Assessing the Efficacy of a Mixed Vaccination Strategy against Rubella in São Paulo, Brazil

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Background. In 1992 a major vaccination strategy against measles-mumps-rubella was introduced in the State of
São Paulo, Brazil. This strategy was based on mathematical models and comprised a pulse vaccination covering all
children aged 1–10 years, followed by the inclusion of this vaccine in the routine calendar at 15 months of age. The
present work reports the evaluation of the efficacy of this mixed vaccination strategy.

Methods. A rubella seroprevalence survey was carried out immediately and one year after the campaign, comprising
4953 children aged 1–15 years.

Results. We show that average rubella seroprevalence increased from 0.40 to 0.97 and that the reported number of
congenital rubella syndrome (CRS) cases dropped dramatically.

Conclusions. The mixed vaccination strategy adopted against rubella has proved to be very effective in reducing the
number of CRS cases in São Paulo.

Keywords: rubella, CRS, vaccination, seroepidemiology, control strategies

Rubella is a viral infection which causes a mild disease. However, it may have a severe impact on the affected
population due to its potential teratogenic effects, which occur in 20–50% of children born to women infected during the first trimester of pregnancy. Therefore, the goal of rubella vaccination should be to pre-
vent the tragic consequences of intrauterine rubella infection (congenital rubella syndrome, CRS). This
could be achieved by one of three distinct strategies, adopted in the US (USA strategy), the UK (UK strat-
egy) and Sweden (mixed strategy). These consist of universal immunization of young children (USA), vac-
cination of prepubertal females (UK), or a mixed strategy combining both the above (Sweden). In addition,
a pulse vaccination in the form of a mass campaign may be adopted.

The aim of the so-called UK strategy, i.e. vaccination
of prepubertal females, is to provide individual pro-	ection against the infection, reducing the proportion of susceptibles of reproductive age and consequently, the
risk of CRS. Contrasting with this, the so-called USA strategy, which proposes the universal vaccination of
young children, aims to reduce circulation of the virus by providing herd immunity in the population. Either of
the above strategies can be effective in reducing the prevalence of CRS, depending basically on vaccination
coverage levels attained. However, it was demonstrated that, for the USA strategy, if the proportion of
immunized susceptibles is below a certain threshold, the incidence of CRS may even increase after vac-
cination. On the other hand, if the threshold is surpassed, this strategy is substantially more effective in
reducing the risk of CRS than the UK strategy.

In some circumstances, it could be advantageous to combine the routine vaccination programme, under a
USA strategy, with a pulse campaign. This is based on
the fact that the introduction of a vaccination scheme causes a disturbance in the system, which will then evolve to another equilibrium. The pulse vaccination would then speed up this approach to a new equilibrium. It provides, moreover, an additional protection for females reaching reproductive ages while the system evolves to the new equilibrium level.\textsuperscript{5}

The State of São Paulo, Brazil, has recently adopted a mixed strategy, based on previous experiences of other countries and also on mathematical models,\textsuperscript{3,6} which demonstrated the optimal age interval to vaccinate children against rubella in order to eliminate the infection from this community to be between 13 and 15 months. Therefore, since July 1992, all children aged 15 months in the State of São Paulo are routinely immunized against measles, mumps and rubella. This age was chosen because this combined vaccine now replaces the second dose of measles vaccine in the former vaccination calendar. Immediately before the introduction of the routine immunization it was decided to carry out a major vaccination campaign with the measles-mumps-rubella vaccine aimed at all children aged 1–10 years, irrespective of previous vaccination status (in Brazil rubella vaccine was not included in the Public Health Service calendar before 1992). This mass vaccination strategy was also designed based on mathematical modelling and had the objective of speeding up attainment of the equilibrium at which we could expect elimination of the disease. Previous mathematical treatments demonstrated that this equilibrium would be achieved by the routine immunization, provided high levels of coverage could be guaranteed at 15 months of age.\textsuperscript{7} However this could take several years to be accomplished, hence the need for the campaign.

The mass vaccination campaign was carried out between 25 April and 5 June 1992. Immediately after the campaign, this vaccine was introduced in the immunization calendar at 15 months of age. In addition, an active epidemiological surveillance programme directed to rubella infection and CRS was established.

In order to assess the impact of this vaccination strategy, we carried out a seroprevalence study in the metropolitan area of São Paulo. The serological survey was chosen because the statistical surveys based on the number of notified cases have shown to serve only as a guide and do not define viral circulation accurately.\textsuperscript{7} Therefore, accurate seroepidemiological investigation is currently considered essential. This is particularly true for rubella virus infections, which are not identified in a great number of cases.

This paper describes the results of seroepidemiological surveys carried out immediately after the mass vaccination, and one year after the introduction of the vaccine in the immunization calendar in São Paulo. This study comprised 4953 children aged 1–15 years. We describe here only the data relative to rubella because the strategy was designed based on rubella seroepidemiological studies. Measles and mumps, as well as the adverse effects due to the vaccine, will be the subject of future publications.

**METHODS**

The study consisted of two phases, one assessing the seroprevalence of rubella-specific antibodies attained immediately after the campaign, and the other the seroprevalence one year after the introduction of the routine vaccination programme.

**The Target Population**

The target population consisted of residents of the city of São Paulo, aged 1–15 years. However, it is very difficult to achieve a representative sample of the entire population of a city with 20 million inhabitants such as São Paulo. Therefore, a sample of this population was randomly taken from schools. The Public Education network of the city of São Paulo is comprised of 2500 schools, including 1000 nurseries, 500 kindergartens and 1000 first grade schools. This corresponds to a population of about 2 million children. In order to avoid a potential selection bias (schoolchildren could have a higher probability of receiving the vaccine) we analysed a sample taken from the community of Caieiras as a whole, a small city in the outskirts of São Paulo, in which only a fraction of children are regularly registered with the education system. Hence the sampling unit in São Paulo was schools and dwellings in Caieiras. We therefore considered the city of Caieiras as a control, used to verify if the population of schoolchildren in the city of São Paulo was representative of children aged 1–15 years from the State of São Paulo. In addition, we had previous studies on rubella seroepidemiology carried out in Caieiras a year before the campaign,\textsuperscript{6} and from São Paulo, carried out in 1987.\textsuperscript{5}

**Sampling**

The sampling units considered for the city of São Paulo were the nurseries, kindergartens and first grade schools from the official Education Council. The sample size for each age a, \( n(a) \), was estimated according to standard methods: \textsuperscript{5}

\[
n(a) = \left[ \frac{Z^2_{1-\alpha/2} P(a) (1-P(a)) \sigma^2}{d^2} \right] t_p
\]  

(1)
where

\[ Z = \text{confidence level, defined as } 95\%, \text{ which implies a value of } 1.96; \]
\[ P(\alpha) = \text{expected proportion of positives to a rubella antibody test for each age } \alpha; \]
\[ d = \text{absolute precision, assigned to } 10\%; \]
\[ e_f = \text{design effect, assigned to } 2, \text{ according to recommendations from WHO for conglomerate sampling}; \]
\[ f = \text{proportion of losses, assigned to } 20\%. \]

The total number of 2208 necessary to represent the population (Table 1) was then randomly assigned to 300 institutions in order to guarantee a homogeneous distribution over the whole city of São Paulo. The institutions to be visited were then systematically drawn from the 2500 available and, in each of them, we assigned a single age. In each institution we randomly drew seven children necessary for the sample. These procedures guaranteed that children were randomly selected for the study. The population from the city of Caieiras was sampled according to the methods described. Table 1 shows the sample size for the study.

**Field Work**

In the first phase, immediately after the mass vaccination campaign, a seroprevalence study was performed in a sample from the population of schoolchildren in the city of São Paulo, and in a sample from the whole population from the city of Caieiras, as described above.

The second phase also consisted of a seroprevalence survey, carried out one year after the introduction of the rubella vaccine in the immunization programme at 15 months of age, which coincided with the end of the vaccination campaign. This second phase aimed to assess the seroprevalence level in the age group covered by the campaign, to estimate the coverage levels attained by the routine vaccination programme, to quantify the impact of this mixed strategy on the prevalence of CRS and to check the theoretical predictions on rubella incidence based on the mathematical models described by Azevedo Neto.

In the period between 19 October and 11 November 1992, we visited 304 schools in the city of São Paulo, sampling 2215 children, and 634 dwellings in the city of Caieiras, sampling 724 children. The age distribution of the children can be seen in Table 2. For each school we drew seven children to participate in the survey. Inclusion criteria included being the age assigned to that school and an explicit authorization from the parents to participate in the study. The children were bled and the

### Table 1: Sample size for the school population from São Paulo and population of children based on dwellings from Caieiras

<table>
<thead>
<tr>
<th>Age</th>
<th>São Paulo</th>
<th>Caieiras</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>183</td>
<td>355</td>
</tr>
<tr>
<td>2</td>
<td>190</td>
<td>220</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>134</td>
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<tr>
<td>4</td>
<td>215</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>226</td>
<td>58</td>
</tr>
<tr>
<td>6</td>
<td>231</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>227</td>
<td>29</td>
</tr>
<tr>
<td>8</td>
<td>215</td>
<td>23</td>
</tr>
<tr>
<td>9</td>
<td>197</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>177</td>
<td>16</td>
</tr>
<tr>
<td>15</td>
<td>148</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>2208</td>
<td>1002</td>
</tr>
</tbody>
</table>

### Table 2: Results of seroprevalence immediately after the campaign

<table>
<thead>
<tr>
<th>Age</th>
<th>São Paulo city</th>
<th>Caieiras city</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample size</td>
<td>Proportion of positives</td>
</tr>
<tr>
<td>1</td>
<td>215</td>
<td>0.912</td>
</tr>
<tr>
<td>2</td>
<td>214</td>
<td>0.991</td>
</tr>
<tr>
<td>3</td>
<td>227</td>
<td>0.969</td>
</tr>
<tr>
<td>4</td>
<td>233</td>
<td>0.979</td>
</tr>
<tr>
<td>5</td>
<td>205</td>
<td>0.990</td>
</tr>
<tr>
<td>6</td>
<td>214</td>
<td>0.986</td>
</tr>
<tr>
<td>7</td>
<td>230</td>
<td>0.983</td>
</tr>
<tr>
<td>8</td>
<td>222</td>
<td>0.986</td>
</tr>
<tr>
<td>9</td>
<td>251</td>
<td>0.972</td>
</tr>
<tr>
<td>10</td>
<td>204</td>
<td>0.980</td>
</tr>
<tr>
<td>Total</td>
<td>2215</td>
<td>0.975</td>
</tr>
</tbody>
</table>

*Adjusted for the study design."
sera analysed by ELISA techniques in order to estimate the age-dependent distribution of seroprevalence of antibody specific against rubella virus.

The second phase was carried out between 20 September and 8 October 1993, when we visited 261 schools in the city of São Paulo, sampling 2044 children. The age distribution of these children can be seen in Table 3. The children were bled and the sera analysed by ELISA techniques in order to estimate the age-dependent distribution of seroprevalence of antibody specific against rubella virus. We assigned the same ages to the same schools in order to guarantee that the second year cohort was different from the first. We also extended the age interval up to 15 years in order to check whether the seroprevalence levels would drop to pre-vaccination time.

**Laboratory Techniques**

In both phases, blood was taken by finger prick with Glucole® lancet, and collected in Whatman No. 1 filter paper. An in-house indirect enzyme immunoassay (ELISA) was used to detect rubella-specific IgG in eluates of blood spotted onto filter papers. Briefly, microtitre plates (Nunc-polysorp Immunoplates, Denmark) were coated with either RA 27/3 rubella virus or control antigen by overnight incubation at 4°C. Virus antigen was extracted from infected Vero cells and control antigen from uninfected Vero cells by treatment with sodium deoxycholate. After washing plates with PBS, control and filter paper eluates diluted 1:50 were dispensed into wells coated with either rubella or control antigen, and incubated for 60 minutes at 37°C. Plates were then washed four times with PBS containing 0.1% Tween 80. Peroxidase conjugated anti-human IgG (Sigma, USA) was added to each well for an incubation period of 40 minutes. After washing plates four times with PBS-Tween, the chromogenic substrate (o-phenylenediamine and hydrogen peroxide) was added to each well for an incubation of 20 minutes at room temperature. Enzymatic reaction was stopped with 2.5 N H2SO4 and the optical density read at 492 nm (OD492). The resultant OD492 of the antigen well minus the OD492 of control well (ΔOD492) was then obtained for each sample. Specimens were considered reactive if they gave a ΔOD492 greater than 0.2. The assay was standardized by comparison with a commercial kit (Enzygnost Rubella, Behring, Germany). It had a sensitivity and specificity similar to that of the commercial kit, which had previously been determined to be 99.26% and 100% respectively, with an overall agreement of 96.9% and a correlation coefficient of 0.9 between optical densities of 220 samples tested for both techniques. Only seven of those gave results near the cutpoint.

**Estimation of the Force of Vaccination**

The coverage levels attained by the routine immunization programme were estimated by the calculation of the force of vaccination, 𝑣(𝛼), for children between 1 and 2 years of age (the target for the programme), assuming that the force of infection for this age interval is close to zero, according to the equation:

\[ v(α) = \frac{S'(α)}{[1-S'(α)]} \]  

where S'(α) is the proportion of seropositive at age α.

From this parameter it is possible to calculate the average age, A1, children are vaccinated, according to the equation:

\[ A_1 = \frac{\int_0^\infty αv(α)X(α)dα}{\int_0^\infty v(α)X(α)dα} \]

where X(α) is the number of seronegative children at age α.

**The Vaccination Strategy Efficacy**

Several measurements have been proposed to estimate the efficacy of a vaccination programme in reducing the susceptibility to a given infection. The efficacy can be estimated by the relative probability of transmission, \[ R \] and the relative attack rate, \[ R_A \]. In this work we will estimate the efficacy, \[ VE_{CRS} \], of the proposed strategy on the attack rates of CRS, by applying the following equation:

\[ VE_{CRS}(i) = 1 - \frac{AR_R(i)}{AR_A(i)} \]

where AR_R(i) are the attack rates for CRS before (i = 0) and after (i = 1) the vaccination.

For this calculation, continuous function was fitted to the relative number of CRS cases.

**RESULTS**

In the period comprising the vaccination campaign, 6850 000 children, representing 96% of the population aged 1–10 years in the State of São Paulo, received a single shot against measles, mumps and rubella. The results of this campaign were also evaluated by vaccination coverage levels as reported in individual immunization documents (vaccination cards). From the
2215 children surveyed in the city of São Paulo, 2039 have information on the vaccination status. From these, 1970 (97%) have been vaccinated during the campaign. In the city of Caieiras, from the 724 children surveyed, 709 had information on the vaccination status. From these, 648 (91.4%) received the vaccination during the campaign. Table 2 summarizes the post-campaign serological results. Figure 1 shows a comparison of both areas surveyed, as related to the post-campaign seroprevalence to rubella. As can be seen, there is no significant difference between the areas. Figure 2 shows the striking increase in the seroprevalence levels after the intervention, indicating the success of the campaign. Table 3 shows the results of the first year.
TABLE 3 Results of seroprevalence one year after the introduction of the vaccine

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sample size</th>
<th>Proportion of positives</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1168</td>
<td>0.690</td>
<td>0.0429</td>
</tr>
<tr>
<td>2</td>
<td>220</td>
<td>0.936</td>
<td>0.0170</td>
</tr>
<tr>
<td>3</td>
<td>205</td>
<td>0.971</td>
<td>0.0119</td>
</tr>
<tr>
<td>4</td>
<td>152</td>
<td>0.974</td>
<td>0.0131</td>
</tr>
<tr>
<td>5</td>
<td>248</td>
<td>0.964</td>
<td>0.0120</td>
</tr>
<tr>
<td>6</td>
<td>155</td>
<td>0.980</td>
<td>0.0113</td>
</tr>
<tr>
<td>7</td>
<td>111</td>
<td>0.981</td>
<td>0.0131</td>
</tr>
<tr>
<td>8-10</td>
<td>25</td>
<td>0.880</td>
<td>0.0602</td>
</tr>
<tr>
<td>11</td>
<td>128</td>
<td>0.976</td>
<td>0.0308</td>
</tr>
<tr>
<td>12</td>
<td>202</td>
<td>0.856</td>
<td>0.0327</td>
</tr>
<tr>
<td>13</td>
<td>180</td>
<td>0.739</td>
<td>0.0381</td>
</tr>
<tr>
<td>14</td>
<td>160</td>
<td>0.758</td>
<td>0.0590</td>
</tr>
<tr>
<td>Total</td>
<td>2014</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After the introduction of the vaccine, Figure 3 illustrates the results of seroprevalence one year after the implementation of the vaccination strategy, superimposed on the projections predicted by the model presented by Massad et al. It can be noted that the model predicts with good accuracy the behaviour of the seroprevalence data.

In order to estimate the seroconversion achieved by the routine programme, we analysed the age group 12 to 24 months. As mentioned, in this age group the force of infection is practically nil (see Souza et al.), so that a seropositive result was due exclusively to vaccination.

Figure 4 shows the seroprofile of this age group fitted to a logistic function. It can be noted from this Figure that the vaccination coverage attained levels which resulted in a seroprevalence above 90%. The force of vaccination, estimated according to equation 2 can be seen in Figure 5. From this parameter, we calculated, according to equation 3, the average age children were vaccinated in the routine scheme, which was 16 months.

In Table 4, we show the reported number of CRS cases in the state of São Paulo, for the years 1992 to 1994.

Confirmed cases refers to those with positive IgM antibodies to rubella, irrespective of clinical findings at birth. Suspected cases are those without serological confirmation, and we also discarded those who gave negative results for IgM.

Data presented in Table 4 were fitted to a continuous function, representing the ratio between the attack rate after and before the intervention, from which, through equation 4, we calculated the time-dependent vaccine efficacy. Results are shown in Figure 6 where it can be seen that the strategy efficacy quickly reaches 100% in the first 3 years after the intervention.

DISCUSSION
This study presents the results of a vaccination strategy which was, for the first time in Brazil, designed
and based on mathematical modelling. It was also the first time that rubella vaccine was introduced in the Public Health immunization calendar.

Originally the pulse vaccination campaign was designed to cover the ages 9 months to 15 years. Actually, this was the strategy adopted by the Federal Ministry of Health in a concomitant campaign against measles. By applying mathematical techniques which demonstrated that coverage of those aged 1–10 years would be sufficient to interrupt the rubella virus spread, the State of São Paulo saved almost 4 million dollars. In addition, by limiting the upper age to 10 years we
avoided the risk of vaccinating pregnant girls, a possible contraindication of the rubella vaccine.  

We have considered the community of Caieiras as a control group for this study, since this is a city with a social structure quite similar to São Paulo, although much smaller. In addition, it is very close to São Paulo and can be taken as a typical borough of this city. As a matter of fact, it was a district of São Paulo until the late 1950s. Therefore, it is fairly representative of the São Paulo community as a whole.

The proportion seropositive, by age, after the campaign is very similar in both populations investigated, which implies that the school population is representative of all children in the age group considered. As a consequence seroprevalence surveillance can be carried out in schoolchildren, with obvious advantages in practical terms.

The high levels of seroprevalence attained after the mass vaccination campaign indicate the success of the strategy adopted, at least from the point of view of the reduction in the proportion of susceptibles remaining after the campaign (see Massad et al.  for details on this point). It is noteworthy in Figure 2 that seroprevalence levels increased more than four times in relation to baseline (pre-campaign) immediately after the pulse vaccination.

One interesting result is that real data fit reasonably well the projections made by the theoretical model, as shown in Figure 3. One expected feature that should be a consequence of the vaccination strategy proposed is the accumulation of susceptibles in age above 10 years. This is exactly what we found one year after the introduction of rubella vaccine. As shown in Figure 3, the theoretical curve of seroprevalence is shifted to the right in age. The difference between the two lines from 11 years upwards is the proportion of remaining susceptibles accumulated in one year. This occurs if, and only if, the force of infection drops to levels close to zero. Our results suggest that this is indeed the case.

The seroprevalence profile attained by children below 20 months of age after the introduction of the routine vaccination programme (Figure 4) can be assumed to be due exclusively to the vaccine. This allowed us to estimate, for the first time to the best of our knowledge, the age-dependent force of vaccination (Figure 5), which is a continuous function for the rate under which children are immunized. As a corollary, we also calculated the average age these children are receiving the vaccine. Considering that the

| Table 4: Reported number of cases of congenital rubella syndrome |
|-----------------|-----|-----|-----|
|                | 1992 | 1993 | 1994 |
| Confirmed      | 16   | 1    | 0    |
| Suspected      | 13   | 5    | 0    |
| Discarded      | 18   | 8    | 1    |
| Total          | 48   | 15   | 1    |

Figure 6. Vaccine efficacy estimated from data in Table 3, according to equation 4 and fitted to a continuous function.
for the programme established 15 months as the age to vaccinate, the average age estimated (16 months) is extremely good. This method could be proposed as an immunization programme evaluation procedure in areas where the force of infection is negligible for the age interval containing the proposed age of vaccination.

Finally, the efficacy of the proposed strategy is attested by Table 4, where we demonstrate the impact on the absolute number of cases of CRS and Figure 6, which demonstrates that the proposed vaccination strategy achieves definite efficacy levels in less than 3 years.

We are still following up this population, and we are convinced that future results will confirm our expectation that CRS will cease to be a public health problem in São Paulo as a result of the adopted immunization strategy.

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