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Comparative seroepidemiology of diphtheria in six European countries and Israel

P. DI GIOVINE[1], G. KAFATOS[2], A. NARDONE[2], N. ANDREWS[2], R.M. ÖLANDER[3], G. ALFARONE[1], K. BROUGHTON[2], D. COHEN[4], B. KRIZ[5], I. MIKOVA[6], D. O'FLANAGAN[7], F. SCHNEIDER[8], I. SELGA[9], L. VALINSKY[10], I. VELICKO[9,11], I. KARACS[12], R. PEBODY[2] AND C. VON HUNOLSTEIN[1]*

[1] *Istituto Superiore di Sanità, Rome, Italy;* [2] *Health Protection Agency, London, UK;* [3] *National Institute of Health and Welfare, Helsinki, Finland;* [4] *Department of Epidemiology and Preventive Medicine, School of Public Health, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel;* [5] *National Institute of Public Health, Centre of Epidemiology and Microbiology, Prague, Czech Republic;* [6] *Department of Medical Microbiology, Regional Public Health Authority, Košice, Slovac Republic;* [7] *Health Protection Surveillance Centre, Dublin, Ireland;* [8] *Department of Microbiology, Laboratoire National de Santé, Luxembourg;* [9] *State Agency 'Public Health Agency', Riga, Latvia;* [10] *Central Laboratories, Ministry of Health, Jerusalem, Israel;* [11] *Swedish Institute for Infectious Diseases Control, Solna, Sweden;* [12] *National Centre for Epidemiology, Budapest, Hungary*

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SUMMARY

Serological surveys for diphtheria were conducted in six European countries including Czech Republic, Hungary, Ireland, Latvia, Luxembourg, Slovakia and one country outside Europe, Israel. For each country, a nationally representative population sample was collected across the entire age range and was tested for antibodies to diphtheria toxin. Although each national laboratory used its preferred assay, the results were all standardized to those of the *in vitro* neutralization test and expressed in international units (IU) which allowed comparative analyses to be performed. The results showed that increasing age is related to a gradual increase in seronegative subjects ($<0{\cdot}01$ IU/ml of diphtheria antitoxin antibodies). This may reflect waning immunity following childhood vaccination without repeated booster vaccinations in adults. Differences in seronegativity were also found according to gender. In subjects aged 1–19 years, geometric mean titres of antitoxin are clearly related to the different vaccination schedules used in the participating countries. Although clinical disease remains rare, the susceptibility to diphtheria observed in these serosurveys highlights the importance of strengthened surveillance.

Key words: Diphtheria, immunoepidemiology, serology.

INTRODUCTION

Before the introduction of mass and routine immunization, diphtheria was a common cause of morbidity and mortality. The disease was characterized by periodic epidemics, with the last major European epidemic occurring during the 1940s. With socioeconomic improvement and the introduction of mass infant immunization during the 1940s and 1950s, there was a marked reduction in the incidence of infection in Europe [1, 2]. The incidence reached an all-time low in 1980 when only 623 cases were reported from the European region of the World Health

* Author for correspondence: Dr C. von Hunolstein, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy. (Email: christina.vonhunolstein@iss.it)

Organization (WHO). At that time, the elimination of diphtheria from the region seemed imminent, and the WHO Regional Committee for Europe endorsed a target of eliminating indigenous cases by 2000 [3]. However, in 1993, epidemic diphtheria re-emerged in the New Independent States (NIS) of the former Soviet Union, beginning in the Russian Federation [4, 5]. Russia had 39 582 (83%) of the 50 412 cases reported by the NIS in 1995 [6, 7]. The highest incidence rates were among adolescents and adults aged 40–49 years [8]. As many cases occurred in adults most of whom would have been previously vaccinated, it seems that immunity as well as antibody levels may have declined following vaccination [9]. Although the reasons for the diphtheria epidemic in the NIS are not fully understood, factors contributing to the epidemic included a large population of susceptible adults, decreased childhood immunization coverage, suboptimal socioeconomic conditions and high population movements [8, 10]. Between 1993 and 2000 several cases of diphtheria were also reported by other European countries, some of which were linked to the epidemic in NIS [11–15]. In almost all cases, the patients were middle-aged individuals that had non-protective levels of antitoxin antibodies [11–15]. As a result of the NIS outbreak, the target of elimination in Europe has been postponed indefinitely.

The epidemic raised many concerns about the risk of potential epidemics or sporadic cases due to travel of unprotected individuals to endemic and epidemic areas. Hence, it became crucial to document the level of immunity to diphtheria in Western Europe through large, population-based serosurveys. In 1996, with funding from the European Commission, the European Sero-Epidemiology Network (ESEN) was established [16]. ESEN surveillance demonstrated that there were large differences in the proportion of adults with insufficient levels of protection to diphtheria in various participating countries. For instance, about 35% of 50- to 60-year-olds were found to be seronegative (antitoxin levels $\leqslant 0{\cdot}01$ IU/ml) in Finland compared to 70–75% in the UK. Although all countries showed high levels of infant vaccine coverage ($>90\%$), the vaccination schedule clearly influenced the antitoxin levels [17]. As an extension of the original ESEN project, the European Sero-Epidemiology Network 2 (ESEN2) was established in 2001. Large population-based serosurveys from six European countries (Czech Republic, Hungary, Ireland, Latvia, Luxembourg, Slovakia) and one country outside Europe, Israel, were tested for antibodies to diphtheria toxin. These serosurveys were used to establish the levels of susceptibility to diphtheria and the effectiveness of the different vaccination programmes in the participating countries.

METHODS

Sera collection

In each country an age-stratified serosurvey of approximately 3300 individuals was collected between 2000 and 2003 and tested for diphtheria antitoxin. The minimum number of samples required to achieve good precision around the age-specific seroprevalence estimates was 100 from yearly age groups of 1–19 years, then 200 samples for 5-year age groups up to age 39 years, then 200 samples for 10-year age groups up to 59 or 69 years and finally, 200 samples for those aged >60 or >70 years [16]. For example, a prevalence estimate of 70% for an age group between 1 and 19 years would give a 95% confidence interval (CI) width of $\pm 9\%$. A 90% prevalence and a 200-sample age group, would give a 95% CI width of $\pm 4{\cdot}2\%$.

Throughout the ESEN2 project, two sampling techniques have been used as in the original ESEN project [16]. Hungary, Ireland and Israel used residual sera collected during routine laboratory testing; Czech Republic, Latvia, Luxembourg and Slovakia used sera collected as part of population-based surveys.

For each serosurvey samples were collected from a wide range of geographical locations within each country. Routine sera from specific sub-populations not representative of the general population (e.g. immunocompromised) were not included [16]. For each serum specimen, age, gender and year of collection was obtained.

Testing and inter-laboratory standardization of diphtheria toxin antibody measurements

Each country undertook diphtheria antitoxin testing in a designated national laboratory.

The assays were performed according to well described procedures [18–21]. Czech Republic, Ireland, Luxembourg and Slovakia used the VERO cell neutralization test (NT); Hungary used a passive haemagglutination assay (PHA); Israel used a double antigen ELISA (DAE) and Latvia used a commercial ELISA kit (Hycor, UK).

To achieve quantitative comparability of assay results between countries, the results of diphtheria

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Table 1. *Assays, correlation with the reference test (NT performed by Finland) and standardization equations*

Country	Assay	R^2	Standardization equation*
Czech Republic	NT	0·96	$y=1{\cdot}18x+0{\cdot}77$
Hungary	PHA	0·74	$y=0{\cdot}12x^2+1{\cdot}05x-0{\cdot}12$
Ireland	NT	0·94	$y=0{\cdot}16x^2+1{\cdot}26x-0{\cdot}15$
Italy	DA-DELFIA	0·86	$y=1{\cdot}12x-0{\cdot}18$
Israel	DAE	0·76	$y=0{\cdot}71x-0{\cdot}15$
Latvia	ELISA	0·90	$y=0{\cdot}04x^2+0{\cdot}74x+0{\cdot}19$
Luxembourg	NT	0·91	$y=0{\cdot}16x^2+1{\cdot}22x-0{\cdot}07$
Slovakia	NT	0·80	$y=1{\cdot}02x-0{\cdot}16$

NT, VERO cell neutralization test; PHA, passive haemagglutination assay; DA-DELFIA, double-antigen delayed time-resolved fluorescence immunoassay; DAE, double antigen ELISA; ELISA, enzyme-linked immunosorbant assay.

* x is the $\log_{10}$ reference country titre; y is the $\log_{10}$ titre of the country being standardized.

antitoxin testing were standardized using a methodology previously developed for the original ESEN project and modified for ESEN2 [22, 23].

In brief, a diphtheria reference centre was established in Italy responsible for collection and testing of a reference panel of approximately 150 samples containing negative, low-positive and positive sera. The participating laboratories tested the standard panel using the *in vitro* assay of their choice, twice: once before and once during the serosurvey testing. Standardization equations were developed by regressing the local results (those tested at the same time as the serosurvey) against those of the NT test performed by Finland. Those assays that had an R^2 (the coefficient of determination) of $\geqslant 0{\cdot}80$ were accepted for the study (Table 1); those with an R^2 as low as 0·74 were also considered, but with caution as some degree of misclassification may have occurred. The standardization equation was calculated to convert each country's serosurvey results to a common unit, thereby allowing international comparisons to be made. In order to be sure that the data could also be compared with the previous ESEN study, it was important to ascertain that the performances of Finland's method remained the same over the period of the two projects. The R^2 and the standardization equation between Finland and Italy ESEN2 is consistent with those obtained in the original ESEN project (0·86 and 0·81, respectively).

All quantitative results were expressed in international units (IU).

The internationally accepted NT cut-off range was used to classify these standardized quantitative results [1]. Antitoxin concentrations, as measured by NT, of $<0{\cdot}01$ IU/ml were classified as negative (i.e. not protective levels of antitoxin, seronegative subjects, not protected subjects). Antibody concentrations of 0·01–0·099 IU/ml were grouped as low-positive (i.e. partial protective levels of antitoxin, not well protected subjects) and antitoxin $\geqslant 0{\cdot}1$ IU/ml as positive (i.e. protective levels of antitoxin, seropositive subjects, protected subjects).

Geometric mean antibody titres (GMTs) were calculated using serum samples with antitoxin levels $\geqslant 0{\cdot}01$ IU/ml.

Vaccine programme structure and coverage

As part of the ESEN2 project, country-specific data were gathered on diphtheria vaccine programmes, vaccine coverage and reported incidence of diphtheria infection. A questionnaire was created by the coordinating centre in London and distributed to the ESEN2 project coordinators in each of the seven participating countries.

RESULTS

Figure 1 depicts the incidence of diphtheria from 1970 up to 2001 in Czech Republic, Hungary and Latvia (note that the scale for Latvia is different). The highest incidence is seen in Latvia where during 1993–2001, a diphtheria epidemic occurred with 1288 cases reported overall, of whom 96 died. Hungary and Czech Republic reported a very low incidence with sporadic cases occurring in 1974–1981 and 1970–1981. In Israel and Slovakia the incidence of diphtheria was reported as very low (0–0·02 cases/100 000 population), Luxembourg reported one case in 1981 and Ireland did not have any cases (data not shown).

Table 2 provides a summary of the diphtheria immunization schedules in use in the participating countries when the sera were collected (2001 for Czech Republic, and Luxembourg, 2000–2001 for Israel and 2003 for all other countries) as well as the year the schedule was introduced and recent changes to the schedule. All countries administered three doses of diphtheria vaccine over the first year of life starting at either 2 or 3 months of age. The scheduling of the booster doses in children was variable across the

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Table 2. *Current diphtheria vaccination programmes and recommendations in participating countries up to 2003*

Since …	Czech Republic 1986	Hungary 1971	Ireland 2002	Israel 1999	Latvia 1998	Luxembourg 1999	Slovakia 1998
Primary series (months)	2, 3, 4	3, 4, 5	2, 4, 6	2, 4, 6	3, 4·5, 6	2, 4, 5	2, 4, 10
Age of boosters (years)	1·5, 5	3, 6, 11	4,12*	1, 7, 13	1·5, 7, 14†	1, 5, 9, 15	2, 5
Military recruits				Yes			
Age shift from infant (D) to adult dose (d)	D only	11 years	12 years	D only	14 years	12 years	D only

* Ireland: prior to 1996 no booster at age 12 years.
† Latvia: prior to 1998, age of boosters (years) 1·5, 9, 15.

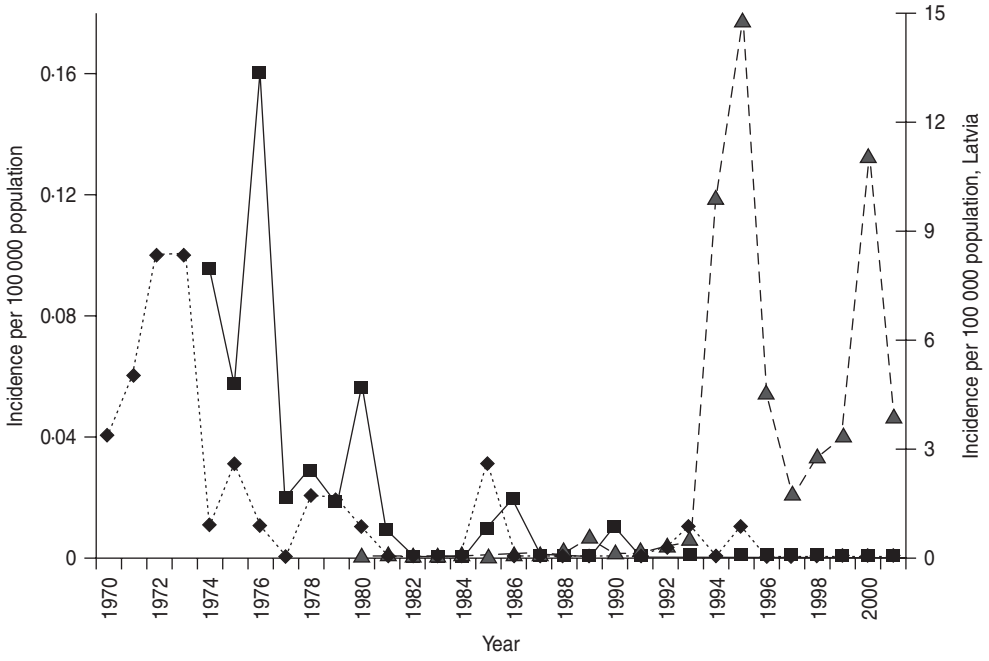

Fig. 1. Incidence of diphtheria per 100 000 population in Czech Republic (···◆···), Hungary (—■—), Latvia (- ▲ -). Note that the scale for Latvia is different.

different countries: the number of boosters scheduled for children was two in Czech Republic, Ireland and Slovakia, and three in the other countries. Booster doses in adults were recommended only in Latvia and Luxembourg. For Czech Republic, Israel and Slovakia there was no switch between infant and adult dosage (low dose of diphtheria toxoid). Vaccine types, i.e. the number of combined antigens to diphtheria toxoid were different from country to country; however, this was not taken into account in the data analysis.

Diphtheria vaccination coverage is reported in Figure 2, together with the standardized age-serological profiles for diphtheria antitoxin. The vaccine coverage for diphtheria vaccine was >95% in Czech Republic, Hungary and Slovakia and >80% in Israel and Latvia. For Luxembourg and Ireland only some of the coverage data were available, with the reported uptake low in certain age groups.

From Figure 2 it is evident that the standardized age-specific serological profile is variable across the different countries, but some common trends are observable (the regression equations used to standardize the serosurvey results into common units can be viewed in Table 1). The majority of individuals aged between 1 and 19 years had serum antibodies against diphtheria toxin above the putative lower protection threshold of 0·01 IU/ml. In Israel, after the age of 2

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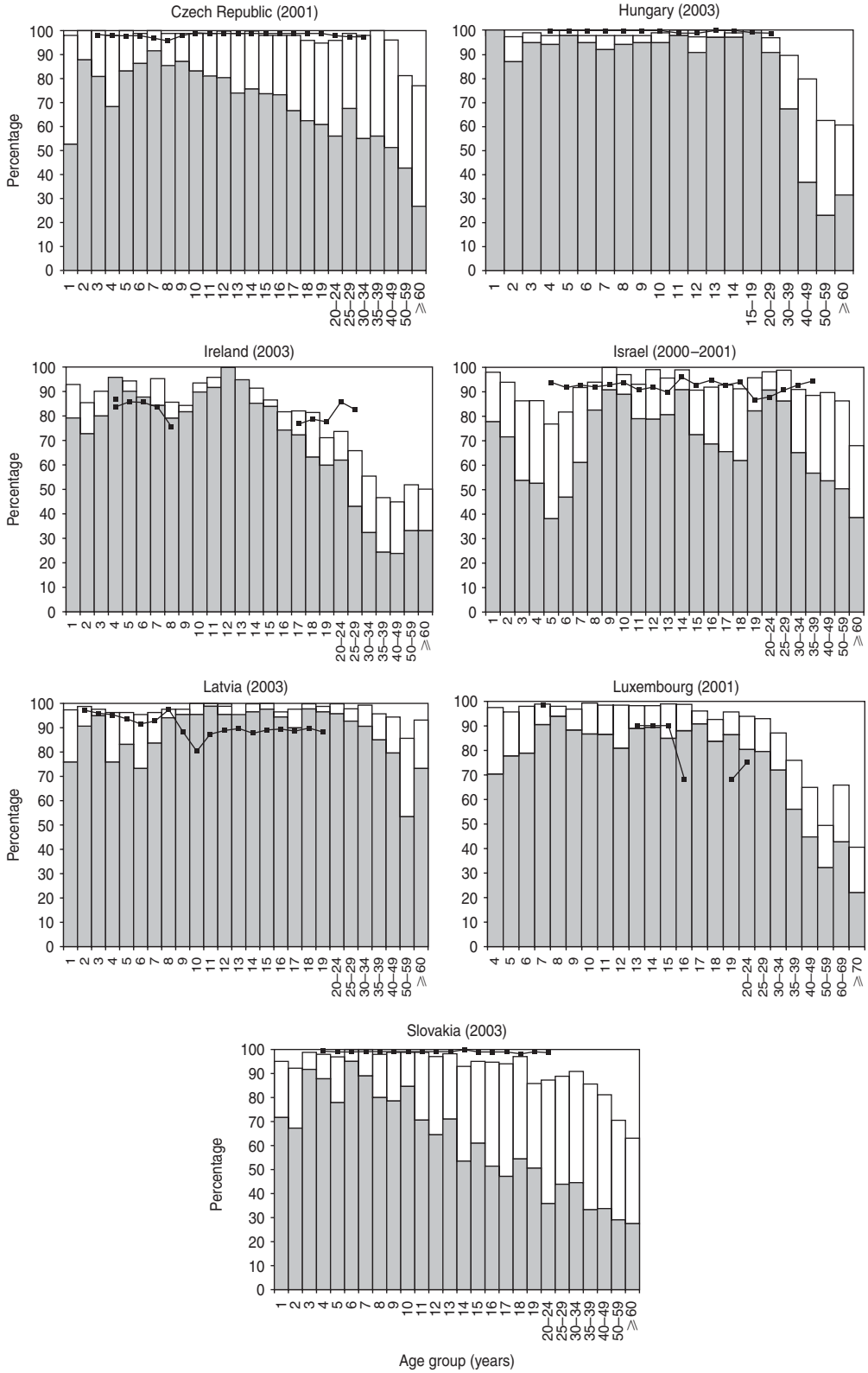

Fig. 2. Standardized age serological profiles. Grey bars (▩) represent the proportion with antitoxin concentrations ⩾0·1 IU/ml, and white bars (□) represent the proportion in each age group with antibody concentration between 0·01 and 0·099 IU/ml. Each bar represents an age group (yearly age groups for 1–19 years, 5-yearly age groups up to 35–39 years, 10-yearly age groups up to 50–59 or 60–69 years, then ⩾60 or ⩾70 years). The year in parentheses after each country is the year in which the sera were collected. The curves represent the percentage of vaccination coverage for diphtheria.

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years, there was a decrease in the proportion of protected children (antitoxin $\geqslant 0{\cdot}1$ IU/ml), that reached a minimum at 5 years ($\sim 40\%$). This was also the point at which the highest proportion of seronegatives was reached ($\sim 20\%$). For Luxembourg no data were available for children aged <4 years.

Diphtheria antibodies rose rapidly in the first year of life, so that in each country, except Czech Republic, more than 70% of 1-year-old children had antibodies $>0{\cdot}1$ IU/ml. In all countries, the quantitative antibody results, in particular the change in the proportion of seropositives, demonstrate the boosting effect provided by re-vaccination of children. The booster dose during military service in young adults can also be clearly discerned in the age serological profile for Israel.

The serological profile of Slovakia shows that the percentage of young subjects with antitoxin levels $>0{\cdot}1$ IU/ml was lower than in the other countries. From the age of 14 years, $<60\%$ were protected. A similar trend was observed in the seroprofile for Czech Republic, but the percentage of positive subjects was higher than in Slovakia. Ireland, compared to the other countries, had the highest proportion of seronegatives from the age of 14 years onwards, while Latvia had the highest percentage of positive individuals in all age groups. Hungary had protected subjects up to age 39 years.

Figure 3 shows the proportion of seronegative individuals (antibody concentrations $<0{\cdot}01$ IU/ml) grouped by age and sex. It is evident that the percentage of non-immune individuals increased with age, except in Latvia. The trend is similar in all countries, but the percentage of seronegatives was very variable. Approximately, $>50\%$ of subjects aged >50 years in Ireland appeared to be seronegative, while in Latvia and in Czech Republic this was only 10% and 20%, respectively. There were also marked differences in seronegatives between males and females in Hungary, Ireland and Luxembourg, with more unprotected females. In Luxembourg, the difference between genders for individuals aged $\geqslant 50$ years is probably due to vaccination of conscripts, since military service for males was compulsory between 1944 and 1967. In Israel, the increase in seropositive subjects in the 19 and 20–24 years age groups may be related to the booster dose given after the age of 18 years during recruitment to the army for both males and females.

The age-specific diphtheria antitoxin GMTs differ widely across countries (Fig. 4). This might be due to the different immunization schedules or diphtheria toxoid dosages in use in the participating countries. The profile of the GMT per country demonstrates the rise and fall in antibody levels which occur after primary vaccination and boosting. The low and more or less stable GMT profile of Czech Republic is due to methodological reasons, as the assay range covered only the antitoxin concentration $<0{\cdot}01$–$1{\cdot}3$ IU/ml.

DISCUSSION

A second large serological survey across a wide age range in six further European countries as well as in Israel has been undertaken to document the current pattern of immunity to diphtheria. In fact, diphtheria has not been eradicated from the world and can be still a health problem, if preventive measures, like childhood vaccination and adult boosting are not properly conducted. A recent example has been provided by the diphtheria epidemic in the Eastern European countries that surprised the scientific community [24]. Only a prompt, concerted and networked activity brought the epidemic under control and reduced morbidity and mortality [8]. More than 115 000 cases and 3000 deaths occurred in the Russian Federation from 1990 to 1997, with a mortality rate higher than 20% at the beginning of the epidemic [10]. Since 2000, the majority of European countries observed sporadic or no cases with the exception of Latvia where cases have continued to occur [25, 26].

The creation, as a consequence of the severe epidemic in the former Soviet Union, of a European Laboratory Working Group on diphtheria [27] and the funding of specific projects both on the microbiological-epidemiological and serological aspects of diphtheria has allowed valuable information to be obtained on this neglected, but still critical disease.

The ESEN and ESEN2 projects were partially funded by the European Commission to coordinate and harmonize throughout Europe the serological surveillance of immunity to vaccine-preventable infectious diseases, one of which was diphtheria. This was achieved by establishing an active, integrated European network of expert laboratory groups involved in the serosurveillance of diphtheria. The seroepidemiology of the original ESEN participating countries (Sweden, Finland, The Netherlands, England & Wales, Germany, Italy) has been previously published [17]. This is now complemented by the addition of six European countries (Ireland, Luxembourg, Czech Republic, Hungary, Slovakia, Latvia) and Israel. The value of these coordinated

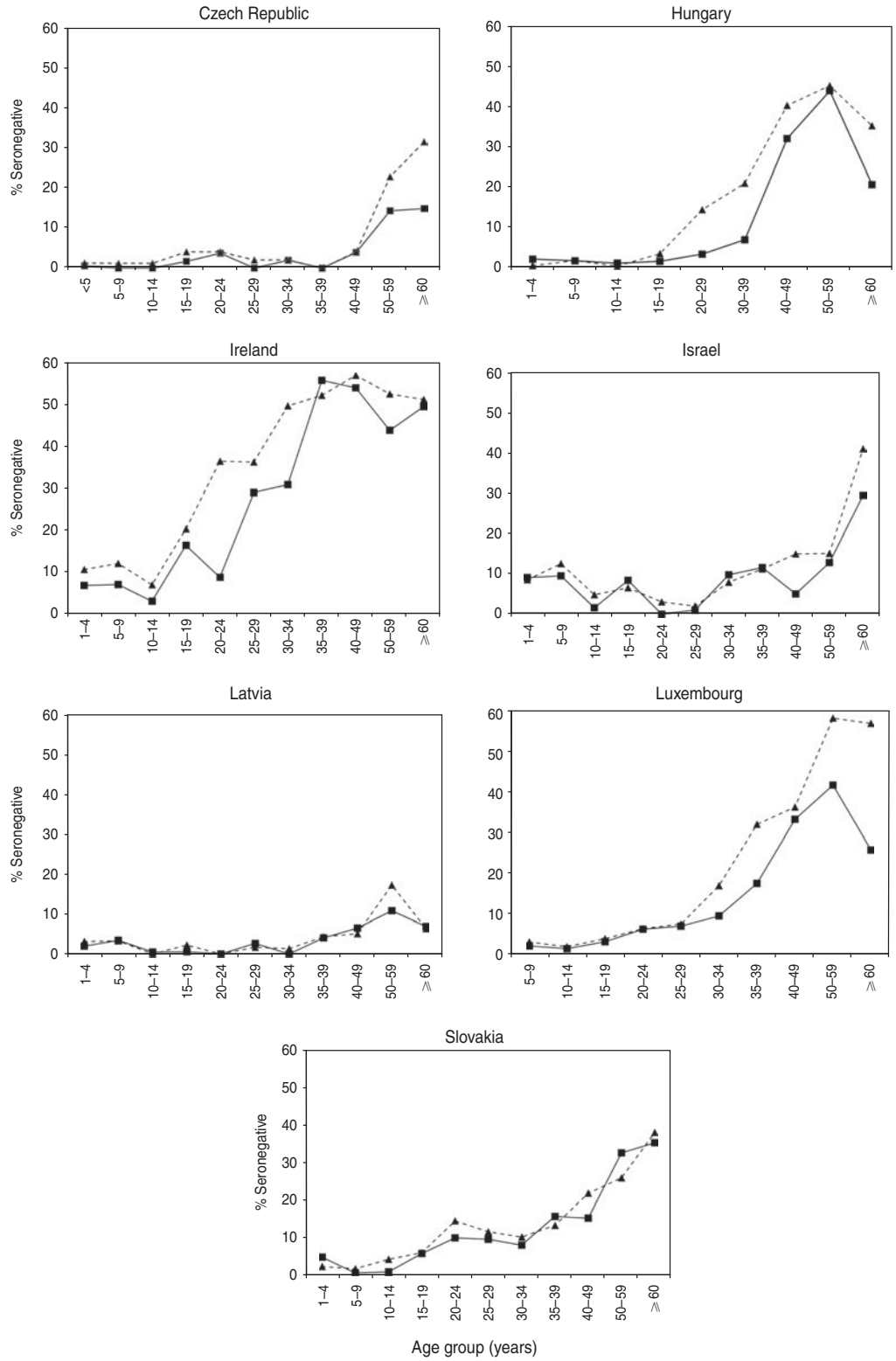

Fig. 3. Proportion of seronegative (antitoxin concentration $<0{\cdot}01$ IU/ml) in each country by sex and age group (5-yearly age groups up to 35–39 years, 10-yearly age groups up to 50–59 years, then $\geqslant 60$ years). ···▲···, Females, —■—, males.

seroprevalence studies is that direct comparisons of data can be achieved using standardization of serological results. The method for standardizing the serological outcomes generally worked well. However, two standardization equations (for Hungary and Israel panel results) were based on low R^2 values

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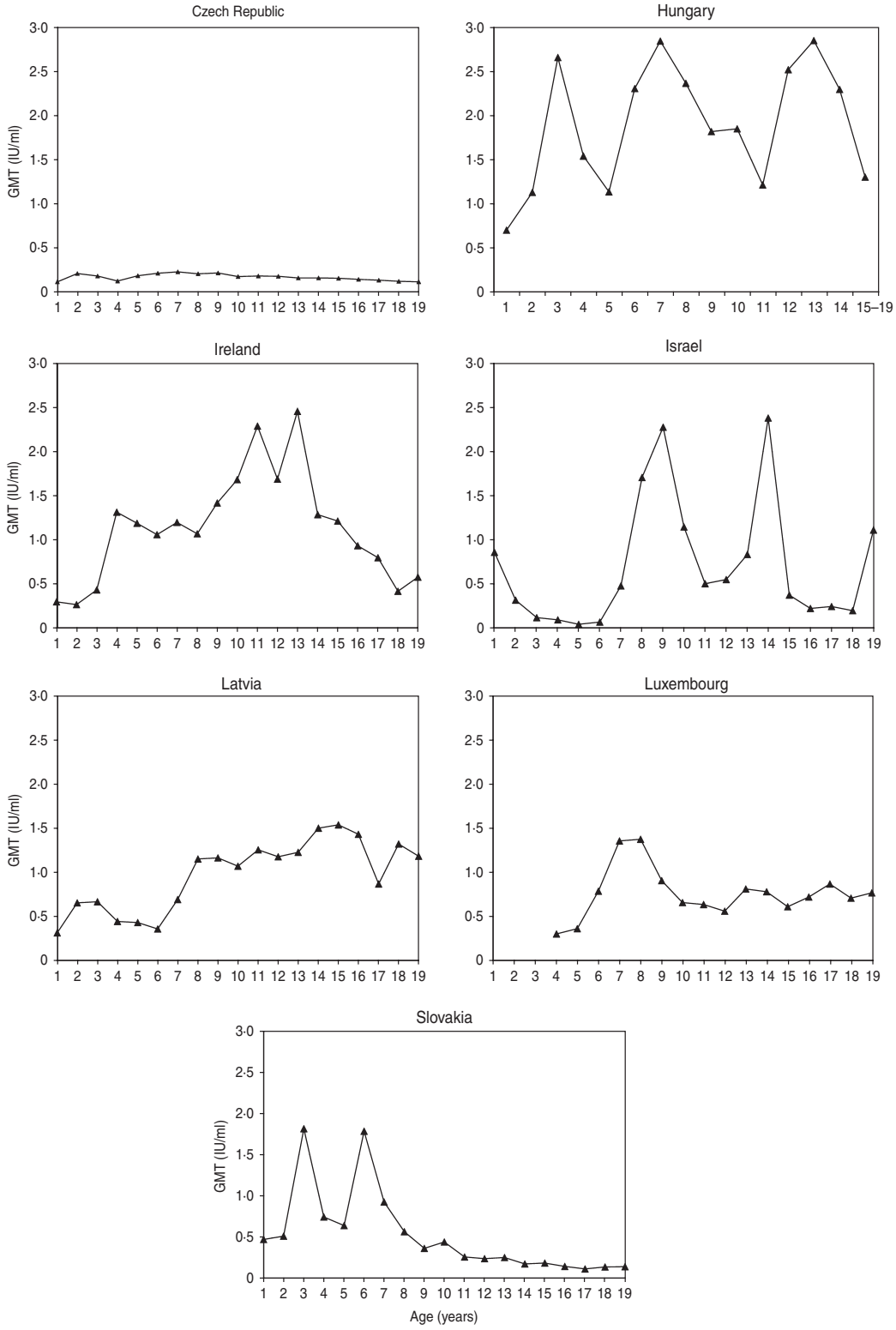

Fig. 4. Geometric mean antibody titre (GMT) in each country for the yearly age groups 1–19 years. GMT has been calculated using samples $\geqslant 0{\cdot}01$ IU/ml.

which may imply that some level of misclassification may have occurred when standardizing the serosurvey results.

Immunity to diphtheria presumably involves both humoral and cell-mediated immunity to multiple antigens of *Corynebacterium diphtheriae*. However,

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only the levels of diphtheria antitoxin are measured. For epidemiological purposes, the minimum protective level is considered to be 0·01 IU/ml of diphtheria antitoxin. The higher level of 0·1 IU/ml is desirable for individual protection [28]. No level of antitoxin provides absolute protection against clinical diphtheria; however, the severity of the illness is proportional to the level of antitoxin [29, 30].

From the serological profiles of the different countries it is evident that the general trend is that children and young people are more protected than adults.

Increase in age groups is related to a progressive decrease of protected subjects and increase in low-positives and seronegatives. Vaccine-derived immunity to diphtheria decreases in the absence of boosters. Differences in seronegatives between female and males indicate the relevance of booster doses administered to adults. Differences can be related to boosters provided during military service or perhaps that males are more likely to have accidents and receive additional boosters of tetanus-diphtheria linked to these events during their lifetime compared to females of the same age. The serological patterns found in this study are quite similar to those found for the original ESEN participating countries [17].

In Latvia, the middle and older age groups show a very low proportion of low-positive and negative subjects. The high levels of protected subjects whose sera were collected in 2003 might be related to natural infection due to the circulation of *C. diphtheriae*, as well as to mass immunization. However, despite the very high percentage of protected subjects and high vaccination coverage, 190 diphtheria cases occurred between 2002 and 2008, with 17 deaths [25] and a further eight cases in 2009–2010 [26]. Most cases were adults but the highest incidence was observed in those aged <10 years. Seventy-two percent of cases occurred in unvaccinated subjects belonging to low-income population and social risk groups.

In order to achieve sufficient herd immunity, a minimum rate of 90% vaccination coverage in children and 75% in adults is required [28]. The immunity, from the seroprofiles, among the young is, in all countries, quite satisfactory, but less so in adults. It is evident from the Latvian experience that unvaccinated subjects (especially when living at low socioeconomic levels) are at high risk when *C. diphtheriae* is circulating in the area. The indigenous transmission of the disease continues in Latvia as shown by a multicentre European screening study for *C. diphtheriae* and *C. ulcerans* in patients with upper respiratory tract infections during 2007–2008 [31]. In all ESEN2 countries, the primary vaccination schedule contained three doses given at intervals of 1 or 2 months. Booster doses were also administered before school entry in all countries. These vaccination schedules guarantee that children have satisfactory protection in all age groups. The absence of pre-school boosters has been shown in Norway and Sweden to be the cause of insufficient protection in children from 7 to 10 years [32, 33]. Differently from the other ESEN2 countries, Czech Republic and Slovakia did not have an adolescent booster in place at the time of serum collection, and this might account for a lower percentage of protected young subjects. Ireland's introduction of a booster at age 12 years after 1996 might explain the high seronegative proportion in adolescents, as this age group would not have been eligible for this booster.

Adults of the ESEN2 participating countries seem to be inadequately protected. As long as vaccination coverage is high, a reduced number of seronegative and low-positive subjects are present; the increase in seronegative subjects can be related to the fact that booster doses are not recommended or, when indicated, not regularly administered. Vaccination coverage is similar in Hungary, Latvia and Slovakia, but the circulation of *C. diphtheriae* might be responsible for the lower percentage of seronegatives in Latvia. Seronegative individuals in epidemic areas or with close contacts to diphtheria cases are at risk of infection [12–14, 25, 28, 31]. However, sporadic cases of diphtheria in adults with low levels of immunity are notified every year by European countries that have not had a diphtheria epidemic since the introduction of mass immunization during the 1950s [34]. The relevance of protective levels of diphtheria antitoxin has been confirmed by an analysis of the factors that contributed to the re-emergence of diphtheria in NIS [7, 8]. This epidemic, primarily affecting adults, demonstrated that diphtheria can still spread explosively in industrialized countries, causing illness and death if a large proportion of adults are susceptible and children are unvaccinated or incorrectly vaccinated [7, 8]. Factors other than immunity are also important to the spread of diphtheria, such as poor socioeconomic conditions, the emergence of invasive and epidemic clones, and the level of circulating diphtheria strains in the community [25, 35]. It would be reassuring to have better immunity in adults in order to reduce the risk of epidemic clusters for a disease for which such a safe and effective vaccine exists [36]. Adult booster

coverage needs to be increased and epidemiological surveillance and laboratory capacity maintained despite the small number of cases. To this end a dedicated surveillance network for diphtheria (DIPNET) was established in Europe, coordinated since November 2006 by the hub at the Health Protection Agency (HPA, London, UK) [37]. At the end of January 2010, the network was transferred to the European Centre for Disease Prevention and Control (ECDC, Stockholm, Sweden) and renamed European Diphtheria Surveillance Network (EDSN) (EDSN ECDC [38]).

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Galazka AM, Robertson SE.** Diphtheria: changing patterns in the developing world and the industrialised world. *European Journal of Epidemiology* 1995; **11**: 107–117.
2. **World Health Organization.** The immunological basis for immunisation. Diphtheria. Update 2009 (http://www.who.int/immunization/documents/ISBN9789241597869/en/index.html). Accessed 21 November 2011.
3. **World Health Organization.** Operational targets for EPI disease. EUR/ICP/CMDS 01, 1996; 14.
4. **World Health Organization.** Expanded programme on immunization. Outbreak of diphtheria. *Weekly Epidemiological Record* 1993; **19**: 132–138.
5. **Hardy IR, Dittman S, Sutter R.** Current situation and control strategies for resurgence of diphtheria in Newly Independent States of the former Soviet Union. *Lancet* 1996; **347**: 1739–1744.
6. **Galazka AM, Robertson SE, Oblapenko P.** Resurgence of diphtheria. *European Journal of Epidemiology* 1995; **11**: 95–105.
7. **Vitek CR, Wharton M.** Diphtheria in the former Soviet Union: reemergence of a pandemic disease. *Emerging Infectious Diseases* 1998; **4**: 539–550.
8. **Dittman S, *et al.*** Successful control of epidemic diphtheria in the States of the former Union of Soviet Socialist Republics: lesson learned. *Journal of Infectious Diseases* 2000; **181** (Suppl. 1): S10–22.
9. **Simonsen O, *et al.*** Fall-off in immunity following diphtheria revaccination – an 8 year follow-up study. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* 1996; **104**: 921–925.
10. **Markina SS, *et al.*** Diphtheria in the Russian Federation in the 1990s. *Journal of Infectious Diseases* 2000; **181** (Suppl. 1): S27–34.
11. **Efstratiou A, *et al.*** Diphtheria in Europe. *Clinical Microbiological and Infection* 1999; **5**: 64.
12. **Lumio J, *et al.*** Diphtheria after a visit to Russia. *Lancet* 1993; **342**: 53–54.
13. **Lumio J, *et al.*** Epidemiology of three cases of severe diphtheria in Finnish patients with low antitoxin antibody levels. *European Journal of Clinical Microbiology and Infectious Diseases* 2001; **20**: 705–710.
14. **Anon.** Diphtheria acquired by US citizens in the Russian Federation and Ukraine – 1994. *Morbidity, Mortality Weekly Report* 1995; **44**: 237–244.
15. **von Hunolstein C, *et al.*** An imported fatal case of diphtheria in Italy. *European Journal of Clinical Microbiology and Infectious Diseases* 1995; **14**: 828–830.
16. **Osborne K, Weinburg J, Miller E.** The European sero-epidemiology network. *Eurosurveillance* 1997; **2**: 29–31.
17. **Edmunds WJ, *et al.*** The sero-epidemiology of diphtheria in Western Europe. *Epidemiology and Infection* 2000, **125**: 113–125.
18. **Miyamura K, *et al.*** Micro cell culture method for the determination of diphtheria toxin and antitoxin titre using VERO cells DI. Studies of factors affecting the toxin and antitoxin titration. *Journal of Biological Standardization* 1974; **2**: 189–201.
19. **Aggerbeck H, Norgaard-Pedersen B, Heron I.** Simultaneous quantization of diphtheria and tetanus antibodies by double antigen, time-resolved fluorescence immunoassay. *Journal of Immunological Methods* 1996; **190**: 171–83.
20. **Kristiansen M, Aggerbeck H, Heron I.** Improved ELISA for determination of anti-diphtheria and/or anti-tetanus antitoxin antibodies in sera. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* 1997; **105**: 843–53.
21. **Walory J, Grzesiowski P, Hryniewicz W.** Comparison of four serological methods for the detection of diphtheria anti-toxin antibody. *Journal of Immunological Methods* 2000; **245**: 55–56.
22. **von Hunolstein C, *et al.*** European sero-epidemiology network: standardisation of the results of diphtheria antitoxin assays. *Vaccine* 2000; **18**: 3287–3296.
23. **Kafatos G, Andrews N, Nardone A.** Model selection methodology for inter-laboratory standardization of antibody titres. *Vaccine* 2005; **23**: 5022–5027.
24. **Galazka A.** The changing epidemiology of diphtheria in the vaccine era. *Journal of Infectious Diseases* 2000; **181** (Suppl. 1): S2–9.
25. **Lucenko I, *et al.*** Diphtheria in Latvia: lesson learnt from DIPNET (http://escaide2009.ecdc.europa.eu/download.cfm-SAVE=2556&LG=1.pdf). Accessed 21 November 2011.
26. **World Health Organization.** (http://apps.who.int/immunization_monitoring/en/globalsummary/timeseries/tsincidencedip.htm). Accessed 21 November 2011.

27. **Efstratiou A, Roure C.** The European Laboratory Working Group on Diphtheria: a global network. *Journal of Infectious Diseases* 2000; **181** (Suppl. 1): S146–151.
28. **Begg N.** Manual of management and control of diphtheria in the European region. The expanded programme on immunization in the European region of WHO. ICP/EPI/038 (B); 1994 (www.who.int/vaccines-documents/DocsPDF05/0602170624_001.pdf). Accessed 21 November 2011.
29. **Ipsen J.** Circulating antitoxin at the onset of diphtheria in 425 patients. *Journal of Immunology* 1946; **54**: 325–347.
30. **Groundström K, *et al.*** What is a protective level of diphtheria antitoxin antibodies. Abstract n. 575, 43rd ICAAC meeting, September 2003, Chicago.
31. **Wagner K, *et al.*** Screening for *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* in patients with upper respiratory tract infection 2007–2008: a multicentre European study. *Clinical Microbiology and Infectious Diseases* 2010; **17**: 519–525.
32. **Skogen V, *et al.*** Immunity to diphtheria among children in Northern Norway and North – Western Russia. *Vaccine* 2001; **19**: 197–203.
33. **Mark A, *et al.*** Immunity and immunization of children against diphtheria in Sweden. *European Journal of Microbiology and Infection* 1989; **8**: 214–219.
34. **European Centre for Disease Prevention and Control.** Annual epidemiological report 2011. Reporting on 2009 surveillance data and 2010 epidemic intelligence data. Vaccine preventable disease, diphtheria (http://ecdc.europa.eu/en/publications/Publications/1111_SUR_Annual_Epidemiological_Report_on_Communicable_Diseases_in_Europe.pdf). Accessed 21 November 2011.
35. **Harnisch JP, *et al.*** Diphtheria among alcoholic urban adults. A decade of experience in Seattle. *Annals of Internal Medicine* 1989; **111**: 71–81.
36. **Quick ML, *et al.*** Risk factors for diphtheria: a prospective case-control study in the Republic of Georgia, 1995–1996. *Journal of Infectious Diseases* 2000; **181** (Suppl. 1): S121–129.
37. **Neal S, Efstratiou A.** DIPNET – establishment of a dedicated surveillance network for diphtheria in Europe. *Eurosurveillance* 2007; **12**: 377–382.
38. **European Centre for Disease Prevention and Control.** (http://ecdc.europa.eu/en/activities/surveillance/EDSN/Pages/index.aspx). Accessed 21 November 2011.