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Immunity against diphtheria and tetanus in German blood donors

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Abstract After the recent diphtheria epidemics in Eastern Europe in the early 1990s, we re-evaluated the diphtheria and tetanus immunity of 321 German blood donors (192 men and 129 women). The mean antitoxin levels of all blood donors in this study, measured by commercial ELISA, revealed a questionable protection (0.1–1.0 IU/ml) against diphtheria. In 1994, 66.4% were without immunity against diphtheria (55.0% in 1997/98), 32.1% (41.5% in 1997/98) showed questionable protection and only 1.5% (3.5% in 1997/98) had protective antitoxin levels. The evaluation of tetanus immunity revealed only 0.5% (1.1% in 1997/98) of the subjects with no protection and 9.1% (8.5% in 1997/98) with questionable protection. For this reason, we conclude that the diphtheria epidemics only lead to an insufficient improvement of the immunization status in a healthy German population.

Keywords Diphtheria · Tetanus · Immunity · Vaccination

Introduction

Since the introduction of immunization against diphtheria and tetanus at the beginning of the last century, the number of infections and deaths caused by these infectious diseases has declined markedly. In the western industrialized countries, diphtheria was only known from imported cases arising in areas where it occurred

endemically (there are still some such areas in Africa, Asia and Eastern Europe [12]). What seemed more menacing was the occasional flaring-up of diphtheria epidemics in neighboring countries such as Sweden in 1986 and the successor states of the former USSR in the early 1990s. Due to introduction of this disease by travelers, diphtheria occurred on repeated occasions in countries such as Finland, Poland and Germany, where diphtheria was considered to have been almost eradicated over the last few decades thanks to immunization programs. This time it was not children who were threatened by diphtheria, as was the case at the beginning of the 20th century [18], but rather the adult population, amongst whom most of the recent cases had now occurred [23, 24]. Because of the fear of new epidemics, many studies were carried out in Europe and North America to evaluate the immunization status of the population [2, 3, 4, 6, 7, 8, 14, 15, 17, 20]. In this context, antitoxin levels against tetanus were often determined as well. Tetanus is still an omnipresent source of danger for mankind because of the ubiquitous presence of *Clostridium tetani* in the environment. In spite of excellent intensive medical care, the mortality rate is still 30–50% [23]. The best protection against tetanus remains vaccination.

The aim of our study was to re-evaluate the status of immunity in a German population in the Lübeck area after the new diphtheria epidemics in Eastern Europe mentioned above. We expected a higher awareness of vaccination and higher antitoxin levels than previously described, especially for diphtheria. Finding different results to those expected, we asked whether there were seasonal fluctuations in antitoxin levels, e.g., due to the common cold in winter time, or other individual fluctuations. In most of the studies, the antitoxin level was determined on only one occasion and the interpretation of the immunization status of the subject was based on that result. To answer these questions, we investigated two to four consecutive samples from 321 blood donors taken in 1994 and one follow-up sample taken at the end of 1997 or at the beginning of 1998 from each of the blood donors.

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Materials and methods

Subjects and samples

A total of 321 blood donors (192 male and 129 female) who regularly donated blood at the Institute of Immunology and Transfusion Medicine, University of Lübeck School of Medicine, at least from 1994 to 1998, were included in this study. The donors were between 18 and 65 years of age and were divided into gender and age groups (Table 1). EDTA-blood samples were collected from two to four consecutive blood donations in 1994 and from one donation at the end of 1997 or the beginning of 1998 from each blood donor. The samples were centrifuged immediately after drawing and the plasma was stored at under -30°C until further processing.

Detection of antibodies

Tetanus and diphtheria antitoxin levels were determined by commercial ELISAs [SERION ELISA classic Diphtherie IgG (quant.) and SERION ELISA classic Tetanus IgG (quant.), Virion-Serion, Würzburg, Germany]. The ELISA procedure was performed as recommended by the manufacturer. The enzymatic turnover was measured photometrically at 405 nm and 620 nm (Anthos Reader 2001, Anthos Labtec Instruments, Salzburg, Austria). The specific antibody concentration was determined by comparisons with standard control sera. Special software was used to simplify and specify the evaluation of the data obtained (Easy.Base4PL-Software, Virion Serion, Würzburg, Germany).

Classification of antitoxin levels

There is some inconsistency in the literature regarding levels considered to confer questionable or adequate protection against diphtheria. In this study, we used the values recommended for ELISA by the STIKO (permanent immunization committee) of the Robert Koch Institute (Berlin, Germany) as a guideline [23]. Antitoxin levels of greater than 1.0 IU/ml were considered to be protective against diphtheria. Levels between 0.1 IU/ml and 1.0 IU/ml were considered to provide questionable protection and values of less than 0.1 IU/ml were considered as not being protective at all. Other values are recommended for immunity against tetanus. Values greater than 0.1 IU/ml were considered to be protective, while those between 0.01 IU/ml and 0.1 IU/ml were designated as questionable, and values below 0.01 IU/ml were considered to be ineffective.

Table 1 Division of blood donors into age groups

Group	Age (years)	Total (male/female)
1	18–29	75 (46/29)
2	30–39	100 (62/38)
3	40–49	83 (52/31)
4	50 and older	63 (32/31)

Table 2 Distribution of samples within the various diphtheria and tetanus immunity levels (the data shown for 1994 are averaged from all samples taken in 1994)

Diphtheria	1994	1997/98	Tetanus	1994	1997/98
	<i>n</i> = 277	<i>n</i> = 282		<i>n</i> = 277	<i>n</i> = 280
< 0.1 IU/ml	184 (66.4%)	155 (55.0%)	< 0.01 IU/ml	1 (0.4%)	3 (1.1%)
0.1–1.0 IU/ml	89 (32.1%)	117 (41.5%)	0.01–0.1 IU/ml	26 (9.3%)	24 (8.5%)
> 1.0 IU/ml	4 (1.5%)	10 (3.5%)	> 0.1 IU/ml	250 (90.3%)	253 (90.4%)

Questionnaire

A questionnaire was sent to all subjects included in this study to find out whether and when they had been immunized against diphtheria and tetanus. The returned questionnaires were evaluated for these aspects, and the results obtained were considered in the evaluation of the ELISA results.

Statistical analyses

Statistical analyses were performed using SPSS 9.0 for Windows. Significances of differences between related spot checks were analyzed by the Friedman Test, those between the various age groups (independent spot checks) were checked by the Kruskal Wallis Test and antibody levels were compared by Mann Whitney U-Test. The coefficients of variation (% CV) were evaluated for diphtheria and tetanus antibody levels. They were calculated in the weakly positive serum range for diphtheria and the positive serum range for tetanus, since the majority of samples detected for diphtheria and tetanus were detected within those respective ranges.

Results

Antitoxin levels against diphtheria and tetanus

The distribution of diphtheria antitoxin levels according to protection criteria in the 321 blood donors is shown in Table 2. The mean antitoxin level of all blood donors corresponded to a questionable protection against diphtheria (0.14 IU/ml in 1994 and 0.21 IU/ml in the follow-up samples of 1997/98). The median (0.06 IU/ml in 1994 and 0.08 IU/ml in 1997/98) was below the mean of the results and revealed no protection in 1994 and in 1997/98. All subjects who had a mean antitoxin level that corresponded to sufficient protection against diphtheria in 1994 and 1997/98 were males. All subjects who had sufficient protection in 1994 also had sufficient protection in 1997/98. For three of the six subjects who did not have a sufficient protection in 1994, but who had it in 1997/98, the questionnaire revealed that they had been vaccinated in the meantime; the remaining three subjects did not return their questionnaires.

The mean of the antitoxin levels against diphtheria dropped with increasing age. The highest level of diphtheria antitoxin was found in age group 1, the lowest in age group 3. However, the antitoxin level in age group 4 was higher than it was in group 3 (Fig. 1). These low antitoxin levels in age group 3 were seen in general as well as when differentiating between the sexes. The statistical analysis also showed a reduced antitoxin level in age group 3 that was significantly lower ($P < 0.05$) in all

quarters of 1994 and in the follow-up sample of 1997/98 than in all the other age groups. The antitoxin level in men's age group 3 was only significantly lower ($P < 0.05$) than the antitoxin level of age group 4 in all quarters of 1994 and lower than the antitoxin level in age groups 1 and 4 in the follow-up sample of 1997/98.

The evaluation of the ELISA results for tetanus immunity also showed that the median value (1.16 IU/ml) was below the mean (1.82 IU/ml) of the antitoxin levels. In contrast to diphtheria immunity, both the median and the mean of the tetanus-antitoxin levels were within the protective range. The results are shown in Table 2 and Fig. 2. The antitoxin levels decreased with increasing age. The highest level was found in age group 1, the lowest in age group 4. The statistical analysis revealed that in all quarters of 1994 and in the follow-up sample of 1997/98, the antitoxin level of age

group 4 was significantly lower ($P < 0.05$) than the antitoxin level of age groups 1 and 2. In 1994 the women showed a significantly lower antitoxin level in age groups 3 and 4 compared to age groups 1 and 2. In the follow-up sample, only the women's antitoxin level of age group 3 was significantly lower than the antitoxin level of age group 2.

Questionnaire

Only 188 of the 321 subjects (58.6%) returned their questionnaire. It was noticeable that the compliance of the subjects in returning their questionnaires rose with increasing age. With 42.6%, age group 1 showed the lowest participation, while in age group 4 the majority participated (76.2%). With the exception of age group 3 and 4, the participation of the female subjects was higher than the participation of the male subjects. Overall 62.0% of the females and 56.3% of the males returned their questionnaire.

The evaluation of the returned questionnaires showed that with increasing age the number of subjects who had booster vaccinations against diphtheria and tetanus within the last 10 years (1987–1997) decreased. Only 36.7% of those returning their questionnaire reported a diphtheria booster vaccination within the time period of 1987 to 1997. The highest rate was found in age group 1 (53.1%), and the lowest in age group 4 (25%). Overall, 58% reported a tetanus booster vaccination, 78.1% in age group 1, 64.3% in age group 2, 46.2% in age group 3 and 50% in age group 4. About 40.4% of the subjects reported their tetanus booster before 1994, only 18.8% did so for diphtheria. Therefore, 81.2% of those who returned the questionnaire received their diphtheria booster vaccination within the period of our interest. We asked, whether this could be seen in antibody levels. In 1997, the antibody level of those who reported a vaccination 1994–1997 was significantly higher ($P < 0.0002$) for diphtheria as well as for tetanus than in the beginning of 1994.

Seasonal fluctuation

Only 138 of the 321 subjects (57 men and 81 women) were evaluated regarding seasonal fluctuation, since four samples in 1994 (one each season) were required as well as a returned questionnaire and one sample in 1997/1998 as a long-term control. The evaluation for seasonal fluctuation was divided into several steps. All blood donors were evaluated together, and they were classified according to gender, age (see Table 1) and both gender and age. There was no seasonal fluctuation detectable. The total % CV of the diphtheria antitoxin levels (as well as the tetanus antitoxin levels) was on average 0.6% in 1994 and 0.6% in the control of 1997/98.

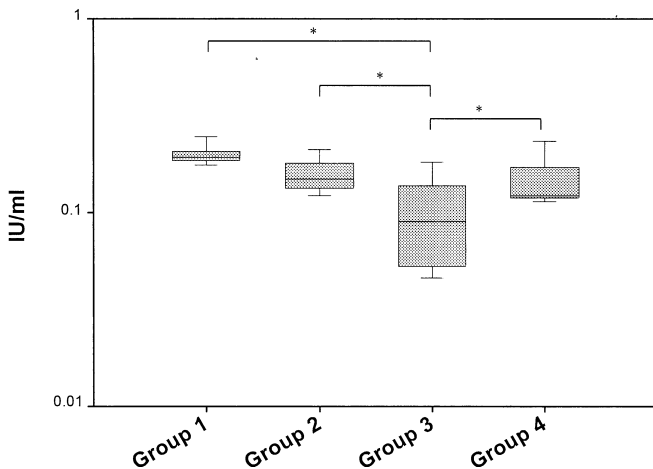


Fig. 1 Comparison of age groups concerning diphtheria antitoxin levels. This graph shows the average diphtheria antitoxin levels of the four different age groups in 1994. * $P < 0.05$

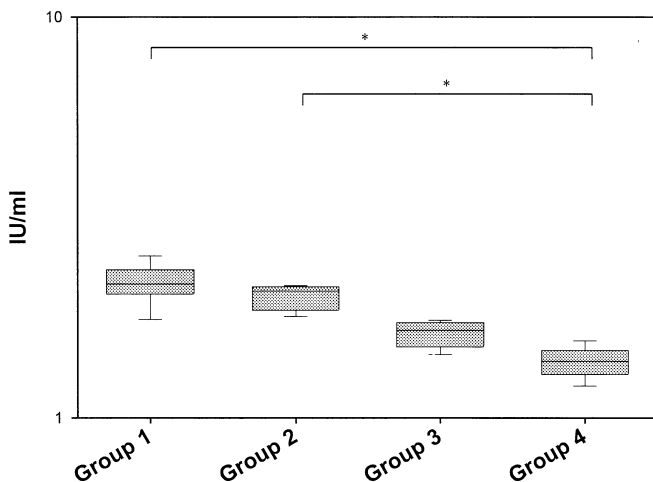


Fig. 2 Comparison of age groups concerning tetanus antitoxin levels. This graph shows the average tetanus antitoxin levels of the four different age groups in 1994. * $P < 0.05$

Discussion

Of our blood donors, 66.4% were without immunity against diphtheria in 1994. These results were comparable to those found by Allerdist et al. [1] in 1981. They detected in 67.5% of their subjects (personnel of hospitals in Hamburg, Germany) non-protective antitoxin titers against diphtheria. The age of the subjects in Allerdist's study was between 15 and 65 years and therefore similar to the population of our study. However, in contrast to our study, 32.5% of Allerdist's subjects showed antitoxin levels within the range considered to be protective, whereas 32.1% of our subjects showed questionable protection and only 1.5% of our subjects had protective antitoxin levels. The results of Klouche et al. [10] showed no protection against diphtheria in only 24% of the evaluated blood donors, questionable protection in 69%, and protective antitoxin levels in 7%. These results are astonishing, given that they also investigated blood donors of the Institute of Immunology and Transfusion Medicine, University of Lübeck School of Medicine, who donated blood between May and November 1993. Therefore this collective is comparable to ours and similar results should have been expected. In 1998, Hasselhorn et al. [5] found no protection in 31.4% of the analyzed subjects (287 healthy adults who were recruited in the course of an occupational health survey by the University Hospital, Freiburg, Germany), questionable protection in 26.5% and antitoxin levels within the protective range in 42.2%. Stark et al. [22] reported in 2001 that 18% of their subjects (2,079 blood donors in Berlin) had no protection against diphtheria, 22% had a questionable protection and 60% of their subjects had protective antitoxin levels against diphtheria. The results of these five different studies showed five different images of immunity against diphtheria in the German population. Klouche et al. [10] also used an ELISA to detect the diphtheria antitoxin, but their test kits were purchased from a different manufacturer. This may have been a causative factor underlying the different results obtained by Klouche et al. [10] and our study concerning the diphtheria immunity. On this question, more research needs to be done to determine the correctness of the different ELISAs for detecting diphtheria antitoxin. In 2000, Kösters et al. [11] compared five different commercial ELISAs for the detection of antibodies against *Bordetella pertussis*. Their conclusion was that adjustments have to be made in standardization, concerning for example the content of antigen and the reference material of the test kits to improve the reliability of the diagnosis and the comparison of different laboratories. This should also be considered for the quantification of diphtheria antitoxin.

Another factor that may influence the comparison between our results and those of the previous studies is the application of different methodologies. One should not forget that significant differences in sensitivity and specificity may exist between different methods when testing the same serum [25]. Therefore, it is questionable

whether the results from numerous studies can be compared when different methods are used. Hasselhorn et al. [5], Allerdist [1], Naumann et al. [19] (3,503 healthy German individuals between 2 and 70 years) and Stark et al. [22] used different methods compared to our study. Hasselhorn et al. [5] and Stark et al. [22] used a toxin neutralization test (TN) and Allerdist [1] as well as Naumann et al. [19] both used indirect hemagglutination (IHA) for the detection of diphtheria antitoxin. Several authors stated the ELISA as an acceptable population screening method for diphtheria antitoxin [16, 25], but where antibody levels below 0.1 IU/ml are found, such samples should be re-determined using one of the neutralization tests. The results of studies employing IHA are controversial. Maheshwari et al. [13] compared IHA and TN for the testing of diphtheria antitoxin in mouse sera and obtained a correlation of 0.91 between the results of these two methods. In contrast, Walory et al. [25] found an unsatisfactorily poor sensitivity in human sera with only 14% correctly diagnosed titers below 0.1 IU/ml for IHA; this led to the authors refusing to use IHA for routine diagnostics in this area. This controversy in discussing different testing methods leads to the next problem in comparing different studies on the matter of population immunity. Different immunity levels are considered to be protective against diphtheria by different authors. Some consider antitoxin levels of greater than 1.0 IU/ml to be protective [10, 23, 25], some use antitoxin levels 10 times lower to determine immunity against diphtheria [1, 5, 19, 22]. Therefore, some authors might consider a person to have protective antitoxin levels against diphtheria [1, 5, 19, 22], whereas others would not [10, 23, 25]. It is also questionable if the testing method used really shows reliable results in the detection range the author is referring to [1, 19].

The elevated antitoxin levels against diphtheria toxin in male subjects compared to female subjects have been described in several studies [2, 8, 20]. This was in general confirmed by our findings.

Naumann et al. [19] and Allerdist [1] described results comparable to our study regarding the drop of antitoxin levels against diphtheria from age group 1 to age group 3 and the marginal rise in age group 4 in similar ways. In the study of Naumann et al., 40.6% of the 14- to 80-year-old subjects did not show protective antitoxin levels against diphtheria. The lowest antitoxin levels were detected in the age group of 20–34 years. Allerdist [1] reported decreasing antitoxin levels in the standard population until the age of 40 years. Both results go along with the results in our study, even though there are differences in the classification of age groups in these studies. Naumann et al. [19] divided the subjects into age groups comprising a range of 15 years, whereas we defined only 10-year groups for our classification. However, it is still clear from all three studies that the subjects born before 1950 have higher antitoxin levels against diphtheria than those born in the 1950s and 1960s. This may be due to naturally acquired immunity during the last big diphtheria

epidemics at the end of World War Two, or due to the large immunization programs that took place at that time.

The results obtained for immunity against tetanus are similar to those seen in a number of previous studies. For example, Klouche et al. [9] found antitoxin titers less than 0.1 IU/ml in 15.5% of their subjects. In our study, only 9.6% of the analyzed subjects had antitoxin levels of less than 0.1 IU/ml. Also, a continuous drop in immunity with ongoing age was detected by Klouche et al. [9]. They analyzed first-time blood donors who visited the Institute of Immunology and Transfusion Medicine at the University of Lübeck School of Medicine between December 1992 and June 1993. Therefore, this collective was similar to that chosen in our study. Several other studies have already described a better immunoprotection of males compared to females [8, 9, 21, 22]. This phenomenon is often ascribed to military service on the one hand, and more injuries to men through their occupation and hobbies on the other. These injuries are often associated with booster vaccinations against tetanus in the course of treatment.

Evaluating the returned questionnaires, we found that 81.2% of the blood donors who reported a diphtheria vaccination within the last ten years were boosted during the period of 1994–1997. This was consistent with a rise of antibody levels within this collective, which were still not within a range that could be considered protective. However, since only 58.6% returned the questionnaire and only 36.7% of these reported a diphtheria booster vaccination, this might explain why the obvious increase in awareness of vaccination in this special collective had such a poor influence on the increase of protection in the whole population (66.4% unprotected in 1994, 55.0% in 1997).

Until now, seasonal fluctuation of IgG antitoxin against diphtheria or tetanus has not been studied. Upon evaluation of the whole collective, no seasonal fluctuation was visible, either for diphtheria or tetanus-antitoxin. The statistically significant differences calculated for both ELISAs lay within the range of the inter- as well as the intra-assay variances given by the manufacturers.

We conclude that the diphtheria epidemics which occurred in Eastern Europe did result in an improved awareness of vaccination in a selected collective, as shown by the returned questionnaires, but did not lead to a sufficient overall protection rate in healthy German adults. The significant difference of received booster vaccinations against diphtheria ($n=69$) and tetanus ($n=109$) within the last 10 years led to the conclusion that, in most cases, the tetanus vaccine is administered regardless of diphtheria immunity.

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