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Immunity to diphtheria in Siena

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SUMMARY

The aim of this study, carried out in 1993, was to evaluate diphtheria immunity in Siena. Diphtheria antitoxin levels were measured by means of the immunoenzymatic test (ELISA) in serum samples of 602 apparently healthy subjects (239 males and 363 females) of all ages residing in Siena. According to widely used criteria, 6% of the total population were susceptible to diphtheria (antibody levels $< 0{\cdot}01$ IU/ml), 71% had basic protection (0·01–0·09 IU/ml) and 23% were fully protected ($\geqslant 0{\cdot}1$ IU/ml). The results suggested that a high proportion of young population had a protective level of immunity against diphtheria, that susceptibility increased with age and a smaller proportion of males (2·9%) than females (8·3%) were unprotected; this difference was statistically significant. Our results suggest that it may be useful to revaccinate adults with low levels of diphtheria toxoid so that the percentage that remains unprotected does not put the community at risk of an outbreak of diphtheria.

INTRODUCTION

The recent outbreaks of diphtheria in Russia [1] and those occurred in Sweden [2, 3] have called attention to the possibility of outbreaks also in countries where vaccination is widely practised and where the disease is considered eradicated, and have pointed out the organism's potential for reintroduction and circulation of toxigenic strains of *Corynebacterium diphtheriae* in the population. In Italy, immunization with diphtheria toxoid has been compulsory for all newborns since 1939. Primary vaccination consists of three doses: a single dose given in the third, fifth and eleventh month of life. Seroepidemiological studies performed in various European countries [4–10] showed that a large proportion of the adult population including younger age groups, is unprotected against the disease. A large proportion of young Italian population has shown a protective level of immunity against diphtheria [11] due to a very high vaccine coverage. Nevertheless diphtheria antitoxin titre has shown a gradual decline in the older generation [12–15]. The aim of this study was to evaluate diphtheria immunity in Siena. This study was a part of a polycentric study that has involved subjects from an open population coming from different Italian cities. In each laboratory the determination of the antibody titre has been carried out using the same immunoenzymatic test (ELISA) in order to study the epidemiological trend of diphtheria immunity among the

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Table 1. *Population studied according to age and sex*

Age group (years)	Males	Females	Total	Mean age (years)	Standard deviation of mean age (years)
0–10	47	37	84	5·36	2·44
11–20	27	43	70	16·51	2·82
21–30	22	68	90	25·9	2·71
31–40	38	70	108	35·37	2·74
41–50	63	67	130	45·61	2·66
51–60	8	24	32	55·62	2·69
> 60	34	54	88	69·57	5·67
Total	239	363	602	35·86	20·17

Italian population, to control the effectiveness of the protection induced by vaccination, to suggest changes in the currently valid vaccination schedule with reference to a possible emergence of risk of disease among different classes of the population.

MATERIALS AND METHODS

Population studied

The study population which included 602 apparently healthy subjects (239 males and 363 females), of all ages coming from an open population residing in Siena, was carried out in 1993. The serum samples had been collected from two public health laboratories that received samples for diagnostic and screening purposes.

Subjects were divided into seven age groups: 0–10, 11–20, 21–30, 31–40, 41–50, 51–60 and > 60 years old (Table 1).

Diphtheria antitoxin evaluation

Blood samples were taken from each subject and sera were stored at −20 °C and later tested for diphtheria antitoxin IgG levels by enzyme linked immunosorbent assay (ELISA Diphtheria IgG; Sclavo Diagnostics, s.r.l., Italy). In this test, plates were sensitized with a purified and inactivated diphtheric toxin. After addition of 100 μl of serum samples diluted at 1:100 in PBS-Tween 20 (0·05%) and BSA (1%) and incubation at 37 °C for 30 min, the plates were washed and 100 μl of an alkaline phosphatase-conjugated goat anti-human IgG solution was added. After incubation and washing, 100 μl of substrate (*p*-nitrophenil-phosphate) was added to the wells, the colour reaction was stopped after 30 min by addition of 25 μl (3 M NaOH) and the resulting absorbances were read spectrophotometrically at 405/620 nm.

Titration of each serum was carried out in duplicate. The titre was expressed in IU/ml using a calibration curve in the range of 0·01–0·16 IU/ml. This curve was obtained using a pool of human positive sera in which the titre was determinated by a rabbit *in vivo* neutralization test carried out in comparison with the WHO Diphtheria anti-toxin equine serum (1st International Standard Statens Seruminstitute, Copenhagen, Denmark). In each analytical section an internal quality control using a titred serum was performed (accepted value 0·04–0·08 IU/ml).

Interpretation of the results

According to widely used definitions, antitoxin concentration below 0·01 IU/ml was considered to indicate susceptibility, 0·01–0·09 IU/ml to provide basic protection against the toxic manifestations of disease, and ⩾ 0·01 UI/ml to be fully protective [10, 11, 16, 17].

RESULTS

Degrees of diphtheria immunity found are shown in Table 2: 6% of the population studied were susceptible to diphtheria, 71% had basic protection and 23% were fully protected. There was a significant age effect on immunity, in fact immunity decreased with increasing age groups.

Figures 1 and 2 show the prevalence, distinguishing age and sex, of subjects with the three different

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Table 2. *Diphtheria immunity by age for both sexes*

Age group (years)	Number of subjects	Subjects with antitoxin level: Susceptible (< 0·01 IU/ml) No.	%	CI 95%	Basic (0·01–0·09 IU/ml) No.	%	CI 95%	Full (> = 0·1 IU/ml) No.	%	CI 95%
0–10	84	1	1·19	0·03–6·46	36	42·86	32·11–54·12	47	55·95	44·7–66·78
11–20	70	0	0	0·00–5·13	29	41·43	29·77–53·83	41	58·57	46·17–70·23
21–30	90	3	3·33	0·69–9·43	65	72·22	61·78–81·15	22	24·44	16–34·64
31–40	108	6	5·55	2–11·56	82	75·9	65·32–82·32	20	18·52	11·5–26·71
41–50	130	15	11·54	6·61–18·35	111	85·38	78·09–90·95	4	3·08	0·84–7·69
51–60	32	5	15·63	5·28–32·79	27	84·38	67·21–94·72	0	0	0–10·89
> 60	88	7	7·95	3·26–15·7	75	85·23	76·06–91·89	6	6·82	2·54–14·25
Total	602	37	6·15	4·38–8·41	425	70·6	66·16–73·64	140	23·25	20·01–26·93

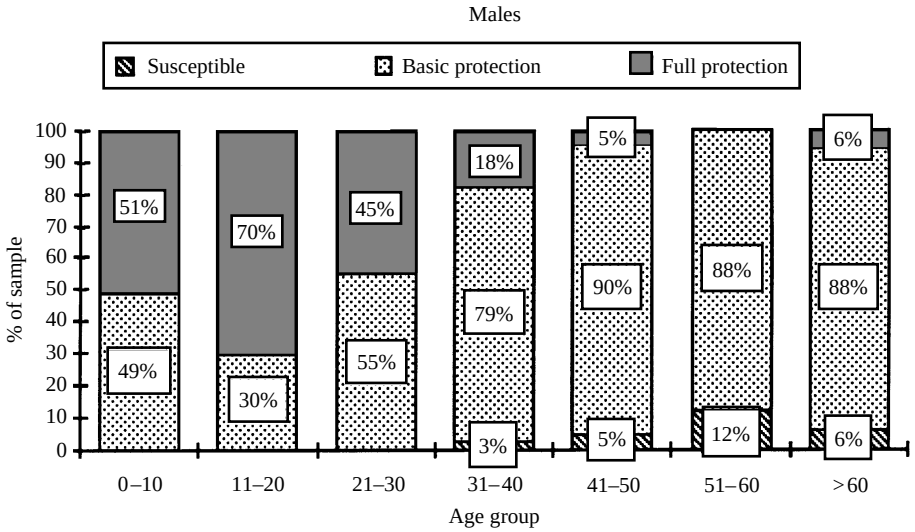

Fig. 1. Age specific prevalence (%) of diphtheria antitoxin levels in males (antitoxin level < 0·01 IU/ml = susceptibility; 0·01–0·09 IU/ml = basic protection, and > = 0·1 IU/ml = full protection).

immunity levels (susceptible, basic protection and full protection). Among the age group 21–30, a greater percentage of males were significantly more fully protected when compared with females (45% males *vs.* 18% females; $\chi^2 = 5{\cdot}535$, $P = 0{\cdot}0186$). For all ages the prevalence of subjects with antitoxin level $\geqslant 0{\cdot}1$ was greater for males (27·6%) than females (20·4%) and this difference was not quite statistically significant ($\chi^2 = 3{\cdot}825$, $P = 0{\cdot}0505$).

There is some evidence of a sex effect (Table 3) because, although similar proportions of males and females belonging to the younger age groups are protected, susceptibility increased among women from 7% in the age group 31–40 to 18% in the age group 41–50, and overall they were less protected than men. The difference is particularly evident and statistically significant among the age group 41–50 (4·8% males and 18% females), ($\chi^2 = 4{\cdot}287$, $P = 0{\cdot}0384$).

For all ages a smaller proportion of males (2·9%) than females (8·3%) were unprotected; this difference was statistically significant ($\chi^2 = 6{\cdot}218$, $P = 0{\cdot}0126$) (Table 3).

DISCUSSION

If we compare the percentages of protected subjects (94%) with the threshold (75%) indicated by Dadswell (18) as sufficient to prevent an outbreak of

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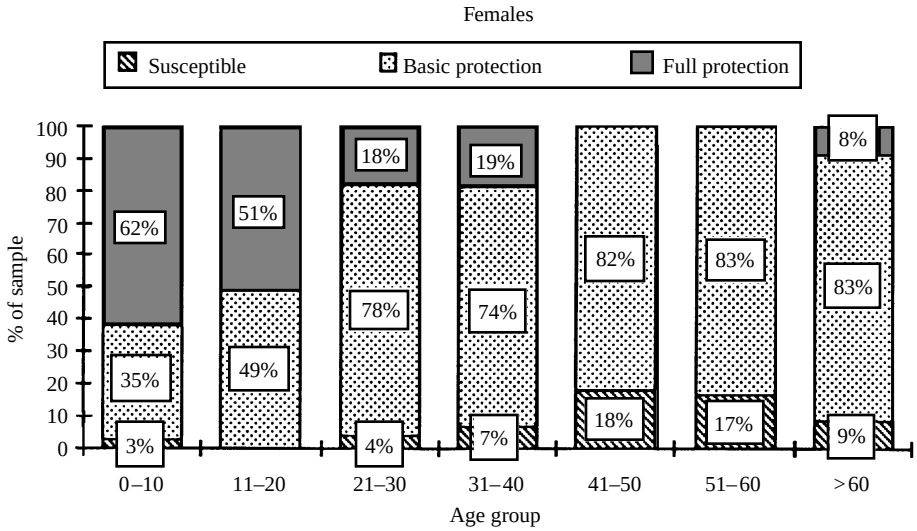

Fig. 2. Age specific prevalence (%) of diphtheria antitoxin levels in females (antitoxin level $< 0{\cdot}01$ IU/ml = susceptibility; 0·01–0·09 IU/ml = basic protection, and $> = 0{\cdot}1$ IU/ml = full protection).

Table 3. *Age-specific prevalence of subjects lacking a protective diphtheria antitoxin level* (*IU/ml* $< 0{\cdot}01$) *according to sex. The overall prevalence was* 2·9% *in males and* 8·3% *in females* ($\chi^2 = 6{\cdot}218$, $P = 0{\cdot}0126$)

Age group (years)	Males				Females			
	Total	No. susc.	%	95% CI	Total	No. susc.	%	95% CI
0–10	47	0	0	0·00–7·55	37	1	2·70	0·07–14·16
11–20	27	0	0	0·00–12·77	43	0	0	0·00–8·22
21–30	22	0	0	0·00–15·44	68	3	4·41	0·92–12·36
31–40	38	1	2·63	0·07–13·81	70	5	7·14	2·36–15·89
41–50	63	3	4·76	0·99–13·29	67	12	17·91	9·1–29·20
51–60	8	1	12·50	0·32–52·65	24	4	16·67	4·74–37·38
> 60	34	2	5·88	0·72–19·68	54	5	9·26	3·08–20·30
Total	239	7	2·93	1·40–7·12	363	30	8·3	6·85–13·98

diphtheria, we can observe that the values obtained in Siena are above the safety limits. Nevertheless we thought it right to point out that the ELISA test which we used could have led to an over-estimation of the subjects really protected, since the antibodies revealed might not always be efficient [19]. For this reason we are conducting research by which we will try to verify the correlation between antibodies bound with the ELISA test and their corresponding neutralizing antibodies; for this purpose culture cells are being used [20].

The high protection level reported here is greater than the 73·3% found in Siena in 1988 [13]; similar results, but with lower levels of protection have been reported in Florence (81·2%) [14]. Results that turned out to be below the ones we achieved, have been reported also in Genoa (60–70%) [15] and in Ferrara (71·8%) [12]. The studies conducted in Siena [13], in Florence [14] and in Genoa [15] have been performed by a passive haemoagglutination assay, while the enzyme-linked immunosorbent assay (ELISA) has been used in Ferrara [12].

Comparing these results with those achieved in other European countries in which a good degree of subjects were found unprotected against the disease, (in Sweden 56·9% of the population between 31 and 40 years old [5] and in Germany 52·2% of the population aged 20–34 were found to be unprotected against diphtheria [17], and in Denmark 36% of the subjects aged 60–69 had a neutralizing antitoxin titre $< 0{\cdot}01$ UI/ml [7]), the epidemiological situation observed in Siena appears more favourable. This may be due to a persistent circulation of *Corynebacterium diphtheriae* up to the 70s [21].

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In Siena a high proportion of the young population have a protective level of immunity against diphtheria: in the 0–10 year age group, 43% had basic protection whilst 56% were fully protected; in the 11–20 year age group, 41% had basic protection whilst 59% were fully protected. This good immunity status may be attributable to the very high vaccine coverage in Italy.

We found susceptibility increasing with age; in fact there was an overall trend of decreasing immunity with increasing age. An age-related increase of unprotected subjects was particularly evident after the 40s in which 12% of the subjects appeared lacking protective immunity (Table 2); this trend has been observed by many other European authors [12–15, 17, 18].

We have reported a gradual tendency of decreasing susceptibility after the 60s that remains unexplained. Nevertheless it's possible that these subjects have an immunological memory such to protect them from a further contact with the microorganism, even though their antibodies might not reach levels higher than 0·01 IU/ml.

A sex effect was observed, in which fewer women (21–30 year age group) were fully protected (18% females *vs.* 45% males), and overall the majority of them were less protected when compared to men (Figs 1, 2). This difference in immunity between sexes after 20–25 years of age has already been observed in other European Countries [8, 10, 22] and can perhaps be explained by diphtheria booster immunization as a consequence of military service.

It is therefore advisable that subjects with low levels of antitoxin who carry out activities that involve frequent or long visits in third world countries, undergo revaccination; the same applies to tourists who travel to such countries.

In agreement with American and European authors, it may be useful to revaccinate adults or rather give them a booster dose with low levels of diphtheria toxoid so that the percentage that remains unprotected does not put the community at risk of an outbreak of diphtheria [17, 23, 24].

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