DIPHTHERIA OUTBREAK IN THAILAND, 2012; SEROPREVALENCE OF DIPHTHERIA ANTIBODIES AMONG THAI ADULTS AND ITS IMPLICATIONS FOR IMMUNIZATION PROGRAMS

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Abstract. An age distribution shift in diphtheria cases during a 2012 outbreak in northeastern of Thailand suggests adults are increasingly at risk for infection in Thailand. Data regarding immunity against diphtheria among the adult Thai population is limited. We review a 2012 diphtheria outbreak in Thailand and conducted a nationwide seroepidemiological survey to determine the prevalence of diphtheria antibodies among Thai adults in order to inform immunization programs. A total of 41 confirmed cases, 6 probable cases and 101 carriers of diphtheria were reported from northeastern and upper southern Thailand. The diphtheria outbreak in northeastern Thailand occurred among adults aged ≥15 years; sporadic cases occurred among children from upper southern Thailand. We conducted a seroepidemiological survey of 890 Thai adults from 4 age groups (20-29, 30-39, 40-49 and 50-59 years) in 7 different geographical areas of Thailand (Chiang Mai, Ratchaburi, Chon Buri, Nakhon Si Thammarat, Phitsanulok, Khon Kaen and Songkhla). Diphtheria toxin antibody levels were measured with a commercially available ELISA test. The seroprotection rate ranged from 83% to 99%, with the highest in eastern Thailand (Chon Buri, 99%) and the lowest in northern Thailand (Chiang Mai, 83%). Diphtheria antibodies declined with increasing age. We recommend one dose of diphtheria-tetanus toxoid (dT) vaccine once after 20 years of age in order to boost the antibody and revaccinations every 10 years to prevent future outbreaks.

Keywords: diphtheria, outbreak, seroprevalence, immunization, adult

INTRODUCTION

Diphtheria is an infection characterized by sore throat, low-grade fever and an adherent membrane on the tonsils, pharynx, and/or nasal cavity. Diphtheria
toxin produced by *C. diphtheriae* can cause myocarditis and polyneuritis (Atkinson *et al.*, 2012). Diphtheria is spread by direct contact or by inhalation of aerosolized secretions from infected individuals. Before the wide-spread use of the diphtheria-tetanus toxoid and whole cell pertussis (DTP) vaccines, the case-fatality rates from respiratory tract diphtheria ranged from 2% to 20%, with an average of 10% for patients who received attentive medical care (Brenzel *et al.*, 2006).

While once common, diphtheria is now rare because of the wide-spread use of the DTP vaccine. The percentage of infants receiving 3 doses of the DTP vaccine increased from 20% in 1980 to 78% in 2005 in developing countries, with substantial variation by district and country (Scheifele and Ochnio, 2009). Thailand began a routine infant immunization program with 2 doses of the DTP vaccine for all infants in 1977 (Tharmaphornplas *et al.*, 2001), and with 3 doses beginning in 1982. After wide-spread use of the vaccine in Thailand, rates of diphtheria among Thais declined substantially from 1,600-2,400 cases per year during 1971-1977 (Tiparat and Kooharat, 2002). By 2000, DTP vaccine coverage in Thailand was estimated to be 97% among infants below one year of age and since then has continually increased (WHO, 2013). However, diphtheria remains a problem in spite of efforts to achieve vaccination coverage, especially in southern Thailand, where vaccination coverage estimates for infants aged ≤12 months were lower compared to other regions (69% in Pattani Province, 74% in Yala Province and 85% in Narathiwat Province) (Tonghong, 2010). Sporadic cases of diphtheria have also been reported in many provinces in recent years. In 1994, a total of 18 cases of diphtheria were identified in an outbreak in Saraburi Province (CDC, 1996). In 1996, a diphtheria outbreak was reported in Buri Ram Province (Pantuksit *et al.*, 2008) with a total number of 31 cases. The annual rate of diphtheria infection in Thailand has gradually increased, amounting to 77 cases per year in 2010 (Tonghong, 2010), the majority of which (71 out of 77) were from southern Thailand. The ages of infected individuals ranged from 7 years to 50 years. The majority were children aged ≤15 years; however, 10% (8 of 77 cases) were among adults. This suggests some age groups are more vulnerable to infection, most likely due to lower vaccine coverage or waning antibody levels in adulthood.

The duration of protective immunity after primary immunization has become a subject of interest. Diphtheria antibody levels among adults aged ≥20 years show a significant age-related decrease in antibody levels with increasing age, reaching a minimum in the 30-39 year old age group, with fewer than half of subjects having protective antibody levels (Pachón *et al.*, 2002; Kurugöl *et al.*, 2011). The concept of herd immunity (National Network for Immunization Information, 2014) states that outbreaks of diphtheria among adults are possible when less than 85% of the population has protective antibody levels. In Thailand, a seroepidemiological survey of diphtheria immunity was conducted among 364 people aged 2-86 years in Nakhon Ratchasima Province (Chasangbong *et al.*, 2004). Although the antibody titers of all 364 people exceeded protective levels (>0.1 IU/ml), the study found that immunity to diphtheria declined with age. Another seroepidemiological survey of diphtheria immunity in Khon Kaen Province, Thailand in 2011 found adults aged 20-50 years were at the highest risk for diphtheria infection (Bansiddhi *et al.*, 2012).
Declining seroprotection rate among adults suggests the potential for future outbreaks of diphtheria. Several studies recommend a booster dose of dT for adults to maintain diphtheria immunity (Ilina, 2000; Kurugöl et al, 2011; Bansiddhi et al, 2012). In Thailand, compliance with the recommendation to have a booster dose of dT among adults has been relatively low. Additional data regarding immunity against diphtheria among Thais of various ages can help guide dT booster vaccine programs among adults. We evaluate a 2012 diphtheria outbreak in Thailand and report a nationwide seroepidemiological study we conducted to determine the prevalence of diphtheria susceptibility among Thai adults.

MATERIALS AND METHODS

Data regarding diphtheria outbreaks in Thailand were retrieved from the Department of Disease Control, Ministry of Public Health. The records of diphtheria cases were reviewed to determine disease patterns and determine the epidemiology. The following case definitions were used by the Committee of the Department of Disease Control: a confirmed case was defined as a case presenting with fever, throat exudate and throat swab culture positive for *Corynebacterium diphtheriae*; a probable case was defined as a person presenting with clinical symptoms of diphtheria or a close contact of a confirmed case; a carrier was defined as someone without clinical symptoms who had a throat culture positive for *Corynebacterium diphtheriae*.

The 2012 outbreak of diphtheria in Thailand

A total of 38 confirmed cases, 5 probable cases and 93 carriers of diphtheria were reported from northeastern Thailand between June 2012 and January 2013. There were also sporadic confirmed cases of diphtheria in the upper south of Thailand during the same period of time as shown in Table 1.

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Confirmed cases</th>
<th>Probable cases</th>
<th>Carriers</th>
<th>Median age (range) (in years)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Northeastern region</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Loei</td>
<td>26</td>
<td>1</td>
<td>68</td>
<td>26 (2-72)</td>
</tr>
<tr>
<td>Phetchabun</td>
<td>5</td>
<td>0</td>
<td>13</td>
<td>33 (20-37)</td>
</tr>
<tr>
<td>Nong Bua Lam Phu</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>12.5 (9-48)</td>
</tr>
<tr>
<td>Nakhon Ratchasima</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>33.5 (33-43)</td>
</tr>
<tr>
<td>Udon Thani</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>Maha Sarakham</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td><strong>Upper southern region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surat Thani</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>9 (8-10)</td>
</tr>
<tr>
<td>Nakhon Si Thammarat</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>1.5 (1-2)</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>6</td>
<td>101</td>
<td>26 (2-72)</td>
</tr>
</tbody>
</table>
A cross sectional study was conducted on 890 randomly selected Thai adults. These were divided into two groups: the first group was comprised of healthy Thai blood donors aged 20-59 years randomly chosen from provincial blood blank centers in each region (299 males and 300 females) in February 2013. The second group was comprised of 291 from individuals aged 20-59 years from Khon Kaen Province, Thailand in June 2011. This second group consisted of individuals presenting for a routine health check-up and acutely ill patients seeking medical care at Chum Phae Hospital, Khon Kaen Province, Thailand. These data were extracted from a previous seroepidemiological survey of diphtheria immunity in Khon Kaen Province (Bansiddhi et al, 2012) using the same ELISA methods. The sample size was chosen based on an assumption of a 90% prevalence of diphtheria antibodies and a significance level of \( p < 0.05 \). These 890 samples were divided into four age groups (20-29, 30-39, 40-49 and 50-59 years) residing in 7 different geographical areas of Thailand (Chiang Mai, Ratchaburi, Chon Buri, Nakhon Si Thammarat, Phitsanulok, Khon Kaen and Songkhla) (Fig 1). All the specimens in this study were kept at the Center of Excellence in Clinical Virology, Chulalongkorn University and Hospital. All personal identifiers, except age and gender, were removed from the data set and specimens. The research protocol was approved by an institutional review board (IRB) at the Faculty of Medicine, Chulalongkorn University and Hospital. The IRB waived the need for consent because the samples were anonymous.

**Laboratory methods**

Diphtheria antibody levels were measured with a solid-phase IgG-specific ELISA (IBL International, Hamburg, Germany) according to a published protocol (Bansiddhi et al, 2012). The data were then classified according to the levels of antitoxin: 0.01-0.099 IU/ml susceptible; 0.1-0.99 IU/ml showed a satisfactory, protective level; 1-1.499 IU/ml showed a high level of immunity; 1.5-2 IU/ml showed a
The antibody levels and personal data were entered into SPSS version 15 (SPSS, IBM, Armonk, NY) by age group and gender.

RESULTS

The 2012 diphtheria outbreak in Thailand

The 2012 diphtheria outbreak in Thailand was first identified in Dan Sai District, Loei Province in June 2012. The first case was a 40-year-old immunocompromised alcoholic male, who subsequently died of acute myocarditis. The second case was a 25-year-old male from a nearby village; subsequent cases were reported in a village close to where the second case lived and then spread to neighboring provinces (Fig 2). In October, diphtheria cases were also reported in upper southern Thailand (Surat Thani and Nakhon Si Thammarat). A total of 6 fatalities from acute myocarditis were reported.

The Ministry of Public Health (Rangseewong and Siriarayaporn, 2013) stated that between June 2012 and January 2013, a total of 38 confirmed cases, 5 probable cases, and 93 carriers of diphtheria were reported from northeastern Thailand (Fig 2). There were also sporadic confirmed cases reported from upper southern Thailand. The majority of confirmed cases in the northeastern outbreak were reported among adults. However, in upper southern Thailand, all the cases occurred among children aged <15 years (Table 1).

Seroprevalence of antibodies against diphtheria toxin

The highest GMT was found among subjects aged 50-59 years (GMT 0.49 IU/ml). Followed by those aged 20-29 and 40-49 years (0.43 IU/ml and 0.38 IU/ml, respectively). The lowest GMT was found among those aged 30-39 years (GMT 0.34 IU/ml). Very high levels of immunity and ≥ 2 IU/ml showed long-lasting immune protection.

Statistical analysis

Geometric mean titers (GMTs) were calculated using the log transformation of serological titers and from taking the antilog mean of the transformed values. Seroprotection rates (SPRs) for each age group were defined as the fraction of the population with a diphtheria antibody level ≥ 0.1 IU/ml.
IU/ml). The overall GMT was 0.40 IU/ml.

The overall seroprotection rate among Thai adults was 90.9%; however, middle-aged adults aged 30-39 years had the lowest seroprotection rate (87.7%) (Table 2). The seroprotection rates in different regions ranged from 83% to 99%, with the highest rate being in Chon Buri (99%) and lowest in Chiang Mai (83%). The group with the lowest seroprotection in 4 provinces was the 30-39 year age group (76% in Phitsanulok, 88% in Nakhon Si Thammarat, 76% in Songkhla, and 90% in Khon Kaen (Bansidhik et al, 2012).

Serological profiles varied by geographical areas but some common themes were noted. In Phitsanulok, Ratchaburi, Chon Buri and Songkhla Provinces, the lowest GMT values were seen in the 30-39 year old age group (0.24 IU/ml, 0.28 IU/ml, 0.37 IU/ml and 0.21 IU/ml, respectively). In Nakhon Si Thammarat the lowest GMT was found in the 20-29 year old age group (0.56 IU/ml); in Khon Kaen, the lowest GMT was found in the 40-49 year old age group (0.36 IU/ml) and in Chiang Mai the lowest GMT was found in the 50-59 year old age group (0.19 IU/ml) (Fig 3). The majority of the studied subjects (632 out of 890, 71%) had diphtheria immunity levels of 0.1-0.99 IU/ml; only 6.6% (59 out of 890) had a long-lasting immunity level (> 2 IU/ml).

**DISCUSSION**

We examined a 2012 diphtheria outbreak and the prevalence of diphtheria immunity in a Thai population. During the 2012 outbreak of diphtheria in the northeast of Thailand, most of the affected individuals were between 15-44 years. This was most likely due to waning vaccine-induced immunity and decreased opportunity to acquire natural immunity.
Fig 3—Percentages of seropositivity and diphtheria antitoxin levels by age group for various provinces during February 2013 in six regions and in June 2011 in Khon Kaen Province, Thailand (Bansiddhi et al, 2012).
The sporadic diphtheria cases in southern Thailand were mostly among those aged \( \leq 15 \) years. Tonghong (2010) found vaccination rates among children aged <12 months in southern Thailand were lower than other areas of Thailand. This finding underscores the need for continued vaccination efforts, especially among economically disadvantaged populations, such as tribal children and children from families living in distant rural areas in southern Thailand.

Since diphtheria among humans is caused by a toxin, carriers may transmit the pathogen to those not yet immunized. Maintaining herd immunity to prevent outbreaks requires complete childhood vaccination and booster dT vaccination in adults (Scheifele and Ochnio, 2009).

In the second part of this study we conducted a nationwide seroepidemiological survey of immunity to diphtheria among Thai adults. Among the studied population, 90.9% had protective immunity to diphtheria; however, the results of our serological study suggested a higher percentage of diphtheria susceptibility among adults aged 30-39 years. The lower percentage of those seroprotected in this age group was probably due to: 1) suboptimal seroconversion among those who received two doses of DTP in infancy during 1977-1982, 2) lack of booster doses during adulthood, and 3) reduction in exposure to circulating \( C. \) diphtheriae. The second lowest seroprotection rate was found among adults aged 40-49 years (91%). This group did not receive the DTP vaccine during infancy.

The seroprotection rates in the Thai population was in our study are higher than in studies from some other countries: a study from Germany in 1993 found 76% of people had protective antibody levels (Klouche et al, 1995), a study from Turkey in 2003 found 30.6% of subjects aged 40-49 years had protective antibody levels (Alp Cavus et al, 2007). No diphtheria outbreaks have been reported in Germany or Turkey as opposed to Thailand; this may be because Thailand’s neighboring countries such as Lao PDR, Cambodia and Myanmar, have a higher incidence of \( C. \) diphtheria, which can spread cross border to Thailand (WHO, 2014).

Several studies have found protective antibody levels decrease with increasing age (Souliou et al, 1997; Di Giovine et al, 2013), except for some elderly populations over 60 years old, where seroprotection rates for diphtheria were higher than in younger age groups. In our study, the percentages of individuals with protective immunity varied by geographical area.

Based on herd immunity (Fine, 1993) \((1-1/R_0)\) with a basic reproductive number \((R_0)\) for diphtheria of 7, diphtheria immunity in at least 85% of the population is required to prevent a diphtheria outbreak. The seroprotection rates in some age groups in our study were lower than this threshold, especially among those aged 30-59 years. Antibodies levels were lower suggesting risk of future outbreaks.

The 2012 diphtheria outbreak in Thailand raises questions about vaccination policy, including the timing a number of booster dT doses required to protect adults. Since 2008, the national vaccine policy for Thailand recommends a DTP-HB (Diphtheria-Tetanus Toxoids–whole cell Pertussis and Hepatitis B) vaccine for infants aged 2, 4 and 6 months, a DTP for children aged 18 months and 4 years, and a booster dT vaccine at the age of 12 years before leaving primary school. This explains the high GMT values found among those aged 20-29 years. However, the
immunity declines over time. The World
Health Organization (WHO) recom-
mends people living in low endemic and
non-endemic areas need a booster every
10 years to maintain life-long protection
(Scheifele and Ochnio, 2009). A study from
the Russian Federation (Brennan et al,
2000) reported adults with an unknown
vaccination history or who are likely to
be immunologically naive to toxigenic
strains of diphtheria should receive at
least 3 doses of dT for protection. In Tha-
land, the current guidelines recommend
a booster dose of dT for those who have
been vaccinated during childhood once
at age 20 years and then every 10 years.
This study further provides evidence for
the importance of adhering to the current
recommendations.

Inadequate immunity to diphtheria
is likely in the near future due to waning
immunity among adults. Adults should
receive dT booster vaccine to maintain
adequate seroprotection levels following
recommendations. For adults with an
unknown history of vaccination, 3 doses
of dT vaccine are recommended. The dT
vaccine may be given during clinic visits
for other reasons, such as for injuries or
during antenatal care in order to prevent
future outbreaks of diphtheria in Thailand.

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