Prevalence of measles antibody among children under 15 years of age in Santa Cruz, Bolivia: implications for vaccination strategies

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Abstract

We conducted a community-based survey in Santa Cruz city, Bolivia, to determine the age-specific prevalence of measles antibodies, determine factors associated with absence of detectable measles antibodies, and to compare results of salivary and serum measles immunoglobulin G (IgG) antibody assays. Serum samples from 1654 children were assayed for measles IgG antibody using the haemagglutination inhibition (HI) assay, and salivary samples were also obtained from 187 children and tested for measles IgG antibody using an antibody capture radioimmunoassay. Reported measles vaccine coverage in children aged 12–35 months was 77% (95% confidence interval [CI], 72–81%). Eighty-seven percent (95% CI 85–89%) had detectable HI antibody, but a high proportion had antibody levels below 200 miu (30–40% of 2–14 years old children). Measles seronegativity was associated with not being vaccinated against measles, a negative history of measles disease, living in the inner city, being a lifetime resident of Santa Cruz, and young age. Of 212 children without detectable measles antibody, 58% had a positive history of vaccination or measles disease, so that historical information was not sufficiently reliable to identify susceptibles. The salivary measles antibody assay was not sufficiently sensitive to be used for population screening; only 54% of 171 salivary samples from children who had detectable serum HI antibody were positive. A mass measles vaccination campaign of all children under 15 years of age is planned in Bolivia in 1994. Although only 7% of school-age children in Santa Cruz were seronegative, the effectiveness of a mass campaign in this age group depends in part on the response to revaccination of children with low, but detectable, antibody levels.

Keywords: measles, seroprevalence, vaccination, Bolivia

Introduction

Measles vaccination is one of the most cost-effective health interventions available, and measles has been identified as a potential candidate for eradication (Hopkins *et al.*, 1982). An increasing number of countries have established targets for the elimination of measles transmission nationally or regionally, and the design and evaluation of optimal strategies for using measles vaccines is a priority research area (Nokes & Cutts, 1993).

Several Latin American countries have recently conducted a mass vaccination campaign of all children aged 9 months to 14 years, and the impact on measles incidence has been dramatic. Although many older children are already immune, mass revaccination is more effective than selective vaccination of children classified as susceptible through a negative history of disease or vaccination, because these histories are not reliable (Preblud *et al.*, 1982; Scott *et al.*, 1984). The development of improved rapid field techniques to measure measles antibody, perhaps using non-invasive techniques such as salivary assays (Perry *et al.*, 1993), would enable developing countries to monitor vaccination programmes more precisely and to predict the need for supplementary strategies such as mass campaigns.

We conducted a serosurvey in Santa Cruz city, Bolivia, to determine the age-specific prevalence of measles antibody and compare results of salivary and serum measles immunoglobulin G (IgG) antibody assays, to compare vaccine coverage and seropositivity rates in the inner and the outer city, and to determine factors associated with the absence of detectable measles antibodies.

Materials and Methods

The setting

Bolivia routinely administers measles vaccine at a recommended age of 9 months at health centres, and supplements this by annual campaigns involving selective vaccination of children 9–59 months of age who have not previously received measles vaccine. In Santa Cruz city, according to routine vaccination reports, it was estimated that 73% of children had received measles vaccine by their first birthday in 1992, and 84% in 1993 (Santa Cruz regional health authority, unpublished data). Of 1314 measles cases reported by city health facilities in 1992–1993, 49% were in children over 5 years of age.

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Survey design

We conducted a community survey in Santa Cruz, stratified by inner city (estimated 1992 population 372 276) and outer suburbs (population 322 340). In each stratum, a cluster sample of 20 administrative units (*Unidades Vecinales*, UV) was selected by sampling with probability proportional to estimated size. We used the detailed maps which are available of the inner city to select randomly 4 blocks from each UV in the sample. For the outer city, we obtained a list of blocks from the administrative authorities in each UV and selected a random sample of 4 blocks. In each block, from a random starting point, interviewers proceeded in a clockwise direction, visiting every household, and registering every child under age 15 in the household until 10 children had been registered. Up to 2 revisits were made if a child was not at home.

At each household with a child aged 0–14 years, we administered a short questionnaire to obtain information on vaccination status and measles disease. With parental consent, we collected a finger-prick blood sample on absorbent paper (M14 0·05 mL microdiluter delivery tester). We collected saliva samples from a subsample of 192 children (all children registered on the last 2 d of the survey), using OraSure™ saliva collection devices (Epitope Inc, Beaverton, Oregon 97005, USA) according to the manufacturer's instructions.

The estimated sample size was 1600 children: 800 in each stratum. With an expected overall measles seropositivity rate of around 85%, this sample size was adequate to estimate the true rate for Santa Cruz with a 95% confidence interval of ±4%, assuming a design effect of 4. The sample size had 80% power to detect a 10% difference in seropositivity rates between the inner and outer city areas.

Serology

Blood samples were left to dry at room temperature,

then transported to Italy on dry ice, where they were tested for measles IgG in microtitre plates using a haemagglutination inhibition (HI) assay. A 12 mm diameter disk was cut from each blood spot and put into a test tube containing 150 μL of 0·15 M phosphate-buffered saline, pH 7·2. The tubes were left for 30 min at room temperature, then centrifuged at 2000 rpm for 10 min to obtain 150 μL of eluate from each disk. Eluates were coded and inactivated at 56°C, then absorbed for 90 min at 4°C with one-third volume of packed green monkey red blood cells. 25 μL of phosphate-buffered saline containing 4 haemagglutinating units of measles haemagglutinin (Behringwerke) were added to 25 μL of each dilution of eluate, shaken, and left overnight at 4°C. 25 μL of a 0·8% suspension of green monkey red blood cells were added to each of the V-bottom wells, left for 90 min at 37°C, and examined. Samples negative by HI assay were tested by measles IgG enzyme-linked immunosorbent assay (ELISA), using a commercially available kit (Enzygnost Measles®, Behringwerke AG, Marburg, Germany). Assays were standardized to the 2nd international standard antimeasles serum (FORSEY, 1992; FORSEY *et al.*, 1991).

Salivary assays

Salivary samples were shipped on ice to the Central Public Health Laboratory, London, UK. Saliva was tested for measles-specific IgG using an established antibody capture radioimmunoassay (PERRY *et al.*, 1993), based on tissue culture-grown virus and a measles-specific monoclonal antibody. Samples were considered positive for measles-specific IgG if the test to negative control (T/N) ratio was ≥2·0. To establish that individual saliva samples were adequate for antibody testing, total salivary IgG concentrations were measured by ELISA using a test previously reported for the quantification of urine IgG levels (CONNELL *et al.*, 1990). Microtitre plates (4-77631, Nunc) were coated with rabbit antibody to human IgG (A424, Dakopatts). Capture of salivary IgG was detected by peroxidase conjugated rabbit anti-human IgG (P406, Dakopatts) followed by tetranitro blue substrate. The IgG concentration was derived from a calibration curve constructed from dilutions of a control serum (Binding Site, catalogue no. GP004). The assay was set up to detect IgG concentrations in the range of 0·1–10 mg/L.

Data analysis

Descriptive analyses were performed using the Epi-Info statistical package (DEAN *et al.*, 1990). Since the total number of children registered per cluster varied, 95% confidence intervals (CI) were calculated using the formula for the analysis of cluster samples, with unequal numbers of children per cluster, given by BENNETT *et al.* (1991). Differences between proportions were tested using the χ^2 test, and Student's *t* test was used with the mean of log-transformed antibody titres to assess differences in geometric mean titres (GMTs) of measles antibody (GMTs were calculated for children with detectable antibody only). We assessed interaction between different variables and strata of the city using classical stratified analyses. Since there was no evidence of significant interaction (at the $P=0·1$ level), we pooled results from both strata and conducted unconditional logistic regression analyses for factors associated with lack of measles antibody, using EGRET (ANONYMOUS, 1991).

Results

General characteristics

A total of 1727 children were registered, 867 in the outer stratum and 860 in the inner stratum. There was no significant difference in sex or age distribution of children in each stratum. Families in the inner city had more rooms per house, but similar total numbers of household members. Most study children had lived all their life in Santa Cruz (12% of the outer, and 14% of the inner, stratum having lived elsewhere). More inner city children had spent more than one month outside Santa Cruz in the last year than outer city children (9·1% vs. 4·2%, $P<0·001$).

Vaccine coverage

Table 1 shows the vaccine coverage among the 280 children aged 12–35 months. Inner city children were more likely to have vaccination records. Coverage for each vaccine was somewhat higher in the inner city, but differences were not significant if a verbal history of vaccination was accepted.

Table 1. Percentage measles vaccination coverage of children aged 12–35 months, Santa Cruz, 1993

	Inner city (n=138)			Outer city (n=142)		
Vaccine*	Card	Verbal history	Total	Card	Verbal history	Total
BCG	41	49	90	32	56	88
Polio 0	40	49	89	30	55	85
Polio 1	43	47	90	35	54	89
Polio 2	40	46	86	30	51	81
Polio 3	35	44	79	26	46	72
DPT 1	44	46	90	34	54	88
DPT 2	40	45	85	30	51	81
DPT 3	35	44	79	25	46	71
Measles	36	42	78	29	46	75
Yellow fever	8	26	34	7	20	27

*BCG = bacillus Calmette–Guérin; DPT = diphtheria–pertussis–tetanus.

Overall, 89% (95% CI, 84–93%) of children aged 12–35 months had received BCG, first dose diphtheria–pertussis–tetanus (DPT) and oral polio vaccines (OPV), 75% third dose DPT and OPV (95% CI 70–80%), 77% measles (95% CI 72–81%), and 31% yellow fever vaccine (95% CI 25–36%).

Reported measles vaccine coverage increased with age, being 11% for children aged less than 9 months, 63% for those 9–23 months old, 83% for those 24–35 months old, 85% for those 36–59 months old, and 90% for school-aged children.

Prevalence of measles antibody

Filter paper samples were obtained from 1727 children; 73 specimens and were mislabelled or unreadable and were rejected. Most sera (201 of 209) which were negative by HI assay were also negative by ELISA, thus all results presented here refer to the HI assay.

Of the total sample, 87% (95% CI 85–89%) had detectable HI antibody, and 58·5% had antibody levels of 200 miu (milli-international units) or higher. The prevalence of antibody, as expected, varied with age (Fig. 1).

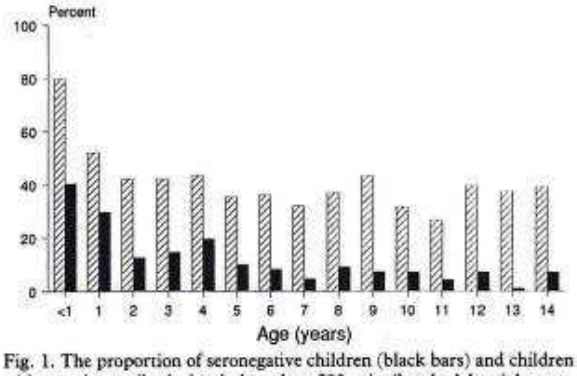

Fig. 1. The proportion of seronegative children (black bars) and children with measles antibody levels less than 200 miu (hatched bars) by age, Santa Cruz, Bolivia, 1993.

Among children aged less than one year, 40% lacked detectable antibody (32% of children aged under 9 months and 60% of children aged 9–11 months). This proportion dropped sharply to 12% of 2 years old children, increased somewhat at 3–4 years old and then fell again.

Among school-aged children, only 7% were seronegative. The percentage with antibody levels below 200 miu was highest (80%) among children less than one year old, but was fairly constant at 30–40% of 2–14 years old children. GMTs were lowest (133 miu) among children aged less than 9 months, but then varied little, between 321–339 miu, in older age groups. GMTs were significantly higher among children with a history of measles disease (398 miu) than among those without (251 miu).

Factors associated with lack of detectable measles antibody

Bivariate analysis showed that younger age, a negative history of measles disease or measles vaccination, being a lifetime resident in Santa Cruz, and (among children aged at least 5 years) not attending school, were significantly associated with susceptibility to measles. Slightly more children lacked detectable antibody in the inner than the outer city (14·3% vs. 11·4% $P=0·07$). The child's sex, number of rooms in the house, number of people sleeping in one room, and travel outside Santa Cruz in the last year, were not associated with measles antibody prevalence (data not shown).

Multivariate analysis showed that the risk factors for lack of measles antibody were a negative history of measles vaccination, a negative history of measles disease, living in the inner city, being a lifetime resident in Santa Cruz, and younger age (Table 2).

history of measles and/or measles vaccination. Of 270 children classified as susceptible according to a negative history of disease and vaccination, 88 (33%) had no detectable antibody. Of 1382 children with a positive history of disease or vaccination, 1258 (91%) had detectable antibody. The 124 (1382–1258) children who would be

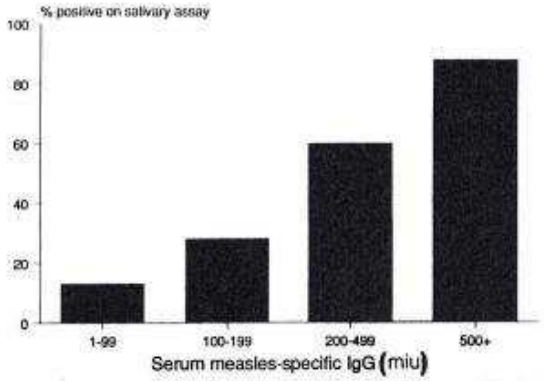

Fig. 2. The proportion of seropositive children with measles-specific IgG antibody in saliva. Serum antibodies measured by haemagglutination inhibition and salivary antibodies by radioimmunoassay.

Table 2. Risk factors for lack of measles antibody on multivariate analysis

Variable	Percentage of sample	Odds ratio Crude	Adjusted	95% CI[a]	*P*
Age group[b]					
5–9 years	33·8	1·65	1·58	0·9–2·7	0·1
36–59 months	16·6	3·79	3·35	1·9–5·8	<0·001
24–35 months	8·2	2·77	2·33	1·2–4·6	0·015
9–23 months	9·7	11·04	7·12	4·0–12·5	<0·001
<9 months	4·8	8·90	2·98	1·5–6·0	0·002
No history of measles vaccination	18·4	4·74	3·55	2·5–5·1	<0·001
No history of measles disease	82·4	2·89	2·14	1·2–3·7	<0·01
Living in inner city	49·9	1·29	1·36	1·0–1·8	0·06
Lived whole life in Santa Cruz	87·1	1·95	1·64	0·9–2·9	0·09

[a]95% confidence interval.
[b]Baseline=10–14 years.

Among children aged 5–14 years, attendance at school was found to be associated with lower susceptibility to measles by bivariate analysis (crude odds ratio [OR] 1·71, $P=0·07$). However, school attenders were more likely to be vaccinated than non-attenders (90·5% vs. 82·4%, $P<0·001$), and after controlling for other variables the adjusted OR was 1·46 (95% CI 0·8–2·7, $P=0·2$).

Relation between a history of measles and measles vaccination and measles antibody detection

Only 17·6% of all children had a history of measles. This proportion increased from 5% at less than 9 months old to 11% of those 9–59 months old and 22% of school-aged children. There was no difference between strata (17·4% in the inner city and 17·7% in the outer city had a positive history).

We assessed the sensitivity and specificity of a history of measles disease among the 302 unvaccinated children in the sample. Of 32 children with a history of measles, 26 had detectable measles antibody. Of 270 children with a negative history of measles, 88 had no detectable measles antibody. The sensitivity and specificity of reported measles were 12·5% and 93·6%, respectively. Thus, the main problem in this population appeared to be under-reporting of measles disease, rather than false positive diagnoses.

To determine the potential for using historical information to identify susceptible children, we evaluated the presence of antibody among children with and without a misclassified as protected formed a large proportion (58%) of the total 212 (124+88) children without detectable antibody, showing that historical information would have low predictive ability to identify susceptibles.

Correlation between salivary assays and HI assays

Salivary samples were obtained from 192 children, 187 of whom had serological results. All saliva samples had detectable total IgG (range 0·5–50 mg/L, GMT 4·14 mg/L (95% CI 3·6–4·8 mg/L)). Salivary total IgG levels were not significantly related to age. The salivary measles antibody assay was relatively insensitive; of 171 children with detectable HI antibody, only 54% were positive by salivary assay. On multiple regression analysis, the salivary measles-specific IgG ratio was positively related to serum HI level ($P<0·001$) (Fig. 2), and inversely related to total salivary IgG ($P<0·01$). There was no false positive result in the salivary assay among the 16 children with no detectable HI antibody.

Discussion

Vaccine coverage in Santa Cruz is high, if a verbal history of vaccination is accepted. Coverage did not differ significantly between the inner and outer cities. Although some measures of socioeconomic status (number of rooms in the household, and household crowding) were higher in the inner city than in the outer city, school attendance did not differ significantly, suggesting that access to social services was similar in the 2 strata.

Most children (87%) in the study had detectable

measles antibody. The proportion of susceptibles was highest in those under 9 months old, but was also high in children aged 9–23 months, reflecting a delay in vaccination; coverage of children 9–23 months old was significantly lower than that of those 24–35 months old.

Lack of measles vaccination was the factor most strongly associated with measles seronegativity. Among children with a history of vaccination, 8·8% were seronegative; this is in the range of the expected proportion of primary vaccine failures after measles vaccination at 9 months of age (DIAZ-ORTEGA *et al.*, 1994), although there may also have been some misclassification of vaccine status. A negative history of measles disease was also associated with lack of measles antibody. Children who had lived outside Santa Cruz were less likely to be seronegative, despite lower reported measles vaccination uptake (19% of those who had lived outside Santa Cruz were unvaccinated compared to 12% of lifetime residents). The proportions reporting measles disease were similar among residents and migrants, but the higher seroprevalence among migrants could reflect higher rates of unreported measles, given the low sensitivity of a history of measles.

The priority for measles control in Santa Cruz is to vaccinate children as soon as possible after they reach 9 months of age, in order to reduce levels of susceptibility among children under 3 years of age, who are most prone to serious disease. Strategies to reduce susceptibility in older children include selective or non-selective 'catch-up' vaccination. Selective vaccination would have lower vaccine costs, since only 16% of children would require vaccination based on their history of prior measles or measles vaccination, but this strategy would miss 58% of children without detectable antibody. Thus, in the absence of rapid field assays which would make selective vaccination of susceptible children a more cost-effective option (GRABOWSKY & MARKOWITZ, 1991), mass revaccination appears to be the only way to reach those who are susceptible.

In an earlier study in the UK, measles-specific IgG was reliably detected in saliva samples following acute measles infection (PERRY *et al.*, 1993). The same assay had low sensitivity in this study, in which most children had acquired measles antibody from vaccination some years previously, rather than from measles infection. Adequate saliva samples were collected from young children using the OraSure™ device. Unexpectedly, the T/N ratio of measles-specific antibody was inversely related to the level of total IgG in saliva samples. Increased local production of IgG in saliva has been demonstrated in some individuals (KORSRUD & BRANDTVAEG, 1980), and this would reduce the proportion of total antibody that is specific and thus reduce the T/N ratio. The total IgG level may therefore not be an appropriate indicator of the quality of saliva samples. The current assay appears to be insufficiently sensitive for population screening, but further work should be done to develop sensitive salivary assays for measles immunity.

Bolivia, like other countries in the Americas, is conducting a mass vaccination campaign of children under 15 years of age in 1994. In São Paulo, Brazil, significant differences in antibody prevalence among attenders and non-attenders at a mass campaign in 1987 were observed with children aged 1–9 years, but not with older children (PANNUTI *et al.*, 1991). In Santa Cruz, only a small proportion of school-aged children were seronegative, but a large proportion had antibody levels below 200 miu, which may not protect fully against measles infection or disease (CHEN *et al.*, 1990). Revaccination may have only a temporary effect on children with low antibody levels (DAI-BIN *et al.*, 1991; MARKOWITZ *et al.*, 1992). The extra value obtained by including school-aged children in a vaccination campaign in Santa Cruz would depend in part on whether children with antibody levels less than 200 miu are susceptible to infection, and on their response to revaccination. Further study of the response of schoolchildren to revaccination is planned in Santa Cruz. The medium-term effect of the mass campaign on measles antibody levels in the target population should be evaluated after 1–2 years.

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