Original article

# Pre-and post-vaccine measles antibody status in infants using serum and oral-fluid testing: an evaluation of routine immunization in Addis Ababa, Ethiopia

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**Abstract**

**Background:** Despite the use of measles vaccine, measles incidence in Ethiopia remains a serious public health concern. Progress towards the control of measles requires a national capacity to measure programme effectiveness. This includes evaluation of vaccine effectiveness in infants attending the routine immunization.

**Objective:** To evaluate the effectiveness of the measles routine immunization activities in Addis Ababa.

**Methods:** This study evaluated pre- and post-vaccine antibodies in children attending for routine measles immunization in Addis Ababa. Infants who presented to 3 health centres between September-November, 1998 for routine measles vaccination were enrolled in the study. In total 296 infants (median age 9 months) provided blood and oral-fluid samples, of which 230 (77%) returned to provide post vaccine samples (median interval of 15 days). Screening of sera was undertaken using commercial indirect ELISA kits, and of oral fluids using an in-house IgM-capture ELISA.

**Results:** Pre-vaccination serology showed 1.4% IgM positive, 2.0% IgG positive, and 97.0% seronegative. Post-vaccination seroprevalence of IgM and IgG was 91.3% and 85.0%, respectively, and 92.9% overall. The seroconversion rate was 92.6% (95%CI 88.2-95.7). Based on oral fluid results, 87.3% (95% CI 82.0-91.4) of children showed specific IgM antibody conversion.

**Conclusion:** These results are in support of the recommended age for measles vaccination in Addis Ababa, and show the merit of oral-fluid IgM screening as a non-invasive alternative to blood for assessing vaccine effectiveness. [*Ethiop.J.Health Dev.* 2003;17(3):149-155]

## Introduction

Worldwide, it is estimated that measles kills some 880,000 children annually, a toll more than any other vaccine-preventable disease. The global plan, established by the World Health Organization (WHO) and United Nations Children's Fund (UNICEF), is to cut this burden by two-thirds between 2000 and 2005, and thereafter to prevent 600,000 measles fatalities annually (1). Half of the total deaths are concentrated in three African countries (Congo, Ethiopia and Nigeria) and one Asian country (India). Progress towards the control of measles requires that countries develop national capacity to measure programme effectiveness by which to assess and refine immunization policy. This includes evaluation of vaccine effectiveness in infants attending routine immunization clinics.

The Expanded Programme on Immunization (EPI) in Ethiopia was launched in 1980 (2,3), and offers a single dose of measles vaccine at 9

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months of age. In 1999 the national measles coverage was 53% with uptake ranging from 7-88% (the highest 88% recorded for Tigray region the lowest 7% recorded for Somali region) in the different administrative regions (Source: Department of Family Health, MOH, 2000).

Research has demonstrated oral fluid to yield detectable levels of immunoglobulins (IgG and IgM antibodies) against a wide variety of infections (4-14) using sensitive and specific antibody capture assays. The detection of measles specific IgM antibodies in oral fluid by antibody capture ELISA (MACELISA) has previously been reported in a study of children who received measles vaccine (14). The position of non-invasive antibody testing for measles grows stronger as emphasis on vaccine programme surveillance increases, accompanied by technical developments in oral-fluid assays (13, 14) and favourable evaluations under a variety of settings (14-17).

The aim of this study is to evaluate pre- and post-vaccine antibody in children attending for routine measles immunization in Addis Ababa. Furthermore, we aim to evaluate the use of oral-fluid testing for measles specific IgM as an alternative to serum assays in estimating vaccine response and effectiveness. For this an enhanced measles MACELISA that incorporates an amplification stage is developed.

**Methods**

***Study population***: Addis Ababa, the capital of Ethiopia, a population of 2,570,004 (density of 4,847.8/km$^2$) (18) settled at 2000-2800m altitude above sea level. Administratively there are 6 'Zones' (each divided 4-7 Weredas), and 28 'Weredas' (=district) each with a population of 45,277-153,688. In 1998/99 measles vaccination was given in 7 hospitals, 17 health centres, 9 health clinics, 20 health posts, 104 out reach sites under the Region 14 Health Bureau. The estimated measles vaccination coverage in 1998/99 (1991 E.C.-Ethiopian Julian Calendar) was 86% with variation in the different 'Zones' and 'Weredas'(63-100%). Measles vaccine consumption for the year was 10,250 vials given to 32,548 children aged under one year (Source: Addis Ababa City Government Health Bureau).

Three of 19 Government health centres were selected for the study: one from the centre of the city (Arada), one in the outer city (Akaki), and the third from an area between the centre and outer city (Wereda 25). All infants who presented to these health centres between September-November, 1998 for routine measles vaccination were eligible for enrolment in the study. All the children had received vaccinations of BCG and OPV at birth, and 3 doses of DPT and OPV according to the recommended schedule of the EPI Global Advisory Group (6, 10 and 14 weeks)(19). Informed consent was obtained from guardians by explaining title of the research project, basic information about the disease and objective of the study, information about coordinators of the project and information about how the work was carried out. Permission for this work was obtained from Addis Ababa City Government Health Bureau (No.1370/172) in 29$^{th}$ August 1998.

***Sample collection***: Blood samples were collected by finger prick using Safety Flow Lancet into Microtainers (Becton Dickinson, Oxford, England). Oral-fluid specimens were collected and processed as described previously (11) by sponge swab collection devices (Oracol: Malvern Medical Developments, Worcester, England) from infants before they received live attenuated measles vaccine (Schwartz strain, SmithKline Beecham Biologicals, Rixensart, Belgium). Mothers were requested to return with their infants two weeks after vaccination for a second oral-fluid and blood sample as recent study demonstrates that the presence of considerable amounts of haemagglutinin-specific antibodies in the serum within the early days (5-10 days) after the onset of rash, which are readily detectable with an ELISA (20). Samples collected were processed at the virology laboratory of the Ethiopian Health and Nutrition Research Institute,

(EHNRI). Specimens were transported on dry ice to the UK for laboratory analysis.

***Serum measles IgM/IgG determination***: Serum samples were screened for measles virus (MV) specific IgM and IgG by using a commercial ELISA kit (Enzygnost for IgM, and IgG; Behring Diagnostics, Marburg, Germany). In the IgG ELISA optical density (OD) readings of <0.100, >0.200 and between 0.100-0.200 obtained from 1:231 IgG serum dilution considered as negative, positive and equivocal respectively. The limit of the IgG detection of the test is 150 mIU/ml, equivalent to an OD of 0.100. Similarly, in the IgM ELISA OD readings of <0.100, >0.200 and between 0.100-0.200 obtained from 1: 42 IgM dilution were considered as negative, positive and equivocal respectively. Serum screening was undertaken at EHNRI.

***Oral-fluid MV IgM detection***: MV specific IgM in oral fluid was determined by MACELISA, which was a modification of the FITC/anti-FITC IgG capture ELISA (GACELISA) described previously (13). A description of these modifications follows. Wells of microtitre plates (Maxisorb "U" wells, Life Technologies, Paisley, UK) were coated with 100µl of a 11.2 g/L solution of rabbit anti-human IgM serum (Dako, Ely, UK) diluted 1:3000 (as this dilution factor was found optimal during developmental (optimisation) stage of the assay) in 0.05M carbonate/bicarbonate buffer, pH 9.6. Plates were then incubated with 100 µl undiluted oral fluid samples after blocking with 200 µl of 5% Sol-u-pro (Dynagel Inc., Calumet City, IL). After addition of measles antigen, anti-measles haemagglutinin monoclonal antibody-FITC conjugate (Chemicon Inc., Temicula, CA., USA) diluted 1:3000 (instead of 1:4000 for GACELISA). Anti-FITC horseradish peroxidase conjugate and TMB were then added and the reaction stopped by adding 100 ul of 0.5M HCl. Oral-fluid MV IgM detection was carried out at Central Public Health Laboratory (CPHL), UK.

OD results from the MACELISA were expressed as T/N ratios (test sample OD /negative control OD) in order to have results fall, from negative to strong positive, represents the "dynamic range" of an assay. The receiver operating characteristics (ROC) curve was generated (21) to determine the appropriate cut-off T/N value (set to the maximum sensitivity and specificity relative to serum results). For assays designed for determining immunity in the population, a more appropriate and accurate cut-off can be set using a statistical techniques such as ROC. Relative to serum IgM results (excluding equivocal results), the MACELISA was 93.6% (190/203) sensitive and 93.4% (284/304) specific, using a T/N ratio of 1.14. T/N ratios less than the cutoff value (1.14) were considered negative in the analyses.

***Statistical analysis***: Analysis was conducted using STATA V7.0 (Stata Corp, College Station, Texas, USA). Comparison between median ages uses the non-parametric Kruskall-Wallace test (with ties). Estimates of the proportions serpositive for either IgM or IgG excludes samples with equivocal results. Overall proportions based on serum specific IgM or IgG results exclude only samples equivocal by both tests. Estimates of seroconversion are derived from the proportion of individuals specific-antibody negative pre-vaccine whose status changes to specific-antibody positive post-vaccination. Proportions seropositive are compared using Fisher's Exact test. Exact binomial confidence intervals are calculated for prevalence estimates.

**Results**

A total of 296 infants were recruited with median age 9 months (m, range 5-40 m) all providing a blood and an oral-fluid sample. The number and median age of individuals attending Akaki, Arada and Wareda 25 clinics were, 86, 9m, 41, 9m and 169, 10m, respectively (median ages did not differ between clinics: $\chi^2_{(2)}$ 5.235, P=0.073). Of the 296 recruits, 230 (77%) returned to provide post-vaccine samples (median interval of 15 days, range 14-46 days) oral-fluid and blood.

Results of the measles-specific IgG and IgM tests on serum samples from pre- and post-vaccinated children are shown in Table 1. Seroprevalence estimates were made excluding equivocal results. Pre-vaccination, 1.4% of children were IgM seropositive and 2.0% IgG positive. Post-vaccination seroprevalence of IgM and IgG was 91.3% and 85.0%.

Table 1: **Seroprevalence of measles antibody status (IgM and IgG) in the pre- and post-vaccinated children in Addis Ababa, Ethiopia 1998.**

| | IgM | | IgG | |
|---|---|---|---|---|
| Results | Pre-vacc. | Post-vacc. | Pre-vacc. | Post-vacc. |
| Positive | 4 | 199 | 6 | 170 |
| Negative | 285 | 19 | 290 | 30 |
| Equivocal | 6 | 9 | 0 | 28 |
| NT[a] | 1 | 3 | 0 | 2 |
| Total | 296 | 230 | 296 | 230 |
| Proportion[b] | 1.38 % | 91.3 % | 2.03 % | 85.0% |

[a] NT = Sample collected but not tested because of insufficient serum
[b] Proportion = Number Positive / Number (Positive + Negative)

Of the 4 pre-vaccination IgM positive samples, 3 were IgG negative (2 aged 9m and 1 age 10m) and one high titre IgG positive (age 9m). All the 4 samples were repeated by Behring serum IgM ELISA and found to remain positive. Of 6 pre-vaccination IgM equivocal results all were IgG negative. Of 6 pre-vaccine sera that were measles-specific IgG positive, 5 had high titres (>900mIU/ml), three of which were aged 9m, one 10m and one 11m. One of the 5 was IgM positive, and one (age 10m) had low level antibody (296mIU/ml) for which the paired post-vaccine sample showed a greater than fourfold rise in titre (ie suggesting this individual had residual maternal antibody).

Of 19 children specific-IgM negative post-vaccination, in pre-vaccination samples 5 were IgG positive and one other was IgM positive. Of 9 post-vaccination IgM equivocal samples, all were IgM and IgG negative pre-vaccine and, post-vaccine, 2 were IgG equivocal and 2 were IgG positive. The time between the pre- and post-sample collection for the 2 IgG positive specimens was 15 and 9 days. Post-vaccine, there were 30 samples negative and 28 equivocal for measles specific-IgG; results which should be interpreted in relation to the short interval (median of 15 days) between vaccination and second sample collection. Of the 30 samples IgG negative post-vaccination, pre-vaccination, none were IgG positive, 1 was IgM positive and 2 had equivocal IgM status.

Overall prevalence and antibody conversion rate for the sera (based on IgG and IgM results) and oral fluid are presented in Table 2. Exact binomial 95% confidence intervals (CI) are shown. There was no significant difference between the three clinics in seroprevalence pre-vaccination (Fisher's Exact P=0.066) or post-vaccination (P=0.752). Compared with serum estimates, oral-fluid prevalence was nearly 4% higher pre-vaccine, and 6% lower post-vaccine, although these differences were not significant (95% confidence intervals on prevalence estimates overlap). The overall antibody conversion rate for serum was 92.6% (200/216), which was higher (but not significantly higher) than for oral-fluid, 87.3% (185/212).

Table 2: **Overall antibody prevalence and conversion rate in vaccine recipients in Addis Ababa, Ethiopia 1998.**

| Sample type | Pre-vaccine % (positive/total) 95% CI | Post-vaccine % (positive/total) 95% CI | Antibody conversion % (converters/total) 95% CI |
|---|---|---|---|
| Serum | 3.04% (9/296) 1.40%-5.69% | 92.9% (208/224) 88.7%-95.9% | 92.6% (200/216) 88.2%-95.7% |
| Oral fluid | 6.76% (20/296) 4.18%-10.2% | 86.9% (199/229) 81.8%-91.0% | 87.3% (185/212) 82.0%-91.4% |

**Discussion**

***Pre- and post-vaccination status of childrenBI***: Assessment of vaccine recipient children (median age 9 months) at three representative vaccine clinics in the city of Addis Ababa, showed an absence of measles-specific IgM and IgG antibody in 99% and 98% of serum samples, respectively. Considering either antibody class, the data suggest that 97% (95% CI 94-98%) of the children attending for routine (9 months of age) measles vaccination would respond to vaccine. Following measles vaccination the seroconversion rate (based on either antibody class) was 93% (95% CI 88-96). Children attending for routine measles vaccination had a median age of 9m; only 5% were less than 9m and 10% were 1 year or above. Together these results indicate a highly successful routine immunization programme. The present seroconversion rate study confirms vaccinating children at age 9 months in the Ethiopia setting is in accordance to the WHO recommendation (22) and previous mathematical model studies made for developing countries (23-25).

A full assessment of whether or not the present age at immunization is optimal should consider the prevalence of residual maternal antibodies and proportion with evidence of recent infection in samples from infants attending for routine vaccination. In this study, of 15 samples that were positive or equivocal for serum measles-specific antibody prior to vaccination, only 1 appeared to indicate the presence of residual maternal antibody (low specific-IgG titre with subsequent four fold rise post-vaccination). In contrast there were 8 pre-vaccine serum samples specific-IgM positive and/or high specific-IgG titre positive, indicative of recent infection (6 aged 9m, and 1 each aged 10 and 11m). These results suggest that at the routine target vaccination age a negligible proportion of infants have residual maternal antibody, but a small but significant proportion (8/296=2.7%) have been exposed to measles virus. This is, at least, an indication of the need to discourage any delay in bringing children for measles vaccination beyond the appointed age.

The present study was primarily designed to investigate the development of measles virus specific IgM antibodies with an average interval of 15 days between receiving vaccination and the collection of second samples. Consequently the results yield a high proportion of serologically negative and equivocal IgG results post-vaccination, compared to IgM data, as IgM antibodies are produced initially followed by IgG. Taking IgM serological data alone yields a serconversion estimate of 90%, only marginally lower than that based on IgG and IgM results.

***Serum/oral fluid comparison***: The detection of oral fluid specific IgM for measles virus by antibody captures ELISA in pre- and post-vaccinated infants has been previously described (14). The present work a new MACELISA test is described that employs a FITC/anti-FITC amplification system (26) to enhance the sensitivity of the oral fluid assay to a level comparable to that of the corresponding 'gold standard' serum testing using a commercial kit. Using this amplification system, adequately sensitive and specific measles and rubella virus specific IgG oral fluid assay methods have been developed (12, 13). The IgM assay developed in this study

demonstrated 93.6% sensitivity and 93.4% specificity, which is comparable to the results of 91% sensitivity and 95% specificity found in a previous oral-fluid testing study (14). Based on oral-fluid samples screened using the MACELISA, an estimate of specific antibody conversion of 87% (82-91) was obtained. Although lower than the overall serconversion rate obtained by Behring ELISA, the result is not significantly lower. With such performance the oral-fluid testing method (MACELISA) would be of use for measuring measles antibody prevalence in pre- and post-vaccine infants.

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