Prevalence of diphtheria toxoid IgG antibodies in children, adolescents and adults in Poland

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Abstract

Background

The recommendation for diphtheria immunization is to apply an effective primary immunization in infancy and to maintain immunity throughout life. Immunity against diphtheria depends primarily on antibody against the diphtheria toxin. The present investigation evaluated the prevalence of IgG diphtheria antitoxin in sera of healthy children, adolescents and adults in Poland.

Methods

A total of 1,387 serum samples collected between 2010 and 2012 from individuals with ages ranging from 1 month to 85 years were investigated. Antibody concentrations were measured with an enzyme-linked immunosorbent assay (Anti-Diphtheria Toxoid ELISA IgG, Euroimmun, Germany).

Results

The results showed that among 1,387 individuals examined, 547 (39.4%) had the level of antidiaphtheria toxoid IgG antibodies below 0.1 IU/ml (36.9% younger than 18 years and 40.5% aged above 18 years old, respectively). The 212 (50.8%) children and 542 (55.9%) adults showed only basic protection (0.1-1.0 IU/mL) and need immediate booster. The high level of anti-diphtheria toxoid IgG antibodies (>1.0 IU/mL) was statistically significant more often diagnosed in children and adolescent (12.2%) than in adults (3.6%) (P<0.05). The proportion of seronegatives (<0.1 IU/mL) in children below 2 years old, adolescents and young adults to 25 years old appears to decreased from 53.5% to 17.4%. However, in older individuals the seronegative proportion tended to increase with age, from 22.7% in adults from the age group 26-30 years old to 67.1% in subjects aged above 60 years old. Characteristically, in individuals aged above 40 years old high levels of anti-diphtheria toxoid IgG antibodies (>1.0 IU/mL) were not seen. We not found statistically significant differences of results in relation to gender.

Conclusions

The presented study showed the inadequate immunity level to diphtheria among Polish population, especially in adults >40 years old and children ≤2 years old. To prevent reemergence of diphtheria information campaign reminding about recommendation
concerning diphtheria booster vaccination in adults should be conducted. Moreover, immunogenicity of the DTP vaccine used in Poland should be verified.

Keywords: diphtheria, IgG antibodies, diphtheria toxin, vaccination
**Background**

Diphtheria is a severe and potentially fatal disease caused by toxin-producing strains of *Corynebacterium diphtheriae*, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*. Before the introduction of active vaccination in 1940’s, diphtheria was endemic in most European countries [1]. Currently, the disease seems to be well controlled in developed countries but it is still endemic in Africa, Asia and Eastern Europe [2]. Humoral immunity against diphtheria depends primarily on formation of specific IgG antibodies against diphtheria toxin, which may be induced by natural infection or passive or active immunization. As diphtheria has become rare, opportunities for acquisition or reinforcing natural immunity have also been reduced [3, 4]. In most European countries diphtheria vaccine is placed in obligatory vaccination schedule. In Poland diphtheria vaccination schedule comprises 7 doses administer in age of 2 months, 3-4 months, 5-6 months, 16-18 months and then 6, 14 and 19 years old. According to World Health Organization data, more than 95% of children are fully vaccinated against the disease in Poland. However, the level of antibodies decreases with time and adults might again become susceptible to diphtheria due to reduced opportunities to boost immunity through subclinical infections. A large pool of susceptible persons creates an epidemic potential what was shown by the last diphtheria epidemic that occurred in the early 1990’s in the countries of the former Soviet Union when over 50 000 cases were recorded at the peak of the epidemic. During this epidemic adolescents and adults were mainly affected, most of whom would have been previously vaccinated [4, 5, 6, 7]. Moreover, during the last decade diphtheria cases due to *C. ulcerans* has increased in Europe. For example, 63% of toxigenic corynebacteria isolated in France in 2002-2008 and in United Kingdom in 2000-2009 belonged to *C. ulcerans* species. The reservoir hosts are domestic cats and dogs [8, 9].

In Poland the last diphtheria case was recorded in 2000 year and the previous cases (9 cases) were recorded in 1996 [10]. In the presented studies we evaluated the immunity status against diphtheria in different age groups of Polish population after the period of over 10 years with no diphtheria incidence in Poland.

**Methods**

**Study population**
A total of 1,387 serum samples were collected to examine the specific anti-diphtheria toxin antibodies. Written informed consent for the use of serum samples for the studies were obtained from participants or, where participants are children, a parent or guardian. The serum bank comprised samples collected between 2010 and 2012, from individuals living in different regions of Poland with ages ranging from 1 month to 85 years (median age was 26 years). Samples from the group aged 0-18 (n=417) were residual sera from diagnostic laboratories, while samples from the adult population (n=970) were residual sera from diagnostic laboratories (n=260) and additionally from routine screening tests of healthy blood donors (n=390), forest workers (n=122) and pregnant women (n=198). Diphtheria vaccination history of the tested individuals was not available. Data on gender were available from 1047 individuals (544 females and 503 males). Precise data on age were not obtained from forest workers and most of blood donors.

**Determination of diphtheria toxoid antibody levels**

Diphtheria toxoid IgG-specific antibody levels were determined using a commercial ELISA Anti-Diphtheria Toxoid ELISA IgG (Euroimmun, Germany) selected in previous studies as the most reliable among tested anti-diphtheria IgG tests [11]. For quantitative evaluation the 4 ready-to-use calibrators - Calibrator 1 (2 IU/mL), Calibrator 2 (1 IU/mL), Calibrator 3 (0.1 IU/mL), Calibrator 4 (0.01 IU/mL) and two control sera (one positive and one negative) were supplied. The concentrations of the of anti-diphtheria toxin antibodies in tested serum samples was read from the standard curve. For the calculation of the standard curve the obtained OD (optical density) of the standards (y-axis, linear) were plotted against their concentration (x-axis, logarithmic) on Excel calculation sheet. The initial dilution of tested sera was 1:101. Samples which showed concentrations above the highest standard were higher diluted. Results of samples of higher predilution were multiplied with the dilution factor. Manufacturer recommended division of the results into five groups: <0.1 IU/mL (indicating immediate basic immunisation), 0.1-1.0 IU/mL (immediate booster), >1.0-1.5 IU/mL (booster after 5 years), >1.5-2.0 IU/mL (booster after 7 years) and >2.0 IU/mL (booster after 10 years).

**Statistical analysis**

The study population was divided into ten age groups: 0-2, 3-5 years, 6-13 years, 14-18 years, 19-25 years, 26-30 years, 31-40 years, 41-50 years, 51-60 years and >60 years. The arithmetic mean titres, standard deviations and geometric mean titres were calculated using Excel
calculation sheet. The statistical significance of the differences was analyzed by Fisher’s exact probability test with Yates’ correction when at least one of the calculated figures was <5. A $P$-value <0.05 was considered significant.

The studies were approved by Bioethics Committee of National Institute of Public Health – National Institute of Hygiene (reference number 2/2013).

**Results**

The distribution of antibodies, arithmetic and geometric mean titres and other statistical parameters in children and adults are presented in Table 1. Among 1,387 individuals examined, 547 (39.4%) had the level of antidiphtheria toxoid IgG antibodies below 0.1 IU/ml (36.9% younger than 18 years and 40.5% aged above 18 years old, respectively). The 212 (50.8%) children and 542 (55.9%) adults showed only basic protection (0.1-1.0 IU/mL) and need immediate booster. In general, the difference in number of seronegatives as well as low positives (0.1-1.0 IU/mL) between individuals aged below 18 years old and adults was not statistically significant (P>0.05). However, the high level of anti-diphtheria toxoid IgG antibodies (>1.0 IU/mL) was statistically significant more often diagnosed in children and adolescent (12.2%) than in adults (3.6%) (P<0.05). The geometric mean titre (GMT) was low both in children (0.141 IU/mL) and in adults (0.102 IU/mL). There were no important differences in diphtheria antibody levels between males and females (P>0.05).

Data presented in the Table 2 show in more detail the distribution of antidiphtheria toxoid IgG titres in healthy individuals of different age groups. The proportion of seronegatives (<0.1 IU/mL) in children, adolescents and young adults to 25 years old appears to decreased from 53.5% to 17.4%. However, in older individuals the seronegative proportion tended to increase with age, from 22.7% in adults from the age group 26-30 years to 67.1% in subjects aged above 60 years old. Characteristically, in individuals aged above 40 years old high levels of anti-diphteria toxoid IgG antibodies (>1.0 IU/mL) were not seen.

A similar picture is seen in Figure 1 and Figure 2, which show the geometric mean concentration of antidiphtheria toxoid IgG antibodies and percentage of subjects with antidiphtheria toxoid antibody levels $\geq$0.1 IU/mL according to age groups, respectively. The effects of the each booster dose are clearly visible, causing the maximum protection level in individuals from group age 19-25 years (GMT=0.274 IU/mL). In older age groups we can see the decrease of GMT values as well as decrease of the percentage of subjects with protection.
level of antidiphtheria toxoid IgG antibodies. The most characteristic is dramatic decrease of protection in individuals aged above 40 years old.

**Discussion**

Although the diphtheria incidence has occurred sporadically in countries of Western Europe, the risk of importation of the disease from endemic regions is increasing together with increase of development of mass tourism to diphtheria endemic countries. Moreover, domestic animals, such as cats and dogs, were identified as a new source of human infections of diphtheria toxin producing *C. ulcerans* [1, 8]. For these reasons a protective level of diphtheria antitoxin antibodies should be maintained in populations.

In this study the overall proportion of susceptible persons was 39.4%. It is alarming as such high percentage of persons without a protective level of anti-diphtheria antibodies creates an epidemic potential. WHO outlined that to achieve sufficient herd immunity a minimum immunity rate of 90% in children and 75% in adults is required [12]. Although >95% children in Poland have received routine primary vaccination with three doses of DTP (the diphtheria-tetanus-pertussis vaccine) and one booster within the first 2 years of life only 46.5% of them revealed antidiphtheria antibodies level ≥0.1 IU/mL. The antibodies level had been increasing with each booster doses and achieved the highest level in young adults (age 19-25 years old) after the last booster administrated in age of 19 years old. It is worth to underline that overall proportion of persons with the high level of anti-diphtheria toxoid IgG antibodies (>1.0 IU/mL), that gives a long-term protection, was only 6,1%. The results differ significantly from the results obtained in the previous study concerning the prevalence of diphtheria immunity in Polish population conducted in 1990’s by Walory et al. [3]. The authors revealed that 23% individuals examined were seronegative, 64% showed basic protection (0.1-1.0 IU/mL) and 13% were highly protected (>1.0 IU/mL). The higher level of diphtheria antitoxin antibodies in Polish population in 1990’s might had been maintained by reinforcing natural immunity and boost immunity through subclinical infections as in 1990’s and earlier diphtheria was a quite common disease in Poland. Such explanation is supported by observation in Latvia where diphtheria is endemic and the prevalence of antibodies to diphtheria toxin is high in all age groups of the population [13].

Studies concerning seroepidemiology of diphtheria in Western Europe revealed that the proportion with serum antibodies to diphtheria toxin rise with age in children, what is in agreement with our findings [14]. But in our studies <50% children in age 0-2 years old had
protective level of antibodies whereas Edmunds et al. [14] showed that >90% of 1-years-olds were seropositive. The differences might be a result of application of various vaccines. Poland is the only European country which uses DTP vaccine containing a whole cells pertussis component. The acellular vaccine is also available but is not refunded by the government therefore overwhelming majority of children are vaccinated using the whole cells DTP. The hypothesis about influence the type of vaccine on diphtheria antitoxin antibodies production in children should be investigated. Low proportion of seropositive children was also identified in the Czech Republic [13]. Our results together with results obtained for Czech population support the opinion of Chironna et al. [15] that basic immunization without booster doses might result unsatisfactory protection among children.

In all European countries where diphtheria seroprevalence studies were conducted, including Poland, the level of antibodies to diphtheria toxin decreases significantly in persons above 40 years old [13, 14, 16, 17, 18, 19, 20, 21]. The most drastic decrease was observed in Poland (this work), Spain and Ireland, reaching even > 67% of seronegative individuals [13, 17].

No significant sex-related differences in the proportion of seropositive individuals were identified in Poland, although, in some countries a marked sex-related differences were observed with higher number of seropositive males. It is probably due to the fact that in those countries military recruits had been vaccinated against diphtheria [18, 19, 20, 21].

Measuring the amount of serum antibodies against diphtheria toxin in individuals is the only way to survey the level of protection in a community. Even though the use of different methods and test kits for determination of the antidiphtheria antibodies level might influence on the obtaining results [11], the comparison of seroprevalence in various European countries clearly demonstrate that a high percentage of adults is not protected to diphtheria. In Poland, similar to several other European countries, booster doses every 10 years in adults are recommended. However, it is difficult to monitor vaccination coverage in adults and, as suggest our results, diphtheria booster vaccination in adults is uncommon. Despite high proportion of seronegative individuals in Polish population, diphtheria cases has not been recorded for over 10 years. It could be supposed that toxigenic corynebacteria do not circulate in the population. But it must be kept in mind that the disease could be imported from endemic regions and cause an outbreak in a susceptible community.

Conclusions
The presented study showed that there is insufficient herd immunity among Polish population what might create an epidemic potential. Before introduction of diphtheria vaccination, the diphtheria was a childhood infection. Currently the disease affects mainly adults [1, 8, 9]. As regards the low level of anti-diphtheria antibodies in persons >40 years old as well as development of tourism to diphtheria endemic regions and the identified new source of infection, which are domestic cats and dogs, it seems to be reasonable to carry out an information campaign reminding about recommendation concerning diphtheria booster vaccination in adults. Special attention should be paid to travelers and persons taking care of cats and dogs. Moreover, due to low seropositives children \( \leq 2 \) years old the immunogenicity of the DTP vaccine used in Poland should be verified. Full protection in the highest possible proportion of the population should help to avoid reemergence of this serious, often fatal infectious disease.

**Competing interest**

The authors declare that they have no competing interests.

**Authors’ contributions**

AAZ designed the study, analyzed the results, reviewed literature and prepare the manuscript; WR selected serum samples, conducted statistical analysis, participated in analysis of results and the manuscript preparation; NR conducted serological tests; MJ contributed in drafting the paper. All the authors have read and approved the final manuscript.

**Acknowledgments**

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**References**


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Table 1. Distribution of seroprotection against diphtheria in the Polish population according to age group

<table>
<thead>
<tr>
<th>Age groups</th>
<th>≤18 years</th>
<th>&gt;18 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of persons</td>
<td>417</td>
<td>970</td>
<td>1.387</td>
</tr>
<tr>
<td>Titre &lt; 0.1 IU/ml</td>
<td>154 (36.9 %)</td>
<td>393 (40.5 %)</td>
<td>547 (39.4 %)</td>
</tr>
<tr>
<td>Titre 0.1-1.0 IU/ml</td>
<td>212 (50.8 %)</td>
<td>542 (55.9 %)</td>
<td>754 (54.4 %)</td>
</tr>
<tr>
<td>Titre &gt;1-1.5 IU/ml</td>
<td>30 (7.2 %)</td>
<td>26 (2.7 %)</td>
<td>56 (4.0 %)</td>
</tr>
<tr>
<td>Titre &gt;1.5-2 IU/ml</td>
<td>18 (4.3 %)</td>
<td>6 (0.6 %)</td>
<td>24 (1.7 %)</td>
</tr>
<tr>
<td>Titre &gt; 2.0 IU/ml</td>
<td>3 (0.7 %)</td>
<td>3 (0.3 %)</td>
<td>6 (0.4 %)</td>
</tr>
<tr>
<td>Arithmetic mean titre (IU/ml)</td>
<td>0.409</td>
<td>0.274</td>
<td>0.310</td>
</tr>
<tr>
<td>Standard deviation (IU/ml)</td>
<td>0.559</td>
<td>0.324</td>
<td>0.414</td>
</tr>
<tr>
<td>Geometric mean titre (IU/ml)</td>
<td>0.141</td>
<td>0.102</td>
<td>0.108</td>
</tr>
<tr>
<td>Minimum (IU/ml)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Maximum (IU/ml)</td>
<td>4.480</td>
<td>1.740</td>
<td>4.480</td>
</tr>
<tr>
<td>Median (IU/ml)</td>
<td>0.210</td>
<td>0.150</td>
<td>0.160</td>
</tr>
</tbody>
</table>
### Table 2. Euroimmun assay results of 965 tested patients according to age group.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Number of tested sera</th>
<th>&lt;0.1 IU/ml</th>
<th>0.1-1.0 IU/ml</th>
<th>&gt;1-1.5 IU/ml</th>
<th>&gt;1.5-2.0 IU/ml</th>
<th>&gt;2.0 IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>114</td>
<td>61 (53.5)</td>
<td>38 (33.0)</td>
<td>10 (8.8)</td>
<td>4 (3.5)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>3-5</td>
<td>100</td>
<td>36 (36.0)</td>
<td>49 (49.0)</td>
<td>7 (7.0)</td>
<td>7 (7.0)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>6-13</td>
<td>133</td>
<td>41 (30.8)</td>
<td>77 (57.9)</td>
<td>9 (6.8)</td>
<td>5 (3.8)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>14-18</td>
<td>70</td>
<td>16 (22.9)</td>
<td>48 (68.6)</td>
<td>4 (5.7)</td>
<td>2 (2.9)</td>
<td>-</td>
</tr>
<tr>
<td>19-25</td>
<td>69</td>
<td>12 (17.4)</td>
<td>52 (75.4)</td>
<td>5 (7.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>26-30</td>
<td>110</td>
<td>25 (22.7)</td>
<td>80 (72.7)</td>
<td>3 (2.7)</td>
<td>2 (1.8)</td>
<td>-</td>
</tr>
<tr>
<td>31-40</td>
<td>145</td>
<td>34 (23.4)</td>
<td>103 (71.0)</td>
<td>6 (4.1)</td>
<td>1 (0.7)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>41-50</td>
<td>80</td>
<td>51 (63.8)</td>
<td>29 (36.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>51-60</td>
<td>70</td>
<td>46 (65.7)</td>
<td>24 (34.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&gt;60</td>
<td>73</td>
<td>49 (67.1)</td>
<td>24 (32.9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 1. Geometric mean concentration of diphtheria toxoid antibodies in the Polish population according to the age groups.
Figure 2. Percentage of subjects with diphtheria toxoid antibody levels \( \geq 0.1 \text{ IU/ml} \) in the Polish population according to the age groups.