
Seroepidemiology of diphtheria and pertussis in Luxembourg in 2000

J. MOSSONG^{1,2*}, L. PUTZ^{1,2}, Z. SHKEDY³ AND F. SCHNEIDER¹

¹ *Laboratoire National de Santé, Luxembourg*

² *CRP-Santé, Luxembourg*

³ *Centre for Statistics, Limburgs Universitair Centrum, Diepenbeek, Belgium*

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SUMMARY

A large serosurvey was carried out in Luxembourg in 2000–2001, to determine the population immunity against a number of vaccine-preventable infections including diphtheria and pertussis. Immunity to diphtheria and pertussis was assessed using an in-house neutralization assay and a commercial ELISA test respectively. Mean pertussis antibody activity decreased from 4 to 8 years of age, reflecting the effects of waning of vaccine-induced immunity. Mean pertussis antibody activity increased during adolescence due to infection in previously vaccinated individuals and levelled out after approximately 20 years of age. For adults >25 years age, a statistically significant 30% difference in mean antibody activity between men and women was observed. The proportion of seronegatives for diphtheria among children and adolescents aged <20 years was 2.5% reflecting the high vaccination coverage. The proportion seronegative for diphtheria tended to increase with age such that 42% of individuals aged >40 years were seronegative. Our study supports the recently introduced acellular pertussis vaccine booster at 6 years to reduce pertussis transmission in school-aged children and adolescents.

INTRODUCTION

The seroepidemiology of pertussis and diphtheria in Luxembourg has mostly been determined by mass vaccination programmes that started in the middle of the last century. In most if not all Western European countries, vaccines against these two bacterial infections are administered to infants in their first year of life, most often combined with tetanus (DTP). In recent years, other components have been added to the vaccine and in many countries, the original whole-cell pertussis vaccine has been replaced by an acellular vaccine, which is thought to cause less adverse events upon immunization while being equally or more immunogenic.

Vaccination led to the virtual elimination of diphtheria in Western Europe during the second half of the 20th century. In Luxembourg the last diphtheria case was recorded in 1981 in an individual who was not vaccinated. However, in the 1990s epidemic diphtheria re-emerged in the newly independent states, which was mainly due to decreasing immunization coverage among infants and children, and adults no longer immune because of waning of vaccine-induced immunity and lack of booster doses [1]. Although the epidemic never spread to western parts of Europe, this episode highlighted the need to assess immunity levels in European countries to determine the potential risk of epidemic diphtheria [2].

The epidemiological situation for pertussis is different. While routine vaccination led to a major reduction of pertussis in infants and young children, widespread transmission of *Bordetella pertussis* still

* Author for correspondence: Dr J. Mossong, Laboratoire National de Santé, PO Box 1102, L-1011 Luxembourg.
(Email: joel.mossong@lns.etat.lu)

occurs. There is increasing evidence that pertussis continues to affect adolescents and young adults who were vaccinated as infants, albeit in a less severe form. This is thought to be due to pertussis vaccine efficacy waning more rapidly than for vaccines against viral childhood infections (e.g. measles). Given that the epidemiology has shifted from a serious disease affecting young children to a milder or more moderate disease affecting older children, adolescents and adults, pertussis booster doses for these older groups are being increasingly considered for inclusion in official vaccination schedules [3]. The booster vaccine for adults and adolescents was only introduced to the vaccination schedule in 2002, so it is unlikely that adolescents were vaccinated prior to this date.

To address the extent to which these important public health questions are relevant in Luxembourg, a large population-based seroprevalence study was carried out in 2000–2001 to determine immunity levels in the population. While overall eight vaccine-preventable infections were studied in the context of the European ESEN 2 project [4], this paper describes the results and implications for pertussis and diphtheria.

METHODS

Study population and survey design

Serum samples were collected prospectively using a multi-tiered study design according to sample specifications of the ESEN 2 project [2]. Samples from children and adolescents were collected from randomly selected primary and secondary schools chosen at random from different geographical regions. The number of primary schools chosen in each region was proportional to the population size of the region: one primary school was selected at random in each of the northern and eastern political regions, two in the central region and three in the southern region. One secondary school was selected from each of the northern/eastern, central and southern regions. Pupils and students or their parents in selected schools were given a leaflet explaining the aims the study including a short description of the vaccine-preventable infections.

Serum samples of adults were obtained from adult volunteer blood donors at the national Red Cross Centre, adult volunteers undergoing compulsory pre-marital testing [prior to marriage, all couples need to undergo the following compulsory testing: syphilis,

tuberculosis (TB) and blood group for males, and toxoplasmosis, rubella, syphilis, TB and blood group for females; an HIV test is offered on a voluntary basis], and adult volunteers coming for routine blood tests at the National Health Laboratory. All study participants were offered test results via a doctor of their choice that could give advice on additional vaccinations if deemed necessary.

Serology

A commercial enzyme-linked immunosorbent assay Serion ELISA classic (Institut Virion/Serion GmbH, Würzburg, Germany) was used to determine IgG antibodies against *B. pertussis*. In this assay, according to the manufacturer, a mixture of pertussis toxin (PT) and filamentous haemagglutinin (FHA) antigens was determined using reference *B. pertussis* anti-serum supplied by the Food and Drug Administration (USA). This assay performed satisfactorily in a recent pan-European study [5] when compared to other quantitative methods. Manufacturer classification of negative, equivocal and positive samples is for activities <20, 20–30 and >30 FDA-U/ml respectively.

Antibodies to diphtheria were determined using a Vero cell toxin neutralization assay [6]. Diphtheria toxin (1000 Lf/ml) was obtained from the Rijksinstituut voor Volksgezondheid en Milieu (Bilthoven, The Netherlands) and the antitoxin reference serum from the Instituto Superiore di Sanita (Rome, Italy). Sera were classified as negative for antibody concentrations <0.01 IU/ml, low positives for concentrations of 0.01–0.1 IU/ml and high positives for concentrations >0.1 IU/ml.

Statistical analysis

Univariate kernel density estimates of the distribution of log₁₀-transformed pertussis antibody activity were calculated using the *k* density function in STATA 8.0 (StataCorp, College Station, TX, USA) with a Gaussian kernel and the optimal bandwidth minimizing the mean integrated square error.

Differences in antibody activity between gender and age groups were tested using a *t* test on equality of log-transformed means.

The log-transformed pertussis antibody activity and diphtheria antibody concentrations were smoothed with a local regression model with a variable smoothing parameter, which was chosen using the cross-validation method [7]. The choice of the

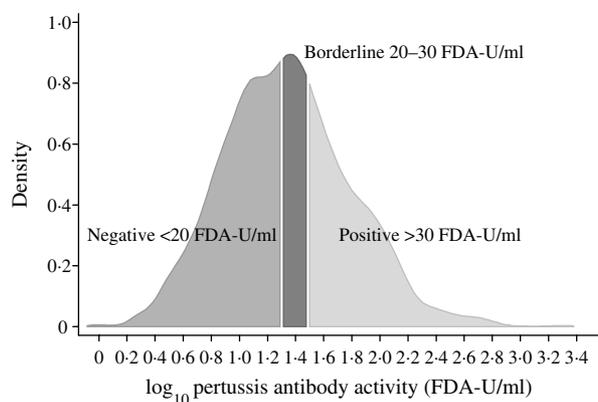


Fig. 1. Kernel density estimate of log-transformed antibody activity against pertussis of all samples as measured by IgG ELISA. Different shades of grey show classification as proposed by ELISA manufacturer.

smoothing parameter is a crucial issue when smoothing data with non-parametric regression models, because it can lead to over- or undersmoothing. Therefore, an automatic data-driven selection method, such as the cross-validation method, is recommended. We used an automatic selection of locally variable smoothing parameter, so that the amount of smoothing at each age is data-driven. The smoothing analysis was carried out in S-PLUS 2000 (MathSoft, Seattle, WA, USA).

RESULTS

Sample collection

2673 serum samples were obtained. One primary school in the eastern region refused to participate in the study. The participation rate in six primary and three secondary schools was 47.2% overall. Actual participation rates were slightly higher, but in a small fraction of volunteer pupils, no sufficient blood sample could be drawn. Participation was highest among 12- to 15-year-olds (>60%) and lowest for young children and the older students in secondary schools. Samples from adults were collected from July 2000 until April 2001, but no data was available on participation rates.

Pertussis

From the estimated kernel density distribution of log-transformed pertussis antibody titres (Fig. 1), it is clear that the distribution of pertussis antibody titres in the Luxembourg population sample has

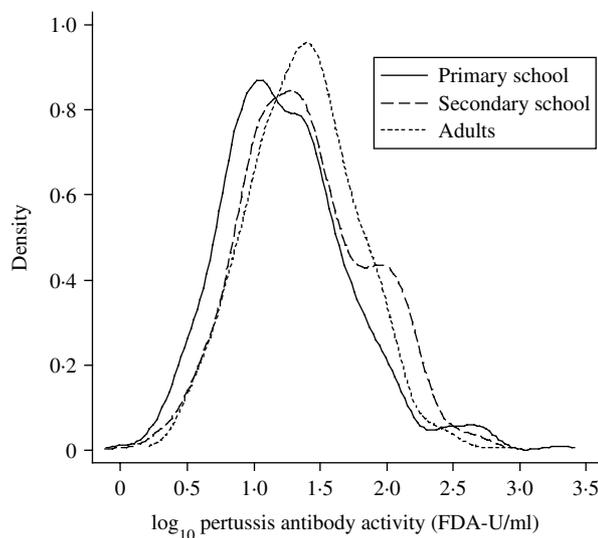


Fig. 2. Comparison of kernel density estimate of the log-transformed antibody activity according to origin of samples: primary school (mean age 8.8 years), secondary school (mean age 16.2 years), adults (mean age 40.7 years).

only one peak and there is no obvious separation of results into positive and negative populations according to the cut-offs given by the manufacturer [8].

However, when stratifying the density distribution of log-transformed antibody titres according to age groups (Fig. 2), some features emerge. Children in primary schools have the lowest antibody levels and the main peak occurs at 1.05, equivalent to ~11 FDA-U/ml. Adolescents in secondary schools have higher titres in general (main peak at 1.29, equivalent to ~19 FDA-U/ml) and there appears to be a sizable subpopulation of individuals with higher titres still (a second peak occurs at 1.95, equivalent of ~89 FDA-U/ml). This probably stems from adolescents with recent or ongoing *B. pertussis* infection. The main peak of adults is highest of the three groups (1.4, equivalent to ~25 FDA-U/ml) probably indicating that a certain level of infection continues to occur after adolescence into adulthood.

A similar picture emerges when smoothed mean antibody activity using local regression techniques are analysed (Fig. 3). For both sexes in the youngest group of the sample, pertussis antibody activity clearly decreased until ~8 years, reflecting the effects of waning of vaccine-induced immunity. Mean antibody activity then increased during adolescence, probably due to ongoing infection in these age groups. For men, mean antibody levels out after ~20 years and stays constant throughout adulthood.

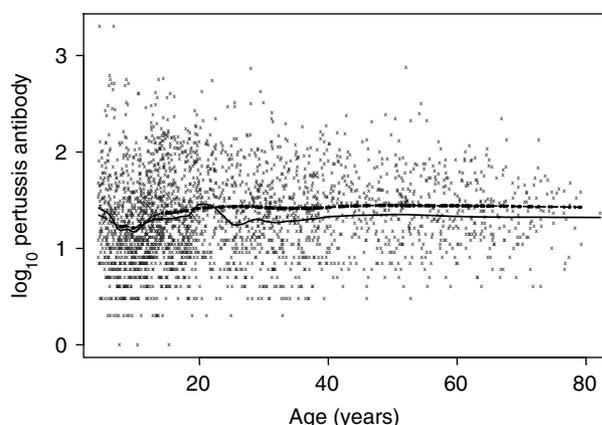


Fig. 3. Scatter of log-transformed pertussis activity as a function of age. Lines indicate smoothed mean local regression for males (---) and females (—).

For women, antibody levels seem to increase somewhat more slowly during adolescence. Moreover, for adults >25 years age, there was a 30% difference in mean antibody activity between sexes with a mean of 21 FDA-U/ml in adult women compared to 27 FDA-U/ml for men ($P < 0.0001$).

Diphtheria

For the youngest age groups, the proportion of high diphtheria seropositives (Fig. 4) appears to increase until the age of 8 years, which is probably due to the administration of a tetanus-diphtheria booster dose at 5–6 years (routine in Luxembourg). The proportion of seronegatives in children and adolescents aged <20 years was low at 2.5%. However, the seronegative proportion tended to increase with age from ~20 years onwards such that 42% of individuals in our sample aged >40 years are seronegative.

A similar picture is seen in Figure 5, which shows the smoothed mean antibody concentrations. The effects of the booster dose are clearly visible at age 5–7 years, and antibody concentrations decrease for both sexes from then on, probably due to insufficient number of booster doses. For individuals aged ≥ 50 years in our sample, there is an interesting difference between sexes which has also been observed in other countries [2] and is probably due to vaccination of conscripts when military service for males was made compulsory from 1944 until 1967.

DISCUSSION

Despite the large size of collected serum bank samples – 2673 serum samples correspond to 0.6%

of the total resident population in Luxembourg – size by itself does not necessarily guarantee that the sample is representative of the population. Selective participation could be an important source of bias when investigating seroprevalence of vaccine-preventable diseases given the low participation rate in some age classes. However, the most important sources of bias, age and gender, were controlled for by the age-stratified study design of the sampling scheme. Schools were chosen at random in different regions to ensure a degree of geographical and social diversity. A feature of the demographical situation in Luxembourg is that a large proportion (35%) of the resident population is of foreign origin. In the school-aged population at least, no difference in participation rates according to nationality was observed, thus excluding nationality as an important source of bias. Other biases could of course still be present (e.g. that parents opposing vaccination would be more likely to oppose participation in the study) but the extent of this is difficult to assess, although it would mean that our estimates of population seroprevalence (particularly for diphtheria) are possibly too high.

Although diphtheria and pertussis are given simultaneously in multivalent vaccine combination during infancy, their seroprevalences in the general population differ substantially. Concentration of antibodies and, thus, immunity to diphtheria is high for children and adolescents in Luxembourg, confirming results from a previous vaccine coverage survey in children [9]. However, our study suggests that large sections of the older adult population are not well protected against diphtheria. This gap of immunity in older adults has been observed in other countries and the proportion seronegative in Luxembourg is similar to that observed in neighbouring France or Germany [2]. The rapid decrease of diphtheria titres after 20 years of age suggests that although 10-yearly booster doses are recommended throughout adulthood, these recommendations are (or have been) poorly followed in practice. If diphtheria is going to re-appear on the European continent as in the early 1990s, vaccination efforts should principally be targeting older age groups which lack necessary protection and are probably most vulnerable to complications of the disease.

For pertussis, our study is clearly limited by the lack of a clear serological correlate of protective immunity against pertussis disease [10]. The cut-off suggested by the manufacturer for negative, equivocal and positive results is problematic due to the lack

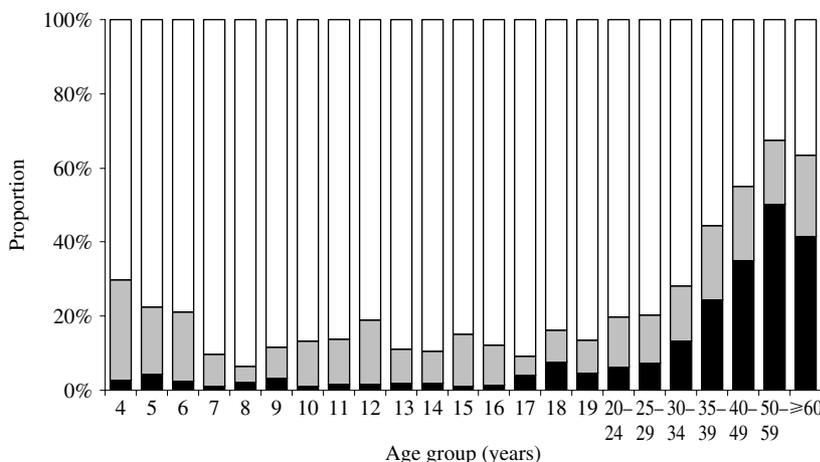


Fig. 4. Serological antibody status for diphtheria as a function of age. ■, Negative; □, low positive; □, high positive.

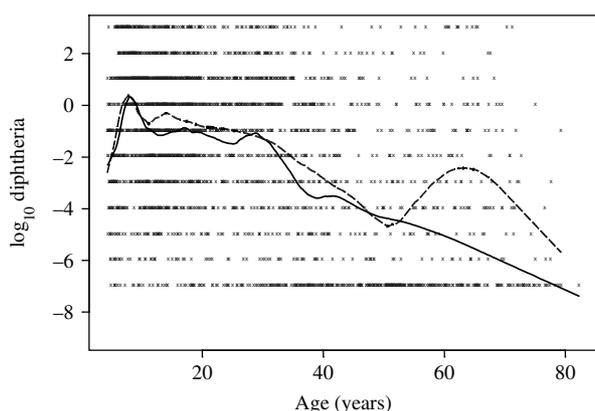


Fig. 5. Scatter of log-transformed diphtheria antibody concentration as a function of age. Lines indicate smoothed mean local regression for males (—) and females (---).

of separate distributions of negative or positive populations, so it is not clear what positive or negative results mean for our particular assay. Although serology cannot reliably distinguish between natural and vaccine-induced immunity for individual titres [11], our data indicate that titres in primary schoolchildren are generally lower than in secondary schoolchildren. A plausible explanation for this difference is that ongoing transmission in these age classes is responsible for boosting titres. Thus, it is likely that pertussis has not been eliminated in Luxembourg, but continues to affect mainly adolescents and young adults. Unfortunately little clinical data are available in Luxembourg to confirm these findings. No pertussis cases were notified in the period 1997–2002, although 10 cases were notified in 2003. However, it is widely recognized that routine notifications underestimate pertussis cases, particularly

in older age groups [10]. Moreover, the quality and ascertainment rate of official case notifications in Luxembourg has not formally been evaluated and, thus, data for pertussis cases are most certainly under-reported and under-diagnosed.

Direct comparison of our data with other countries' serological profiles is potentially problematic and would require a rigorous statistical standardization into common units [5]. This work is currently in progress as part of the ESEN 2 project [12]. Nevertheless, the seroepidemiological situation in Luxembourg is comparable to other Western European countries with a history of high DTP coverage. In a recent study based on the original ESEN data, Pebody and colleagues have found that high titre sera are more likely to occur in older age groups in countries with a history of high immunization coverage [13]. On the other hand for countries with lower coverage (West Germany and Italy), high titre sera were found more frequently in younger age groups.

Following the introduction of acellular booster doses in older age groups by some European countries [14], the Conseil Supérieur d'Hygiène, the national board advising the government on vaccination policies, decided in 2002 to include an acellular pertussis vaccine booster in the vaccination schedule at 5–7 years and every 10 years thereafter. Results from our study support this action and it will be interesting to see the long-term effects of this booster dose. Our study, which was conducted before the introduction of this pertussis booster, will provide a valuable baseline against which to measure the impact of the acellular booster doses on antibody levels in the population.

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DECLARATION OF INTEREST

None.

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