How Many Doses of Diphtheria Toxoid Are Required for Protection in Adults? Results of a Case-Control Study among 40- to 49-Year-Old Adults in the Russian Federation

Muireann Brennan,1 Charles Vitek,1 Peter Strebel,1 Wendy Wattigney,1 Kris Bisgard,1 Sergei Brisgalov,2 Vera Bragina,3 Valery Pyanikh,4 and Melinda Wharton1

1National Immunization Program, Centers for Disease Control and Prevention, Atlanta, Georgia; Sanitary-Epidemiologic Surveillance Division, Ministry of Health, 2Vladimir Oblast, 3Novgorod City, 4Novgorod Oblast, Russian Federation

During the Russian diphtheria epidemic of the 1990s, adults had an unexpectedly high rate of disease. A retrospective, matched case-control study was done to measure the effectiveness of one, two, or three or more doses of diphtheria toxoid against diphtheria among 40- to 49-year-old Russians. Thirty-nine diphtheria case-patients and 117 controls were studied. Previous vaccinations were included if one dose was received within the previous 10 years. Five cases (13%) and 33 controls (28%) had received three or more doses of vaccine. The matched odds ratio was 0.3 (95% confidence interval, 0.1–0.9) for three or more doses compared with no doses, which was a vaccine effectiveness of 70% (95% confidence interval, 10–90). A trend existed toward milder disease with increasing doses (χ² test for trend, P = .02). The results suggest that Russian adults, who were unlikely to have acquired immunity to diphtheria through immunization or natural infection, required at least three doses of diphtheria toxoid for reliable protection against disease.

In 1990, the largest diphtheria epidemic in more than three decades began in the Russian Federation and soon spread to the rest of the former Soviet Union [1]. By December 1997, >114,000 cases and almost 3000 deaths had been reported in the Russian Federation [2]. In contrast to diphtheria epidemics in the prevaccine era, in which disease among young children predominated, in this epidemic, most cases and deaths occurred among adults. In 1993, 68% of cases and 78% of deaths in the Russian Federation were among those ≥14 years old [3]. Adults who were 40–49 years had the highest mortality rate (1.3/100,000 population), and 55% of all fatalities occurred among adults in this age group.

During the epidemic, the Ministry of Health of the Russian Federation (MOH) modified the recommendations for the vaccination of adults several times. Until 1993, adult vaccinations had been limited to groups traditionally considered by public health authorities to be at increased risk. In November 1993, one booster dose was recommended for all adults who had no documentation of a dose within the previous 10 years. By 1995, three doses were recommended for all adults without documentation of receipt of a primary series. Implementing the recommendation for three doses proved difficult, especially as the incidence of diphtheria began to decline. Overall, the proportion of adults who received at least one dose within the previous 10 years increased from 20% in 1993 to ~75% in December 1995 [4].

Serologic studies of diphtheria antitoxin levels carried out in the Russian Federation and Ukraine showed differences in susceptibility to diphtheria among different age cohorts of adults that corresponded to the observed differences in the incidence of disease and in mortality rates [5, 6]. These age-specific differences in susceptibility are most likely due to differences in the proportion of adults in each age group who were immune either through receipt of a three-dose primary series of diphtheria toxoid vaccine in childhood or through exposure to natural infection [7].

Although serologic data were used to formulate the vaccination recommendations, no published data demonstrating the effectiveness of adult vaccination against disease were available. In 1997, the MOH and the Centers for Disease Control and Prevention conducted a study to determine the effectiveness of various numbers of doses of diphtheria toxoid against clinical disease.

Methods

Study population. Novgorod is located in northwestern European Russia and has a population of ~250,000. Kovrov is located in central European Russia and has a population of ~100,000. The study was conducted in these cities because of previously successful collaborative diphtheria projects. In addition, a pilot study showed that adult immunization records were well maintained in Novgorod and that nearly all adults were registered in polyclinics [8].

Immunization records and population registration. In the two...
study sites, the only sources of primary medical care for adults were state-run adult polyclinics and medical posts at work sites. The catchment population of each polyclinic was subdivided geographically. Each area was served by a physician who maintained census logbooks, which registered households by their location. Diphtheria vaccinations administered to adults were also recorded in the logbooks. A system of individual adult immunization cards was instituted in 1995. These cards recorded age, place of residence, and place of work. For all cases and controls, the logbooks, immunization cards, and work-place records (even if the work place was closed) were sought for information on date of birth and dates of all known doses of diphtheria toxoid vaccine. In addition, we reviewed case investigation reports, which included vaccination status, that were compiled by local epidemiologists. Diphtheria vaccinations received by cases and controls were included in the analysis if given >14 days prior to onset of illness in the case-patient. No information on prior history of diphtheria was obtained for either cases or controls.

Cases. All confirmed diphtheria cases among 40- to 49-year-old adults that were reported between December 1995 and December 1996 in the cities of Kovrov and Novgorod were eligible for inclusion in the study. In the Russian Federation, diphtheria reporting is thought to be very complete [9]. Epidemiologists investigated all cases of possible diphtheria and compiled individual case reports. Patients with pharyngitis routinely had samples cultured for Corynebacterium diphtheriae. Those from whom toxigenic C. diphtheriae were isolated were considered confirmed cases whether a pharyngeal pseudomembrane was observed or not. A staging system based on the extent of the pseudomembrane and neck edema was used to determine disease severity [10]. In our analysis, cases without associated neck edema were classified as mild, and cases associated with neck edema or other toxic manifestations, such as cardiac arrhythmia or neuropathy, were classified as severe.

Controls. Each case was matched with 3 controls who were within 2 years of age and from the same geographic division as the case. The area physician’s logbook was used to select controls. A case entry was located in the logbook, and then study personnel sequentially counted 10 records down from the case. If the tenth entry was a 40- to 49-year-old non-case, this person was selected as a control. If not, the first subsequent non-case in this age group was selected as a control. Study personnel repeated the process, beginning with the previously chosen control, until 3 controls were identified. The interval of 10 was used to avoid selection of multiple controls from the same household.

Although we did not plan to match cases and controls by sex, they were matched by sex at one study site due to a misunderstanding. For this reason we were unable to evaluate sex as a risk factor for diphtheria. Vaccination status. The records of immunizations received during childhood were not available for most adults, and only vaccinations given in adulthood were used for analysis. All previous doses of diphtheria toxoid received during adulthood were counted if at least one dose had been received within the previous 10 years. Vaccination doses were classified as zero for persons who had not received a dose within the preceding 10 years and as one, two, or three or more doses, according to the total number of doses received during adulthood up to 14 days prior to the onset of diphtheria in the case.

We defined an adult as unvaccinated if they had not received a dose of diphtheria toxoid within the previous 10 years, because serum antitoxin levels, which are required for protection against toxigenic diphtheria, decline steeply after vaccination [11] and the World Health Organization recommends that adults receive a dose of diphtheria toxoid every 10 years. In addition, 2 cases and 7 controls were classified as having zero doses because no vaccination records were available and information on vaccination was missing from the case investigation forms. For controls, the reference date for disease onset was the date of onset of disease in the matched case. The time since receipt of the last dose of vaccine was categorized into two intervals: 0–4 years (the reference group) and ≥5 years.

Data analysis. The ages of cases and controls were compared by use of a t test. Conditional logistic regression was used to estimate matched odds ratios (ORs) for one, two, and three or more doses of vaccine compared with zero doses. Three design variables were created for vaccination status, using reference cell coding [12]. Restricting analysis to case-control sets for which the case was vaccinated, we used conditional logistic regression to estimate the risk of disease associated with the length of time between disease onset in the case and the most recent dose of diphtheria vaccine received within the preceding 10 years. A possible association between disease severity and vaccination status (zero vs. one or two doses vs. three or more doses) was determined using a χ² test for trend (Epi Info, version 6; CDC, Atlanta). We used SAS software (SAS Institute, Cary, NC) to analyze other data. Assuming the OR approximated the relative risk, we calculated the estimated vaccine effectiveness (VE) as VE = 1 – OR.

Results

Case investigation records and vaccination records were obtained for 39 (95%) of 41 eligible cases. These 39 cases and their 117 matched controls were enrolled in the study. Women predominated among cases (69%) (table 1). Compared with controls, cases had received fewer doses of diphtheria toxoid:

Table 1. Selected characteristics of 40- to 49-year-old diphtheria case-patients and their matched controls, Kovrov and Novgorod City, Russian Federation, December 1995 to November 1996.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 39)</th>
<th>Controls (n = 117)</th>
<th>P</th>
<th>Matched odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>44.1 ± 3.0</td>
<td>44.3 ± 3.1</td>
<td>0.80</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Male</td>
<td>12 (31)</td>
<td>35 (30)</td>
<td>.94</td>
<td>1.2</td>
<td>0.5-2.8</td>
</tr>
<tr>
<td>Female</td>
<td>27 (69)</td>
<td>82 (70)</td>
<td></td>
<td>0.7</td>
<td>0.2-2.7</td>
</tr>
<tr>
<td>No. of doses</td>
<td></td>
<td></td>
<td></td>
<td>0.3b</td>
<td>0.1-0.9</td>
</tr>
<tr>
<td>0</td>
<td>17 (44)</td>
<td>42 (36)</td>
<td></td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>1</td>
<td>13 (33)</td>
<td>29 (25)</td>
<td></td>
<td>1.2</td>
<td>0.5-2.8</td>
</tr>
<tr>
<td>2</td>
<td>10 (26)</td>
<td>13 (11)</td>
<td></td>
<td>0.7</td>
<td>0.2-2.7</td>
</tr>
<tr>
<td>≥3</td>
<td>5 (13)</td>
<td>33 (28)</td>
<td></td>
<td>0.3b</td>
<td>0.1-0.9</td>
</tr>
<tr>
<td>Time since last dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4 years</td>
<td>19 (76)</td>
<td>50 (96)</td>
<td></td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>≥5 years</td>
<td>6 (24)</td>
<td>2 (4)</td>
<td></td>
<td>12.7</td>
<td>1.5-106.6</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) unless otherwise specified. CI, confidence interval.

a With at least 1 dose received within previous 10 years.
b Vaccine effectiveness ≥70%; 95% CI, 10–90.
c Case-control sets where case vaccination status was 0 removed, leaving 25 and 52 controls for analysis.
17 cases (44%) had received zero doses, compared with 42 controls (36%; table 1). However, only 5 cases (13%) had received three or more doses, compared with 33 controls (28%). The matched OR was 0.3 for three or more doses, compared with zero doses (95% confidence interval [CI], 0.1–0.9), giving an estimated VE of 70% (95% CI, 10–90). Neither one nor two doses were significantly protective against disease.

Among 25 cases and their 52 matched controls who had received at least one dose within the last 10 years, cases were more likely than controls to have received their last dose ≥5 years prior to disease onset. Six cases (24%) and 2 controls (4%) received their last dose ≥5 years previous (matched OR, 12.7; 95% CI, 1.1–107.0). Because of the small sample size, it was not possible to examine the relationship between the time since the last dose and the number of doses received. Among 39 cases, 28 (72%) were classified as having mild disease and 11 (28%) as having severe disease (table 2). Of 17 cases with zero doses, 9 (53%) had mild disease and 8 (47%) had severe disease; there was a significant trend toward milder disease with increasing number of doses (χ² test for trend for zero doses vs. one or two doses and three or more doses, P = .02). No severe case had received three or more doses of diphtheria toxoid.

### Discussion

The diphtheria outbreak in the Newly Independent States of the former Soviet Union raised major questions on vaccination policy. One challenging issue was the number of doses required for protection of adults, particularly for adults who had been children when the circulation of natural infection had declined and when immunization coverage rates were still low [1]. Childhood vaccination coverage against diphtheria rapidly increased in the former Soviet Union in the second half of the 1950s, followed by a dramatic decline in the incidence of disease. Adults who were 40–49 years old in 1995 were born during 1946–1955 and grew up during a period in the Russian Federation when routine childhood immunization had begun but coverage was not complete and circulation of C. diphtheria was diminishing. In 1995, these adults were the least likely of any age group to be immunologically protected by either natural infection or vaccination.

After three doses of diphtheria toxoid vaccine given in infancy, 94%–100% of children have diphtheria antitoxin antibody levels ≥0.1 IU/mL and are considered immune [13, 14]. Although vaccine-induced immunity wanes with time, revaccination with a single dose of toxoid can restore protective antibody levels. Studies suggest that adults who were not immunized in childhood require three doses for reliable protection; after two doses of vaccine, many of these adults do not produce protective levels of circulating antitoxin but virtually all will produce protective levels after three doses [15–17].

Previous studies have shown Russian-produced diphtheria toxoid vaccine to be immunogenic and effective in children and immunogenic in adults. In the 1980s, Russian studies demonstrated that >90% of children who had received four doses of diphtheria toxoid in the first 2 years of life had levels of antibody consistent with protection against disease [18, 19]. Studies in the 1990s among children confirmed the effectiveness of vaccination with Russian-produced vaccine against diphtheria [20]. A study done in Ukraine using Russian-produced diphtheria toxoid found that 89% of 18- to 67-year-old adults with unknown primary immunization history had antibody levels >0.1 IU/mL 1 month after revaccination with a single dose [6].

Our study supports the results of previous studies regarding the need for three doses among unprimed adults and extends findings that demonstrate the clinical effectiveness of Russian-produced vaccine. Although childhood vaccination histories were unavailable, our study subjects were 40–49 years of age, the age group in Russia with the highest likelihood of being immunologically naive to diphtheria. Thus, our study supports the public health effectiveness of the recommendation by the Russian MOH for three doses of diphtheria vaccine for adults in this age group with an unknown childhood vaccination history.

Our study did not show any significant protective effect of one or two doses. This is somewhat surprising as, even in this high-risk age group, at least some subjects were likely to have been vaccinated in childhood or to have acquired immunity from previous infection. In the immunogenicity studies conducted in Ukraine, some adults in the 40- to 49-year-old age group did respond to one dose of diphtheria toxoid with a rise in serum antibody levels to protective levels. Our inability to show a protective effect against clinical diphtheria from one or two doses may be due to a higher proportion of unprimed adults in our study population, a rapid decline in antibody after one or two doses, or, most likely, to the small number of cases. Our study did suggest, however, that one or two doses protected against severe disease.

Our study also found that a period of ≥5 years since receipt of the last dose of diphtheria toxoid was significantly associated with increased risk of disease. Previous immunogenicity studies [15] have suggested that the lower potency toxoid used for the primary series of vaccination in adults may result in a shorter duration of protection. However, our sample size did not allow

<table>
<thead>
<tr>
<th>No. of doses</th>
<th>Mildb (n = 28)</th>
<th>Severe (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9 (53)</td>
<td>8 (47)</td>
</tr>
<tr>
<td>1</td>
<td>11 (86)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>2</td>
<td>3 (75)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>≥3</td>
<td>5 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Note:** Data are no. (%)  

a With at least 1 dose received within previous 10 years.  

b Mild vs. severe disease and dose (0, 1 or 2, 3), P = .02, χ² test for trend.
us to distinguish between time since last dose and number of doses because both variables are highly correlated.

Our conclusion that adults in high-risk age groups should receive at least three doses of diphtheria toxoid for protection against disease may be particularly relevant in developing nations in the future. In the past, most children in these countries acquired immunity through exposure to cutaneous C. diphtheriae or, less commonly, through exposure to respiratory disease. The improvements in vaccination coverage and rising socioeconomic levels in many developing countries in the past 2 decades appear to be reducing the circulation of C. diphtheriae among some populations and leading to an accumulation of immunologically unprimed individuals, and many persons in these age groups are likely to lack accurate records of childhood vaccination. Outbreak-control guidelines should recommend three doses of diphtheria toxoid for these groups of adults. In addition, routine adult immunization guidelines should include three doses for the primary immunization of adults who are likely to be immunologically naive and booster doses at least every 10 years for those known to be previously immunized. Maintenance of high levels of immunity throughout the adult population will prevent similar outbreaks of diphtheria in the future.

References