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Prevalence and correlates of diphtheria toxoid antibodies in children and adults in Israel

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ABSTRACT

A seroepidemiological study was performed to evaluate immunity to diphtheria and to determine the correlates of diphtheria toxoid antibody levels among children and adults in Israel. In total, 3185 sera from an age-stratified sample of children and adults, obtained in 2000–2001, were tested for diphtheria toxoid antibodies by an in-house double-antigen ELISA. A level of ≤0.01 IU/mL (no immune protection or seronegativity) was found in 168 (5.3%) of the 3185 subjects, 639 (20.1%) had antibody levels of 0.011–0.099 IU/mL (basic immunity or low seropositivity), and 2378 (74.7%) had antibody levels ≥0.1 IU/mL (full protection or seropositivity). Seronegativity increased significantly in subjects aged >50 years, reaching levels of 9.7%, 12.6% and 18.9% in the groups aged 50–54, 55–59 and >60 years, respectively (p 0.001), with rates of basic immunity following a similar pattern. Subjects born abroad had higher seronegativity rates than those born in Israel (7.7% vs. 4.9%; p 0.019). No difference in diphtheria toxoid antibody levels was found according to other demographical variables, such as gender, Jewish or Arab ethnicity, urban or rural settlements, and the subjects' place of residence. The level of immunity to diphtheria among children and adults in Israel was satisfactory, with the exception of individuals aged >50 years. The risk of diphtheria outbreaks is low, but sporadic cases may occur among individuals lacking basic immunity against the disease.

Keywords Antibiotics, diptheria, immunity, Israel, seropositivity, toxoid

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INTRODUCTION

The introduction of vaccination programmes against diphtheria worldwide has been effective in the control of the disease, but the recent outbreaks of diphtheria in the republics of the former Soviet Union demonstrated that re-emergence of diphtheria can occur when population immunity is not maintained [1–4]. Risk-factors for a diphtheria epidemic include the presence of a large number of susceptible individuals (low immunisation coverage and inappropriate primary immunisation), lack of immunity in the elderly, socio-geographically clustered members of communities who refuse vaccination, and large-scale population movements [1–4]. The diphtheria epidemics in the republics of the former Soviet Union raised concerns regarding the level of immunity to diphtheria in Europe, and prompted many European countries to conduct seroprevalence studies.

In Israel, comprehensive childhood immunisation against diphtheria was introduced in 1951 and has been maintained with *c.* 90% coverage [5]. The current vaccination schedule comprises four doses of diphtheria toxoid (25 limit of floculation units (Lf) each), at ages of 2, 4, 6 and 12 months, administered together with tetanus and pertussis vaccines, and two subsequent doses (4 Lf), at ages of 7 and 13 years in combination with tetanus toxoid. The booster injection at the age of 13 years was introduced in 1999. Since

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1990, male and female recruits of the Israeli Defence Force have also received a booster vaccine against diphtheria, combined with tetanus toxoid (diphtheria low dose, 2 Lf).

The incidence of diphtheria has decreased dramatically in Israel, from 100 to 150 cases/100 000/year in 1951–1955 to only five reported cases of diphtheria in the whole population during the last two decades [5,6] (L. Moerman, personal communication). However, seroepidemiological studies in Israel during the 1990s showed significant pockets of lower immunity among older age groups and immigrants from the former Soviet Union, thus increasing the risk of re-emergence of diphtheria in Israel [7,8].

The objectives of the present study were to provide an update of the diphtheria immune status in a large sample of children and adults in Israel, to determine correlates of antibody levels to diphtheria toxoid, and to reassess the risk of occurrence of cases of the disease.

MATERIALS AND METHODS

Study design and sampling

A cross-sectional seroprevalence study was undertaken using stored serum samples (n = 3185) collected by the Israel Centre for Disease Control during 2000–2001. The serum bank comprised samples from all regions of Israel, from both males and females, with ages ranging from 3 weeks to 79 years. Samples from the group aged 0–18 years were residual sera from diagnostic laboratories, while samples from the adult population (>18 years) were residual serum samples from routine screening tests of healthy blood donors. Both sources excluded blood samples taken from cases with suspected immunological disorders. Blood donations in Israel are voluntary. Samples were selected randomly from the serum bank using a stratified sampling design. The target numbers of samples were 100 per year of age for those aged 1–19 years, 200 samples for each 5-year age group up to the age of 35–39 years, 200 samples for each 10-year age group up to the age of 50–59 years, and 100 samples for those aged ≥60 years. All samples were anonymised and linked, via a unique study number, to demographical information recorded at the time of specimen collection. Variables included patient age, gender, town of residence, country of birth (categorised into Israel or other countries), place of origin, as determined by the father's country of birth, i.e., second-generation Israeli, 'Western' (Europe and the Americas) or 'Eastern' (Africa, Asia and the Middle East), and ethnicity (Jewish or Arab). Actual data on ethnicity were collected only for the younger age groups (0–18 years). Therefore, a new variable was defined, i.e., 'modified ethnicity', which was deduced from the place of residence (coded as Jewish, Arab, or mixed). There was good agreement between actual ethnicity and modified ethnicity when cross-referenced in a population where the actual ethnicity was known.

Determination of diphtheria toxoid antibody levels

Sera were stored at −20°C until tested. An in-house double-antigen ELISA (DAE) was used to determine diphtheria toxoid antibody levels as described previously [9], with minor modifications. Diphtheria toxoid (Statens Serum Institute, Copenhagen, Denmark; Batch D22-1) was used for biotin labelling and microplate coating [9]. Pre-diluted (1:20) sample sera, standards and controls, in dilution buffer, were added to the plates. Three two-fold serial dilutions were made for the initial dilution of the serum samples and controls, and six two-fold serial dilutions of the 1st International Diphtheria Antitoxin Reference (DI 93) Equine (0.04 IU/mL) were included in each plate. Medium- and high-level controls, consisting of pooled human sera of 0.3 IU/mL and 3 IU/mL, respectively, were included in each test. Following the detection steps, the $OD_{450\ nm}$ was determined with an Emax Precision Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). Data were transferred online to SOFTmaxPRO software (Molecular Devices). The antibody concentration of each sample was calculated in relation to the standard, assuming a linear relationship between log antibody concentration and log absorbance. The final antibody concentration was calculated from the weighted mean value of the three dilutions.

Immunity to diphtheria was defined as: 'no immunity or seronegativity', a diphtheria antibody level of ≤0.01 IU/mL; 'basic immunity or low seropositivity', 0.011–0.099 IU/mL; or 'full protection or seropositivity', ≥0.1 IU/mL [10].

All assay components, toxoid coating, biotin-labelled toxoid, horseradish peroxidase–streptavidin, standards and controls, were calibrated and validated to the optimal concentration. Assays were performed by two technicians who were unaware of the subjects' characteristics and other test results. Criteria for approval of results required that: standard and sample curves were linear; r^2 was >0.95; at least two dilution points could be used; standard and sample lines were parallel, with slopes <0.9 and >0.5, and with <50% difference between the sample and reference slope; the geometric mean of the control sera did not exceed $\pm 2 \times$ SD; the absorbance of the standard (highest concentration) was >1.2 OD to guarantee a low quantification limit (<0.007 IU/mL); and the blank was <0.1 OD. Samples with results that did not meet these criteria were retested at a higher dilution (1:100) or a lower dilution (1:10) as appropriate.

The validity of the DAE for detection of diphtheria antibodies has been demonstrated by comparison with the toxin neutralisation test on Vero cells [9]. Quality control assurance of the performance of the DAE with the above methodology was conducted within the framework of the European Seroepidemiological Network (ESEN 2). A panel of 150 sera were tested as above, and then independently by two reference laboratories: (i) National Public Health Institute, Helsinki, Finland, by DAE and toxin neutralisation test on Vero cells; and (ii) Istituto Superiore di Santia, Rome, Italy, by the double-antigen delayed time-resolved fluorescence immunoassay (DA-DELFIA). The 150-serum panel comprised 37 samples negative for diphtheria toxoid antibodies (≤0.01 IU/mL), 45 equivocal samples (0.011–0.099 IU/mL), and 68 positive samples (≥0.1 IU/mL).

Statistical analysis

Prevalence rates, geometric mean concentrations (GMCs) and 95% CIs were calculated. Age- and gender-standardised

seroprevalence estimates were also computed using the direct method, with the total Israeli population, according to the Statistical Abstracts of Israel 2000 [11], taken as the reference population. Chi-square or Fisher's exact tests were used to analyse the association of the various demographical correlates with the prevalence rate of diphtheria toxoid antibodies, while differences in GMCs of diphtheria toxoid antibodies between groups were tested for statistical significance by Student's *t*-test. Data were analysed using SPSS v.11.5 software (SPSS Inc., Chicago, IL, USA).

RESULTS

The mean age of the 3185 subjects tested for diphtheria toxoid antibodies was 21 ± 16.67 years (range 3 weeks to 79 years), with 47.3% females; 89.1% of all subjects were Jews, and 86.6% were born in Israel. Based on the father's country of birth, the majority (47.7%) were second-generation Israelis, 29.7% were of Western origin, and 22.67% were of Eastern origin.

A diphtheria anti-toxoid level of ≤0.01 IU/mL (no immune protection or seronegativity) was found in 168 (5.3%) of the 3185 subjects, while 639 (20.1%) had anti-toxoid levels of 0.011–0.099 IU/mL (basic immunity or low seropositivity) and 2378 (74.7%) had anti-toxoid levels ≥0.1 IU/mL (full protection or seropositivity). The age- and gender-standardised rates for no immune protection, basic immunity and full protection were 7.0%, 22.3% and 70.7%, respectively.

Seronegativity rates increased significantly in subjects aged >50 years, reaching levels of 9.7%, 12.6% and 18.9% in the groups aged 50–54, 55–59 and >60 years, respectively (p 0.01; Fig. 1). The seronegativity rates decreased significantly in the groups aged 10–14 years (p 0.0001) and 20–24 years (p 0.0128) following the booster vaccination of all the population at the age of 7 years, and of male and female subjects recruited to military service after the age of 18 years (Fig. 1). The rates of basic immunity (0.011–0.099 IU/mL) followed the same pattern described above. The age groups with the highest seronegativity rates were also those with the lowest GMC of diphtheria toxoid antibodies measured among subjects with antibody levels of ≥0.1 IU/mL (Fig. 2).

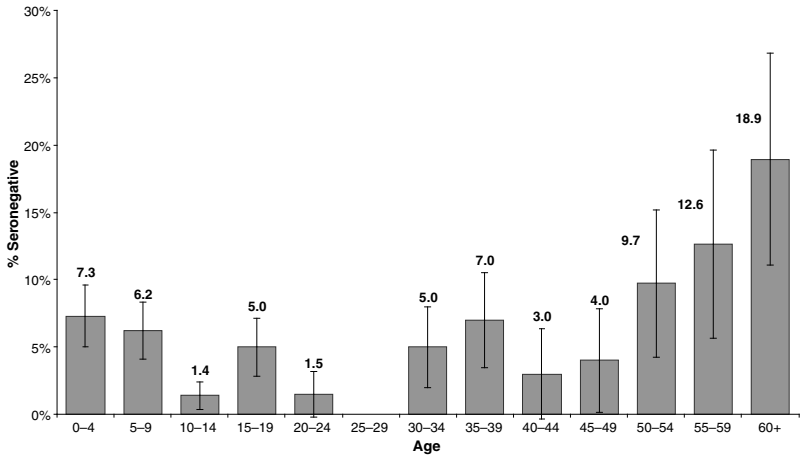

Fig. 1. Percentage of subjects with diphtheria toxoid antibody levels ≤0.01 IU/mL (seronegative). Vertical bars indicate 95% CIs.

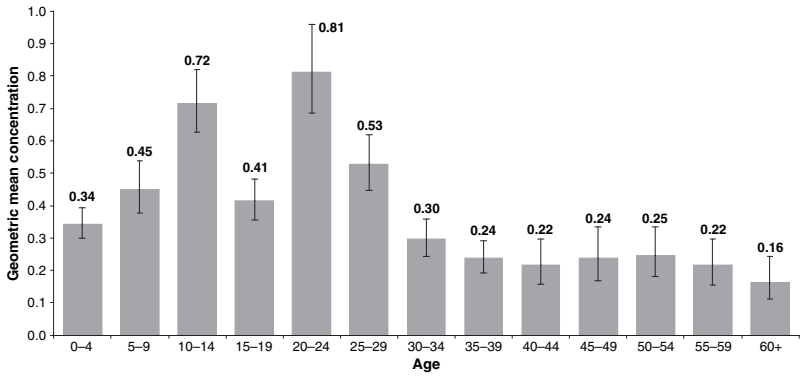

Fig. 2. Geometric mean concentration of diphtheria toxoid antibodies among subjects with individual antibody levels >0.01 IU/mL. Vertical bars indicate 95% CIs.

Table 1. Correlates of diphtheria toxoid antibody seronegativity

	Total tested	Seronegative to diphtheria		p
		n	%	
Gender				
Male	1679	89	5.3	0.94
Female	1506	79	5.2	
Country of birth				
Israel	2758	135	4.9	0.02
Other countries	427	33	7.7	
Country of origin				
Israel	1075	49	4.6	0.09
Eastern	508	28	5.5	
Western	669	47	7.0	
Ethnicity[a]				
Jewish	2798	146	5.3	0.95
Non-Jewish	342	18	5.2	
Area of residence				
North Israel	908	36	4.0	0.13
Central Israel	1731	97	5.6	
South Israel	534	32	6.0	
Type of settlement				
Urban	2691	138	5.1	0.64
Rural	449	26	5.8	

[a]Modified ethnicity (see definition in Materials and Methods).

Subjects born abroad had higher seronegativity rates than those born in Israel (7.7% vs. 4.9%, respectively; p 0.07; Table 1). When analysed separately, the same trend of association was found for both children and adults, but statistically significant differences were observed only for adults (8.4% (24/287) for those born abroad vs. 4.9% (54/1108) for those born in Israel; p 0.03). No statistically significant differences in the seronegativity rates of diphtheria toxoid antibodies were found according to gender, between Jewish and Arab subjects, between urban and rural settlements, or according to the subjects' area of residence (Table 1).

DISCUSSION

The diphtheria toxoid antibody levels were examined in the present study using the double-antigen ELISA, a recently developed in-vitro method that shows good correlation with the toxin neutralisation test performed on Vero cells [9]. Overall, 5.3% (range 0–18.9%) of the study population had an antibody level ≤0.01 IU/mL (no immune protection to diphtheria), while 20.1% had antibody levels of 0.011–0.099 IU/mL (basic immunity or low seropositivity), and 74.7% had antibody levels ≥0.1 IU/mL (full protection or seropositivity). The age- and gender-standardised rates for the corresponding levels of immunity were similar to the crude rates, thereby demonstrating a good representation of the general population of Israel in terms of age and gender.

The gradual increase in seronegativity rates with age, together with the lowest GMC of diphtheria toxoid antibodies being found among subjects with anti-toxoid levels >0.01 IU/mL in these age groups, reflect waning immunity following childhood vaccination without repeated booster vaccinations in adults. A previous study of a random sample of male Israeli reserve soldiers serving in 1983 showed a similar increase in seronegativity with age, with statistically significant differences between the groups aged 25–35 years and 41–51 years [7]. Similar trends in immunity to diphtheria have been reported from European countries and the USA [12,13]. Decreasing immunity with age among adults has been observed in almost all studies, regardless of the variation in testing procedures, the differences in vaccination and booster schedules, and the analysis methods. However, the seronegativity rates detected among adults aged >50 years in the present study (range 9.7–18.9%) were lower than those identified in the same age groups in various other European countries participating in the European Seroepidemiological Network Project no. 1 (seronegativity rates of *c.* 22–50%) [12]. This difference could be associated, at least in part, with the nature of the adult volunteers in the present study (i.e., blood donors), who might have complied better with vaccination schedules in the past.

The booster injection of diptheria toxoid given after the age of 18 years, which is the time of recruitment to the army for both males and females, is most probably associated with the decrease in the seronegativity rates seen in the groups aged 20–24 years and 25–29 years, respectively. For the groups aged >18 years, blood donors were the source of the sera examined. Many blood donors among the young adult age groups are soldiers serving in the Israel Defence Force, with far fewer Arab and orthodox Jewish citizens than their respective proportions in the total Israeli population. It is therefore possible that the observed reduction in the seronegativity rate for the group aged 20–24 years would not be found in these sub-populations that do not serve in the army. It has been shown that the seronegativity rates in adults of countries with compulsory military service are lower than in

countries with no vaccination programmes for adults [12]. In countries with compulsory military service for men, but not for women, the gender-related differences in the proportion of seronegatives have been attributed to the additional booster dose of diphtheria vaccine given to men following their induction to the army [12]. In Israel, large numbers of young women are conscripted to military service and are also given a booster dose of diptheria vaccine. This probably explains why no significant gender-related difference in the proportion of subjects without basic immunity to diphtheria was found among adults. Most of the Arab population and part of the orthodox Jewish religious community do not serve in the army and therefore do not receive the booster injection given to recruits since 1990. To ensure a good level of immunity to diphtheria in all Israeli young adults, an additional dose of diphtheria vaccine has been given since 1999 to all adolescents in Israel at the age of 13 years. This booster injection, coupled with that given to military recruits, will probably lead to a further reduction in the seronegativity rate and an increase in the proportion of the population with full protection against diphtheria.

Subjects born abroad had higher seronegativity rates than those born in Israel. This could be the result of lower immunisation coverage in childhood, different paediatric vaccination programmes, an inferior quality of vaccine, or lack of repetitive booster injections in the countries of birth. No significant differences in immunity to diphtheria were detected among different subgroups, e.g., Jewish and Arab subjects, subjects living in urban or rural settlements, and subjects living in different areas of the country.

In the last two decades, three sporadic cases of culture-proven diphtheria have been documented in Israel, in 1988, 1996 and 1999, respectively [6]. All three patients were children, aged 4.5, 6 and 10 years, respectively, who belonged to orthodox families and who did not receive diphtheria vaccine. The two younger patients died, despite antibiotic and supportive treatment [6]. In two of the three cases, carriers of toxigenic *Corynebacterium diphtheriae* were detected among asymptomatic contacts. Two additional cases of diphtheria have been identified, the first in 1999, in a subject aged 18 years with a full history of vaccination against diphtheria but suffering from severe cystic fibrosis, and the second in 2002, in a male aged 54 years with an unknown vaccination history (L. Moerman, personal communication). The occurrence of sporadic cases of diphtheria every few years, as well as the presence of asymptomatic carriers of toxigenic *C. diphtheriae*, demonstrates that toxigenic strains of *C. diphtheriae* are still present and circulating in Israel. Subjects with complete lack of immune protection to diphtheria will remain at risk of developing the disease when exposed to *C. diphtheriae*. The risk of the introduction of cases of disease or healthy carriers of toxigenic *C. diphtheriae* to Israel will increase as susceptible individuals travel to endemic areas.

Based on the findings of this study, the level of immunity to diphtheria in the Israeli population is probably sufficient to prevent outbreaks of the disease occurring following sporadic cases of disease. Although the proportion of subjects lacking basic immunity to diphtheria increases after the age of 50 years and reaches 18.9%, this proportion is lower than that detected in similar serosurveys in other European countries [12]. Despite the high rates of adult susceptibility, no sustained chains of transmission occurred in these countries following documented importations of cases of diphtheria [12–14]. During the large diphtheria epidemic in Russia between 1990 and 1998, more than 10 million individuals travelled between Finland and Russia, or vice versa. This resulted in only ten imported cases of diphtheria in Finland, with no secondary spread to health-care workers or other close contacts [14].

Between 1989 and 1994, *c.* 600 000 immigrants from the former Soviet Union arrived in Israel. Immunity against diphtheria was assessed in a sample of 992 males aged 17–49 years [8]. The seronegativity rate in the group aged 35–49 years was 18.2%, much higher than the overall rate of 5.3% in the present study. The difference between the seronegativity rates of the two populations could be even greater, since the indirect ELISA used to examine the levels of diphtheria antibodies among immigrants from the former Soviet Union is known to generate more false-positive values around the low concentration of 0.01 IU/mL. Only 9% of the immigrants from the former Soviet Union had diphtheria antibody levels >0.1 IU/mL, compared with 64.3% of the blood donors in the present study. The different nature of the two study populations (immigrants vs. blood donors) could also contribute to the differences in the level

of diphtheria toxoid antibodies. Despite the large pockets of susceptibility in the immigrant population from the former Soviet Union, and the possibility that toxigenic *C. diphtheriae* strains from the countries of origin were introduced into Israel, no cases of diphtheria were identified among the new immigrants. None of the five reported sporadic cases of diphtheria had an obvious link to new immigrants from the former Soviet Union.

For many close-contact infections, including diphtheria, the transmission potential among children is the most important determinant of the overall rate of transmission caused by day-care and school-related mixing patterns [12]. The number of susceptible children is likely to be a critical determinant of the epidemic potential. The level of immunity among children (age group 0–14 years) in the present study was high (95%), and it is assumed that this provides the necessary level of herd immunity to prevent epidemic transmission of *C. diphtheriae* if sporadic cases of disease occur. The three cases of diphtheria that occurred among unvaccinated subjects during the last two decades were not followed by secondary cases of disease [6]. The preventive measures adopted in all these events were prompt and adequate, but the level of protective immunity of the population against diphtheria probably also had a critical role in preventing further transmission of the pathogen.

In conclusion, the data generated by the present serosurvey revealed a satisfactory level of immunity to diphtheria among children and adults in Israel, with the exception of individuals aged >50 years, for whom the seronegativity rates increase continuously with age. The risk of diphtheria outbreaks is low, but sporadic cases may occur. Active immunisation remains the most important means of prevention of both outbreaks and sporadic cases. Improved vaccine coverage of travellers to endemic or epidemic areas, as well as the recommendation of administration of a booster dose of vaccine to adults every 10 years, should be implemented.

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