Diphtheria antitoxin response to DTP vaccines used in Swedish pertussis vaccine trials, persistence and projection for timing of booster

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Abstract

Data from two Swedish pertussis vaccine trials with various combination vaccines were used to compare anti-diphtheria antitoxin concentrations over time between different vaccines, vaccine lots and vaccine schedules. The immune responses were measured with a validated ELISA method.

Results are given for 1326 children, born 1992, that were recruited to the placebo (DT)-controlled Trial I which used a 2, 4, 6 month schedule. Two DTP acellular and one DTP whole cell vaccine were used. No DT boosters were given until 5 years of age.

Trial II recruited children born 1993–94 and compared three DTP acellular vaccines with one DTP whole cell vaccine. Results are given for 306 children in a 2, 4, 6 month schedule and for 531 children in a 3, 5, 12 month schedule. The latter schedule gave significantly higher diphtheria antitoxin concentrations post third dose.

The various DTP acellular vaccines and an ineffectual DTP whole cell vaccine gave lower antitoxin concentrations than both an efficacious DTP whole cell vaccine and the DT vaccine. The larger differences in antigen response between vaccines was reduced in the course of time. Generally, an initial rapid decline of antitoxin concentration was followed by a slower decline; the change typically occurring when the antitoxin concentration reached 0.13–0.16 EU/ml. The time needed to reach this level was between 6 and 10 months based on the initial vaccine response.

A “best-fit” combined exponential regression model was used to predict the optimal timing for booster vaccinations against diphtheria.

Our data support a 3, 5, 12 month schedule followed by a fourth dose 4–5 years after the third dose, depending upon the vaccine used. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: DTP-vaccines; Diphtheria antitoxin response; Persistence; Booster

1. Background

Studies on seroprevalence and longitudinal examination of antitoxin concentrations have been used to follow immunity against diphtheria [1–10]. According to the WHO recommendations IgG concentrations ≥0.1 IU/mL are considered as protective and 0.01–0.09 IU/mL may be regarded as giving basic immunity when assessed by neutralisation methods [11,12]. But the protection is not absolute [13–15]. Ipsen [14] showed a significant relationship between antitoxin titer in acute serum and clinical course of diphtheria disease in vaccinated children. To maintain herd immunity and prevent outbreaks of diphtheria it is...
believed that a minimum of 75% of adults and 90% of children should have antitoxin concentrations of 0.01 IU/mL or more [6,16]. In Sweden there were 36 cases with culture-positive diphtheria disease, 6 fatalities and in addition 72 healthy carriers between 1984 and 1988; thereafter no case of diphtheria has been reported [17, and unpublished data].

In Russia the recommended age for the second booster dose was changed from 6 to 10 years in 1986. A recent diphtheria epidemic in that country showed the highest age-specific incidence rates in children age 7–10 years. A booster vaccine dose given at 6–8 years improved clinical protection in the Russian population during the recent epidemic [18].

Earlier seroprevalence studies in Sweden had indicated a high proportion of seronegatives among children [3] and adults [2]. Preliminary data from the European SeroEpidemiology Network for standardisation (ESEN) show that Sweden substantially differed from other European countries in seroprevalence for diphtheria antitoxin. Only about 80% of 5–9 year old children had antibody concentrations above 0.02 EU/mL and very few had levels above 0.1 EU/mL (unpublished data). The present Swedish situation reflects a vaccination programme with three doses of DT containing 15 Lf diphtheria toxoid given in infancy followed by a late DT booster dose containing 7.5 Lf diphtheria toxoid at 10 years of age.

It was therefore of particular relevance to re-evaluate the Swedish childhood vaccination schedule in connection with two recent randomised vaccine efficacy trials [19,20]. The present study was a post hoc analysis and not planned as part of the original design. Both NIAID-sponsored trials — called Trial I and II — were conducted to evaluate absolute or relative efficacy of various pertussis vaccines (P) combined with diphtheria and tetanus toxoids. The vaccine preparations contained variable amounts of diphtheria (D) and tetanus (T) toxoids and adjuvants. In this article we report data on the anti-diphtheria response and the decline of antibody concentrations post three doses of DT or DTP vaccines. None of the children reported had received a diphtheria booster vaccine after 12 months of age during up to 45 months of follow-up after the third dose. A special subgroup obtained a DTP booster about 5 years after the third dose.

The aims of the present study were:
1. To compare the antitoxin responses between various vaccines, vaccine schedules, vaccine lots and the influence of maternal antibodies by means of an ELISA assay.
2. To analyse the time-related decline of postvaccination antitoxin concentrations in infancy and to fit a mathematical model to predict the status for booster vaccination.
3. To study the antitoxin response after a fourth dose of diphtheria toxoid as a five-component acellular DT vaccine (DTPa5) given 52–65 months after the third dose of DTPa5 vaccine.

2. Subjects and methods

2.1. Vaccines

The amount of diphtheria toxoid in the vaccines evaluated in Trial I and Trial II are outlined in Table 1. The toxoid content listed for each vaccine is based on the manufacturer’s determination of the antigen in terms of Lf (limit of flocculation). In both trials the diphtheria toxoid content was the same for the DTPa2 and the DTPa5 vaccines. The DTPa2 composition was similar also for the other vaccine components while the DTPa5 vaccine was reformulated to a higher PT and FHA content in Trial II compared to Trial I [19,20]. In Trial II two different lots were used for each vaccine.

2.1.1. Trial I [19]

Trial I was a placebo (DT)-controlled randomised vaccine efficacy trial that enrolled 9829 children born in 1992 and vaccinated them at 2, 4, and 6 months of age at 14 study sites. In almost all children preplanned blood samples were collected at pre-determined ages at 1 year, and later at either 2 or 3 years of age. These sera were stored for later use but not all of them were analysed.

Pre-scheduled serum samples for immunogenicity studies were collected systematically at one study site, Linköping. The Linköping children (n = 651) were bled at 2 months of age before the first dose and thereafter at 7, 13 and 29 months of age, i.e. 1, 7 and 23 months after dose 3.

In Linköping and two other sites 235 DT-placebo recipients were also bled before and after catch up Pa vaccination after termination of Trial I. Seventy-four of the 235 children were bled at five different occasions after the third DT dose.

Acute and convalescent blood samples were obtained during episodes of suspected pertussis [19] and were analysed together with pre-planned sera collected at 1–3 years of age. The acute and convalescent blood samples analysed for antitoxin in the present study were selected among all available study samples from suspected, but laboratory negative, episodes of pertussis. This selection of samples was done to increase sample sizes at the pre-determined ages, and to cover also other age intervals after the third vaccine
dose, in particular the changeover from rapid to slow decline.

Table 2 gives the origin of the 4939 blood samples from 1326 Trial I children used in the present analysis. The main statistical analyses of diphtheria antitoxin decline utilised 4288 of 4939 samples.

2.1.2. Trial II [20]

In Trial II, enrolling children born in 1993 and 1994, pre-vaccination sera were taken at 2 months of age, for children in a 2, 4 and 6 months schedule (n = 306). Sera were also obtained at 7 and 13 months of age, corresponding to 1 and 7 months after dose 3. For children in the alternative 3, 5 and 12 months schedule (n = 531) the pre-vaccination sera were taken at 3 months of age. Sera were obtained at 7 months of age, 2 months after dose 2, or at 13 and 19 months of age, corresponding to 1 and 7 months after dose 3. Only pre-planned sera for children with both a pre- and a post-vaccination sample after three doses were used for statistical analyses.

Table 2
Samples in Trial I used for analysis of diphtheria antitoxin determinations

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Origin of sample</th>
<th>DTPa2</th>
<th>DTPa5</th>
<th>DTPw-C</th>
<th>DT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose 1, Linköping</td>
<td>180</td>
<td>180</td>
<td>134</td>
<td>157</td>
<td>651</td>
<td></td>
</tr>
<tr>
<td>Sera taken after the third trial dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month after dose 3, Linköping</td>
<td>180</td>
<td>180</td>
<td>134</td>
<td>157</td>
<td>651</td>
<td></td>
</tr>
<tr>
<td>1 year of age, Linköping</td>
<td>180</td>
<td>180</td>
<td>134</td>
<td>157</td>
<td>651</td>
<td></td>
</tr>
<tr>
<td>1 year of age, other study sites</td>
<td>143</td>
<td>154</td>
<td>97</td>
<td>126</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td>2 years of age, other study sites</td>
<td>66</td>
<td>82</td>
<td>50</td>
<td>59</td>
<td>257</td>
<td></td>
</tr>
<tr>
<td>2.5 years of age, Linköping</td>
<td>180</td>
<td>180</td>
<td>134</td>
<td>157</td>
<td>651</td>
<td></td>
</tr>
<tr>
<td>3 years of age, other study sites</td>
<td>71</td>
<td>66</td>
<td>44</td>
<td>67</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td>Acute sample, other study sites</td>
<td>142</td>
<td>151</td>
<td>94</td>
<td>125</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>Convalescent sample, other study sites</td>
<td>87</td>
<td>105</td>
<td>61</td>
<td>75</td>
<td>328</td>
<td></td>
</tr>
<tr>
<td>Before “catch-up” dose, Linköping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After “catch-up” dose, Linköping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of sera taken after the third trial dose</td>
<td>1049</td>
<td>1098</td>
<td>748</td>
<td>1393</td>
<td>4288</td>
<td></td>
</tr>
<tr>
<td>Total number of children</td>
<td>323</td>
<td>337</td>
<td>231</td>
<td>435</td>
<td>1326</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 summarizes the number of serum samples from Trial II used for analysis in this article.

2.2. Booster vaccination

Sixty children in the DTPa5 group in Trial I received a fourth 0.5 mL dose of DTPa5 vaccine at about 5 years of age median 63 months (range 57–70 months). The interval from the third dose was median 57 months (range 52–65 months). A pre-booster blood sample was taken and a post-booster sample was drawn 7–11 days after vaccination.

2.3. ELISA method for analysis of diphtheria antitoxin antibodies

An indirect ELISA method was used to measure the concentration of diphtheria antitoxin IgG antibodies [21,22]. In brief, purified diphtheria toxin of specific activity 2700 Lf/mg protein nitrogen was used as the antigen. PVC ELISA plates were coated with 0.1 mL per cup of a solution of toxin containing 2 Lf per mL. Eight two-fold dilutions of sera were used, starting from an initial dilution of 1 + 24 in the buffer.

As reference antitoxin we used, a standardised preparation of human antitoxin immunoglobulin (IgG), kindly provided by Dr S. Varallay, Swiss Serum Institute at Berne.

Absorbance values between 0.1 and 2.0 were used to plot the curves. The lower limit of the assay procedure was set at 0.02 ELISA units (=EU/mL) with an equivalence to 0.02 IU/mL when a minimum of three points were taken for evaluation as determined and validated by the rabbit intradermal toxin neutralisation test [23].

The validation studies had shown good agreement between the ELISA assay method and toxin neutralisation test in the range 0.02–5.0 IU/mL [22]. In this study all sera assigned a value of not less than 0.02 EU/mL were used for the analyses.

2.4. Statistical methods

Diphtheria antitoxin levels for a given duration after the third trial dose were summarised in tables in terms of percentiles and geometric means. Reverse cumulative distributions were produced separately for each vaccine group. Statistical comparisons of distributions of antitoxin levels between vaccines, between schedules, between trials, and between lots were performed with the Kolmogorov–Smirnov two-sample test. A p-value < 0.05 was considered statistically significant.

The persistence of the antitoxin levels over time was described by different statistical models using all 4288 post dose 3 sera available (Table 2). In each model the total numbers of samples for a given vaccine arm was grouped in 30-days intervals after the third trial dose.

In the first statistical model the percentage of children, in each 30-days interval, with an antitoxin level above 0.02 and 0.1 EU/mL was calculated.

In the second model a combined exponential mathematical regression model was fitted to describe the decline of the antitoxin level as a function of time.

An alternative third statistical model used to describe the persistence of antitoxin concentrations over time had its origin in former works by Jensen [24] and Scheibel et al. [25]. Jensen studied the antitoxin curve after active immunization with a single injection of purified and concentrated diphtheria antitoxin with special reference to the duration of the antitoxin immunity. In later works Scheibel et al. used a mathematical formula slightly modified by Bentzon,

\[ Y_t = Y_0 \left( \frac{C}{c + t} \right) \]

where \( Y_t \) is the antitoxin concentration at time \( t \), \( Y_0 \) the titer at time 0 (the maximum titer) and \( C \) a parameter estimated from available data.

We modified the Bentzon model to suit logarithmic data. In our hands the early post-vaccination sera were taken to define the maximum antitoxin concentration and the parameter of the statistical regression model was estimated with data from a subset of 74 DT recipients with both an early post-vaccination blood sample and four later samples within 3.5 years post third dose.

Methods for estimating parameters in non-linear regression were used in the different regression models.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Serum samples used in Trial II for analysis of antidiphtheria antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 4, 6 months schedule</td>
<td>3, 5, 12 months schedule</td>
</tr>
<tr>
<td>Pre-vaccination</td>
<td>7 months</td>
</tr>
<tr>
<td>DTPa2</td>
<td>67</td>
</tr>
<tr>
<td>DTPa3</td>
<td>80</td>
</tr>
<tr>
<td>DTPa5</td>
<td>80</td>
</tr>
<tr>
<td>DTPw-E</td>
<td>75</td>
</tr>
</tbody>
</table>
Fig. 1. Post-vaccination IgG anti-diphtheria antibody concentrations. (a) Two-component acellular DTPa2, (b) three-component acellular DTPa3, (c) five-component acellular DTPa5, and (d) whole-cell DTPw-E. Reversed cumulative distribution curves are given at 7 months in the 3, 5, and 12 months schedule in Trial II (---), at 7 months of age in the 2, 4, and 6 months schedule in Trial II (-----), at 7 months in the 2, 4, and 6 months schedule in Trial I (----), and at 13 months in the 3, 5, and 12 months schedule in Trial II (------). Intercepts of distribution curves with the 50% line correspond to median concentrations. The x-axis is logarithmic. Intercepts of distribution curves with the 0.1 EU/mL line give the percentage of children being well protected according to WHO standards. The number of analysed samples at each point in time are given in Table 2 for Trial I data, and in Table 3 for Trial II data.
3. Results

3.1. Immunogenicity 1 month post-vaccination, in the 2, 4 and 6 months vaccine schedule (Trial I and Trial II)

IgG diphtheria antitoxin post-vaccination concentrations 1 month after the third trial dose at 7 months of age are given as geometric means (GM), with confidence intervals and percentiles (Tables 1 and 5 and Fig. 1a–d). The DTPw-E-group had the highest GM-value (3.97 EU/mL) 1 month post-vaccination, followed by the DT-group (1.05 EU/mL) and in decreasing order DTPa5, DTPa2 and DTPw-C.

The DTPw-E and the DT vaccines gave levels above 0.1 EU/ml — considered as indicative of long-term protection [11] — in 100% of the children. For the DTPa2 and the DTPa5 vaccines two children (1.1%) in each vaccine group did not reach 0.1 EU/ml and one of the two children in the DTPa5 group had a post-vaccination level below 0.02 EU/ml. The corresponding numbers for the DTPw-C vaccine were six children out of 134 (4.5%) who had a post-vaccination level below 0.1 EU/ml — two of the six children had a post-vaccination level below 0.02 EU/ml.

3.2. Variation with composition and lot

To analyse the lot to lot variation, antitoxin levels were compared between Trials I and II for the DTPa2 and the DTPa5 vaccines (Table 1). The geometric mean for the DTPa2-group was 0.64 EU/mL in Trial I and 0.58 EU/mL in Trial II. The similar results obtained for DTPa2 in the two trials can be used as an accuracy control of the assay over time. The corresponding results for DTPa5 were 0.85 EU/mL in Trial I and a significantly lower geometric mean 0.66 EU/mL ($p < 0.05$) in Trial II in which a vaccine with higher concentration of PT and FHA was used.

In Trial II there was a statistically significant difference 1-month post-vaccination between the two DTPw-E lots, but not for the DTPa vaccines.

3.3. Influence of maternal antibodies on post-vaccination levels

Pre-vaccination concentrations at 2 months of age were taken to represent maternal antibodies. High maternal antibodies (for this analysis defined as $\geq 0.05$ EU/mL in the pre-vaccination sample) were found in 25% of the pre-vaccination samples ($n = 651$) from Trial I. The samples were divided in three subgroups according to the pre-vaccination level of antitoxin concentrations (Table 4). It is evident from the table that there is a relationship between the level of maternal antibodies pre-vaccination and the post-vaccination level measured 1 month after dose 3.

Post-vaccination antibody levels in each vaccine group were also compared by Kolmogorov two sample test between children with less than 0.05 EU/mL before vaccination and children with 0.05 EU/mL or more. For DTPa5, DTPw-C and DT recipients the group with “low” anti-toxin levels pre-vaccination had significantly higher post-vaccination levels compared to the group with “high” pre-vaccination levels ($p < 0.001$). The differences between the post-vaccination levels were not statistically significant ($p = 0.30$), however, for DTPa2 recipients.

There were similarly in Trial II for children vaccinated according to the 2, 4 and 6 months schedule significantly higher ($p < 0.001$) post-vaccination antitoxin levels for the subgroup with “low” pre-vaccination levels compared to the group with “high” levels. Also in the 3, 5 and 12 months schedule the post-vaccination levels after the second and the third dose were higher ($p < 0.05$) in the subgroup with “low” pre-vaccination levels compared to the other group (data not shown).

3.4. Comparison between 2, 4 and 6 and 3, 5 and 12 months vaccination schedules

We compared the antitoxin levels obtained at 7 months of age in the 2, 4 and 6 months schedule with

<table>
<thead>
<tr>
<th>Lf/dose</th>
<th>Pre-vaccination antibodies (EU/mL)</th>
<th>DTPa2 ($n = 180$)</th>
<th>DTPa5 ($n = 180$)</th>
<th>DTPw-C ($n = 134$)</th>
<th>DT ($n = 157$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt; 0.02$</td>
<td>365 (56%)</td>
<td>0.68 (0.58–0.79)</td>
<td>1.02 (0.85–1.22)</td>
<td>0.79 (0.68–0.92)</td>
<td>1.18 (1.07–1.29)</td>
</tr>
<tr>
<td>0.02–&lt; 0.05</td>
<td>124 (19%)</td>
<td>0.63 (0.50–0.80)</td>
<td>0.83 (0.68–1.02)</td>
<td>0.64 (0.48–0.85)</td>
<td>0.87 (0.66–1.13)</td>
</tr>
<tr>
<td>$\geq 0.05$</td>
<td>162 (25%)</td>
<td>0.50 (0.41–0.61)</td>
<td>0.44 (0.33–0.60)</td>
<td>0.33 (0.20–0.55)</td>
<td>0.66 (0.49–0.90)</td>
</tr>
</tbody>
</table>

$^a$ Number of children and percent are given for three levels of pre-vaccination antitoxin levels. Geometric mean value and 95% confidence interval in parenthesis ($n = 651$).
the 3, 5 and 12 months schedule (Fig. 1a–d) for the various vaccine groups. The antitoxin levels at this age were much lower in children that had received only two vaccine doses compared to children that had received three doses. However for the DTPw-E, DTPa5 and the DTPa3 groups at least 90% of the children attained the level of 0.1 EU/mL post two doses, but the percentage was lower among DTPa2 recipients. After the third dose of vaccine practically all vaccinees had attained an antibody level over 0.1 EU/mL irrespective of the vaccine or schedule used (Fig. 1a–d).

The 3, 5 and 12 schedule gave significantly higher concentration 1 and 7 months after dose 3, than after dose 3 in the 2, 4 and 6 schedule.

3.5. Decline of antitoxin concentrations

Serum samples from Trial I were used for analysis of persistence of antitoxin (Table 2). The samples covered 1–32 months after the third and last vaccine dose for DTP recipients and 1–45 months for placebo (DT) recipients.

A subgroup of 74 DT-recipients in Trial I was bled at five occasions mean 1, 7, 23, 34 and 38 months after the third vaccine dose (Fig. 2). About 5% of the children in the DT group had concentrations below 0.1 EU/ml 7 months post-vaccination. At 23 months post-dose 3 this number had increased to 65%. The reverse cumulative distribution curves for the 23 and the 34, 38 months samples were apparently similar. However an additional 5–10% of the children were below 0.1 EU/ml at 34 and 38 months. Seemingly there was a much slower decline of antitoxin concentrations in the DT-group between 2 and 3 years post dose 3 compared to the decline during the first year. The larger differences in GM-values between the DT- and the three DTP-groups observed after 1 month in Trial I was reduced at 23 months after the third dose (Table 5). However, some differences could be noted at the various percentile levels. Corresponding data were not available for DTPw-E in Trial II which had given the highest antitoxin concentrations at 1 month post-vaccination.

Two statistical methods based on all serum samples (n = 4.288) were used to describe the persistence of the antitoxin levels over time. In the first statistical method the percentage of children, in each 30-day interval, after the third dose with an antitoxin concentration above 0.02 and 0.1 EU/mL were calculated. The results are presented in Fig. 3. For the DT-group the proportion of children with concentrations below the 0.1 EU/mL level was 30–35% after 1 year, 65–70% after 2 years and around 80% after 3 years.

In the second statistical method individual serum concentrations were summarised per 30-day interval as the geometric mean for the interval (Fig. 4a–d). Fig. 4a–d show for each vaccine an initial rapid decline followed by a slower one. The duration of the period of “rapid” decline seemed to depend on the level observed 1 month after dose 3. The “shift” to the much slower decline seemed to occur when the geometric mean level was in the range of 0.13–0.16 EU/mL for all vaccines.

We tried to find a mathematical model that could describe the observed pattern of decline of antitoxin

![Fig. 2](image2.png)

Fig. 2. Longitudinal data of IgG anti-diphtheria antibody concentrations in 74 children with 5 samples each following three doses of DT in a 2, 4, and 6 months schedule, Trial I. Reversed cumulative distribution curves are given at 1 month (---), 7 months (- - - -), 23 months (-), 34 months (-----), and 38 months after dose 3 (----). Intercepts of distribution curves with the 50% line correspond to median concentrations. The x-axis is logarithmic. Intercepts of distribution curves with the 0.1 EU/mL line give the percentage of children being well protected according to WHO standards.

![Fig. 3](image3.png)

Fig. 3. Percent of children with IgG anti-diphtheria antibody concentrations $\geq 0.02$ EU/ml (fine lines), and $\geq 0.1$ EU/ml (bold lines), respectively, at different intervals from dose 3 in a 2, 4, and 6 months schedule in Trial I. Two-component acellular DTPa2 (●---●), five-component acellular DTPa5 (□-□-□), whole-cell DTPw-E (▲-▲-▲), and DT (+---+).
levels as a function of time. All the simple models failed accurately to depict the observed data. Therefore a combined exponential regression function was tried and found to fit the data. A good agreement between the observed geometric means and the two functions was found when the duration after the third dose was first divided in two intervals after which two separate exponential functions were estimated. In Figs. 4a–d the curve “Q” suited well to describe the first “rapid” decline and the curve “R” the “slower” decline that followed thereafter. The mathematical functions for the regression lines are given in Table 6.

The geometric means and the predictives with the experiential model were then compared with the formula given by Bentzon [25]. One component of the formula was the maximal concentration of antitoxin that was reached within 2–4 weeks after the injection. Since we lacked early post-vaccination sera for most of the children in our material we had to restrict our comparison to a subgroup of children. We chose the DT group of 74 children who all had a 1 month post-vaccination serum and four later samples within 3.5 years after the third dose. Our results for the subset of 74 children in the DT group are given in Fig. 5. All 370 sera were used for the estimation by the Bentzon model and the values of 1-month post-vaccination sera were defined as maximum. In this figure our exponential formulas for the DT group (Table 6) have also been used. As can be seen the lowest portion of the rapid decline in the observed geometric means that was seen in Fig. 4d for all 1.393 sera in the DT group, was not observed here. This is due to the fact that we lacked data in the interval between 7 and 23 months for the subgroup with 74 children. As a consequence, the Bentzon model overestimated the predicted means in the interval 8–22 months post dose 3 compared with the geometric means actually observed and also to the predictions with the exponential model. However, in the other intervals there was a good agreement with the observed geometric means and the Bentzon model.

### Table 5

IgG diphtheria antitoxin post-vaccination concentrations for each vaccine group in Trial I, percentiles, at 1, 7 and 23 months (in mean) after dose 3

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Subjects</th>
<th>Month</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>90</th>
<th>GM EU/mL</th>
<th>95% c.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTPa2</td>
<td>180</td>
<td>1</td>
<td>0.247</td>
<td>0.390</td>
<td>0.645</td>
<td>1.086</td>
<td>1.517</td>
<td>0.64</td>
<td>0.58–0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>0.044</td>
<td>0.082</td>
<td>0.140</td>
<td>0.241</td>
<td>0.366</td>
<td>0.14</td>
<td>0.12–0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>&lt;0.02</td>
<td>0.027</td>
<td>0.052</td>
<td>0.087</td>
<td>0.113</td>
<td>0.041</td>
<td>0.035–0.049</td>
</tr>
<tr>
<td>DTPa5</td>
<td>180</td>
<td>1</td>
<td>0.260</td>
<td>0.545</td>
<td>0.893</td>
<td>1.450</td>
<td>2.556</td>
<td>0.85</td>
<td>0.74–0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>0.065</td>
<td>0.117</td>
<td>0.196</td>
<td>0.325</td>
<td>0.645</td>
<td>0.19</td>
<td>0.17–0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>&lt;0.02</td>
<td>0.030</td>
<td>0.056</td>
<td>0.119</td>
<td>0.208</td>
<td>0.055</td>
<td>0.046–0.065</td>
</tr>
<tr>
<td>DTPw-C</td>
<td>134</td>
<td>1</td>
<td>0.211</td>
<td>0.331</td>
<td>0.623</td>
<td>1.280</td>
<td>1.910</td>
<td>0.61</td>
<td>0.51–0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>0.038</td>
<td>0.071</td>
<td>0.144</td>
<td>0.260</td>
<td>0.453</td>
<td>0.13</td>
<td>0.11–0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>&lt;0.02</td>
<td>0.038</td>
<td>0.065</td>
<td>0.124</td>
<td>0.213</td>
<td>0.058</td>
<td>0.047–0.070</td>
</tr>
<tr>
<td>DT</td>
<td>157b</td>
<td>1</td>
<td>0.488</td>
<td>0.677</td>
<td>1.200</td>
<td>1.594</td>
<td>2.310</td>
<td>1.05</td>
<td>0.95–1.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>0.122</td>
<td>0.298</td>
<td>0.444</td>
<td>0.698</td>
<td>0.29</td>
<td>0.25–0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>23</td>
<td>0.021</td>
<td>0.040</td>
<td>0.072</td>
<td>0.116</td>
<td>0.225</td>
<td>0.066</td>
<td>0.056–0.079</td>
</tr>
</tbody>
</table>

a For study children with three trial doses and serum samples taken at the three occasions.
b Including the 74 children bled at five occasions presented in Fig. 2.

### Table 6

Mathematical functions fitting data in Fig. 4a–d, where \( D \) = Duration in days for each interval since dose 3 in Trial I, and predicted duration until \( \approx 50\% \) of the children have less than 0.02 EU/mL

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Interval since Dose 3</th>
<th>Regression model</th>
<th>Predicted duration in years</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTPa2</td>
<td>0– &lt; 180 days</td>
<td>( Y = 0.8583 \exp(-0.0102^D) )</td>
<td>2.9</td>
</tr>
<tr>
<td>DTPa2</td>
<td>180–960 days</td>
<td>( Y = 0.1855 \exp(-0.0021^D) )</td>
<td>3.5</td>
</tr>
<tr>
<td>DTPa5</td>
<td>0– &lt; 240 days</td>
<td>( Y = 1.0829 \exp(-0.0088^D) )</td>
<td>4.2</td>
</tr>
<tr>
<td>DTPa5</td>
<td>240–960 days</td>
<td>( Y = 0.1994 \exp(-0.0018^D) )</td>
<td>6.2</td>
</tr>
<tr>
<td>DTPw-C</td>
<td>0– &lt; 180 days</td>
<td>( Y = 0.8491 \exp(-0.0014^D) )</td>
<td>2.9</td>
</tr>
<tr>
<td>DTPw-C</td>
<td>180–960 days</td>
<td>( Y = 0.1680 \exp(-0.0014^D) )</td>
<td>3.5</td>
</tr>
<tr>
<td>DT</td>
<td>0– &lt; 300 days</td>
<td>( Y = 1.3322 \exp(-0.0080^D) )</td>
<td>4.2</td>
</tr>
<tr>
<td>DT</td>
<td>300–1.350 days</td>
<td>( Y = 0.1224 \exp(-0.0008^D) )</td>
<td>6.2</td>
</tr>
</tbody>
</table>
3.6. Antitoxin concentrations after booster vaccination with a DTPa5 vaccine

The GM of the pre-booster diphtheria antitoxin at 50 months after dose 3 was 0.031 (0.026–0.038) EU/mL suggesting a continuous slow decline from 23 months when the GM was 0.055 EU/mL (Table 5). In this material 43 of 60 children (72%) had a pre-booster level higher than 0.02 EU/mL. The GM 7–11 days after the booster was 0.65 (0.32–0.92) EU/mL.

One child had a level below 0.02 EU/mL 7 days after the booster dose, and five children (8.3%) all sampled at 7 days had not reached 0.1 EU/mL at the time of the post-booster sample.

4. Discussion

Relatively few published studies about the duration of diphtheria immunity after vaccination have dealt with longitudinal serological data [1,2,6,11,25]. In this paper we have used material from two pertussis vaccine trials to describe the decline of antitoxin levels over time. One cohort of children vaccinated at 2, 4 and 6 months of age was followed up to the age of 5 years.

A direct comparison of antitoxin concentrations between laboratories may be misleading. Therefore the analyses have focused trends rather than absolute values. In our studies the highest post-vaccination concentrations were found for DTPw-E (32 Lf/mL) and the DT (15 Lf/mL)-vaccines whereas the DTPa vaccines (25 Lf, 25 Lf and 15 Lf/mL) gave lower levels. However, the DTPw-C (6.65 Lf) gave similar results as

![Figure 4. IgG anti-diphtheria antibody decline after dose 3 in a 2, 4, and 6 months schedule in Trial I. Observed geometric mean (GM) concentrations EU/mL (—). Predicted GM concentrations are given based on data until 6–10 months after dose 3 (Q– – –), and later than 6–10 months after dose 3 (R), for (a) the two-component acellular DTPa2, 1049 samples, (b) the DTPw-C, 748 samples, (c) the five-component acellular DTPa5, 1098 samples, and (d) the DT, 1393 samples. The different cut-off points at 6–10 months after Dose 3 for each vaccine are indicated by a vertical line in the four graphs.](image-url)
the DTPa-vaccines. All children in the DT group and 95–99% in the DTP vaccine arms had antitoxin levels taken to indicate protection (≥0.1 EU/mL) 1 month after vaccination. In the DTPw-C group the concentration was less than 0.02 EU/mL for two children.

Our data are in alignment with previous publications [26] that a more widely spaced immunisation schedule was significantly more immunogenic than an accelerated one, at least in the short term. Although the levels were lower at 7 months in the 3, 5, and 12 month schedule than in the 2, 4 and 6 schedule over 90% of the children attained 0.1 EU/mL after two doses with DTPw-E, DTPa5 and DTPa3. Thus the protection was adequate after the initial two doses during the extended schedule. Similar differences between schedules were reported when the UK schedule was changed from a 3, 5 and 9 month schedule to one with injections at 2, 3 and 4 months of age [26].

As reported by other investigators the presence of maternal antibodies influences the antibody response [26]. Children with high levels, defined as 0.05 EU/mL, in the pre-vaccination sample had significantly lower 1-month post-vaccination concentrations than those with lower levels for most of the vaccines. There was also an inhibitory effect on the response to immunisation in the interval 0.02–0.05 EU/mL as compared to levels below 0.02. The 25 Lf dose in DTPa2, however, seemed to overcome the effect of maternal antibodies.

The proportion of children with concentrations below 0.1 EU/mL increased from 4 to 35% at 7 months after dose 3 to 65–85% at 23 months (Fig. 3). After that the proportion seemed to increase a further 10% during the following year. After 2–3 years the differences between vaccines were reduced. Simonsen et al. [27] reported a continuous fall-off of antitoxin concentrations after revaccination, a 12.2-fold decrease in the geometric mean over 8 years. Other studies report a 20–30% annual decrease [1,28].

As shown earlier [24,25,27] the decline of antibodies by time could be divided in two phases. The initial rapid response may reflect a simultaneously activated immune system and a synchronised half-life of immunoglobulin. The second slower phase may represent a less active and less synchronised production of immunoglobulin by memory cells. The shift occurred between 6 and 10 months after the third dose depending upon the initial response given by the vaccine. A strong initial response is linked to a later shift.

A mathematical regression model that was developed gave good agreement between two exponential functions and the observed geometric means at different intervals after the third dose. This model was compared with a historical model first presented by Jensen [24] and later modified by Bentzon [25]. They had both early post-vaccination samples representing the maximum of the curve and later samples available from all children. For most of the children in our trial we did not take an early post-vaccination sample and therefore we had to restrict the comparison to a subset of 74 children in the DT-group for whom we had both an early post-vaccination sample and four later scheduled samples.

The overestimation in the interval between 8 and 22 months after dose 3, when the Bentzon model was used, is due to the fact that we lack the data in that interval for the 74 children. In the other intervals the agreement between the two mathematical models was good. Finally we did note that the geometric mean was close to the median of the distribution. Predicted from the exponential models this means, to take an example, that 3.5 years after the third DTPa5 dose in a 2, 4 and 6 months schedule at about 50% of the children are predicted to have more than 0.02 EU/mL. Compared to the pre-booster levels in the booster vaccination with a DTPa5 vaccine at about 4–5 years after dose 3 the mathematical model underestimates the rate of children with levels higher than 0.02 EU/mL. Nevertheless this means that a majority of Swedish school children 7–10 years of age may lack internationally recommended antitoxin concentrations. On the other hand all children in our booster study had a good antibody response after an additional DTPa5 dose at 5–5.5 years of age indicating an adequate immune memory for the antigen. This is in accordance with other investigations [29,30] and the predicted results agree with the observed results of the ESEN-study with DT-vaccine.

A general problem with serology, clearly demon-
strated by external quality assessment programs, is the lack of comparability between laboratories. Values of measurement from the same panel may differ substantially also when the concentrations are expressed as IU/mL calculated against an international calibrator. Different assay methods may measure different antibodies.

The toxoid preparations used in the present investigation were of different purity, i.e. the minimum requirement being of 50% purity (1500 Lf/mg protein nitrogen), thus giving rise also to antibodies to the contaminating proteins upon vaccination. The ELISA system is known to detect also low levels of non-neutralising IgG [31,32], particularly in the interval 0.02–0.1 IU/mL [11,33–36]. Our assay was therefore validated against the intradermal rabbit neutralisation test [23] and the vero-cell assay [5,37]. Active toxin, 90% purified, instead of toxoid was used as coating antigen to minimise the difference to the functional assays. If there were at least three points on the ELISA curve [22] the correlation with the in vivo test was acceptable above 0.02 IU/mL. The inhibitory effect of maternal antibodies above 0.02 EU/mL supports the use of 0.02 EU/mL as the lower limit of detectable seroimmunity for our ELISA assay. If the WHO-criteria for immunity were strictly used our data may give a possible overestimate. This deviation rather strengthens the potential impact on vaccination policy.

The similarity between ELISA-results for the DTPa2 analysed over a 2–3 year period in two different Swedish trials indicated good accuracy of the assay over time, Table 1 and Fig. 1a. The good agreement between our results and those by Jensen and Scheibel who used an in vivo neutralising antibody assay also supports that our ELISA-technique is equally reliable for surveillance programs.

In conclusion our studies on diphtheria antitoxin persistence based on ELISA arbitrary units after primary vaccination at 2, 4 and 6 months suggested that the duration of serorelated immunity was around or even less than 5 years. Applying the data to the 3, 5 and 12 months schedule may suggest that also that schedule would not provide recommended seroimmunity in all children until a fourth dose is given. In Sweden the fourth dose is currently given at 10 years of age. The levels were lower when the toxoids were combined with an acellular pertussis vaccine than after vaccination with DT or a potent whole cell vaccine. If antitoxin concentrations were used as the only marker for immunity the data support the European recommendation of a booster dose at school entry [16,38]. This conclusion is also supported by available epidemiological information from Russia indicating that at 5–7 years of age only duration of time since last dose of diphtheria vaccine was significantly associated with disease [18].

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References


