Serosurvey of measles, mumps and rubella antibodies in Saudi children

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ABSTRACT

Objective: A serosurvey study to evaluate the proportion of children with antibodies against diseases targeted by the Expanded Program of Immunization in the Kingdom of Saudi Arabia.

Methods: Using multistage sampling techniques, we collected samples and sent them for laboratory assay from the following age groups; 100 samples at 6 months, 12 months, 18 months, 6 years, 13 years, and 17 years. We conducted the study from September 2001 to February 2002. We assayed sera for measles, rubella, and mumps antibodies in the measles-mumps-rubella reference laboratory in Germany, using enzyme immunoassay and plaque neutralization (PN) as a backup test for equivocal and negative samples. We only carried out a backup test for measles samples.

Results: The age group of 6 months had the highest proportion with negative measles antibodies. After adding the backup test (PN), the proportions of children with protective measles antibody were; 64% at 6 months, 87% at 12 months, 91% at 18 months, 75% at 6 years, 96% at 13 years, and 98% at 17 years. Rubella antibody positivity rates (>7 IU) were 28% at 6 months, 49% at 12 months, 97% at 18 months, 98% at 6 years, and 100% at 13 years. While positivity rates in mumps were 14% at 6 months, 29% at 12 months, 59% at 18 months, 64% at 6 years, and 75% at 13 years.

Conclusion: The unexpected low proportion of children with protective level at 6 years, despite being vaccinated with 2 measle doses is an important phenomenon. This reflects the interference between the first and the second measles dose. The Ministry of Health decided to conduct a catch up campaign targeting 1st through 3rd grade primary schools, who did not catch the mass campaign conducted in 2000. Also, this supports the decision taken by the ministry to change the measles immunization schedule to MMR at 12 months and a second dose at 6 years of age.

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The Expanded Program of Immunization (EPI), in the Kingdom of Saudi Arabia (KSA), started more than 2 decades ago, with a coverage rate exceeding 95%. However, it should be noted that, even with this very high coverage, susceptibles will continue to accumulate rapidly as vaccines are not 100% effective. We can divide the history of the measles immunization policy into 3 phases.[1] The first phase in the 1980s, using the Schwartz measles vaccines, with the problem of vaccine failure, due to the persistence of maternal antibodies.[2] In the second phase, a 2-dose schedule was introduced in the 1990s, using Edmonston-Zagreb at 6 months and measles-mumps-rubella (MMR) at 12 months.[3] In the third phase, the MMR campaign was conducted in 1998-2000 to upgrade the measles preventive strategy to the elimination phase. In 2001, the measles immunization schedule was

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changed to 2 MMR doses at 12 months and preschool. Those changes in immunization policy were made according to the changing pattern of measles epidemiology.[4] We know that immunity after vaccination lasts for a shorter time than that acquired after contact with the wild virus. The decreased natural boosting effect due to the absence of circulating wild organism is an additional negative effect on the persistence of post-vaccination antibody.[5-7] Accordingly, additional doses and campaigns were needed to maintain reasonable protection levels. We need these additional activities to upgrade the preventive strategies to a level of elimination, or eradication. This dynamic situation needs close observation to monitor the persistence of antibodies, and the proportion of children with protective levels against the targeted diseases, to optimize the vaccination schedule to cope with the new epidemiological pattern of the diseases. The aim of this serosurvey is to generate antibody prevalence data for national immunization policy evaluation, and to discover and fill the gaps or windows of susceptibility between different age groups.

Methods. This is a national study to evaluate the serological pattern of diseases targeted by the EPI in KSA in different age groups. In a cross-sectional study, 100 children at of the ages of 6 months, 12 months, 18 months, 6 years, 13 years, and 17 years were recruited to evaluate the antibody levels against diseases targeted by the immunization. The results of MMR will be presented in this paper. A multi-stage sampling technique was used to draw the sample. In the first stage, the Kingdom was divided into 5 main regions, northern, southern, eastern, western, and central regions. In each region, one health province was randomly selected. The following provinces were selected; Tabuk (northern), Gizan (southern), Al-Gatif (eastern), Madinah (western), and Al-Qassim (central). In the second stage, 20 primary health care centers (PHCCs), including schools in their vicinity, were selected randomly. A sample distribution in each region was carried out according to the proportion of the total population living in that region, 5 PHCCs from Al-Qassim, 2 from Tabuk, 3 from Gizan, 3 from Al-Gatif, and 7 from Madinah. In the third stage, 20 blood samples for each PHCC from each age group, 6 months, 12 months, 18 months, 6 years, 13 years (1st year intermediate school), and 17 years (last year of the secondary school) were collected. These age groups were selected on their importance in checking the level of inherited immunity from the mothers, and the level of protectivity against the studied diseases in older age groups. A sub-sample of 100 sera was selected randomly from each group and sent for laboratory assay. The sample size was calculated to give the proportion of children with protective levels against measles with 95% confidence and 80% power; taking into consideration that the lowest expected proportion of children with protective levels for measles is 65% at the age of 6 months[2] with a null hypothesis value of 50%. Fieldwork was conducted during the period; September 2001 to February 2002. The serological assay was carried out in 2002. A commercial enzyme immunoassay (EIA, Dade Behring, Germany) was used to detect the virus-specific IgG against MMR virus according to the instructions of manufacturers as described previously.[8] The quantitative antibody values for measles and rubella were expressed in International Units (IU), and for mumps in titres. The cut off values used for measles were <0.15, 0.15-0.35 and >0.35 IU/ml for negative, equivocal and positive, for mumps titres, <1:230, 1:230-1:500 and >1:500 for negative, equivocal and positive, while for rubella <4, 4-7 and >7 IU/ml were the cut off for negative, equivocal and positive. Sera with negative and equivocal antibody values for measles detected by EIA were retested by the plaque neutralization test (PNT). The PNT was performed as described previously.[8] Two fold serial dilutions of serum were mixed with measles virus strain L16 (20–30 pfu), incubated for one hour at 37°C, and inoculated onto the monolayer of VERO cells. The PNT titre was the reciprocal serum dilution, which reduced the plaque numbers by ≥50%. Titres ≥1:2 were considered to be a positive result and corresponded with ≥0.04 IU/ml. Assay results were entered in an excel file format and transformed into SPSS file format for statistical analysis. Data are presented as mean, geometric mean of titer and proportions with protective levels. Chi-square and ANOVA were used for statistical analysis.

Results. Five hundred and ninety-seven samples from the 6 age groups were tested by EIA. Equivocal results were repeated. One hundred and nine negative and equivocal were retested by PN tests. As expected, age group of 6 months had the highest proportion with negative antibody (43%), as measured by EIA **(Table 1).** The age group, 6 years shows an unexpected low positivity rate, 48%, and with the lowest mean antibody among all groups. Measles mean antibody were as follows; 0.84 IU/ml for the age group of 6 months, 1.5 for 12 months, 1.16 for 18 months, 0.59 for 6 years, 1.9 for 13 years, and 2.1 for 17 years. After the backup test of PN, the proportion of children with protective levels reached, 64% at 6 months, 87% at 12 months, 91% at 18 months, 75% at 6 years, 96% at 13 years, and 98% at 17 years. The protective level is assigned to the value 0.2 IU/ml by PN, while positive level by

Table 1 - Qualitative measles antibody level (EIA) by age group.

Age group	Antibody Positive (>350 mlU) n (%)	Negative (<150 mlU) n (%)	* (150-350) n (%)	Total
6 months	46 (47)	42 (43)	10 (10)	**98 (16.4)**
12 months	65 (65)	28 (28)	7 (7)	**100 (16.8)**
18 months	80 (80.8)	9 (9)	10 (10.2)	**99** -
6 years	48 (48)	31 (31)	21 (21)	**100 (16.8)**
13 years	89 (89)	5 (5)	6 (6)	**100 (16.8)**
17 years	91 (91)	3 (3)	6 (6)	**100 (16.8)**
Total	**419 (70.2)**	**118 (19.8)**	**60 (10.1)**	**597 (100)**

* - Results were repeated, EIA - enzyme immunoassay

Table 2 - Proportion with positive antibody titer (>7 IU/ml) and mean antibody titer according to different age groups.

Age group	Positive n (%)	mean ± SD	GMT
6 months	98 (28.6)	25 ± 49	4
12 months	100 (49)	52 ± 69	9
18 months	99 (97)	149 ± 118	105
6 years	100 (98)	81 ± 62	59
13 years	100 (100)	83 ± 64	62
Total	**497 (74)**	**78 ± 86**	**26**

GMT - geometric mean of titer

Table 3 - Mumps antibody titers in different age groups.

Age group	Mean	Median	GMT
6 months	431	115	175
12 months	551	115	266
18 months	1185	670	633
6 years	1367	745	625
13 years	1444	985	875

GMT - geometric mean of titer

PN is 1:2 titer or 0.02-0.04 IU/ml. For rubella, a peak of the antibody titer is seen at the age of 18 months (149 IU/ml), 6 months after giving the MMR vaccine **(Table 2).** The mean antibody level at 12 months is significantly higher compared to antibody titer at 6 months (p=0.001). The proportion of children with positive mumps antibody was 14% at 6 months, 29% at 12 months, 65% at 18 months, 59% at 6 years, and 75% at 13 years. A similar pattern was noticed for the antibody titer **(Table 3).**

Discussion. We carried out the lab work in the current study in the MMR reference center in Germany, which is a part of the European Sero-Epidemiology Network (ESEN). The ESEN project was established to harmonize the sero-epidemiology of 5 vaccine preventable infections including MMR in 8 European countries. This involved achieving comparability both in the assay results from testing in different centers, and also sampling methodology. Standardization of EIA results was achieved through the development of common panels of sera by designated reference centers.[9]

We conducted the current study before changing of the measles schedule to 2 MMR doses at 12 months and 6 years. So, in our study children were vaccinated with Edmonston-Zagreb measles vaccine at 6 months and MMR at 12 months. Children at 13 and 17 years of age also received another MMR dose during the campaign in 1998-2000. Measles antibodies at 6 months represent mainly maternal antibodies as we collected samples before giving the first dose of measles vaccine. The low measles mean antibody at 6 years **(Table 1)** and the relatively high proportion of children without the protective antibody (25%) was also reported during the evaluation of the MMR campaign in 2000.[10] This is a window of susceptibility in children not vaccinated during the last MMR campaign. The low mean antibody at 18 months (after the second MMR dose given at 12 months), 1.16 IU/ml, compared to 1.54 IU/ml at 12 months, reflects impairment in antibody response to the dose given at 12 months. This phenomenon was reported in other studies.[11] Absence to exposure to natural infection may be a contributing factor.[12] At the time of the study, outbreaks were reported especially in Madinah. The low measles antibody at 6 years compared to 12 years was also reported during the evaluation of the MMR campaign in KSA.[10,13] Children at 13 years and 17 years showed a higher proportion of children with protective levels as they were vaccinated also in the last campaign and due to possible natural exposure to the wild virus in the community. Each age group represents a different cohort with different determinant factors including exposure to natural infection.

The age groups of 6 and 12 months were not vaccinated against rubella; accordingly, the

antibody titre represents either maternal antibody or exposure to infection **(Table 2).** The increase of proportion of children with positive titre from 28% at 6 months to 49% at 12 months represents exposure to natural infection, knowing that the mean also increased from 25 IU/ml to 52 IU/ml. This should be further investigated, as circulating rubella virus is a big hazard to pregnant women. The proportion of children with positive rubella antibody is satisfactory after vaccination; 97% at 18 months, 98% at 6 years, and 100% at 13 years, which is comparable to previous studies.[10] We know that children in the 18 months and 6 years age group were vaccinated with one MMR dose at 12 months, while 13-year-old children were vaccinated with 2 doses of MMR, the last one was given during the last MMR campaign in the year 2000.

In this study, seronegativity was higher at the age of 6 months, and mumps antibodies can be attributed mainly to the persistence of maternal antibodies. However, although blood samples were collected at 12 months before giving MMR, seropositivity was higher, 29% compared to 14% at 6 months. This may reflect exposure to natural infection. Seronegativity dropped to 21% at 18 months and 30% at 6 years, as they were vaccinated with one MMR at 12 months. The positivity rate at 18 months, after receiving the MMR at 12 months, was low compared to another study carried out in 1996, where the positivity rate was 93%. The lab work was carried out in the same lab.[14] Children at the age of 13 years were given a second dose of MMR during the campaign in 2000. This explains the higher seropositivity. Seronegativity at 13 years of age in our studies is comparable to published data from the USA. In army recruits in the USA, antibody seronegativity for mumps ranged from 12.3-15.6%.[15,16]

This serosurvey supported the action taken by the Ministry of Health in changing the immunization policy against measles to 2-MMR doses at 12 months and 6 years. To close the window of susceptibility to measles at school entry, a catch up campaign was conducted targeting those not vaccinated in the 2000 campaign. The catch-up campaign was carried out in the year 2003.

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