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Low prevalence of diphtheria immunity in the population of Florence, Italy

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Abstract. A seroepidemiological study was conducted in 1994 on a representative sample of the population of Florence in order to verify the immunity coverage against diphtheria. Subjects were divided according to sex and age class. Sera from each selected class were at least 1.5/1000 of the residing population. Diphtheria antitoxin was titrated using a quantitative ELISA test. The results show an overall adjusted prevalence of diphtheria immunity (≥ 0.01 IU/ml) equal to 63.7%. Subjects of younger age classes have good protection levels (85.5% immune under 30 years), while only half individuals aged ≥ 50 years have antibody titres ≥ 0.01 IU/ml. Full protection (antibody titre ≥ 0.1 IU/ml) was detected only in a very small proportion of those aged ≥ 40 years. Our data show (1) how a recrudescence of diphtheria could theoretically take place in older subjects living in Italy, and (2) stress the importance of periodical re-vaccination of adults.

Key words: Antibody coverage, Diphtheria antitoxin, General population, Italy

Introduction

Diphtheria is a humanly transmitted infectious disease with a worldwide distribution. It is mainly transmitted by air droplets from nasopharyngeal secretions, and humans are the only host and reservoir for the bacterial agent. For this reason, the elimination of the infection is theoretically possible through the use of an effective vaccine. During the last few decades, the aim of vaccination programmes was to provide widespread immunization, starting with young children. Indeed, the latter had the highest attack rate for diphtheria in the pre-vaccination era, and this is still the case in countries where active immunization is not yet widespread [1–4].

Vaccination with diphtheria toxoid protects only against the phage-mediated toxin and not against infection by the C. diphtheriae organism [5]. Nevertheless, past experience has shown that mass immunization can also confer a sort of herd immunity through the elimination of selective advantage for toxigenic C. diphtheriae transmission in vivo [6]. Moreover, the circulation of non-toxigenic C. diphtheriae capable of lysogenic conversion to toxigenicity, when infected by bacteriophages, has probably not been reduced to the same degree in well vaccinated populations.

In Europe, following the occurrence of sporadic cases, a recrudescence of the disease was reported in the former Soviet Union.

In Russia, an epidemic began in 1990. A total of 15,211 cases of the disease were registered in 1993 and 34,408 in the first eleven months of 1994. In the Ukraine, 5,312 cases were reported between January 1993 and November 1994 [7]. The infection also spread to Latvia, Lithuania, Belarus, Norway and Poland, where subjects in the 30–50 year age-group were mainly infected. In addition, other European countries such as Denmark, Sweden, Germany, Portugal, Turkey and England also reported sporadic cases of the disease [8, 9].

With regard to Italy, where diphtheria vaccination with aluminium-containing toxoids has been mandatory since 1939, in the 1950s and 1960s, the number of reported cases came to several thousand/year, while between 1973 and 1982 the cumulative number of cases dropped to 1,427. Since the mid 1980s, the number of cases has dropped to a few per year. Indeed, with reference to the last six years for which data are presently available (1988–1993), the Health Authorities have been notified of only 3 cases of diphtheria [10].

The present epidemiological picture explains how diphtheria could be re-introduced to Western European countries through immigrants or travelers coming from areas where the infection is highly endemic or where epidemics are underway [11–13], considering that a recurrence of the disease can theoretically occur when the antibody coverage of populations drops below the threshold level which is set approximately at 70% [5, 14, 15].

On the basis of the above, the aim of our study

was to determine diphtheria immunity in a representative sample of the population of Florence stratified by sex and age.

Subjects and methods

Subjects. Census data were used in order to stratify the population of Florence according to age (ten-year interval classes) and sex. In 1994, serum samples from subjects living in Florence who had applied to different labs for routine blood examinations were collected and at least 1.5/1000 resident individuals of each selected class were checked. A total of 641 sera were obtained. Samples were frozen and stored at –20 °C until antibody testing was performed.

Laboratory tests. Samples were tested with a newly developed immunoassay (E.L.I.S.A. Diphtheria IgG Test, Sclavo Diagnostics s.r.l., Siena, Italy). The solid phase of the kit was covered with purified and inactivated diphtheria toxin. In brief, 100 µl of diluted specimens (1:100) were dispensed into the micro-wells and incubated at 37 °C for 30 minutes. After washing, 100 µl of anti-human IgG antibodies (goat) conjugated with alkaline phosphatase (ALP) were added and incubated at 37 °C for 30 minutes. Following a further washing, 100 µl of a para-nitrophenil-phosphate (p-NPP)-containing substrate was dispensed and incubated at 37 °C for 30 minutes. The development of a yellow colour from the reaction:

p-NPP (+ALP) → p-nitrophenol (yellow)

was stopped by adding 25 µl of 3M NaOH.

Optical density was measured at 405 nm by a spectrophotometer (Sclavo Reader SR 400 FW).

The calibration curve for the quantitative determination of antibodies was drawn using a set of 5 calibrators supplied by the producer. The latter were obtained by titrating pools of human sera against the reference diphtheria anti-toxin standard (horse immune globulin) by an 'in vivo' neutralization assay in rabbits. The 5 calibrators contained 0.01 IU/ml (standard 1), 0.02 IU/ml (standard 2), 0.04 IU/ml (standard 3), 0.08 IU/ml (standard 4) and 0.16 IU/ml (standard 5) of diphtheria antitoxin, respectively. All standards were tested in duplicate.

The antibody concentration of each sample was directly calculated in International Units/ml using a software package connected to the reader.

All sera, with diphtheria antitoxin > 0.16 IU/ml, were serially diluted in sample diluent and re-tested.

Statistical analysis. The minimum protection level of diphtheria antitoxin was set at 0.01 IU/ml. Results were classified according to degrees of antitoxin concentrations: < 0.01 IU/ml (susceptible); 0.01–0.09 IU/ml (basic protection); ≥ 0.1 IU/ml (full protection) [15–17]. Differences in antibody coverage with increasing age were evaluated using the Chi-square test with continuity correction and the Chi-square test for linear trend.

Results

Of the 641 subjects studied, 436 (68%) had a diphtheria antitoxin titre ≥ 0.01 IU/ml. In particular, 301 of them (47%) showed basic protection and 135 (21%) were fully protected. The adjusted percentage of individuals in the population of Florence, with basic or full protection, taking into account sex and age group, was 63.7%.

The analysis of results by age in Figure 1, shows that the prevalence of immune subjects (basic plus full protection) progressively declines from 89.6% for the 0–9 year age-group, to 48.9% for the 60–69 year age-group, while it increases to 53.8% for the ≥ 70 year age-group. The Chi square test for linear trend was highly significant ($\chi^2 = 72.28$; $p < 0.0001$).

With regard to full protection, the difference between the 10–19 year age-group and the 20–29 yr. one, is statistically significant ($\chi^2 = 4.57$; $p = 0.0325$). The percentage of individuals with diphtheria antitoxin titres ≥ 0.1 IU/ml sharply declines in the 40–49 year age-group, and shows a highly significant difference for the 30–39 year age-group ($\chi^2 = 12.95$; $p = 0.0003$). The overall prevalence of fully protected individuals is low (< 5%) in subjects ≥ 50 years.

Males show an overall higher prevalence of immunity (216/301; 71.8%) than females (220/340; 64.7%). However, this difference is not statistically significant ($\chi^2 = 3.34$; $p = 0.0678$).

The degrees of diphtheria immunity as determined by sex and age are reported in Table 1. The prevalence of immunity is not significantly different in males and in females, except for the ≥ 70 year age-group (males = 75%, females = 40.3%, $\chi^2 = 9.31$; $p = 0.0023$).

Discussion

In 1985 it was envisaged that by the year 2,000 diphtheria would be eliminated from Europe [18]. Such a goal seemed possible until a few years ago. Recent outbreaks of the disease in Russia, the Ukraine and the other countries previously mentioned, will probably set back that date. The present epidemic in Eastern Europe together with the increase in migrations, business travel and tourism, highlight the need for both strict epidemiological surveillance as well as the adoption and application of preventative measures if a further spread of diphtheria in Western Europe is to be avoided.

Indeed, diphtheria had, until very recently, been regarded as a solved problem in many industrialized

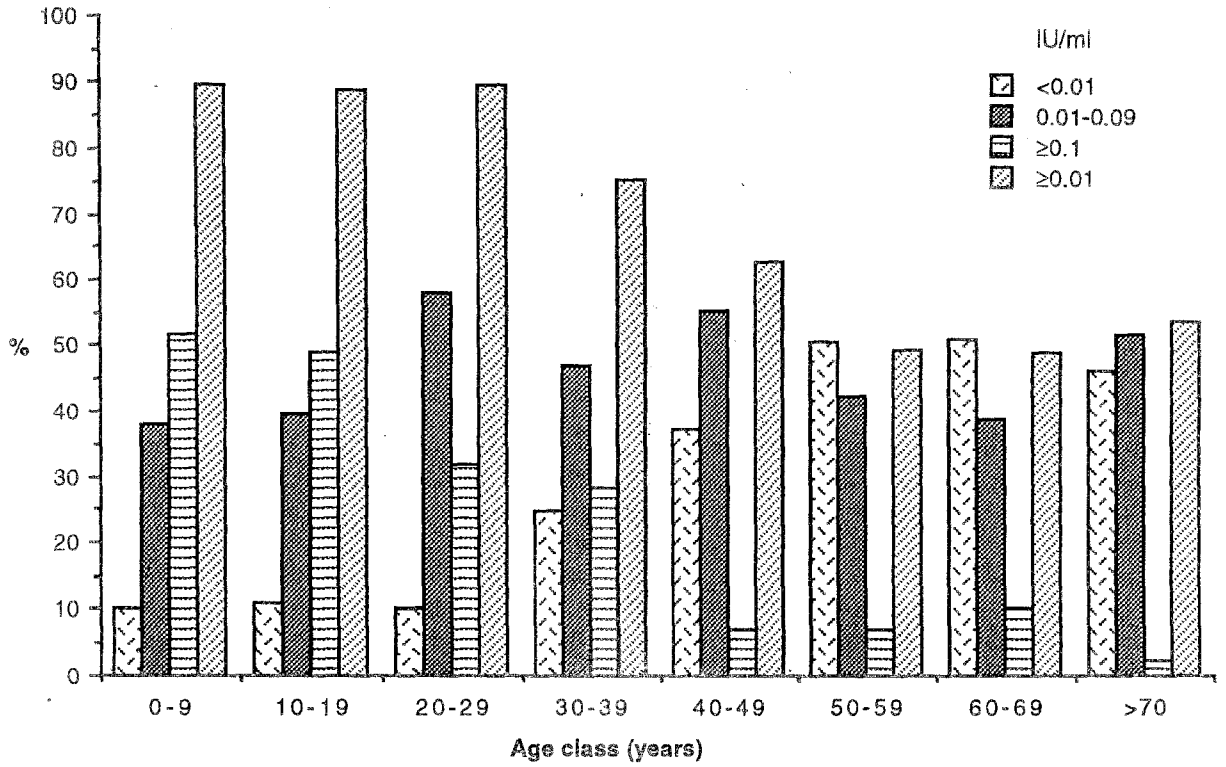

Figure 1. Diphtheria immunity (susceptibility, basic protection, full protection, basic + full protection) according to age class.

Table 1. Prevalence of diphtheria immunity in the population of Florence by age and sex

Age group (years)	Males (n = 301)			Females (n = 340)		
	< 0.01 IU/ml	0.01–0.09 IU/ml	≥ 0.1 IU/ml	< 0.01 IU/ml	0.01–0.09 IU/ml	≥ 0.1 IU/ml
0–9 (n = 58)	3 (11.2%)	12 (44.4%)	12 (44.4%)	3 (9.7%)	10 (32.3%)	18 (58.0%)
10–19 (n = 63)	1 (3.0%)	15 (45.5%)	17 (51.5%)	6 (20.0%)	10 (33.3%)	14 (46.7%)
20–29 (n = 88)	2 (4.4%)	28 (62.2%)	15 (33.3%)	7 (16.3%)	23 (53.5%)	13 (30.2%)
39–39 (n = 81)	11 (27.5%)	19 (47.5%)	10 (25.0%)	9 (22.0%)	19 (46.3%)	13 (31.7%)
40–49 (n = 83)	19 (47.5%)	19 (47.5%)	2 (5.0%)	12 (27.9%)	27 (62.8%)	4 (9.3%)
50–59 (n = 85)	23 (57.5%)	17 (42.5%)	0 (0.0%)	20 (44.5%)	19 (42.2%)	6 (13.3%)
60–69 (n = 90)	17 (42.5%)	17 (42.5%)	6 (15.0%)	29 (58.0%)	18 (36.0%)	3 (6.0%)
> 70 (n = 93)	9 (25.0%)	27 (75.0%)	0 (0.0%)	34 (59.7%)	21 (36.8%)	2 (3.5%)
Total (n = 641)	85 (28.2%)	154 (51.2%)	62 (20.6%)	120 (35.3%)	147 (43.2%)	73 (21.5%)

countries. Undoubtedly, the virtual elimination of the disease was dependent on the improvement of social conditions, nutrition and housing and on the implementation of effective immunization programmes [19]. High levels of immunity to the diphtheria toxin seem to be responsible, not only for individual protection, but also to ensure that selective forces can avoid lysogenic conversion of non-toxigenic strains of *C. diphtheriae*, which normally circulate in immunized populations [5]. It is worth noting that the sharp decline in diphtheria incidence registered in the USA and in some European countries during the 1970s (which was out of proportion with the actual number of immune subjects), together with the concomitant decrease in the isolation of toxigenic strains of *C. diphterhiae*, stress the importance of diphtheria antitoxin for herd immunity [5, 20].

What is far too often forgotten is that immunity, after vaccination, is short-lived in the absence of further antigenic stimulations [15, 21, 22]. This factor, together with the drop in vaccination coverage registered in some countries, has led to the creation of large cohorts of susceptible individuals, especially in adult population. For such reasons, the World Health Organization has recommended that the serological immunity to diphtheria in all age groups

be determined using standard international measurements [23].

Our seroepidemiological study on a representative sample of the population of Florence is part of a national multicentre study aimed at providing (1) data on immunity to diphtheria in Italy, and (2) practical prevention guidelines.

A survey coordinated by the National Institute of Health (Istituto Superiore di Sanità) and based on the cluster sampling method was recently conducted in order to verify compliance with mandatory vaccinations in 7 Italian regions. Children aged 2 years living in Tuscany turned out to have a 95.2% mean compliance with the 3 basic doses of diphtheria-tetanus toxoids (95% CI = 91.6%–98.9%) [24]. According to these data, the lack of protection in a considerable number of younger subjects can only partially be explained by the few individuals who still had to complete the basic vaccination course (age: < 1 year). In reality, we can hypothesize that full compliance with mandatory vaccinations is lower in Florence than the mean reported for Tuscany. Alternatively, it is possible that an inappropriate conservation of vaccines (i.e. freezing) occurred in some cases, thus causing a decrease in the immunogenicity of the diphtheria toxoid. However, it must not be forgotten that individual variation in the response to the vaccine is possible and could have contributed to determine our observation.

If we consider data on the prevalence of subjects with full protection, we can observe a gap between the 10–19 year and the 20–29 year age-groups, and more specifically, between the 30–39 year and the 40–49 year age-groups. Both differences are, in fact, statistically significant.

Ther overall rapid decline in diphtheria immunity can be attributed to the lack of the natural 'booster effect' following the strong reduction of *C. diphtheriae* circulation in our population. In reality, a mean of 27,000 diphtheria notifications/year were reported in Italy between 1931 and 1940, and cases were still about 20,000/year between 1941 and 1950. The full implementation of mass vaccination after the end of the Second World War, together with the improved social conditions, led to a steady decline of the disease starting from the 1950s [10].

No major change in diphtheria vaccination policy occurred in Italy since 1939, except for the age of mandatory immunization that shifted from the second to the first year of life in 1981.

Simonsen reports that, among vaccinees born at the time when diphtheria occurred frequently in Denmark, serum antitoxin concentrations relative to time from vaccination were significantly higher than among those who were born later. These observations were explained by the effect of natural immune stimulation upon vaccination response and the maintenance of immunity after vaccination [15]. For that reason, the 15% and 13% prevalence of fully protected subjects in males aged 60–69 years and in females aged 50–59 years, respectively, can probably be ascribed to repeated *C. diphtheriae* infections during childhood.

Our results show that only the younger population of Florence is sufficiently immune to diphtheria.

Indeed, the global adjusted prevalence of immune subjects is below 70%. Thus, even if, in Italy, diphtheria has practically disappeared over the last few years [10], the situation could change dramatically in the near future.

In conclusion, our research highlights the need for some practical steps to be undertaken to improve the level of diphtheria immunity. Precise and updated information must be supplied to general practitioners on the need for periodical re-vaccination of adults with the combined tetanus and diphtheria toxoids. A wider use of the Td vaccine is the only way to protect subjects > 30 years from a possible recrudescence of the infection, which is more likely to occur as a result of travel to highly endemic or epidemic areas. Furthermore, an effort must be made by public health authorities to inform the population of just how important it is to maintain full compliance with childhood mandatory vaccinations, even in the absence of reported cases for long periods of time. Indeed, maintaining high levels of vaccination coverage plays a key role (1) in the control of diphtheria, and (2) in eliminating it from Europe in the not too distant future.

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