

Diphtheria-neutralizing antibody levels in healthy adults from Rio de Janeiro, Brazil

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In Brazil, until 2004, the immunization policy against diphtheria involved childhood vaccination with no official routine booster dose administered after 15 years of age. This study assessed functional antibody levels against diphtheria among blood donors. A total of 140 blood samples were collected, and diphtheria antitoxin levels were evaluated by Vero cell neutralization test. The mean age of the population was 34 years old (range: 18-61 years); 37.8% females and 62.2% males. Overall, 30.7% (95% CI: 23.4-38.7) individuals presented neutralizing antitoxin antibody titers < 0.01 IU/ml; 42.1% (95% CI: 34.1-50.4) showed values between 0.01-0.09 IU/ml and, 27.1% (95% CI: 20.2-34.9) had ≥ 0.1 IU/ml. In the subgroup of individuals with history of diphtheria immunization during childhood (85%), a number of 28.5% showed unprotective levels of circulating neutralizing antibody (< 0.01 IU/ml). Despite the continuous progress of immunization programs directed to Brazilian population, currently healthy adults remain susceptible to diphtheria.

Key words: diphtheria - diphtheria antitoxin - Vero cells assay - Rio de Janeiro - Brazil

The vast territory and unfavorable economic conditions presented by most regions of our country make difficult the notification of diphtheria cases to Public Health authorities and remittance of suspect isolates for bacteriological confirmation and toxigenicity tests to reference laboratories. Local outbreaks have been reported indicating gaps or failure in vaccine coverage (Mattos-Guaraldi et al. 2003).

Western Europe epidemics illustrated the potential susceptibility of adults to diphtheria in the vaccine era. Although the World Health Organization recommends evaluation of vaccine-induced immunity among both children and adults, a very few serosurveys have been done outside of North American and Western Europe continents (Damasco et al. 2005). Nationwide seroepidemiological studies are necessary to elaborate effective strategies to maintain immunity against diphtheria in adults, including periodic booster doses and immunization of selected age groups (Galazka & Robertson 1996, Galazka 2000a,b).

In Brazil, the National Immunization Program was established in the early 1970s. Although diphtheria control was only achieved in the 1990s, 640 diphtheria cases were notified with an incidence coefficient (IC) of 0.45/100,000

inhabitants at the end of decade. During 2004, 19 cases of diphtheria were reported in the country with an IC of 0.01/100,000. Since 1970s, the vaccine coverage to tetanus-diphtheria toxoid (Td) increased from 66 to 94%. Since 2004, Brazilian immunization policy against diphtheria recommends childhood vaccination at 2, 4, and 6 months of age and booster doses including in adolescents and adults (Brasil 2004). However, there is not much information about regular immunization coverage and immunity of Brazilian adult population (Funasa 2002). In 2002, the Public Health Agency of Rio de Janeiro (SES-RJ 2002) stated a shifting in the age distribution of cases of diphtheria to persons over 15 years of age. A change in epidemiological aspects of diphtheria has been also recently observed in São Paulo (Casagrande et al. 2005). Current information also indicated the circulation of *Corynebacterium diphtheriae* in our population, including cancer patients (Mattos-Guaraldi et al. 2001a) and healthy vaccinated adults (Mattos-Guaraldi et al. 2001b). Recent investigations on the prevalence of IgG anti-diphtheria toxin levels in blood donors from Rio de Janeiro showed that only 30% of adults were fully protected. However, investigation on the circulating neutralizing toxin antibody levels in the vaccinated population remains necessary, especially for individuals with titers < 0.1 IU/ml of specific IgG (Damasco et al. 2005). The aim of this study was to assess functional antibody levels against diphtheria toxin among healthy blood donors living in Rio de Janeiro.

The survey analyzed 140 sera from a random sample of 240 blood donors of Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro (Hupe/Uerj) collected from July to October 2002. The study protocol was approved by The Institutional Review Board of

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the University Hospital, and blood donors were included in the study after signing a written informed consent. Healthy adults included individuals from 18 to 61 years old, stratified by age and sex: median age of 34 years; 53 (37.8%) female, and 87 (62.2%) male. A total of 119 (85.5%) subjects reported basic childhood diphtheria immunization. Serum samples were frozen and stored at -70°C until performance of diphtheria toxin antibody test.

Serum samples were tested for specific anti-diphtheria toxin neutralizing antibodies using a microtitre plate as previously described (Mills et al. 2003). Briefly, Vero cells were grown in modified Eagle's medium (MEM) supplemented with 10% fetal calf serum. A volume of 50 μl of serial twofold dilutions of undiluted test sera or standard serum for diphtheria toxin, initially diluted at 1/200 (equine antiserum from Instituto Vital Brazil, RJ, Brazil; 1000 IU/ml of antibody to diphtheria toxin) were added to the plates. Following incubation of plates with diphtheria toxin (80 pg/50 μl) at 37°C for 1h, 100 μl of Vero cells (2.5×10^5 cells/ml) were added to each well. The neutralizing effects of antibodies were evaluated by growth of Vero cells after 4 days of incubation at 37°C . Neutralizing antibody titer was defined as the highest dilution of serum neutralizing toxin killing of 50% Vero cells (CD_{50}). Viability of cells was determined, by the MTT assay (Efstratiou et al. 1998). Neutralizing antibody levels were expressed as IU/ml calculated from the standard serum and were categorized according to internationally accepted ranges: < 0.01 IU/ml (non-protective), between 0.01 to 0.09 IU/ml (basic protection), ≥ 0.1 IU/ml (full protection). The lowest protective level of serum neutralizing antibody to diphtheria is considered as > 0.01 IU/ml (Galazka 1993).

Data analysis was carried out using the Epi Info™ software program version 6.03 developed by the Centers for Disease Control and Prevention (CDC, Atlanta, GA, US).

Diphtheria antitoxin production, primarily of IgG type, can be induced by absorption of native toxin during clinical infection or in the carrier state, or by immunization with diphtheria toxoid (Walory et al. 2000). Protection against diphtheria is mainly due to the development of neutralizing toxin antibodies. It is believed that a circulating diphtheria antitoxin level of 0.01 IU/ml, as determined by the neutralization test in animals or in cell culture, provides clinical immunity against disease. The outcome of revaccination of adults depends on several factors, in-

cluding the immunization schedule, potency and time since the last dose of toxoid (Galaska 1993).

In developing countries where diphtheria is endemic, the process of maintaining immunity usually operates through natural mechanisms, including frequent skin infections caused by *C. diphtheriae*. Nowadays, adults might become susceptible to diphtheria due to reduced opportunities of sub clinical infections. Since diphtheria infection may also occur among previously vaccinated persons (Mattos-Guaraldi et al. 2001b), the immunity gap observed among adults should be closed by regular diphtheria boosters (Oluabunwo et al. 2005).

Preliminary studies on IgG diphtheria antitoxin levels determined by means of an ELISA showed basic immunity in 66.7 and 90.9% of children (0-14 years) and teenagers (15-20 years), respectively (Filyard et al. 2001). The lack of information on the immunity status against diphtheria in Brazilian adults prompted us to analyse IgG levels anti-diphtheria toxoid in healthy individuals aged 18-61 years. The results indicated that only 30.7% of individuals were fully protected (Damasco et al. 2005). Herein, data presented in the Table illustrated the distribution by age of antitoxin neutralizing antibody levels among Rio de Janeiro citizens blood donors. Of note, only 27.1% of individuals showed antibody levels ≥ 0.1 IU/ml considered to be protective against diphtheria. Most individuals (42.1%) had antibody levels between 0.01-0.09 IU/ml and are considered partially protected against the disease. However, a great proportion (30.7%) of individuals remains vulnerable to disease due to a lack of specific antibody (< 0.01 IU/ml).

Among unprotected individuals, 26.6% were from the age group of 18-30 years. The high percentage of individuals (72.8%) presenting diphtheria antitoxin levels < 0.1 IU/ml suggests a significantly higher risk of diphtheria among adults in our community. Nevertheless, the current strategy of mass immunization during childhood may indeed be sufficient to prevent major outbreaks due to generation of memory cells. Despite this hypothesis, epidemic diphtheria has re-emerged on a massive scale in the Newly Independent States (NIS) of the former Soviet Union, beginning in 1990 and affecting 15 countries by the end of 1994, with more than 175,000 reported cases and 5000 deaths before immunization campaigns controlled the epidemic. Diphtheria epidemics also spreaded to neighbouring countries in Europe, the Middle East and

TABLE
Neutralizing-antibody levels to diphtheria toxin in blood donors stratified by age

Age groups (years)	Titers (IU/ml)								
	< 0.01			0.01-0.09			> 0.1		
	n	%	CI 95%	n	%	CI 95%	n	%	CI 95%
18 - 30 (n = 60)	16	26.6	16.6-38.8	28	46.6	34.3-59.2	16	26.6	16.6-38.8
31 - 40 (n = 45)	13	28.8	17.2-43.3	20	44.4	30.4-59.0	12	26.6	15.3-40.9
41 - 50 (n = 24)	10	41.6	23.4-61.7	8	33.3	16.8-53.6	6	25.0	10.8-44.9
51 - 61 (n = 11)	4	36.3	12.7-66.3	3	27.2	7.4-57.8	4	36.3	12.7-66.3
Total (n = 140)	43	30.7	23.4-38.7	59	42.1	34.1-50.4	38	27.1	20.2-34.9

Asia. According to literature, control of diphtheria epidemics in vaccine era requires high levels of immunity among all age groups of the population (Galazka 2000a,b, Ohuabunwo et al. 2005).

The heterogeneous selection criteria for the participants and serological methods used for determining immunity to diphtheria make difficult to establish reliable comparisons of data from different epidemiological studies (Corbeira et al. 1999). However, data achieved in other countries in which antitoxin antibody concentrations were measured by neutralization test (Kjedlsen et al. 1988, Rappuoli et al. 1993, Mathei et al. 1997, Edmunds et al. 2000, Marlovits et al. 2000, McQuillan et al. 2002), demonstrated a low percentage of immunity against diphtheria in healthy adults, as observed in this study.

The present evaluation of neutralizing antibodies showed that the highest percentage (41.6%) of subjects susceptible to diphtheria was in the age group of 41-50 years old. During epidemic in Eastern Europe the highest mortality rate (62%) was also detected in this age group (Rakhmanova et al. 1996, Brennan et al. 2000, Ohuabunwo et al. 2005).

Questionnaires answered by blood donors revealed that 119 (85%) had history of basic diphtheria immunization during childhood. In this group, 28.5% and 42% showed diphtheria neutralizing antibody titers < 0.01 IU/ml and between 0.01-0.09 IU/ml, respectively. Only 29.4% of subjects showed protective levels of neutralizing antibodies to diphtheria (≥ 0.1 IU/ml).

We believe that the vast territory and unfavorable economic conditions presented by most regions of our country turn difficult the notification of cases to Public Health authorities and remittance of suspect isolates for bacteriological confirmation and toxigenicity tests to reference laboratories. Local outbreaks have been reported indicating gaps or failure in vaccine coverage (Mattos-Guaraldi et al. 2001b).

In general, diphtheria has become rare in immunized populations despite the lack of protective antibody titers in large proportion of the adult population. Some authors questioned the validity of accepted cut-off values for diphtheria protective titers. They believe antibodies wane with time after complete basic immunization while immunological memory persists, discarding the need for adult boosting (Mathias 1985). Nevertheless, during the diphtheria resurgence in parts of Eastern Europe, 60-70% of reported cases were among persons aged 15 years and older (Galazka & Robertson 1996). Therefore, if immunological memory generated by vaccination in childhood is enough to protect adult individuals, without additional booster doses, needs further investigations.

This seroepidemiologic survey indicates a low prevalence of diphtheria neutralizing antibody levels in healthy adults from Rio de Janeiro, Brazil, with 30.7% (95% CI: 23.4-38.7) of individuals presenting titers < 0.01 IU/ml. The existence of susceptible adults creates an epidemic potential in our community and reinforces the need of introduction and surveillance of Td vaccination for adults in order to ensure adequate diphtheria neutralizing antibody levels.

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