

Immunity to diphtheria and tetanus in England and Wales

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Abstract

The immunity profile of the English and Welsh population to diphtheria and tetanus has been determined by measuring diphtheria and tetanus antitoxin levels for 3088 and 3142 sera, respectively. Time-resolved fluorimetric immunoassay — DELFIA was used to measure diphtheria antitoxin levels and an in-house, indirect ELISA to measure tetanus antitoxin levels. More than 80% of those aged between 2 and 20–24 years had protective diphtheria antitoxin levels of 0.01 IU/ml, or greater, and more than 80% of those aged between 4 and 35–39 years had protective tetanus antitoxin levels of 0.1 IU/ml, or greater. Only 29% and 53% of those aged 60 and over were protected against diphtheria and tetanus. Two increases of diphtheria antitoxin levels greater than 0.1 IU/ml and tetanus antitoxin levels greater than 1.0 IU/ml were apparent, starting at 4 and 14 years of age, which correspond with the policy of giving a diphtheria and tetanus toxoid booster on school entry and a tetanus plus low-dose diphtheria toxoid (recently introduced) booster to school leavers. This is the first comprehensive study of diphtheria and tetanus immunity in the English and Welsh population and shows that the accelerated schedule of immunisation, introduced in 1990, has effectively primed immunological memory against both these antigens and that boosting at school entry and at school leaving is effective in increasing levels of immunity. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Immunisation against diphtheria, which commenced on a national scale in the early 1940s, has been a tremendous success, and now, diphtheria is a very rare disease in England and Wales [1,2]. Unlike diphtheria, tetanus is not an epidemic disease, but is acquired through inoculation of wounds with spores of *Clostridium tetani*. Neonatal tetanus has been of very low incidence in England and Wales [3] and before 1950 active immunisation with tetanus toxoid was virtually restricted to military personnel. Only in 1964 was universal active immunisation officially sanctioned in Eng-

land and Wales [4]; although, tetanus vaccination had been performed as part of childhood immunisation schedules before this time [5].

In recent years, in England and Wales there have been a number of alterations and additions to the national vaccination programme [6] which could have a significant impact on the immunity of the population to diphtheria and tetanus. With the aim of improving levels of vaccine uptake, an accelerated schedule of diphtheria, tetanus and pertussis (DTP) immunisation was implemented in the early 1990s [7]. In 1994, a low dose diphtheria component was added to the school leavers dose of tetanus toxoid [8]. Very recently, the traditionally used whole cell component of DTP has been replaced by acellular components [9] and meningococcal C conjugate vaccination has been introduced [10]. The meningococcal C conjugate vaccination programme is targeted at pre-school entry and school

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leaving and is of particular interest because the vaccines used contain either mutant diphtheria toxin, i.e. CRM₁₉₇ [11] or tetanus toxoid [12].

The object of this study is to show the immunity profile of the English and Welsh population to diphtheria and tetanus using an appropriately selected set of sera. Such an initiative is timely because it will provide a baseline against which to evaluate the impact of the recently introduced meningococcal C conjugate vaccine immunisation programme, particularly in those aged under 18 years.

2. Materials and methods

2.1. Study population and study design

The survey was carried out on residual sera from 18 laboratories which had been used for microbiological or biochemical investigations and then submitted to the Public Health Laboratory Service Seroepidemiology Unit during 1996. The minimum number of sera collected per age group was determined from power calculations using age specific estimates of sero-prevalence of antibody to various vaccine preventable infections including diphtheria [13]. The target number of samples were 100 from yearly age classes 1–19 years of age, 200 samples from five yearly age classes up to 35–39 years, 200 from 10 yearly age classes up to 50–59 and 200 samples from those over the age of 60 years. The sera were frozen and stored at –20°C until diphtheria and tetanus antitoxin testing was performed. Freezing and thawing of sera was kept to a minimum and thawed sera were stored at 4°C for the minimum length of time.

2.2. Diphtheria antitoxin measurement

Diphtheria antitoxin was measured by a time-resolved fluorimetric immunoassay system, commonly known as DELFIA (dissociation-enhanced lanthanide fluorescence immunoassay). This technique has been shown to be as sensitive and specific as Vero Cell Assay [14], an *in vitro* neutralisation technique considered appropriate for measuring diphtheria immunity [15]. In brief, DELFIA microtitration plates (Wallac Oy, Turku, Finland) were coated (100 µl/well) overnight at 4°C, with diphtheria toxoid (Batch H, Statens Serum Institut, Copenhagen, Denmark) diluted 1:1000 in 0.05 M carbonate buffer, pH 9.6. The plates were then washed four times with DELFIA wash buffer (DELFIA wash concentrate diluted 1/25, cat. no. 1244-114, Wallac Oy) using a DELFIA Platewash, 1296-06 (Wallac Oy). Serum was tested, in duplicate, at a dilution of 1 in 3 by adding 33–66 µl of DELFIA assay buffer (cat. no. 1244-111, Wallac Oy) in relevant

wells in the microtitre plates. A Standard (Diphtheria Antitoxin, Equine, 3rd British Standard, NIBSC, South Mimms, UK) at final concentrations ranging from 0.3 to 0.002 IU/ml was run on each plate together with low, medium and high internal quality controls. The plates were incubated for 2 h at room temperature on a shaker (SO5, Stuart Scientific, Redhill, Surrey, UK) at 150 rpm and then washed four times. Europium labelled diphtheria toxoid tracer (batch H, diluted 1 in 10,000 in assay buffer) was then added. Europium labelling of the diphtheria toxoid was performed, on behalf of Wallac, UK, by Dr G. Barnard, Department of Chemical Pathology, Southampton General Hospital, Southampton, UK. The plates were incubated and then washed, as previously described. DELFIA Enhancement Solution (cat. no. 1244-105, Wallac Oy) at 100 µl/well was then added and the plates shaker incubated for 5 min before reading. The plates were read using a 1234 DELFIA Fluorimeter and the data handled by 1224 MultiCalc, version 2.5.

2.3. Tetanus antitoxin measurement

Tetanus antitoxin was measured by an in-house, indirect ELISA which is based on that previously described by Melville-Smith et al. [16]. The ELISA has been, satisfactorily, evaluated against the gold standard, mouse neutralisation test and a commercial assay (unpublished observations). Nunc-immuno plates (F96, Maxisorp, Life Technologies, Renfrewshire, UK) were coated overnight at 4°C, with tetanus toxoid (NIBSC, South Mimms, UK) diluted to 0.5 LF/ml in carbonate buffer, pH 9.6. At the same time a set of negative control wells were coated with a solution of 1.0% Marvel (Premier Brands, UK) in carbonate buffer, pH 9.6. The following day, coated plates were washed three times with phosphate buffered saline pH 7.4 (PBST) containing 0.05% Tween 20 (Sigma, Poole, Dorset, UK) using a Denley microtitre (Wellwash 4) plate washer (Labsystems, Helsinki, Finland). All the wells were then blocked for 1 h at 37°C with 1% Marvel in PBST and then washed three times with PBST. Sera for testing were diluted in sample buffer (0.1% Marvel in PBST) and tested at dilutions of 1:50, 1:100, 1:200, 1:400, 1:800 and 1:1600. Negative control wells were loaded with sera at 1:50 dilution only. Standard tetanus antitoxin (Antitetanus Immunoglobulin, Human 26/488, NIBSC, South Mimms, UK) was tested at concentrations of 0.025, 0.012, 0.006, 0.003, 0.0016 and 0.0008 IU/ml in each plate and, in addition, two quality control sera (one high and one low) were run on each plate. After loading, the plates were incubated for 2 h in a humid chamber, at 37°C and then washed three times with PBST buffer. Conjugate (rabbit anti-human horse radish peroxidase, Sigma

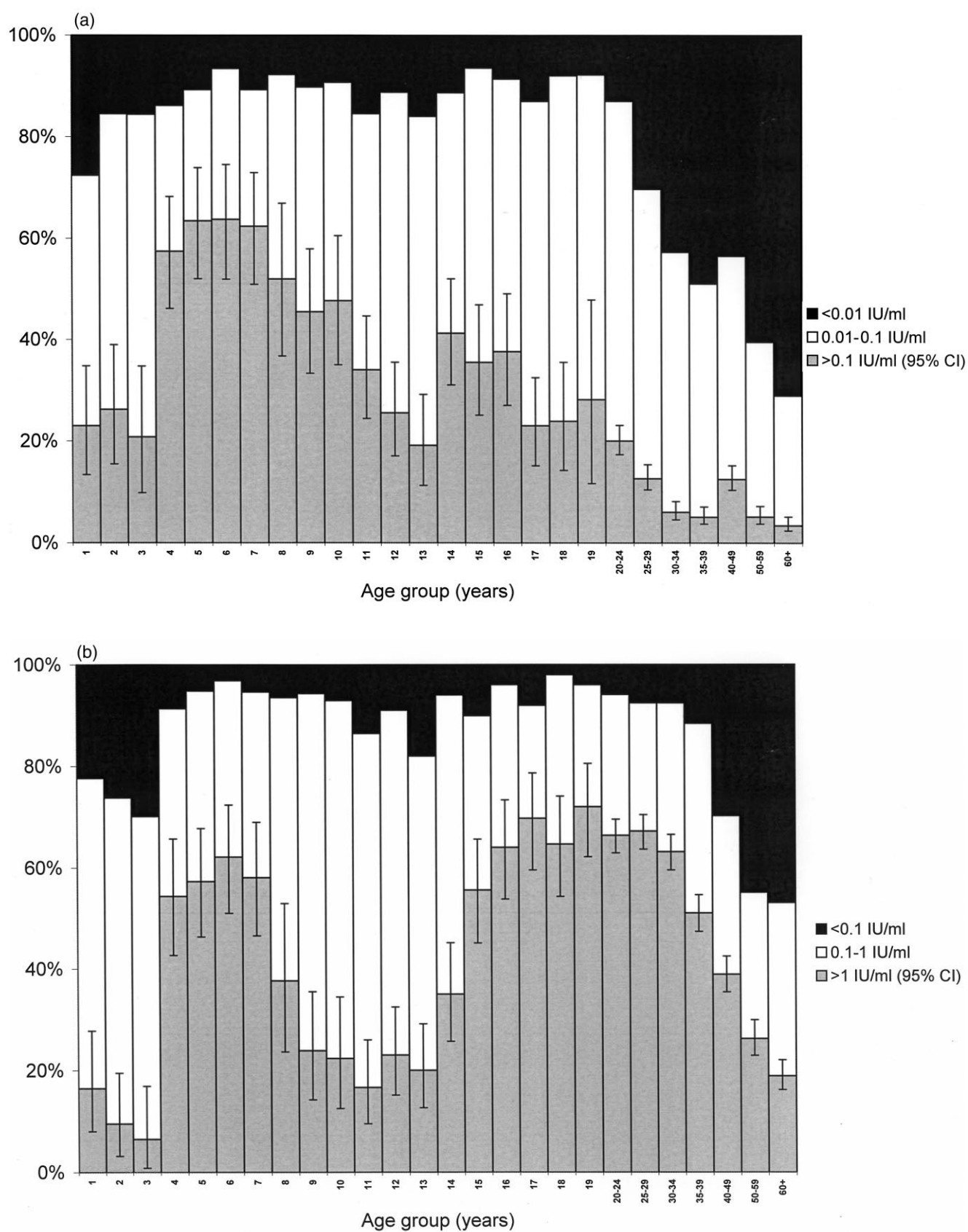


Fig. 1. (a) Diphtheria antitoxin (IU/ml) distribution with age in England and Wales, 1996. (b) Tetanus antitoxin (IU/ml) distribution with age in England and Wales, 1996.

A8792) was added and after incubation, at 37°C for 1 h, plates were washed three times with PBST and then three times with phosphate buffered saline, pH 7.4. Substrate (ABTS tablets, Sigma A9941 dissolved in citric acid buffer) activated immediately before use with hydrogen peroxide was added and after 20–25 min at room temperature the plates were read at 405 nm using an Anthos plate reader, model 2001. Standard curves were drawn for each plate and optical densities of test sera dilutions falling within the linear part of the curve were interpolated. The results were expressed as IU/ml.

2.4. Statistical methods

Diphtheria and tetanus log titres were compared using linear regression and by calculating the correlation coefficient. Differences in geometric mean titres between males and females were assessed using the two-tailed z-test. Changes in serological profiles by age were interpreted with the aid of 95% confidence intervals on the proportions >1 IU/ml for tetanus and >0.1 IU/ml for diphtheria.

3. Results

Diphtheria and tetanus antitoxin levels were obtained for 3088 and 3142 sera, respectively. The shortfall from a total of 3300 which should have been tested, according to the study protocol was due to insufficient volumes, inappropriate serum samples (grossly haemolysed, contaminated) and non-specific antibody reactivities.

Fig. 1 shows the distribution of (a) diphtheria antitoxin levels and (b) tetanus antitoxin levels by age group. For diphtheria, we have considered antitoxin levels less than 0.01 IU/ml to denote susceptibility, antitoxin levels between 0.01 and 0.1 IU/ml to provide basic protection, and antitoxin levels greater than 0.1 IU/ml to be fully protective. For tetanus, antitoxin levels less than 0.1 IU/ml denote susceptibility, antitoxin levels of 0.1–1.0 IU/ml are protective and levels in excess of 1.0 IU/ml are considered as giving long term protection. More than 80% of those aged between 2 and 20–24 years had some protection against diphtheria; however, there was a progressive decline in protection from 70% in the 25–29-year age group to 29% in the age group of 60 years and above. In the 1-year age group, 72% had some protection against diphtheria increasing to 84% in the 3-year age group.

More than 80% of those aged between 4 and 35–39 years had protective tetanus antitoxin levels; however, there was a progressive decline in protection from 70% in the 40–49-year age group to 53% in the age

group of 60 years and above. Between 74 and 78% of 1–3-year olds tested had protective tetanus antitoxin levels. On comparison of the diphtheria and tetanus antitoxin profiles in Fig. 1, two increases of diphtheria antitoxin levels greater than 0.1 IU/ml and tetanus antitoxin levels greater than 1.0 IU/ml are apparent, starting at 4 and 14 years of age.

The geometric mean titres (GMT) of diphtheria and tetanus antitoxins by age and sex are given in Fig. 2(a) and (b), respectively. For both females and males, the diphtheria and tetanus antibody profiles show two increases, beginning at 4 and 14 years. There is a discrepancy between male and female GMTs for teenagers and adults in relation to tetanus, as it is evident that adolescent and adult males have significantly higher anti-tetanus GMTs than females in the same age groups ($P < 0.01$). There is no difference between the GMTs for males and females for tetanus in children ($P = 0.17$) or for diphtheria in adults ($P = 0.96$) or children ($P = 0.13$).

In Fig. 3, the correlation of diphtheria and tetanus antitoxin titres in selected age groups (1–3, 4–13, 14–19, and 20+ years) is shown. There is a strong positive correlation between individuals' antibody titres against diphtheria and tetanus toxin for all age groups, although the correlation is less clear in adults; the correlation coefficients for the four age groups are 0.35, 0.65, 0.43 and 0.3, respectively. The slope of this relationship is much steeper in the 4–13-year age group compared to the other age groups indicating that there are considerable numbers of individuals with both high diphtheria and tetanus antitoxin levels.

4. Discussion

This is the first survey to measure both diphtheria and tetanus antibody levels in a large age and sex stratified set of serum samples collected from England and Wales. The diphtheria antitoxin titres obtained have been classified, according to established criteria [17], levels of less than 0.01 IU/ml indicate susceptibility, levels of 0.01 IU/ml to 0.1 IU/ml provide protection against the toxic manifestations of disease and levels greater than 0.1 IU/ml are fully protective. For the tetanus antitoxin titres, there is a lack of established criteria for interpreting antitoxin levels; we have chosen levels of less than 0.1 IU/ml as susceptible, levels of 0.1–1.0 IU/ml as protective and levels in excess of 1.0 IU/ml as highly protective. Some studies [18] use an antitoxin titre of less than 0.01 IU/ml to indicate susceptibility, particularly when this is measured using an *in vivo* neutralisation assay. A higher cut-off (0.1 IU/ml) for tetanus susceptibility has been adopted, in view that an ELISA has been used [19] and that there is considerable clinical evidence [20] that tetanus can

occur in individuals possessing tetanus antitoxin levels greater than 0.01 IU/ml.

Less than 50% of the population tested, aged 50 years and above, had some protection against diphtheria and less than 20% of those aged 25 years and above had full protection against diphtheria. Previous studies [21,22] have also shown low levels of diphtheria immunity in older adults in England and Wales. Since October 1994, an extra booster dose of diphtheria toxoid [8] has been combined with tetanus toxoid, which is routinely given to 15–19-year olds, in an attempt to improve levels of immunity to diphtheria in adults. This action was prompted by concern about

the epidemics of diphtheria in eastern Europe and the waning of vaccine induced immunity in adults in the UK. The effect of this policy is apparent in both Figs. 1 and 2 in which levels of diphtheria immunity are seen to rise at 14 years. The increase in diphtheria antitoxin levels is not as pronounced as the increase in tetanus antitoxin levels which is to be expected as only a low-dose diphtheria toxoid is given. There is a significant difference in the level of tetanus immunity between females and males aged 16–39 years which probably reflects a higher uptake of booster vaccination following injury or as a result of military service. Other studies [23] have also shown a significant

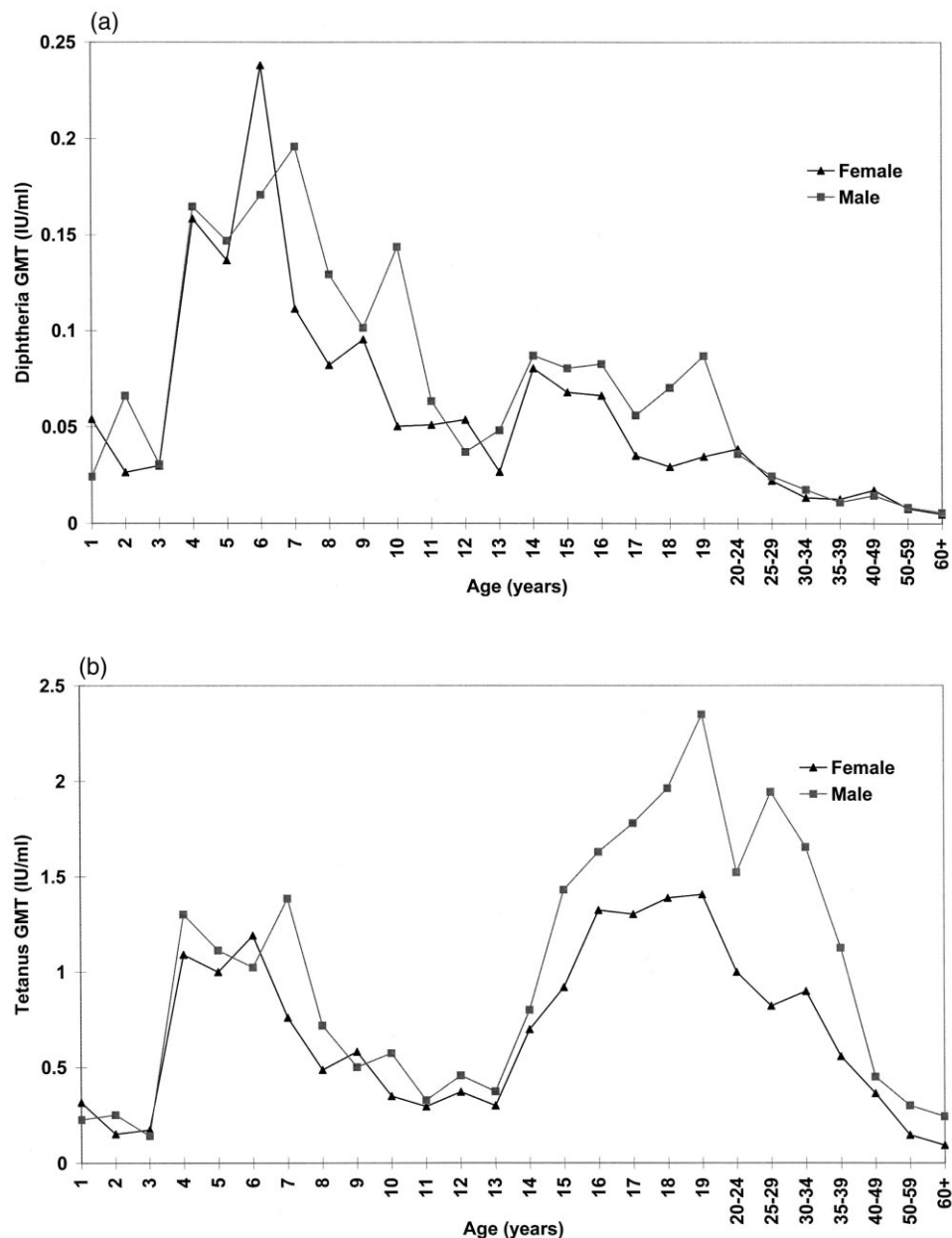


Fig. 2. (a) Diphtheria GMTs by age and sex. (b) Tetanus GMTs by age and sex.

difference between tetanus antitoxin levels in men and women. A considerable number of the elderly have antitoxin levels below the protective threshold and this age group is facing utmost risk from tetanus [24]. A case can be made for giving an additional tetanus booster in late middle age [25]; however, the benefits of such a policy have to be weighed against the costs and feasibility of such an undertaking together with the potential for considerable local reactions associated with receiving more than five doses of tetanus toxoid [26]. Improving levels of diphtheria immunity in the elderly has also been advocated [27]; however, because of the virtual disappearance of the disease in the UK [28], the addition of further boosters does not appear to be necessary.

In 1968, a single national immunisation schedule was introduced [29] which recommended three doses of triple vaccine be given at 3, 5 and 9 months of age and, in relation to diphtheria and tetanus immunisation there has been little change in this procedure, until 1990, when it was superseded by an accelerated schedule in which triple vaccine was given at 2–4 months of age [7]. It is evident from this study that the “accelerated schedule” produces an effective immunological memory response for both diphtheria and tetanus as shown by the high antitoxin levels following pre-school vaccination. Very recently [30] vaccination of babies aged 2–4 months with meningococcal C conjugate vaccines has commenced. The protein conjugate

in such vaccines is either mutant diphtheria toxin, i.e., CRM₁₉₇ [11] or tetanus toxoid [12] and it will be important to determine the impact that these will have on immunity to diphtheria and tetanus. Clinical trials data (Miller, unpublished) with these vaccines in pre-school (3–4-year olds) and adolescents (13–17-year olds) clearly show that CRM₁₉₇ induces a high booster response to diphtheria and, similarly, that the tetanus toxoid conjugates induce a high response to tetanus. The responses are equivalent in magnitude to those induced by Td or DT booster doses. Repeating this study on a regular basis will enable the overall impact of changes in immunisation policy, such as the introduction of meningococcal C conjugate vaccination or modification of pertussis containing vaccines, to be monitored and, if necessary, acted upon.

The collection of national immunity profiles, particularly if the data obtained are standardised, can be of benefit in international collaborative studies. The diphtheria immunity component of this study was performed as part of a European wide initiative, the European Sero-epidemiology Network (ESEN) sponsored by the BIOMED 2 programme of the European Community. The aim of ESEN is to coordinate and harmonise the serological surveillance of immunity to vaccine preventable diseases, in participating countries, and the data generated will assist with the evaluation and rationalisation of vaccine policy throughout Europe [31].

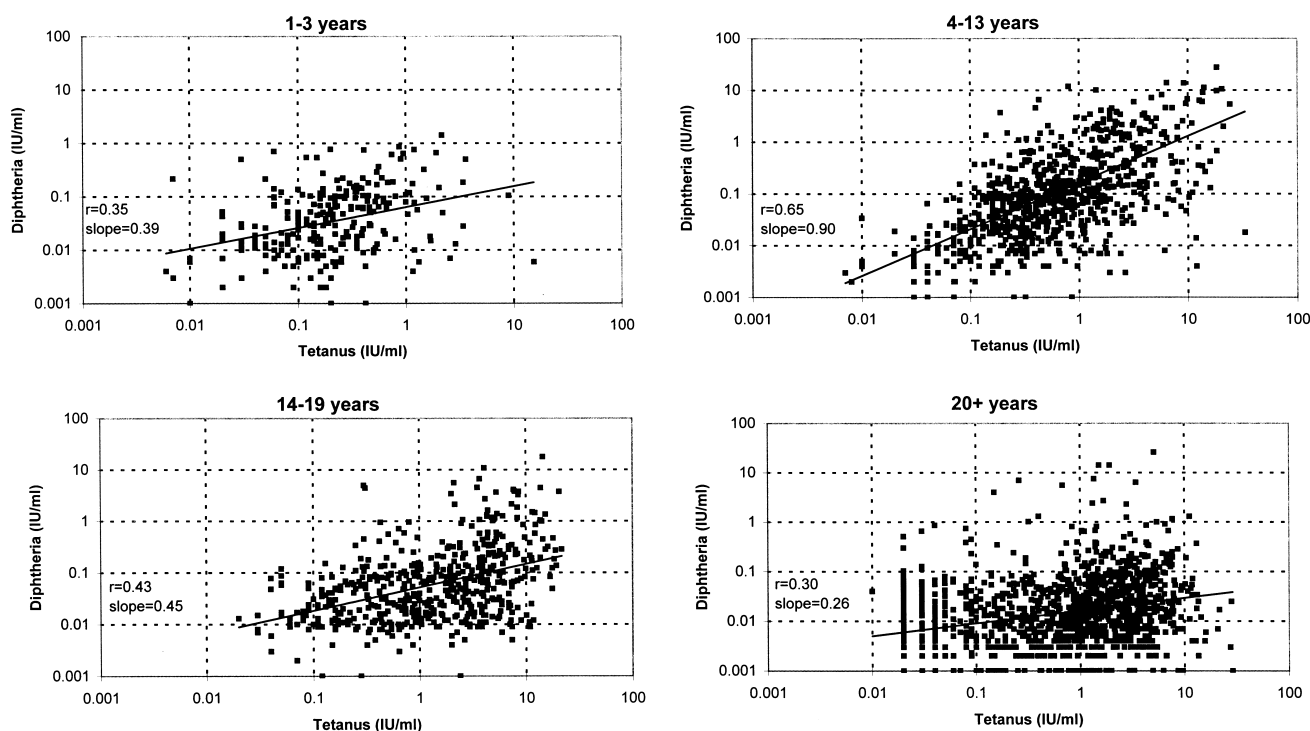


Fig. 3. Scatterplots of diphtheria and tetanus antitoxin results (IU/ml) with regression lines and correlation coefficients (r) in four age groups.

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