A SEROSURVEY TO IDENTIFY THE WINDOW OF VULNERABILITY TO WILD-TYPE MEASLES AMONG INFANTS IN RURAL MALI

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Abstract. As infants lose maternally derived antibody, they experience a period when antibody levels are insufficient to protect against measles yet may interfere with immunization. In Kangaba Mali, sera were collected from 89 2–8-month-old infants and 32 9–10-month-old infants without a history of measles or vaccination; post-vaccination sera were collected from 24 of the 9–10-month-old infants 3–5 weeks after receiving measles vaccine. Measles antibody was measured by plaque reduction neutralization (PRN) and enzyme linked immunosorbent assay. At two months of age, 30% had protective PRN titers; among six-month-old infants, none had protective titers. Prior to vaccination, 16% of 9–10-month-old infants exhibited protective titers; all demonstrated protective titers post-vaccination. The early onset of the window of vulnerability in Kangaba infants likely reflects the changing ecology of measles in Africa. Ways to protect these vulnerable infants against measles must be devised.

INTRODUCTION

Despite the availability of an inexpensive and highly effective attenuated vaccine, in 2000, the World Health Organization (WHO) estimated that measles caused 770,000 deaths, most of which occurred in Africa. A notable proportion of cases and deaths occur in infants younger than nine months of age, before reaching the age at which measles vaccination is currently recommended in developing countries. Several factors conspire to allow measles to persist as a public health problem, particularly in the developing world. First, routine vaccine coverage of infants is low, especially in remote rural districts and urban slums where access to immunization services is often impeded. Second, the current parenteral attenuated vaccine does not elicit high antibody levels when administered to infants less than nine months of age. Finally, while maternal antibodies at adequate titer can protect infants early in life, multiple factors influence the quantity and quality of antibodies that cross the placenta. As a result, many young infants experience several months during which the titer of maternal antibodies falls below a level that can protect them against wild-type measles virus, but may interfere with antibody production in response to attenuated measles vaccine virus. During this window of vulnerability, young infants may develop severe or fatal measles if exposed to wild-type virus. In the early 1990s, vaccination of six-month-old infants with high titer measles vaccine was explored as a strategy to overcome maternal antibodies and enable successful immunization of infants during this window. However, excess mortality among female infants led to abandonment of this approach.

The primary strategy now being pursued to control the measles disease and mortality burden in developing countries, including in young infants, is based on mass immunization campaigns that target older infants and children with current vaccines. By drastically diminishing the incidence in this population, it is hoped that young infants will be indirectly protected. In Mali, where during the early 1990s approximately 231,000 measles cases and approximately 13,850 deaths occurred annually, the Ministry of Health of Mali and the WHO in 1998 and 1999 organized mass immunization campaigns with parenteral measles vaccine for urban children 9–59 months of age. These campaigns, which were carried out in association with National Immunization Days performed as part of the Polio Eradication Initiative, diminished the incidence of measles by 95% in vaccinated areas. Another measles vaccination campaign conducted in 2001 included children up to 14 years of age, and a national campaign conducted in late 2004 targeted children 9–59 months of age. While effective, such campaigns are expensive and temporarily tie up many immunization service resources.

An adjunct control strategy being pursued aims to help by immunizing with a new generation of measles vaccine infants in developing countries who are less than six months of age. In support of this strategy, the Bill and Melinda Gates Foundation has sponsored initiatives to develop a measles vaccine that can be safely administered to young infants during the window of vulnerability. The new vaccines may supplement what can be accomplished with the current measles vaccines.

Information on the prevalence and magnitude of neutralizing antibody titers at different time points during infancy are needed to help guide the development of these new vaccines and to monitor the impact of mass immunization campaigns on the serologic status of infants. Notably, the most recent serosurveys to study the window of vulnerability in developing countries using the gold standard plaque reduction neutralization (PRN) assay were conducted in the 1980s and 1990s when most mothers had antibodies from natural infection rather than vaccination and when the incidence of measles was higher. To elucidate the window of vulnerability among infants in Kangaba, Mali and to better understand the changing epidemiology of measles, we performed a PRN assay serosurvey of infants living in a typical rural village setting in the Koulikoro region. We also used this opportunity to evaluate, in parallel, a simpler enzyme-linked immunosorbent assay (ELISA) for IgG antibodies to measles virus.

MATERIALS AND METHODS

Study site. Mali, a land-locked west African country, is divided into nine administrative regions containing 58 cercles.
(districts). Kangaba cercle, with a population of 80,923 in the Koulikoro Region, is located approximately 100 km southwest of the capital (Bamako). In 2002, local health authorities reported that measles vaccine coverage was 77% and there were no measles case notifications to the national surveillance office from Kangaba cercle during this time period. However, measles notification data are not considered adequately reliable because the surveillance system is limited. The overall seroprevalence of human immunodeficiency virus (HIV) in Mali is 1.7% but is thought to be lower in rural areas such as Kangaba.

The population of Kangaba cercle is spread over 60 villages and health care is provided at 10 health centers. This study was performed at two community health centers that provide primary health care (including routine immunizations) for the villages of Salamalê and Kangaba. Health care workers at each center are expected to document immunizations on a card, which is given to the parent, and in a clinic logbook where all vaccines administered at the facility are recorded. In most instances the vaccination card also lists the child’s date of birth or the age of first contact with health care personnel, usually for vaccination with bacille Calmette-Guérin.

Study review and informed consent. The Ethics Committee of the University of Mali Medical School (Faculté de Medicine, Pharmacologie et Odontostomatologie) and the Institutional Review Board of the University of Maryland, Baltimore reviewed and approved the study protocol. A written version of the consent form was available in French, the official language of Mali. In addition, because the literacy rate in Mali is only 31%, audiotapes of the consent form in the local languages (Bambara and Malinké) were prepared by the National Literacy and Applied Linguistics Education Office.

Administrative community approval for the study was obtained through meetings with the prefect of the cercle, the mayor, and local leaders. The study and its requirements were explained at each of these meetings and the consent audiotape was played for the local leaders. After obtaining community approval, personnel from the local health center visited the area and invited parents of age-eligible infants to consider having him or her participate in the study. Interested parents received a verbal description of the study and listened to the audiotape of the consent form. To document individual informed consent, the parent or guardian placed a fingerprint on the written French version.

Subject selection and serum collection. We conducted a cross-sectional survey of healthy infants who were 2, 4, 6, 8, and 9–10 months of age, without a history of previous measles vaccination (as documented on the vaccination card), clinical measles infection (an illness characterized by fever, rash, and coryza as reported by the parent or guardian), or receipt of blood products in the previous month. An additional eligibility requirement for all six-month-old participants was that they had to have received the three recommended doses of diphtheria-tetanus-pertussis vaccine. The reason was that a parallel serosurvey of tetanus antitoxin was also planned. After obtaining informed consent and ensuring subject eligibility, a single blood sample was collected from all infant subjects 2–8 months of age. The 9–10-month-old infants had two blood specimens drawn, the first prior to measles vaccination and the second 3–5 weeks thereafter.

Two to four milliliters of whole blood were obtained from each participant at each time point. After centrifugation, serum was aliquoted and stored in a liquid nitrogen dry shipper for transport to Bamako. One aliquot of each sample was then shipped to the Applied Immunology Unit at the Center for Vaccine Development (CVD) in Baltimore, Maryland for analysis; the second aliquot remained at CVD-Mali. Serum samples were frozen and thawed no more than three times.

Plaque reduction neutralization assay. Serum samples were incubated with 100 plaque-forming units of wild-type (Edmonston strain) measles virus for one hour at 37°C in an atmosphere of 5% CO2 and then plated onto confluent (~90% density) monolayers of Vero cells (American Type Culture Collection, Manassas, VA) in 12-well plates, in an adaptation of the method of Albrecht and others.26 After incubation for one hour, the serum-virus mixture was removed and the cells were overlaid with 2× minimum essential medium with 2% fetal bovine serum and Sea Plaque Agarose (Cambrex Bio Science Rockland Inc, Rockland, ME) (2 mL/well) and incubated for five days. Wells were stained with neutral red (Gibco Invitrogen Corp., Grand Island, NY), incubated overnight, and the number of plaques in each well was counted. International standard serum (66/202) from the WHO was measured in parallel with the samples, thereby permitting expression of PRN antibody in miU/mL. Samples with undetectable neutralizing antibody (titer < 10 by PRN) were arbitrarily assigned a value of 6.25 miU/mL, the equivalent of a titer of 5 by PRN. Titers ≥ 200 miU/mL were considered protective.27–29

Measles-specific IgG ELISA. Briefly, 96-well plates were coated with measles virus lysate (Advanced Biotechnologies Inc, Columbia, MD) at a concentration of 5 μg/mL in carbonate buffer, pH 9.0, for three hours at 37°C, and blocked overnight with 10% milk (Nestle USA Inc., Glendale, CA) in phosphate-buffered saline (PBS). After incubation, plates were washed 6 times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBST). Serum samples were tested in two-fold dilutions in 10% dried milk in PBST (PBSTM). Plates were incubated for one hour at 37°C and washed as described above. Specific IgG against measles virus was detected with peroxidase-labeled goat anti-human Fcγ chains (ICN, Irvine, CA) diluted 1:5,000 in PBSTM. The secondary antibodies were incubated for one hour at 37°C. The substrate solution used was tetramethylbenzidine microwell peroxidase (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD). After incubation for 15 minutes in the dark, the reaction was stopped by the addition of 100 μL of 1 M H2PO4 and the optical density at 450 nm OD450 was measured in an ELISA micro plate reader (Multiskan Ascent; Thermo Labsystems, Franklin, MA). Sera were run in duplicate and negative and positive control sera were included in each assay. Linear regression curves were plotted for each serum sample and titers were calculated (through equation parameters) as the inverse of the serum dilution that produces an OD of 0.2 above the blank (ELISA units/mL). Samples with < 5 ELISA units/mL were considered to have undetectable levels of antibody. Measles-specific titers were also expressed in miU/mL by interpolating regression-corrected OD values of serum samples in the curve of the WHO standard for anti-measles serum 66/202.30 The limit of sensitivity of the assay was 5 ELISA units/mL or 0.20 miU/mL.

Data analysis. Geometric mean titers (GMTs) of serum PRN antibody and measles-specific IgG were calculated for each age group. In addition, the proportion of infants in each
age group with a neutralizing antibody titer \( \geq 200 \text{ mIU/mL} \) (considered to be the protective level) were calculated. Titers measured by PRN and ELISA were log-transformed and the correlation coefficient was calculated using Small Stata 8 (Stata Corporation, College Station, TX).

RESULTS

A total of 149 serum samples were collected from 125 2–10-month-old infants; 52% were males. All participants provided a baseline blood sample. Pre-vaccination and post-vaccination samples were obtained from 24 (75%) of the 32 9–10-month-old infants before and 21–24 days after measles vaccination. During routine Good Clinical Practice quality control of case report forms, errors in calculation of age were noted for three infants who had blood samples taken; since they did not meet the inclusion criterion, they are excluded from analysis. One infant’s serum sample was not shipped to Baltimore for analysis.

Titers of PRN antibody by age are shown in Table 1 and presented graphically in Figure 1. Among two-month-old infants, the youngest age group tested, 95% had detectable antibody but only 30% exhibited protective titers (\( \geq 200 \text{ mIU/mL} \)). Similarly, whereas 90% of the four-month-old infants had detectable antibody, only three subjects (15%) had a protective titer (with two being convincingly above the protective threshold). By age six months, none of the 30 infants exhibited protective titers; moreover, < 40% had detectable antibody. This age group manifested the GMT nadir of PRN. Among 9–10-month-old infants prior to vaccination with measles vaccine, 50% had detectable antibody and approximately 16% already exhibited protective titers of measles PRN antibody. Notably, one month following administration of measles vaccine, 100% of subjects reached protective titers.

Antibody titers measured by IgG ELISA are shown in Table 1 and Figure 2. The age-specific seroepidemiologic curve of IgG ELISA antibody (Figure 2) closely paralleled that of PRN antibody. Results obtained by ELISA correlated well with the PRN assay results (\( r = 0.93, P < 0.001 \)) and were reproducible between runs (coefficient of variation < 5%, \( r = 0.93, P < 0.001 \)). The ELISA had a sensitivity of 100% in being able to detect antibody in all infants who had detectable PRN titers. Among all infants who had PRN titers \( \geq 200 \text{ mIU/mL} \), 87% also had measles-specific IgG levels \( \geq 200 \text{ mIU/mL} \) measured by the ELISA. All infants with less than protective levels of neutralizing antibody (i.e., < 200 mIU/mL) had measles-specific IgG ELISA titers < 200 mIU/mL. The ELISA was less sensitive than PRN in detecting infants who reached protective levels one month post-vaccination as only 88% of the infants exhibited such levels by ELISA versus 100% by PRN.

DISCUSSION

The window of vulnerability to wild-type measles infection among infants in Kangaba, Mali, measured by PRN begins at two months of age and extends to nine months of age when it is recommended that the current measles vaccine should be administered in developing countries. There are few publications describing the seroepidemiology of measles during infancy in developing countries in recent years. Moreover, most published studies measured measles antibody by methods other than the gold standard PRN and were completed in the 1980s and 1990s when mothers were more likely to have measles antibodies derived from natural infection than from vaccination.\(^{9,11,24,25,28,31}\) In only two studies, one in the Congo\(^2\) and the other in Bangladesh,\(^2\) was neutralizing antibody measured, albeit by different techniques. Although

![Figure 1. Scatterplot of plaque reduction neutralizing (PRN) antibody titers and geometric mean titer (GMT) measured in different age groups of Malian infants. The horizontal line represents the cutoff for protection (200 mIU/mL). Ages are in months. Pre = pre-vaccination; Post = post-vaccination.](image)

<table>
<thead>
<tr>
<th>Infant age (months)</th>
<th>No.</th>
<th>% with detectable antibody* (95% CI)</th>
<th>GMT by PRN (mIU/mL)</th>
<th>Range (mIU/mL)</th>
<th>% with PRN ( \geq 200 \text{ mIU/mL} )† (95% CI)</th>
<th>GMT by ELISA (mIU/mL)</th>
<th>Range (mIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20</td>
<td>95 (75–100)</td>
<td>85</td>
<td>6–684</td>
<td>30 (12–54)</td>
<td>67</td>
<td>6–362</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>90 (68–99)</td>
<td>55</td>
<td>6–1,464</td>
<td>15 (3–38)</td>
<td>22</td>
<td>2–341</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>36.7 (20–56)</td>
<td>10</td>
<td>6–40</td>
<td>0 (0–12)</td>
<td>7</td>
<td>1–41</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>52.6 (29–76)</td>
<td>16</td>
<td>6–183</td>
<td>0 (0–18)</td>
<td>9</td>
<td>2–170</td>
</tr>
<tr>
<td>Pre-vaccination§†</td>
<td>32</td>
<td>50 (32–68)</td>
<td>20</td>
<td>6–2,926</td>
<td>15.6 (5–33)</td>
<td>12</td>
<td>2–1,690</td>
</tr>
<tr>
<td>Post-vaccination§</td>
<td>24</td>
<td>100 (86–100)</td>
<td>2,997</td>
<td>375–18,074</td>
<td>100 (86–100)</td>
<td>805</td>
<td>141–3,146</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td></td>
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</tbody>
</table>

* PRN = plaque reduction neutralization; CI = confidence interval; GMT = geometric mean titer; ELISA = enzyme-linked immunosorbent assay.
† Neutralizing antibody level > 6.25 mIU/mL.
‡ Protective level.\(^{2,25}\)
§ Refers to receipt of measles vaccine. Infants in the pre-vaccination group were 9–10 months old and those in the post-vaccination group were 10–11 months old.
This could explain why 16% of the 9–11-year-olds may be a contributing factor. Also, if there is less exposure to wild-type measles virus, in some infants they may modify the severity of measles disease upon exposure to wild type virus. Boosting from wild-type measles virus will decrease even further if follow-up mass immunization campaigns are carried out every few years. Consequently, we conclude that infants are not receiving large amounts of antibody transplacentally and may be more vulnerable to wild-type measles infection at an earlier age than previously observed. As further expansion of the window would be expected, this trend should be monitored in other areas of Mali, as well as in other African and Asian countries to document serologically the change in the ecology of measles virus and the impact of this change on the susceptibility of young infants.

Among Kangaba infants 2–9 months of age prior to vaccination, 50% (61 of 121) had low titers of neutralizing antibody (> 6.25 mIU/mL but < 200 mIU/mL). Although these low antibody levels are unlikely to prevent infection due to wild-type measles virus, in some infants they may modify the severity of measles disease upon exposure to wild type virus.37,38 This could explain why 16% of the 9–10-month-old infants had neutralizing antibody levels in the protective range yet did not have a history of clinical measles infection. Few notifications of measles cases have been reported in Kangaba during the past two years. However, the official notification data must be viewed with caution because the surveillance system is weak, and it is known from epidemiologic studies in other areas of rural Mali that transmission is continuing among infants less than one year of age and children more than five years of age.21 Thus, wild-type measles virus transmission is also likely to be ongoing in Kangaba. Alternatively, it is possible that the PRN titers detected in 16% of the 9–10-month-old infants are the consequence of measles vaccination in this age group administered without proper record keeping. Indeed, a measles vaccination campaign had been held in Kangaba as recently as one week prior to the conducting of this study. Measles vaccinations administered under campaign conditions in Mali are not typically recorded on infant immunization cards. Consequently, the vaccination cards examined as part of the eligibility criteria may not have reflected vaccination received during campaigns.

The window of vulnerability age span comprises a heterogeneous population of infants, where many infants may have low or undetectable antibody levels, leaving them at risk of clinical illness due to wild-type infection and others may have protective PRN levels. Because of this variability and the immaturity of the immune system, vaccination of six-month-old infants with standard dose vaccine is not recommended except during epidemics. While the standard vaccine induces T cell responses in infants as young as six months of age,4,39,40 strategies for some vaccines under development are aiming to vaccinate even younger infants so that protection afforded by maternal antibodies may be bridged with that induced by vaccine.22 Our data provide invaluable information for the design of future vaccine clinical trials and suggest that in Mali a measles vaccine is needed that is safe and can induce antibody responses in infants as young as two months of age.

The immune response that best correlates with protection from measles is the presence of neutralizing antibodies. The PRN assay is considered the gold standard in measuring neu-
neutralizing antibodies and a titer ≥ 120 was associated with protection from disease. With the aid of a WHO standard serum, investigators can quantify neutralizing antibodies in mIU/mL, thereby permitting comparison of results among laboratories. Since the PRN assay is a rigorous, expensive and time-consuming test, a simpler, sensitive, and more economical serologic test would be more appropriate for use in developing country laboratories. A practical alternative would be the ELISA, if the assay is sufficiently sensitive and specific and correlates well with PRN. Several ELISA kits that measure measles antibody are available commercially. While these kits are easy to use, they are generally not sufficiently sensitive to detect low levels of antibody and are therefore not optimal for seroprevalence surveys such as the one described herein.

The ELISA that we developed for use in this serosurvey proved to be 100% sensitive in detecting measles neutralizing antibodies relative to the PRN and there was a significant correlation between the two assays (r = 0.93). At a level of 200 mIU/mL, the ELISA had a sensitivity of 87% and a specificity of 100% in identifying individuals who had protective titers of neutralizing antibody measured by PRN. The ELISA was able to quantify different levels of antibody among those with PRN titers < 20, supporting the ability of this assay to measure low levels of antibody. Lastly, 88% of all vaccinated infants had a titer > 200mIU/mL, demonstrating that this assay is able to identify appropriate immune responses to measles vaccination. Although this ELISA is not available as a commercial kit, several large lots of measles antigen from a commercial source have given repeatable and consistent results. These attributes support the notion that this ELISA could be established in developing country laboratory settings for use with seroepidemiologic surveys and measurement of vaccine-induced immune responses.

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