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Age-specific seroprevalence of poliomyelitis, diphtheria and tetanus antibodies in Spain

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SUMMARY

In 1996, a seroepidemiological study was undertaken in Spain, with the main aim of estimating the population's immunity against poliomyelitis, tetanus and diphtheria. A population-based cross-sectional study was conducted, covering the population aged 2–39 years. The sample was stratified by age and rural–urban environment, and informed consent obtained to take blood specimens from subjects attending phlebotomy centres. The study included 3932 persons and the prevalence of antibodies against all three types of poliovirus exceeded 94% across all age groups. From a high of 96% in subjects under the age of 15 years, immunity against diphtheria steadily declined to a low of 32·3% in subjects aged 30–39 years. Similarly, tetanus antitoxin concentrations indicating basic protection were present in 98–9% of the under-14 years age group; thereafter, immunity declined, until reaching 54·6% in the 30–39 years age group.

INTRODUCTION

In Spain, mass immunization of the child population by means of oral poliomyelitis vaccine (OPV) campaigns began in 1963. These campaigns covered cohorts born in the preceding 7 years and attained coverages of 80–95% in the initial years. In 1965, these campaigns were expanded to incorporate vaccination against diphtheria, tetanus and pertussis (DTP) for all children under the age of 3 years. In 1996, the year in which the study was undertaken, the vaccination schedule recommended in Spain included a primary series of three doses of OPV and DTP, starting at 2–3 months of age and administered at intervals of 8 weeks; the schedule was completed by three booster doses of OPV at ages 15–18 months, 6 and 14 years, a booster dose of DTP at age 15–18 months and two booster doses of tetanus at ages 6 and 14 years.

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The incidence of these vaccine-preventable diseases covered by such vaccination declined sharply following the initiation of these vaccination campaigns. The last reported cases of wild poliovirus occurred in the south of Spain in 1988, with the detection of four poliovirus type 1 cases among an unvaccinated low socio-economic child population [1]. The last two cases of diphtheria were reported in 1986, and annual incidence of tetanus in 1996 stood at 0·11 per 100 000 population (43 cases) [2].

Knowledge of the effects of vaccination and the duration of immunity following the use of different vaccination schedules is inadequate. Different methods have been used, generally based on the study of specific population groups in which it is easy to obtain information, even though biases added to the study [3].

One way of obtaining an accurate picture of the population's immunity status is serum analysis, with a suitably representative collection of sera. In 1996, the Spanish Ministry of Health planned and conducted a nationwide seroepidemiological study to evaluate the

efficacy of the immunization programme, to ascertain its coverage and to calculate the efficacy of the vaccines used [4].

This paper analyses the results obtained in that study, which examines the immunity against the three poliovirus serotypes and the tetanus and diphtheria toxoids, vaccination coverage, and OPV- and DTP-vaccine efficacy.

METHODS

A cross-sectional study was conducted, based on a survey of the Spanish population (except for Catalonia), age range 2–39 years, who had attended public primary care phlebotomy centres in the period April–July 1996. All immunodeficient individuals or non-permanently residents of the local public health district were excluded from the study. In Spain, public health-care coverage extends to approximately 95% of the population.

The study design involved 14 independent samples, one for each of the seven age groups, and in each age group, two strata, rural and urban. Age groups were determined in accordance with modifications to the existing immunization programme, and were as follows: 2–5, 6–9, 10–14, 15–19, 20–24, 25–29 and 30–39 years.

A sample size of 270 individuals for each age group was chosen on the assumption of a 90% prevalence of antibody presence, a 4% sampling error and a 5% level of statistical significance. The initial sample size was corrected for a design effect of 1·25, based on a previous study which served as the reference study [5].

A three-stage sampling design was used [6–8]. In the first stage, the total estimated sample was distributed in proportion to the size of the population in the respective regions. Within each such region, it was then subdivided into rural–urban strata ($>50\,000$ inhabitants). For assignment by region and stratum we used the 1991 Population Census issued by the National Statistics Institute (Instituto Nacional de Estadística, INE). In the second stage, simple random sampling was used to choose the primary sampling units (PSU), i.e. basic public health districts or peripheral phlebotomy centres (Centros de Extracción Periférica). In the third stage, a constant number of basic units was selected in each PSU, using systematic random-start sampling.

All subjects who participated in the study completed a questionnaire that addressed: age, date of birth, sex and town or city of residence; and vaccination status, as recorded on the vaccination card or other accrediting document indicating type of vaccine administered, number of doses received, date of administration of the last dose and history of disease.

Informed consent was obtained from all subjects, a blood specimen taken and the following laboratory tests then performed: Neutralizing antibody titres against poliovirus types 1, 2 and 3 were determined. Sera with neutralizing antibody titres of $\geqslant 2$ were regarded as positive. Total antibody against diphtheria was determined by passive haemagglutination. Sera containing concentrations equal to or exceeding 0·01 international units/ml (IU/ml) were regarded as positive. Total antibodies against tetanus was determined by passive haemagglutination, with qualitative assessment based on two different cut-off points, namely, 0·01 IU/ml as indicating basic and 0·1 IU/ml as indicating complete protection. Briefly, equal volumes of a suspension of tanned sheep red blood cells (RBC) (2·5%) and pretitrated diphtheria and tetanus toxoids unadsorbed (Statens Seruminstitut, Copenhagen) in PBS pH 7·2 were incubated for 15 min at room temperature, with agitation at regular intervals. The sensitized RBC were then washed by centrifugation by using PBS supplemented with normal rabbit serum (PBS-NRS). The RBC suspension was finally adjusted to 0·4%. A suspension of non-sensitized RBC was prepared simultaneously, to be used as control. Prior to testing, serum samples were inactivated by heating at 56 °C for 30 min, and treated with a suspension of fresh sheep RBC, for removing non-specific agglutinins. In three different microplates, double dilutions of the previously treated samples were made, using PBS-NRS (volume 0·05 ml) as diluent. The corresponding RBC suspension (diphtheria, tetanus and control) (0·05 ml per well) was added to each plate. The plates were then shaken, incubated for 2 h and then read. The titre was the highest serum dilution showing agglutination with the sensitized RBC, in the absence of agglutination with the control RBC [9].

In the data analysis, weighted seroprevalences were computed. Seroprevalences and measures of association were calculated with the SUDAAN computer software package [10]. Odds ratio (OR) was used as a measure of association to assess the effect of risk factors on prevalence of infection.

Vaccination coverages were calculated on the basis of an inspection of vaccination cards, which all children surveyed aged 2–12 years were asked to present. The vaccination card provides information on the type

Table 1. *Vaccination coverage by vaccine type and dosage, and by birth cohort*

Vaccine dosage	Vaccination coverage (%) (95% CI)*		
	Birth cohorts		
	1991–94	1987–90	1984–86
OPV†			
3 doses	96 (94–97)	95 (93–97)	94 (92–96)
4 doses	87 (82–92)	91 (87–95)	90 (87–93)
5 doses	—	61 (56–66)	81 (75–87)
Diphtheria			
3 doses	96 (95–97)	96 (94–97)	93 (91–95)
4 doses	87 (82–92)	89 (86–93)	89 (85–92)
Tetanus			
3 doses	96 (95–97)	96 (95–97)	94 (93–96)
4 doses	87 (82–92)	92 (90–95)	91 (88–94)
5 doses	—	62 (57–67)	83 (77–89)

* 95% CI, lower and upper 95% confidence intervals.
† OPV, oral poliomyelitis vaccine.

and number of doses of vaccine received, as well as the place and date of vaccination. Vaccine efficacy was estimated in children aged 2–5 years and was taken to be the percentage of vaccinated children with serum antibodies at the date of the survey [11].

RESULTS

A total of 3932 persons, consisting of 2085 urban and 1847 rural residents was sampled. Due to small serum samples being available from some subjects, between 8% and 12% of persons were excluded from the study according to the infection to be studied. A total of 38 persons (1%) refused to participate in the survey.

Overall, vaccination cards were produced by 96% of children (98% in rural and 94·3% in urban settings). Those failing to produce cards was most evident in the 10–12 years age group (96·4% in rural and 89·1% in urban settings).

Vaccination coverage was assessed on the basis of the vaccination schedule in use in Spain in 1996. Table 1 shows vaccination coverage by vaccine type and dosage received. While coverages in the primary series were 95% for all cohorts, a lower coverage was detected in the booster doses, becoming more pronounced with age of administration.

Table 2 shows estimated vaccine efficacy by vaccine type and dosage, bearing in mind that the primary series encompasses three doses and that the fourth dose is the booster dose given at 18 months.

Table 2. *Oral poliomyelitis, diphtheria and tetanus vaccine efficacy among children ages 2–5 years*

Vaccine against	Sample size	Vaccine efficacy	95% CI*
Poliovirus 1 (3 doses)	41	97·6	85·6–99·9
Poliovirus 1 (4 doses)	387	98·4	96·5–99·3
Poliovirus 2 (3 doses)	41	100	98·3–100
Poliovirus 2 (4 doses)	386	99·2	97·5–99·8
Poliovirus 3 (3 doses)	41	100	89·3–100
Poliovirus 3 (4 doses)	385	96·9	94·5–98·3
Diphtheria (3 doses)	37	94·6	80·5–99·1
Diphtheria (4 doses)	384	99·0	97·2–99·7
Tetanus (3 doses)	36	97·2	83·8–99·9
Tetanus (4 doses)	387	98·4	96·4–99·3

* 95% CI, lower and upper 95% confidence intervals.

Prevalence of antibodies against the three types of poliovirus exceeded 94% across all age groups, with a slightly greater percentage of the population possessing protective antibodies against poliovirus 2 and a lower percentage possessing protective antibodies against poliovirus 3 (Table 3). The under-15s, the age at which presentation of the disease is most frequent, had a 99% protection against poliovirus 1 and 2 and a 94·4% protection against poliovirus 3. No significant differences were observed when the analysis was broken down by environment (urban–rural setting) or by sex.

Of the total sample, 6·8% (246) of subjects were susceptible to one or more types of poliovirus, and 0·4% (14) were susceptible to all three types of poliovirus. In the latter instance, the 14 persons concerned were above the age of 19 and had no vaccination history, with the single exception of one girl, age 5 years, who had a record of vaccination and presence of antibodies against other antigens.

The population with immunity against diphtheria is depicted in Table 4. Percentage protection against diphtheria antitoxin was 96% in subjects under 15 years of age. Above this age, protection registered a marked fall, with the 30–39 years age group displaying low levels (32·3%). This drop in protection is a consequence of the loss of diphtheria toxoid antibodies over time (i.e. time elapsed since the last dose), due to the absence of any booster dose subsequent to the dose administered at 18 months of age. No differences in antitoxin percentages were detected when the analysis was broken down by rural–urban setting or sex.

Figure 1 shows the levels of protection against tetanus toxoid, by age group. For ages older than 25

Table 3. *Percentage of the population with immunity against poliovirus 1, 2 and 3, and 95% confidence intervals, by age group*

		Poliovirus 1			Poliovirus 2			Poliovirus 3		
Age group (years)	Sample size	Sero-prevalence	95% CI*	Design effect	Sero-prevalence	95% CI	Design effect	Sero-prevalence	95% CI	Design effect
2–5	452	98·3	96·7–99·9	1·0	99·6	99·0–100	0·6	97·5	95·4–99·6	1·2
6–9	466	99·5	98·9–100	0·6	98·7	97·2–100	1·5	97·6	95·8–99·4	1·1
10–14	487	99·5	98·9–100	1·1	99·2	98·2–100	1·5	94·4	92·0–96·8	1·4
15–19	525	98·4	97·2–99·6	1·3	99·2	98·4–100	1·2	97·3	96·0–98·6	1·0
20–24	589	95·8	93·7–97·9	1·5	97·1	95·5–98·7	1·3	94·2	92·2–96·2	1·1
25–29	535	94·6	92·3–96·7	1·2	97·6	95·9–99·3	1·6	94·7	92·6–96·8	1·1
30–39	561	95·7	93·7–97·7	2·2	98·2	97·1–99·1	1·7	94·7	92·6–96·8	2·2

* 95% CI, lower and upper 95% confidence intervals.

Table 4. *Percentage of the population with immunity against diphtheria, by age*

Age group (years)	Sample size	Sero-prevalence	95% CI*	Design effect
2–5	439	95·6	90·0–100	4·7
6–9	454	96·7	94·7–98·7	1·0
10–14	472	86·1	82·3–89·9	1·5
15–19	502	67·0	60·6–73·4	2·5
20–24	516	58·7	52·8–64·6	1·9
25–29	516	59·5	53·8–65·2	1·6
30–39	528	32·3	27·1–37·5	2·7

* 95% CI, lower and upper 95% confidence intervals.

years, immunity, both basic and complete, decreased. Differences between men and women were in evidence from age 20 years upwards. Men registered a higher probability of having protective antibodies against tetanus toxin (Table 5). Table 6 shows the percentage of the population with basic protection, by sex. The level of protection remained above 94% until age 30 years, but fell to 73·8% in the 30–39 years age group. In women, the percentage of antibodies remained steady at 94% until age 19 years, after which it declined to 78·2% at age 29 years, and further still to 47·4% at ages 30–39 years. These differences were due to the fact that men receive a booster dose against tetanus toxoid on entering military service.

DISCUSSION

As compared to other strictly population-based studies [12, 13], our study design (cross-sectional, based on a general population sample drawn from public health-care centres) resulted in a minimum non-response rate and facilitated the task of obtaining information and serum samples from participants. Furthermore, any selection bias that may have arisen can be regarded as negligible, since Spain's National Health System is available free of charge to the entire population and, at the time of the study, afforded health-care coverage of close on 95%.

In our study, a very high percentage (96%) of the population under the age of 12 years had vaccination cards, indicating that they had access to the health-care system and that they had received a dose of some vaccine. Moreover, this high percentage enabled us to obtain a reliable calculation of vaccination coverage, namely, 95% for the primary series. A worrying finding is the decrease in vaccination coverage observed for booster doses, although part of this decline is attributed to a failure to update the vaccination cards rather than to any real fall-off in coverage.

As there is a correlation between concentration of antibody and clinical protection, this study allows vaccine efficacy to be indirectly inferred for all three infections studied. Furthermore, the fact that this type of study is not conducted at the same time as administration of the vaccine and, as a consequence, is unable to exert any degree of control over vaccine handling procedures, means that vaccine efficacy can be estimated under real conditions [11].

After three doses, oral poliomyelitis vaccine was observed to induce seroconversion of close on 100% (97·6–100), a figure similar to that reported elsewhere [14, 15]. No significant increase in neutralizing antibodies was in evidence after the fourth dose. The decrease observed with age is similar a decline in the antibody level seen in other studies [16–18].

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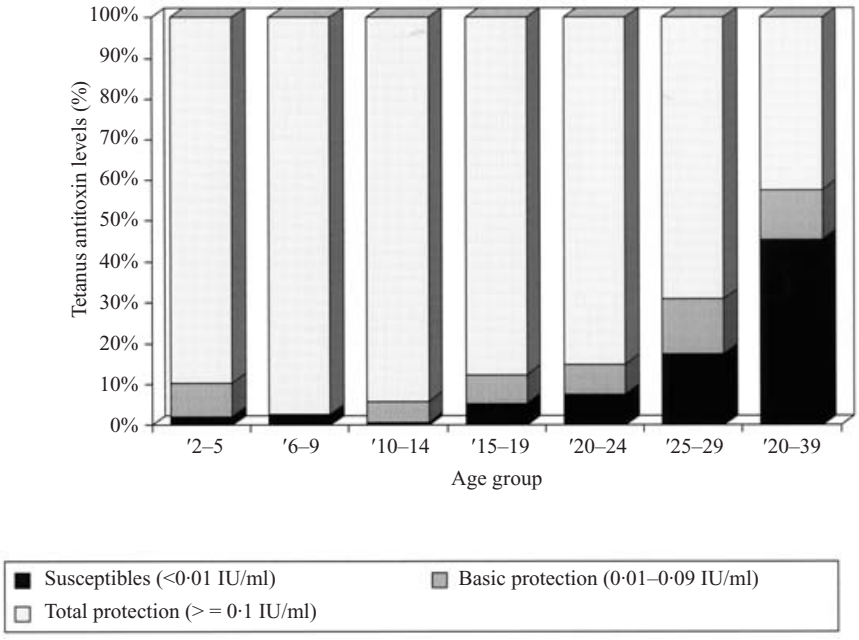

Fig. 1. Levels of protection against tetanus, by age group.

Table 5. *Tetanus: association with seroprevalence by age group, with a breakdown by setting and sex*

	Rural*/urban		Women*/men	
Age group (years)	Odds ratio	95% CI†	Odds ratio	95% CI
2–5	0·81	0·15–4·3	0·83	0·15–4·5
6–9	6·85	0·72–65·6	2·46	0·76–7·9
10–14	0·22	0·02–2·3	2·23	0·23–21·7
15–19	2·12	0·50–8·9	1·65	0·41–6·5
20–24	0·98	0·45–2·2	4·07	1·32–12·5
25–29	2·06	1·10–3·8‡	4·45	1·66–11·9
30–39	0·76	0·50–1·2	3·13	1·84–5·3

* Reference group.
† 95% CI, lower and upper 95% confidence intervals.
‡ Statistical significance.

Among subjects under 5 years old, the efficacy of the diphtheria and tetanus toxoids is high following administration of three doses (94·6 and 97·2%, respectively) and rises still further after administration of the fourth dose (to 99%). These findings are in line with similar results reported in other studies [19, 20].

This study shows that the Spanish population in the 2–39 years age group is well protected against poliomyelitis, with antibody prevalence of over 94% against all three types of poliovirus and across all age groups. The highest antibody prevalence is against poliovirus 2, yet the differences compared with the other two serotypes are not significant, findings which are similar to those reported elsewhere [18, 21].

The risk of reintroduction of the virus leading to poliomyelitis epidemic is low in Spain, in view of the fact that, while the threshold of susceptibles needed to give rise to an epidemic is estimated at 13–18% [22], the highest level of susceptibles detected in any age group in our study was below 6%.

The last case of wild virus poliomyelitis to be detected in Spain occurred in 1988. In 1997, acute flaccid paralysis (AFP) surveillance was introduced at a national level, in line with the strategy recommended by the World Health Organization for obtaining certification of eradication of poliomyelitis [23]. Of cases reported as AFP, two have been confirmed as OPV-vaccine-related poliomyelitis; both cases involved recipients of a first dose of vaccine, and no cases have been detected among contacts [24].

Immunization with diphtheria toxoid induces protection that wanes after the elapse of 7–13 years [25]. In Spain, diphtheria vaccine was introduced in 1965 and practically all persons included in this study were born after the introduction of the vaccine.

It should be pointed out that in the period leading up to the study, the last booster dose of diphtheria toxoid was routinely administered at 18 months of age. The last reported cases of diphtheria occurred in 1982–6. The study shows that the under-10 population enjoys an over 95% vaccination coverage and

Table 6. *Tetanus immunity (basic protection), by age group and sex*

	Males				Females			
Age group (years)	Sample size	Sero-prevalence	95% CI*	Design effect	Sample size	Sero-prevalence	95% CI	Design effect
2–5	248	97·6	94·9–100	1·1	184	98·0	95·9–100	0·7
6–9	244	98·6	96·6–100	1·3	202	96·8	94·1–99·5	1·0
10–14	229	99·6	98·8–100	1·0	244	99·0	97·9–100	0·8
15–19	190	96·3	93·6–99·0	1·0	319	94·0	87·6–100	6·8
20–24	164	97·4	94·7–100	1·3	367	90·1	86·6–93·6	1·4
25–29	139	94·1	88·8–99·4	1·7	383	78·2	72·5–83·9	1·7
30–39	153	73·8	64·6–83·0	2·7	386	47·4	41·7–53·1	2·2

* 95% CI, lower and upper 95% confidence intervals.

a like degree of protection against diphtheria toxoid. From ages 10–14 years upwards, protection begins to decline, falling to 67% in the 15–19 years age group and, further still, to 32% in the 30–39 years age group. Although these results may possibly be underestimated owing to the passive haemagglutination technique used, we nevertheless feel this to be unlikely in view of the pretreatment given to the samples [25]. Furthermore, recent studies which covered a Spanish population of similar characteristics but used the neutralization test in Vero cells [26] or a commercial ELISA test [27], have reported a lower degree of protection against diphtheria.

The results of serological studies suggest a higher percentage of susceptibles in the adult population. Results for the population aged 30–39 years vary from 95% of the immune population in Sienna, Italy [28], to 77·6% in women and 87·3% in men in Sweden born in the period 1956–65 (in our survey, the 30–39 years age group was born in the period 1956–66) [21], down to 20% in Greece [29]. Several reasons can be found to explain these different results, including, among others, different vaccination schedules in the respective countries, vaccination during military service, unknown effects of natural exposure to *Corynebacterium diphtheriae* toxin, different serological criteria to classify persons as immune, and different laboratory methods [19, 25].

In Spain, the loss of immunity with age detected in our study is basically due to the absence of a booster dose after 18 months of age. Recently, a fourth booster dose and revaccination with Td have been recommended. Nevertheless, presentation of diphtheria cases in Spain arising from loss of immunity is not to be expected, since resurgence of diphtheria is seen as being influenced by a number of factors, including, among others, the existence of susceptible child cohorts resulting from low coverages attained in childhood immunization programmes [30].

Vaccination with tetanus toxoid is known to solely protect the person who receives the vaccine: it does not afford indirect protection against other persons. Consequently, prevalence of tetanus toxoid antibodies can be used as a parameter to assess the effects of vaccination. In Spain, vaccination against tetanus toxoid was included in the combined DTP vaccine in 1965, along with diphtheria toxoid and *Bordetella pertussis*. In contrast to diphtheria toxoid, tetanus toxoid is administered as a booster dose at ages 6 and 14 years, as well as to all males entering military service. Practically all study subjects had received at least six doses of tetanus toxoid, which explains why the decrease in immunity took place very much later and to a lesser extent than that observed for immunity against diphtheria.

While subjects under the age of 20 years registered levels of basic protection of over 95%, protection fell to 85% among the under-30 years age group and, further still, to 64% among the over-30 years age group.

From age 20 years upwards, sex-based differences in immunity against tetanus were in evidence, with men being observed to enjoy greater protection, a difference that increased with age. This is due to the fact that women had received no booster dose after the age of 14, whilst men had received new doses on entry into military service.

On the basis of the results yielded by this study, Spain can be said to have had an effective immunization programme in place for many years, with high coverage for all vaccines administered.

With respect to diphtheria, the high percentages of protection against the toxoid attained after the primary series must be maintained if the ensuing loss detected at adult ages is to be prevented. In 1998, the

Spanish Public Health Authorities amended the immunization programme by introducing a booster dose with DTP or DT at ages 4–6 years, accompanied by the recommendation to revaccinate the entire population with Td vaccine every 10 years in lieu of the anti-tetanus vaccination that had previously been recommended.

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