

DIPHThERIA EPIDEMIOLOGY IN THE UK AND EUROPE

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Abstract

A resurgence of diphtheria (*Corynebacterium diphtheriae*) occurred in the former Soviet Union in the 1990s. Concerted control measures brought about a decline in cases, however some endemic transmission has continued and increasingly *C. ulcerans* cases have been reported in some Western European countries. Questions existed regarding risk factors for infection, availability of diphtheria antitoxin (DAT) treatment, circulation of potentially toxigenic *Corynebacteria*, and UK population immunity.

Surveillance data from the World Health Organization European Region, Diphtheria Surveillance Network (DIPNET) and UK were analysed. In addition, 47 countries provided information regarding their DAT treatment supplies. To examine circulation of *Corynebacteria*, throat swabs were screened across ten countries. UK diphtheria immunity was assessed by serosurvey, and vaccination coverage data from nine London Primary Care Trusts (PCTs) were analysed by ethnicity.

During 2000-2009 *C. diphtheriae* cases declined across the European Region. *C. ulcerans* cases (associated with domestic animals) outnumbered *C. diphtheriae* (associated with travel to endemic areas) in DIPNET countries outside the former Soviet Union. There was a clear protective effect of vaccination. The case fatality rate for respiratory diphtheria was lower in Latvia than in other DIPNET countries. Global shortages of DAT were highlighted. Screening identified endemic transmission of toxigenic *C. diphtheriae* in Latvia and Lithuania, and circulation of non-toxigenic strains in several countries. UK population immunity had increased since the last serosurvey in 1996; in 2009 75% of the population had at least basic protection. Low childhood vaccination coverage in London related partly to the size of ethnic groups within a PCT but also to completeness of data records.

Surveillance and screening datasets likely missed some cases/isolates due to lost clinical and/or laboratory expertise. These skills need to be retained and high vaccination coverage levels achieved, as well as records accurately maintained. A DAT alternative is needed, with improved availability and access.

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1. Introduction

Pathogenesis and disease

Diphtheria is caused by toxin-producing strains of *Corynebacterium diphtheriae*, and more rarely *Corynebacterium ulcerans*, or *Corynebacterium pseudotuberculosis*. *C. diphtheriae* is spread from person to person via respiratory droplets, contaminated fomites, or direct contact with infected skin lesions. The classic presentation is a sore throat with a swollen 'bull neck' appearance and a membrane, comprised of fibrin, epithelial cells, bacteria and polymorphs, which obstructs the airway and makes it difficult to breathe. A cutaneous presentation, typically 'rolled edge' ulcers, is more common in tropical areas of the world. Respiratory disease has a high (5-10%) case fatality rate (CFR) (Begg, 1994).

C. diphtheriae is classified into biovars (*mitis*, *gravis*, *intermedius*, or *belfanti*), according to colony morphology, and ribotypes based on genetic fingerprinting of genes coding for (16s and 23s) ribosomal RNA.

Epidemiology of diphtheria in the UK and World Health Organization (WHO) European region

Diphtheria was a much feared disease of childhood until the advent of antibiotics, general improvements in living conditions, and crucially, the introduction of national diphtheria immunisation programmes in the European Region (Figure 1) in the 1940s and 50s, marked a steep decline in cases.



Figure 1. World Health Organization European Region and DIPNET member countries (*map produced using country borders from thematicmapping.org (2013) and the QGIS Application (QGIS Development Team, 2014)*)

In 1984 the WHO set a target for the elimination of indigenous diphtheria in the European Region for the year 2000 (Begg, 1994). This target was almost within sight when a major resurgence occurred in the countries of the former Soviet Union, from where between 1990 and 1998 more than 157,000 cases and 5,000 deaths were reported (Dittmann et al., 2000). At its peak, in 1994 and 1995, the epidemic accounted for more than 85% of diphtheria cases reported worldwide (Figure 2) (World Health Organization, 2013).

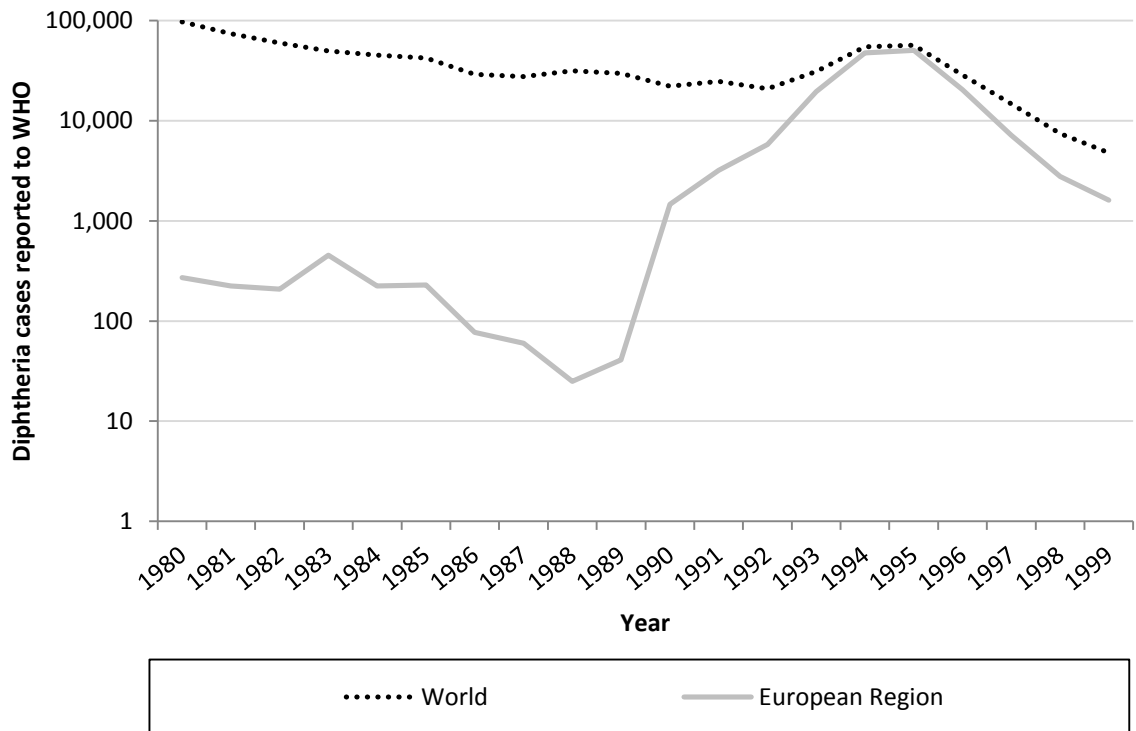


Figure 2. Diphtheria cases reported to the World Health Organization 1980-1999 (source: World Health Organization (2013))

A number of factors contributed to the epidemic (Dittmann et al., 2000, Vitek and Wharton, 1998); the break-up of the former Soviet Union led to health services being disrupted, including supplies of vaccine and diphtheria antitoxin (DAT) treatment. In addition there were large scale population movements, including military personnel and refugees from neighbouring endemic countries, possibly resulting in the introduction of epidemic strains. Furthermore, immunity in the adult population in the vaccine era had waned in the absence of exposure to disease, leading to a susceptible adult population (reflected in the age distribution of cases and deaths). There was also an extensive list of (mostly inappropriate) contraindications to vaccination in the childhood immunisation advice at that time (Tatochenko and Mitjushin, 2000), and a lower dose vaccine had been used for some primary immunisations (Vitek and Wharton, 1998) which meant that there were children within the population who were insufficiently protected.

A plan for co-ordinated action to control the epidemic was developed by WHO and the United Nations Children's Fund (UNICEF), in collaboration with several other organisations. It involved initiating mass immunisation, as well as early detection and management of cases and contacts (Dittmann et al., 2000). In addition, a microbiological network, the European Laboratory Working Group on Diphtheria (ELWGD) was formed in 1993 to assist with supplies of reagents and improve training and skills that had been lost (Efstratiou and Roure, 2000). The epidemic was largely brought under control, with case numbers steadily declining from 1995 onwards. However, endemic transmission continued in some countries within the region, raising questions about population residual susceptibility.

Spread to countries outside the former Soviet Union during the epidemic was fortunately limited (Eskola et al., 1998), and case numbers in the rest of the European Region at this time remained low. However, the epidemic resulted in heightened awareness and increased screening practices. Partly as a consequence of this, around the same time sporadic cases of diphtheria caused by *C. ulcerans* were increasingly reported from Western European countries and the United States (Communicable Disease Surveillance Centre, 2000, Lartigue et al., 2005, Schuegger et al., 2009, Tiwari et al., 2008). *C. ulcerans* was first isolated from human throat lesions in 1926 by Gilbert and Stewart (Gilbert and Stewart, 1926). It has historically been associated with contact with dairy animals and/or the consumption of raw milk or dairy products (Bostock et al., 1984, Hart, 1984). However, some of the more recent cases have not had this exposure history, raising questions about reservoirs of infection and transmission of this organism.

Diphtheria cases from the UK and other Western European countries are most commonly reported as individual case reports. However, over recent decades a sizable enhanced surveillance database of UK cases has been developed. Furthermore, in 2007-2010 the Diphtheria Surveillance Network (DIPNET) a project involving 25 member countries (Figure 1) was funded by the European Commission (Neal and Efstratiou, 2007). The development of this network enabled the amalgamation of ten years of case-based data from member countries, as well as cross-country collaboration on other projects. The case-based data included information about vaccination status, disease

presentations, outcomes and risk factors. Diphtheria vaccination is known to be highly effective in preventing diphtheria symptoms. However, information about vaccination status had not previously been formally analysed with respect to the varying severity of clinical presentations observed in recent diphtheria cases.

Respiratory diphtheria requires treatment with DAT and antibiotics (Begg, 1994). DAT acts against the toxin and must be administered quickly, before the toxin has had a chance to bind to receptors, whilst antibiotics are needed to clear the infection. DAT is a preparation of immunoglobulins or immunoglobulin F(ab')₂ fractions produced from the immunisation of horses, it has a shelf-life of approximately two years. Problems with sourcing DAT from international suppliers had been anecdotally reported. In addition there had been a number of informal requests for loan of DAT from one country to another when cases had arisen. It was apparent that some countries lacked a supply themselves, but the full picture in terms of stocks of treatment, current producers and possible alternatives was unknown. Even in the UK, where stocks of DAT are maintained in several centres across the country, not all cases receive this treatment, though the extent of use amongst cases had not been formally reviewed. During the epidemic, the availability of DAT supplies was shown to dramatically influence CFRs (Dittmann et al., 2000). In the post-epidemic era, where supplies of DAT are variable across the European Region, CFRs had not previously been calculated. In addition, how CFRs currently compared in the UK to those of the pre-vaccine era was unknown.

A barrier to maintaining a current stock of DAT within a country, given various other competing priorities, is the low perception of risk either of imported cases or endemic circulation. Although surveillance data can give an indication of the circulation of disease-causing organisms in a population, it does not provide a complete picture. As well as issues with case recognition and ascertainment, there may be asymptomatic carriage. This may be particularly relevant in vaccinated populations because the diphtheria vaccine targets the toxin rather than the organism itself. Non-toxigenic strains are unlikely to be detected in the absence of specific screening procedures. There have been reports of non-toxigenic strains causing severe disease (Romney et al., 2006), but in general, because the clinical presentations of diphtheria are toxin-mediated, they do not give rise to the same public health concerns. Yet it is possible for a non-

toxigenic strain to carry the toxin gene but not express it (non-toxigenic toxin-bearing (NTTB) strains) (Groman, 1984). In addition, in some circumstances the toxin gene can be transmitted via a bacteriophage to a non-toxigenic strain so that it becomes toxigenic (Uchida et al., 1971, Freeman, 1951). Hence non-toxigenic strains provide a reservoir from which toxigenic organisms can potentially arise (De Zoysa et al., 2005). Some studies have previously attempted to assess carriage of toxigenic and non-toxigenic organisms in specific populations, but full methodology was often not available and carriage rates varied (Alexandrou-Athanasoulis et al., 2006, Von Hunolstein et al., 2003, Lucenko et al., 2006). The carriage of these organisms in a population has important public health implications, particularly, given our knowledge of the outbreak in the former Soviet Union, if there are susceptible adult populations (as have been reported in studies from Europe and elsewhere (Edmunds et al., 2000, Di Giovine et al., 2013) and areas of low childhood immunisation coverage.

Diphtheria immunity and vaccination coverage in the UK

Immunity to diphtheria is acquired either through vaccination or natural infection (though this does not always confer immunity). In the UK, some older individuals have natural immunity but most immunity is now vaccine-acquired. The diphtheria vaccine is highly effective (estimated effectiveness for three or more doses 97% (95% confidence interval 94-98%) (Bisgard et al., 2000)). In the UK, diphtheria vaccine is currently given as part of a 5-in-1 vaccine that also protects against tetanus, pertussis, polio and *Haemophilus influenzae* type b (DTaP/IPV/Hib), and is scheduled at 2, 3 and 4 months of age (primary course) (Public Health England, 2014a). Following this a pre-school booster is scheduled between 3 years 4 months and five years of age (DTaP/IPV), and a school leaving (also known as 'adolescent') booster around 14 years of age (Td/IPV) (Public Health England, 2014a).

Diphtheria immunity in England was assessed at the time of the epidemic in the former Soviet Union, using samples collected in 1991 (Miller et al., 1994). Overall 67% of the population at this time had full (>0.1 IU/mL) immunity which, in combination with good national childhood immunisation coverage, did not give cause for concern. However, the susceptible population was expected to

increase with the gradual replacement of natural immunity (and natural boosting) by less long-lasting vaccine-induced immunity in older people. A seroepidemiology study undertaken in England and Wales using sera from 1996 found that at that time only 16% of the total population had full (≥ 0.1 IU/mL) diphtheria immunity (Maple et al., 2000). The diphtheria component was added to the school leaver booster in 1994 in order to boost immunity in adulthood. In addition, since 1992, glycoconjugate vaccines, containing either tetanus toxoid or CRM₁₉₇ (a non-toxicogenic natural variant of diphtheria toxin), have been included in the UK schedule for infants in routine and catch-up programmes. Immune responses to CRM₁₉₇ have been shown in trials to significantly increase diphtheria antitoxin levels (Burrage et al., 2002). An increase in population immunity from these changes was therefore expected but had not been previously assessed.

Immunity and vaccination coverage are regularly monitored at a national level in the UK, and vaccination coverage is published quarterly and annually at Primary Care Trust (PCT) level (Public Health England, 2014b). However, the vaccination coverage data received at the national centre are aggregated, and consequently do not enable further scrutiny. Overall good coverage statistics can mask pockets of unimmunised children within a PCT if coverage varies across different populations within a PCT. Maintaining high vaccination coverage can be challenging, particularly where populations are mobile, and/or language barriers exist. Vaccination coverage by ethnicity for diphtheria-containing vaccines was previously assessed for broad ethnic groups nationally (for children born 2000-2001) as part of the Millennium Cohort Study, which found that children of Black Caribbean mothers were more likely to be unimmunised than those of other ethnic groups (Samad et al., 2006). In addition, a study in Manchester (of children born 2002-2007) found that white infants were least likely to be vaccinated with primary vaccines (Baker et al., 2011). London is an increasingly ethnically diverse city, encompassing both long-established and new migrant populations, including those arriving from diphtheria-endemic regions of the world. Identification of low coverage within particular groups of children within London could enable appropriate targeting of immunisation resources.

This thesis aims:

- To describe the epidemiology of diphtheria in the UK (1986-2008) and WHO European Region (2000-2009), including trends, risk factors for infection, and influences on disease severity and case fatality.
- To explore the evidence base for the use of DAT in the UK, and issues relating to its supply internationally.
- To gain an understanding of the circulation of both toxigenic and non-toxigenic organisms, including *C. ulcerans*, in endemic and non-endemic countries within the WHO European Region.
- To examine the susceptibility of the UK population in terms of both:
 - Diphtheria immunity levels, taking into account changes to the UK vaccination schedule
 - Childhood vaccination coverage within different ethnic groups in London.

2. Publications

1. Wagner, K.S., White, J.M., Crowcroft, N.S., De Martin, S., Mann, G., Efstratiou, A. (2010) '**Diphtheria in the United Kingdom, 1986-2008: the increasing role of *Corynebacterium ulcerans*.**' *Epidemiology & Infection*, 138(11) pp. 1519-1530.
Contribution: preparation of surveillance data, review of literature, data analysis and drafting of manuscript
2. Wagner, K.S., White, J.M., Lucenko, I., Mercer, D., Crowcroft, N.S., Neal, S., Efstratiou, A., on behalf of the Diphtheria Surveillance Network. (2012) '**Diphtheria in the postepidemic period, Europe, 2000-2009.**' *Emerging Infectious Diseases*, 18(2) pp. 217-225.
Contribution: co-ordination of surveillance data collection from DIPNET member states, data analysis and drafting of manuscript
3. Wagner, K.S., Stickings, P., White, J.M., Neal, S., Crowcroft, N.S., Sesardic, D., Efstratiou, A. (2009) '**A review of the international issues surrounding the availability and demand for diphtheria antitoxin for therapeutic use.**' *Vaccine*, 28(1) pp. 14-20.
Contribution: survey design and distribution, collation of responses, data analysis, drafting of manuscript.
4. Wagner, K.S., White, J.M., Neal, S., Crowcroft, N.S., Kuprevičienė, N., Paberza, R., Lucenko, I., Jöks, U., Akbaş, E., Alexandrou-Athanassoulis, H., Detcheva, A., Vuopio, J., von Hunolstein, C., Murphy, P.G., Andrews, N., Members of the Diphtheria Surveillance Network (DIPNET) and Efstratiou, A. (2011) '**Screening for *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* in patients with upper respiratory tract infections 2007-2008: a multicentre European study.**' *Clinical Microbiology and Infection*, 17(4) pp. 519-525.
Contribution: study co-ordination, data collection, analysis and drafting of manuscript

5. Wagner, K.S., White, J.M., Andrews, N.J., Borrow, R., Stanford, E., Newton, E., Pebody, R.G. (2012) '**Immunity to tetanus and diphtheria in the UK in 2009.**' *Vaccine*, 30(49) pp. 7111-7117.
Contribution: data analysis and drafting of manuscript

6. Wagner, K.S., van Wijgerden, J.C.J., Andrews, N., Goulden, K., White, J.M. (2014) '**Childhood vaccination coverage by ethnicity within London between 2006/2007 and 2010/2011.**' *Archives of Disease in Childhood*, 99(4) pp. 348-353.
Contribution: study design, co-ordination, data collection, analysis and drafting of manuscript

The full texts of the publications listed above can be found in Section 6.

3. Contribution to knowledge and scholarship

Epidemiology of diphtheria in the UK and WHO European Region

Trends

Between 2000 and 2009 the number of diphtheria cases reported to the WHO European Regional Office continued to decline from a peak in 1995 (Figure 3). Diphtheria incidence in the European Region decreased by 95% from 1.82/million population in 2000 to 0.07/million population in 2009. Most (85%) cases reported from the European Region during 2000-2009 were from Russia and Ukraine. However, Latvia (a country with a population of only two million) had the highest annual incidence in the European Region during 2000-2009. In 2009, although only six symptomatic cases were reported (compared to over 250 in 2000 when an outbreak occurred in the military), Latvia remained the only country with an incidence rate greater than 1 per million population.

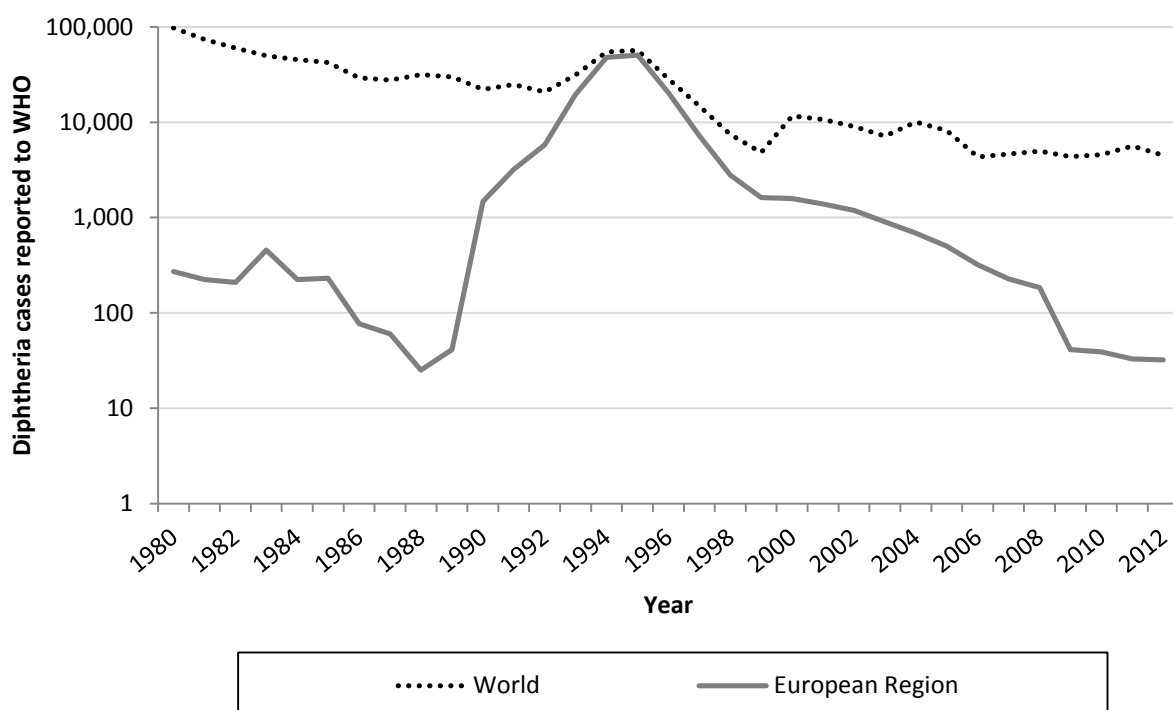


Figure 3. Diphtheria cases reported to the World Health Organization 1980-2012 (source: World Health Organization (2013))

The DIPNET surveillance database included case-based data relating to infections (symptomatic and asymptomatic) with toxigenic strains of *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* for its 25 member countries for 2000-2009. The Baltic States (Estonia, Latvia and Lithuania) were included within DIPNET, but the other 22 countries within the network were from outside the former Soviet Union. Estonia and Lithuania reported four and six symptomatic *C. diphtheriae* cases, respectively, at the beginning of the surveillance period (2000-2002), Lithuania also reported two further symptomatic cases in 2008.

Fewer than sixteen symptomatic (ranging from mild symptoms to classic respiratory diphtheria) cases each year were reported overall from the 22 DIPNET countries excluding the Baltic States during 2000-2009 (Figure 4). Twelve DIPNET countries reported zero cases during this time period.

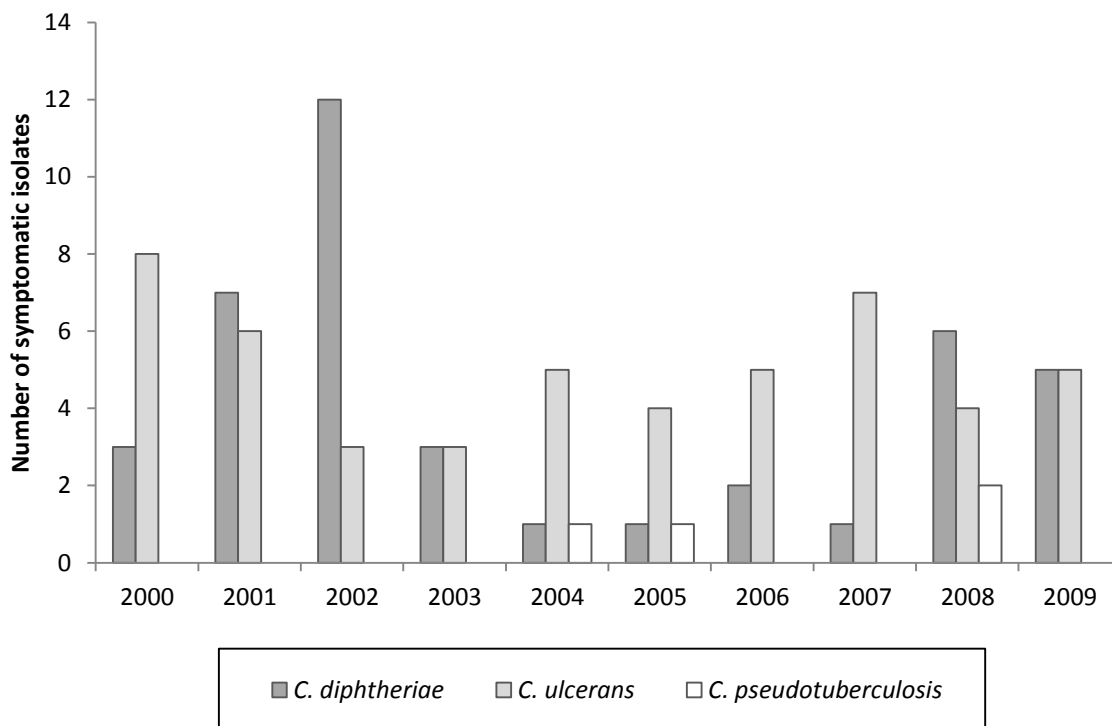


Figure 4. Isolates from symptomatic cases reported by DIPNET member countries excluding the Baltic States, 2000-2009 (data from Publication 2, Table 1)

Symptomatic *C. ulcerans* cases outnumbered symptomatic *C. diphtheriae* cases for DIPNET countries excluding the Baltic States during 2000-2009. The majority (87%, 46/53) of *C. ulcerans* reports were from France, Germany and the UK. Cases of *C. pseudotuberculosis* remained very rare throughout the European Region.

In the UK, between one and nine symptomatic cases of diphtheria were recorded each year between 1986 and 2008. *C. ulcerans* cases were more common than *C. diphtheriae* cases in the UK between 2004 and 2008.

The majority of *C. diphtheriae* isolates 2000-2009 from the epidemic region with a known biovar were biovar *gravis*. In contrast, the majority of UK *C. diphtheriae* biovars (1986-2008) were biovar *mitis*.

As well as the basic counts of cases described above, twelve countries from the epidemic region provided monthly reports to the WHO Regional Office for Europe with more detailed information such as patient age, sex, and outcome. These data were analysed for the period 2000-2009 (and 2002-2009 to exclude the military outbreak that occurred in Latvia in 2000). Detailed case-based data for the same time period for DIPNET countries, including Latvia, were available from the DIPNET surveillance database. UK enhanced surveillance data concerning cases from 2000-2009 were included within the DIPNET surveillance database, and also analysed separately for the period 1986-2008. Within the DIPNET and UK databases, information was recorded concerning risk factors/exposures, disease symptoms, vaccination histories and outcomes. These data enabled the study of common risk factors for infection, as well as disease severity and case fatality.

Risk factors for infection

In the epidemic region, toxigenic *C. diphtheriae* cases were reported across all age groups with most cases reported in teenagers and adults. However, the severity of infection depended on immunity; the greatest risk of death was in those too young to be vaccinated and older adults (≥ 40 years) unvaccinated or with waning immunity. More than 60% (123/196) of symptomatic cases in Latvia during 2002-2009 were in females, a similar bias was observed in the epidemic region as a whole for adult cases (≥ 20 years of age) during 2002-

2009. This could relate to the increased exposure of women to infection in their roles as caregivers in occupational and/or domestic settings, and/or the increased immunity in men because of vaccination during military service. Unemployment was identified as a risk factor amongst the Latvian cases, reflecting the association of diphtheria with low socio-economic conditions.

In DIPNET countries outside of the epidemic region, *C. diphtheriae* cases were commonly associated with recent return from travelling abroad, contact with travellers, or recent migration from endemic areas. The sex distribution was even in symptomatic *C. diphtheriae* cases for DIPNET countries excluding Latvia, and for the UK individually. Similarly, in the UK, the main risk factor for *C. diphtheriae* infection was travel to an endemic country, and although there was a wide age range, the mean and median age of cases were both <25 years. Three cases of laboratory-acquired *C. diphtheriae* in the UK highlighted both the importance of maintaining immunisations for occupational exposure, and safe laboratory practice.

C. ulcerans cases in those DIPNET countries that detected this organism most commonly occurred in older adults (59% (29/49) of cases were ≥45 years of age), predominantly females. The cases reported during 2000-2009 had not travelled abroad, and did not have traditional risk factors such as consumption of raw milk or contact with dairy animals. Ninety-four percent (32/34) of cases for which information was available had had contact with domestic animals. All recent (between 2003 and 2008) cases of *C. ulcerans* in the UK had had contact with domestic cats or dogs, however, the organism was only isolated from one dog from which one of these cases had been in contact (animals were only swabbed for five cases). An identical strain was also isolated from a patient and dog with which the patient had been in contact in France (non-toxigenic strain), and from a patient and their pig in Germany.

Insufficient information was available to determine common risk factors for *C. pseudotuberculosis* infection from the four cases that arose in DIPNET countries during 2000-2009.

Disease severity and case fatality

C. diphtheriae cases reported from former Soviet Union countries generally had respiratory presentations; apart from one cutaneous case these were the only presentations (excluding asymptomatic carriers) reported from Latvia during 2000-2009. In DIPNET European countries outside the former Soviet Union both cutaneous and respiratory presentations were reported for *C. diphtheriae* and *C. ulcerans* (as well as one *C. diphtheriae* case with bacterial endocarditis). For *C. diphtheriae* the cutaneous cases reflected acquisition in tropical areas of the world (cutaneous *C. ulcerans* cases did not have a history of travel abroad). Most (15/17, 88%) *C. diphtheriae* cutaneous cases in DIPNET countries excluding Latvia during 2000-2009 were biovar mitis whereas most (17/28, 61%) respiratory cases with a known biovar were biovar gravis. The majority (3/4) of *C. pseudotuberculosis* cases had cutaneous presentations.

The classic respiratory presentation with pseudomembrane did not arise in any UK cases who were fully vaccinated (though cannot be ruled out in immunised individuals as did occur in fully vaccinated persons in other DIPNET countries). The most common presentation amongst UK cases was respiratory disease; typically a sore throat in a partially immunised individual, although occasionally such cases were fully immunised. Vaccination showed a significant protective effect with respect to severity of infection across all DIPNET data; fully vaccinated cases in general had milder disease than unvaccinated cases.

All five fatal cases in the UK during 1986-2008 were unimmunised. In addition, most patients (74%) and infants (93%) who died within the epidemic region during 2000-2009 were unvaccinated. Case fatality rates were highest for those with classic respiratory presentations, but were also high when those with any respiratory symptoms were included in the denominator (Figure 5). The CFR was significantly higher for respiratory diphtheria in DIPNET countries excluding Latvia, compared to in Latvia. DIPNET countries excluding Latvia are less familiar with identifying and treating the disease and in some cases did not have the resources/procedures in place to detect and respond to cases appropriately. The CFR in the UK for the period 1986-2008 was similar to that in the pre-vaccine era in England and Wales, though smaller numbers meant there was less certainty around the recent estimate.

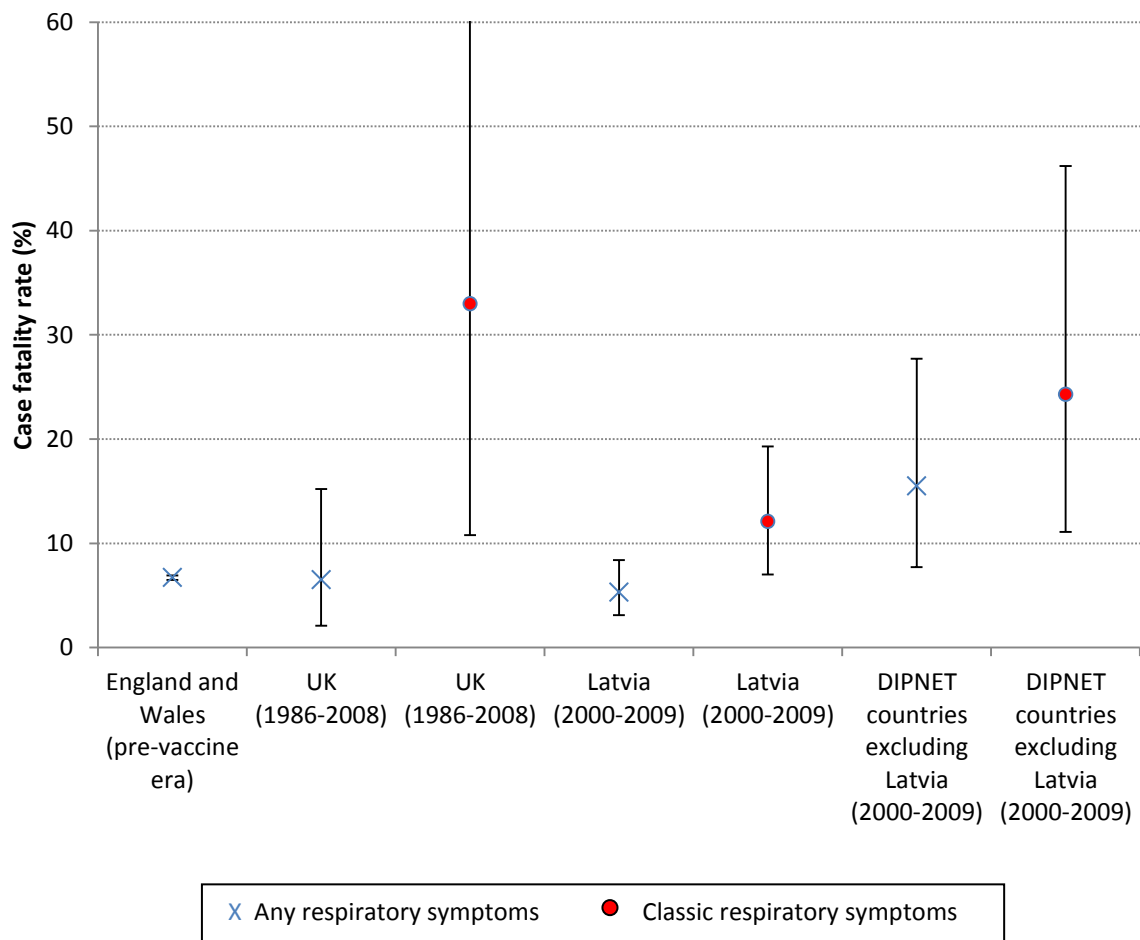


Figure 5. Case fatality rates with 95% confidence intervals for UK, Latvia and DIPNET countries excluding Latvia (data from England and Wales notifications (Public Health England, 2014c), and Publications 1 and 2)

Evidence base for the use of DAT in the UK, and issues relating to its supply

Information regarding receipt of DAT is requested on the UK enhanced surveillance form for diphtheria, which is completed in the event of a case. Information is requested regarding whether or not DAT was administered, as well as the dose and date of administration (although completion of the latter two fields is poor). These data were available for the analysis of UK cases reported between 1986 and 2008.

UK guidelines for the management and control of diphtheria recommend DAT for respiratory presentations, the dose depending on the site, the degree of

toxicity and the duration of illness (Bonnet and Begg, 1999). However, although DAT is available in the UK, the majority of respiratory cases reported between 1986 and 2008 did not receive this treatment (Figure 6). Of the 60 UK cases with respiratory symptoms that did not receive DAT, most (57/60) recovered. Many of these cases were fully or partially immunised (23/60 (38%) were fully immunised, 10/60 partially immunised, 7/60 unknown immunisation status, and only 10/60 unimmunised). Given that no fully vaccinated cases developed the severest disease presentation, even without DAT treatment, the benefits of administration of DAT, for mild, fully immunised, cases (when is it not clinically indicated) need to be weighed against potential side-effects. DAT can cause hypersensitivity reactions which manifest either immediately as an anaphalactic reaction and/or a few days later as serum sickness (the symptoms of which can include generalised erythema, urticaria and itching) (Public Health England, 2013).

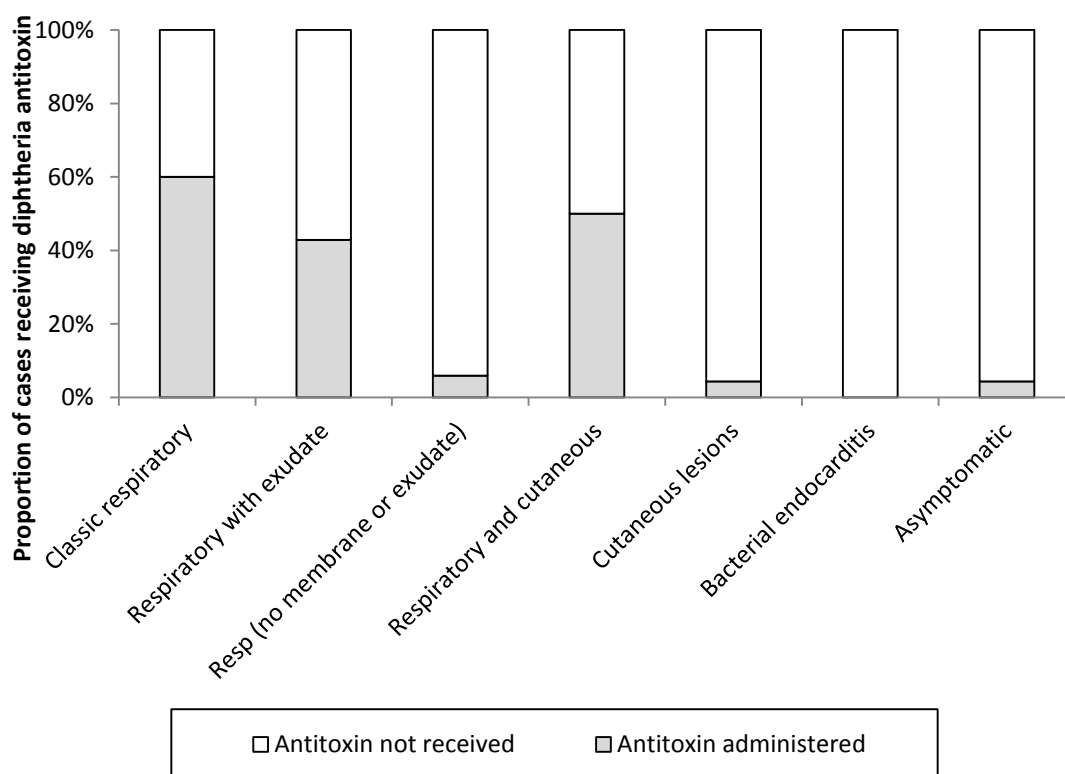


Figure 6. Proportion of UK cases administered diphtheria antitoxin 1986-2008, by clinical presentation (data from Publication 1, table 3)

DAT was administered to nine cases with classic respiratory symptoms (six unvaccinated, two partially vaccinated and one with unknown vaccination status), two of whom died. There were three fatalities among the six patients with these symptoms (five unvaccinated, one partially vaccinated) who did not receive DAT. Timely administration of DAT is dependent on prompt recognition and diagnosis, and often, because of unfamiliarity with the disease, correct diagnosis was delayed; even when DAT was administered it was often at a late stage (DAT has been shown to be ineffective if administered after the second day of diphtheritic symptoms (Logina and Donaghy, 1999)).

The global availability of DAT was assessed by means of an international survey, sent to 57 countries in total. Of 47 countries where the status of DAT stocks was known for 2007-2008, only 27 (57%) held a current stock of DAT (including the four countries that produce and supply internationally), the remainder had no stock, or an expired stock. Various arrangements were in place for the holding of stocks, from national level through to district/hospital supplies. Most stocks were obtained internationally, though three countries (Turkey, Bulgaria and Japan) had their own internal suppliers. The four international producers of DAT identified at the time of the survey were based in Brazil, Croatia, India and Russia. However, at the time of writing, DAT was not available from the Croatian or Brazilian producers, further reducing the international availability of this treatment. As yet there are no internationally available alternatives to the liquid preparation, which requires refrigeration, and expires after 2-3 years (although a freeze-dried version is used in Japan). Several countries which had reported cases in the eight years prior to the survey did not hold current stocks, or had stocks which were close to expiry. A central European or other international stock might be a possible approach but this option has not been fully explored and, even if possible, may not enable timely enough access to treatment. Ideally a non-animal based alternative (without the side-effects of horse serum and easier to produce) would be developed.

Circulation of both toxigenic and non-toxigenic organisms in endemic and non-endemic countries within the WHO European Region

In order to gain an understanding of the circulation of potentially toxigenic strains, ten countries (Bulgaria, Estonia, Finland, Greece, Italy, Ireland, Latvia, Lithuania, Turkey, and England (representing the UK)) participated in a screening study. During the study period (between December 2007 and June 2008) routinely submitted throat swabs were screened for potentially toxigenic *Corynebacteria*. The only one of these countries with known endemic transmission of toxigenic strains was Latvia.

During the screening period two toxigenic strains were identified in Latvia which, when added to the seven non-toxigenic strains also identified, gave a combined carriage rate for Latvia of 2.5 per 1,000 (lower than the carriage rate of 3.7 per 1,000 population from a Latvian study conducted during 2002-2006 (Lucenko et al., 2006)). Toxigenic strains (two cases and two carriers) were also detected in Lithuania in persons with no history of travel or contact with travellers, suggesting the presence of endemic transmission in this country also. The last reported case of diphtheria in Lithuania had been in 2002. The two cases identified in Lithuania during the screening study were unlinked and had classic respiratory presentations. At least one of these cases would not have been detected in the absence of the study, highlighting the impact of this laboratory screening exercise on case ascertainment. The toxigenic strains isolated in Latvia and Lithuania were ribotype Sankt-Petersburg, one of the major epidemic clones. Furthermore, two of four non-toxigenic strains isolated in Lithuania during the study period were non-toxigenic toxin gene bearing strains, indicating additional circulation of the toxin gene beyond the toxigenic strains detected.

Carriage rates of non-toxigenic strains amongst swabs screened (from patients with sore throats) ranged from 0-4.0 per 1,000 swabs screened (Figure 7). However, ascertainment appeared to be related to laboratory training as strains were more frequently detected in laboratories that had had recent training in diphtheria diagnostics (Estonia, Latvia, Turkey, UK).

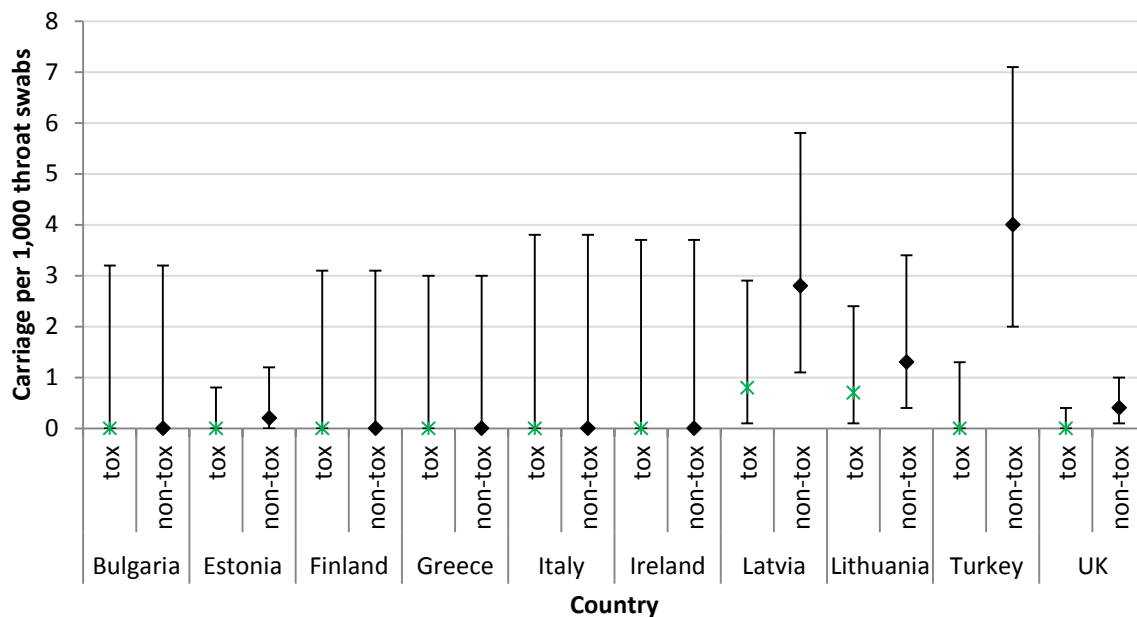


Figure 7. Carriage rates of toxigenic (tox) and non-toxic (non-tox) strains for patients with sore throats in participating countries (data from publication 4, table 2)

Although the study was limited by the sample sizes achieved in each country, the study indicated overall that circulation of non-toxic strains appears largely limited to *C. diphtheriae*; only one non-toxic *C. ulcerans* and one non-toxic *C. pseudotuberculosis* were detected in total during the study compared to 26 non-toxic *C. diphtheriae* strains. In countries with endemic circulation of toxigenic strains, circulation of non-toxic strains is also occurring. However, non-toxic strains are also circulating to a greater (Turkey) or lesser (Estonia, UK) extent in non-endemic countries. Endemic transmission of toxigenic strains and non-toxic toxin gene bearing strains continues in two of the former Soviet Union countries included within DIPNET.

Susceptibility of the UK population

Residual sera from routine diagnostic testing, representing the entire ranges of age and most geographical regions of the population of England, were assayed using multiplexed fluorescent bead assay to quantify IgG antibodies to diphtheria toxoid. The proportions susceptible (antitoxin level <0.01 IU/mL), with basic protection (0.01-0.099 IU/mL) and with full protection (≥ 0.1 IU/mL)

were calculated. The proportions protected were then standardised by age and sex to the UK population.

Population-level diphtheria immunity in 2009 was observed in accordance with the UK vaccination schedule. The highest proportions fully protected occurred in the early years of life, during and in the years directly following administration of the primary course and pre-school booster, as well as in the (approximately ten) years subsequent to administration of the school leaver booster. Overall, after adjusting for age group, the anti-diphtheria IgG geometric mean concentration for males was 26% higher than for females (95% confidence interval 9-46%, $p=0.001$), a finding that could not be fully explained.

Geometric mean concentrations of diphtheria IgG antibodies were significantly higher in 2009 compared to 1996 for ages 1-3 years, due, in part, to a boosting effect of the pneumococcal conjugate vaccine (PCV) (containing CRM₁₉₇) which has been included in the UK schedule since September 2006, when there was also a catch-up to two years of age. Meningococcal conjugate vaccines also utilising CRM₁₉₇ were introduced into the UK infant immunisation schedule from 1999 with a catch-up to 18 years of age.

Lowest immunity levels were observed in the youngest age group (which included those too young to have received primary immunisations), in children aged 10-11 years (prior to administration of the school leaver booster) and also in adults (>35 years). These older adults are not scheduled to receive any further routine diphtheria vaccines (though may receive diphtheria as part of the tetanus booster in the event of a tetanus-prone injury if they are not already fully immunised). This may be more of a concern for *C. ulcerans* infection which has an older case distribution than *C. diphtheriae* in the UK. However, the numbers of *C. ulcerans* cases are small, and immunity would be expected to improve in adults as increasingly those moving in to the older age groups will have received diphtheria vaccine as a school leaver booster.

Overall, 75% of the UK population had at least basic protection against diphtheria (≥ 0.01 IU/mL) in 2009, an increase when compared to 60% in 1996 ($p<0.001$). The proportion fully protected (≥ 0.1 IU/mL) was 41% in 2009 (compared to 16% in 1996, $p<0.001$). This increase related to both the addition

of CRM₁₉₇ containing glycoconjugate vaccines to the UK schedule, and the inclusion of diphtheria vaccine in the school leaver booster.

The serosurvey results, whereby increases in immunity correspond to UK immunisation programme changes, reflect good national immunisation coverage. However, coverage in London is lower than nationally. In order to examine London coverage in more detail, data for children born April 2001 to March 2010 were extracted from the Child Health Information Systems (CHISs) of nine London Primary Care Trusts. Vaccination coverage of diphtheria-containing vaccines was assessed at first and second birthdays (primary vaccinations) as well as fifth birthday (primary vaccinations and pre-school booster).

Limited data fields were available from the CHIS. Of those available in the system, not all were well completed, for example nationality was available for less than 2.5% of extracted records. However, ethnicity was better recorded, and enabled further analysis.

Overall, across the nine London PCTs included in the study, consistently good coverage of the primary course (>88% at first birthday, >89% at second birthday) was achieved across the five largest ethnic groups. Coverage of the preschool booster at fifth birthday was >65% across the five largest ethnic groups. Although some of the smallest ethnic groups had good coverage, the lowest coverage in each cohort was among the smaller ethnic groups and those with unknown ethnicity. Adjusting for gender, deprivation, PCT and year of birth did not substantially change the ethnicity patterns in coverage. No particular ethnic groups were found to have consistently poor coverage across all PCTs. An interaction was found between PCT and ethnicity for all three age cohorts ($p < 0.001$). This was most pronounced for the white-Polish ethnic group, and related to population size. Where white-Polish populations were larger within a PCT, coverage was closer to the average for the PCT, but two PCTs with smaller populations had lower than average coverage for this ethnic group (Figure 8, first birthday as example).

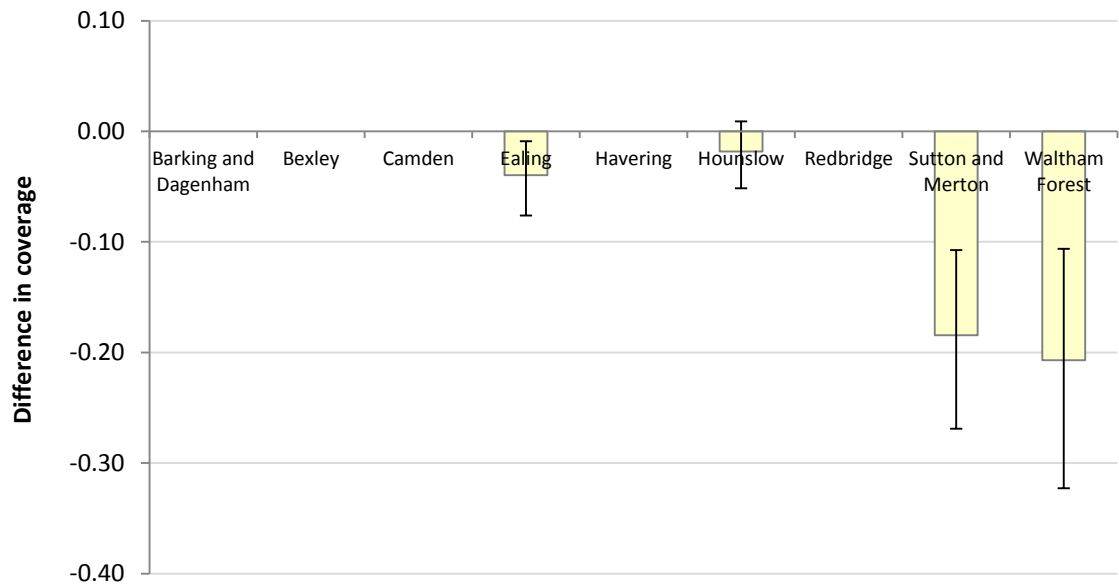


Figure 8. Difference in coverage at first birthday between the average for each PCT* (excluding those with unknown ethnicity) and coverage within the white-Polish ethnic group in each PCT (error bars indicate 95% confidence intervals)

**Note: All nine participating PCTs are included in figure 8, but data are displayed only for those with ≥ 50 children in the white-Polish group*

Deprivation scores were assigned based on geographic coding of area of residence. Deprivation was not found to be a strong indicator of coverage overall and for most ethnic groups there was no relationship between deprivation and coverage. However, interactions between ethnicity and deprivation were significant for each cohort. A trend of reducing coverage by increasing deprivation was observed across each age cohort for the White-British (Figure 9, first birthday as example) and not known ethnic groups only.

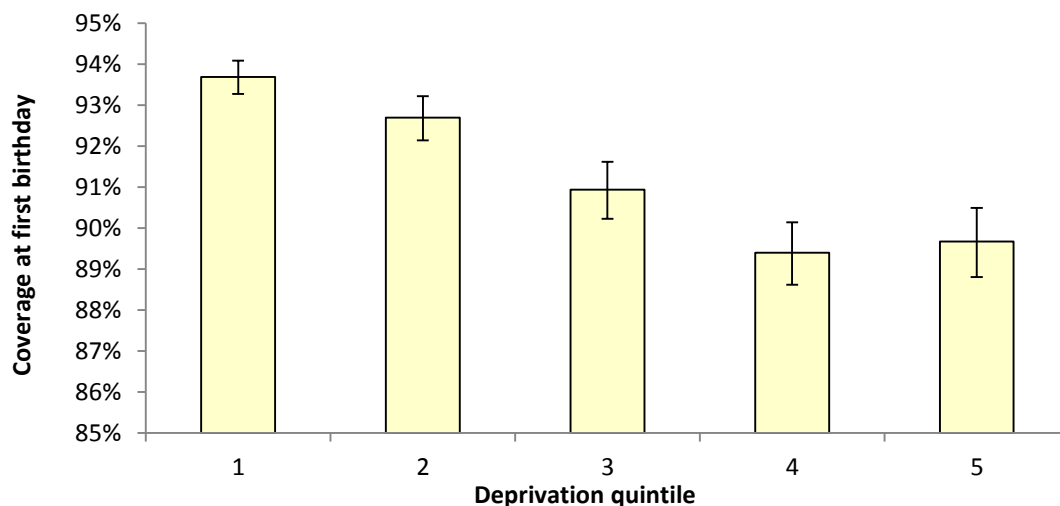


Figure 9. Vaccination coverage at first birthday by deprivation quintile (1=least deprived, 5=most deprived) for the white-British ethnic group (error bars indicate 95% confidence intervals)

The opposite trend was seen for Indian (Figure 10, first birthday as example) and white-Other/mixed/unspecified at first and second birthdays only. Trends were not seen for other ethnicities.

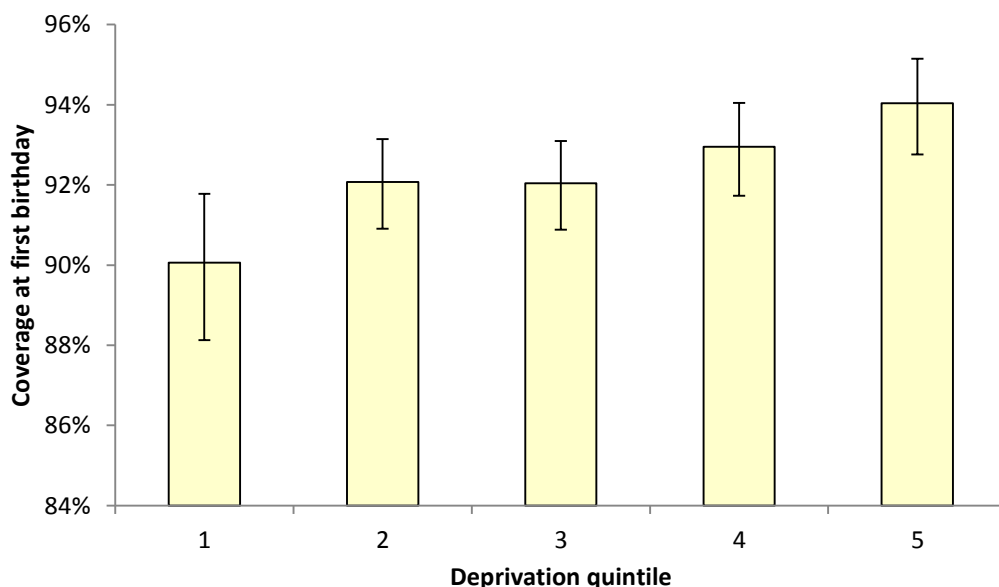


Figure 10. Vaccination coverage at first birthday by deprivation quintile (1=least deprived, 5=most deprived) for the Asian or Asian British - Indian ethnic group (error bars indicate 95% confidence intervals)

For all age cohorts, children who were not assigned to a GP in the CHIS had lower vaccination coverage than children with a GP practice code recorded. This was not a surprising finding, as not registering in general practice is generally considered to be problematic in terms of access to healthcare, but this had not previously been demonstrated with available data (a search of Pubmed (21/09/2013) with the following terms did not find any studies that had reported childhood vaccination status with respect to unregistered children: ((registration AND ("general practice" OR GP OR "child health"))) OR (unregistered AND children)) AND (vaccination OR immunisation OR immunization OR vaccine).

Recorded vaccination coverage appeared to be strongly associated with the general level of record keeping for a child, in so far as those children with records complete in other areas (for example with ethnicity completed, with their record linked to a maternal record, and assigned to a GP practice on the system), were more likely to have vaccinations recorded. Incomplete records could simply have been out of date (e.g. relating to children who have since moved out of the area), or they could relate to children still living within the PCT who are genuinely out of touch with the health system and consequently missing out on vaccinations and other areas of healthcare. Either way, records should be checked and removed/children followed up if vaccination coverage is to be accurately monitored and improved.

4. Critical reflection of methodological issues and indication of the future direction of research

Epidemiology of diphtheria in the UK and WHO European Region

Trends

Trends in diphtheria incidence in the WHO European Region were assessed from surveillance data submitted to the WHO European Regional Office by its member states. Annual reporting of aggregate case numbers is a requirement of all 53 member states. The WHO case definition for diphtheria includes only classic respiratory diphtheria cases resulting from infection with toxigenic *C. diphtheriae* (Begg, 1994); these data therefore enabled monitoring of severe infections with the potential for epidemic spread.

DIPNET member countries submitted case-based data to DIPNET for the period 2000-2009. The DIPNET case definition includes infections caused by toxigenic *C. ulcerans* and *C. pseudotuberculosis*, as well as *C. diphtheriae*; enabling monitoring of the full range of diphtheria cases detected by its 25 member countries.

The WHO case definition includes a requirement for a confirmed case (as opposed to a 'possible' or 'probable' case) to have both a respiratory presentation with pseudomembrane and to be laboratory confirmed (Begg, 1994), so the specificity is high. Similarly, the DIPNET case definition requires that confirmed cases are either laboratory confirmed (with various clinical presentations possible), or have a classic respiratory presentation and an epidemiological link to a laboratory confirmed case. Fifty-three Latvian cases not fitting the DIPNET case definition (without laboratory confirmation) were also included because they were in the national dataset; the inclusion of these cases was reliant on the experience of the Latvian clinicians involved in the diagnosis. The inclusion of cases laboratory confirmed only by PCR detection of the tox gene (as opposed to demonstration of phenotypic toxigenicity using the Elek test) highlighted a discrepancy between some countries' definitions/laboratory procedures for confirming diphtheria and the European (DIPNET and WHO) standards. Given that these cases were symptomatic it is

likely that they were toxigenic strains, but the possibility of infection with NTTB strains cannot be excluded.

Despite each DIPNET member country, excluding Latvia, submitting only small numbers of cases, there was often a lack of clarity in the data. Numbers submitted to DIPNET often differed from those submitted to WHO or the European Centre for Disease Control for the corresponding years (after taking into account differences in case definitions (Begg, 1994, European Commission, 2012)). Discrepancies were queried with individual countries and resolved, but suggested there were limitations with record keeping and/or communication within some countries. This can sometimes be caused by poor communication between epidemiological and laboratory personnel (perhaps due to physical separation and sometimes institutional cultural barriers).

The use of national surveillance data from each member country meant that WHO and DIPNET data were comprehensive in terms of their data collections, but did not rule out under-ascertainment (or record keeping errors) in individual country surveillance systems. Zero annual case numbers, as reported by several DIPNET countries, can be an indicator of the success of control programmes, but can also result from under-ascertainment. This is more relevant in countries where diphtheria cases are very rare, and laboratory skills are lost, and for *C. ulcerans* in particular because these cases often do not fulfil the standard laboratory screening criteria for diphtheria (such as travel to an endemic area). In general the *C. ulcerans* cases reported to DIPNET were from countries with particular interests in the infection and/or those with highly developed laboratory and epidemiological surveillance systems. Given the financial constraints and lack of routine screening for Corynebacteria in most European countries, as well as unfamiliarity with the clinical disease, the surveillance systems in some countries may not be sensitive for diphtheria. However, rather than meaning that large outbreaks were occurring undetected during the study periods (though there was some undetected endemic transmission in Lithuania), it is likely that occasional isolated cases were sometimes missed/not correctly diagnosed as diphtheria.

In the UK not all laboratories routinely screen for diphtheria, and it is therefore possible that, although some cases with milder or atypical symptoms were

detected, others may have been missed. Diphtheria is notifiable in the UK (to Local Authority and ultimately national level) under the Health Protection (Notification) Regulations 2010 (Public Health England, 2014c). However, case finding through medical literature proved useful in the detection of two additional *C. ulcerans* cases in the UK that had not been reported to the national centre through standard channels. Literature searching may also be useful in the surveillance of other rare infections, where unfamiliarity with organisms and procedures can lead to cases not being reported appropriately. The use of several data sources (notifications, death registrations, laboratory reports, case follow-up forms, literature searching) ensured that the UK data presented were as comprehensive as possible.

It might be beneficial to develop a set of standards against which a country's surveillance system for diphtheria could be measured so that these can be taken into account when viewing reported case numbers. Surveillance indicators developed for the surveillance of other vaccine preventable diseases that might be appropriate include; the proportion of confirmed cases with complete surveillance information, the number of cases of suspected disease that are reported, investigated and ruled out as cases, and the interval between date of symptom onset and public health notification (Roush, 2011).

For diphtheria surveillance an approach relating to a country's laboratory screening policies might be a relatively straightforward measure (e.g. if all throat swabs nationally are screened for diphtheria a system would be considered very sensitive, if screening is carried out by a few sentinel laboratories it would be moderate and if screening is only carried out at the specific request of a clinician it would be least sensitive).

C. ulcerans detection might also provide a means of assessing sensitivity. It seems reasonable to assume that this organism is present to a similar extent in domestic animals in most countries; hence a similar incidence of cases would be expected. Those countries that regularly detect cases could therefore be considered to have sensitive surveillance systems relative to those that have not detected *C. ulcerans*.

For endemic *C. diphtheriae* countries it might be possible to calculate some indicators to give a guide to surveillance system sensitivity. If we assume that,

in populations with similar vaccination coverage, for each severe case of diphtheria that is identified, there will also likely be a certain number of milder cases and asymptomatic carriers. In Latvia during 2000-2009 overall, approximately five milder respiratory cases/asymptomatic carriers were detected for every two classic respiratory diphtheria cases. In contrast in Lithuania for the same period four milder respiratory cases/asymptomatic carriers were reported with seven classic respiratory diphtheria cases. If the Latvian ratio of 5:2 is applied to the Lithuanian data we would have expected approximately 17 milder respiratory cases/asymptomatic carriers to have been reported in the Lithuanian dataset rather than four, suggesting the surveillance system in Lithuania missed several cases. This approach is based on the theory that countries who only detect the severest cases of diphtheria have relatively insensitive surveillance systems. It is complicated however by assumptions that population immunity and conditions for disease transmission are similar in both countries.

New and/or re-emerging threats from *C. diphtheriae* can potentially be better understood through the application of the latest molecular genomic technologies. Ribotyping was the typing method used in the 1990s epidemic to characterise *C. diphtheriae* strains (Popovic et al., 2000), and remains the most affordable method in use, particularly within developing countries. Multilocus Sequence Typing (MLST) is now being increasingly used, along with other methods, but there is currently no generally accepted standard method for strain characterisation (ribotyping therefore, still remains the gold standard). Whole genome sequencing however, has the potential to provide greater clarity, both in terms of our understanding of circulating strains, their origins, and spread, and the pathogenicity mechanisms that could enable particular strains to become epidemic strains.

The genomes of a range of *C. diphtheriae* strains have been sequenced. The toxin gene itself (in particular the active A subunit) is very stable (the B subunit is more variable (Nakao et al., 1996)), hence the long-term success of vaccine and antitoxin treatment. However, it is possible for more than one copy to be inserted into a bacterial chromosome resulting in increased toxin production (Rappuoli et al., 1983a, Rappuoli et al., 1983b). Pathogenicity islands that can be transferred horizontally have also been identified, the majority of which

encode subunits of adhesive pili, used for bacterial adhesion to host tissues (Mokrousov, 2009, Trost et al., 2012). To date little antimicrobial resistance in *C. diphtheriae* has been reported (apart from reports of macrolide resistance in South East Asia (Kneen et al., 1998), rifampicin resistance in Russia (Maple et al., 1994), and some multi-drug resistance in strains isolated in Brazil (Pereira et al., 2008) and Canada (Mina et al., 2011)), but it is feasible that genes encoding antimicrobial resistance mechanisms could be transferred horizontally (indeed an integron containing drug resistance gene cassettes framed by insertion sequences has already been identified within a *C. diphtheriae* biovar *mitis* genome (Barraud et al., 2011)), possibly from other species, providing further challenges for the treatment of this disease.

Risk factors for infection

For analyses beyond counts of case numbers, additional data fields were available from detailed monthly reporting to the WHO Regional Office for Europe, DIPNET enhanced surveillance, and UK enhanced surveillance.

Although four of the 16 countries asked to participate in WHO monthly surveillance did not do so, data were still available on a sizable number of cases. In addition the data collection undertaken as part of the DIPNET surveillance project created a database of European cases with common data fields, providing an opportunity to analyse larger numbers of cases than is usually possible in the post-vaccine era. Even so, for non-endemic countries these numbers were still relatively small (and for two cases the data were limited further by country-specific confidentiality restrictions preventing release of case details). Only limited case details were available for more than 200 of the Latvian cases provided to DIPNET. More than half of these cases with missing information were from the military outbreak and therefore represented a different case-profile (in terms of age and gender) from the rest of the Latvian dataset, but the remainder could have informed the profiling of cases that arose in the general population.

Completion of fields such as 'risk group' and 'veterinary contact' depended to a certain extent on a country's interpretation/investigations around a case as well as their recording of this information for past cases. The vaccination status field would have had different methods of completion (e.g. patient recall vs

documented records) for different cases, and in some instances was assumed based on a patient's age and country of birth. In addition different vaccination schedules in different countries meant that the classification of partially/fully vaccinated may have differed with respect to the number of doses received in some instances. In the UK completeness of surveillance forms, and vaccination histories, was variable. A standardised questionnaire was only in use since 1995, and questions on companion animals (for *C. ulcerans*) were only included from 2003 onwards. Since the quality of any analysis is dependent on the accuracy of the data on which it is based, variations in data collection methodology and missing data were potentially serious limitations. However, these data provided the only means of studying this rare disease in current European populations, and were sufficiently complete to enable several common themes to be observed. The availability of comprehensive DIPNET and UK surveillance forms should improve data recording for future cases. In addition in the UK an electronic information management system (HPZone) is now utilised by Public Health England to record information on cases and incidents. HPZone is used in both local health protection units and the national surveillance centre enabling viewing of data between local and national teams. It records all possible cases at local level and documents the risk assessment and laboratory and epidemiological investigations, improving data quality in the last five years.

Cases of diphtheria due to *C. ulcerans* were shown to have a range of presentations, including classic respiratory diphtheria, and the majority of *C. ulcerans* cases reported by DIPNET countries had had contact with domestic animals. However, there remain some unanswered questions relating to the spread of this organism. It has been demonstrated to have a wide host range (Seto et al., 2008) which includes companion animals, and identical organisms have been isolated from human cases and the domestic pets with which they have been in contact (Hogg et al., 2009, Berger et al., 2011). In addition, a study in Japan found that carriage of *C. ulcerans* in healthy domestic dogs was 7.5% (44/583; 42 toxigenic and three non-toxigenic strains were identified in 44 dogs (from one dog both toxigenic and non-toxigenic organisms were isolated)) (Katsukawa et al., 2012). Another carriage study in which swabs from the oropharynx of healthy cats and dogs in rescue centres were screened for

Corynebacteria is also underway in North West England (only preliminary results published to date (National Consortium for Zoonosis Research, no date)). But the direction of transmission (animal to human and/or human to animal) and whether the organism can be passed from human to human are aspects around which there is still uncertainty. These two questions may only be further understood should specific case scenarios arise. Even then, the testing of animals in the event of a case in the UK continues to be problematic, not least because some cases have been exposed to many animals. There is currently no guidance relating to the testing or treatment of animals associated with human toxigenic *C. ulcerans* cases. Questions exist regarding for example, who should cover the costs of screening animals, as well as the course of action should a toxigenic strain be identified in a companion animal (whether or not the animal should be treated to eliminate carriage, and if so which treatment to use as the antibiotics used in human treatment are not suitable for animals). Swabbing of companion animals is therefore not always conducted.

Disease severity and case fatality

Classification of cases caused by toxigenic strains into categories relating to their disease severity (classic respiratory, mild diphtheria/severe pharyngitis, asymptomatic) and vaccination status (vaccinated, partially vaccinated, unvaccinated) enabled a test for trend to be applied, which gave an overview of the relationship between vaccination status and disease severity across the DIPNET dataset. In the UK dataset, the relationship between vaccination status and disease severity was analysed as a simple comparison of the proportions of cases vaccinated vs partially/unvaccinated presenting with classic respiratory diphtheria which also demonstrated a clear effect.

The classification of disease severity into different groupings also enabled the calculation of case fatality rates for classic respiratory symptoms as well as any respiratory symptoms. Cases with classic respiratory diphtheria are difficult to treat, even if DAT is readily available, as it may be too late for the DAT treatment to be effective. This may explain the similarities in the case fatality rates for classic respiratory diphtheria in the UK, Latvia and DIPNET countries excluding Latvia.

Case fatality rates for any respiratory symptoms can vary across different populations for a number of reasons. For cases to be included in the surveillance dataset requires that they are recognised and correctly diagnosed as diphtheria. Lack of diagnosis of mild cases could raise the apparent case fatality rate if only the severest disease is recognised as diphtheria. Only countries that have staff competent in the laboratory diagnosis of diphtheria, the resources available to perform the testing, and the policies in place to initiate screening will reliably detect milder cases of disease.

A high case fatality rate, as well as demonstrating the seriousness of an infection, indicates that a large proportion of cases have low immunity levels and for whatever reason are not prevented from progressing to severe disease by medical treatment. Appropriate medical treatment may not be administered if the disease is not correctly diagnosed in time, or if DAT is not available. As observed during the epidemic in the former Soviet Union, the availability of DAT can have a dramatic impact on CFRs. In Russia, where DAT was always available, the CFR was approximately 3%, compared to >20% at the start of the epidemic in the Newly Independent States (excluding the Russian Federation) where supplies of DAT and antibiotics were limited (Dittmann et al., 2000). In recent years prompt treatment of cases has been problematic for countries that do not maintain a supply of DAT.

Evidence base for the use of DAT in the UK, and issues relating to its supply internationally

Fields relating to DAT administration for UK cases were poorly completed, in particular those relating to the timing of administration and dose of DAT. Because the impact of DAT treatment is heavily dependent on timing, it was therefore difficult to draw strong conclusions from the data regarding the effectiveness of DAT treatment. However, the fact that <50% of severe cases (respiratory symptoms with membrane or exudate) received DAT does indicate that even in the UK, where DAT is readily available, there were difficulties surrounding the treatment of diphtheria cases. These include timely diagnosis, as well as the acceptability of DAT. Data concerning the treatment of cases were not collected across DIPNET countries but would have been of value to contribute to this analysis.

As well as improving completion of fields relating to DAT administration on surveillance forms for UK cases, it may be possible to link records for requests for DAT from issuing centres to provide confirmation of timings in relation to onset of symptoms. These data are also now better recorded through HPZone (the electronic patient management system now in use in the UK, described above) so recent data should allow improved analyses to be undertaken.

The DAT survey allowed assessment of the extent of DAT supplies and shortages. Invitation to complete the survey, beyond DIPNET members, was primarily dependent on contact details being available for an appropriate person within a particular country. Expanding the survey, through the assistance of the WHO to Russian-speaking countries enabled wider participation. However, there were still many countries not reached by the survey, and it would have been particularly interesting to understand the situation in diphtheria-endemic countries outside of the European Region. Even without wider participation, the results demonstrated the challenges in the supply of this product. The review has already been of value to those advocating for a supply of DAT in their country, as well as those needing information about current suppliers.

Further development is needed to explore alternatives to equine DAT. Promisingly, a human monoclonal antibody was recently identified which binds to the receptor-binding domain of the diphtheria toxin and completely protected guinea pigs from intoxication in an *in vivo* model (Sevigny et al., 2013). Additional testing is planned to explore its safety and efficacy for development as a human treatment.

As well as being used for diphtheria therapy, DAT is also a component of the Elek test used for the laboratory confirmation of diphtheria. A review has recently been undertaken to explore access to DAT for both therapeutic and diagnostic purposes; it further emphasises the need for alternatives and/or a central stockpile (Both et al., 2014).

Circulation of both toxigenic and non-toxigenic organisms, including *C. ulcerans*, in endemic and non-endemic countries within the WHO European Region

Carriage of potentially toxigenic organisms in ten DIPNET countries was assessed by screening routinely submitted (throat) swabs from people with sore

throats. Given that a sore throat is a symptom of respiratory diphtheria, this had the advantage of focussing study resources on populations from which these organisms were most likely to be isolated, but the carriage rates did not then apply to the populations as a whole.

The sample size calculated for the study (for the number of swabs each country was required to screen) was based on the prevalence of 3.7 per 1,000 population from a previous screening study in Latvia. Latvia has the highest incidence of diphtheria in the European region, so the sample size may have been too small to detect organisms in other countries. Even so, more than half of the participating countries (Bulgaria, Finland, Greece, Italy, Ireland and Latvia) screened fewer than 2,700 samples; there was therefore insufficient power in these studies, hence the wide confidence intervals on the zero estimates.

The ten countries that participated in the study ranged from countries within Europe that routinely report cases (Latvia, UK), to countries that had not reported any cases in the seven years immediately preceding the study (Bulgaria, Greece, Ireland). Central Europe was not represented, but there was no evidence to suggest that carriage would be different in Central Europe compared to in other countries included in the study. Within countries, the study relied on voluntary participation of laboratories; as such the areas served did not always represent the general population of that country (for example, no London laboratories participated from the UK). The age of the populations sampled in each country also varied, with some countries including a large number of swabs from children's hospitals (these were the only swabs included for Greece). Given that immunity levels vary with age, and that children often have higher immunity than adults, this may have influenced the low/zero carriage rates for these countries. The higher number of females compared to males in the study as a whole may simply have related to higher consultation rates amongst females in general practice.

The study relied on the national policies and procedures in place in individual countries for the submission of throat swabs. Clinicians in different countries, and possibly also within countries, have different criteria for submitting a throat swab for laboratory investigation. This depends on the policies within different

countries (in some countries additional costs are incurred for transporting samples), as well as a clinician's criteria/knowledge for clinical suspicion.

All countries participating in the study processed throat swabs for potentially toxigenic *Corynebacteria* according to their standard protocols and WHO guidelines. Ideally a standard study laboratory protocol would have been used. However, most countries base their laboratory protocol on WHO guidelines, so protocols across countries should have been similar. Furthermore, all organisms reported in the study were confirmed at the Health Protection Agency's Respiratory and Systemic Infections Department, UK.

A DIPNET international external quality assurance study was conducted just after the study period had ended, in which all ten countries from the carriage study participated (Neal and Efstratiou, 2009). It found that only 6/34 centres produced acceptable results for all six specimens, and many centres could not isolate the target organism. Training workshops had been conducted in Turkey and Estonia just prior to the start of the study; both of these countries identified non-toxigenic strains, suggesting laboratory competence in some of the other participating countries (that did not identify any isolates) was an issue.

Although surveillance forms were completed for patients identified as infected with toxigenic strains, those carrying non-toxigenic strains were not followed up. Since no public health action is taken around detection of non-toxigenic strains, follow-up may have caused undue concern in these patients. However, it would have been interesting to know the vaccination status of those carrying non-toxigenic strains, if they had any other symptoms (beyond a sore throat), and if they had a recent history of travel abroad.

As described above, this study was limited by several factors which should be taken into account when viewing the results. Ideally the study would have included laboratory training prior to the screening period, a common laboratory screening protocol, funding for personnel as well as laboratory media, wider participation and greater sample sizes from participating countries. However, it was limited by the resources available. Despite these limitations the demonstration of endemic transmission in Lithuania was an important finding with direct public health implications. In addition, although carriage estimates within several countries were uncertain (given the wide confidence intervals),

pooling the results gives a carriage rate across the ten participating DIPNET member countries, for both toxigenic and non-toxigenic strains combined, of 1.1 per 1,000 swabs screened (95% confidence interval 0.8-1.6). The study also serves as a baseline from which the methodology can be developed for future assessments of carriage.

Following the screening study, Lithuania increased its laboratory screening practices. It would be interesting to know the impact of this change in relation to detection of strains (both toxigenic and non-toxigenic). Since the screening study was undertaken in 2007, Lithuania has reported only one further toxigenic *C. diphtheriae* case to WHO (in 2011) (World Health Organization, 2013). Unfortunately austerity measures resulting from the financial crisis across Europe will have adversely affected screening practices/surveillance and thus any increases in screening immediately following the study may not have been long in duration.

The UK has a large migrant population which includes increasingly populations from eastern European countries (Latvia and Lithuania joined the EU in 2004). Detection of strains as part of screening undertaken in the UK, either in specific migrant populations, or routinely if country of birth data are collected, could potentially indicate continuing/increased transmission in countries with more limited laboratory resources.

Susceptibility of the UK population

Diphtheria immunity levels, taking into account changes to the UK vaccination schedule

National vaccination coverage is documented over time, and the low numbers of diphtheria cases reported nationally, along with high recorded vaccination coverage suggest that population immunity is high, and consequently the diphtheria vaccine in use in the UK is effective. However, a serosurvey allows direct measurement of the population immunity afforded by the vaccination programme.

The sera used in the serosurvey represented most geographical regions of England, as well as a range of ages. The results were standardised to the UK population as a whole to give a measure of overall immunity for the UK. This

seemed reasonable given that the vaccination schedule applies to the whole of the UK. Vaccination coverage is generally higher in Scotland than England though, so UK immunity may have been very slightly underestimated in this study.

Unfortunately individual vaccination histories of the patients whose sera were included in the survey were not available (in contrast to collections e.g. at the National Institute for Public Health and the Environment, RIVM in the Netherlands) so to what extent they matched the UK vaccination schedule, and represented national vaccination coverage could not be determined.

The residual sera used in the study were collected typically from patients presenting with symptoms requiring diagnostic testing. However, sera from patients known to be immunocompromised were excluded from the archive collection; previous studies using this sampling base have shown it to be representative of the wider population (Osborne et al., 2000).

The international standard correlates of protection for diphtheria (whereby antitoxin levels <0.01 IU/mL denote susceptibility, antitoxin levels 0.01-0.099 IU/mL provide basic protection, and antitoxin levels ≥ 0.1 IU/mL are fully protective) were derived from studies of patients with diphtheria and relate to protection from severe disease (World Health Organization, 2009) (in contrast to those for tetanus, which lacks established criteria).

The multiplexed fluorescent bead assay was used to measure antitoxin levels in 2009, a different method to that used in 1996. The multiplex assay enabled a large number of samples to be run rapidly against multiple antigens (in this case *Haemophilus influenzae* type b, diphtheria, and tetanus). This method was more cost effective, and also used less serum volume than the methods used in 1996 (indirect enzyme linked immunosorbent assay (ELISA) and dissociation enhanced lanthanide fluorescence immunoassay (DELFI)). Standardisation of a panel of samples from 1996 enabled the results from 1996 and 2009 to be compared despite the use of different laboratory methods at the two time points.

The UK does not schedule any diphtheria booster immunisations for adults (several European countries offer ten yearly boosters although compliance is not known as reliable coverage data is not available). Immunity in older age

groups could be further explored, particularly in relation to countries that offer additional boosting to understand not only the differences in adult immunity levels (for smaller age groups than studied here), but also how widely these boosters are taken up by adults in countries where they are offered.

Given that there are variations in vaccination coverage across the country, it may also be of interest to explore regional variations in immunity, which it was not possible to do within this serosurvey data.

Childhood vaccination coverage within different ethnic groups in London

Childhood vaccination coverage data were extracted from CHISs in nine London PCTs, using a common script and analysed by ethnicity.

The study took place during 2011/2012, around the time of a major re-organisation of the NHS including the abolishment of PCTs (childhood vaccination coverage is now assessed by Local Authority (as well as by PCT to allow for continuity with historical data)). Participation in the study was voluntary and relied on interest within each PCT; completion was challenging given the absence of specific funding, and the organisational changes occurring in PCTs at that time. However, the nine PCTs that participated represented several geographical locations across London. A comparison of Greater London Authority projections overall for the same time period (children aged 0-4 years in 2009, and equivalent calendar years for children born 2005-2009 from the study dataset) found that the proportion of Black and Minority Ethnic groups in the study dataset was 51%, whilst the proportion of Black and Minority Ethnic groups for the same time period for Greater London was similarly 53%. In terms of vaccination coverage, the study PCTs came from all four quartiles of 2010/2011 London PCT COVER data for diphtheria-containing vaccines at first, second and fifth birthdays. Therefore, although not specifically selected for the study, the participating PCTs did appear representative of Greater London PCTs as a whole.

Participation of a greater number of London PCTs would have improved the sample sizes, which could have been beneficial for observing coverage in smaller ethnic groups. Participation beyond London could also have expanded the range of ethnic groups to include those not concentrated in London.

However, even with only nine London PCTs participating, the dataset was large (over 300,000 records), and included a range of ethnicities, including those most common in the UK as a whole (White British, Indian, Pakistani).

The study was limited to London PCTs using the RiO CHIS because the extraction script was written for this system by one of the study authors. Participation by PCTs using other systems would have been possible but would have depended on the interest and skills of particular data managers familiar with those systems. Some PCTs expressed interest in participating but did not feel able to extract the data, highlighting a drawback of CHISs that have a limited range of options available for extractions and are difficult to query. RiO is now being used more widely beyond London in the south of England (Evenstad, 2014, Todd, 2014) so a future study could have wider participation.

An advantage of using RiO data was that these data were routinely collected on a large scale over several years. The RiO system contains documented immunisation data for each child, which should be more accurate than data based on maternal recall of vaccinations (as used by the Millennium Cohort study of childhood vaccination coverage (Samad et al., 2006)).

Diphtheria-containing vaccines (as opposed to other vaccines) were chosen as the measure of vaccination coverage because they have been routine for many years, and are generally well accepted. For this reason they can also to a certain extent provide a proxy for the level of contact a child has had with the UK health system. The vaccination status of each child was calculated by taking into account all diphtheria-containing vaccines recorded for that child. This allowed vaccines not routinely administered in the UK but containing equivalent dosage to be included. The method of assigning vaccination status was more rigorous than some COVER extractions in so far as every dose was required to be recorded, rather than only the final dose.

Although the exact date of vaccination was available, only month and year of birth were extracted for confidentiality reasons. It was therefore not possible to calculate if the vaccination was received 'by first birthday' exactly. However, the calculation was generous, counting those vaccines received up to 13 months rather than exactly 12 months. Given that the primary course is scheduled between 2 and 4 months of age, the numbers fully vaccinated at 13 months

would not have been expected to differ greatly from the number fully vaccinated at 12 months.

Partially immunised and completely unimmunised were included in the same grouping (termed 'not fully immunised'). Although partial immunisation would provide some protection, the goal of the immunisation programme is full vaccination. The COVER programme monitors completion of the primary course, hence that was the measure used for this study (the number/proportion of children fully vaccinated is also the requirement for annual country reports to the WHO). However, it might be of interest to further explore the data and determine the proportions partially immunised for each ethnic group to improve understanding of the breakdown of vaccination status, in particular of children in ethnic groups with low overall coverage.

The study was limited to the data fields available for extraction in the RiO CHIS. Consequently there may have been variables missing from the model that might affect vaccination coverage. However, the variables that were included (gender, deprivation, PCT and fiscal year of birth) did not show much evidence of confounding the ethnicity effect. Although other variables may explain the differences, it could be argued that these are part of the profile of ethnicity so the unadjusted coverage is still important. In other published analyses additional factors were studied such as family size, maternal smoking, maternal education and lone parenthood (Samad et al., 2006, Baker et al., 2011). But although the study based in Manchester included some of these other measures, their principle finding related to deprivation (Baker et al., 2011), which was a measure included in this study.

Despite the number of fields available being limited, the data in the fields included in the model was near complete; gender (>99.9% complete), deprivation (98% complete), PCT (100% complete) and fiscal year of birth (100% complete). In the ethnicity data a category was included for 'not known' to display the characteristics of records with missing information in this regard.

There were insufficient data fields available for extraction to assess the individual deprivation score of each child; deprivation was therefore assessed according to the geographical area of residence, Lower Super Output Area (LSOA). Given that there were on average 1,600 people resident in each LSOA

in 2010 (Office for National Statistics, 2011) and that deprivation can vary in close proximity in London areas, there would have been some inaccuracies in this method. However, LSOA was the smallest geographical area available for use in the study given that postcodes needed to be removed (for confidentiality reasons) before the data left the PCT. The Income Deprivation Affecting Children Index was chosen (as opposed to other geographical measures of deprivation) because it relates to children, the subject of this study. It is a ranking based on the percentage of children aged 0-15 years in each LSOA living in families that are income-deprived (Local Government Association, 2014).

Multivariable logistic regression enabled the effect of other variables (gender, deprivation, PCT and year of birth) on coverage to be taken into account. Interactions were examined based on *a priori* reasoning. An interaction between PCT and ethnicity was examined because different PCTs may take different approaches to targeting coverage in particular ethnic groups. In addition, an interaction between ethnicity and deprivation was examined because the Manchester study had identified that for white infants, lower coverage was significantly associated with living in a deprived area, but for black infants or black British infants and Pakistanis, there was no significant association between deprivation and immunisation (Baker et al., 2011).

No record of immunisation indicates that either the child did not receive the immunisations, or any immunisations received were not recorded. Populations moving in to a PCT may not have had their immunisation records transferred across. This process is largely done manually as these data cannot always be automatically transferred between systems. This could give a falsely low coverage result. If demographic data are also missing from the record, and the record is not linked to a maternal record this suggests missing data could be the main issue (rather than lack of receipt of vaccines). Children without immunisations recorded may also be children who have moved out of the system but have not had their record deleted. Without clarifying these data issues at the PCT-level, the true vaccination coverage cannot be determined. Information about country of birth and date of arrival in the UK (where relevant) would be useful to further understand some of these issues.

It may be possible to link child health system records with birth registrations data to see what proportion of records match to UK birth details for the same period. This may help to further understanding of populations with low immunisation coverage/without GP practice codes recorded on the system if children are shown either to have been born outside their current area of residence or if their birth was not registered in the UK.

Another option is to improve country of birth recording at the GP practice level. Some GP systems have the facility for the country of birth field to be specifically added in to their standard data-capture screen. Encouraging collection of this information within GP practices could improve the data on CHISs, as well as enabling GPs to take country of birth related health issues into account when considering patient care (Public Health England, no date).

A national reconciliation exercise of CHIS and GP systems is currently being undertaken to assess the potential for children to be registered with GPs but not known to CHIS systems. It is thought this will particularly highlight children who have moved in from abroad. It also includes an analysis of the systems in place for the transfer of data between GP/CHIS and CHIS/CHIS.

Whilst combining data across the nine participating PCTs enabled the analysis of overall coverage for different ethnic groups across London, the study also provided a means for each individual PCT to explore their coverage data. Individual analyses were fed back to each participating PCT providing breakdowns of coverage by ethnicity, and numbers of unregistered children, so that these data could be used for further investigations at a local level.

In conclusion, the studies described here, although limited to varying extents by issues relating to ascertainment and/or record keeping, have provided a valuable update on the current epidemiology of diphtheria in the UK and European Region. Importantly, they have also highlighted the need to maintain laboratory and clinical expertise in this area, and to continue striving for good population immunity throughout the European Region.

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6. Publications (full text)

REVIEW ARTICLE

Diphtheria in the United Kingdom, 1986–2008: the increasing role of *Corynebacterium ulcerans*

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SUMMARY

Diphtheria is an uncommon disease in the UK due to an effective immunization programme; consequently when cases do arise, there can be delays in diagnosis and case-fatality rates remain high. We reviewed 102 patients with infections caused by toxigenic corynebacteria (an average of four per year) reported in the UK between 1986 and 2008: 42 *Corynebacterium diphtheriae*, 59 *C. ulcerans* and one *C. pseudotuberculosis*, as well as 23 asymptomatic carriers. Five fatalities were reported, all in unvaccinated patients. The major risk factor for *C. diphtheriae* infection continued to be travel to an endemic country. *C. ulcerans* infections became more common than *C. diphtheriae* infections in the UK; they were associated with contact with companion animals. The occurrence of indigenous severe *C. ulcerans* infections and imported *C. diphtheriae* cases highlights the need to maintain UK routine vaccination coverage at the 95% level in the UK, as recommended by the World Health Organization.

Key words: *Corynebacterium*, diphtheria, epidemiology, immunization, vaccine-preventable diseases.

INTRODUCTION

Diphtheria, historically one of the most feared diseases of childhood, is now uncommon in the UK due to national immunization since the 1940s (Fig. 1). Since 1990, UK diphtheria vaccination coverage at age 2 years has exceeded 90%, rising to 94% from the beginning of the 21st century, close to the World Health Organization (WHO) 95% target. Diphtheria vaccine is made from inactivated diphtheria toxin

and protects individuals from the effects of toxin-producing corynebacteria. Three *Corynebacterium* spp. can potentially produce diphtheria toxin; *C. diphtheriae* (associated with epidemic diphtheria and spread from person-to-person via respiratory droplets and close contact), *C. ulcerans* and *C. pseudotuberculosis* (both less common globally and traditionally associated with farm animal contact and dairy products). The classic and most severe presentation of diphtheria is a respiratory disease with a swollen ‘bull neck’ and strongly adherent pseudo-membrane, which obstructs the airways. Patients with less severe respiratory disease can present with a sore

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Table 1. UK vaccination schedule for diphtheria-containing vaccines

Age	Vaccine
2, 3, 4 months	DTaP/IPV/Hib (diphtheria, tetanus, acellular pertussis, polio, <i>Haemophilus influenzae</i> group b antigens)
3 years and 4 months to 5 years	dTaP/IPV or DTaP/IPV (diphtheria, tetanus, acellular pertussis and polio)
13–18 years	Td/IPV (tetanus, diphtheria and polio)
<ul style="list-style-type: none"> ● All travellers to epidemic or endemic areas should ensure that they are fully immunized according to the UK schedule. Additional doses of vaccines may be required according to the destination and the nature of travel intended. Where tetanus, diphtheria or polio protection is required and the final dose of the relevant antigen was more than 10 years ago, Td/IPV should be given. ● Individuals who may be exposed to diphtheria in microbiology laboratories and clinical infectious disease units should be tested and, if necessary, given a booster dose of a diphtheria-containing vaccine. An antibody test should be performed at least 3 months after immunization to confirm protective immunity and the individual should ideally be given a booster dose at 10-year intervals thereafter. ● Diphtheria vaccine may also be given as part of the combined Td/IPV vaccine given to individuals presenting with a tetanus-prone injury. 	

Source: Green Book 2006 [1].

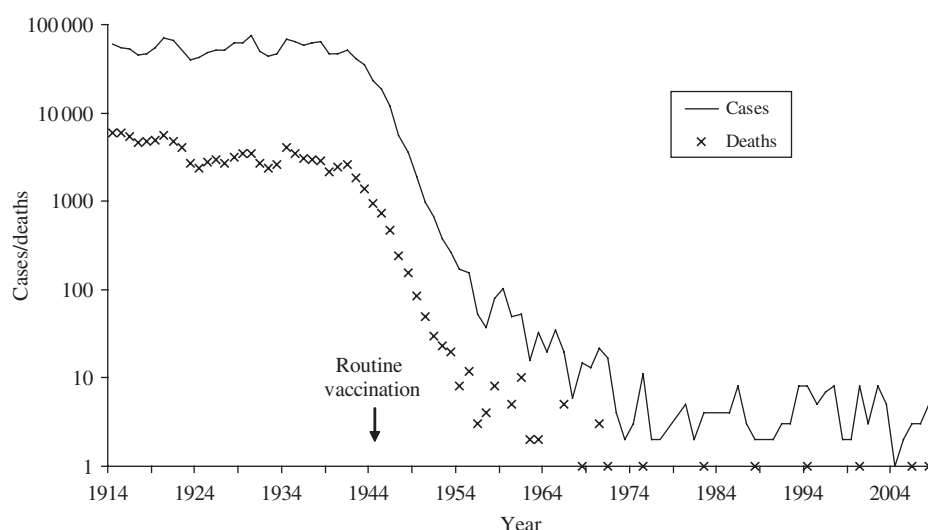


Fig. 1. Diphtheria notifications and deaths in England and Wales 1914–2008 (notifications up to 1985, laboratory-confirmed cases 1986–2008).

throat. Diphtheria can also cause cutaneous infection, characterized by ‘rolled edge’ ulcers, which are more common in tropical areas of the world.

Diphtheria vaccine is currently scheduled in the UK as shown in Table 1. An accelerated schedule (2, 3, 4 months) for infant immunization replaced an extended schedule [2] (3, $4\frac{1}{2}$ –5, $8\frac{1}{2}$ –11 months) in the early 1990s [3], and the low-dose diphtheria component was added to the school-leaver dose of tetanus toxoid (Td) in 1994 (Td/IPV since 2004) [4]. A total of five doses of a diphtheria-containing vaccine at appropriate intervals are considered to give satisfactory long-term protection in most circumstances.

UK guidelines for the control of diphtheria, and laboratory diagnosis of infections caused by

Corynebacterium diphtheriae and *C. ulcerans* were published in 1999 [5]. A clinical case of respiratory diphtheria requires rapid administration of diphtheria antitoxin (a concentrated immunoglobulin preparation prepared from horse serum, that neutralizes circulating toxin), as well as antibiotics to clear the bacterial infection. Antibiotics of choice are erythromycin, azithromycin, clarithromycin, or penicillin, all of which are active *in vitro* against *C. diphtheriae* and *C. ulcerans*. Administration of diphtheria vaccine is recommended during convalescence because diphtheria infection does not always confer immunity.

This paper summarizes all cases of diphtheria and other related infections caused by toxigenic corynebacteria that have been reported in the UK

during the last 23 years, highlighting key trends and characteristics of the disease, as well as its changing epidemiology.

METHODS

Information concerning diphtheria cases in the years 1986–2008 was obtained from the following routine sources:

- Statutory notifications to the Office for National Statistics up to 1996, which then transferred to the Communicable Disease Surveillance Centre, now the Health Protection Agency (HPA) Centre for Infections (CfI).
- Death registrations to the Office for National Statistics.
- Laboratory reports from the WHO Collaborating Centre for Diphtheria and Streptococcal Infections, Respiratory and Systemic Infections Department.
- Case follow-up information from the HPA CfI Immunization, Hepatitis and Blood Safety Department.

In addition a literature search was carried out across Medline, EMBASE and Scopus databases during July–September 2008 in order to identify any additional cases not reported through routine surveillance. The search strategies covered titles and abstracts of English-language publications from 1985 to 2008 and comprised key-word combinations of ‘diphtheria’, ‘Corynebacterium AND diphtheriae’, ‘Corynebacterium AND ulcerans’, ‘Corynebacterium AND pseudotuberculosis’.

In the UK, toxigenicity testing of suspect isolates from local laboratories is carried out by the WHO Collaborating Centre for Diphtheria and Streptococcal Infections, Respiratory and Systemic Infections Department (RSID) at the HPA in London. Historically, the Elek test has been used since 1940 to assess toxin production; prior to 1991, toxigenicity was also assessed by an *in vivo* subcutaneous test, and since 1993 PCR has been used to detect the presence of the toxin gene. However, the gold standard phenotypic test is the Elek test [6]. Case follow-up is prompted by either a report of a toxigenic *Corynebacterium* isolate from the RSID, a direct communication with a consultant in communicable disease control or a clinician involved in the management of a suspected case, or notification of a suspected case of diphtheria to the local authority. Follow-up of all

toxigenic isolates (cases and carriers) of *Corynebacterium* spp. has been standardized since 1995 using a questionnaire to ascertain the patient’s clinical and immunization history, travel history and contact with travellers, exposure to raw dairy produce and domestic animals (*C. ulcerans* only) and management of the case and contacts. Information about contact with companion animals (cats/dogs) for *C. ulcerans* cases has been included on follow-up forms since 2003.

Here, a confirmed case is defined according to the Diphtheria Surveillance Network (DIPNET, www.dipnet.org) case definition (see Appendix) whereby a toxigenic isolate of *C. diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis* has been isolated from the patient with an appropriate clinical presentation. An asymptomatic carrier is defined as having a toxigenic isolate with no symptoms. In this paper cases are further grouped according to the severity of their disease, the most severe presentation being classic respiratory diphtheria with pseudomembrane.

Statistical analyses involved χ^2 tests using Stata statistical software, release 8.0 (StataCorp, USA).

Patients were assigned to four groups according to their vaccination status:

- *Fully immunized for age* [have received all scheduled vaccinations appropriate for their age (and vaccination schedule of their time), if they have a history of recent travel to an endemic area or work in a laboratory handling diphtheria this includes receipt of appropriate booster immunizations].
- *Partially immunized* (have received some scheduled vaccinations but not all appropriate for their age, or have not received appropriate booster vaccinations for travel/occupation).
- *Vaccination history not known or not reported*.
- *Unimmunized* (if no history was available, patients born prior to 1940 were assumed to be unimmunized).

RESULTS

During the 23-year period 1986–2008, there were 125 toxigenic *Corynebacterium* isolates; *C. diphtheriae* (62), *C. ulcerans* (62) and *C. pseudotuberculosis* (1). The data for *C. diphtheriae* and *C. ulcerans*, clinical presentation and immunization status are summarized in Table 2 (data for *C. pseudotuberculosis* are described later). The analysis includes two cases of toxigenic *C. ulcerans* which had not been routinely

Table 2. *Toxigenic C. diphtheriae and C. ulcerans isolates by clinical presentation and immunization status*

Immunization status at the time of infection/clinical presentation	<i>C. diphtheriae</i>					<i>C. ulcerans</i>					Grand total <i>C. diphtheriae</i> and <i>C. ulcerans</i> (%)
	Fully immunized for age	Partially immunized	Vaccination history not known or not reported	Unimmunized	Total (%)	Fully immunized for age	Partially immunized	Vaccination history not known or not reported	Unimmunized	Total (%)	
Classic respiratory presentation with adherent pseudomembrane observed in tonsils, pharynx, or larynx		1*		4* (includes 2 fatal cases)	5 (8%)		2	1	7 (includes 3 fatal cases)	10 (16%)	15 (12%)
Respiratory presentation with exudate†		2		1	3 (5%)	3			1	4 (6%)	7 (6%)
Respiratory presentation (sore throat) with no pseudomembrane or exudate	5	4	4	1	14 (23%)	16	1	16	4	37 (60%)	51 (41%)
Respiratory and cutaneous lesions‡			1	3	4 (6%)					—	4 (3%)
Cutaneous lesions	4	4	2	5	15 (24%)			6	2	8 (13%)	23 (19%)
Other (bacterial endocarditis)	1				1 (2%)					—	1 (1%)
Asymptomatic§	8		11	1	20 (32%)	2		1		3 (5%)	23 (19%)
Total	18	11	18	15	62	21	3	24	14	62	124

* One patient from each of these groups also had cutaneous lesions but has been assigned to this group since this is the most serious presentation.

† Observation of tonsillar exudate, although not a solid membrane, could indicate the early stages of membrane formation.

‡ Toxigenic organism isolated from both sites.

§ May have been swabbed due to another illness or may be a contact (with no symptoms) of a confirmed case.

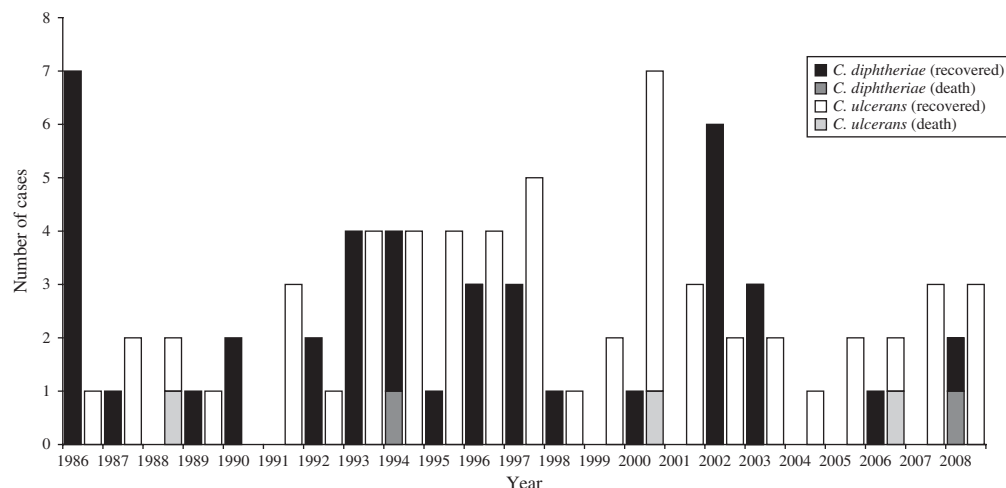


Fig. 2. Toxigenic cases of diphtheria and outcome in the UK, 1986–2008.

reported but were detected through the literature search [7, 8].

Fifteen cases of classic respiratory diphtheria with pseudomembrane were reported between 1986 and 2008, none of whom were fully vaccinated (Table 2). The most frequent presentation among UK cases is respiratory disease; typically a sore throat in a fully or partially immunized individual. Twenty-nine patients presented with cutaneous lesions, six of whom also had respiratory symptoms (including two with a pseudomembrane). One patient with toxigenic *C. diphtheriae* infection presented with bacterial endocarditis. Vaccination history was frequently unavailable in the follow-up notes for *C. ulcerans* cases (particularly earlier cases), and for asymptomatic carriers of *C. diphtheriae*. However, based on all cases with available data, the protective effect of vaccination could be demonstrated since none of the 39 fully vaccinated cases were recorded as presenting with classic respiratory diphtheria with pseudomembrane, whereas 14 of the 43 unvaccinated/incompletely vaccinated cases presented with these symptoms ($P < 0.001$).

The following analysis excludes the asymptomatic patients listed in Table 2, these patients are described separately later. Between one and nine symptomatic cases of diphtheria were recorded each year in the UK between 1986 and 2008 (Fig. 2); an average of four cases per year. The yearly incidence rates ranged from 0.0141 (in 1986) to 0.0017 (in 2004) cases per 100 000 population. In the last 10 years *C. ulcerans*, rather than *C. diphtheriae*, has been the predominant cause of diphtheria in the UK. Forty per cent of the *C. diphtheriae* cases were reported from the London

region, whereas the *C. ulcerans* cases were distributed more evenly across the country. The predominant toxigenic *C. diphtheriae* biotype in the UK during 1986–2008 was var. *mitis* (81% of cases), followed by var. *gravis* (17%) and var. *intermedius* (one case only).

The majority of *C. ulcerans* cases (76%) were female whereas the sex distribution was even for *C. diphtheriae* (Fig. 3). In addition *C. ulcerans* cases were generally older than *C. diphtheriae* cases with mean and median age for *C. ulcerans* cases of 38 years compared to 15 years (mean) and 21.5 years (median) for *C. diphtheriae* cases.

C. diphtheriae risk factors

The main risk factor for acquisition of toxigenic *C. diphtheriae* was travel to the Indian sub-continent, Africa or South East Asia (Table 3). Only eight cases had no history of travel or contact with a traveller recorded, three of which were laboratory-acquired infections. All three cases of laboratory-acquired diphtheria were due to toxigenic *C. diphtheriae* and occurred in separate incidents. The first in 1987 concerned a senior registrar in medical microbiology who had handled a non-toxicogenic isolate of *C. diphtheriae* and a toxigenic control strain. The patient was known to have received childhood immunizations and presented with a severe sore throat with white exudate on both tonsillar beds. The second case in 1997 concerned an experienced medical laboratory scientific officer (MLSO) who became infected with a toxigenic strain of *C. diphtheriae* while handling a sample distributed by the National External Quality Assessment Scheme for microbiology in a non-containment

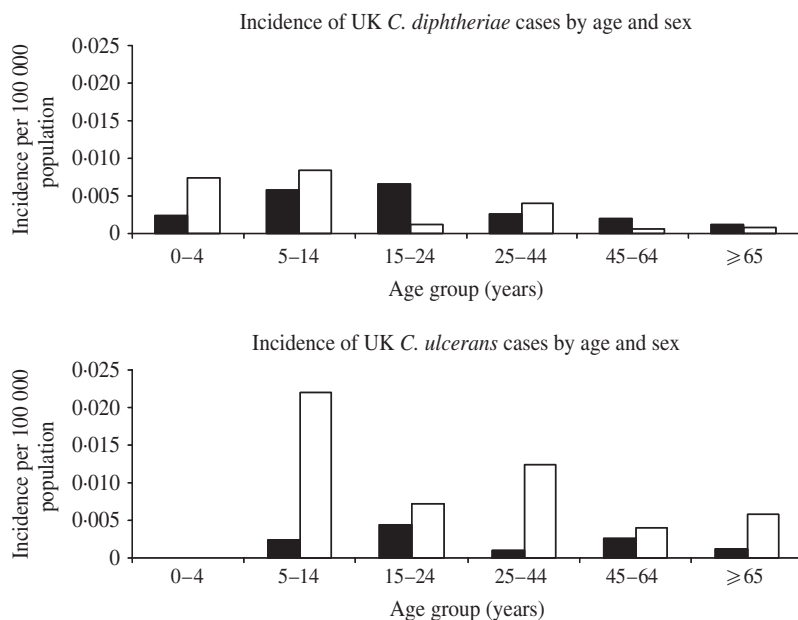


Fig. 3. Distribution of diphtheria cases by organism, age and sex. ■, Males; □, females.

facility [20]. The MLSO was known to have received childhood immunizations and developed severe tonsillitis. The third case in 2003 also occurred in a laboratory worker handling liquid samples of a toxigenic strain on an open bench in a microbiology laboratory.

C. ulcerans risk factors

Only eight *C. ulcerans* cases had a history of travel abroad within the 3 months prior to the onset of their infection. Seven of 59 (12%) *C. ulcerans* cases were recorded as having consumed raw milk or dairy products, one of these also had contact with cattle. One further case, who had previous contact with a range of animals, was also recorded as having had contact with cattle. All 13 cases reported between 2003 and 2008 had made contact with domestic pets (cats and dogs) [24–27]. In recent years domestic cats and dogs in contact with five UK cases were swabbed but in only one case was *C. ulcerans* isolated, that case was from dogs that the patient had been in contact with; the strain was identical to that found in the patient [28].

Case management

Six of 15 classic respiratory presentations with pseudomembrane did not receive diphtheria antitoxin (Table 4). Antitoxin was not administered to three of these cases because the disease was not recognized in time [7, 8, 23], for the other three an explanation

was not available. Most cases (77% of those with treatment known) were prescribed appropriate antibiotics (erythromycin, azithromycin, clarithromycin, or penicillin) although the precise timing of administration was not available. In total, only 18/96 cases that recovered (8%) are recorded as receiving diphtheria vaccine during convalescence.

Deaths

There were five fatal cases (two *C. diphtheriae*, three *C. ulcerans*) between 1986 and 2008, a case-fatality rate in patients with respiratory symptoms of 6%. The deaths all occurred in unvaccinated patients. Clinical presentations and treatments are detailed in Table 4; in each of the fatal cases the disease was not immediately recognized as diphtheria and there were consequent delays in administration of appropriate treatment. The fatality rate in patients with classic respiratory diphtheria (including all fatal cases) was 33%. The death of a school-aged child in 2008, due to *C. diphtheriae* infection, was only diagnosed at post-mortem [23]. The presentation was consistent with laryngeal diphtheria, not recognized at the time of treatment. The other *C. diphtheriae* fatality occurred in 1994; a 14-year-old male patient, recently returned from Pakistan, who presented with pharyngitis, a unilateral pharyngeal swelling, bull neck and respiratory distress [14]. A pseudomembrane was visible during attempts to drain what was initially considered to be quinsy. In view of the patient's respiratory

Table 3. *Origin of infection for toxigenic cases C. diphtheriae 1986–2008 in the UK*

Origin of infection	No. of cases
History of travel	32
Bangladesh [9, 10]	10
South East Asia [11]	(one also Nepal) 6
Africa [9, 12, 13]	6
Pakistan [9, 14, 15]	5
India [16]	3
Other [17–19]	2
Contact with traveller	(Greece and Pakistan) 2
Laboratory acquired [20]	3
No history of travel* [21–23]	5
Total†	42

* One case report describes contact with a family member who had travelled to Africa, returning approximately 1 month before the child became ill [23]. This contact was swabbed and tested negative for *C. diphtheriae*, although this does not exclude the possibility of earlier carriage of the organism.

† This total excludes the 20 asymptomatic infections which are described later.

distress he was intubated and ventilated but subsequently developed complete heart block and renal failure. Antitoxin and high-dose intravenous penicillin were administered, but this was delayed due to late diagnosis. The three deaths from *C. ulcerans* were all in elderly (>70 years) females. The first (in 1988) presented with sore throat, painful cough and difficulty breathing, and died the same day without receiving antitoxin [7]. She had stridor, and yellowish mucus covering the fauces and palate. At autopsy the entire respiratory tree from the upper part of the larynx to the small bronchi was found to be covered by a thick yellowish membrane. The second fatal case (in 2000) was admitted to hospital with a pharyngeal membrane and died of pneumonia 10 days after admission (no antitoxin was administered) [29]. The most recent fatality from *C. ulcerans* (in 2006) was hospitalized with a 2-day history of malaise, sore throat and a change in the sound of her voice. On the day of admission she had difficulty breathing and said that she felt her throat was closing. A preliminary diagnosis of angio-oedema was made, related to her recent treatment with an angiotensin-II receptor antagonist, and the patient was treated accordingly. However, her condition deteriorated and a diagnosis of diphtheria was made when a greyish-white membrane was observed across the pharynx during a tracheostomy. She received diphtheria antitoxin

(4 days after onset of first symptoms) and antibiotics but died from her infection [27].

Transmission and carriage

Only one cluster of symptomatic cases, comprising four unimmunized family members, was identified during the study period. This cluster of cases caused by *C. diphtheriae* var. *mitis* occurred in 1986, in a family of recent immigrants from Bangladesh. The 14-month-old index case and 6-year-old sibling had both classic respiratory and cutaneous diphtheria. Another 3-year-old sibling had classic respiratory diphtheria, and a 9-year-old sibling had respiratory diphtheria. Their 47-year-old father was found to be an asymptomatic carrier. Extensive investigation of almost 250 contacts identified no further cases. A total of 20 asymptomatic carriers of toxigenic *C. diphtheriae* were recorded between 1986 and 2008; eight were fully immunized, one was unimmunized, and for the remaining 11 the immunization histories were unknown. The carriers fell into three main groups:

- Contacts of an index case, thought to have acquired infection through contact with an index case in the UK (three index cases, eight carriers).
- Fellow travellers of a case or carrier; may have acquired the infection abroad from the same source as the index case or carrier, or through contact with the index case (three index cases, four fellow traveller carriers).
- Patients that had recently returned from travel abroad and were seeking medical attention for an unrelated condition ($n=7$, four of whom were siblings from the same family).

In addition, an asymptomatic carrier of toxigenic *C. diphtheriae* was identified when screened as a contact of a patient infected with a non-toxigenic *C. diphtheriae* strain; contact-tracing would not usually be carried out in response to non-toxigenic infections.

Of the 16 unrelated cutaneous cases four (25%) had infected contacts, while two (10%) of the 20 unrelated isolations of *C. diphtheriae* from the throat had infected contacts (patients with both respiratory and cutaneous diphtheria excluded), the difference in these percentages was not significant ($P=0.37$).

Two unrelated *C. ulcerans* cases each had an infected asymptomatic contact. In 1996 toxigenic *C. ulcerans* was isolated from a 20-year-old male who

Table 4. Treatment prescribed to toxigenic *C. diphtheriae* and *C. ulcerans* cases by presentation (fatal cases indicated in parentheses)

Presentation	Antitoxin administered	Appropriate antibiotic	Late-appropriate antibiotic	Not known	Inappropriate/not prescribed	Total
Classic respiratory (with pseudomembrane)	Yes	4	4 (2)	1		9
	No	2		1 (1)	3 (2)	6
Respiratory with exudate	Yes	2		1		3
	No	4				4
Respiratory (sore throat) with no pseudomembrane or exudate	Yes	2	1			3
	No	29	1	16	2	48
Respiratory and cutaneous lesions	Yes	2				2
	No	1	1			2
Cutaneous lesions	Yes	1				1
	No	9	4	7	2	22
Bacterial endocarditis	Yes					0
	No	1				1
Asymptomatic	Yes			1		1
	No			22		22
Total	Yes	11	5 (2)	3	0	19
	No	46	6	46 (1)	7 (2)	105
	Total	57	11	49	7	124

presented with a sore throat, and also from his asymptomatic 18-year-old sibling. They both lived in a rural area but had no contact with cattle or raw dairy produce, no data regarding domestic pets were recorded. In 1998 toxigenic *C. ulcerans* was isolated from a 35-year-old male who presented with respiratory diphtheria with a pseudomembrane. The organism was also isolated from his asymptomatic 11-year-old son. Five household dogs were swabbed but *C. ulcerans* was not isolated. Details concerning the third asymptomatic *C. ulcerans* case in Table 2 are not available. The absence of any apparent source of infection for the first two incidents raised the possibility of person-to-person transmission.

Management of contacts was generally undertaken with advice from the Centre for Infections and, from 1999, with reference to UK published guidance [5] and hence was consistent with respect to swabbing of contacts, offering diphtheria vaccine, and prescribing prophylactic antibiotics (macrolides) where necessary.

C. pseudotuberculosis

In addition to the *C. diphtheriae* and *C. ulcerans* cases described above, one case of toxigenic *C. pseudotuberculosis* was reported in 2008. This was the only reported isolation of toxigenic *C. pseudotuberculosis* from a human in the UK during the study period. The organism was isolated from the aortic root vegetation

of an injecting drug user with endocarditis. *C. pseudotuberculosis* is typically associated with contact with cattle, sheep and goats [30]; however, this patient had no history of animal contact and no possible source of infection was identified.

DISCUSSION

Diphtheria vaccine is highly effective, and good immunization coverage in the UK has resulted in very few cases of diphtheria being reported over the last 23 years. Although infection has been reported in vaccinated or partially vaccinated individuals, severe or fatal cases have been limited to the unvaccinated, and this analysis demonstrates the protective effect of vaccination. The main risk factor for *C. diphtheriae* infection remains travel, or contact with someone who has recently travelled, to an endemic area; asymptomatic carriers of *C. diphtheriae* can pose a threat to unimmunized individuals [31]. Individuals intending to travel abroad (particularly to the Indian sub-continent, South East Asia or Africa) should ensure they have received all childhood immunizations as well as booster vaccinations appropriate for their destination [1, 32]. These data also highlight the importance of ensuring those with potential occupational exposure to toxigenic organisms follow UK recommendations and are fully protected by vaccination [1], and strongly emphasize the importance of

microbiology laboratory workers undertaking procedures in containment facilities [33].

The epidemiology of diphtheria in the UK appears to be changing with the majority of toxigenic isolates in recent years associated more often with *C. ulcerans* than *C. diphtheriae*. Travel does not appear to be a major risk factor for *C. ulcerans*.

C. ulcerans is a veterinary pathogen and infection in humans was traditionally associated with the consumption of raw milk or dairy products (cow and goat) [34–36]. The last outbreak of milk-borne diphtheria reported in the UK was in 1943 (prior to the introduction of the national immunization programme) [34]. However, many cases reported in this paper had no association with raw milk products or farming communities suggesting another source. Although there is no direct evidence of person-to-person transmission for *C. ulcerans*, this route of transmission was considered following events in the USA and UK in the mid 1990s. In 1997 the US Center for Disease Control and Prevention reported a case of membranous pharyngitis caused by toxigenic *C. ulcerans* in which it recommended people exposed to the index case should be treated along similar lines to cases exposed to toxigenic *C. diphtheriae*, because it was considered there was inadequate information about human-to-human transmission [37]. The 1999 UK guidelines for the control of diphtheria were also changed to include the recommendation that anyone who has been in close contact with a case of diphtheria caused by toxigenic *C. diphtheriae* or *C. ulcerans* (whatever the clinical presentation) in the previous 7 days should be considered as potentially at risk [5]. This was based on the US recommendation and the report of two asymptomatic *C. ulcerans* contacts in the UK in 1996 and 1998. However, domestic cats and dogs have recently been proposed as potential sources of human infection [38, 39]. Identical strains were reported from a UK patient and dogs that the patient had been in contact with [28]; further studies in this area would be of benefit in order to elucidate the transmission route. The reason for the bias in *C. ulcerans* cases towards females is unclear although it may be related to the greater tendency for females to consult a general practitioner [40], or could be related to pet ownership habits if domestic animals are indeed a reservoir of *C. ulcerans*. However, it is thought that about half of households in the UK own pets [41]. It is important to note that these analyses are based on small numbers of cases so this discussion is only speculative.

Maintaining high immunization coverage across the UK is essential given the occurrence of sporadic, and apparently indigenous, *C. ulcerans* cases; clinicians could use routine consultations as opportunities to check the immunization status of elderly patients who may not have received diphtheria immunizations during childhood, and of adult patients born before 1980 who would not have been offered a routine booster dose of diphtheria at school-leaving age (introduced in 1995).

Despite being clinically indicated, several of the cases reported in this paper did not receive antitoxin treatment. In some this was due to the delay in diagnosis; antitoxin has been shown to be ineffective if administered after the second day of diphtheritic symptoms [42]. In the UK, antitoxin can only be obtained from one of nine issuing centres, coordinated by the HPA, CfI [1]. Antitoxin is given on clinical diagnosis but, as it is an animal blood product, treatment can have severe side-effects so the benefits and risks need careful consideration. Most patients were prescribed appropriate antibiotics although the information available was not always detailed so only limited conclusions can be drawn. The low percentage of patients recorded as receiving a diphtheria vaccine booster during convalescence may be due to this section of the follow-up questionnaire being under-completed if vaccine is generally given after the questionnaire has been returned, or it might highlight a gap in convalescent care.

Cutaneous infection has previously been reported to be more contagious than respiratory diphtheria [43–45]; although the data reported in this paper appear to support this, the numbers are too small to adequately test this hypothesis. As well as infection of contacts of cutaneous diphtheria cases, toxigenic organisms were isolated from the throats of six patients with cutaneous infection, suggesting autoinfection.

The presentation of bacterial endocarditis due to toxigenic *C. diphtheriae* is unusual, and is more commonly reported as due to non-toxigenic [46–48] rather than toxigenic strains [49, 50]. As the fatal cases demonstrated, even severe diphtheria can be unrecognized, or the diagnosis delayed, as most clinicians are unfamiliar with the disease. Case ascertainment may be particularly high in the UK due to the expertise and interest of the London-based WHO Collaborating Centre for Diphtheria & Streptococcal Infections. In addition, some UK laboratories routinely screen all throat swabs for corynebacteria, and hence detect mild and atypical infections.

In the UK, although diphtheria is a statutory notifiable disease, where reporting is supposed to be on clinical suspicion, the majority of notifications relate to non-toxigenic strains [51]. The Centre for Infections can offer advice regarding case management and the reference laboratory provides full species confirmation and toxigenicity testing (full details are provided on the HPA website [52]). The toxin test is the most important component of microbiological diagnosis and it is of concern that in one paper reporting a fatal *C. ulcerans* case the toxin result was not described and it was not reported to the HPA (although the strain was assumed to be toxigenic because of the pathology described) [7]. For those cases reported to the HPA CfI, the quality of follow-up data was variable and comprised a combination of notes, microbiology reports, and fully/partially completed follow-up forms. Data for recent years was generally more complete due to the use of a standardized follow-up form. It is important to continue to improve the quality and completeness of the surveillance data, particularly for a rare disease such as diphtheria where analyses are based on small numbers of cases. We also recommend the use of literature-searching for unreported cases as good practice in investigations for other rare diseases which may not always be routinely reported. Vaccination histories, particularly for immigrants and elderly patients are often difficult to obtain and hence were sometimes based on assumptions relating to the country of origin and the age of the patient. Despite these limitations the data available has allowed analysis of recent trends and presentations which should be of interest to vaccine policy makers, public health specialists and clinicians encountering a case in the future.

APPENDIX

EU Case Definition for National Diphtheria Surveillance

Community Decision of 19 March 2002 (under 2119/98/EC).

Modified version (by A. Efstratiou, N. Crowcroft, J. White, on behalf of DIPNET, November 2002).

Clinical description

Clinical picture compatible with diphtheria, i.e. an upper respiratory tract illness characterized by sore throat, low grade fever, and an adherent membrane of the tonsils, pharynx or nose or non-respiratory

diphtheria; cutaneous, conjunctival, otic and genital lesions.

Laboratory criteria for diagnosis

Isolation of diphtheria toxin-producing corynebacteria from a clinical specimen.

Case classification

Possible: Not applicable.

Probable case: A clinically compatible case that is not laboratory confirmed and does not have an epidemiological link to a laboratory-confirmed case.

Confirmed case: A clinically compatible case that is laboratory confirmed with the isolation of a toxigenic strain of *C. diphtheriae*, *C. ulcerans*, or *C. pseudotuberculosis* or has an epidemiological link to a laboratory-confirmed case.

Confirmed case (other): Non-respiratory/cutaneous diphtheria cases with isolation of toxigenic strains, or cases not meeting the specified clinical criteria but with isolation of toxigenic strains (e.g. mild respiratory diphtheria, or respiratory diphtheria with absence of membrane).

Asymptomatic carriers: Asymptomatic carriers (any anatomical site) with toxigenic strains.

Cases with non-toxigenic *C. diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis* should not be reported.

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DECLARATION OF INTEREST

None.

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Diphtheria in the Postepidemic Period, Europe, 2000–2009

Karen S. Wagner, Joanne M. White, Irina Lucenko, David Mercer, Natasha S. Crowcroft, Shona Neal, and Androulla Efstratiou, on behalf of the Diphtheria Surveillance Network¹

Diphtheria incidence has decreased in Europe since its resurgence in the 1990s, but circulation continues in some countries in eastern Europe, and sporadic cases have been reported elsewhere. Surveillance data from Diphtheria Surveillance Network countries and the World Health Organization European Region for 2000–2009 were analyzed. Latvia reported the highest annual incidence in Europe each year, but the Russian Federation and Ukraine accounted for 83% of all cases. Over the past 10 years, diphtheria incidence has decreased by >95% across the region. Although most deaths occurred in disease-endemic countries, case-fatality rates were highest in countries to which diphtheria is not endemic, where unfamiliarity can lead to delays in diagnosis and treatment. In western Europe, toxigenic *Corynebacterium ulcerans* has increasingly been identified as the etiologic agent. Reduction in diphtheria incidence over the past 10 years is encouraging, but maintaining high vaccination coverage is essential to prevent indigenous *C. ulcerans* infections and reemergence of *C. diphtheriae*.

In 1994, following success of widespread vaccination programs earlier in the century, diphtheria was proposed as a candidate for elimination in the World Health Organization (WHO) European Region; the goal was for elimination of indigenous diphtheria by 2000 (1). However, during the 1990s, when this goal seemed within

sight, several factors caused a resurgence of diphtheria to epidemic proportions in the newly independent states of the former Soviet Union. There were a large number of unnecessary contraindications to vaccination in guidance for these countries at that time, which led to reductions in adequate vaccination coverage in children. This problem was exacerbated by mistrust in vaccinations among health professionals and the public and by use of low-dose formulation vaccine for primary vaccinations. Waning immunity in the adult population, large-scale population movements caused by breakup of the former Soviet Union, disruptions in health services, and lack of adequate supplies of vaccine and antitoxin for prevention and treatment in most affected countries provided conditions under which diphtheria could spread (2,3). At the peak of the epidemic in 1995, there were >50,000 cases reported in the WHO European Region (2). Intensive vaccination strategies brought the disease under control in most countries, but some endemic transmission still continues.

Clinical diphtheria is caused by toxin-producing corynebacteria. Three species (*Corynebacterium diphtheriae*, *C. ulcerans*, and *C. pseudotuberculosis*) can potentially produce diphtheria toxin. *C. diphtheriae* is the most common of potentially toxigenic species and is associated with epidemic diphtheria and person-to-person spread. The organism has 4 biovars (gravis, mitis, intermedius, and belfanti). *C. ulcerans* is historically associated with cattle or raw dairy products, and, although it is rarely reported, its incidence has increased slightly in some countries in western Europe and in the United States in recent years (4–6). *C. pseudotuberculosis* rarely infects humans and is typically associated with farm animals (7).

¹Additional members of the Diphtheria Surveillance Network who contributed data are listed in online Technical Appendix 1 (wwwnc.cdc.gov/EID/pdfs/11-0987-Techapp1.pdf).

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Currently, no direct evidence has been found of person-to-person spread of *C. ulcerans* or *C. pseudotuberculosis*.

Classical respiratory diphtheria is characterized by formation of a gray-white pseudomembrane in the throat that is firmly adherent (8). A swollen, bull-neck appearance caused by inflammation and edema of soft tissues surrounding lymph nodes is associated with severe illness and higher death rates (8). In progressive disease, the toxin can bind to cardiac and nerve receptors and cause systemic complications. Milder respiratory disease may manifest as a sore throat, most commonly seen in patients who are fully or partially vaccinated. In some tropical areas, cutaneous symptoms, characterized by rolled-edge ulcers, are more common. Patients may have both cutaneous and respiratory disease. The purpose of this study was to analyze diphtheria data for Europe during 2000–2009.

Methods

Case-based diphtheria surveillance data from each of 25 Diphtheria Surveillance Network (DIPNET) member countries (Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Ireland, Italy, Latvia, Lithuania, the Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden, Turkey, and the United Kingdom) for 2000–2007 were submitted retrospectively to the coordinating center in the United Kingdom during 2008. Data for 2008 and 2009 were obtained in August 2009 and September 2010 from the DIPNET online database, which was launched in September 2007.

We analyzed cases meeting the DIPNET case definition (isolation of a toxigenic strain or clinically compatible case with an epidemiologic link to a laboratory-confirmed case) (online Technical Appendix 2, wwwnc.cdc.gov/EID/pdfs/11-0987-Techapp2.pdf). In addition, 48 cases without laboratory confirmation and pseudomembrane (mild diphtheria/severe pharyngitis) and 5 cases with unknown manifestations were included for Latvia because these cases had been recorded in the national dataset. For most cases, toxigenicity was confirmed by using the Elek phenotypic test (9). However, in some cases, toxigenicity was evaluated only by detection of the toxin gene with PCR. We assumed that all cases in this dataset were toxigenic (toxin producing) because the number of cases without Elek confirmation was small and referred to symptomatic cases. Data fields collected included year; organism; biovar; and patient age, sex, clinical manifestations, vaccination status, veterinary contact, risk group, and outcome. Further strain characterization (ribotyping) was available for a limited number of isolates as part of a screening study in 10 DIPNET countries (10).

Cases were assigned to 5 clinical manifestation groups. These groups were classic respiratory diphtheria with

pseudomembrane (the most serious form of the disease); mild diphtheria/severe pharyngitis (respiratory symptoms without the pseudomembrane); cutaneous (toxigenic organism isolated from skin lesions); other (e.g., toxigenic organism isolated from blood); and asymptomatic (carriers of toxigenic organisms, usually contacts of a confirmed case-patient).

Additional information concerning countries in the WHO European Region that are not DIPNET member countries was provided by the WHO Regional Office for Europe. Twenty-five of 53 member states of the WHO European Region are members of DIPNET. WHO European Region countries (including DIPNET members) report total cases annually to the WHO Regional Office for Europe through the WHO/United Nations Children's Fund Joint Reporting Form, which is the global annual data survey of WHO member states for vaccine-preventable diseases and immunization program indicators. In addition, 16 countries in 2003 (Figure 1) were asked to prospectively participate in monthly surveillance and provide more detailed information (e.g., pathogen biovar; patient age, sex, and outcome; and carriers among contacts). Twelve countries currently provide monthly reports to WHO Regional Office for Europe through this system. The only major source of cases that has not participated in the monthly reporting system (but

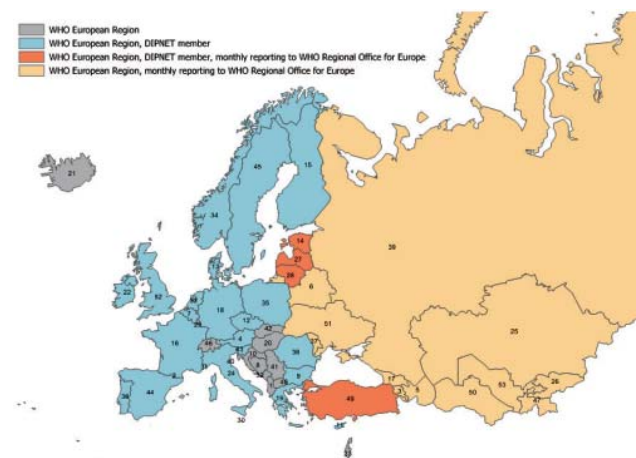


Figure 1. Diphtheria Surveillance Network (DIPNET) and World Health Organization (WHO) European Region countries. 1, Albania; 2, Andorra; 3, Armenia; 4, Austria; 5, Azerbaijan; 6, Belarus; 7, Belgium; 8, Bosnia and Herzegovina; 9, Bulgaria; 10, Croatia; 11, Cyprus; 12, Czech Republic; 13, Denmark; 14, Estonia; 15, Finland; 16, France; 17, Georgia; 18, Germany; 19, Greece; 20, Hungary; 21, Iceland; 22, Ireland; 23, Israel (neighboring countries not shown); 24, Italy; 25, Kazakhstan; 26, Kyrgyzstan; 27, Latvia; 28, Lithuania; 29, Luxembourg; 30, Malta; 31, Monaco; 32, Montenegro; 33, the Netherlands; 34, Norway; 35, Poland; 36, Portugal; 37, Republic of Moldova; 38, Romania; 39, Russian Federation; 40, San Marino; 41, Serbia; 42, Slovakia; 43, Slovenia; 44, Spain; 45, Sweden; 46, Switzerland; 47, Tajikistan; 48, Former Yugoslav Republic of Macedonia; 49, Turkey; 50, Turkmenistan; 51, Ukraine; 52, United Kingdom (Great Britain and Northern Ireland); 53, Uzbekistan.

does report annually) is the Russian Federation. Rates per 1 million person-years were calculated by using population estimates derived from the Population Division of Economic and Social Affairs of the United Nations Secretariat (11).

Statistical Analyses

Proportions were compared by using χ^2 or Fisher exact tests, as appropriate, in Stata statistical software version 7.0 (StataCorp LP, College Station, TX, USA). For assessment of a trend for variables in ordered groups (vaccinated, partially vaccinated, unvaccinated) and severity of disease (classic respiratory, mild diphtheria/severe pharyngitis, asymptomatic), the Wilcoxon test for trend in Stata (12) was used. This test enabled nonparametric analysis across these groups.

Results

Overall, across the WHO European Region, the number of cases of diphtheria has substantially decreased since the epidemic in the 1990s (Figure 2). Data on clinically confirmed cases and toxigenic isolates of *C. diphtheriae* and *C. ulcerans* reported to DIPNET during 2000–2009 are shown in Tables 1 and 2, respectively. Member countries that are not listed reported no isolates. Data are analyzed separately for Latvia, where diphtheria is endemic.

Diphtheria-Endemic Countries in WHO European Region

During 2000–2009, Latvia reported the highest annual incidence rate of diphtheria in the European Region each year and a 10-year incidence rate of 23.8 cases/1 million person-years. This rate was $\approx 7\times$ higher than in countries with the next highest 10-year incidence: i.e., Georgia (3.5), Ukraine (3.3), and the Russian Federation (3.0). However, during this time, 4,304 (>61%) of 7,032 cases in the WHO European Region were reported from the Russian Federation, and 2 countries, the Russian Federation and Ukraine, accounted for 83% of all cases.

Over the past 10 years, diphtheria incidence decreased by >95% across the region (from 1.82/1 million population in 2000 to 0.07/million in 2009), including in Latvia (from 111.22/million in 2000 to 2.67/million in 2009). In 2009, Latvia was the only country in the region that had not yet achieved the elimination benchmark of an incidence <1 case/million population (Figure 2).

Most cases reported to WHO through the monthly surveillance system were in teenagers and adults. However, the major risk groups for death have been infants (too young for complete primary vaccination) and adults ≥ 40 years of age (unvaccinated or with waning immunity). Although risk did not differ by sex in cases in children, during 2002–2009, $\approx 2\times$ as many cases were reported in women ≥ 20 years of age than in men (510 [64%] vs. 292

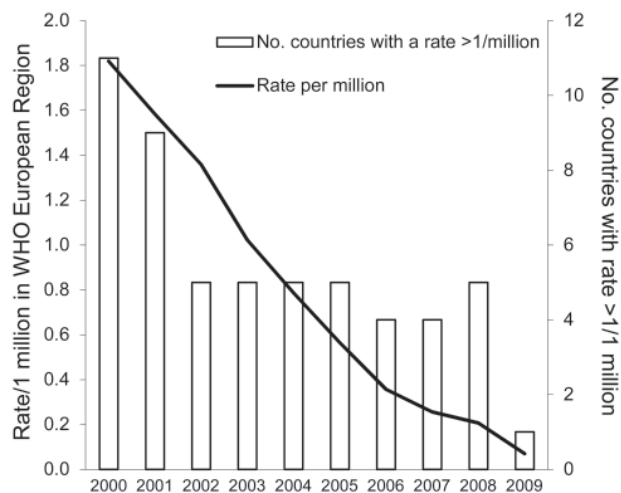


Figure 2. Diphtheria cases per 1 million population in the World Health Organization (WHO) European Region and number of countries with a rate >1 cases/1 million population, 2000–2009.

[36%], respectively). Most (75%) case-patients reported in the European Region were at least partially vaccinated, but most (74%) case-patients and (93%) infants who died were unvaccinated). *C. diphtheriae* biovar *gravis* was the predominant strain (60%–80%). Of isolates from Latvia (Table 1), 355 (99%) of 358 with a known biovar were *gravis* and 3 (1%) were *mitis*.

Clinical manifestations and vaccination status for cases from Latvia (all *C. diphtheriae*) reported to DIPNET are shown in Table 3. Most (340/341) case-patients with symptoms had respiratory manifestations, and 141 (41%) of 340 respiratory case-patients had classic diphtheria symptoms. Vaccination showed a significant protective effect with respect to severity of infection ($p < 0.001$ by test for trend).

For symptomatic cases for 2002–2009 (excluding the military outbreak in 2000 and cases from 2001 for which limited information was available) the highest overall incidences were in children 0–4 and 5–15 years of age and adults 45–64 years of age; lower incidence rates were observed in other age groups (Figure 3). Most (123/196, 63%) symptomatic cases during those years were in female patients.

The second most common risk factor (after military service) identified among symptomatic case-patients in Latvia was unemployment (60 case-patients). Information was not available regarding connections of case-patients to other countries of the former Soviet Union.

Non-Disease-Endemic Countries (DIPNET)

Clinical manifestations and immunization status for case-patients with toxigenic *C. diphtheriae* and *C. ulcerans*

Table 1. Toxigenic *Corynebacterium diphtheriae* isolates and epidemiologically linked cases and deaths reported by DIPNET member countries, Europe, 2000–2009*

Characteristic	Patient description†	No. toxigenic isolates or clinical cases with epidemiologic link (no. deaths)										
		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	
Country												
Estonia	Symptomatic	2	2	0	0	0	0	0	0	0	0	0
	Asymptomatic	1	7	0	0	0	0	0	0	0	0	0
	Total	3	9	0	0	0	0	0	0	0	0	0
Finland	Total	0	2 (1)	0	0	0	0	0	0	0	0	0
France	Total	0	0	1	0	1	0	1	1 (1)	1	0	0
Germany	Total	1	2	4	0	0	1	0	0	0	2	0
Latvia	Symptomatic	145	0	45	26	20	20	32	18	29	6	
	Asymptomatic	61	24	15	22	2	2	11	5	12	3	
	Not known	119	91	0	0	0	0	0	0	0	0	
	Total	325 (9)	115 (5)	60 (3)	48 (2)	22 (1)	22 (2)	43 (6)	23 (1)	41 (2)	9 (1)	
Lithuania	Symptomatic	2	0	4	0	0	0	0	0	2	0	
	Asymptomatic	0	0	1	0	0	0	0	0	2	0	
	Total	2	0	5 (1)	0	0	0	0	0	4 (1)	0	
Norway	Symptomatic	0	0	0	0	0	0	0	0	3	0	
	Asymptomatic	0	0	0	0	0	0	0	0	1	0	
	Total	0	0	0	0	0	0	0	0	4	0	
Sweden	Total	0	0	0	0	0	0	0	0	0	1	
Turkey	Symptomatic	1	3	1	0	0	0	0	0	0	0	
	Asymptomatic	2	0	0	0	0	0	0	0	0	0	
	Not known	1	4	1	1	0	0	0	0	0	0	
	Total	4 (1)	7 (3)	2 (1)	1	0	0	0	0	0	0	
United Kingdom	Total	1	0	6	3	0	0	1	0	2 (1)	2	
Total known symptomatic patients	NA	152	9	61	29	21	21	34	19	37	11	
Total (all countries)	NA	336 (10)	135 (9)	78 (5)	52 (2)	23 (1)	23 (2)	45 (6)	24 (2)	52 (4)	14 (1)	
Total known symptomatic patients, excluding Latvia	NA	7	9	16	3	1	1	2	1	8	5	
Total, excluding Latvia	NA	11 (1)	20 (4)	18 (2)	4	1	1	2	1 (1)	11 (2)	5	

*DIPNET, Diphtheria Surveillance Network; NA, not applicable. A total of 89 cases were clinically diagnosed without microbiological confirmation (76 in Latvia, 11 in Turkey, and 2 in Lithuania).

†If only total is displayed for a country, all patients were symptomatic.

isolates and epidemiologically linked cases reported by 24 DIPNET member countries, excluding Latvia, during 2000–2009 are shown in Table 4. Vaccination had a significant protective effect with respect to severity of infection ($p = 0.001$ by test for trend).

C. *diphtheriae* Isolates

Isolates of *C. diphtheriae* were sporadically reported in the 24 DIPNET member countries, excluding Latvia. Each year, 0–6 symptomatic cases of toxigenic *C. diphtheriae* infection were reported by each country (53 cases during 2000–2009). For each case-patient, 0–4 asymptomatic contacts were reported (14 in the 10-year period). Of 60 isolates with a biovar recorded during 2000–2009, a total of 32 were gravis and 28 were mitis. Seventeen cutaneous cases, 35 respiratory (24 classic respiratory) cases, and 1 case with other manifestations were reported. Most (15/17, 88%) cutaneous cases were caused by biovar mitis, and most (17/28, 61%) respiratory cases with a known biovar were caused by biovar gravis. Sixteen of 17 patients with cutaneous disease had recently

returned from traveling, had contact with travelers, or were recent immigrants from a disease-endemic area, as was the situation for 12 of 35 patients with respiratory disease. One case-patient with bacterial endocarditis had contact with a relative who had recently traveled to Pakistan. For case-patients with *C. diphtheriae* symptomatic infection, sex distribution was even. A higher incidence rate was observed in male patients 0–4 years of age (Figure 3), but this finding was influenced by 6 cases reported in Turkey during 2001–2003.

C. *ulcerans* Isolates

A total of 4–8 isolations of toxigenic *C. ulcerans* were reported by DIPNET member countries each year (53 [50 symptomatic] during 2000–2009). Of these cases, 51% were reported by the United Kingdom, 19% by Germany, and 17% by France. Of the symptomatic cases for which patient sex/age group were known, 38 (78%) of 49 were in female patients and 29 (59%) of 49 were in patients ≥ 45 years of age. Incidence rate was higher in female patients than in than male patients (0.014/1 million person-years vs.

Table 2. Isolates of toxigenic *Corynebacterium ulcerans* and patient deaths reported by DIPNET member countries, Europe, 2000–2009*

Characteristic	Patient description†	No. toxigenic isolates (no. deaths)									
		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Country											
France	Total	0	1	0	1	3	0	2	1	0	1
Germany	Total	1	1 (1)	0	0	1	2	1	2	0	2
Italy	Total	0	0	1	0	0	0	0	0	0	0
The Netherlands	Total	0	1	0	0	0	0	0	1	0	0
Romania	Asymptomatic	0	0	1	0	0	0	0	0	0	0
	Total	0	0	1	0	0	0	0	0	0	0
Sweden	Symptomatic	0	0	0	0	0	0	0	0	1	0
	Not known	0	0	0	0	1	0	1	0	0	0
	Total	0	0	0	0	1	0	1	0	1	0
United Kingdom	Total	7 (1)	3	2	2	1	2	2 (1)	3	3	2
No. symptomatic patients	NA	8	6	3	3	5	4	5	7	4	5
No. isolates	NA	8 (1)	6 (1)	4	3	6	4	6 (1)	7	4	5

*DIPNET, Diphtheria Surveillance Network; NA, not applicable

†If only total is shown for a country, all patients were symptomatic.

0.004/1 million person-years). Eleven cutaneous cases, 38 respiratory (14 classic respiratory) cases, and 1 case with other manifestations were reported. Ninety-four percent of case-patients for which information was available had contact with domestic animals. Traditional risk factors such as consumption of raw milk products were not reported, and no patients had a recent history of travel. One of the 2 case-patients infected with *C. ulcerans* who died in the United Kingdom had an identical strain of *C. ulcerans* to that isolated from a dog with which the patient had been in contact (14). A similar finding was observed in France for a nontoxigenic case reported in 2003 (5,15). In 2007, identical strains were isolated from a patient infected with *C. ulcerans* and her pig in Germany (16).

C. pseudotuberculosis Isolates

Four case-patients with diphtheria caused by toxigenic *C. pseudotuberculosis* were reported: 1 in France in 2005 and 1 in 2008, 1 in Germany in 2004, and 1 in United Kingdom in 2008. Three of these patients had cutaneous manifestations (1 was unvaccinated, 2 had an unknown vaccination status) and 1 (partially vaccinated) had bacterial endocarditis. To our knowledge, none of these infected patients died. Animal contact (with a calf) was recorded for

only 1 patient (1 had no history of animal contact and 2 had an unknown history of animal contact).

Deaths Caused by Diphtheria

During 2000–2009, a total of 32 deaths caused by diphtheria were reported in Latvia, and 13 deaths (10 caused by *C. diphtheriae* and 3 caused by *C. ulcerans*) (Tables 1, 2) were reported by the remaining 24 DIPNET countries. Overall, patients with respiratory disease and a pseudomembrane had a significantly higher case-fatality rate (CFR) than patients with respiratory disease without a pseudomembrane (14.6% vs. 1.3%; $p < 0.001$). For case-patients in Latvia, the CFR was 5% for patients with any respiratory symptom (including classic manifestations) and 12% for patients with classic respiratory symptoms. Of 18 case-patients in Latvia who died, 14 were ≥ 40 years of age and 4 were ≤ 7 years of age; all were unvaccinated.

Nine of 13 patients who died of diphtheria in DIPNET countries excluding Latvia had classic respiratory diphtheria symptoms, and 2 had severe pharyngitis (2 had unknown manifestations). All 3 deaths caused by *C. ulcerans* (2 in the United Kingdom and 1 in Germany) were in elderly (>75 years of age) patients (unvaccinated or vaccination status unknown). Two of the patients infected with *C.*

Table 3. Vaccination status of case-patients and clinical manifestations of toxigenic *Corynebacterium diphtheriae* infections and epidemiologically linked cases without laboratory confirmation, Latvia, Europe, 2000–2009*

Vaccination status	Classic diphtheria (with membrane)	Mild diphtheria/ severe pharyngitis	Cutaneous	Asymptomatic	Not known	Total
Full	64†	118	0	71	0	253
Partial	1	3	0	5	0	9
Unvaccinated	74	70	1	18	0	163
Not known	2	8	0	63	210	283
Total	141	199	1	157	210	708

* $p < 0.001$ by test for trend (vaccination status and disease severity).

†Includes 52 fully vaccinated case-patients with classic respiratory diphtheria (with membrane) from an outbreak in the military in 2000. The outbreak comprised 145 symptomatic case-patients and 25 asymptomatic contacts. A total of 96% of these case-patients and contacts were 18–23 years of age at the time of diagnosis. Spread of disease was traced to use of a communal drinking cup (13).

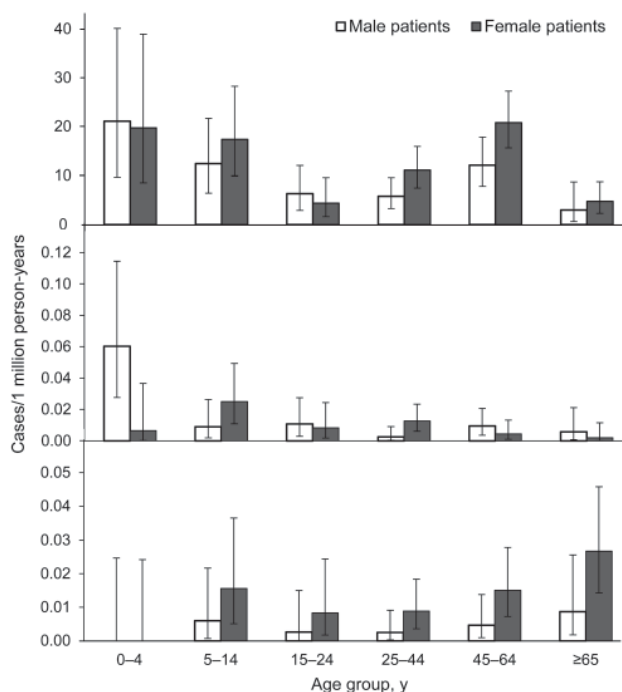


Figure 3. Diphtheria incidence per 1 million person-years for Latvia (*Corynebacterium diphtheriae*, 2002–2009) and the remaining 24 Diphtheria Surveillance Network (DIPNET) countries (*C. diphtheriae* and *C. ulcerans*, 2000–2009). Error bars indicate 95% CIs. The period 2002–2009 excludes the military outbreak in 2000 and cases from 2001 for which limited information was available.

diphtheriae who died were unvaccinated infants (1 from Mayotte and 1 from Finland). The infant in Finland died at 3 months of age in 2001 after recent contact with visitors from Russia (17). Six other children died: an unvaccinated school age child in the United Kingdom (18) and 5 children <7 years of age in Turkey (vaccination status unknown). Two adults in Lithuania (ages 45–64 years; vaccination status unknown) also died. The CFR for patients with any respiratory symptoms reported for patients infected with toxigenic *C. diphtheriae* or *C. ulcerans* in regions where diphtheria was not endemic was 15%; CFR was 24% among patients with classic respiratory diphtheria.

The difference between CFRs for respiratory diphtheria cases in Latvia and member countries excluding Latvia (5% and 15%, respectively) was significant ($p = 0.002$). The difference between CFRs for classic respiratory diphtheria in Latvia and the member countries excluding Latvia (12% and 24%, respectively) showed borderline significance ($p = 0.06$).

Any case-patients without symptoms recorded who died likely had respiratory diphtheria. However, because symptoms were also not available for several surviving case-patients for whom clinical manifestations were less

certain, all case-patients for whom clinical manifestations were unavailable were excluded from analysis.

Discussion

Substantial progress has been made in controlling diphtheria across Europe since the epidemic in the 1990s, but diphtheria has not disappeared as a serious public health threat. After major disruption to a mass vaccination program, recovery time is lengthy, and pockets of unvaccinated persons can remain because recovery is not necessarily homogeneous.

The protective effect of vaccination in preventing progression to severe disease is clear. However, 64 patients in Latvia recorded as fully vaccinated had classic respiratory diphtheria symptoms. Most of these patients were infected during a military outbreak in 2000 and would have been scheduled for primary vaccinations during the 1980s, when changes in vaccines, vaccination policy, medical practice, and public acceptance led to less intensive vaccination of children in the former Soviet Union. Beginning in 1980, Soviet vaccination recommendations enabled use of an alternative primary vaccination schedule against diphtheria that recommended 3 doses of a lower-potency vaccine (19). The classification of fully/partially vaccinated relies on specific interpretation of a country. Since the 2000 outbreak, greater attention has been given to checking vaccination records of new recruits into the Latvian military, and booster vaccinations are given where appropriate.

Lower CFRs for respiratory diphtheria in disease-endemic areas compared with those in nonendemic areas highlight how lack of familiarity with a rare disease can affect diagnosis and treatment. As the incidence of diphtheria has decreased, so has the practice of routine laboratory screening (20). No DIPNET member country routinely screens all throat swab specimens for corynebacteria, although sentinel screening of all throat swab specimens is conducted in Denmark, Ireland, and the United Kingdom. All other DIPNET countries (and outside sentinel screening areas) perform screening only at the request of the clinician or if the laboratory identifies particular criteria for screening from information accompanying a swab specimen (DIPNET, unpub. data). This practice has resulted in a loss of laboratory expertise and the opportunity for infections to go undetected because only clinically indicated swab specimens are tested; thus, milder cases or those with unusual manifestations may be missed.

A recent DIPNET external quality assurance evaluation of 6 simulated throat specimens found that only 6 of 34 international centers produced acceptable results for all 6 specimens; many centers could not isolate the target organism (21). In some poor countries, screening can be limited by cost of laboratory reagents, and problems

Table 4. Vaccination status of case-patients and clinical manifestations of toxigenic *Corynebacterium diphtheriae* and *C. ulcerans* infections and epidemiologically linked cases without laboratory confirmation, DIPNET cases excluding Latvia, Europe, 2000–2009*

Vaccination status	Classic respiratory diphtheria (with membrane)	Mild respiratory diphtheria/severe pharyngitis	Cutaneous	Other	Asymptomatic	Not known	Total
Full	4	17	2	1†	2	0	26
Partial	5	3	7	0	0	0	15
Unvaccinated	14	3	4	0	1	0	22
Not known	15	10	15‡	1§	12	11¶	64
Total	38	33	28	2	15	11	127

*DIPNET, Diphtheria Surveillance Network. $p = 0.001$ by test for trend (vaccination status and disease severity).

†Bacterial endocarditis (*C. diphtheriae*, fully vaccinated)

‡One cutaneous case-patient also had a sore throat.

§Isolation from blood (*C. ulcerans*, vaccination status not known).

¶Includes 2 case-patients infected with *C. diphtheriae* who died and are assumed to have respiratory symptoms without specific details available.

have also occurred in obtaining Elek reagents and media (21). During a recent screening study across 10 countries in Europe, toxigenic organisms were isolated in Latvia and Lithuania (10). At least one of these cases in Lithuania would not have been correctly diagnosed in the absence of the screening study. In addition to the potential for missed or late diagnoses, in areas where diphtheria is not endemic, diphtheria antitoxin treatment is not always available, which can have serious consequences. A recent international survey highlighted global shortages of diphtheria antitoxin (22). Information about administration and timing of antitoxin treatment was not collected for this analysis, but studying such timing in relation to differing CFRs would be useful.

Higher incidence rates of *C. diphtheriae* among women in disease-endemic countries could be caused by several factors. Women more commonly work as caregivers in domestic and health care settings, consultation rates are usually higher among women, and men are more likely to have received diphtheria vaccine during military service.

Although the United Kingdom, France, and Germany regularly report isolations of toxigenic *C. ulcerans*, it is unlikely that this organism is present only in these countries. The ability to detect *C. ulcerans* could indicate the capability of a country to detect potentially toxigenic organisms and provide an indicator of good surveillance. Detection of mild diphtheria cases (any toxigenic organism) is another potential indicator of good surveillance. *C. ulcerans* appears to have a wide host range and has been isolated from many domestic and wild animals, including the killer whale and lion (nontoxigenic strain) (23). During 2002 and 2003, toxigenic *C. ulcerans* strains isolated from domestic cats in the United Kingdom were found to have the predominant ribotypes observed among human clinical isolates, which suggests that cats could be a potential reservoir for human infection (24). Identical *C. ulcerans* strains have been isolated from diphtheria patients and dogs in France and the United Kingdom (14,15). The presence of this organism reinforces the need to maintain high vaccination levels in all countries. Higher incidence

of infection among elderly women could be related to pet ownership habits, in combination with low or waning immunity.

Vaccination coverage for diphtheria is assessed annually in many countries in Europe by using a range of methods, including computerized vaccination registers, survey methods, administrative methods, or a combination (25). These methods will provide varying degrees of accuracy in coverage estimates, which makes countries difficult to compare. Coverage for vaccination with diphtheria-tetanus-pertussis 3 vaccine (third dose of diphtheria, tetanus, pertussis vaccine) in early childhood in 2009 was >90% for most (85%) countries in the European Region, and 66% of countries (including Latvia, Lithuania, Turkmenistan, and the Russian Federation) reported coverage $\geq 95\%$ (26). Coverage in Ukraine decreased from 98% in 2006 and 2007 to 90% in 2008 and 2009. Austria, Denmark, Georgia, and Moldova recorded diphtheria-tetanus-pertussis 3 vaccine coverage <90%. Azerbaijan and Malta had the lowest coverage (73% for both countries) in the European Region in 2009.

Following high-profile vaccine-scare stories in some countries in eastern Europe, such as the Russian Federation and Ukraine, anti-vaccination groups have gained strength by using television, the Internet, and other media for publicity (27); this activity could seriously affect vaccination coverage. Adult diphtheria immunity can be increased through scheduled booster vaccinations every 10 years (e.g., as in Austria, Belgium, Bulgaria, Cyprus, Estonia, Finland, France, Germany, Greece, Latvia, Norway, Portugal, and Romania) or as part of a combined tetanus and low-dose diphtheria vaccine given for tetanus-prone injuries. In Latvia, annual adult vaccination coverage surveys are undertaken, but in most countries adult coverage is rarely assessed. Seroprevalence studies have indicated that many adults in some countries have immunity levels below the protective threshold (28). Gaps in immunity in the adult population contributed to the resurgence of diphtheria in eastern Europe during the 1990s.

Trends in diphtheria cases in Europe are encouraging, but continued striving for improved vaccination coverage is essential. Diphtheria has a socioeconomic component; outbreaks are typically seen in marginalized groups. In the current economic climate, more socially deprived groups that are vulnerable to infection will emerge. The economic crisis may also threaten supplies of vaccine and antitoxin and delivery of immunization programs. Because reductions in finances can limit the capacity for surveillance, decreases in case reporting need to be interpreted with caution. Every effort must be made to maintain high diphtheria vaccination coverage.

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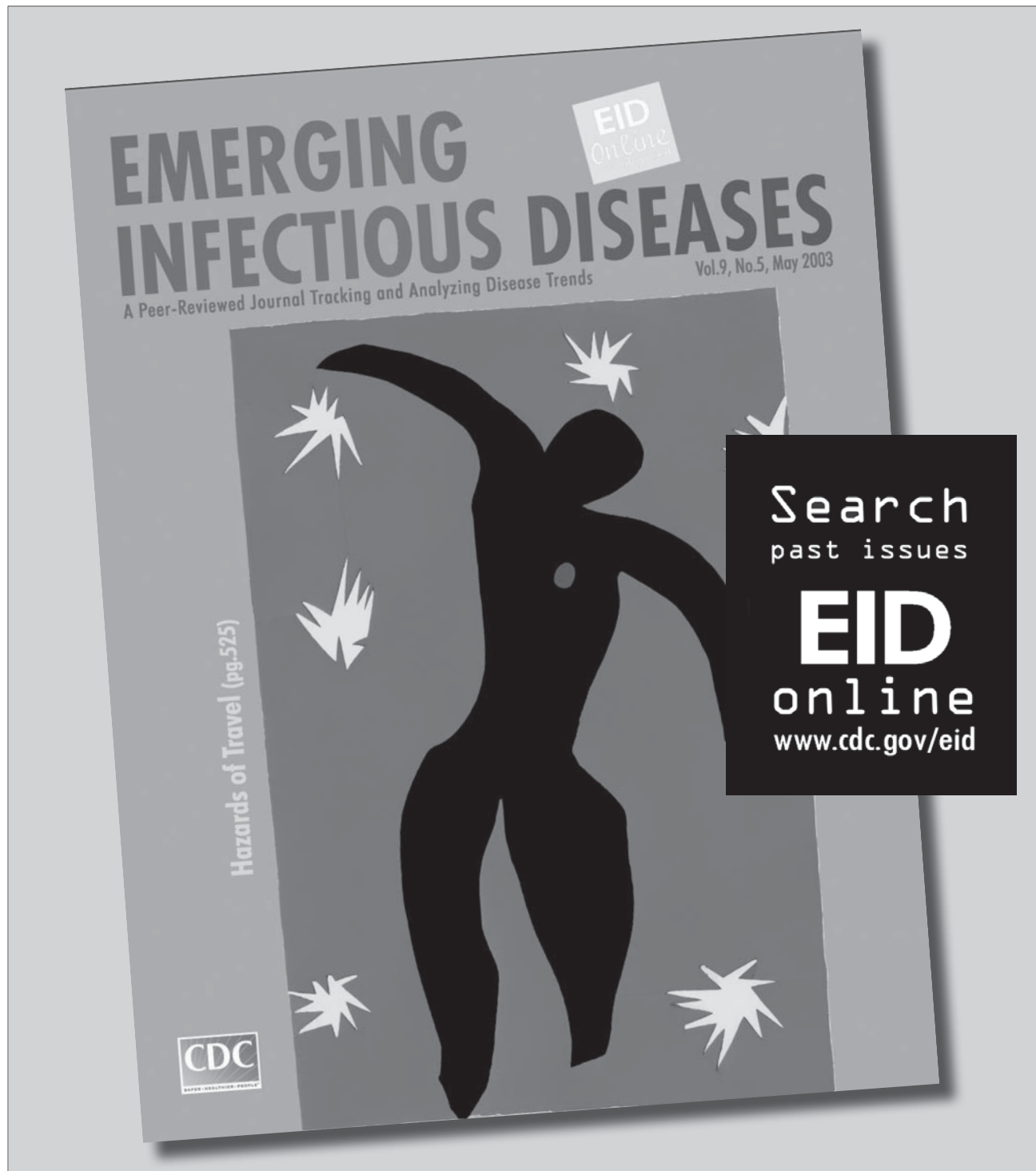
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Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the US Department of Health and Human Services.



Diphtheria in the Postepidemic Period, Europe, 2000–2009

Technical Appendix 1

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Diphtheria in the Postepidemic Period, Europe, 2000–2009

Technical Appendix 2

European Union Case Definition for National Diphtheria Surveillance, Community Decision of March 19, 2002 (under 2119/98/EC), Modified Version.

Androulla Efstratiou, Natasha S. Crowcroft, and Joanne M. White, on behalf of Diphtheria Surveillance Network, November 2002

Clinical Description

Clinical picture compatible with diphtheria, i.e., an upper respiratory tract illness characterized by sore throat, low-grade fever, and an adherent membrane of the tonsils, pharynx, or nose or nonrespiratory diphtheria; cutaneous, conjunctival, otic, and genital lesions.

Laboratory Criteria for Diagnosis

Isolation of diphtheria toxin–producing corynebacteria from a clinical specimen.

Case Classification

Possible: Not applicable

Probable: A clinically compatible case that is not laboratory confirmed and does not have an epidemiologic link to a laboratory-confirmed case.

Confirmed: A clinically compatible case that is laboratory confirmed with the isolation of a toxigenic strain of *Corynebacterium diphtheriae*, *C. ulcerans*, or *C. pseudotuberculosis* or has an epidemiologic link to a laboratory-confirmed case.

Confirmed (other): Nonrespiratory/cutaneous diphtheria cases with isolation of toxigenic strains, or cases not meeting the specified clinical criteria but with isolation of toxigenic strains (e.g., mild respiratory diphtheria, or respiratory diphtheria with absence of membrane).

Asymptomatic carriers: asymptomatic carriers (any anatomical site) with toxigenic strains.



Review

A review of the international issues surrounding the availability and demand for diphtheria antitoxin for therapeutic use

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Immunoglobulin

ABSTRACT

Diphtheria treatment requires early administration of diphtheria antitoxin (DAT), an immunoglobulin preparation that neutralises circulating diphtheria toxin. Here, we review issues relating to the supply and use of DAT and assess its availability by means of an international survey. Results showed that several countries do not currently hold DAT stockpiles due to low prevalence, and hence perceived risk of diphtheria, and/or difficulties in obtaining DAT supplies. The potential for importation of cases into any country exists globally, since diphtheria remains endemic in many regions. It is therefore important that DAT be readily available – particularly since waning diphtheria immunity has been observed among adult populations in countries with good vaccination coverage. Options for diphtheria therapy are discussed.

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1. Diphtheria

Diphtheria is an acute bacterial disease with a considerable case fatality rate caused by toxigenic strains of corynebacteria. Diphtheria toxin (DT) is the major virulence factor for these organisms, and contributes to the formation of a pseudomembrane in the nasopharynx of affected individuals. The colonising organisms are rarely found outside the local area of infection but the toxin is

absorbed into the circulatory system where, when disseminated, it is able to cause systemic complications such as myocarditis and neuritis [1]. Three toxin-producing species have been identified; *Corynebacterium diphtheriae* is most commonly associated with communicable disease in humans, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* are both less common in humans globally and are traditionally associated with contact with farm animals or dairy products. Recent cases of *C. ulcerans* have been associated with companion animals [2–4]. Toxigenic *C. diphtheriae* and *C. ulcerans* can cause both classic respiratory and systemic diphtheria, as well as other clinical presentations such as cutaneous diphtheria, which is more common in tropical areas of the world. Toxigenic *C. pseudotuberculosis* infections are usu-

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Fig. 1. Bleeding of a diphtheria horse, Statens Serum Institut, Copenhagen, 1904. With permission from Statens Serum Institut.

ally associated with lymphadenitis [5]. The DT gene is carried by a family of closely related bacteriophages (corynebacteriophages) that can integrate into the bacterial chromosome and convert non-toxic, non-virulent strains into toxic, highly virulent species [6,7]. However, transformation of a non-toxin-producing strain to a toxigenic organism is believed to occur rarely in nature.

2. Diphtheria toxin

Diphtheria toxin is synthesised and secreted as a single polypeptide, pro-enzyme that is cleaved and reduced *in vivo* to produce a toxic protein consisting of A and B fragments [8]. The B subunit contains the receptor binding and translocation domains of the toxin and the first step in the intoxication of eukaryotic cells by DT is the binding of toxin to specific cell surface receptors [9]. The receptor for DT was identified as the heparin-binding epidermal growth factor-like growth factor precursor (pro-HB-EGF) [10,11]. After binding of the toxin B subunit to the receptor, the toxin is internalised by receptor-mediated endocytosis. The low pH within the endosome causes a conformational change in the toxin molecule, facilitating translocation of the catalytically active A subunit of the toxin into the cytoplasm [12]. Once inside the cytoplasm, the A subunit, an ADP-ribosyltransferase, exerts its cytotoxic action by ADP-ribosylating elongation factor 2 (EF-2) thereby inhibiting cellular protein synthesis. The toxin has an estimated lethal dose for humans of $\leq 0.1 \mu\text{g}/\text{kg}$ [13]. The DTs of *C. diphtheriae* and *C. ulcerans* have been shown to be 95% identical; differences between these two DTs are mainly located in the translocation and receptor-binding domain of the B subunit. In contrast to *C. diphtheriae* DT, the DT of *C. ulcerans* seems to be much more heterogeneous [14].

3. Diphtheria therapy

Whilst diphtheria is an increasingly rare disease in the majority of developed countries, when cases do arise they can be severe and require a rapid and robust public health response. Case fatality rates worldwide remain high (>10%) [15]; a recently reported case fatality ratio (CFR) for Latvia for 2002–2007 was 9% [16]. Outside endemic areas CFRs can be even higher; delays in diagnosis and hence appropriate treatment have been reported [17]. The most effective treatment for diphtheria is early administration of

diphtheria antitoxin (DAT), along with appropriate antimicrobial therapy to eliminate the corynebacteria from the site of infection thus stopping ongoing toxin-production. The protective effect of DAT has also been demonstrated *in vitro* and *in vivo* for *C. ulcerans* and is a treatment option for diphtheria caused by *C. ulcerans* [18]. However, in practice DAT is given based on clinical diagnosis, usually prior to laboratory confirmation [19]. DAT is a preparation of immunoglobulins or immunoglobulin F(ab')₂ fractions produced from immunisation of horses, that neutralises circulating DT. Emil von Behring won the first Nobel Prize for medicine in 1901 for his work on “Serum Therapy in Therapeutics and Medical Science” where he noted the importance of early use of diphtheria serum in order to achieve successful “detoxication of the bacillus poison” [20]. The antitoxin will only neutralise circulating toxin which has not bound to tissue; it is therefore critical that DAT is administered as soon as a presumptive diagnosis has been made without waiting for bacteriological confirmation [1]. A study of fifty patients with diphtheritic polyneuropathy in Riga, Latvia found antitoxin to be ineffective if administered after the second day of diphtheritic symptoms [21]. Aside from improved methods to refine or purify the equine serum, little has changed in diphtheria serotherapy since its introduction in the late 19th century and its continued use today, over 100 years later.

4. Diphtheria antitoxin supplies

Historical documents suggest that even in the pre-vaccine era the supply of DAT could be problematic, particularly in remote areas. ‘The Serum Run of 1925’ describes life-saving supplies of antitoxin being urgently ‘mushed’ across the snow by huskies in Alaska to reach a diphtheria epidemic in Nome [22]. Later, in an account of nursing during World War II, Barbara Brooks Tomblin describes problems with the supply of DAT and waiting ‘as long as forty hours’ for it to arrive [23].

In the early 1900s many countries (Denmark (Fig. 1), France [24], Germany [25], Canada [26], USA (Fig. 2) and UK [27] to name a few) produced their own therapeutic antitoxin preparation from horses. Fig. 1 shows the bleeding of a horse for production of diphtheria antitoxin at the Statens Serum Institut in Copenhagen, Denmark in 1904. Except for the director, the complete staff of the institute were present in the photograph. The description accompanying the

Too Old to Work but Not Too Old to Give the Record Yield of Diphtheria Antitoxin at the Otisville Laboratory.

Fig. 2. Headline from The New York Times, 7 June 1914.

photograph stated that when bleeding, usually eight pots of blood were drawn corresponding to about eight litres. The bleeding had no immediate effect on the horse and in the following days the horses were usually more lively and playful; about 1 month later the horse could be bled again. Fig. 2 shows an extract from the New York Times describing the employment of a retired cleaning-cart horse at the Otisville Laboratory, New York. Following the introduction of mass vaccination in the 1940s/1950s and the consequent decline in cases of diphtheria, several countries stopped manufacturing their own supplies, some relatively recently. Diphtheria antitoxin for therapeutic use was manufactured in the USA until 1996, after which time supplies were imported from France (until production there was stopped in 2002) and more recently from Brazil [28]. Companies in Australia (Commonwealth Serum Laboratories Ltd.), Poland (Biomed Serum and Vaccine Manufacturers Ltd.) and Switzerland (Berna Biotech Ltd.) previously supplied several countries internationally but have recently ceased production of DAT (last stocks expired during 2007–2008). There are a number of factors contributing to the depletion of traditional sources of equine DAT, including economic viability (due to reduced demand and the need to manufacture pure products), the poor reputation of the product based on the rates of adverse reactions to old un-purified products, and public objection to the use of horses as blood donors [29]. Consequently, the supply of equine DAT for human therapeutic use has become increasingly problematic in recent years. Even in the UK, a small country where antitoxin has been held in 10 sites, it has proved challenging to transport antitoxin to more remote areas in a timely manner (Health Protection Agency duty doctor personal communication).

The potential consequences of a limited supply of DAT were highlighted during the resurgence of diphtheria that occurred in the Newly Independent States (NIS) of the former Soviet Union. During this outbreak in the 1990s there were shortages of vaccine, DAT, and antibiotics across the NIS (except in the Russian Federation) [30]. At the time of the disintegration of the former Soviet Union in 1991, all NIS relied on supplies of vaccine and DAT from Russia, and most lacked the financial resources to procure them from the international market. At the start of the epidemic, due to lack of DAT and delayed treatment, the CFR was very high (>20%) [30]. Once the international community made available DAT and antibiotics from 1995, the CFR fell to around 5–10%. In Russia, where DAT was always available, the CFR was approximately 3%. Regional and secular differences in CFRs in Uzbekistan during the diphtheria epidemic 1993–1996 may have been related to DAT availability and use. For example, in 1994, Qashqadaryo and Surkhondaryo Oblasts (which reported the majority of cases), had CFRs of 9.6% and 21.5% respectively, and during this time 79 (93%) of 85 cases in Qashqadaryo and 46 (50%) of 93 cases in Surkhondaryo received DAT. In Surkhondaryo Oblast, DAT supply was severely limited during the latter half of 1994, and the CFR rose from 16% (the national average) for the first 6 months of 1994 to 26% in the latter 6 months [31].

Today, the threat of diphtheria, even in countries with good coverage in their childhood immunisation programmes, has not disappeared. As the resurgence of diphtheria in the NIS demonstrated, it is possible for this disease to re-emerge in previously low-prevalence countries under particular conditions for example, gaps in childhood vaccination coverage combined with waning immunity in adults [32,33]. A number of seroepidemiology studies have reported sizable proportions of adults with immunity levels below the putative protection threshold in countries with high

childhood immunisation coverage [34–39]. In addition, the potential for a case to be imported into any country, either as a national acquiring an infection abroad or a new arrival/visitor to the country, will always exist whilst diphtheria is endemic in some parts of the world. It is important that in these situations a supply of DAT can be identified and the product distributed quickly.

5. Survey methods

As part of a work package assessing the surveillance and incidence of diphtheria, a questionnaire was developed by the UK Health Protection Agency, the lead partner of the European Commission funded Diphtheria Surveillance Network (DIPNET), to enquire about diphtheria surveillance practices within the 25 DIPNET member countries. It included a brief section on national facilities for maintaining stocks of DAT and was completed by the member countries in October 2007. Following the responses to this initial questionnaire, a more detailed questionnaire about DAT was developed with the assistance of colleagues at the National Institute for Biological Standards and Control. In February 2008 this DAT questionnaire was circulated to the 11 DIPNET member countries identified from the previous questionnaire as maintaining a stock of antitoxin, and to an additional 20 DIPNET collaborating countries, as well as 12 countries within the WHO EURO region not covered by DIPNET. Completed questionnaires were received from all DIPNET member countries, 12/20 DIPNET collaborating countries, and two countries from the WHO Euro region not included within DIPNET. In October 2008 a Russian translation of the questionnaire was sent to six of the countries within the original distribution list from whom a response had not been received; this resulted in five additional returned questionnaires, giving an overall total (including the original DIPNET responses) of 44 returned questionnaires from 57 countries.

6. Survey results

The results in Fig. 3 are based both on responses to the initial DIPNET questionnaire and the second DAT questionnaire. Responses to the survey were not received from Brazil, Russia and Croatia but it is assumed that these countries have a national stock because they are known to produce and supply DAT to other countries. Of the 47 countries where the status of DAT stocks was known for 2007–2008, 57% hold a current stock of antitoxin (this includes countries that produce and supply internationally). In the majority of countries the Ministry of Health is responsible for maintaining the DAT stocks and these are held at national level, which may involve distribution to regional holding sites. In Latvia, Kyrgyzstan and Kazakhstan stocks are held at all levels of the health system from national centres down to district hospitals and local health centres. Some countries specified that rather than a national stock, it is the responsibility of each state (Germany) to hold a stock, or that a limited amount of DAT is known to be available in at least one hospital (Austria).

All countries that maintain a stock of DAT use equine DAT. Expiry dates of stock at the time of surveying ranged from recently expired (2007) to 2015 (Japan) though most countries with current stock have expiration dates in 2009 or 2010. The Japanese antitoxin is a freeze-dried preparation, hence the long shelf life compared to the other stocks which are liquid preparations and typically have a shelf life of 2–3 years (NIBSC unpublished observations). Some countries are supplied internally by either a national institute (Turkey), state-owned company (Bulgaria) or private company (Japan); these organizations may be able to supply internationally in the future but were not currently supplying any of the other countries surveyed. Three countries have stocks (one expired, two expiry dates

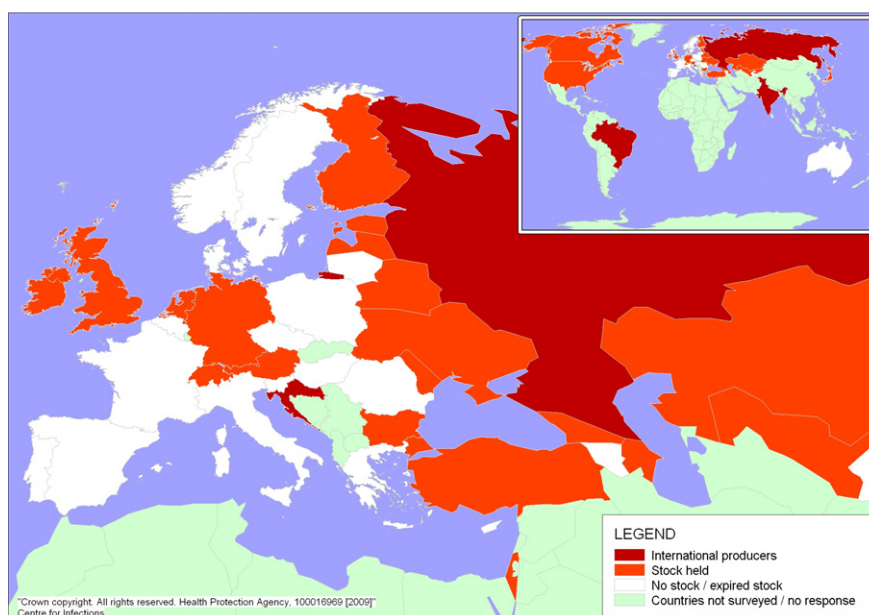


Fig. 3. Distribution of stocks and international suppliers of DAT for therapeutic use: 2007–2008 (European region with global inset). The four countries displayed in dark red in this figure produce DAT and supply internationally, those shown in lighter red (23) held a stock of DAT at the time of survey (either produced within their country or obtained from one of the four countries identified on the map as supplying internationally), and those with no stock or expired stock (20) are in white. Countries that were not surveyed or did not respond are shown in green. *Note 1:* in Tajikistan DAT was used during 1991–1999 thanks to humanitarian aid from the WHO/EURO. Currently, a patient can buy DAT by prescription at a private pharmacy. *Note 2:* in Australia, patients may gain access to DAT through the Special Access Scheme (SAS) which refers to the arrangements which provide for the import and/or supply of an unapproved therapeutic good for a single patient on a case by case basis.

Table 1
International suppliers of DAT amongst countries surveyed: 2007–2008.

Name of company	Location of company	Additional countries supplied
Mikrogen www.microgen.ru	Russia	Azerbaijan, Belarus, Estonia (some hospitals), Georgia,** Kazakhstan, Kyrgyzstan, Latvia, Moldova, Ukraine
Institute of Immunology Inc., ^a www.imz.hr	Croatia	Finland, Switzerland, Germany, Estonia
Instituto Butantan*** www.butantan.gov.br	Brazil	USA, Canada, UK, Israel
Serum Institute Ltd. www.seruminstitute.com	India	Uzbekistan

2009 updates: **Georgia is no longer supplied by Mikrogen and hence no longer has a stock of DAT. ***As of November 2008 and February 2009 France and Ireland respectively have sourced supplies of DAT from Instituto Butantan. *Note:* It is important to clarify that it is not known whether or not all of the companies listed in the above table conform to European and/or International standards. Of the countries that use external suppliers of DAT, only the UK, Ukraine and Belarus test the stock for potency in a national control laboratory.

^a The Institute of Immunology Inc also supplies more widely through a Canadian company (Intervax Ltd.) which supplies United Nations Agencies affiliated to the World Health Organization (Intervax Ltd. email communication).

in 2008) from an Australian company (Commonwealth Serum Laboratories Ltd.) which is no longer producing DAT; all three reported recent difficulty in finding a new supplier. Four current international suppliers were identified as shown in Table 1.

Forty countries answered the question asking if they had had difficulties recently in obtaining a supply of DAT; 50% (10 with a stock, 10 without a stock) had experienced recent difficulties. Six of the 20 countries that had not experienced difficulties obtaining stocks were countries without stocks where cases have not been recently reported, and no attempt to source DAT has been made. Thirty-eight of the 39 countries (97%) that answered the question about websites thought it would be useful to maintain a central list of current suppliers on a website. The websites of choice were (in order of preference) DIPNET, ECDC (European Centre for Disease Prevention and Control), EMEA (European Medicines Agency), with additional suggestions of WHO and ESCMID (European Society of Clinical Microbiology and Infectious Diseases) websites.

7. Discussion

This survey highlights both the range and absence of DAT stocks across participant countries. Importantly, those countries with the current highest incidence of infection such as Latvia and India do

hold stocks and these are maintained at national, hospital and even family doctor level. Countries which have reported sporadic cases in the last 8 years such as the UK, Germany and Turkey hold stocks of antitoxin at the state or national level. However, there are several countries, including some which have reported cases in the last 8 years which do not hold stocks, or hold stocks which are close to expiry. This is of public health concern – particularly considering the requirement for early administration of DAT when disease is suspected. In Australia, the Special Access Scheme described for importation of a therapeutic good is likely to be too time-consuming to be of benefit in the event of a case. The survey has identified a need for easy access to information about current suppliers of this product, as well as the need to raise awareness globally of the importance of maintaining stocks. The survey was limited to the WHO Euro region and some DIPNET collaborating countries outside of this region; it would be interesting to know the situation regarding supplies of DAT in other WHO regions, particularly in endemic areas.

Lack of a DAT supply can increase the likelihood of mortality as demonstrated during the shortages in the NIS epidemic [31]. In Lithuania in 2007, a case of classical respiratory diphtheria was reported however, Lithuania does not currently hold a stock of DAT so antitoxin treatment was not available for this patient, who sub-

sequently died (DIPNET unpublished data). In 2008, a suspected case of toxigenic *C. ulcerans* was identified in Ireland (although toxigenicity was never confirmed and the patient improved on antibiotic treatment alone). However, this resulted in an initiation of discussions with the UK regarding possible mobilisation of DAT supplies from the UK Northern Ireland stock if required, since the Irish stock had expired 6 months previously. Since then Ireland has procured new stock. Also in 2008, a cluster of diphtheria cases occurred in Oslo, Norway, in an unimmunised family returning from a visit to Latvia [40]. Norway does not maintain a stock of antitoxin, DAT treatment was not administered to the cases though the possibility of receiving DAT from the Staten Serum Institute in Copenhagen, Denmark was discussed (although Denmark does not currently maintain stockpile either). In November 2008 a case of diphtheria was diagnosed in France and it took 4 days for DAT to be delivered from the Instituto Butantan in Brazil after failed efforts to obtain this treatment from neighbouring countries. Currently it is the responsibility of each individual country to supply treatment for diphtheria in the event of a case, which (as demonstrated by these examples) may necessitate negotiations with neighbouring countries if the case occurs in a country that does not hold DAT stocks. During the NIS epidemic, WHO Euro involved governmental and non-governmental organizations such as the United States Agency for International Development, European Community Humanitarian Office, and International Federation of Red Cross in the initiation of an effort, monitored by the Interagency Immunization Coordination Committee, to mobilize the needed materials (i.e. vaccine, syringes, needles, DAT and antibiotics) [30]. There has been some discussion at European level regarding centralising stocks of essential medicines, specifically in the context of pandemic flu, but as yet this does not exist on a practical level (personal communication 2008: ECDC). This approach could however present difficulties in terms of timely transportation of DAT to the case and funding issues surrounding the cost of maintaining the central stock. The potential for current suppliers to increase production of DAT in the event of an epidemic has not been assessed here. For those countries that have experienced difficulties in sourcing a supplier of antitoxin it would be useful to maintain a list of DAT producers on a readily accessible website such as DIPNET, ECDC or the EMEA website. Countries can also email DIPNET (dipnet@hpa.org.uk) for more detailed information although DIPNET cannot recommend or endorse particular suppliers.

It may also be useful to review the current specifications for DAT for therapeutic use (European Pharmacopoeia 1000 IU/ml). This may be addressed as part of the current review of the WHO manual for the management and control of diphtheria (see Appendix A). Any relaxation in these specifications may be useful in the short term for emergency situations where product that has recently expired is available immediately and there is likely to be a delay in obtaining a replacement product in-date. However, this would not address the larger issue of maintaining adequate supplies on a global scale.

The problems in obtaining equine DAT together with the potential for adverse side effects such as serum sickness (which was reported to affect 9% of recipients receiving DAT in the US between 1940 and 1950 [41]) mean alternative therapies for diphtheria should be investigated. The use of an antitoxin preparation from human rather than horse blood (as has been the case for tetanus) would be more satisfactory in terms of limiting the risk of hypersensitivity reactions but may not be economical or practical on a large scale. In an American study reported by Sgouris et al. in 1969, a human DAT immunoglobulin preparation was produced which raised the antibody titre in subjects with less than 0.001 units per ml of serum to protective levels (0.01 IU/ml) without any local or general reactions occurring. However, only one percent of the outdated human plasma units that were tested as source material for this

production had sufficient antitoxin for fractionation [42]. A similar study in 1979 using selected blood donations to the Australian Red Cross Transfusion Services did not yield sufficient concentrations of antitoxin to allow use for therapy of established disease, only for prophylaxis in asymptomatic contacts of diphtheria [43]. It should be noted though that since these studies were carried out (prompted by the epidemic in the Newly Independent States) diphtheria immunisation strategies in adults have been reviewed in many countries in order to try to improve diphtheria immunity among older age groups [44]. Additionally the CRM₁₉₇ protein (a non-toxic variant of diphtheria toxin) is now used as a conjugate for several new vaccines (*Haemophilus influenzae* type b, meningitis C and pneumococcal vaccines) which may have a boosting effect [45]; consequently future diphtheria antibody levels may be higher in routine blood donations than they were in these early studies; further studies would be required to confirm this. However, considering that the dose of DAT required for even mild cases of diphtheria is 10,000 IU and the fact that normal human IgG for intravenous use (IVIG) has a DAT potency of approximately 3 IU/ml (NIBSC, unpublished observations), a volume in excess of 3 l of product would be required, which makes rapid and early administration of this kind of antitoxin preparation difficult for therapeutic use (see Appendix A for guidelines on administration of DAT). For severe cases, in excess of 13 l would be required to achieve the recommended dose of 40,000 IU, which is impractical. Recent guidelines issued by the UK Health Protection Agency for the treatment of tetanus do recommend the use of a human normal immunoglobulin preparation where specific tetanus immunoglobulin cannot be obtained. However, for tetanus, the antitoxin potency of IVIG is approximately 20 IU/ml (NIBSC unpublished observations) and the recommended treatment dose of 5000–10,000 IU can be achieved using 250–500 ml of antitoxin infused over a period of 3–6 h [46]. Therefore, if similar or even higher DAT levels could be achieved in a human-derived product the treatment dose of 10,000–40,000 IU could be achieved. Research in Russia during the NIS epidemic found that in an emergency situation it is possible to select donors for specific anti-diphtheria plasma among convalescent patients (approximately half of patients may be considered as donors), and that booster vaccination of convalescent diphtheria patients leads to enhanced antibody titres [47]. By selection of high-titre donors for DAT from human plasma pools (as is done for anti-D and anti-HepB IgG) and assuming an enrichment factor of 10 following purification, it may be possible to produce a product containing 50 IU/ml. However, even in this scenario antitoxin volumes of up to 800 ml would be required for treatment of severe cases of diphtheria. Furthermore, there is a global shortage of serum for immunoglobulin production which is threatening all supplies [48] and the higher economic costs involved in producing these types of product may be prohibitive. Production of human or humanized antibodies is a forward looking therapy for many toxin mediated diseases [49–51] and it would be desirable to also consider such products for diphtheria, if sufficient market can be identified.

There are also possibilities for non-antitoxin based therapy of diphtheria which could be explored. One example is the use of soluble receptor analogues for blocking of the DT receptor. Possible options include using the mature form of HB-EGF, although a truncated form of the protein would be necessary to avoid the potent mitogenic (and hence tumourigenic) effects of wild type HB-EGF [52]. It may also be possible to use other competitors for DT-receptor binding such as the non-toxic mutant of DT, CRM₁₉₇ which is licensed for human use in conjugate vaccines. Studies have shown that this mutant toxin can bind to the pro-HB-EGF receptor and prevent the mitogenic activity of the receptor [53]. In each of these examples, the diphtheria antidote would need to be administered early as is the case for DAT and the economics of manufacturing and supplying these materials may also be prohibitive.

Another possible future option may be the use of an extracorporeal device together with specific or non-specific adsorbents to remove circulating DT from the blood of affected individuals (reviewed in [54,55]), although, as with the other treatments, therapy would need to be started rapidly after preliminary or presumptive diagnosis of disease. Other drawbacks for this potential therapy include the need for invasive techniques.

8. Conclusions

One of the most critical aspects of current antitoxin therapy for diphtheria (and potential future therapies) is the requirement for rapid administration of the antidote. Toxin must be neutralised prior to binding to its receptor (or prevented from binding to the receptor by competition) resulting in a narrow therapeutic window. Current diphtheria therapy, based on the administration of DAT, is effective but compromised by a difficulty in maintaining supply of the therapeutic product. Until equally effective alternative therapies are identified and brought into use, the focus remains on how to ensure antitoxin therapy can be supplied in a timely manner to patients with diphtheria. The use of a freeze-dried antitoxin preparation, as is the case in Japan, would allow for an extended shelf life for the product, making it easier to maintain an in-date stock. However, there may be a reduced incentive for production of a relatively low-demand product that has an extended shelf life. If large scale production of antitoxin with a long shelf life on a rolling contract could be negotiated on a European or global scale this would facilitate the maintenance of individual country stocks. In the meantime information about current international suppliers of DAT will be made available and countries without stocks are urged to procure this treatment. The scarcity of DAT stock piles also emphasises the importance of maintaining high diphtheria vaccine coverage in all countries.

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Appendix A. European and international standards for the production of DAT

Relevant European guidelines concerning the production of DAT are listed below:

1. Minimum requirements for potency are prescribed in the Ph Eur monograph for Diphtheria Antitoxin (01/2008:0086) <http://www.pharmacopoeia.co.uk/ixbin/bp.cgi?tab=search&a=query&all=&q=&title=diphtheria+antitoxin> [restricted website: login required].
2. European Medicines Agency (EMA): Note for Guidance on Production and Quality Control of Animal Immunoglobulins and Immunosera for Human Use <http://www.who.int/bloodproducts/publications/EMA-animal%20sera.pdf>.
3. Ph Eur monograph for Immunoserum for Human Use, Animal (01/2008:0084) <http://www.pharmacopoeia.co.uk/ixbin/bp>.

[cgi?r=7b1NI5Bj39O&id=4575&a=display&tab=search](http://www.pharmacopoeia.co.uk/ixbin/bp.cgi?r=7b1NI5Bj39O&id=4575&a=display&tab=search) [restricted website: login required].

A.1. Administration of DAT

Guidelines for the administration of DAT are described in the WHO manual for the management and control of diphtheria (ICP/EPI 038 (B), 1994) (<http://www.who.int/vaccines-documents/DocsPDF05/0602170624.001.pdf>). Prior to administration, tests to exclude hypersensitivity of the patient to horse serum should be carried out. DAT should be given according to the manufacturer's instructions, the dosage depending on the clinical condition of the patient. Concurrent administration of antimicrobial treatment is also essential to halt toxin-production. This manual is currently being reviewed and updated under the auspices of DIPNET.

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Screening for *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* in patients with upper respiratory tract infections 2007–2008: a multicentre European study

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Abstract

Diphtheria is now rare in most European countries but, when cases do arise, the case fatality rate is high (5–10%). Because few countries continue to routinely screen for the causative organisms of diphtheria, the extent to which they are circulating amongst different European populations is largely unknown. During 2007–2008, ten European countries each screened between 968 and 8551 throat swabs from patients with upper respiratory tract infections. Six toxigenic strains of *Corynebacterium diphtheriae* were identified: two from symptomatic patients in Latvia (the country with the highest reported incidence of diphtheria in the European Union) and four from Lithuania (two cases, two carriers); the last reported case of diphtheria in Lithuania was in 2002. Carriage rates of non-toxigenic organisms ranged from 0 (Bulgaria, Finland, Greece, Ireland, Italy) to 4.0 per 1000 (95% CI 2.0–7.1) in Turkey. A total of 28 non-toxigenic strains were identified during the study (26 *C. diphtheriae*, one *Corynebacterium ulcerans*, one *Corynebacterium pseudotuberculosis*). The non-toxigenic *C. ulcerans* strain was isolated from the UK, the country with the highest reported incidence of cases due to *C. ulcerans*. Of the eleven ribotypes detected, Cluj was seen most frequently in the non-toxigenic isolates and, amongst toxigenic isolates, the major epidemic clone, Sankt-Petersburg, is still in circulation. Isolation of toxigenic *C. diphtheriae* and non-toxigenic *C. diphtheriae* and *C. ulcerans* in highly-vaccinated populations highlights the need to maintain microbiological surveillance, laboratory expertise and an awareness of these organisms amongst public health specialists, microbiologists and clinicians.

Keywords: Corynebacteria, diphtheria, screening

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Introduction

In the European region, diphtheria is rarely suspected in patients presenting with an upper respiratory tract infection due to the success of widespread immunization programmes. The disease is caused by toxin-producing *Corynebacterium* species: *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, or very rarely *Corynebacterium pseudotuberculosis*. In vaccinated or partially-vaccinated individuals, diphtheria can

present simply as a sore throat without the classic pseudo-membrane; clinically, the disease may not be suspected, or can be confused with other more common conditions such as severe streptococcal sore throat [1]. Most European laboratories no longer routinely screen throat swabs for corynebacteria, resulting in a loss of laboratory capability in this field [2]. It is therefore often difficult to differentiate between surveillance systems that report low numbers because there are genuinely few cases and surveillance systems that have low sensitivity.

In the 1990s, a dramatic resurgence of diphtheria occurred in the newly-independent states of the former Soviet Union. Many factors are considered to have contributed to the epidemic: reductions in vaccination coverage, numerous contraindications to vaccination, increased adult susceptibility, large-scale population movements, and a lack of adequate supplies for prevention and treatment in most affected countries [3,4]. Intensive vaccination strategies helped to bring the resurgence under control in most areas; however, of the countries participating in the present study, relatively high numbers of cases (an average of 28 symptomatic cases each year between 2002 and 2006) are still being reported in Latvia, predominantly from the capital city, Riga. No cases of diphtheria were reported to the Diphtheria Surveillance Network (DIPNET; <http://www.dipnet.org>) from Bulgaria, Estonia, Finland, Greece, Ireland and Turkey in the 5 years preceding this study (2002–2006). One case of toxigenic *C. ulcerans* was reported in Italy in 2002 and five isolates of *C. diphtheriae* were reported in Lithuania in 2002, but none subsequently. The UK reported between one and eight toxigenic isolates (including respiratory/cutaneous infections and asymptomatic carriage) of *C. diphtheriae* and/or *C. ulcerans* each year between 2002 and 2006.

Carriage rates in highly-vaccinated populations are expected to be low; a strong statistical association has been demonstrated between carriage of corynebacteria and non-protective levels of antitoxin antibodies [5]. European studies conducted in the last decade have documented carriage rates of 0.5 per 1000 (for toxigenic *C. diphtheriae* within routine throat swabs from Greek children) [6], and 0.7 per 1000 population (for non-toxigenic *C. diphtheriae* in an Italian population with sore throats) [7]. A Latvian study, which screened 38 157 throat swabs from both healthy and non-healthy individuals between 2002 and 2006, generated 140 *C. diphtheriae* isolates; 86% were toxigenic strains giving a carriage rate for *C. diphtheriae* in Latvia (both toxigenic and non-toxigenic organisms) of 3.7 per 1000 population [8]. Of the countries participating in this study, only clinicians in Latvia routinely request screening for corynebacteria when submitting a throat swab. In the UK, routine screening for

corynebacteria is only undertaken by selected laboratories; in the remaining participant countries, screening would only be undertaken to investigate a suspected case, although some countries (Lithuania, Ireland) have increased their screening practices subsequent to the present study being undertaken.

Widening membership of the European Union has led to significant migration of Eastern European populations to live and work in many parts of Western Europe. The present study attempts to determine the current prevalence of potentially toxigenic corynebacteria in different European populations to help with the interpretation of any future changes in the epidemiology of these infections in Europe.

Materials and Methods

Ten countries participated in this screening study, representing Baltic (Estonia, Latvia, Lithuania), Northern (Finland), Western (Ireland, UK), Southern (Italy, Greece) and Eastern (Bulgaria, Turkey) European countries.

Between December 2007 and June 2008, participating laboratories in each country processed throat swabs routinely received from patients with upper respiratory tract infections for potentially toxigenic corynebacteria, regardless of any other clinical indication. The exact screening periods for each individual laboratory varied in the range 1–5 months. The number of participating laboratories in each country ranged from one (in Finland) to 16 (in Greece) (Table 1).

Information on symptoms, vaccination history, travel history, and management of the case and contacts was completed for each patient in whom a toxigenic strain was identified using a case follow-up questionnaire. Patients from whom a non-toxigenic strain was isolated were not followed-up.

Statistical analysis

It was calculated that a minimum sample size of 2700 swabs per country was required to estimate, with reasonable precision, a prevalence similar to that previously seen in Latvia (described above) of 3.7 per 1000 population (a 95% CI length of <5 per 1000) [8]. Exact 95% CIs for carriage rates were calculated and the effects of country, age and sex were investigated in univariable analyses using Fisher's exact test and, in multivariable analyses, by logistic regression, using STATA software, version 8.0 (Stata Corp., College Station, TX, USA).

Laboratory analysis

All participating countries processed throat swabs for potentially toxigenic corynebacteria according to their standard

TABLE 1. Participating laboratories and regions in each country

Country	Number of laboratories	Areas served by participating laboratories
Bulgaria	4	Sofia area
Estonia	11	Whole country
Finland	1	Helsinki area
Greece	16	Ten laboratories from the Greater Athens area, other laboratories from Central and Northern Greece, Thessalia, and Crete
Italy	3	Rome, Perugia and Palermo
Ireland	3	Dublin North, Dublin South West, and part of Cork
Latvia	2	Whole country
Lithuania	13	Kaunas, Panevezys, Alytus, Vilnius, Siauliai, Marijampole and Klaipeda counties
Turkey	12	Adana, Ankara, Diyarbakir, Erzurum, Izmir, Istanbul, Samsun and Sanliurfa provinces
UK	12	Central and South Manchester, South and West Bristol, Cambridge, West Suffolk, Leicestershire, Southampton, East Birmingham, Newcastle, North East Derbyshire and part of South Yorkshire, Mid Essex, West Norfolk and Fenland, Bedfordshire

protocols and WHO guidelines [9]. Most countries performed primary screening using Hoyle's tellurite at the local laboratory level, and suspect colonies were sent to the country's reference centre for further confirmation of identification and toxigenicity.

At the end of the screening period, all *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* isolates identified during the study were sent to the HPA Respiratory & Systemic Infections Department in London, UK, for confirmation and molecular typing (ribotyping) [10].

Results

The number of swabs examined by each country during the screening period ranged from 968 (Italy) to 8551 (UK) (Table 2). Generally, more throat swabs were screened from females than males. Swabs submitted from children's hospitals were included for Estonia, Ireland, Lithuania, Latvia and Turkey; in Greece, only children were screened.

Toxigenic *C. diphtheriae* strains were isolated in Latvia and Lithuania, giving carriage rates of 0.8 per 1000 (95% CI 0.1–2.9) and 0.7 (95% CI 0.1–2.4), respectively. Carriage rates of toxigenic strains were zero in all other countries, although the upper 95% CI ranges varied from 0.4 per 1000 in the UK to 3.8 per 1000 in Italy. Toxigenic *C. diphtheriae* carriage rates did not significantly differ by country, age or sex.

Non-toxigenic *C. diphtheriae* carriage estimates ranged from 0 (Bulgaria, Finland, Greece, Italy, Ireland) to 4.0 per 1000 in Turkey (95% CI 2.0–7.1). In the multivariable analysis (including all countries), non-toxigenic *C. diphtheriae* carriage rates varied between countries ($p < 0.001$), sex ($p = 0.03$) and age ($p = 0.03$); however, after excluding Turkey, which had a cluster of seven males and two females aged 5–14 years, there was no difference by sex ($p = 0.14$) but differences remained between countries ($p < 0.001$) and there

was some evidence, although not significant, of a difference by age ($p = 0.05$), with the highest rates in the 15–44 years age groups.

Toxigenic cases: additional information from follow-up questionnaires

Both Latvian cases were 14 year olds presenting with sore throats; one (CaseLV2) also had a fever. CaseLVI had completed primary diphtheria vaccinations, whereas CaseLV2 had received only two doses (in 2002 and 2003, respectively). In Latvia, primary diphtheria vaccination is scheduled at 3, 4 and 6 months of age, with boosters at 18 months, 7 and 14 years. The cases were unlinked and there was no history of travel or known risk factors identified. Both patients received antibiotics. No diphtheria antitoxin was administered due to the mild clinical course, although Latvia does maintain a stock. Close contacts for both cases (two for CaseLVI, 37 for CaseLV2) were negative for *C. diphtheriae*. Contact tracing swabs are not included in Table 2.

Both Lithuanian cases presented with classic respiratory diphtheria with a pseudomembrane; neither had a history of travel, nor a link to another confirmed case. The fatal case was an unvaccinated 61-year-old woman (CaseLT1) who lived in crowded conditions with inadequate nutrition. She presented with a sore throat, pseudomembrane and fever, swelling and oedema of the neck, and submucosal or skin petechial haemorrhages; she also had underlying autoimmune thyroiditis and grade 4 aortic atherosclerosis. Eighty contacts were swabbed, two of whom were carriers of toxigenic *C. diphtheriae* (CarrierLT1 and CarrierLT2); one unimmunized and the other with vaccination status unknown. The second Lithuanian case, a 15-year-old female (CaseLT2), was immunized (completed primary immunization, last high dose booster of diphtheria was received in 2001, next booster would be scheduled at 15–16 years of age) with no other known risk factors. All twenty-two close contacts for

TABLE 2. Number of swabs screened from patients with sore throats by country, age and sex, with *Corynebacterium diphtheriae* carriage rates

Country	Bulgaria	Estonia	Finland	Greece	Italy	Ireland	Latvia	Lithuania	Turkey	UK
Screening period	December 2007 to April 2008	December 2007 to February 2008	January 2008 to February 2008	January 2008 to June 2008	December 2007 to March 2008	December 2007 to April 2008	December 2007 to March 2008	December 2007 to April 2008	January 2008 to June 2008	December 2007 to April 2008
Males (age group in years)										
0-4	163	573	51	355	20	223	234	363	234	714
5-14	170	487	209	292	61	119	169	400	524	720
15-24	45	370	82	-	45	38	205	207	100	700
25-44	95	305	116	-	73	47	223	167	235	947
45-64	32	135	26	-	23	30	191	69	141	360
65+	14	69	2	-	12	27	101	30	24	185
Unknown	-	36	-	-	116	-	-	-	41	90
Males total	519	1975	486	647	350	484	1123	1236	1299	3716
Females (age group in years)										
0-4	176	451	58	308	29	175	169	324	215	568
5-14	136	500	201	260	63	126	176	363	497	776
15-24	55	468	142	-	79	59	226	286	150	1123
25-44	163	570	232	-	155	95	331	437	311	1499
45-64	74	360	61	-	45	30	309	254	204	566
65+	36	131	18	-	38	17	146	88	38	229
Unknown	-	50	-	-	209	-	-	-	57	74
Females total	640	2530	712	568	618	502	1357	1752	1472	4835
Country total	1159	4505	1198	1215	968	986	2480	2988	2771	8551
Toxicogenic <i>Corynebacterium diphtheriae</i> isolates	-	-	-	-	-	-	2	2	-	-
Toxicogenic <i>C. diphtheriae</i> carriage per 1000 (95% CI)	0.0 (0.0-3.2)	0.0 (0.0-0.8)	0.0 (0.0-3.1)	0.0 (0.0-3.0)	0.0 (0.0-3.8)	0.0 (0.0-3.7)	0.8 (0.1-2.9)	0.7 (0.1-2.4)	0.0 (0.0-1.3)	0.0 (0.0-0.4)
Non-toxicogenic <i>C. diphtheriae</i> isolates	-	1	-	-	-	-	7	4	11	3
Non-toxicogenic <i>C. diphtheriae</i> carriage per 1000 (95% CI)	0.0 (0.0-3.2)	0.2 (0.0-1.2)	0.0 (0.0-3.1)	0.0 (0.0-3.0)	0.0 (0.0-3.8)	0.0 (0.0-3.7)	2.8 (1.1-5.8)	1.3 (0.4-3.4)	4.0 (2.0-7.1)	0.4 (0.1-1.0)

CaseLT2 were negative for *C. diphtheriae*. Both patients received antibiotics [CaseLT1 Cefuroxime (1 day) then imipenem and cilastatin sodium (½ day), CaseLT2 Cefazolin (15 days) then Gentamicin (10 days)]. The carriers received antibiotics (erythromycin) and diphtheria vaccine. Neither case received diphtheria antitoxin; Lithuania does not currently hold a stock of diphtheria antitoxin because of procurement difficulties [11].

Microbiological characterization of isolates

All toxigenic isolates were biotype *gravis*; one Latvian strain was not available to ascertain the ribotype and the other (CaseLV2) was Sankt-Petersburg; all four Lithuanian strains were also Sankt-Petersburg (Table 3). Biotyping and ribotyping was also performed on the non-toxigenic isolates: 12 of 26 *C. diphtheriae* were biotype *var gravis*, ten of 26 were *var mitis*, and two of 26 were *var belfanti* (two were not avail-

able for further characterization). Of the 86 ribotypes that have been previously identified and validated from over 25 countries, Cluj was detected in Latvia and Turkey, Buzau in the UK, Moskva in Lithuania, Romania in Estonia, and Lithuania and Lyon in Turkey. A new ribotype was also identified in Turkey, which matched closest to Constantine. The non-toxigenic *C. ulcerans* isolate detected in the UK was ribotype U4 (a different nomenclature to *C. diphtheriae* ribotyping) [12]. The *C. pseudotuberculosis* isolate from Latvia did not undergo ribotyping. The non-toxigenic isolates were also tested for the presence of the diphtheria toxin gene; the two isolated from Lithuania (ribotype: Moskva) were toxin-gene positive, all the others were negative. These two strains are designated as non-toxigenic toxin-gene bearing strains (NTTBs); the gene is present but the toxin is not expressed and are thus negative when examined in the Elek phenotypic test [9].

TABLE 3. Isolates detected during screening period

Country	Age group (years)	Sex	Organism	Biotype	Ribotype	Comment
Toxigenic isolates						
Latvia	5–14	M	<i>Corynebacterium diphtheriae</i>	<i>var gravis</i>	NA	CaseLV1
Latvia	5–14	F	<i>C. diphtheriae</i>	<i>var gravis</i>	Sankt-Petersburg	CaseLV2
Lithuania	45–64	F	<i>C. diphtheriae</i>	<i>var gravis</i>	Sankt-Petersburg	CaseLT1
Lithuania	25–44	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Sankt-Petersburg	CarrierLT1 (Contact of CaseLT1) - not included in the screening study as asymptomatic
Lithuania	15–24	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Sankt-Petersburg	CarrierLT2 (Contact of CaseLT1) - not included in the screening study as asymptomatic
Lithuania	15–24	F	<i>C. diphtheriae</i>	<i>var gravis</i>	Sankt-Petersburg	CaseLT2
Country	Age group (years)	Sex	Organism	Biotype	Ribotype	Tox PCR result
Non-toxigenic isolates						
Estonia	15–24	F	<i>C. diphtheriae</i>	<i>var belfanti</i>	Romania	Negative
Latvia	0–4	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Cluj	Negative
Latvia	5–14	M	<i>C. diphtheriae</i>	<i>var mitis</i>	Cluj	Negative
Latvia	15–24	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Cluj	Negative
Latvia	15–24	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Cluj	Negative
Latvia	45–64	M	<i>C. diphtheriae</i>	NA	NA	NA
Latvia	15–24	F	<i>C. diphtheriae</i>	NA	NA	NA
Latvia	25–44	F	<i>Corynebacterium pseudotuberculosis</i>	–	–	Negative
Latvia	45–64	F	<i>C. diphtheriae</i>	<i>var gravis</i>	Erlabrunn	Negative
Lithuania	15–24	M	<i>C. diphtheriae</i>	<i>var mitis</i>	Moskva	Positive; NTTB
Lithuania	25–44	M	<i>C. diphtheriae</i>	<i>var mitis</i>	Otchakov	Negative
Lithuania	25–44	F	<i>C. diphtheriae</i>	<i>var mitis</i>	Moskva	Positive; NTTB
Lithuania	25–44	F	<i>C. diphtheriae</i>	<i>var belfanti</i>	Romania	Negative
Turkey	5–14	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Cluj	Negative
Turkey	5–14	M	<i>C. diphtheriae</i>	<i>var mitis</i>	Cluj	Negative
Turkey	5–14	M	<i>C. diphtheriae</i>	<i>var mitis</i>	St Albans	Negative
Turkey	5–14	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Cluj	Negative
Turkey	5–14	M	<i>C. diphtheriae</i>	<i>var mitis</i>	Cluj	Negative
Turkey	5–14	M	<i>C. diphtheriae</i>	<i>var mitis</i>	Cluj	Negative
Turkey	5–14	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Cluj	Negative
Turkey	45–64	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Lyon	Negative
Turkey	5–14	F	<i>C. diphtheriae</i>	<i>var mitis</i>	Closest to Constantine/NT	Negative
Turkey	5–14	F	<i>C. diphtheriae</i>	<i>var gravis</i>	Cluj	Negative
Turkey	45–64	F	<i>C. diphtheriae</i>	<i>var gravis</i>	Lyon	Negative
UK	25–44	M	<i>C. diphtheriae</i>	<i>var mitis</i>	Pamiers	Negative
UK	25–44	M	<i>C. diphtheriae</i>	<i>var gravis</i>	Buzau	Negative
UK	15–24	F	<i>C. diphtheriae</i>	<i>var gravis</i>	Buzau	Negative
UK	5–14	M	<i>Corynebacterium ulcerans</i>	–	–	Negative

NA, not available; NT, new type; NTTB, non-toxigenic toxin-gene bearing.

Discussion

This is the first large multicentre European screening study to be undertaken for corynebacteria, with throat swabs collected and screened during a 7-month period. The differing numbers of swabs screened by each country were influenced by population size, consulting rates, and the different probabilities of a throat swab being taken for patients presenting with a sore throat. None of the countries with sample sizes below 1300 detected any *C. diphtheriae* or *C. ulcerans*, suggesting that there may have been insufficient power in these studies to detect the low levels of carriage found in the other countries.

All participating countries schedule at least five doses of diphtheria vaccine in their vaccination programmes, although the composition (low/high dose) and administration age varies. Vaccination coverage estimates for participating countries are high; estimates for Latvia and Lithuania over the last decade show over 90% coverage at 2 years of age for the first three doses of DTP (diphtheria, tetanus, pertussis) vaccine, with 98% and 95% coverage respectively reported in 2007 (http://www.who.int/immunization_monitoring/en/globalsummary/timeseries/tscoveredtp3.htm; accessed 30 July 2009). It should be noted, however, that different methods of assessment of coverage are employed in different countries and, although the overall coverage may be high, pockets of low coverage can still exist. A large-scale seroepidemiology study conducted across seven European countries between 1995 and 1998 found that 70–75% of adults aged 50–60 years from the UK had diphtheria antitoxin antibody titres below the putative lower protection threshold compared to approximately 35% of Finnish adults of the same age [13]. The proportion of seronegative adults (aged 30 years and above) in Italy was approximately 28% at the time of the study, and expected to increase. Waning immunity with age coupled with proportions of unvaccinated adults can lead to a susceptible population in older age groups.

Training workshops were conducted just prior to this study in Turkey and Estonia, and during the final stages of the study in Latvia; these countries all detected *C. diphtheriae* strains. In the UK, three of four non-toxigenic organisms detected were isolated by the West Suffolk microbiology laboratory, which screened the second largest number of swabs and was the only laboratory from which a microbiologist had recently attended a diphtheria diagnostics workshop. The other non-toxigenic *C. diphtheriae* was isolated from a UK laboratory that has a routine screening policy for corynebacteria. The absence of a screening policy and a lack of recent

training in other centres may have resulted in an overall under-estimation of carriage rates nationally.

One of the major clones causing the 1990s epidemic in the European region was a toxigenic *C. diphtheriae* var *gravis*, ribotype Sankt-Petersburg [14]. Recent studies have shown that this ribotype is still circulating and causing disease in Russia, Belarus and Latvia [15,16]. This screening study detected Sankt-Petersburg isolates from Latvia and Lithuania. In addition, ribotyping of concurrent isolates from Latvia that had caused diphtheria-like disease revealed the Sankt-Petersburg ribotype, highlighting the persistence of a highly successful and virulent clone. The majority of the ribotypes seen amongst the non-toxigenic isolates are more commonly associated with toxigenic isolates (Cluj, Moskva, Otchakov, Pamiers and St Albans) [10]; some have also been detected recently from Belarus (Cluj and Moskva) [16]. These data illustrate that persistent ribotypes are still circulating, and the bacterial population is evolving despite high vaccine coverage, resulting in a *C. diphtheriae* population that remains diverse enough to cause both epidemic and sporadic diphtheria.

Antibiotic susceptibility testing was not undertaken on isolates sent to the reference centre; these tests are usually undertaken locally. No unusual findings were reported to the co-ordinating centre, although this information was not specifically requested. It may be interesting to explore this area in a future study; however, the incidence of antibiotic resistance amongst potentially toxigenic corynebacteria is low [17].

The identification of two cases of diphtheria from Lithuania, neither of whom had any history of travel or contact with travellers, indicates that toxigenic *C. diphtheriae* is circulating within Lithuania. Examination of throat swabs for diphtheria is usually funded by the state in Lithuania but, in some cases, transportation of swabs requires payment, which may reduce the submission of samples to diagnostic laboratories; this could have influenced the lack of cases reported in recent years. A similar situation exists in Latvia. One of the toxigenic *C. diphtheriae* strains detected in Lithuania would have been missed in the absence of this screening study, highlighting the importance of screening for these organisms.

The present study has shown that NTTBs are circulating in Lithuania; these strains have the potential to become toxigenic and cause more serious illness [18,19]. The isolation of nine non-toxigenic *C. diphtheriae* strains in Turkey in geographically unrelated 5–14 year olds may not be unusual; in the UK, non-toxigenic *C. diphtheriae* strains are often isolated from unlinked young adults with a preponderance of females and may reflect consultation rates in the general population [20]. There is currently no direct evidence of person-to-person transmission of *C. ulcerans* or *C. pseudotuberculosis*, so

there is less public health concern surrounding the isolation of these zoonotic organisms, particularly non-toxigenic strains. Although not collected as part of the present study, the Latvian patient from whom non-toxigenic *C. pseudotuberculosis* was isolated was known to have had contact with cats and dogs and had also consumed untreated milk products.

The results of the present study, particularly the finding of toxigenic *C. diphtheriae* in Lithuania, highlight the importance of routine screening or further 'snapshot' studies within the European Region. In addition, they reinforce the need to achieve and maintain high vaccination coverage across the European region, as well as to maintain laboratory expertise in this specialized area. One of four cases identified in the study was fatal, demonstrating the severity of this disease in unimmunized patients, and the need to remain vigilant and aware of its possible clinical presentations. Larger studies in the future are essential for providing improved estimates of carriage in European countries, and for monitoring any changes in the circulation of these organisms against these baseline data.

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Transparency Declaration

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Immunity to tetanus and diphtheria in the UK in 2009

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ABSTRACT

Introduction: This study aimed to estimate the immunity of the UK population to tetanus and diphtheria, including the potential impact of new glycoconjugate vaccines, and the addition of diphtheria to the school leaver booster in 1994.

Methods: Residual sera ($n = 2697$) collected in England in 2009/10 were selected from 18 age groups and tested for tetanus and diphtheria antibody. Results were standardised by testing a panel of sera ($n = 150$) to enable comparison with a previously (1996) published serosurvey. Data were then standardised to the UK population.

Results: In 2009, 83% of the UK population were protected (≥ 0.1 IU/mL) against tetanus compared to 76% in 1996 ($p = 0.079$), and 75% had at least basic protection against diphtheria (≥ 0.01 IU/mL) in 2009 compared to 60% in 1996 ($p < 0.001$). Higher antibody levels were observed in those aged 1–3 years in 2009 compared to 1996 for both tetanus and diphtheria. Higher diphtheria immunity was observed in those aged 16–34 years in 2009 compared to 1996 (geometric mean concentration [GMC] 0.15 IU/mL vs. 0.03 IU/mL, $p < 0.001$). Age groups with the largest proportion of susceptible individuals to both tetanus and diphtheria in 2009 were <1 year old (>29% susceptible), 45–69 years (>20% susceptible) and 70+ years (>32% susceptible). Low immunity was observed in those aged 10–11 years (>19% susceptible), between the scheduled preschool and school leaver booster administration.

Discussion: The current schedule appears to induce protective levels; increases in the proportions protected/GMCs were observed for the ages receiving vaccinations according to UK policy. Glycoconjugate vaccines appear to have increased immunity, in particular for diphtheria, in preschool age groups. Diphtheria immunity in teenagers and young adults has increased as a result of the addition of diphtheria to the school leaver booster. However, currently older adults remain susceptible, without any further opportunities for immunisations planned according to the present schedule.

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1. Introduction

The current UK immunisation policy recommends five doses of tetanus and diphtheria toxoid; an accelerated primary course at ages 2, 3 and 4 months (given as DTaP/IPV/Hib vaccine), followed by booster doses at age 3 years 4 months to 5 years (pre-school booster, DTaP/IPV vaccine) and between 13 and 18 years of age (school leaver booster, Td/IPV vaccine) [1]. Vaccination coverage

of primary immunisations evaluated at one and two years has remained at around 91–95% in the UK since the beginning of the 1990s [2]. Assessment of the coverage of the preschool booster started in 1999/2000 and remained stable, between 78% and 82%, during the following decade, before increasing to 86% in 2009/2010. Vaccination coverage of the school leaver booster is unclear (data are collected only as number of doses given). For adults who have completed the five dose schedule there are no scheduled boosters for tetanus and diphtheria. Prior to 2002 a tetanus-containing vaccine was recommended following presentation of a tetanus prone wound if the last tetanus vaccine was received more than ten years previously, although a survey of accident and emergency departments in 2004 found that this practice was still continuing contrary to Department of Health guidance [3]. Currently vaccination should occur following presentation of a tetanus prone injury to health

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services if the patient is not already fully immunised [1]. Opportunities for additional vaccination may occur during a travel health consultation for example for those who are going to live or work in diphtheria epidemic or endemic areas (the same UK policy is also followed by the military), or for occupational reasons (e.g. if working in a microbiology laboratory) [1]. A recent survey of vaccination policies across 29 EU/EEA countries reported that tetanus and diphtheria vaccines are recommended to all adults in 22 and 21 countries respectively although only six countries have data on coverage of tetanus adult boosters, and five on diphtheria coverage [4]. The UK is one of the few European countries where routine adult booster doses are not recommended; other countries may therefore find the UK experience of interest in relation to their own policy.

Clinical cases of either disease are now rare in the UK. Tetanus has occurred mainly in unimmunised older adults [5] with 17/27 cases in the last five years being aged >45 years. A cluster of 25 tetanus cases was reported in 2003/04 among young adult injecting drug users [6] and sporadic cases are occasionally reported in this risk group (three cases in the last five years). Toxigenic *Corynebacterium diphtheriae* infection reported in the UK is usually acquired overseas in countries where the disease is still endemic and is transmitted from person to person via respiratory droplets and close contact [7]. In contrast, toxigenic *Corynebacterium ulcerans* is a zoonotic infection, and although traditionally associated with exposure to cattle, raw milk or dairy products, in recent years has been associated with contact with companion animals [7–10]. Five classic respiratory diphtheria cases were reported in the UK in the last decade, four of whom were aged >45 years.

Since 1992, glycoconjugate vaccines containing tetanus toxoid (TT) or CRM₁₉₇ (a non toxigenic natural variant of diphtheria toxin) carrier proteins have been introduced into routine and catch-up immunisation programmes in the UK (Appendix A). In clinical trials administration of TT or CRM₁₉₇ glycoconjugate vaccines has increased immunity to tetanus or diphtheria respectively [11–13]. In the Netherlands, increased tetanus antitoxin antibody levels have been observed in some age groups following the introduction into the national immunisation programme and catch-up campaign of meningococcal serogroup C glycoconjugate (MCC) vaccine, using TT as the carrier protein [14,15].

In 1994, low dose diphtheria toxoid (d) was added to the school leaving booster in the UK (which previously only contained tetanus and polio vaccine). This action was prompted by the epidemics of diphtheria in eastern Europe and the concern about waning of vaccine induced immunity of adults in the UK. Gaps in immunity have previously been identified in older adults in the UK; in 1996 only 53% and 29% of those aged >60 years were protected against tetanus and diphtheria respectively [16]. Other European countries have also identified lower immunity to tetanus and diphtheria in older adults [17–19].

Given these programme changes since the previous tetanus and diphtheria seroepidemiologic study undertaken in England and Wales in 1996 [16], there is uncertainty about the current immunity profile. Consequently, this study was undertaken to estimate the immunity of the UK population to tetanus and diphtheria, and interpret the findings in order to inform vaccination policy.

2. Methods

2.1. Serum samples

Serum samples representing the entire ranges of age and most geographical regions of the population of England were selected from the Health Protection Agency (HPA) seroepidemiology collection. Briefly, participating NHS and HPA laboratories submit

residual sera from routine diagnostic testing to the HPA Seroepidemiology Unit. All samples are anonymised, a unique identity number is assigned and details of age, gender and geographical location are collated on a database. Approximately 150 samples were randomly selected from each of 18 age groups (total $n = 2697$), in order to allow the proportions protected within each age group to be estimated with 95% confidence intervals (CIs) to within $\pm 8\%$. The majority of samples with valid results had a sample date between January and December 2009 (98%, 2640/2688 for tetanus, 98%, 2641/2689 for diphtheria), with the remainder from January to February 2010.

2.2. Standardisation panel

In addition, a panel of 150 sera (50 selected randomly from each of those which had full, basic protection and susceptible results) from the original 1996 samples were tested using the same multiplexed fluorescent bead assay as the main 2009 serum survey. These results were then used to standardise the 2009 data to enable comparisons with 1996 results. For the 1996 sera, antibody to TT was originally measured by an in house, indirect enzyme linked immunosorbent assay (ELISA) and antibody to diphtheria toxin was measured by a time resolved fluorimetric immunoassay system commonly known as DELFIA (dissociation enhanced lanthanide fluorescence immunoassay) [16].

2.3. Serology

All serum samples were assayed in the Vaccine Evaluation Unit (VEU) at the HPA Public Health Laboratory, Manchester, using a multiplexed fluorescent bead assay to quantify IgG antibodies to tetanus and diphtheria toxoid, based upon previously published methodology [20]. Similar methods have also been used in the VEU to quantify antibodies to meningococcal serogroups A, C, W135 and Y [21] and multiple pneumococcal serotypes [22].

2.4. Data analysis

Standardisation of 2009 data with 1996 data via the selected 1996 panel of 150 sera was conducted using methodology previously described [23]. Panel results from 1996 were plotted against those obtained in 2009 to derive standardisation equations, which were applied to the 2009 quantitative results.

Geometric mean concentrations (GMC) were calculated for each age group for 1996 and 2009, apart from <1 year olds in 1996 as immunity in this age group was not assessed at that time. In addition, GMCs were calculated for males and females separately. Changes in serological profiles by age were interpreted with the aid of 95% CIs on the proportions. For comparison of GMCs for males and females for each age group the Bonferroni correction for multiple comparisons was used, so that only significant differences where $p < 0.0028$ were accepted (0.05/18, since there were 18 age groups).

For tetanus, antitoxin levels <0.1 IU/mL denote susceptibility, antitoxin levels of 0.1–1.0 IU/mL are protective and levels >1.0 IU/mL are considered as giving long term protection as per the previous 1996 study [16,24]. For diphtheria, antitoxin levels <0.01 IU/mL denote susceptibility, antitoxin levels 0.01–0.099 IU/mL provide basic protection, and antitoxin levels ≥ 0.1 IU/mL are fully protective, as per the international standard [25].

For both the 2009 data and the previous 1996 results, the proportions protected were standardised by age and sex to the 2009 and 1996 UK populations respectively [26]. Although samples were only collected in England, the vaccination schedule applies to the

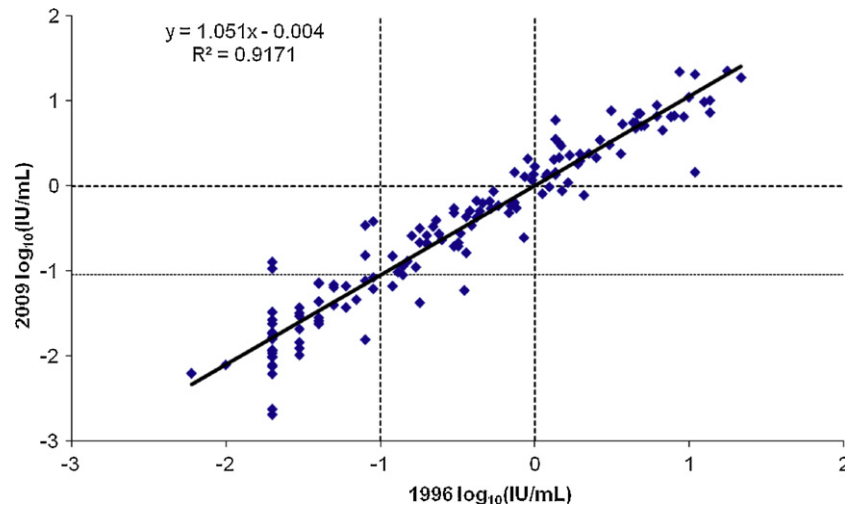


Fig. 1. Comparison of tetanus 1996 sample panel results for 1996 (in-house indirect ELISA) and 2009 (multiplexed fluorescent bead assay).

whole of the UK therefore the results should be generalisable to the UK.

2.5. Ethical approval

National Research Ethics Service approval for the seroepidemiological surveillance of the National Immunisation programme of England and Wales, REC number 05/Q0505/45 was granted by the Joint University College London/University College London Hospital Committees on the Ethics of Human Research.

3. Results

3.1. Standardisation

The 2009 panel results regressed well against the 1996 reference results (R^2 was 0.92 for tetanus, 0.78 for diphtheria). The standardisation line was linear for tetanus (Fig. 1) and quadratic for

diphtheria (Fig. 2). The effect of standardisation of the 2009 panel results to the 1996 unitage was the same qualitatively as changing the categories described above to <0.088 IU/mL (susceptibility) and >0.99 IU/mL for tetanus (long term protection), and <0.021 IU/mL (susceptibility) and >0.098 IU/mL (full protection) for diphtheria, for the 2009 data. The horizontal dashed lines on Figs. 1 and 2 indicate the cut off values for 2009 data standardised against the 1996 data.

3.2. Tetanus results

In 2009, 83% of the UK population was protected (≥ 0.1 IU/mL) against tetanus (vs. 76% in 1996, $p = 0.079$), and 44% had long term protection (>1 IU/mL) (vs. to 39% in 1996, $p = 0.277$). In 2009, the proportion with long term protection increased throughout early childhood to 57% at aged 5 years (Fig. 3). The proportion with long term protection then declined from age 5 to 10–11 years before a second increase was observed for teenagers, peaking at age 25–34

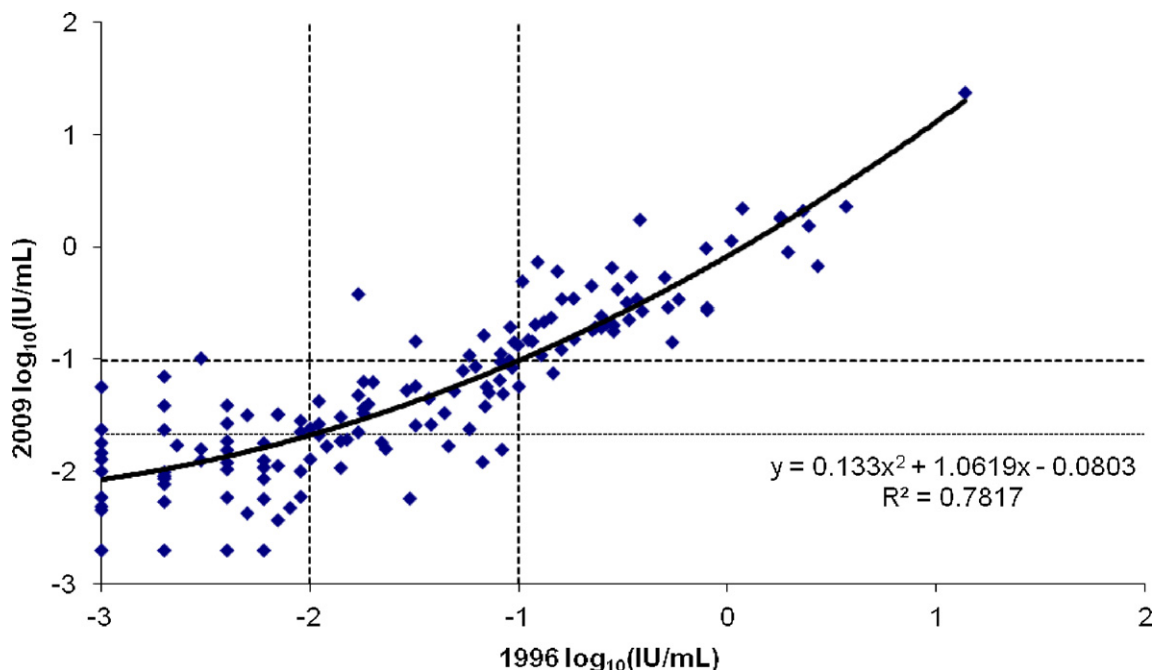


Fig. 2. Comparison of diphtheria 1996 sample panel results for 1996 (DELFI A) and 2009 (multiplexed fluorescent bead assay).

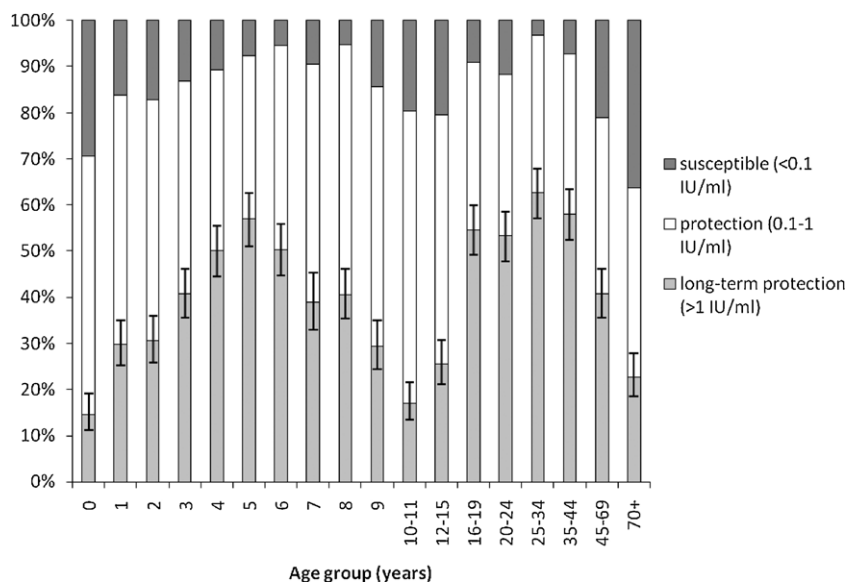


Fig. 3. Tetanus antitoxin distribution by age group in England, 2009. Error bars indicate 95% confidence intervals for long-term protection.

years (63%), then declining with age. The age group specific pattern of tetanus immunity in 2009 resembled that observed in 1996 (Fig. 4), however, higher antibody levels were observed in the 1–3 (GMC 0.49 IU/mL vs. 0.20 IU/mL, $p < 0.001$) and 35–69 years age groups in 2009 compared to 1996, and lower antibody levels were observed in the 12–19 year olds in 2009 compared to 1996.

The largest proportions susceptible in 2009 were observed in the age groups 70+ years (36%), and <1 year (29%). Similarly, the lowest proportions with long term protection were in those aged <1 year (15%), 10–11 years (17%) and 70+ years (23%). Those with the highest proportions susceptible in 1996 were aged 45+ years (>43%). In 1996, the lowest proportions with long term protection were aged 1–3 years (<16%), 10–11 years (19%), and 70+ years (15%).

Overall, after adjusting for age group, the anti-TT IgG GMC for males was 26% higher than for females (95% CI 12–42%, $p < 0.001$) in 2009, compared to 49% higher in 1996 (95% CI 34–65%, $p < 0.001$). There was some interaction between age group and gender for tetanus in both 2009 ($p = 0.0015$, test for interaction) and 1996 ($p = 0.0219$, test for interaction). In 2009, the anti-TT IgG GMC was significantly higher for males in the 70+ years age group ($p = 0.0011$), and almost significantly higher for males in the 45–69 years age group ($p = 0.0029$), when the Bonferroni correction was applied. In 1996, the GMC for males was almost significantly higher

for those aged 16–19 years ($p = 0.0029$), and significantly higher for adults aged 25–44 years ($p < 0.001$).

3.3. Diphtheria results

In 2009, 75% of the UK population had at least basic (≥ 0.01 IU/mL) diphtheria protection (vs. 60% in 1996, $p < 0.001$), and 41% had full (≥ 0.1 IU/mL) diphtheria immunity (vs. 16% in 1996, $p < 0.001$). The proportion fully protected in 2009 remained stable (64–71%) between ages 1 and 9 years, declining afterwards to a low of 44% fully protected amongst those aged 10–11 years (Fig. 5). The proportion fully protected increased again for teenagers and young adults, before declining in older adults. In contrast, in 1996 immunity declined from age 6 years onwards (Fig. 6).

Higher diphtheria antibody levels were observed in those aged 1–3 years in 2009 compared to 1996 (GMC 0.20 IU/mL vs. 0.03 IU/mL respectively, $p < 0.001$). In 2009 the largest proportions susceptible were observed in the age groups <1 year (37%), 35–44 years (27%), 45–69 years (41%) and 70+ years (33%). In 1996 the proportions susceptible in these corresponding age groups in adults were larger (ranging from 47 to 70%). In addition, in 1996, 37% of those aged 25–34 years were susceptible. The lowest proportions fully protected in 2009 were those aged 35+ years; the proportion

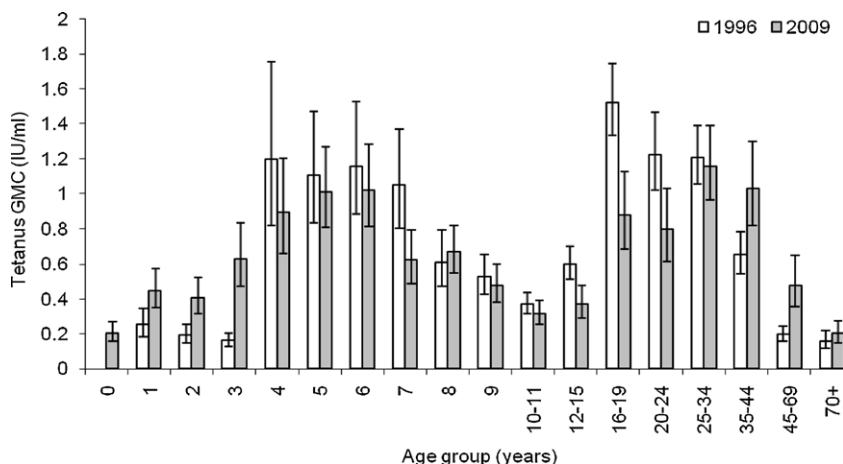


Fig. 4. Tetanus GMCs by age group, 1996 and 2009 results. Error bars indicate 95% confidence intervals.

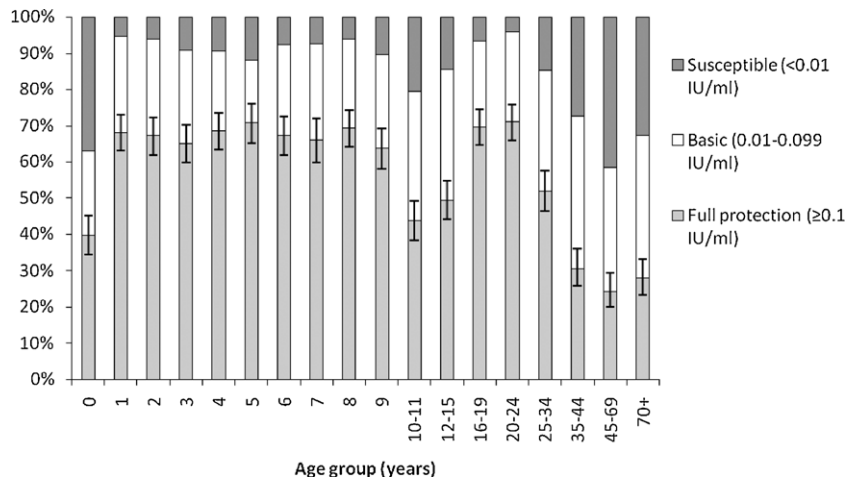


Fig. 5. Diphtheria antitoxin distribution by age group in England, 2009. Error bars indicate 95% confidence intervals for full protection.

fully protected ranged from 24 to 31% in these older age groups (vs. <10% in 1996).

Overall, after adjusting for age group, the anti-diphtheria GMC for males was 26% higher than for females (95% CI 9–46%, $p = 0.001$), compared to 16% higher in 1996 (95% CI 3–30%, $p = 0.011$). There was no significant interaction between gender and age group in 2009 ($p = 0.8200$, test for interaction), unlike in 1996 ($p = 0.01$, test for interaction). The anti-diphtheria IgG GMC for males and females was not significantly different across any age groups in 2009 when the Bonferroni correction was applied. In 1996, male teenagers 16–19 years had a higher GMC than females ($p < 0.001$).

4. Discussion

This is the second large scale sero-survey undertaken in the UK to assess immunity of the general population to tetanus and diphtheria. The key findings of this study include the increase between 1996 and 2009 in overall diphtheria population immunity, the identification of higher tetanus and diphtheria antibody levels in males, a decrease in tetanus antibody levels in teenagers and young adults in 2009 compared to 1996, an increase in antibody levels between 1996 and 2009 for tetanus and diphtheria in preschool age groups and diphtheria in teenagers and young adults, as well as gaps in immunity to both infections in the youngest and oldest age groups and those aged 10–11 years.

There are several potential limitations to the study. Firstly, in contrast to diphtheria, for tetanus established criteria for interpreting antitoxin levels are lacking, however, using the same criteria as the previous serosurvey has enabled comparisons. Secondly, different assays were used in 1996 and 2009, but the results standardised well and this methodology has previously been applied when comparing results from different laboratories [27–29]. Thirdly, this study used residual sera, which has been collected typically as a result of patients presenting with symptoms requiring diagnostic testing. However, serum from patients known to be immunocompromised is excluded from the archive collection, and previous studies using this sampling base have shown it to be representative of the wider population [30]. Routine vaccination coverage has been relatively stable so any changes in immunity over time due to changes in coverage would be too slight to be detected in these data.

Diphtheria antibody levels for the UK population are now above the >70% level generally considered protective [31,32]. This increase in immunity can largely be attributed to the addition of diphtheria to the school leaver booster vaccine, as well as the introduction of glycoconjugate vaccines (in particular PCV). Improvements in opportunistic vaccination in older individuals following the introduction of routine immunisation in the 1940s (reflected in the higher immunity in the 2009 cohorts aged 45+ years) may also have contributed.

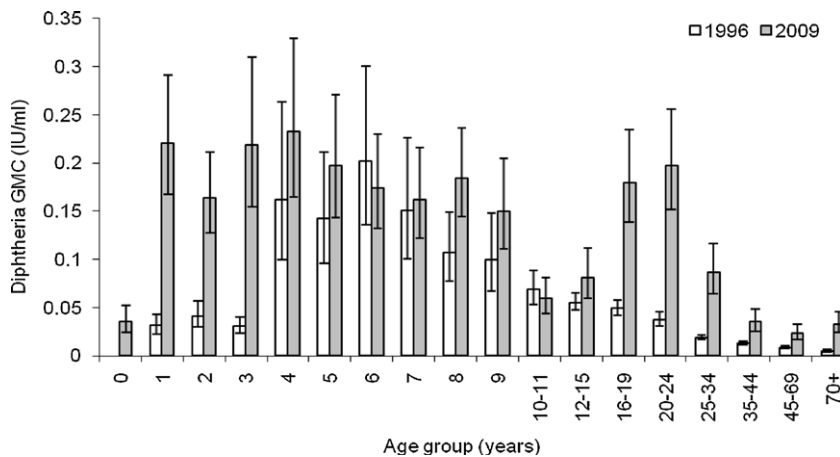


Fig. 6. Diphtheria GMCs by age group, 1996 and 2009 results. Error bars indicate 95% confidence intervals.

Higher tetanus immunity in males has previously been attributed to males presenting more commonly with tetanus prone injuries and receiving tetanus toxoid for this reason, as well as active immunisation of males taking part in military service, until conscription ended in the UK in 1960. The lower tetanus immunity in teenagers and young adults in 2009 compared to 1996 may be due to the change in the tetanus toxoid content of the school leaver booster which occurred in 2004. The Td/IPV vaccine currently used in the UK contains half the international units of tetanus toxoid of the Td vaccine which was previously in use. Over time, this can be expected to also reduce immunity in older adults as teenagers and young adults with lower immunity induced by the current school leaver booster move into the older age groups. The reason for overall higher diphtheria immunity in males is not known but has been reported previously [33,34].

Both the 1996 and 2009 cohorts aged 1–3 years would have received the Hib component of their primary immunisations conjugated with a TT carrier protein which does not explain the increase in antibody between the two surveys. The 2009 cohort would also have received MCC (TT or CRM₁₉₇ conjugate) at 3, 4 and 13 months which may explain the observed higher tetanus immunity in the 1–3 year olds in 2009 compared to 1996 (as also observed in the Netherlands [14,15]). The higher diphtheria immunity in those aged 1–3 years in 2009 compared to 1996 is likely due to the introduction of PCV (containing CRM₁₉₇) in 2006 which only the 2009 cohort for these ages would have received (at 2, 4 and 13 months), in addition to MCC vaccine as described above.

The increase in diphtheria immunity in teenagers and young adults is mainly due to the addition of low dose diphtheria toxin to the school leaver booster from 1994. All those in the 16–24 years age groups in 2009 and approximately half of those in the 25–34 years age group would have been scheduled to receive this vaccine. Teenagers and young adults in the 2009 cohort up to age 31 years would also have received MCC vaccine (CRM₁₉₇ or TT conjugate) from late 1999 to 2002 in catch-up campaigns [35]. However, the impact of this glycoconjugate vaccine on diphtheria immunity at this age appears negligible, as evidenced by the low immunity in those aged 10–11 and 12–15 years who would also have received MCC vaccine in 2000.

For both diphtheria and tetanus, the interval between the preschool booster and school leaver booster appears to leave those aged 10–11 years exposed. This relates particularly to a drop in full protection; levels of basic protection are less markedly affected. Babies aged <1 year include those midway through or about to start their primary immunisations which explains the lower immunity in this age group. As national tetanus immunisation was introduced (routinely for children between 1956 and 1961, initially in some areas as a monovalent vaccine and nationally in 1961 as part of DTP vaccine) and cumulative coverage improved, a greater proportion of adults aged 35–69 years in the 2009 cohort received vaccine than the corresponding age groups in 1996. Similar improvements were observed for diphtheria and increasingly, cohorts moving into the adult age groups will have received diphtheria in the school leaver booster. However, adults currently in these older age groups remain exposed.

5. Conclusions

The current tetanus and diphtheria vaccine schedule appears to protect well; increases in the proportions protected/GMCs were observed for the ages scheduled to receive vaccinations according to the UK schedule for both tetanus and diphtheria. This is supported by surveillance data which shows that few cases of either disease are reported each year.

As observed in previous studies, males generally have higher immunity than females, particularly older adults in the case of tetanus immunity, relating to the receipt of vaccine for tetanus-prone injuries and possible vaccination during military service for those aged 70+ years. The unintentional added benefit of glycoconjugate vaccines is observed in the higher diphtheria immunity in preschool ages in 2009 compared to 1996. Higher antibody levels are also observed for tetanus in these age groups, though this is less pronounced. A clear impact of the change to including low dose diphtheria toxoid in the school leaving booster from 1994 was observed on diphtheria immunity in teenagers and young adults; this should continue to improve immunity in adults. The pattern of immunity for diphtheria now more closely resembles that of tetanus which, with polio, has been part of the school leaver booster vaccine for several decades.

The age groups with lowest immunity are babies <1 year, and, as also identified in 1996, children approaching the age for administration of the school leaver booster for whom immunity from the primary series is waning. In addition, adults too old to have received routine immunisations have low antibody levels, though there have been some improvements since 1996. Those aged <1 year and 10–11 years will have opportunities for further routine tetanus and diphtheria immunisation. In contrast, although immunity in adults should gradually improve as those moving into older age groups will increasingly have received routine immunisations, including the school leaver booster, currently there is a gap in immunity in older adults which is not addressed by the present UK schedule. Given that immunity is known to wane with time, further boosting in adulthood may be of value for older adults. However, although surveillance data shows that the severest infections are in older, unimmunised adults, the number of cases of tetanus and diphtheria reported in the UK remains very small.

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Contributions: KW carried out the main data analysis, interpretation of data and drafting of the article. JW contributed to the conception and design of the study, interpreted the data, and critically appraised drafts. NA contributed to the conception and design of the study, conducted the statistical standardisation, advised on statistical methods and critically appraised drafts. RB contributed to the conception and design of the study, and critically appraised the drafts. ES contributed to the acquisition of data from the laboratory side, and commented on the draft. EN contributed to the acquisition of data from the laboratory side, and commented on the draft. RP contributed to the conception and design of the study, interpretation of data and critically appraised drafts. All authors have approved the final version. *Conflict of interests:* RB, ES and EN perform contract research on behalf of the Health Protection Agency for Baxter Biosciences, GSK, Merck, Novartis, Pfizer, Sanofi Pasteur and Sanofi Pasteur MSD.

Appendix A. Glycoconjugate vaccine additions to the UK immunisation schedule since 1996

Vaccine	Glycoconjugate	Date introduced	Age groups of 2009 cohort offered glycoconjugate
Meningococcal serogroup C (MCC) glycoconjugate vaccine	CRM ₁₉₇ and TT conjugate vaccines have been available although approximately 80% of MCC vaccine used was conjugated with the CRM ₁₉₇ carrier protein	Autumn 1999	0–31 years
Pneumococcal glycoconjugate vaccine (PCV) to protect against seven serotypes of <i>Streptococcus pneumoniae</i>	CRM ₁₉₇	September 2006	0–5 years
Hib/MCC booster doses for <i>Haemophilus influenzae</i> type b and <i>Neisseria meningitidis</i> serogroup C	TT	September 2006	1–4 years
Hib glycoconjugate catch-up campaigns	TT	2003 and 2007–2009	4–10 years

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Childhood vaccination coverage by ethnicity within London between 2006/2007 and 2010/2011

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ABSTRACT

Objectives To assess childhood vaccination coverage at first, second and fifth birthdays by ethnicity in London between 2006/2007 and 2010/2011 and identify factors relating to lower coverage.

Design Data concerning receipt of diphtheria-containing vaccines were extracted from child health information systems (CHISs) and sent to the Health Protection Agency.

Setting Nine London Primary Care Trusts (PCTs).

Participants Records for 315 381 children born April 2001–March 2010.

Main outcome measures Receipt of a full primary course of diphtheria-containing vaccines at first and second birthdays, and a primary course and preschool booster at fifth birthday.

Results Consistently good vaccine coverage of the primary course (>88% at first birthday, >89% at second birthday) was achieved across the five largest ethnic groups. Coverage of the preschool booster at fifth birthday was >65% across the five largest ethnic groups. Lowest coverage was observed in smaller ethnic groups. Deprivation was not a strong indicator of coverage overall, and for most ethnic groups there was no relationship between deprivation and coverage. Coverage was significantly lower in children not assigned to a general practitioner practice in the CHIS.

Conclusions Smaller, less well-established ethnic groups within a PCT may require specific targeting to ensure children are fully immunised and to improve record keeping. Unregistered children need particular attention and may be missed by current scheduling processes in London. In order to monitor the impact of the current National Health Service (NHS) reorganisation on inequalities in access to healthcare data on country of birth, in addition to ethnicity, should be available for analysis.

INTRODUCTION

The UK childhood immunisation programme currently includes a 5-in-1 vaccine that protects against diphtheria, tetanus, pertussis, polio and *Haemophilus influenzae* type *b* (DTaP/IPV/Hib) offered at 2, 3 and 4 months of age (primary course) and a preschool booster between 3 years 3 months and 5 years of age (dTaP/IPV or DTaP/IPV).¹ Diphtheria-containing vaccines have been routine for decades and are uncontroversial, thus providing an indication of primary care access in children.

Child health information systems (CHISs) are managed by child health departments and one of their many functions is to schedule and record the immunisations given to children,² although this varies locally³; in London, general practitioners

What is already known on this topic

- The UK has a universal childhood immunisation programme with overall high vaccine coverage rates; these are lower in London where there is also an increasingly ethnically diverse population.
- Previous studies have identified differences in vaccination coverage by ethnicity, but the capacity for monitoring this using data routinely collected within London has not been assessed.
- Reducing inequalities in childhood immunisation has been identified as a priority by the National Institute for Clinical Excellence.

What this study adds

- In general, the largest ethnic groups have good vaccination coverage, but newer, smaller communities within a PCT may need particular attention.
- Improvements in record keeping and transfer of information are associated with improvements in reported vaccination coverage.
- Children not registered with a general practitioner, or without up-to-date GP practice details in the child health information system, have lower recorded vaccination coverage and are at risk of missing out on key primary care initiatives.

(GPs) are often responsible for scheduling. Since 2006, the majority of Primary Care Trusts (PCTs, statutory bodies responsible for ensuring the availability of health services in a geographical area) in London have transferred to RiO CHIS. Vaccination coverage across the UK is currently monitored by the Cover of Vaccination Evaluated Rapidly (COVER) programme.⁴ Coverage is not uniform throughout the country; in particular coverage in London is lower,^{5 6} but recent increases, correlated with the introduction of National Health Service (NHS) London's Immunisation Improvement Programme, have reduced the gap between London and the rest of England.⁷

In 2010, 34% of people living in London were estimated to have been born abroad and 56% of children born in London were born to non-UK

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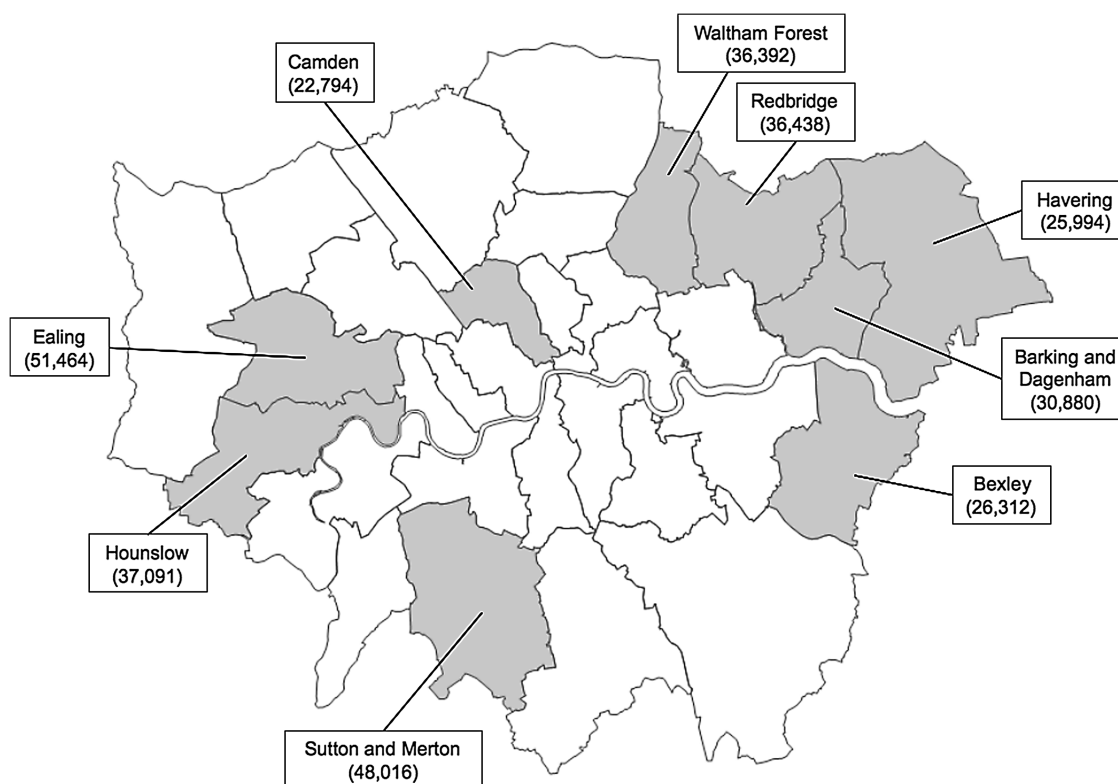


Figure 1 Participating Primary Care Trusts, London, including the total number of records extracted from each trust for analysis (number of children born April 2001–March 2010).

born mothers.^{8–9} London has an increasingly ethnically diverse population.¹⁰ Reducing differences in immunisation uptake among children is a priority identified by the National Institute for Clinical Excellence.¹¹ This study explored the capacity for routine CHIS data in London to monitor vaccination coverage by ethnicity.

METHODS

Nine London PCTs, who responded to a request at a London Immunisation Network (a group of London immunisation coordinators) meeting and via email, participated (figure 1). Participation was voluntary. The study PCTs represented a range of London PCTs in terms of vaccination coverage (coming from all four quartiles of 2010/2011 London PCT COVER data for diphtheria-containing vaccines at first, second and fifth birthdays).

A script written for the RiO CHIS was used to extract the following fields for each PCT's responsible population (children registered with a GP in the PCT or unregistered children living within the PCT's geographical boundary): month and year of birth, date of receipt of each diphtheria-containing vaccine (see appendix 1—web only), gender, ethnic group, nationality, post-code and GP practice code. Where the child's record was linked to a maternal record, basic demographic data were extracted from the mother's record.

In total records for 315 381 children born April 2001–March 2010 were extracted. There were 185 534 children born April 2005–March 2010 (first birthday cohort), 180 477 born April 2004–March 2009 (second birthday cohort) and 164 000 born April 2001–March 2006 (fifth birthday cohort).

To maintain anonymity, postcodes were replaced by lower super output area codes¹² prior to sending to the Health Protection Agency. Deprivation scores for each area were

assigned using the Income Deprivation Affecting Children Index 2010.¹³ The following deprivation quintiles were created: 0–0.130 (least deprived), 0.131–0.250, 0.251–0.340, 0.341–0.440 and 0.441–0.960 (most deprived).

Vaccination status was computed for each child at each age evaluated; either fully immunised or not fully immunised (including completely unimmunised and partially immunised). Only month and year of birth were extracted, therefore a child born in April 2005 was considered to have received a dose within 1 year (by 'first birthday') if the vaccine was received between April 2005 and April 2006 inclusive. Fully immunised status was assigned at first and second birthdays if at least three primary immunisations (vaccines containing high-dose diphtheria; 'D') were recorded within 1 and 2 years, respectively. Fully immunised was assigned at fifth birthday if at least four high-dose diphtheria-containing vaccines were recorded, with the fourth (or subsequent) dose received between ages 3 and 5 years. The fourth (or subsequent) dose could also be the low-dose diphtheria dTap/IPV vaccine. This coding is more conservative than some COVER extractions, which may only require the final vaccine ('Part 3' for a primary course or pre-school booster within the appropriate age range for fifth birthday assessment) to be recorded for a child to be considered fully immunised.

Individual RiO ethnicity categories were used for larger groupings and combined into broader categories for those with small numbers of children (see appendix 2—web only).

Statistical methods

Analyses were conducted in STATA SE/V.12.0 statistical software. Crude coverage proportions with exact 95% CIs were calculated for each variable. Multivariable logistic regression was used to determine whether ethnicity differences were due to other

available data on gender, deprivation, PCT and year of birth. We also tested for interactions in the model between ethnicity and PCT and ethnicity and deprivation.

RESULTS

Overall, vaccination coverage was 87% at first birthday, 87% at second birthday and 60% at fifth birthday. An increase in coverage over the study period was seen for all cohorts by year of birth. In general, individual PCT coverage in this study was similar or lower than COVER data for the same period due to more conservative coding; the coverage in this data set also reflects low coverage in London.

Nationality was available for <2.5% of all children. Ethnicity was recorded for 66% (121 657) of children at first birthday, 64% (115 941) at second birthday and 56% (91 877) at fifth birthday. To examine representativeness of recorded ethnicity data, known ethnicity data for children born 2005–2009 in each PCT were compared with Greater London Authority ethnicity projections for children aged 0–4 years in 2009.¹⁴ The proportion in black and minority ethnic (BME) groups in the study data set was within 5% of the Greater London Authority projected BME proportion for all PCTs except one, which had a higher proportion of BME children in the data set (63%) than projected (49%). Overall, the study PCTs had a similar proportion of BME children (51%) to the projection for Greater London (53%).

Consistently good coverage of the primary course (>88% at first birthday, >89% at second birthday) was achieved across the five largest ethnic groups. Coverage of the preschool booster at fifth birthday was >65% across the five largest ethnic groups (figure 2). Although some of the smallest ethnic groups had good coverage, the lowest coverage in each cohort was among the smaller ethnic groups and those with unknown ethnicity. Adjusting for gender, deprivation, PCT and year of birth did not substantially change the ethnicity patterns in coverage (model details in appendix 3—web only). There was evidence of interaction between PCT and child ethnicity for all three age cohorts ($p<0.001$); this was most pronounced for white-Polish populations and related to the size of the white-Polish population within a PCT. Where white-Polish populations were larger (410 and 383 children), coverage at first birthday in this group (90% and 88%, respectively) was closer to the average for the PCT. Two PCTs with smaller white-Polish populations (77 and 140 children; all other PCTs had <50 children in this group) had significantly lower coverage at first birthday in their white-Polish populations (67% and 69%, respectively) than the average for their PCT. Similar interactions relating to the white-Polish population size were observed at second and fifth birthdays.

Gender was recorded for >99.9% of children overall. There was no difference in coverage between males and females at first birthday, although coverage was fractionally higher (<1%) for females at second and fifth birthdays ($p<0.01$).

Deprivation scores were assigned to 98% (309 552) of records overall. Coverage across quintiles ranged between 86% and 88% at first birthday, between 87% and 88% at second birthday and between 59% and 63% at fifth birthday, lower coverage in general relating to higher deprivation. Interaction between ethnicity and deprivation was significant in each cohort ($p<0.001$). Across each age cohort, a trend of reducing coverage by increasing deprivation was seen only for white-British and Not known groups. The opposite trend was observed for Indian and white-Other/Mixed/Unspecified at first and second birthdays only. Trends were not seen for other ethnicities.

At the time of data extraction 14 022 (4.4%) children in the data set were not assigned to a GP (ie, did not have a GP practice code recorded because they were unregistered, moving between practices or records were not current). The proportion of children not assigned to a GP in each PCT at the time of data extraction ranged from 1.1% to 7.0%. Vaccination coverage was 52% versus 88% in children without a GP practice code versus those with a GP practice code at first birthday, 55% versus 89% at second birthday and 21% versus 63% at fifth birthday. Significant differences in coverage between children with and without a GP practice code assigned were seen across all PCTs in all three cohorts. Overall, 2.2% of white-British children were not assigned to a GP practice compared with 4.2% of non-white-British and 5.8% of children without ethnicity recorded.

Maternal records

Overall 42% (131 077) of records in the data set were linked to a maternal record. This varied considerably by age and PCT. At first birthday, 112 306 (61%) child records were linked to a maternal record, 87 998 (49%) at second birthday and 32 395 (20%) at fifth birthday. At first birthday, linkage to a maternal record varied across PCTs from 16% to 87%. However, in all PCTs the proportion of children linked to a maternal record improved over time, sometimes dramatically (eg, in one PCT from 2% (children born 2005/2006) to 61% (children born 2009/2010)). Overall, vaccination coverage at first birthday was higher for linked (90.8%) children versus unlinked (81.3%) children ($p<0.001$) (further subanalyses of maternal data for linked children were not conducted as they were not considered representative). As linkage improved over time for each PCT, the difference in coverage between linked and unlinked children became more pronounced. Of those children in the data set with ethnicity recorded, 51% were linked to a maternal record compared with 27% of children without ethnicity recorded.

DISCUSSION

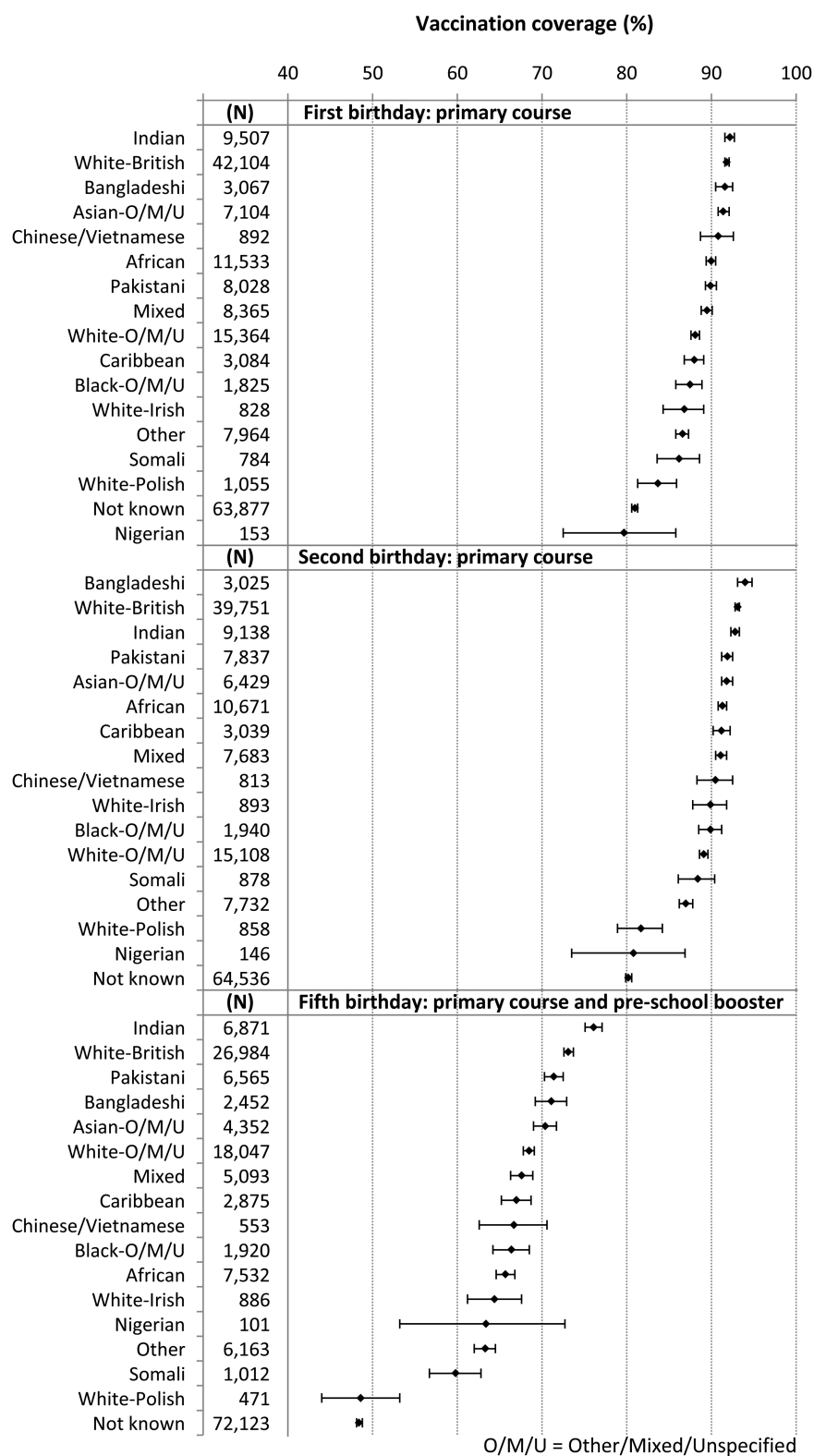
Main findings

The largest ethnic groups in each cohort had good vaccination coverage. Ethnic groups with lowest coverage were generally smaller and those with unknown ethnicity. Interactions between PCT and ethnicity were observed for a minority of ethnic groups, for white-Polish populations (for whom migration to the UK has increased since Poland joined the European Union in 2004) this related to the size of the population in a PCT. Differences in coverage between ethnic groups were not explained by adjustment for gender, deprivation, PCT or year of birth. Deprivation was not a strong indicator of coverage overall, and for most ethnic groups there was no relationship between deprivation and coverage. Data completeness was a key factor in determining the vaccination coverage recorded (as evidenced by the low coverage in children with unknown ethnicity and those not linked to a maternal record). Children not assigned to a GP in the CHIS had lower vaccination coverage than those with a GP practice code recorded. Routinely collected data from the RiO CHIS can be used for basic analysis of vaccination coverage by ethnicity, with adjustment for certain factors.

Strengths and limitations of study

This is the first study to explore the capacity for data routinely collected within the RiO system to provide vaccination coverage data by ethnicity for London and includes smaller ethnic groups such as Somali and white-Polish. However, some ethnicities, for

Figure 2 Vaccination coverage by ethnicity for children at first birthday (born April 2005–March 2010), second birthday (born April 2004–March 2009) and fifth birthday (born April 2001–March 2006), with 95% CIs.



example, Romanian, are not captured individually. In other published analyses, additional factors have been studied such as family size, maternal smoking, maternal education and lone parenthood,^{15 16} but this study was restricted to CHIS fields. Deprivation in this study was assigned based on postcode, relating deprivation to a geographical area.

Although vaccination is unlikely to be recorded incorrectly, no record of vaccination could reflect failure to immunise or

failure to record. This is more likely where immunisations were given in other geographical areas and would lead to lower measured coverage in those who move into the PCT, particularly at an older age. This may therefore explain the lower coverage in population groups more likely to have moved since birth,¹⁷ including recent migrants to the UK. Failure to record vaccination is also often associated with missing demographic data. It is difficult to disentangle true improvements in coverage from

improvements in data quality. Data quality issues relating to information migrated from legacy systems have previously been identified.¹⁸ The lower vaccination coverage observed among children without a link to a maternal record may result from data relating to the mother and immunisations either not being added or transferred across to the current PCT records for children born outside the PCT (either in another PCT or abroad). Other information such as a child's ethnicity may also not have been transferred across. Obtaining a vaccination history from children arriving from abroad can be problematic; even if the vaccines received abroad are known, they may not be coded within the CHIS and so may be omitted from the record. Children who are not linked to a maternal record may also be those who are living with relatives, possibly moving frequently, or are looked-after children. More information such as country/area of birth/previous residence would aid understanding.

Comparison with other studies

Improvements in vaccination coverage over time, differences in coverage between PCTs and lower coverage of the preschool booster at fifth birthday compared with coverage of primary vaccines at first and second birthdays were expected.⁵ Previously, the Millennium Cohort Study identified differences in coverage by ethnicity.¹⁶ A study in Manchester found that white infants were least likely to be vaccinated with primary vaccines, and that for white infants (as found here) lower coverage was significantly associated with living in a deprived area.¹⁵ For black infants or black British infants and Pakistanis, there was no significant association between deprivation and immunisation.¹⁵

Conclusions and policy implications

We have shown that monitoring coverage by ethnicity is possible and could be used to identify groups with low recorded immunisation coverage. Such findings should be explored to determine whether there is a genuine need to improve coverage or a need to improve data quality. London's population is highly mobile making it challenging to maintain accurate health records as children move across PCT boundaries and change GP, particularly in the first year of life when the primary vaccine course is offered. The absence of data could indicate less contact with the health system, both in terms of opportunities for immunisation and maintaining records. Children in London are invited for vaccination by GPs; those not registered with a GP are at serious risk of missing out on immunisations and need particular attention. Registration with a GP can be particularly low among certain migrant populations.¹⁹

Although it is encouraging to see data completeness and vaccination coverage improving, the NHS is currently undergoing a major reorganisation and previous experience has shown that reorganisation negatively impacts on the quality of data²⁰; this is therefore likely to continue to present challenges. However, it is also an opportunity to influence the CHIS service specification to ensure that fields such as ethnicity and country of birth are accurately recorded. Directors of Public Health in Local Authorities will have a duty to scrutinise and challenge the NHS for how well and how equitably it provides immunisation services—coverage by ethnicity should be one of the key metrics by which this role is undertaken.

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Contributors Contributors (specific author contributions, please note that contributions of other persons are listed in the acknowledgements). KSW (guarantor) had the idea for the study, designed the study, coordinated the collection of data, conducted the analysis and interpretation of results and drafted and revised the manuscript. JCvW designed the study, wrote and piloted the data extraction script, provided data from Ealing and critically appraised the manuscript drafts. NA provided statistical guidance with respect to the planning of the study, analysis and interpretation of the study data and critically appraised the manuscript drafts. KG designed the study and critically appraised the manuscript drafts. JCvW designed the study, interpreted the study results and critically appraised the manuscript drafts.

Competing interests None.

Ethics approval This study involved secondary use of non-identifiable data from health records; under the revised governance arrangements for research ethics committees (RECs) REC review was not required. Management permission for research within each PCT was obtained via research and development offices.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data submitted to the study by individual PCTs are available to those PCTs.

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Appendix 1: Vaccine codes and application of coding of fully/not fully immunised status

A = acceptable vaccine for primary and/or pre-school booster within coding framework

B = acceptable vaccine only for pre-school booster (4th or higher dose) within coding framework

C = not counted within coding framework

Vaccine code*	Vaccine description	Application of coding
D_P	Diphtheria primary course	A
DT_P	Diphtheria/Tetanus Primary Course	A
DTaP_P	Diph/Tetanus/Acellular Pertussis Primary	A
DTaPIPvH	Dip/Tet/Pert/Polio/Hib Primary (< 10yrs)	A
DTaPIPvH4	Dip/Tet/Pert/Polio/Hib Primary part 4	A
DTP_P	Dip/Tet/Pert Primary course	A
DT_B	Dip/Tet preschool booster	A
DTaP_B	Diph/Tetanus/Acellular Pertussis Booster	A
dTaIPV	Dip/Tet/Pert/Polio Preschool booster	B
DTaPIPvHiB	Dip/Tet/Pert/Polio/HiB Preschool booster	A
DTP_B	Dip/Tet/Pert Pre-School Booster	A

DTPH	Dip/Tet/Pert/Hib	A
DTPP4	Dip/Tet/Pert/Polio/ Primary 4	A
SUP_D	Supplementary Diphtheria	A
SUP_DT	Supplementary Diphtheria/Tetanus	A
SUP_DTP	Supplementary Dip/Tet/Pert	A
Td/IPV_B	Dip/Tet/Polio Booster (> 10yrs)	C
Td/IPV_B10	Dip/Tet/Polio Booster	C
Td/IPV_P	Dip/Tet/Polio Primary course (> 10yrs)	C
TDL_B	Low dose Diph/Tet School Leavers Booster	C

*_P = primary, _B = booster

Note: several vaccine codes/combinations were possible within this study because the remit was to assess coverage of diphtheria-containing vaccines. Diphtheria vaccine is administered globally and children arriving from abroad may have received vaccines different from those that are scheduled in the UK. In addition, there have been some changes in scheduling/supply within the UK so a number of options are possible*. However, the majority of children within this study dataset born on or after September 2004 received DTaPIPvH (DTaP/IPV/Hib) as their first (96% of first doses received), second (98% of second doses received), and third (97% of third doses received) vaccine. Prior to September 2004 first, second and third doses received were usually DTPH (approximately 70%) or DTP_P (approximately 18%). The majority of children evaluated at fifth birthday had dTaPIPv (61%) recorded as their 4th vaccine (there is no 'DTaP/IPV' code in RiO; this code is used for both dTaPIPv and DTaPIPv), 29% received DTaPIPvHiB.

*The current accelerated primary schedule in the UK has been in place since 1990, but before 2004 a vaccine containing whole cell pertussis (DTwP-Hib) and a separate oral polio vaccine (OPV) were used (Chief Medical Officer, Chief Nursing Officer, Chief Pharmaceutical Officer. New vaccinations for the childhood immunisation programme [letter] 10th Aug 2004. Department of Health.

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Appendix 2: Ethnicity codes and grouping categories [abbreviation]

Grouping	Code	CodeDescription
White-British	A	White - British
	CA	White - English
	CB	White - Scottish
	CC	White - Welsh
	CD	White - Cornish
White-Polish	CP	White - Polish
White-Irish	B	White - Irish
White-Other/Mixed/Unspecified	C2	White - Northern Irish
	C3	White - Other/Unspecified
	CE	White - Cypriot (part not stated)
	CF	White - Greek
	CG	White - Greek Cypriot
	CJ	White - Turkish Cypriot
	CH	White - Turkish
	CK	White - Italian
	CL	White - Irish Traveller
	CM	White - Traveller
	CN	White - Gypsy/Romany
	CQ	White - All Republics of former USSR
	CR	White - Kosovan
	CS	White - Albanian
	CT	White - Bosnian

	CU	White - Croatian
	CV	White - Serbian
	CW	White - Other Republics of former Yugoslavia
	CX	White - Mixed White
	CY	White - Other European
	C	White - Any other background
Mixed	D	Mixed - White & Black Caribbean
	E	Mixed - White & Black African
	F	Mixed - White & Asian
	G	Mixed - Any other mixed background
	GA	Mixed - Black and Asian
	GB	Mixed - Black and Chinese
	GC	Mixed - Black and White
	GD	Mixed - Chinese and White
	GE	Mixed - Asian and Chinese
	GF	Mixed - Other/Unspecified
Asian or Asian British-Indian [Indian]	H	Asian or Asian British - Indian
	LB	Asian or Asian British - Punjabi
Asian or Asian British-Pakistani [Pakistani]	J	Asian or Asian British - Pakistani
	LC	Asian or Asian British - Kashmiri
Asian or Asian British-Bangladeshi [Bangladeshi]	K	Asian or Asian British - Bangladeshi
Asian or Asian British-	L	Asian or Asian British - Any other

Other/Mixed/Unspecified		background
	LA	Asian or Asian British - Mixed Asian
	LD	Asian or Asian British - East African Asian
	LF	Asian or Asian British - Tamil
	LG	Asian or Asian British - Sinhalese
	LE	Asian or Asian British - Sri Lanka
	LH	Asian or Asian British - British
	LJ	Asian or Asian British - Caribbean Asian
	LK	Asian or Asian British - Other/Unspecified
Black or Black British-Caribbean [Caribbean]	M	Black or Black British - Caribbean
Black or Black British-African [African]	N	Black or Black British - African
Black or Black British-Nigerian [Nigerian]	PC	Black or Black British - Nigerian
Black or Black British-Somali [Somali]	PA	Black or Black British - Somali
Black or Black British- Other/Mixed/Unspecified	PB	Black or Black British - Mixed
	P	Black or Black British - Any other background
	PD	Black or Black British - British
	PE	Black or Black British - Other/Unspecified
Other Ethnic Groups- Chinese/Vietnamese [Chinese/Vietnamese]	R	Other Ethnic Groups - Chinese
	SA	Other Ethnic Groups - Vietnamese
Other Ethnic Groups-Other [Other]	S	Other Ethnic Groups - Any Other Group

	SB	Other Ethnic Groups - Japanese
	SC	Other Ethnic Groups - Filipino
	SD	Other Ethnic Groups - Malaysian
	SE	Any Other Group
	SK	Other Ethnic Groups - Arab
	SL	Other Ethnic Groups - North African
	SM	Other Ethnic Groups - Other Middle East
	SN	Other Ethnic Groups - Israeli
	SO	Other Ethnic Groups - Iranian
	SP	Other Ethnic Groups - Kurdish
	SQ	Other Ethnic Groups - Moroccan
	SR	Other Ethnic Groups - Latin American
	SS	Other Ethnic Groups - South/Central American
	ST	Other Ethnic Groups - Maur/SEyc/Mald/StHelen
	SG	Other Ethnic Groups - Hindu
	SJ	Other Ethnic Groups - Sikh
	SF	Other Ethnic Groups - Buddhist
	SI	Other Ethnic Groups - Muslim
Not known	Z	Not Stated
	ZZ	Not Known
	ZZZ	Information not yet obtained
	ZZZ	Refused
	Z	

Appendix 3a:**Multivariable logistic regression model results for vaccination coverage at first birthday**

Factor	Sub-group	OR (95% CI)
Ethnicity	White-British	1.00 (baseline)
	White-Polish	0.39 (0.33-0.47)
	White-Irish	0.57 (0.46-0.7)
	White-Other/mixed/unspecified	0.66 (0.62-0.71)
	Mixed	0.8 (0.74-0.87)
	Asian or Asian British-Indian	0.94 (0.87-1.03)
	Asian or Asian British-Pakistani	0.78 (0.71-0.84)
	Asian or Asian British-Bangladeshi	1.05 (0.92-1.2)
	Asian or Asian British-Other/mixed/unspecified	0.91 (0.83-1)
	Black or Black British - Caribbean	0.67 (0.59-0.75)
	Black or Black British-African	0.82 (0.76-0.88)
	Black or Black British-Nigerian	0.3 (0.2-0.45)
	Black or Black British-Somali	0.46 (0.37-0.57)
	Black or Black British - Other/mixed/unspecified	0.63 (0.54-0.73)
	Other Ethnic Groups-Chinese/Vietnamese	0.92 (0.72-1.16)
	Other Ethnic Groups-Other	0.55 (0.51-0.6)
	Not known	0.35 (0.33-0.36)
Gender	Male	1.00 (baseline)
	Female	1.03 (1-1.06)
	Unknown	0.06 (0.03-0.14)
Deprivation	Quintile 1	1.00 (baseline)
	Quintile 2	0.86 (0.82-0.9)
	Quintile 3	0.81 (0.77-0.85)
	Quintile 4	0.8 (0.76-0.84)
	Quintile 5	0.8 (0.76-0.84)
PCT	1	1.00 (baseline)
	2	1.13 (1.05-1.21)
	3	0.9 (0.85-0.97)
	4	1.37 (1.29-1.45)
	5	1.58 (1.47-1.7)
	6	1.38 (1.3-1.47)
	7	1.16 (1.09-1.23)
	8	0.57 (0.54-0.61)
	9	1.08 (1.02-1.14)
Year of birth	2005-2006	1.00 (baseline)
	2006-2007	1.08 (1.03-1.13)
	2007-2008	1.11 (1.06-1.16)
	2008-2009	1.4 (1.34-1.47)
	2009-2010	1.43 (1.37-1.5)

Number of observations = 181,719 (2% of records did not have a deprivation score assigned)

Overall model significance: LR chi2(34) = 5770, p<0.001

Log likelihood = -66743 Pseudo R2 = 0.041

Hosmer-Lemeshow fit test with 10 groups: chi2(8) = 91.0, p<0.001

Appendix 3b:**Multivariable logistic regression model results for vaccination coverage at second birthday**

Factor	Sub-group	OR (95% CI)
Ethnicity	White-British	1.00 (baseline)
	White-Polish	0.25 (0.21-0.3)
	White-Irish	0.58 (0.46-0.73)
	White-Other/mixed/unspecified	0.56 (0.52-0.59)
	Mixed	0.79 (0.73-0.87)
	Asian or Asian British-Indian	0.79 (0.72-0.87)
	Asian or Asian British-Pakistani	0.75 (0.68-0.82)
	Asian or Asian British-Bangladeshi	1.18 (1.01-1.39)
	Asian or Asian British-Other/mixed/unspecified	0.79 (0.71-0.87)
	Black or Black British - Caribbean	0.74 (0.64-0.85)
	Black or Black British-African	0.75 (0.69-0.81)
	Black or Black British-Nigerian	0.24 (0.15-0.36)
	Black or Black British-Somali	0.42 (0.33-0.52)
	Black or Black British - Other/mixed/unspecified	0.66 (0.56-0.77)
	Other Ethnic Groups-Chinese/Vietnamese	0.71 (0.55-0.91)
	Other Ethnic Groups-Other	0.43 (0.39-0.46)
Gender	Not known	0.25 (0.24-0.26)
	Male	1.00 (baseline)
	Female	1.05 (1.02-1.08)
Deprivation	Unknown	0.03 (0.01-0.07)
	Quintile 1	1.00 (baseline)
	Quintile 2	0.86 (0.82-0.91)
	Quintile 3	0.82 (0.78-0.86)
	Quintile 4	0.82 (0.78-0.86)
PCT	Quintile 5	0.85 (0.81-0.89)
	1	1.00 (baseline)
	2	0.91 (0.85-0.98)
	3	0.85 (0.79-0.91)
	4	1.35 (1.27-1.44)
	5	1.75 (1.62-1.89)
	6	1.33 (1.24-1.41)
	7	1.05 (0.99-1.12)
	8	0.46 (0.44-0.49)
9	1.15 (1.08-1.23)	
Year of birth	2005-2006	1.00 (baseline)
	2004-2005	0.69 (0.66-0.72)
	2006-2007	1.08 (1.03-1.14)
	2007-2008	1.13 (1.07-1.18)
	2008-2009	1.49 (1.42-1.56)

Number of observations = 176,739 (2% of records did not have a deprivation score assigned)

Overall model significance: LR chi2(34) = 8953, p<0.001

Log likelihood = -61931 Pseudo R2 = 0.0674

Hosmer-Lemeshow fit test with 10 groups: chi2(8) = 105.3, p<0.001

Appendix 3c:**Multivariable logistic regression model results for vaccination coverage at fifth birthday**

Factor	Sub-group	OR (95% CI)
Ethnicity	White-British	1.00 (baseline)
	White-Polish	0.34 (0.28-0.41)
	White-Irish	0.67 (0.58-0.78)
	White-Other/mixed/unspecified	0.77 (0.73-0.8)
	Mixed	0.81 (0.76-0.87)
	Asian or Asian British-Indian	1.13 (1.06-1.21)
	Asian or Asian British-Pakistani	0.94 (0.89-1)
	Asian or Asian British-Bangladeshi	1.11 (1.01-1.22)
	Asian or Asian British-Other/mixed/unspecified	0.89 (0.83-0.95)
	Black or Black British - Caribbean	0.8 (0.73-0.87)
	Black or Black British-African	0.74 (0.7-0.78)
	Black or Black British-Nigerian	0.66 (0.44-1)
	Black or Black British-Somali	0.56 (0.49-0.64)
	Black or Black British - Other/mixed/unspecified	0.76 (0.69-0.84)
	Other Ethnic Groups-Chinese/Vietnamese	0.74 (0.62-0.89)
	Other Ethnic Groups-Other	0.65 (0.61-0.69)
	Not known	0.4 (0.38-0.41)
Gender	Male	1.00 (baseline)
	Female	1.03 (1.01-1.05)
	Unknown	0.14 (0.08-0.24)
Deprivation	Quintile 1	1.00 (baseline)
	Quintile 2	0.9 (0.87-0.93)
	Quintile 3	0.87 (0.84-0.9)
	Quintile 4	0.86 (0.83-0.89)
	Quintile 5	0.89 (0.86-0.93)
PCT	1	1.00 (baseline)
	2	1.15 (1.09-1.21)
	3	0.59 (0.56-0.62)
	4	1.14 (1.09-1.19)
	5	1.62 (1.54-1.71)
	6	1.16 (1.11-1.22)
	7	1.14 (1.09-1.19)
	8	0.78 (0.75-0.82)
	9	1.01 (0.96-1.06)
Year of birth	2005-2006	1.00 (baseline)
	2001-2002	0.53 (0.51-0.54)
	2002-2003	0.55 (0.54-0.57)
	2003-2004	0.61 (0.59-0.63)
	2004-2005	0.78 (0.76-0.81)

Number of observations = 161,356 (2% of records did not have a deprivation score assigned)

Overall model significance: LR chi2(34) = 12216, p<0.001

Log likelihood = -102070 Pseudo R2 = 0.0565

Hosmer-Lemeshow fit test with 10 groups: chi2(8) = 151.6, p<0.001