Immunization coverage and immunity to diphtheria and tetanus among children in Hyderabad, India

Manoj V. Murhekara,*, Sailaja Bitragunta a, Yvan Hutina a, Anita Ckakravarty b, Hitt J. Sharma c, Mohan D. Gupte a,c

a Field Epidemiology Training Programme, National Institute of Epidemiology, Chennai, India
b Department of Microbiology, Maulana Azad Medical College, New Delhi, India
c Serum Institute of India Ltd, Pune, India

Accepted 30 December 2008
Available online 5 February 2009

KEYWORDS
Diphtheria; Tetanus; Immunity; Immunization

Summary  Background: The Indian state of Andhra Pradesh accounted for 50% diphtheria and 3% tetanus cases reported globally during 2005. During 2003–2006, there was a rising trend of diphtheria in Hyderabad, the state capital, whereas there was no major change in trend of tetanus cases. We estimated coverage of diphtheria and tetanus vaccine among children aged ≤6 years and immunity against these diseases among school children aged 7–17 years in Hyderabad.

Methods: Using lot quality assurance sampling method, we surveyed children aged 12–23, 18–36 and 54–72 months to estimate coverage of three primary doses and first and second boosters of diphtheria and tetanus vaccine respectively. We conducted a sero-survey among children aged 7–17 years studying in randomly selected schools in Hyderabad. We tested sera for antibodies against diphtheria and tetanus.

Results: Primary vaccination coverage was <80% in four of the seven circles of Hyderabad while booster coverage was <80% in entire city. Of the 2419 children sero-surveyed, 56% and 64% were immune to diphtheria and tetanus respectively (titre ≥0.1 IU/ml). Booster coverage and immunity against these diseases was lower among Muslims.

Conclusions: It is necessary to improve booster coverage especially among Muslims. Vaccinating school children at school entry and periodic boosters thereafter will increase immunity among children.

© 2009 The British Infection Society. Published by Elsevier Ltd. All rights reserved.

Introduction

The universal immunization programme (UIDP) has been implemented in India since 1985. 1 The programme emphasizes universal coverage of six vaccines including Bacillus Calmette Guerin (BCG), three doses of diphtheria–pertussis–tetanus...
(DPT) and oral polio vaccine (OPV) and measles vaccine among infants. High level of immunity acquired from full course of diphtheria and tetanus vaccination wanes in late childhood and adolescence. As a result, periodic boosters are necessary for adequate maintenance of protection. Under UIP, after the primary vaccination, two booster doses of diphtheria and tetanus vaccines, one at 18 months (DPT) and other between 54 and 72 months of age (DT) are administered. Besides these booster doses, two doses of tetanus toxoid (TT) are administered to school children at the age of 10 and 16 years under the school health programme.

Despite improvement in the coverage of primary vaccination over last two decades, vaccine-preventable diseases remain an important cause for morbidity and mortality among children in many states in India. During the year 2005, India contributed 23% of the 15,561 tetanus cases and 71% of the 8229 diphtheria cases reported globally. The incidence rates for tetanus in the city ranged from 0.7 to 1.1 per 100,000 during 2003–2006 without any major change in the trend. On the other hand, diphtheria rates increased from 11 per 100,000 population in 2003 to 23 per 100,000 in 2006. The disease was most common among children aged 5–19 years, females and Muslims. Overall, the persistence of tetanus and rise in diphtheria led us to question the level of immunity in the population.

Vaccination coverage rate is frequently considered as a surrogate measure of protection, while the serum level of protective antibodies is a more objective measure. Considering the large number of cases of diphtheria and tetanus reported from Hyderabad, we conducted a study to estimate (1) the coverage of diphtheria and tetanus vaccine among children aged six years or less and (2) the immunity against these diseases among school children aged 7–17 years.

Methods

Vaccination coverage survey

Sampling and sample size
We surveyed Hyderabad city to estimate the coverage of three primary doses of DPT, first booster of DPT and second booster of DT vaccine among children of 12–23 months, 18–36 months and 54–72 months, respectively. Hyderabad is divided into seven circles (municipal administrative sub-divisions) with population ranging between 124,433 and 1,003,625. The city is further divided into 35 ‘wards’ and divisions) with population ranging between 124,433 and 1,03,625. A stratified sample was drawn from all the seven circles. In each of the seven circles, we selected 11 clusters with a probability proportional to size. We considered a ‘block’ as cluster. In each cluster, we sampled nine children for each of the three age groups (Total: 99 children for each age group in each circle). We then used lot quality assurance sampling (LQAS) method to test hypothesis with respect to the coverage.

In Hyderabad, the administrative coverage for primary vaccination was 100% from 1995 to 2004 and 98% in 2005–2006 [Govt. of Andhra Pradesh, unpublished data]. In addition, in 2005, coverage for first booster dose was 89% [Govt. of Andhra Pradesh, unpublished data]. We considered 20% as upper threshold of proportion of incompletely immunized children (acceptable level of vaccination coverage = 80%) and 8% as the lower threshold (desired level of vaccination coverage = 92%). Using LQAS tables, with an alpha error of 1% and a power of 80%, the lot sample size was 94 for a critical number of incompletely vaccinated children (d) acceptable of nine. Hence, for each lot, we excluded randomly five of the 99 children selected to keep the size as 94. For each age group and in each stratum, if the number of incompletely vaccinated children among the 94 was nine or less, we accepted the null hypothesis that the proportion of incompletely vaccinated children was less than 20% (vaccination coverage ≥ 80%). If the number of incompletely vaccinated children exceeded nine, we rejected the null hypothesis and considered the vaccination coverage is less than 80%.

Data collection
We first attempted to ascertain vaccination status using vaccination cards. If the card was not available and the mother reported that the child received the vaccine from urban health centers, we used urban health centre records. When the card was not available and mother reported that the child received the vaccine from outside of the urban health centers, we used the mother’s statement. Children aged 12–23 months who received three primary DPT vaccines, those aged 18–36 months who received three primary DPT vaccines and first DPT booster and those aged 54–72 months who received three primary DPT vaccines, first DPT and second DT booster were considered as completely vaccinated. We also collected information about religion and reasons for non-vaccination, when applicable.

Data analysis
First, we conducted LQAS analysis for each circle and age group as per LQAS tables. Second, we calculated the overall coverage of complete immunization in Hyderabad city for the three age groups after weighting for age-specific population size in each circle. We also calculated the overall coverage by religion and sex.

Immunity against diphtheria and tetanus

Study population and sampling
We conducted a sero-survey covering a population of 138,777 children aged 7–17 years studying in the 817 government schools in Hyderabad [Govt. of Andhra Pradesh, unpublished data]. We randomly selected three government schools [one primary (standard 1–5), one secondary school (standard 6–10) and one junior college (standard 11–12)] from each of the seven circles of the city. From each circle, we sampled a minimum of 30 children in each of the single year age categories between 7 and 17 years.
Data collection
We collected information regarding age, sex, religion and place of residence from the school children. We collected 2 ml of blood from the selected children aseptically and stored the sera at −20 °C until tested.

Laboratory testing
For the quantitative estimation of diphtheria and tetanus antibody levels, we tested the sera using commercially available enzyme linked immunosorbent assay (Diphtheria toxoid IgG ELISA, Demeditec Diagnostics GmbH, Lise-Meitner, Kiel, Germany and tetanus toxoid IgG ELISA, Demeditec Diagnostics GmbH, Lise-Meitner, Kiel, Germany). These ELISA kits are calibrated using international standards (WHO reference preparation 00/496 for diphtheria and WHO reference preparation 76/589 for tetanus). Both the assays have been shown to correlate well with other ELISAs [coefficient of correlation of 0.94 with Immunolab Diphtheria IgG test, Virotech, Germany (Cat No. EC129.00) and coefficient of correlation of 0.897 with Immunozym Tetanus IgG test, Progen Bioteknik, Germany (Cat No. 7702010)].

We classified school children into categories of immunity to tetanus and diphtheria according to internationally accepted criteria.2,8,9 We considered children with antibody concentrations of 0.1 IU/ml or more as fully immune, those with levels in the range of 0.01 to <0.1 IU/ml as partially immune and those with levels below 0.01 IU/ml as non-immune.

Data analysis
We calculated the proportion of children who were immune, partially immune and not-immune according to age, sex and religion. We tested the difference in proportion using χ² test. We calculated χ² for linear trend to assess the increase in proportion of children immune to diphtheria and tetanus with age. We also fitted a linear regression model to estimate the increase in the proportion of immune children with age.

Human subject protection
The ethics committee of National Institute of Epidemiology, Indian Council of Medical Research, Chennai approved the study. We obtained written informed consent from the parents of children and permissions from the education and health departments of government of Andhra Pradesh and from the Principals of the selected schools to conduct the study.

Results

Vaccination coverage survey

Table 1 Number of incompletely vaccinated individuals among the 94 children in each of the seven circles of Hyderabad, by age group, Andhra Pradesh, India 2006.a

<table>
<thead>
<tr>
<th>Circle</th>
<th>Age groups</th>
<th>12–23 monthsb</th>
<th>18–36 monthsb</th>
<th>54–72 monthsb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>46</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>50</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>24</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>39</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>37</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>37</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>29</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

a Numbers in bold exceed the critical number nine, suggesting that coverage in that circle cannot be considered as exceeding 80% as per the lot quality assurance sample analysis.
b Children aged 12–23 months who received three primary DPT vaccines, those aged 18–36 months who received three primary DPT vaccines and first DPT booster and those aged 54–72 months who received three primary DPT vaccines, first DPT and second DT booster were considered as completely vaccinated.

Reasons for non-vaccination
There were 765 children who were not completely vaccinated in three age groups. We interviewed 194 (25%) mothers of such children. The information about the reasons for non-vaccination from the remaining mothers was not forthcoming. Of the 194 mothers whose children were not vaccinated, 93 (48%) did not have adequate information about vaccination, 62 (32%) faced obstacles in vaccinating their children and 39 (20%) lacked motivation. Among the 93 mothers who lacked information, 46 (50%) were not aware of the need to return for the next dose, 29 (31%) were not aware of the need for immunization and 18 (19%) were not aware of the time and place of immunization. Among the obstacles faced by mothers of incompletely vaccinated children, 21 (35%) children were ill and not brought for vaccination and 37 (46%) mothers were too busy or had family problems. Of the 39 mothers who lacked motivation, 23 (59%) postponed vaccination and 7 (18%) believed in rumors of adverse events.

Overall coverage
591 of the 658 children aged 12–23 months had received primary immunization (coverage: 90%, 95% confidence [CI] = 86–93). 397 of the 658 children aged 18–36 months had received the first booster (fourth dose coverage: 60%, 95% CI = 54–66) whereas 235 of the 658 children aged 54–72 months had received the second booster (fifth dose coverage: 36%, 95% CI = 27–40) (Table 2). The coverage for primary and booster doses did not differ among males and females (primary immunization: 88% vs. 92%, p = 0.08; first booster: 59% vs. 62%, p = 0.4, second booster: 34% vs. 37%, p = 0.38. Compared with others, Muslims had a coverage that did not differ for primary vaccination (91% vs. 87%, p = 0.11). However, the coverage among Muslims was significantly lower for the fourth (63% vs. 54%, p = 0.03) and fifth dose (41% vs. 24%, p = 0.0000) (Table 2).
Immunity against diphtheria and tetanus

Overall immunity
We collected blood samples from 2419 children. Of these, 1344 (55.5%, 95% CI 54–58) and 1546 (64%, 95% CI 62–66) were immune to diphtheria and tetanus respectively. 142 (5.9%) were non-immune to diphtheria whereas only one child was non-immune to tetanus. The geometric mean titres (GMT) of antibodies against diphtheria and tetanus were 0.12 IU/ml (95% CI 0.11–0.13) and 0.33 IU/ml (95% CI 0.30–0.35) respectively (Table 3).

Immunity by sex, religion and circles
The proportion of children immune to diphtheria and tetanus were significantly lower among female (p = 0.0000) and Muslim (p = 0.0000) children (Table 3). Immunity to diphtheria in different circles of the city ranged from 44% to 61% whereas 47–77% school children in different circles were immune to tetanus (data not shown).

Diphtheria and tetanus anti-toxin levels according to age
At seven years of age, 50% and 60% of children were immune to diphtheria and tetanus respectively (Fig. 1). There was linear increase in proportion of children immune against diphtheria ($\chi^2$ trend = 12.8, p = 0.0004) and tetanus ($\chi^2$ trend = 10.2, $p = 0.001$) with age. The proportion of immune children in successive age years increased by 1.3%, (regression coefficient = 0.013, 95% CI = 0.007–0.020) for diphtheria and 1% for tetanus (regression coefficient = 0.01, 95% CI = 0.004–0.017). The GMTs against diphtheria and tetanus increased from 0.099 IU/ml and 0.25 IU/ml at the age of seven years to 0.13 IU/ml and 0.42 IU/ml at the age of 17 years respectively. For both the antigens, the GMTs were lower among Muslims (Fig. 2). One hundred and thirty (92%) of the 142 children non-immune to diphtheria were below 15 years of age.

Discussion
The overall coverage of primary immunization was adequate in Hyderabad whereas the coverage for booster doses was low. A large proportion of children studying in government schools did not have adequate protective antibody levels against diphtheria and tetanus. Coverage of booster doses as well as the immunity against these diseases was significantly lower among Muslims.

Serologic data on vaccine-preventable diseases are useful to evaluate the impact of immunization programs, and to identify susceptible subgroups. About two-third of school children in the city were immune to tetanus, while half

<table>
<thead>
<tr>
<th>Sex</th>
<th>Fully vaccinated for age (%)</th>
<th>Vaccinated for age (%)</th>
<th>Vaccinated for age (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>338</td>
<td>88</td>
<td>344</td>
</tr>
<tr>
<td>Female</td>
<td>320</td>
<td>92</td>
<td>314</td>
</tr>
<tr>
<td>Religion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muslim</td>
<td>223</td>
<td>87</td>
<td>225</td>
</tr>
<tr>
<td>Non-Muslim</td>
<td>435</td>
<td>91</td>
<td>433</td>
</tr>
<tr>
<td>Overall</td>
<td>658</td>
<td>90</td>
<td>658</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. tested</th>
<th>Diphtheria antibody level, IU/ml (%)</th>
<th>Tetanus antibody level, IU/ml (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.01</td>
<td>0.01–0.09</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1097</td>
<td>3.7</td>
</tr>
<tr>
<td>Females</td>
<td>1322</td>
<td>7.6</td>
</tr>
<tr>
<td>Religion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muslim</td>
<td>777</td>
<td>8.4</td>
</tr>
<tr>
<td>Non-Muslim</td>
<td>1642</td>
<td>4.7</td>
</tr>
<tr>
<td>Overall</td>
<td>2419</td>
<td>5.9</td>
</tr>
</tbody>
</table>

* Geometric mean titres.
were immune to diphtheria. A large proportion of the children non-immune against diphtheria had antibody concentrations ranging between ≥0.01 and <0.1 IU/ml indicating partial immunity. The low immunity against tetanus and diphtheria among school children is consistent with the findings of low booster coverage. Though the partially immune children are immune to clinical disease, the duration of protection is usually considered as short-term. It is therefore necessary to vaccinate these children against diphtheria and tetanus.

The results of the LQAS survey also indicated that the coverage of primary immunization was less than 80% in three of the seven circles. The coverage of first and second boosters was low in all the circles of the city. Immunity to diphtheria and tetanus was also significantly lower among Muslim and female children. Low immunity among Muslims is consistent with low coverage of booster.

Lower immunity among Muslim children could either be related to a poor offer of vaccine by the health services or to a poor demand of vaccine in the community. In the Indian state of Uttar Pradesh, occurrence of poliomyelitis cases among Muslims was attributed to low community acceptance of pulse polio immunization. However, our data indicates that the primary vaccination coverage was identical among Muslim and non-Muslim in Hyderabad. This suggests that initially, the demand for vaccine is identical in all communities but that the health system is not able to retain the same demand in the Muslim community. Lower immunity among female school children suggests lower vaccination coverage among them in the past. However, the results of the coverage survey indicated that the disparity between immunization coverage among gender are declining. Similar findings were also observed during the last two National Family Health surveys.

Diphtheria immunity among the school children increased with age, though the increase in proportion of immune children every year was small. In the absence of any school-based vaccination programme for diphtheria, the increase in diphtheria immunity could be due to natural immunity acquired through apparent and inapparent infections. Unlike diphtheria, natural immunity does not have any practical importance in controlling tetanus.

Under the school health programme, two doses of tetanus toxoid are administered to school children at the age of 10 and 16 years. Besides, tetanus toxoid is also administered routinely after any minor injury. These vaccinations may explain the persistence of immunity as well as the increase in geometric mean antibody titres against tetanus with age. This could also be the reason for larger proportion of children being non/partially immune to diphtheria compared to tetanus. A very small increase in the proportion of children immune to tetanus (10%) between 7 and 17 years however indicates a lower coverage of school health programme in Hyderabad. During 2006, the coverage of TT at 10 and 16 years of age was 40.2% and 41.9% respectively (Govt. of Andhra Pradesh, unpublished data).

In India, the performance of universal immunization programme is measured in terms of coverage of six primary vaccines among infants. Although booster delivery is included in the vaccination schedule, coverage is not routinely monitored. As a consequence, children who drop out for booster doses are not followed up. The Hyderabad health system functions with sub-optimal number of field staff. Overworked health workers do not spend sufficient time communicating with the mothers about the next dose of vaccine. While the immunization schedule is printed on vaccination cards and on the walls of all the health centers, this is not a sufficient message...
for illiterate mothers. Lack of awareness regarding the need for booster and various obstacles were the main reasons cited by the mothers of incompletely vaccinated children. These factors need to be corrected through interpersonal communication between health providers and mothers. Minor childhood ailments considered as obstacles to immunization by the mother could also be addressed by educating mothers.

Our study had certain limitations. First, Vaccination cards were available for only 1169 (59%) of 1974 children surveyed. As health system does not need immunization cards once the vaccination schedule of an infant is complete, there is a low retention of cards leading to poor documentation. We tried to address this limitation by cross checking the vaccination status with health care worker’s records and by probing vaccination history through a link with the child’s milestones. However, this limitation could have resulted in an over or underestimation of the vaccination coverage. Second, we did not collect immunization history from the school children as the parents could not recall the history of immunization. In the absence of such information, it was not possible to compare the immunity levels according to vaccination status. Third, for calculation of sample size for LQAS survey, we used uniform thresholds for the proportion of incompletely vaccinated children for the three age groups.

Our study indicated that the coverage of booster doses and immunity against diphtheria and tetanus was low in Hyderabad, especially among Muslims and females. On the basis of these findings, we can propose a number of recommendations. First, there is a need to improve the coverage for boosters especially among Muslims and females. As 91% of the children in Hyderabad attend primary schools (Govt. of Andhra Pradesh, unpublished data), a high coverage of second booster could also be achieved by vaccinating the children at school entry. Second, the awareness of mothers about the importance of booster doses must be improved. Such efforts should be especially conducted among Muslims. Third, tetanus toxoid administered to school children at 10 and 16 years of age could be replaced with adult type of combined tetanus-diphtheria vaccine. Fourth, coverage of boosters should be considered as performance indicator to improve the immunization programme. The diphtheria situation in India also warns of a long-term potential for the re-emergence of vaccine-preventable diseases in other countries with low vaccination coverages. It also reinforces the need of examination of the global diphtheria control strategy including continued investment in improved vaccines.

Funding source

The study was partially funded by Serum Institute of India Ltd., Pune, India.

Acknowledgements

We gratefully acknowledge the help provided by Dr. G.S. Namjoshi, Assistant Medical Director and Dr. S.S. Parekh, Manager, Medical Affairs, Serum Institute of India Ltd, Pune during the sero-survey.

References