Seroconversions in unvaccinated infants: further evidence for subclinical measles from vaccine trials in Niakhar, Senegal

John Bennett, Hilton Whittle, Badara Samb, Badara Cisse, Francois Simondon and Peter Aaby

Background

Increases in measles antibodies without rash illnesses have been documented in previously vaccinated children exposed to measles cases. The phenomenon has been incompletely evaluated in young unvaccinated infants with immunity of maternal origin.

Methods

Monthly cohorts of newborns were prospectively randomized to vaccine and placebo control groups during a trial of high-titre vaccines in Niakhar, Senegal. Measles antibodies were assayed in blood samples of enrolled children collected at 5 months old, when controls received a placebo injection, and at 10 months, when the placebo group was given measles vaccine. Intensive prospective surveillance for measles was conducted throughout the trial.

Results

One-fifth (n = 53) of the placebo controls seroconverted, with known exposure to a measles case in only three of them. None of the seroconverters developed a measles-like rash. Sixteen-fold or greater increases in titres were noted in about one-quarter of them. Compared with placebo controls who did not seroconvert, seroconverters were more likely to have had exposure to a measles case and to travel, more likely to be boys than girls, and had significantly lower baseline antibody titres. Measles was endemic in the study area throughout the trial. Seroconversions did not adversely effect subsequent nutritional indices or mortality.

Conclusions

Although laboratory errors and inadvertent injection of vaccine rather than placebo may have played some role, they do not fully explain the above observations, which are consistent with subclinical measles in the seroconverters. The possible role of subclinical measles in occult transmission, its potential effect on the type and duration of subsequent immunity, and its impact on response to primary vaccination need to be determined.

Keywords

Measles, subclinical, infants, seroconversions

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Subclinical measles is evidenced by increasing antibodies in the absence of measles-like rashes, and has been documented in previously vaccinated children exposed to measles cases. Similar events may be hypothesized in infants with immunity of maternal origin which declines as infants grow older. An opportunity to further assess such responses in infants was provided by a placebo control group enrolled during a trial of high-titre measles vaccine in Niakhar, Senegal.

Methods

Beginning in 1987, 24 successive monthly cohorts of newborns in a rural area of Senegal were enrolled in a trial of high-titre measles vaccines. Children were assigned at birth to receive either a high-titre vaccine or a placebo injection at 5 months old. Children in the placebo group had blood samples collected for measles antibody determinations at 5 and 10 months old, and were given a standard dose of measles vaccine at 10 months old after the second blood sample was obtained. Intensive, prospective surveillance for measles cases was conducted in the study area throughout the trial. Each compound
was visited by experienced, trained health workers on a weekly basis. Mothers were asked about illnesses in their children. All measles-like rash illnesses were further evaluated by a physician. This system was thus considered likely to be highly sensitive in detecting measles-like rashes.

Antibody titres were determined by a previously described haemagglutination inhibition (HI) technique\(^6\) with a starting dilution of 1:2 and capability of detecting concentrations of \(\geq 62.5 \, \text{MIU}\) in undiluted sera. For calculation of geometric mean titres (GMT), titres \(\leq 1:2\) were assumed positive in undiluted sera (i.e. at a 1:1 dilution). Assays were run periodically as specimens were received from cohorts 1 through 18. Assays were not run on specimens from cohorts 19–24.

All specimens from placebo controls showing twofold or greater increases between the 5 and 10 month samples were deemed to have seroconverted. Seroconverters were then compared with children in the placebo group who had stable or declining antibody titres between 5 and 10 months. Children showing increases \(\geq 4, 8\), and 16-fold were also compared with those who had no increase in titres.

Data was locally recorded in Database III+ files (Ashton-Tate), which were subsequently converted into Epi Info\(^7\) files for analysis. Conditional logistic regression was performed with LOGISTIC\(^8\) and anthropometric analysis with EPINUT, a program available within Epi Info\(^7\) that adjusts for sex and exact age.

### Results

Dropout rates for the 971 children randomized at birth to the control group were 65% and 50% for attendance at the 5 and 10 month sessions, respectively. Measles antibody titres were determined at both 5 and 10 months old for only 254 (26%) of the children initially randomized to the placebo control group; 146 (57.5%) showed declining titres, 55 (21.7%) stable titres (of which 43 had undetectable titres at both 5 and 10 months), and 53 (20.9%) had twofold or greater increases in titre. About one-third of these were serosensitive at 10 months old, with seropositivity significantly more frequent in boys than girls (51/125, 40.8% versus 35/129, 27.1%, \(P = 0.03\) by \(\chi^2\)). In contrast, increases in measles antibody titres from 5 to 10 months were shown for the seroconverters in Table 1. Nearly half of them had 16-fold or greater increases. Thirty-nine of the 53 seroconverters had no detectable antibody at 5 months.

Measles cases (\(n = 62\)) occurred in the study area in all but five of the months encompassed by the vaccination phase for cohorts 1–18, with cases occurring during the 5 months interval between placebo injection and collection of 10 month blood samples for each seroconverting control.

Comparisons of health status, exposures and host factors of seroconverters and placebo controls who did not show increases in titres are presented in Table 2. Health status (mortality and nutritional indices) did not differ significantly between groups, and there were no measles-like rashes among any of the children in the placebo group.

The average population in the compound, and frequency of exposure to vaccinated children within the compound of residence were similar between the two groups. However, seroconverters were more likely to be exposed to measles cases. Four children had specific known exposure to a measles case before collection of the blood sample at 10 months, and three seroconverted.

### Table 1: Placebo controls with increasing measles antibody titres from 5 to 10 months old

<table>
<thead>
<tr>
<th>Increase (fold)</th>
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<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>Total</th>
<th>Percentage (%) with increase</th>
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<tbody>
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<td>0</td>
<td>2</td>
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<td>0</td>
<td>15</td>
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<td>1</td>
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<td>2</td>
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<td>Total</td>
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<td>53</td>
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Each of these seroconverters had negative baseline specimens, and reciprocal titres of 4, 16, and 128 in samples collected at 10 months of age. The fourth exposed child was seropositive at a 1:16 dilution at 5 months, and seronegative at 10 months. Seroconverters also travelled more frequently from their homes in the 5 months following placebo injections.

Boys were more likely than girls to seroconvert, and baseline titres at 5 months were significantly lower among seroconverters. However, baseline titres at 5 months did not differ significantly between the sexes (reciprocal GMT for boys and girls 3.0 and 3.4, respectively, \(P = 0.63\) by ANOVA). The average interval between collection of blood specimens at 5 and 10 months was 150 days for girls and 151 days for boys (\(P = 0.53\) by ANOVA), indicating comparable duration of exposure for boys and girls.

Logistic regression revealed increasing baseline titres were inversely related, while male sex, more frequent trips, and known exposures to measles cases were positively related to the risk of seroconversion with odds ratios of 0.33, 2.7, 1.5, and 30.4, respectively, and \(P\) values of \(<0.0001\), 0.009, 0.015, and 0.033, respectively (likelihood ratio statistic). No significant interactions were noted, and no other factors contributed significantly when added to a model containing these four variables.

<table>
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The reproducibility of the HI assay was further evaluated. Triplicate readings by three observers on 15 samples showed...
identical outcomes in 41 instances, and readings within ±1 dilution in the remaining four instances, suggesting little interobserver variation in reading the same samples. Further, all samples collected at 10 months that revealed eightfold or greater increases were re-assayed, with confirmation of eightfold or greater increases in about three-quarters of the samples that were re-tested. In addition, none of the 58 samples rerun to check reproducibility showed 16-fold or greater increases.

Antibody levels were not systematically measured after vaccination. However, 64 of the children were included in a serosurvey conducted 5–6 years after vaccination. The geometric mean titre for nine children who seroconverted from 5 to 10 months of age in Haitian infants prior to the introduction of measles vaccination showed increasing prevalence by month, increasing from about 10% positive at 6 months old to 58% at 10 months that revealed eightfold or greater increases in titre in samples collected at 5 and 10 months old.

Discussion

The data derived from our placebo-controlled study are consistent with the notion that unrecognized exposure to measles virus may result in seroconversions without measles-like rashes in young infants in endemic areas. Baseline titres of seroconverting controls were often below putative 'protective' levels, but measles-like rashes did not occur along with the seroconversions, and were notably absent in the three cited instances where a seronegative infant seroconverted after known specific exposure to a measles case. The basis for protection against measles-like rashes in the seronegative infants who seroconverted is uncertain, but most likely derived from antibodies of maternal origin that were present in concentrations below the threshold of the assay.

The classic studies of Krugman et al. in the early 1960s first suggested the possible existence of this phenomenon. During a longitudinal study of measles immunity during the first year of life, 6 of 107 infants had fourfold or greater increases in titre following 'known or unknown' exposure to measles. Modified disease or subclinical infection without measles-like rashes accompanied these seroconversions. Measles antibodies were not detectable in these children before the titre increases, but antibodies of maternal origin were nonetheless postulated to have been present in sufficient amount to suppress disease without suppressing infections.

Studies in Guinea-Bissau of 24 infants exposed to measles when younger than 6 months revealed high enzyme immunoassay (ELISA) antibody titres in five following exposure. Of these five children, two had clinical measles, two had no symptoms of illness, and one manifested only fever following exposure. There were no deaths.
to about 40% at one year old.\textsuperscript{11} Histories of measles were present only in an estimated 15% of children who were seropositive at one year old. Children whose mothers had low titres were significantly more likely to acquire measles antibodies. Weight-for-age (expressed as percentage of the international reference median values) did not differ significantly between seropositive (HI $\geq 1:10$) and seronegative children at 6, 7, 8 or 9 months, but was significantly less for seropositive children who were 10 months old. Length-for-age did not differ significantly among children $\leq$10 months in age.

Trials of measles vaccine in the pre-vaccine era frequently noted neutralizing antibodies among children with no histories of measles. Such observations were noted in 8/24 (33.3%) of children aged 10 months to 32 years,\textsuperscript{12} 223 of 425 (52.5%) and 115 of 348 (33.0%) school children aged 5–10 years in two separate surveys\textsuperscript{13} and in a ‘considerable portion’ of 101 institutionalized children.\textsuperscript{14}

A previous report from our study site also noted detectable HI antibodies to measles in 32% of 313 unvaccinated children who were older than one year and who had no histories of measles.\textsuperscript{15}

Illnesses other than measles-like rash illnesses were not systematically studied by us in connection with seroconversions. The available data on height and weight at 10 months, and deaths, suggest that such illnesses, if they did occur in seroconverters, were mild and generally pursued a benign course. Further, children who were seropositive at 10 months old averaged 82.1% of the reference median for weight-for-age, while seronegatives averaged 80.8% ($P = 0.59$ by ANOVA). Thus, our observations differ from those in Haitian children cited above,\textsuperscript{11} perhaps because measles cases were included among the Haitian seropositives, whereas none of our children who were seropositive at 10 months had experienced measles.

The risk of seroconversion without measles-like rash increased with increasing number of trips taken to Dakar and other towns. Such travel would increase the likelihood of exposure to measles virus, and the significant association of travel, as well as the increased frequency of known exposures to measles cases, assist in establishing the plausibility of subclinical measles in these infants. Travel might also increase the risk of receiving measles vaccine elsewhere, although the standard policy in Senegal at the time was to vaccinate at 10 months old, and none of the seroconverting placebo controls reported receipt of vaccine between 5 and 10 months. Travel did not increase the risk of seroconversion in the high titre vaccinees, perhaps because a much greater proportion seroconverted following vaccination, leaving less opportunity to observe such as response.

Placebo control girls in our study were more likely to be seronegative at 10 months old, and seroconverted significantly less frequently than boys between 5 and 10 months. Baseline titres were similar for the sexes at 5 months, however, suggesting that sex-specific differences in responses rather than baseline titres, may have been the main determinant of the titre differences seen at 10 months. Boys did not travel more frequently than girls (average trips 0.37 and 0.50, respectively, $P = 0.62$ by ANOVA), but more frequent or intense exposures of boys may nonetheless have occurred. Boys may also have responded more frequently to comparable exposures. Available data does not allow the relative importance of these two most obvious explanations to be established.

We cannot exclude the erroneous administration of vaccine rather than placebo nor misidentification of specimens as explanations for some of the antibody responses without measles-like rashes. Erroneous vaccine injection is attractive as a mechanism for antibody increases without rash. However, the administration of vaccines at 5 months of age was closely supervised, and repeat assays of samples showed credible reproducibility. Further, more than 20% of the placebo controls seroconverted, which seems far too frequent to be likely to be plausibly explained by such errors. Finally, incorrect injections should not have affected significantly more boys than girls nor have occurred more frequently in those exposed to measles cases, and the fact that sex was not a predictor of response in the high-titre vaccine groups argues against inadvertent vaccination as a major factor underlying seroconversions in the placebo controls.

It is also conceivable that seroconverting controls without measles-like rashes may have acquired vaccine virus as a result of exposure to contacts who had received measles vaccines. Spread of vaccine virus to susceptibles following standard doses has never been demonstrated. Further, although not specifically studied in any of the other high-titre vaccine trials, our findings do not suggest more frequent exposure of seroconverting controls than non-converters to children who had been given either high-titre or standard titre vaccines.

Variation in the reproducibility of the assay may also have been responsible for some seroconversions. This might be especially likely in the 15 instances where baseline and 10-month samples differed only by one dilution. However, findings remained unaltered even with very much stricter criteria for seroconversion, including 16-fold increases, where reproducibility becomes a very unlikely explanation for the observed titre changes. The consistently strong influence of baseline titres on seroconversion provides a biological basis for the observed responses, and argues against the seroconversions being solely artifactual.

The epidemiological implications of our findings and related data from other studies are uncertain. It appears that seroconversions can occur in unvaccinated infants unattended by rash and consequent to unrecognized exposures. Such responses might be expected to blunt subsequent responses to initial vaccination at later ages, and to complicate estimates of time of decay of maternal antibodies. Although antibody responses to vaccine might subsequently be impaired, such infants might nonetheless possess enhanced protection against measles consequent to respiratory tract mucosal exposure to wild measles virus. Replication of measles virus presumably accompanies these seroconversions, and it is possible that infants with subclinical measles could transmit measles virus to their contacts, thus participating in occult chains of transmission. Further studies of these interesting possibilities are warranted.

Acknowledgements

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References


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