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The prevalence of diphtheria immunity in healthy population in Poland

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SUMMARY

The degree of seroprotection against diphtheria in Poland was evaluated by determination of IgG antibodies to *Corynebacterium diphtheriae* toxin (IgG-DTAb). The study population consisted of 4829 healthy subjects aged from 1 day to 85 years from 7 regions of Poland. Serum samples collected between 1996 and 1998 were assayed for IgG-DTAb antibodies using a toxoid enzyme immunoassay. Neutralization of toxin in Vero cells was performed as a reference method with the WHO standard for human diphtheria antitoxin. The study revealed a lack of seroprotection (IgG-DTAb $< 0{\cdot}1$ IU/ml) in 23% of individuals, basic seroprotection ($0{\cdot}1$–$1{\cdot}0$ IU/ml) in 64%, and effective seroprotection ($> 1{\cdot}0$ IU/ml) in 13%. The non-protected group consisted of non-vaccinated children below 2 months of age (10%), individuals between 2 months and 18 years old (20%) and greater than 19 years old (70%). Of the adults, 32% were seronegative, 63% had basic seroprotection and only 5% were fully protected; 43% of adults between 30 and 64 years, who had not been vaccinated at least during the previous 10 years were not protected against diphtheria. The geometric mean titre (GMT) of IgG-DTAb was $0{\cdot}25$ IU/ml in the total population. Age-related GMTs differed significantly from each other and were higher ($0{\cdot}44$ IU/ml) in individuals from 2 months to 18 years old, compared with $0{\cdot}14$ IU/ml and $0{\cdot}17$ IU/ml in children under 2 months and adults, respectively. No significant difference was found in the GMTs of men and women in all age groups. We conclude that the currently used vaccination programme in Poland is highly effective and assures protection against diphtheria in the majority of the population in the 10-year period following the last booster. However, a significant proportion of adults between 30 and 64 years lack protection and this indicates a need for booster immunization for this group.

INTRODUCTION

Diphtheria is a serious infectious disease affecting children and adults. The introduction of vaccination programmes worldwide has been effective in its control but in recent years diphtheria has re-emerged particularly in countries of the Russian Federation and some former Soviet Union states with an increase in morbidity and mortality [1, 2]. Importation of diphtheria cases to several other European countries, such as Finland, Germany and Norway have been reported [3–6] and some cases identified in Poland have been linked to spread from neighbouring countries [7].

Humoral immunity against diphtheria depends primarily on formation of specific IgG antibodies against diphtheria toxin (IgG-DTAb), which may be induced by natural infection or passive or active

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immunization. Naturally and artificially acquired specific IgG-DTAb cannot be distinguished by existing techniques. It is believed that a IgG-DTAb level of 0·01 IU/ml, as evaluated in neutralization tests in animals or cell cultures, provides seroprotection against the disease [8]. However, the exact level of anti-toxin antibodies that ensures complete protection against diphtheria is not precisely defined [9] but in general a level of 0·1 IU/ml is considered to be protective [10, 11]. Monitoring of specific IgG-DTAb in the population is therefore an important means of monitoring the effectiveness of a vaccination programme and controlling the potential risk of diphtheria infection. To this end, we evaluated the immunity against diphtheria in healthy Polish populations of different age groups using a standardized ELISA test for IgG-DTAb and a toxin neutralization assay.

MATERIALS AND METHODS

Study population

Between 1996 and 1998 a total of 4829 serum samples were collected from healthy individuals, ranging from neonates to persons over 65 years of age, living in seven regions of Poland: western (707), northern (324), central (594), north-eastern (479), eastern (529), southern (866), south-eastern (1330). The study population was divided into three age groups: < 2 months, $\geqslant 2$ months to 18 years, and $\geqslant 19$ years. Individuals with acute infection, cancer, autoimmune disease, primary or secondary immune deficiency and receiving immunosuppressive treatment, were excluded from the study. After collection, sera were immediately frozen at -25 °C and thawed just prior to the analysis.

Determination of diphtheria antibody

Toxin neutralization. Neutralizing serum antibody titres to diphtheria toxin were determined in Vero cell culture [12, 13]. Serial twofold dilutions of serum were mixed in 50 μl quantities with 50 μl of diphtheria toxin (0·0002 Lf/ml, Sera & Vaccines Plant, Cracow, Poland) and incubated for 1 h at room temperature; 50 μl of a suspension of Vero cells (2, 5×10^5 cells/ml) were then added to each well. The plates were gently shaken, sealed with polyethylene and incubated at 37 °C for 6 days. A colour change from red to yellow indicated the absence of metabolic inhibition by the toxin due to its neutralization by anti-toxin antibodies. Serum titres were expressed in International Units (IU/ml) by reference to the WHO human anti-toxin (1·5 IU/ml) standard (National Institute for Biological Standards and Control, Hertfordshire, UK). This served as the reference method against which the Toxoid ELISA was compared for 100 serum samples.

Toxoid ELISA. Anti-diphtheria toxin antibodies in all samples were determined by enzyme immunoassay using the Toxoid ELISA test [14]. Diphtheria toxoid (10 μg/ml protein) was bound to microtitre plates and a calibration curve for each plate was performed with the WHO anti-toxin standard.

Serum titres were performed in triplicate and interpreted as follows: negative or lack of seroprotection ($< 0{\cdot}1$ IU/ml), basic or moderate seroprotection (0·1–1·0 IU/ml) and high seroprotection ($> 1{\cdot}0$ IU/ml) [9, 15, 16].

Statistical analysis

The results were analysed using Microsoft Office 98 and Statistica PI for Windows. The difference between geometric mean titres was compared by the Student test. According to international epidemiological standards, a population with more than 30% of individuals with non-protective titres against *C. diphtheriae* was considered at risk of diphtheria.

RESULTS

Correlation between NT Vero and Toxoid ELISA tests

There was a high degree of correlation between the antibody titres determined by the 2 assays ($r = 0{\cdot}81$; $P < 0{\cdot}0001$). For samples with an antibody concentration of $< 0{\cdot}1$ IU/ml the correlation coefficient was 0·5 ($P < 0{\cdot}001$) and 0·74 ($P < 0{\cdot}0001$) for samples of $\geqslant 0{\cdot}1$ IU/ml. Overall, the immunoassay gave values of 94% for both sensitivity and specificity against the reference method.

Immunity against diphtheria in the Polish population

For the population under study, the GMT of diphtheria anti-toxin IgG-DTAb was 0·25 IU/ml. The distribution of GMTs and other statistical

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

Age groups	< 2 month	2 months–18 years	> 19 years	Total
Number of persons	265	2122	2442	4829
Titre < 0·1 IU/ml	109 (41 %)	228 (11 %)	787 (32 %)	1124 (23 %)
Titre 0·1–1·0 IU/ml	146 (55 %)	1404 (66 %)	1528 (63 %)	3078 (64 %)
Titre > 1·0 IU/ml	10 (4 %)	490 (23 %)	127 (5 %)	627 (13 %)
Arithmetic mean titre (IU/ml)	0·26	0·71	0·31	0·48
Standard deviation (IU/ml)	0·28	0·74	0·37	0·60
Geometric mean titre (IU/ml)	0·14	0·44	0·17	0·25
Minimum (IU/ml)	0·011	0·005	0·003	0·003
Maximum (IU/ml)	1·55	8·89	5·32	8·89
Median (IU/ml)	0·16	0·56	0·18	0·29

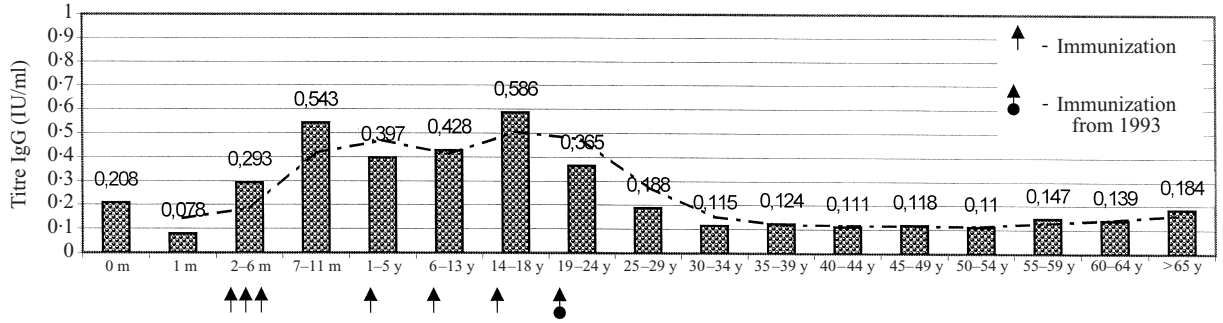

Fig. 1. Geometric mean titres of diphtheria antibody in the Polish population.

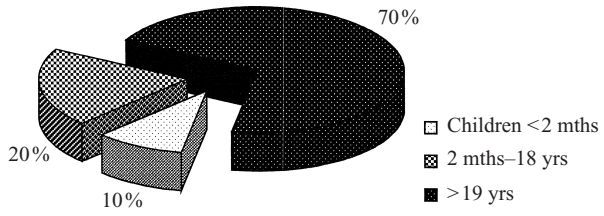

Fig. 2. Percentage of Polish population not immune protected against diphtheria.

parameters of anti-toxin levels in different age groups are presented in Table 1 and the GMTs of the total population are shown in Fig. 1. Among 4829 individuals examined, 1124 (23 %) were seronegative and lacked protective immunity against diphtheria. The majority, 3078 (64 %), showed basic protection and only 727 (13 %) were highly protected. The non-protected group consisted of children younger than 2 months of age (10 %), those between 2 months and 18 years (20 %) and adults over 19 years (70 %) (Fig. 2).

In children under 2 months of age ($n = 265$), the GMT was 0·14 IU/ml and 41 % were not protected against diphtheria. In individuals aged 2 months to 18 years ($n = 2122$), 23 % were highly protected, 66 % had basic protection and only 11 % ($n = 228$) were seronegative (Table 1). The GMT in this age group was 0·44 IU/ml. Among adults, 32 % ($n = 2442$) were not protected, 63 % had basic protection and only 5 % were highly protected (Fig. 3). The GMT for adults was 0·17 IU/ml. There was a significant difference between the GMT in adults and children less than 2 months of age and those aged from 2 month to 18 years ($P < 0·0001$). The lowest GMT (0·12 IU/ml) as well as the highest percentage of non-protected individuals (43 %) were found in adults aged from 30 to 64 years, which represented a risk group sensitive to diphtheria infection (Fig. 3).

DISCUSSION

The introduction of a worldwide immunization programme has led to a considerable reduction in the incidence of diphtheria. However, in many countries vaccination is limited to childhood and adolescence, which does not ensure life-long protection during adulthood. In Poland, immunization against diphtheria is a part of the National Vaccination Programme and consists of a 4-dose primary schedule given at 2, 4, 6, and 18 months of life in the combined

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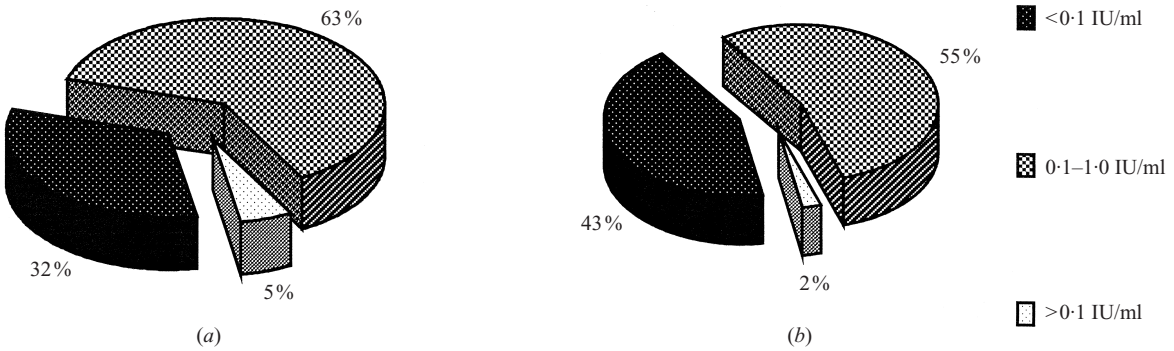

Fig. 3. Distribution of diphtheria antibody levels in persons < 19 years (*a*) and 30–64 years (*b*).

diphtheria, tetanus and pertussis vaccine (DTP) followed by three boosters with a combined diphtheria and tetanus vaccine (Td) given at 6, 14 and 19 years of life. The last dose at 19 years was introduced in 1993.

In this study we have shown that the currently used vaccination programme in Poland is highly effective and ensures protective anti-diphtheria IgG-DTAb levels in the majority of the population for 10 years after the last booster dose. We have also found that there is insufficient protection against diphtheria among adults, as only 57% of individuals older than 19 years had a protective level of antibodies.

Comparison of the immune status against diphtheria between different countries is complicated because of the use of different vaccination programmes, different vaccines and various dosing schedules. The selection criteria for population studies vary and so do the methods utilized for the determination of diphtheria antibodies. Nevertheless, the conclusion of most studies is that immunity to diphtheria in adult populations is not satisfactory. In the majority of countries national vaccination programmes involve only children, because of the historical importance of diphtheria in this group. Immunized children acquire high levels of protective antibodies that persist for a variable period of time, but if boosters are not given during adolescence, immunity declines over time and many adults become susceptible to *C. diphtheriae* infection [17].

Jenum and colleagues [18] compared anti-toxin neutralizing antibodies in Norwegian (243 persons) and Russian populations (227 persons) and found that 44% and 40% respectively had antibody levels below 0·1 IU/ml. In adults aged between 30 and 60 years, however, the percentage protection in Norway was markedly higher than in Russia. Both of these countries use different vaccination schedules and vaccine types. In comparison to the Polish population, the degree of immunity in Norway and Russia is significantly lower and this may lead to epidemiological problems in this region.

A German study of more than 2000 individuals showed that about 90% of persons over 30 years of age had protective antibodies [19]. However, two other German surveys reported lower seroprotection rates in the 20–40-years-old population [20, 21]. This was supported by Klouche [16] who, using a standard IgG-DTAb ELISA, found that 24% of northern Germans were not protected. The average anti-toxin titre was 0·39 IU/ml with children having the highest titres; no individuals over 50 years of age had a diphtheria antibody level over 1·0 IU/ml. In comparison with Poland, the German Vaccination Programme uses a reduced number of doses without a booster at 19 years. Nevertheless, the percentage of non-protected persons in Poland is broadly similar to that in Germany but the number of individuals with high protective titres in Poland is almost twofold higher. Moreover, in Poland the percentage of persons protected gradually decreases from age 25 compared with age 11 in Germany. Recently, Hasselhorn and colleagues [22] reported that 46% of German adults (19–54 years) lacked immunity to diphtheria, a figure comparable with the 43% of non-protected adults in Poland.

Susceptibility to diphtheria is clearly widespread among European adults as 54% of individuals over 40 years of age and 67% of the over 65 age group in a French survey were considered not protected [23] and only 23% of adults in Italy had seroprotective levels of antibody with 22% of the 20–30-year-old group having inadequate levels [24, 25]. Similar low frequencies of protected adults were reported from Spain [26]. Other studies from Nordic countries [27–29] and in the United States [30], repeatedly showed that the lowest level of seroprotection was

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found in people over 50 years of age. In Denmark, Kjeldsen and colleagues [29] demonstrated two risk groups for diphtheria infection; the first being adult females aged between 30 and 39 years with a seroprotection rate of about 32% and the second aged 60–69 with 26% protection. Males are better protected against diphtheria in Denmark due to immunization during military service. A shortened vaccination schedule (3, 5, 12 months) was introduced in Sweden in 1979 and recent studies attest to the fact that the majority of the Swedish population have only low immunity to diphtheria and the rate of protection for 10 year olds did not exceed 52% [31, 32]. A number of earlier studies from Poland, England and elsewhere also showed inadequate protection rates in adults of 20–50 years [10, 33] and 30–60 years [11, 27, 34–37].

In conclusion, outbreaks of diphtheria in recent years in the Russian Federation, Belarus and Ukraine have drawn attention to the re-emergence of this disease. They clearly show the need for the continuation of the National Vaccination Programme in Poland and of monitoring protective antibody levels in the population. In the light of our results and previous data from Poland and other European countries, we conclude that adults in the population have inadequate immune protection against diphtheria and booster vaccination of these individuals at 10-year intervals may be necessary.

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REFERENCES

1. World Health Organization. Expanded programme on immunization. Outbreak of diphtheria. Wkly Epidemiol Rec 1993; **19**: 134–8.
2. World Health Organization. Expanded programme on immunization. Diphtheria epidemic. Wkly Epidemiol Rec 1994; **34**: 253–8.
3. Galazka AM, Robertson SE. Diphtheria: changing patterns in the developing world and the industrialized world. Eur J Epidemiol 1995; **11**: 107–17.
4. Hardy IRB, Dittmann S, Sutter RW. Current situation and control strategies for resurgence of diphtheria in newly independent states of the Soviet Union. Lancet 1996; **347**: 1739–44.
5. Dittmann S. Epidemic diphtheria in the newly independent states of the former USSR – situation and lessons learned. Biologicals 1997; **25**: 179–86.
6. CDC. Diphtheria epidemic – new independent state of the former Soviet Union, January 1995-March 1996. MMWR 1996; **45**: 693–7.
7. World Health Organisation. Expanded programme on immunization. Recrudescence of diphtheria. Wkly Epidemiol Rec 1993; **36**: 261–4.
8. World Health Organisation. The immunological basis for immunisation. Module 2: Diphtheria. Geneva, 1993.
9. Ipsen J. Circulating antitoxin at the onset of diphtheria in 425 patients. J Immunol 1946; **54**: 325–47.
10. Galazka A, Kardymowicz B. Immunity against diphtheria in adults in Poland. Epidemiol Infect 1989; **103**: 587–93.
11. Cellesi C, Zanchi A, Michelangeli C, et al. Immunity to diphtheria in a sample of adult population from central Italy. Vaccine 1989; **7**: 417–20.
12. Kreeftenberg JG, Gun van der J, Marsman IR, et al. An investigation of a mouse model to estimate the potency of the diphtheria component in combined vaccines. J Biol Stand 1985; **13**: 229–34.
13. Kreeftenberg JG, Gun van der J, Hendriksen CFM. Potency determination in mice of diphtheria toxoid in vaccines, by serum neutralisation of diphtheria toxin in Vero cell cultures. Laboratory methods for the testing for potency of diphtheria (D), tetanus (T), pertussis (P) and combined vaccines. World Health Organization. BLG/92.1. Geneva, 1992.
14. Walory J, Grzesiowski P, Hryniewicz W. Comparison of four serological methods for the detection of diphtheria anti-toxin antibody. J Immunol Meth 2000; **245**: 55–65.
15. Scheibel I, Bentzon MW, Christensen PE, Biering A. Duration of immunity to diphtheria and tetanus after active immunization. Acta Pathol Microbiol Scand 1966; **67**: 380–92.
16. Klouche M, Luhmann D, Kirchner H. Low prevalence of diphtheria antitoxin in children and adults in Northern Germany. Eur J Microbiol Infect Dis 1995; **14**: 682–5.
17. Simonsen O, Kjeldsen K, Bentzon MW, Heron I. Susceptibility to diphtheria in populations vaccinated before and after the elimination of indigenous diphtheria in Denmark. Acta Pathol Microbiol Immunol Scand C 1987; **95**: 225–31.
18. Jenum PA, Skogen V, Danilova E, Esklid A, Sjursen H. Immunity to diphtheria in Northern Norway and Northwestern Russia. Eur J Clin Microbiol Infect Dis 1995; **14**: 794–8.
19. Riger J, Kuhlmann D. Diphtheria immunity in the German population. Gesundheitswesen, 1994; **56**: 667–71.
20. Naumann P, Hagedorn HJ, Paatz R. Diphtheriae immunitat und ihre epidemiologische bedeutung. Deutsch Med Wochenschr 1983; **108**: 1090–6.

21. Hasselhorn HM, Nubling M, Tiller FW, Hofmann F. Factors influencing immunity against diphtheria in adults. Vaccine 1998; **16**: 70–5.
22. Hasselhorn HM, Nubling M, Tiller FW, Hofmann F. Diphtheria booster immunization for adults. Deutsch Med Wochenschr 1997; **122**: 281–6.
23. Ballerau F, Schrive I, Fisch A. A multicentre serosurvey on diphtheria immunity in a French population of 1004 subjects. Eur J Epidemiol 1998; **14**: 499–503.
24. Gasparini R, Pozzi T, Fragapane E, et al. Immunity to diphtheria in Siena. Epidemiol Infect 1997; **119**: 203–8.
25. Mastroeni I, Patti AM, Santi AL, et al. Diphtheria antitoxin levels in the 14–30 years age group in Italy. Eur J Epidemiol 1998; **14**: 683–6.
26. Garcia-Corbeira P, Dal-Re R, Garcia-de-Lomas J, Aguilar L. Low prevalence of diphtheria immunity in the Spanish population: results of a cross-sectional study. Vaccine 1999; **17**: 1978–82.
27. Kerttula Y, Nors T, Kuronen T, Turpeinen T. Immunity to diphtheria in Helsinki in 1975. Scand J Infect Dis 1980; **12**: 37–9.
28. Christenson B, Bottiger M. Serological immunity to diphtheria in Sweden in 1978 and 1984. Scand J Infect Dis 1986; **18**: 227–33.
29. Kjeldsen K, Simonsen O, Heron I. Immunity against diphtheria and tetanus in the age group 30–70 years. Scand J Infect Dis 1988; **20**: 177–85.
30. Crossley K, Irvine P, Warren JB, Lee BK, Mead K. Tetanus and diphtheria immunity in urban Minnesota adults. JAMA 1979; **242**: 2298–300.
31. Mark A, Christenson B, Strandell A, Wickbom B, Bottiger M. Immunity and immunization of children against diphtheria in Sweden. Eur J Clin Microbiol Infect Dis 1989; **8**: 214–9.
32. Bjorkholm B, Granstrom M, Taranger J, Wahl M, Hagberg L. Influence of high titers of maternal antibody on serologic response of infants to diphtheria vaccination at three, five and twelve months of age. Pediatr Infect Dis J 1995; **14**: 846–50.
33. Maple PA, Efstratiou A, George RC, Andrew NJ, Sesardic D. Diphtheria immunity in UK blood donors. Lancet 1995; **345**: 963–5.
34. Rappuoli R, Podda A, Giovannoni F, et al. Absence of protective immunity against diphtheria in large proportion of young adults. Vaccine 1993; **11**: 576–7.
35. Wirz M, Puccinelli M, Mele C, Gentili G. Immunity to diphtheria in the 4–70 year age group in Italy. Vaccine 1995; **13**: 771–3.
36. Commodo N, Bonnani P, Lonnostro A, et al. Low prevalence of diphtheria immunity in the population of Florence. Eur J Epidemiol 1996; **12**: 251–5.
37. Ballereau VF, Schrive I, Fisch A, et al. Immunité antidiphtérique de la population française adulte d'après une enquête sérologique multicentrique. Bull Epidémiol Hebdomadaire 1995; **15**: 65–7.