Prevalence of diphtheria and tetanus antibodies among adults in Singapore: a national serological study to identify most susceptible population groups

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ABSTRACT

Background In view of waning antitoxin titres over time after the last vaccine dose against diphtheria and tetanus, we determined the immunity levels in adults to identify most susceptible groups for protection in Singapore.

Methods Our study involved residual sera from 3293 adults aged 18–79 who had participated in a national health survey in 2010. IgG antibody levels were determined using commercial enzyme-linked immunosorbent assay.

Results Overall, 92.0% (95% confidence interval [CI]: 91.1–92.9%) had at least basic protection against diphtheria (antibody levels 0.01 IU/ml), while 71.4% (95% CI: 69.8–72.9%) had at least short-term protection against tetanus (antibody levels 0.1 IU/ml). The seroprevalence declined significantly with age for both diseases; the drop was most marked in the 50- to 59-year age group for diphtheria and 60- to 69-year age group for tetanus. There was a significant difference in seroprevalence by residency for diphtheria (92.8% among Singapore citizens versus 87.1% among permanent residents; P = 0.001). The seroprevalence for tetanus was significantly higher among males (83.2%) than females (62.4%) (P < 0.0005).

Conclusions It may be of value to consider additional vaccination efforts to protect older adults at higher risk for exposure against diphtheria and tetanus, particularly those travelling to areas where diphtheria is endemic or epidemic.

Keywords booster, immunity, seroprevalence, vaccination

Background

Diphtheria is a bacterial infection caused by toxigenic Corynebacterium diphtheriae. It is often spread from person to person via the respiratory route and occasionally by direct contact with infected skin lesions.1 With the successful introduction of mass childhood immunization programme against diphtheria, there has been a drastic reduction in its morbidity and mortality in countries with high vaccination coverage.1 A total of 4490 cases of diphtheria worldwide were reported to the World Health Organization (WHO) in 2013, compared with 97 164 cases in 1980.2

Tetanus is an acute infection that usually results from contamination of a wound with soil containing spores of the bacterium Clostridium tetani, which produces an exotoxin that affects the brain and nervous system.3 There has been a marked decline in mortality from tetanus with the introduction of tetanus toxoid in routine childhood immunization. The worldwide number of tetanus cases was higher than that of diphtheria, with a total of 10 392 cases reported in 2013, compared with 113 936 cases in 1980.4

In Singapore, the disease burden of diphtheria was high in the early 1900s, with morbidity rate of 11.3–50.8 per 100 000 population and mortality rate of 0.9–4.4 per 100 000
population during the period 1954–64. Neonatal tetanus was also a serious disease with high mortality rate. Vaccination against diphtheria commenced in 1938 and was made mandatory by law in April 1962 for infants and children below 7 years of age. Vaccination against pertussis and tetanus started later in 1959. The current national childhood immunization schedule comprises a total of five doses to protect against diphtheria, pertussis and tetanus (DPT): three doses of diphtheria, tetanus and acellular pertussis (DTaP) vaccine are administered to infants at 3, 4 and 5 months, followed by the first booster of DTaP vaccine at age 18 months, and the second booster of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine at age 10–11 years. Since 1988, young men aged 17–21 entering the national service are given one booster dose of tetanus toxoid. Between 2003 and 2012, the coverage of the primary vaccination against DPT in the National Childhood Immunisation Programme (NCIP) ranged from 95 to 97% in children at 2 years of age, while the coverage under the first booster dose ranged from 84 to 91%. With the successful implementation of the NCIP, the last indigenous case of diphtheria involved a 41-year-old local resident with multiple co-morbidities was reported in 1992. Neonatal tetanus has virtually disappeared with the last case reported in 1994. However, sporadic cases of tetanus among unvaccinated adults continued to occur.

A serological survey to measure the population immunity against diphtheria and tetanus was conducted between 1982 and 1984. Of 425 healthy persons between 6 months and over 40 years of age, 94.6% had protective diphtheria-neutralizing antitoxin (titre >0.01 IU/ml). However, 14.3% of adults above 40 years of age had no protective immunity against diphtheria toxin. In the case of tetanus, 19.3% had no immunity (antitoxin titre <1:10), and among adults above 30 years of age, 38–47.9% were not immune.

In another survey conducted among 600 apparently healthy children and adults between 6 months and over 40 years of age in 1993, 95.5% possessed antitoxin immunity against diphtheria (≥0.01 IU/ml). Overall, 90.7% of the population possessed immunity against tetanus (particle agglutination antitoxin titre >1:16), which was a significant increase from 80.7% in 1982–84. The antitoxin prevalence was 99.6% among children below 5 years of age, while a progressive decline in immunity with age was observed from 98.6% in children aged 5–9 to 50.0% in those aged 40 and older.

The high level of immunity against diphtheria among the childhood population was confirmed in a national pediatric seroprevalence survey (NPSS) conducted on 1200 children aged 1–17 in 2008–10. The overall immunity level against diphtheria (≥0.1 IU/ml using commercial enzyme-linked immunosorbent assay [ELISA] kits, IBL-America, Minneapolis, MN, USA) was 99.4% (95% confidence interval [CI]: 98.8–99.7%).

There is potential for outbreaks of diphtheria in susceptible population groups, especially in the era of accelerating scale of global travel when those with inadequate protection travel to endemic or epidemic regions. We undertook a national serological study in 2010 to estimate the immunity levels of the Singapore adult population to diphtheria and tetanus, and discuss the implications of the findings on vaccination policy. We sought to identify most susceptible population groups who may require additional vaccination efforts to improve their immunity.

**Materials and methods**

Residual sera collected from the National Health Survey (NHS) in 2010 were used for our serological study. The NHS 2010 was a population-based, cross-sectional survey conducted by the Ministry of Health (MOH) to determine the prevalence of chronic diseases and lifestyle-related risk factors among Singapore residents aged 18–79. The survey population was selected by a combination of disproportionate stratified sampling and systematic sampling. Fieldwork for the NHS 2010 was conducted at five government primary care clinics and one community club geographically well spread out across Singapore over a 3-month period from 17 March to 13 June 2010. Ethical approval was given by the Ethics Committee of the Health Promotion Board, Singapore (Reference Number 006-2010). Only sera from NHS participants who had given informed consent to allow use of their residual sera for further research were included in this serological study. Personal identifiers of the NHS participants were permanently removed, so as to ensure strict anonymity of these participants.

A total of 4337 eligible Singapore residents aged 18–79 participated in the NHS 2010, giving a response rate of 57.7%. The weighted respondent sample of 4337 participants of the NHS 2010 was similar to the Singapore resident population in socio-demographic profile. Residual sera from 3293 (75.9%) of these respondents were included in our serological study, and their socio-demographic profile and that of the Singapore resident population aged 18–79 were also found to be similar.

IgG antibody levels against diphtheria and tetanus were determined in the study sample of 3293 sera using commercial test kits: anti-diphtheria toxoid enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Germany) and anti-tetanus toxoid ELISA (Euroimmun, Germany), respectively. For diphtheria, antitoxin levels <0.01 IU/ml denote susceptibility, levels of 0.01–0.099 IU/ml indicate basic protection (i.e. giving basic immunity) and levels ≥0.1 IU/ml show full
protection. For tetanus, antitoxin levels \( \leq 0.1 \) IU/ml denote susceptibility, levels of \( 0.11-1.0 \) IU/ml indicate short-term protection and a booster dose is recommended after 3 years, and levels \( >1.0 \) IU/ml show long-term protection. Similar definitions for diphtheria and tetanus were adopted in a study to estimate the population immunity in the UK.

The \( \chi^2 \) test or Fisher’s exact test, where appropriate, was used to test for group differences. The Mantel–Haenszel \( \chi^2 \) test for trend was used to evaluate the difference in seroprevalence across the age groups. Statistical analysis was performed using the statistical software package, SPSS Statistics software, version 19.0 (IBM, USA). Statistical significance was taken at \( P < 0.05 \).

**Results**

**Diphtheria**

In 2010, among the adult population aged 18–79, 8.0% (95% CI: 7.1–8.9%) were susceptible to diphtheria (<0.01 IU/ml), 32.5% (95% CI: 30.9–34.1%) had basic protective immunity (0.01–0.099 IU/ml) and 59.5% (95% CI: 57.8–61.2%) were fully immune (≥0.1 IU/ml).

The proportion with at least basic protection against diphtheria (≥0.01 IU/ml) was 92.0% (95% CI: 91.1–92.9%), and it decreased significantly from 99.1% in subjects aged 18–29 to 85.5% in those aged 50–59 (test for trend, \( P < 0.0005 \)) (Table 1). The seroprevalence ranged from 87.7 to 92.7% among the elderly aged 60–79. The proportion with full protection (≥0.1 IU/ml) declined significantly from 87.6% in subjects aged 18–29 to 57.0% in those aged 40–49 years and remained stable (39.9–47.4%) in the older age groups (Fig. 1).

The seroprevalence (≥0.01 IU/ml) in males (93.7%) was not significantly different from that of females (90.8%) (\( P = 0.39 \)). Its seroprevalence among Singapore citizens (92.8%) was significantly higher than that of permanent residents (87.1%) (\( P = 0.001 \)). Among the three main ethnic groups, Chinese had a significantly lower seroprevalence (91.0%) compared with that of Indians (96.2%) (\( P = 0.03 \)) and Malays (94.5%) (\( P = 0.01 \)). The difference in seroprevalence between Malays and Indians was not statistically significant (\( P = 0.38 \)).

**Tetanus**

About 28.6% (95% CI: 27.1–30.2%) were susceptible (≤0.1 IU/ml), 32.6% (95% CI: 30.9–34.1%) had short-term protective immunity (0.11–1.0 IU/ml) and 38.8% (95% CI: 37.2–40.5%) had long-term protective immunity (≥1.0 IU/ml).

The proportion with at least short-term protection against tetanus (≥0.1 IU/ml) was 71.4% (95% CI: 69.8–72.9%), and it declined significantly from 95.6% in subjects aged 18–29 to 61.5% in those aged 50–59 and subsequently halved to 33.4% in the 60- to 69-year age group (test for trend, \( P < 0.0005 \)) (Table 1). In particular, the proportion with long-term protection against tetanus (≥1.0 IU/ml) declined significantly from 64.7% in subjects aged 18–29 to 15.5% in those aged 60–69, followed by a further slight decline to 12.4% in the 70- to 79-year age group (Fig. 2).

The proportion with at least short-term protection against tetanus (≥0.1 IU/ml) among males (83.2%) was significantly higher than that of females (62.4%) (\( P < 0.0005 \)). About 54.3% of males had long-term protection against tetanus (≥1.0 IU/ml), compared with 27.1% in females (\( P < 0.0005 \)). Further analysis of this category by age group revealed that the difference was most marked in the 30- to 39-year age group; 71.3% in males compared with 31.8% in females (\( P < 0.0005 \)).

**Table 1** Prevalence (%) of diphtheria and tetanus antibodies by demographic characteristics among 3293 adults aged 18–79 in Singapore, 2010

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>No. of subjects</th>
<th>% With least basic protection against diphtheria (≥0.01 IU/ml)</th>
<th>% With at least short-term protection against tetanus (≥0.1 IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>3293</td>
<td>92.0</td>
<td>71.4</td>
</tr>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–29</td>
<td>664</td>
<td>99.1</td>
<td>95.6</td>
</tr>
<tr>
<td>30–39</td>
<td>682</td>
<td>94.7</td>
<td>82.0</td>
</tr>
<tr>
<td>40–49</td>
<td>730</td>
<td>91.0</td>
<td>77.0</td>
</tr>
<tr>
<td>50–59</td>
<td>684</td>
<td>85.5</td>
<td>61.5</td>
</tr>
<tr>
<td>60–69</td>
<td>341</td>
<td>87.7</td>
<td>33.4</td>
</tr>
<tr>
<td>70–79</td>
<td>192</td>
<td>92.7</td>
<td>31.1</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1420</td>
<td>93.7</td>
<td>83.2</td>
</tr>
<tr>
<td>Female</td>
<td>1873</td>
<td>90.8</td>
<td>62.4</td>
</tr>
<tr>
<td>Residencya</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Singapore citizens</td>
<td>2830</td>
<td>92.8</td>
<td>71.8</td>
</tr>
<tr>
<td>Permanent residents</td>
<td>457</td>
<td>87.1</td>
<td>68.9</td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Chinese</td>
<td>2432</td>
<td>91.0</td>
<td>70.8</td>
</tr>
<tr>
<td>Malay</td>
<td>420</td>
<td>94.5</td>
<td>70.0</td>
</tr>
<tr>
<td>Indian</td>
<td>316</td>
<td>96.2</td>
<td>82.6</td>
</tr>
<tr>
<td>Othersb</td>
<td>125</td>
<td>94.4</td>
<td>58.4</td>
</tr>
</tbody>
</table>

*Numbers do not add up to 3293 due to non-response, such as refusals.

Ethnic group of ‘Others’ comprises all persons other than Chinese, Malays and Indians. They include Eurasians, Europeans, Arabs, Japanese, etc.
The seroprevalence among Singapore citizens (71.8%) with at least short-term protection (≥0.1 IU/ml) was not significantly different from that of permanent residents (68.9%) (P = 0.20). The seroprevalence among Indians (82.6%) was significantly higher compared with that of Chinese and Malay (P < 0.0005), while the difference between Chinese (70.8%) and Malays (70.0%) was not statistically significant (P = 0.73).

**Discussion**

**Main findings of this study**

**Diphtheria**

Our study showed that overall, 92.0% of the Singapore adults aged 18–79 had at least basic protection against diphtheria (≥0.01 IU/ml) and 59.5% had full protection (≥0.1 IU/ml). When we combined our results with those of the NPSS
In Singapore, the seroprevalence of diphtheria declined significantly with age. The proportion susceptible increased from 0.9% in the 18–29 year olds to 5.3% in the 30–39 year olds, 9.0% in the 40–49 year olds and 14.5% in the 50–59 year olds. This has also been observed in seroprevalence studies conducted in European countries where the level of antibodies to diphtheria toxin decreased significantly in adults above 40 years old.20–26 The most drastic increase in the proportion of seronegatives among adults above 40 years of age (<0.1 IU/ml) was observed in a study in Poland during the period 2010–12; the seronegative rate increased from 23.4% in the 31- to 40-year age group to 63.8% in the 41- to 50-year age group.27 Sharp increases in susceptibility in those above 40 years of age were also seen in Ireland20 and Spain.23

In our study, a significantly higher proportion of permanent residents were found to be susceptible to diphtheria compared with Singapore citizens (12.9 versus 7.2%; P = 0.001). This could be partly attributed to increases in immigration from diphtheria endemic countries.

Despite the overall high level of herd immunity of the Singapore population, it is important to maintain a high degree of vigilance against diphtheria. Adults constitute a high proportion of the diphtheria cases in countries such as the UK28 and the USA.29,30 Majority of these cases have acquired their infection overseas, which indicates the need for travellers, in particular older adults, who are going to endemic or epidemic regions to be fully immunized so as to reduce the risk of reintroducing diphtheria upon their return. There is an increased risk of continuous introduction of toxigenic strains of toxigenic C. diphtheriae due to globalization of trade and travel. In addition, the organism can be presented as an asymptomatic infection in pharyngeal, aural, nasal and cutaneous lesions, which continues to pose a threat to susceptible children and adults.

### Tetanus

For tetanus, our study showed that 71.4% of Singapore adults aged 18–79 had at least short-term protection against tetanus (antibody levels >0.1 IU/ml) and 38.8% had long-term protective immunity (>1.0 IU/ml). The seroprevalence declined significantly with age, with the drop most marked in the 60- to 69-year age group. We also found a statistically significant difference by gender in the proportion with at least short-term protection against tetanus. The significantly higher proportion with at least short-term protection against tetanus among males than females (83.2 versus 62.4%; P < 0.0005) is likely to be due to the additional booster dose of tetanus toxoid administered to young men entering national service, as well as males receiving additional tetanus boosters during wound treatment in their lifetime compared with females as they are more prone to accidents due to their nature of work or type of activities.

### What is already known on this topic

The duration of protection after a person receives the primary series of DTaP vaccine is ~10 years.31 While some adults may have protection for life, most have antitoxin levels that only approach the minimal protective level by 10 years after the last dose due to waning antitoxin titres.1,3

A booster dose of tetanus toxoid and reduced diphtheria toxoid (Td) vaccine for adults is recommended every 10 years, with the first dose combined with pertussis using Tdap vaccine if it has not been given previously, in a number of developed countries including USA,32 Canada33 and Australia.34 In the UK, further tetanus booster doses are not recommended for those who have received five doses of tetanus-containing vaccine since they would have long-lasting protection, unless a high-risk injury has occurred.35

### What this study adds

Based on the results of this study, the drop in the proportion with least basic protection against diphtheria was most marked in the 50- to 59-year age group, while the most drastic decline in the proportion with at least short-term protection against tetanus was in the 60- to 69-year age group, nearly halved that of the 50- to 59-year age group. The most susceptible population groups with no protective antibody were those aged 50–59 for diphtheria (14.5%) and those aged 70–79 for tetanus (68.9%).

In conclusion, the findings of declining seroprevalence with age for diphtheria and tetanus in our study inform policymakers, public health practitioners and clinicians on the levels of immunity in adults against these two diseases. The current national childhood immunization schedule is deemed to offer adequate protection against diphtheria and tetanus with high vaccination coverage in children. This is also supported by surveillance data with no indigenous cases of diphtheria and few cases of tetanus reported in the past two decades. Adults aged 50 and older are found to be most at risk in this study. Given the waning immunity over time, it...
may be of value to consider additional vaccination efforts to protect older adults at higher risk for exposure against diphtheria and tetanus. Those travelling to areas where diphtheria is endemic or epidemic are advised to consult doctors regarding vaccinations required or recommended before embarking on their trips, so as to minimize their risk of acquiring infections overseas. Following the findings from our observational study, analytical studies would be required if there are new recommendations to the current vaccination programme.

**Limitations of this study**
As different methods and test kits were used to determine the anti-diphtheria and anti-tetanus antibody levels, the findings from various seroprevalence studies may not be comparable. Unlike diphtheria, there are no well-established criteria for interpreting tetanus antitoxin levels. Moreover, low tetanus antitoxin levels do not necessarily indicate susceptibility.

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**References**


