

SHORT REPORT

Prevalence of IgG diphtheria antitoxin in blood donors in Rio de Janeiro

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SUMMARY

The lack of information on the immunity of adults in Brazil against diphtheria prompted us to analyse sera from 234 blood donors aged 18–61 years (30·3% females and 69·7% males). IgG diphtheria antitoxin levels determined by means of an ELISA, validated by toxin neutralization test in Vero cells, showed that 30·7% (95% CI 25·0–37·1) of the population was fully protected (≥ 1 IU/ml). The highest percentage of subjects fully protected was in the 31–40 years age group. Most of the subjects with uncertain or no protection (< 1 IU/ml) were found in the 18–30 years age group (43·8%, OR 2·18, $P=0\cdot01$). Antitoxin levels were not influenced by the increase in age. Males were more protected than females (80·5%, OR 0·44, $P=0\cdot01$). The prevalence of 30% of individuals fully protected against diphtheria in blood donors in Rio de Janeiro supports the fact that immunity to diphtheria among healthy Brazilian adults is inadequate. To avoid diphtheria epidemics in the future the immunity among adults should be raised in the coming years.

The recent epidemics in Eastern Europe serve as a warning that diphtheria may re-emerge in susceptible populations [1]. It is generally accepted that when more than 30% of a population are unprotected against diphtheria there is a risk of epidemic diphtheria occurring in that population [2]. Many of the poorest countries in the world do not yet provide vaccination after infancy. The World Health Organization (WHO) recommends that nations should document the level of vaccine-induced immunity among children, adolescents and adults [3]. Such information is needed to assess the susceptibility of populations to diphtheria in countries without epidemic spread of the disease and to determine the

epidemiological effect of widespread vaccination in developing countries. Unfortunately, very few sero-surveys have been achieved outside of North America and Western Europe. Although diphtheria is thought to be declining in Brazil [4], the disease remains endemic in various states and as in other countries the lack of immunity in older (> 15 years old) individuals is a cause for concern [5, 6]. The present investigation evaluated the prevalence of IgG diphtheria antitoxin in sera by a cross-sectional study of blood donors from Rio de Janeiro.

The survey was carried out on 234 serum samples collected from blood donors of Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro (HUPE/UERJ) from July to October 2002. The study protocol was approved by The Institutional Review Board of the University Hospital, and blood donors were included in the

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Table. Prevalence of IgG diphtheria antitoxin levels according to age group

Age groups (years)	Antitoxin antibody titre (IU/ml)								
	<0.1			0.1–0.9			≥1		
	<i>n</i>	%	95% CI	<i>n</i>	%	95% CI	<i>n</i>	%	95% CI
18–30 (<i>n</i> =90)	8	8.89	4.1–17.2	63	70.00	59.2–79.9	19	21.11	13.4–31.2
31–40 (<i>n</i> =78)	13	16.67	9.5–27.1	29	37.18	26.7–48.9	36	46.15	34.9–57.8
41–50 (<i>n</i> =44)	10	22.73	11.9–38.3	24	54.55	38.9–69.3	10	22.73	11.9–38.2
51–61 (<i>n</i> =22)	3	13.64	3.58–35.9	12	54.55	32.6–74.9	7	31.82	14.7–54.8
Total (<i>n</i> =234)	34	14.53	10.4–19.8	128	54.70	48.0–61.1	72	30.77	25.0–37.1

n, sample number; CI, confidence interval.

study only after written informed consent. Donors included individuals aged 18–61 years, stratified by age and sex: median age was 34.6 years; 71 (30.3%) female and 163 (69.7%) male. A total of 201 (83.7%) subjects reported basic childhood diphtheria immunization. Of 163 males, 39.8% had a history of military service. Serum samples were frozen and stored at -70°C until tested.

Diphtheria toxin IgG-specific antibody titres were determined by means of an enzyme-linked immunosorbent assay [7–9] using the ELISA commercial kit with titres (IU) calibrated against the WHO standard NIBSC 91/534 purchased from Immuno-Biological Laboratories GmbH (Hamburg, Germany). The titres were classified in three groups: <0.1 IU/ml, between 0.1 IU/ml and 0.9 IU/ml and ≥ 1.0 IU/ml to indicate unprotected, partially protected, and fully protected individuals respectively.

A total of 140 serum samples were tested for specific anti-diphtheria toxin neutralizing antibodies using a Vero cell assay as previously described [10]. The correlation of the ELISA values with functional immunity was analysed by the SPSS Program for Windows 12.0 (SPSS Inc., Chicago, IL, USA). The χ^2 test and Fisher's exact test were used to compare the probability of antibody titres <1.0 IU/ml between categories of the studied variables. Statistical analyses were conducted with Epi-Info statistical software version 6.03 (CDC, Atlanta, GA, USA).

The ELISA test for diphtheria antibody determination has been frequently used in seroepidemiological surveys with results mainly reproducible when antibody levels are >0.1 IU/ml [2, 8, 9, 11–13]. In this study, validation of the ELISA revealed a high specificity and good predictive value for evaluation of full protection. The ELISA showed 96% (98/102)

specificity, 55.3% (21/38) sensitivity, 84% [21/25, 95% confidence interval (CI) 78–90] and 85% (98/115, 95% CI 79–91) positive and negative predictive values respectively, with correlation between neutralization test (antibody levels ≥ 0.1 IU/ml) and ELISA (titres ≥ 1 IU/ml) corresponding to $\kappa=0.575 \pm 0.081$ ($P<0.001$).

In Brazil, in the late 1990s, the incidence of diphtheria was 0.13 cases per 100 000 for 94% coverage of children. However, local outbreaks have been reported indicating gaps or failure in vaccine coverage [4]. In theory, over 90% of children and more than 75% of adults ought to be protected to prevent diphtheria outbreaks [7]. The heterogeneous selection criteria for the participants and serological methods used for determining immunity make it difficult to establish reliable comparisons of data from different studies worldwide [9]. However, in comparison with data reported from other countries [11, 12, 14–19], a high percentage (39.5–79.3%) of Brazilian adults were found to be unprotected.

Data presented in the Table show the distribution of IgG diphtheria antitoxin titres in sera of healthy individuals of different age groups: 14.5% (95% CI 10.4–19.8) had titres <0.1 IU/ml; 54.7% (95% CI 48.0–61.1) had titres between 0.1 and 0.9 IU/ml and 30.7% (95% CI 25.0–37.1) had levels ≥ 1.0 IU/ml. In the latter group, 21.1, 46.1, 22.7 and 31.8% of fully protected individuals were from the age groups 18–30, 31–40, 41–50, and 51–61 years respectively. The overall prevalence of 30.7% of healthy adults (blood donors) with full serological protection against diphtheria is in accordance with data (20.7–60.5%) from previous surveys performed in Europe and North America [8, 11, 12, 14, 15]. In contrast to observations from other countries [16], protection did not decrease

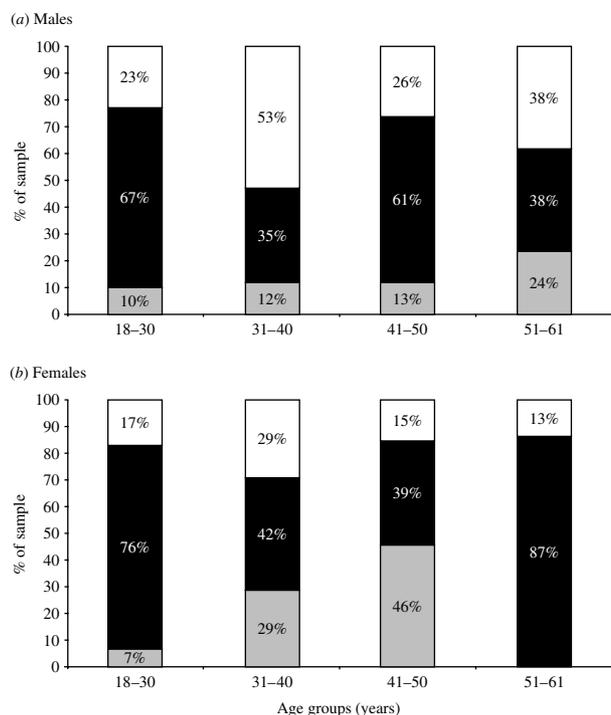


Fig. Age-specific prevalence (%) of diphtheria antitoxin levels in (a) males; (b) females. Antitoxin level: <0.1 IU/ml = susceptible (□); $0.1-0.9$ IU/ml = basic protection (■); ≥ 1.0 IU/ml = full protection (▒).

with an increase in age. This pattern of immunity probably reflects an endemic level of diphtheria in Brazil, similar to other developing countries [20]. Antitoxin titres ≥ 1.0 IU/ml were most prevalent in subjects aged ≥ 31 years [73.6%; odds ratio (OR) 0.46, 95% CI 0.24–0.88, $P=0.01$], especially in the 31–40 years age group (46.1%; 95% CI 34.9–57.8). Interestingly, the lowest prevalence of protective immunity was observed in the 18–30 years age group (26.4%; OR 2.18, 95% CI 1.14–4.19, $P=0.01$). Data suggest that booster vaccination for adolescents and adults remains necessary to enhance collective immunity and prevent the occurrence of local outbreaks of diphtheria among Brazilian adults.

The prevalence of individuals with different immunity levels grouped by age and sex is presented in the Figure. A gender effect was observed, in which the overall majority of females were less protected when compared to males (OR 2.25, 95% CI 1.10–4.64, $P=0.01$). This difference in immunity between sexes has also been observed in other countries [8, 21]. The highest number of males (53%) and females (29%) fully protected (antitoxin titres ≥ 1.0 IU/ml) was found in the 31–40 years age group. Only 29.3%

donors with history of military service were fully protected. In contrast to some studies [22], differences in immunity between sexes could not be explained by diphtheria booster immunization as a consequence of military service. However, routine administration of combined tetanus-diphtheria vaccines after injury, which occur more frequently in men [14], remains a possible explanation. Some authors have also pointed to a possible difference in response to immunization, suggesting that vaccination is less efficient and of shorter duration in women [8, 21].

Diphtheria antitoxin production, primarily of IgG type, can be induced by natural toxin during infection or in the carrier state, or by immunization with diphtheria toxoid. The antitoxin stimulated by immunization is believed to persist at protective levels for 10 years or more [23]. Epidemiological studies in Europe and North America have shown that after years of large-scale vaccination and improved hygiene there has been a change in the age group susceptible to diphtheria. Globally, the level of antibodies to diphtheria toxoid has dropped (<0.1 IU/ml) among the adult population [1, 23, 24] and it is widely accepted that antitoxin levels of 1.0 IU/ml and above are associated with long-term protection [25].

Thus, the prevalence of 30% of individuals fully protected against diphtheria in blood donors in Rio de Janeiro supports the fact that immunity to diphtheria among healthy Brazilian adults is inadequate, according to WHO criteria to prevent diphtheria outbreaks. Data corroborate the alarming results of similar serosurveys performed in industrialized countries indicating the requirement of re-vaccination of adolescents and adults as a general practice.

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