or, in samples containing less than 0.1 optical-density unit, a measles antibody concentration of 120 mIU per milliliter or more as measured on the PRNT.

randomly assigned to aerosolized vaccine and 79% (796 of 1003) of the children who were randomly assigned to subcutaneous vaccine (Fig. S3 in the Supplementary Appendix). The case-based surveillance system in the trial villages identified 772 confirmed cases of measles from November 1, 2009, through December 31, 2011.

LABORATORY ASSAYS

On the basis of both ELISA and PRNT results from day 28 samples obtained from children in Vadu, the positive predictive value of the ELISA cutoff value of 0.1 optical-density units, as compared with PRNT, was 96% (241 of 250 samples). The negative predictive value was 69% (34 of 49

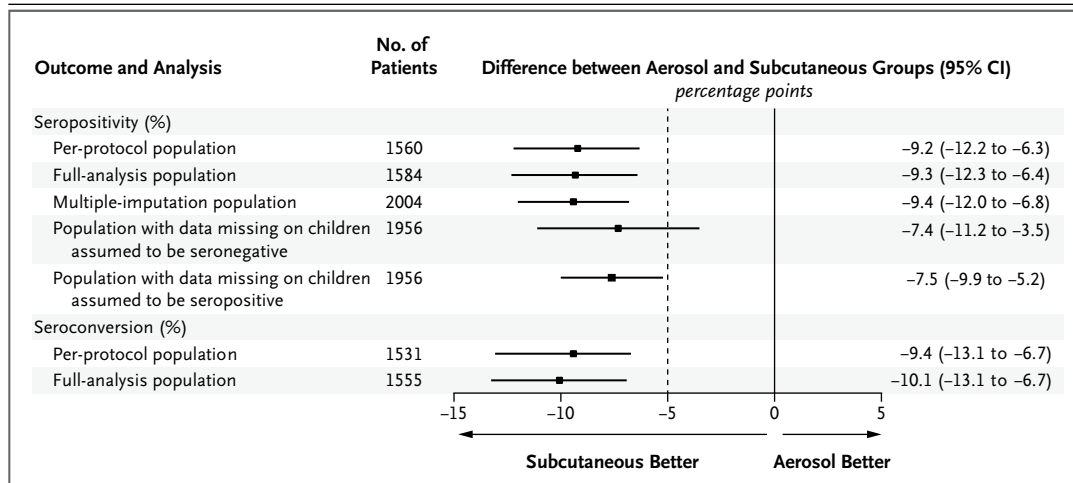


Figure 2. Immunogenicity Outcomes at Day 91 in the Aerosolized and Subcutaneous Vaccine Groups.

Rates of seropositivity measured in the per-protocol, full-analysis, and multiple-imputation sets and in the two other sensitivity analyses are shown. Seroconversion was measured in children who were seronegative for measles antibody at day 0 and had a valid result from a serum sample at day 91. The dashed line indicates the noninferiority margin of 5 percentage points, the squares indicate point estimates, and the I bars indicate 95% confidence intervals.

samples); this confirmed the need to test samples that were negative on ELISA with the use of PRNT. All samples that were seronegative according to PRNT at baseline were also seronegative on ELISA.

IMMUNOGENICITY

At day 91, a total of 662 of the 775 children in the per-protocol population who received aerosolized vaccine (85.4%; 95% CI, 82.5 to 88.0) and 743 of the 785 who received subcutaneous vaccine (94.6%; 95% CI, 92.7 to 96.1) were seropositive, a difference of -9.2 percentage points (95% CI, -12.2 to -6.3). The results, based on the confidence intervals, did not show noninferiority of aerosolized vaccine to subcutaneous vaccine at the 5 percentage-point margin. Aerosolized vaccine, as compared with subcutaneous vaccine, resulted in statistically inferior rates of seropositivity (Fig. 2). After multiple imputation and with seroconversion as the outcome, results in the per-protocol and full-analysis data sets were similar (Table S6 in the Supplementary Appendix). In a sensitivity analysis in which the results of damaged specimens were classified as being either all seronegative or all seropositive, the differences between aerosolized and subcutaneous vaccine were smaller but still did not show noninferiority of the aerosolized vaccine. Among children who were seropositive at day 91,

geometric mean concentrations were similar in the two groups (ratio of the concentration in the aerosol group to that in the subcutaneous group, 1.05; 95% CI, 0.99 to 1.11) (Table S6 in the Supplementary Appendix). None of the measured factors were very strongly associated with a lack of seroconversion after the receipt of aerosolized vaccine (Table S7 in the Supplementary Appendix).

Among children in Vadu, geometric mean concentrations increased at each time point up to 364 days after vaccination in both the aerosol and subcutaneous groups (Fig. 3). Between-group differences in the proportion of children in Vadu who were seropositive (the percentage of children who were seropositive in the aerosol group minus the percentage of children who were seropositive in the subcutaneous group) were as follows: -0.5 percentage points (95% CI, -5.9 to 4.5) on day 0, -24.4 percentage points (95% CI, -36.3 to -11.8) on day 28, -11.2 percentage points (95% CI, -18.3 to -3.9) on day 91, and 1.5 percentage points (95% CI, -8.7 to 11.2) on day 364.

SAFETY

Serious Adverse Events

None of the children died during the trial or the follow-up period. By day 91, a total of 38 children who had received aerosolized vaccine and 31 who had received subcutaneous vaccine had

had a serious adverse event (Table 2, and Tables S8 and S9 in the Supplementary Appendix). The data and safety monitoring board did not judge any of the serious adverse events to be probably or most probably associated with vaccination (Table S8 in the Supplementary Appendix).

Adverse Events

In total, 3776 adverse events from any cause (1952 in the aerosol group and 1824 in the subcutaneous group) were recorded up to 91 days after vaccination, mostly during three home visits in the first 14 days (Table 2, and Tables S9 and S10 in the Supplementary Appendix). Adverse-event profiles in the two groups were similar, but coryza was more common among children who received aerosolized vaccine (Table 2) ($P=0.02$). Five adverse effects (rash, coryza, cough, diarrhea, and fever) that were reported in 4 episodes in 2 children in the aerosol group and 13 episodes in 3 children in the subcutaneous group were judged as being probably or most probably related to the vaccination.

DISCUSSION

Aerosolized vaccine against measles, as compared with subcutaneously administered vaccine, resulted in statistically inferior levels of seropositivity at 91 days in children who were 9.0 to 11.9 months of age. Profiles of adverse events were similar in the two groups, and adverse events associated with vaccination were rare.

The difference of 9 to 10 percentage points in seropositivity between the aerosolized and subcutaneous vaccine was greater than the prespecified noninferiority margin of 5 percentage points. The narrow noninferiority margin reflected the need to elicit a high level of seropositivity for antibodies against measles, given the established record of the licensed subcutaneous vaccine.⁷ The main limitation of our trial was that we did not have measles antibody results for nearly 20% of the samples; although we followed almost all participants to day 91, a batch of specimens was damaged during transport. We do not believe that this biased our results, however, because these data were missing at random, sensitivity analysis with the use of multiple imputation showed that our findings were robust, and the sample size was adequate because the enrollment target allowed for 20% loss to follow-up.

The proportion of children who were seropositive 91 days after they received aerosolized vaccine against measles was 85% (95% CI, 83 to 88); this proportion was also lower than expected. Inhibitory maternal antibodies and immature immune systems could have reduced responses to the measles vaccine,⁷ but only 2% of the children had measles antibodies at enrollment, and seropositivity was similar in older and younger children. Furthermore, seropositivity in children after receipt of the subcutaneous vaccine was in line with published data.⁷

The proportion of children who were protected against measles by aerosolized vaccine could be higher than observed because cell-mediated immunity is not captured by measurement of neutralizing antibodies. There is no consistent evidence, however, that cell-mediated immunity is relatively more important after aerosol vaccination than after subcutaneous vaccination.^{10,25} Prolonging the duration of nebulization to increase the retained dose of aerosolized vaccine could increase levels of both neutralizing antibodies and cellular immune responses.²⁵ In the subgroup of children tested in Vadu, the geometric mean concentrations increased between

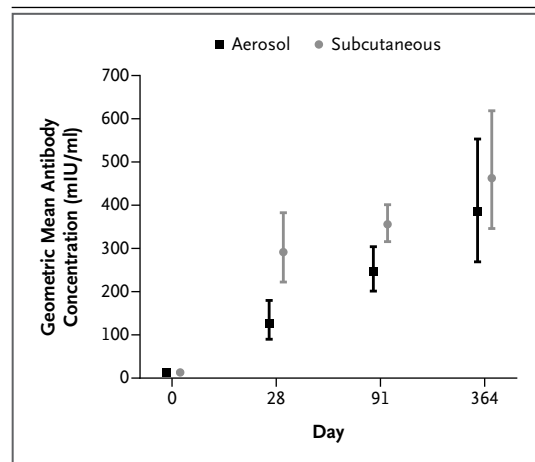


Figure 3. Geometric Mean Concentrations of Antibodies against Measles, According to Vaccine Group.

Shown are the geometric mean concentrations of antibodies to measles virus as measured on the PRNT in children in Vadu who were randomly assigned to aerosolized or subcutaneous vaccine against measles. Specimens were available at days 0, 28, 91, and 364. Observations from day 0 and day 91 include all children who were enrolled in the trial; observations from day 28 and 364 are from randomly selected children. Circles and squares indicate point estimates, and I bars indicate 95% confidence intervals.

Table 2. Adverse Events up to Day 91.*

Event	Aerosolized-Vaccine Group (N=1002)			Subcutaneous-Vaccine Group (N=998)		
	No. of Adverse Events	No. of Children with Event†	% (95% CI)	No. of Adverse Events	No. of Children with Event†	% (95% CI)
Type of event						
Any						
Total	1952	644	64.3 (61.2–67.2)	1824	609	61.0 (57.9–64.1)
Probably or most probably related to vaccination	4	2	0.2 (0.02–0.7)	13	3	0.3 (0.1–0.9)
Serious						
Total	38	38	3.8 (2.7–5.2)	32	31	3.1 (2.1–4.4)
Probably or most probably related to vaccination	0	0	0 (0.0–0.4)	0	0	0 (0.0–0.4)
Events of any severity						
Anorexia	21	19	1.9 (1.2–3.0)	22	20	2.0 (1.2–3.1)
Conjunctivitis	8	8	0.8 (0.4–1.6)	4	4	0.4 (0.1–1.0)
Coryza‡	594	441	44.0 (40.9–47.1)	510	386	38.7 (35.6–41.8)
Cough	331	264	26.3 (23.6–29.2)	331	269	27.0 (24.2–29.8)
Crying	39	34	3.4 (2.4–4.7)	30	29	2.9 (2.0–4.2)
Diarrhea	366	282	28.1 (25.4–31.0)	368	286	28.7 (25.9–31.6)
Difficulty breathing	5	4	0.4 (0.1–1.0)	2	2	0.2 (0.02–0.7)
Fever	363	287	28.6 (25.9–31.6)	362	274	27.5 (24.7–30.3)
Irritability	33	29	2.9 (2.0–4.1)	28	24	2.4 (1.6–3.6)
Local reaction	2	2	0.2 (0.02–0.7)	0	0	0 (0.0–0.4)
Malaise	12	11	1.1 (0.6–2.0)	16	14	1.4 (0.8–2.3)
Rash	34	33	3.3 (2.3–4.6)	29	27	2.7 (1.8–3.9)
Seizure	1	1	0.1 (0.0–0.6)	0	0	0 (0.0–0.4)
Shivering	0	0	0.0 (0.0–0.4)	0	0	0 (0.0–0.4)
Vomiting	114	96	9.6 (7.8–11.6)	100	91	9.1 (7.4–11.1)
Wheeze	2	2	0.2 (0.02–0.7)	1	1	0.1 (0.0–0.6)
Others	27	18	1.8 (1.1–2.8)	21	11	1.1 (0.6–2.0)

* CI denotes confidence interval.

† Each child could have more than one adverse event

‡ P=0.02 for the between-group difference.

day 91 and day 364 in children in both trial groups. Vaccine-induced antibody levels tend to decrease over time, but natural boosting from circulating measles virus can result in sustained or increasing antibody levels.²⁶ The case-based surveillance system showed that measles virus was circulating throughout the trial area,²² so natural boosting of antibody levels in children in both trial groups might have attenuated the difference in seropositivity by day 364.

The results of this trial are relevant for the planning of future research to determine the

ways in which aerosolized vaccine against measles could still contribute to further policies and goals for measles immunization worldwide.^{1,18} First, aerosolized vaccine against measles might be effective in older children. In countries that have high levels of measles vaccine coverage or that are in the elimination phase, primary vaccination at 12 months of age is recommended. Second, a second dose of a measles-containing vaccine is now recommended to protect children who did not have a response to the first dose and to maintain the 95% level of population im-

munity required to eliminate measles.²⁷ By 2014, a total of 145 WHO member states had introduced a two-dose measles vaccination strategy.⁴ Aerosolized vaccine against measles induced higher and more sustained levels of serologic protection than subcutaneous vaccine when administered as a second dose in school-age children in South Africa.^{28,29} Third, if aerosolized vaccine increases the coverage of primary vaccination, this strategy could be more cost-effective than two-dose vaccination at lower levels of coverage.³⁰

Data are lacking to evaluate the effectiveness and cost-effectiveness of aerosolized vaccine against measles as a primary dose in children older than 12 months of age and as a second dose in children younger than 5 years of age. Data are also lacking to evaluate the effectiveness and cost-effectiveness of aerosolized vaccine containing both measles and rubella virus. In

this randomized, controlled trial, a primary dose of aerosolized vaccine against measles in children 9 months of age or older was immunogenic, but at the prespecified margin, the aerosolized vaccine was inferior to the subcutaneous vaccine with respect to the rate of seropositivity.

The views expressed in this article are those of the authors and do not necessarily represent the decisions, policies, or views of the WHO.

Supported by a grant (21537) from the Bill and Melinda Gates Foundation.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the health center staff, field staff, parents or guardians, and children at the trial sites in Pune for their contributions; Dr. Thomas Gsponer from the Institute of Social and Preventive Medicine, University of Bern, who oversaw the initial statistical analyses and performed additional statistical analyses; Dr. Bernard Cohen from Public Health England, who provided support to set the laboratory standards for the trial and oversaw the laboratory in India; Drs. Teresa Aguado and Marie-Paule Kiény from the WHO for their expert advice; and the data and safety monitoring board and the product development group (members listed in the Supplementary Appendix).

APPENDIX

The authors' full names and academic degrees are as follows: Nicola Low, M.D., Ashish Bavdekar, D.N.B., Lakshmanan Jeyaseelan, Ph.D., Siddhivinayak Hirve, Ph.D., Kavitha Ramanathan, M.Sc., Nicholas J. Andrews, Ph.D., Naseem Shaikh, Ph.D., Ramesh S. Jadi, Ph.D., Arunachalam Rajagopal, M.C.A., Kevin E. Brown, M.D., David Brown, F.R.C.Path., James B. Fink, Ph.D., Oommen John, M.D., Pippa Scott, Ph.D., A. Ximena Riveros-Balta, B.Sc., Michel Greco, M.B.A., Rajeev Dhere, Ph.D., Prasad S. Kulkarni, M.D., and Ana Maria Henao Restrepo, M.D.

The authors' affiliations are as follows: the Institute of Social and Preventive Medicine, University of Bern, Bern (N.L., P.S.), and the World Health Organization (WHO), Geneva (A.X.R.-B., A.M.H.R.) — both in Switzerland; the Department of Pediatrics, King Edward Memorial Hospital Research Centre (A.B.), the National Institute of Virology (N.S., R.S.J.), the Serum Institute of India (R.D., P.S.K.), and Shirdi Sai Baba Hospital (S.H.), Pune, the Department of Biostatistics, Christian Medical College, Vellore (L.J., K.R., A.R.), and the WHO Regional Office for South-East Asia, New Delhi (O.J.) — all in India; the Statistics Unit (N.J.A.) and Virus Reference Department (K.E.B., D.B.), Public Health England, London; Aerogen, Galway, Ireland (J.B.F.); and Sainte-Foy-lès-Lyon, France (M.G.).

REFERENCES

1. Global vaccine action plan 2011-2020. Geneva: World Health Organization, 2013 (http://www.who.int/immunization/global_vaccine_action_plan/GVAP_doc_2011_2020/en).
2. Katz SL, Enders JF. Immunization of children with a live attenuated measles virus. *AMA J Dis Child* 1959;98:605-7.
3. Simons E, Ferrari M, Fricks J, et al. Assessment of the 2010 global measles mortality reduction goal: results from a model of surveillance data. *Lancet* 2012; 379:2173-8.
4. Measles deaths reach record lows with fragile gains toward global elimination. Geneva: World Health Organization, 2014 (<http://www.who.int/mediacentre/news/notes/2014/measles-20140206/en/index.html>).
5. Omer SB, Hiremath GS, Halsey NA. Respiratory administration of measles vaccine. *Lancet* 2010;375:706-8.
6. Garrison LP Jr, Bauch CT, Bresnahan BW, Hazlet TK, Kadiyala S, Veenstra DL. Using cost-effectiveness analysis to support research and development portfolio prioritization for product innovations in measles vaccination. *J Infect Dis* 2011; 204:Suppl 1:S124-S132.
7. The immunological basis for immunization series. Module 7: measles update 2009. Geneva: Department of Immunization, Vaccines and Biologicals, World Health Organization, 2009 (http://whqlibdoc.who.int/publications/2009/9789241597555_eng.pdf).
8. Fernández-de Castro J, Kumate-Rodríguez J, Sepúlveda J, Ramírez-Isunza JM, Valdespino-Gómez JL. Measles vaccination by the aerosol method in Mexico. *Salud Publica Mex* 1997;39:53-60. (In Spanish.)
9. Wong-Chew RM, Islas-Romero R, García-García Mde L, et al. Immunogenicity of aerosol measles vaccine given as the primary measles immunization to nine-month-old Mexican children. *Vaccine* 2006;24:683-90.
10. Wong-Chew RM, Islas-Romero R, García-García Mde L, et al. Induction of cellular and humoral immunity after aerosol or subcutaneous administration of Edmonston-Zagreb measles vaccine as a primary dose to 12-month-old children. *J Infect Dis* 2004;189:254-7.
11. Cutts FT, Clements CJ, Bennett JV. Alternative routes of measles immunization: a review. *Biologicals* 1997;25:323-38.
12. Hiremath GS, Omer SB. A meta-analysis of studies comparing the respiratory route with the subcutaneous route of measles vaccine administration. *Hum Vaccin* 2005;1:30-6.
13. Low N, Kraemer S, Schneider M, Restrepo AM. Immunogenicity and safety of aerosolized measles vaccine: systematic review and meta-analysis. *Vaccine* 2008; 26:383-98.
14. Henao-Restrepo AM, Greco M, Laurie X, John O, Aguado T. Measles aerosol vaccine project. *Procedia Vaccinol* 2010;2: 147-50.
15. Workbook for investigators. Geneva: World Health Organization Special Pro-

- gram for Research and Training in Tropical Diseases, 2002 (<http://www.who.int/tdr/publications/documents/investigator.pdf>).
16. Handbook: good laboratory practice: quality practices for regulated non-clinical research and development. Geneva: World Health Organization Special Program for Research and Training in Tropical Diseases, 2001 (<http://www.who.int/tdr/publications/training-guideline-publications/good-laboratory-practice-handbook-ver1/en>).
17. International Institute for Population Sciences (IIPS). District level household and facility survey (DLHS-3), 2007-08: India. Mumbai, India: IIPS, April 2010 (http://www.rchiips.org/pdf/india_report_dlhs-3.pdf).
18. World Health Organization. Measles vaccines: WHO position paper. *Wkly Epidemiol Rec* 2009;84:349-60.
19. International Network for the Demographic Evaluation of Populations and Their Health (INDEPTH). 2013 (http://www.indepth-network.org/Profiles/vadu_hdss_2013.pdf).
20. Cohen BJ, Doblas D, Andrews N. Comparison of plaque reduction neutralisation test (PRNT) and measles virus-specific IgG ELISA for assessing immunogenicity of measles vaccination. *Vaccine* 2008;26:6392-7.
21. Bonhoeffer J, Bentsi-Enchill A, Chen RT, et al. Guidelines for collection, analysis and presentation of vaccine safety data in pre- and post-licensure clinical studies. *Vaccine* 2009;27:2282-8.
22. Bose AS, Jafari H, Sosler S, et al. Case based measles surveillance in Pune: evidence to guide current and future measles control and elimination efforts in India. *PLoS One* 2014;9(10):e108786.
23. Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. *Stat Med* 1998;17:873-90.
24. van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. *Stat Med* 1999;18:681-94.
25. Wong-Chew RM, García-León ML, Espinosa-Torres Torrija B, et al. Increasing the time of exposure to aerosol measles vaccine elicits an immune response equivalent to that seen in 9-month-old Mexican children given the same dose subcutaneously. *J Infect Dis* 2011;204:426-32.
26. Whittle HC, Aaby P, Samb B, Jensen H, Bennett J, Simondon F. Effect of sub-clinical infection on maintaining immunity against measles in vaccinated children in West Africa. *Lancet* 1999;353:98-102.
27. Gay NJ. The theory of measles elimination: implications for the design of elimination strategies. *J Infect Dis* 2004;189:Suppl 1:S27-S35.
28. Dilraj A, Cutts FT, de Castro JF, et al. Response to different measles vaccine strains given by aerosol and subcutaneous routes to schoolchildren: a randomised trial. *Lancet* 2000;355:798-803.
29. Dilraj A, Sukhoo R, Cutts FT, Bennett JV. Aerosol and subcutaneous measles vaccine: measles antibody responses 6 years after re-vaccination. *Vaccine* 2007;25:4170-4.
30. Beutels P, Gay NJ. Economic evaluation of options for measles vaccination strategy in a hypothetical Western European country. *Epidemiol Infect* 2003;130:273-83.

Copyright © 2015 Massachusetts Medical Society.