

## Seroprevalence and determinants of diphtheria, tetanus and poliomyelitis antibodies among adults in Berlin, Germany

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### Abstract

The immunity levels against diphtheria, tetanus and poliomyelitis were investigated among blood donors ( $n = 2079$ ) in Berlin. Of all participants, only 60% had full, long-term protection against diphtheria, 72% against tetanus, 87% against poliomyelitis type 1, 77% against poliomyelitis type 2 and 73% against type 3. There was a striking decrease of tetanus and diphtheria immunity levels by age. Immunity levels against tetanus were higher among males, whereas females were better protected against poliomyelitis. After adjusting for confounding effects in logistic regression diphtheria immunity in those aged  $<40$  years was significantly higher in participants from East-Berlin, whereas the immunity levels against poliomyelitis were higher in West-Berlin. These differences reflect the different vaccination policies in East-Germany and West-Germany before 1989. There is a need to improve the immunity levels of the adult population in Berlin. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords:* Diphtheria; Tetanus; Poliomyelitis; Immunity

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### 1. Introduction

Diphtheria, tetanus and poliomyelitis are still major public health problems in many developing countries. The recent diphtheria epidemic in the Newly Independent States and the outbreaks of poliomyelitis in Finland, Israel or the Netherlands showed that susceptible populations also exist in industrialised regions [1–4]. In order to achieve adequate levels of herd immunity and to prevent such outbreaks it is necessary to monitor the immunity levels of the general population and to identify and vaccinate insufficiently protected groups. Several surveys in industrialised countries revealed that substantial proportions of the adult population may lack immunity against diphtheria, tetanus and poliomyelitis. However, there are limitations in some of these studies due to small or non-representative samples, or because the

gold standard tests for antibody detection were not used.

Before the unification in 1989, there existed different vaccination policies between West-Germany and East-Germany. In former East-Germany, vaccination against diphtheria, tetanus, poliomyelitis and a few other preventable diseases was mandatory in children [5]. This included a primary series of immunisations against diphtheria-pertussis-tetanus (DPT) in the 3rd, 4th and 5th month, a DPT booster in the 3rd year and a DT booster in the 8th year of life. A further tetanus booster was given at age 16. Beginning in the 3rd month the children received monovalent oral vaccines against the three types of the poliomyelitis virus 4 weeks apart. At age 8 the trivalent oral polio vaccine was given. In 1988, the vaccination coverages at age 1 year were 98% for DPT, 98% for poliovirus type 1, 97% for polio type 2 and 94% for polio type 3 [6]. In West-Germany, vaccinations for children were strongly recommended but voluntary. The proposed schedule for the primary series of the DPT vaccination was the same as in East-Germany.

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Boosters were recommended for the 2nd year (DPT) and for the 6th to 8th year (Td). The primary series of the polio vaccination consisted of two doses of trivalent oral polio vaccine given 6 weeks apart beginning after month 3 and a third dose in the 2nd year. A trivalent booster was recommended for age 10. Public vaccination campaigns for older children and adults were organised by the district public health services for poliomyelitis on a voluntary basis. All military recruits received a booster against tetanus. This situation before 1989 allowed us to investigate the potential impact of the different policies on immunity levels in the population.

The objective of our study was to assess the antibody levels for diphtheria, tetanus and poliomyelitis in a large sample of blood donors and to identify determinants of immunity.

## 2. Materials and methods

### 2.1. Study population

Healthy unremunerated adults (aged 18–70) were recruited at different blood donation sites in East-Berlin and West-Berlin. Four permanent donation sites (2 in East-Berlin and 2 in West-Berlin) and a few mobile sites were chosen for the recruitment of blood donors. We aimed at recruiting about 2,000 blood donors equally distributed with respect to place of residence (East-Berlin or West-Berlin) until 1989 and to the four age groups <30, 30–39, 40–49, and  $\geq 50$  years. The individuals were consecutively recruited into the study and the recruitment was continued until the intended frequency distributions by age, gender and site of residence had been achieved. Informed consent was obtained and a serum sample from each participant was stored at  $-70^{\circ}\text{C}$ . The routinely collected data on age and gender could be extracted from the blood donors' records at the Red Cross. Additional information on the place of residence before 1989 and on vaccinations against diphtheria, tetanus and poliomyelitis in the previous 10 years was obtained by a self-administered standardised questionnaire. The response rates were 94% in East-Berlin and 91% in West-Berlin.

### 2.2. Laboratory methods

Diphtheria antitoxin levels were assessed by a micro-cell-culture-neutralising test. Serial dilutions of sera were tested for their capacity to neutralise the effect of diphtheria toxin on cultured monkey-kidney cells (vero cells) [7, 8]. Tetanus antitoxin levels were determined by enzyme-linked immunosorbent assay (ELISA). Diluted sera were incubated in microtitre plates coated

with tetanus toxoid. The antitoxins were then detected by goat anti-human-immunoglobulin conjugated to alkaline phosphatase. *P*-Nitrophenylphosphate was added and the colour reaction was photometrically measured at 405 nm. Diphtheria and tetanus antitoxin levels were expressed in international units (IU) according to the WHO standard and were classified according to international standards: <0.01 IU/ml, no protection; 0.01–0.1 IU/ml, uncertain, short-term protection and  $\geq 0.1$  IU/ml, full protection. In this paper, antitoxin levels <0.1 IU/ml are referred to as insufficient protection.

Neutralising antibodies against poliovirus type 1 (strain Mahony), type 2 (strain Lansing) and type 3 (strain Leon) were measured by a microneutralisation assay. Approximately 1000 tissue culture infective doses (TCID<sub>50</sub>) of each virus and serial dilutions of serum were incubated for 3 days, and examined microscopically. A neutralising activity at a dilution of  $\geq 1:8$  was regarded as protective.

The laboratory methods applied in this study can be regarded as internationally accepted standard methods [9–11].

### 2.3. Statistical analysis

Associations between the antibody levels and categorical variables such as gender, site of residence and age group were assessed by  $\chi^2$ -test or  $\chi^2$ -test for trend. To adjust for confounding effects logistic regression analysis was performed. For this purpose, the outcome variables were used as binary variables for the protection (full protection versus incomplete protection) against diphtheria, tetanus and poliomyelitis. To adjust for potential mutual confounding effects all three explanatory variables (age, gender, site of residence) were included in the logistic regression models. Adjusted odds ratios with 95% confidence intervals (CI) were calculated. Interaction was also assessed in the logistic regression analysis. Models were fitted using age as an ordered categorical variable (<30 years, 30–39, 40–49,  $\geq 50$ ), and as a continuous variable. The model which gave the best fit according to the likelihood ratio statistic was chosen as the final model.

## 3. Results

Of the 2079 blood donors, 63% were males. Until 1989, 1127 participants had lived in East-Berlin and 952 in West-Berlin. The study participants (mean age 39 years, standard deviation 11 years, range 18–70 years) were distributed in the four age groups as follows: 500 (<30 years), 593 (30–39 years), 500 (40–49 years) and 486 ( $\geq 50$  years).

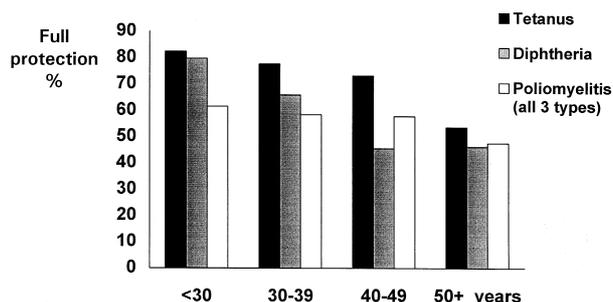


Fig. 1. Immunity levels against tetanus, diphtheria and poliomyelitis in blood donors in Berlin by age group.

Overall, 40% had insufficient immunity levels against diphtheria (antitoxin level  $<0.01$  IU/ml 18%, 0.01–0.1 IU/ml 22%) and 28% against tetanus (antitoxin level  $<0.01$  IU/ml 1%, 0.01–0.1 IU/ml 27%). 13% of the participants lacked immunity against poliovirus type 1, 23% against poliovirus type 2 and 27% against poliovirus type 3. Only 57% of the blood donors were protected against all three poliomyelitis types and 4% were protected against neither type.

### 3.1. Gender, age and immunity levels

Full protection against tetanus was found in 63% of the females, and in 78% of the males ( $p < 0.0001$ ). These differences were observed throughout all age groups. Conversely, females were significantly more likely to be protected against each of the three types of poliomyelitis (type 1: females 89%, males 85%, type 2: 81% versus 75%, type 3: 77% versus 70%, all  $p < 0.01$ ). 62% of the females and 53% of the males were fully protected against all three types ( $p < 0.001$ ). This difference was only present in the two higher age groups. Antibody levels against diphtheria did not differ significantly by gender.

For all three diseases, there was a significant inverse association between the immunity levels and age with all  $p$ -values  $< 0.0001$  in the  $\chi^2$  tests for trend (Fig. 1).

For example, in the age group  $<30$  years, 80% were fully protected against diphtheria and 82% against tetanus. These proportions decreased to 46 and 54%, respectively, in the age group  $\geq 50$  years. Protection against all three types of the poliovirus decreased significantly from the age group  $<30$  years (62%) to the age group  $\geq 50$  years (48%) ( $p < 0.0001$ ).

### 3.2. Site of residence and immunity levels

The levels of immunity against diphtheria were significantly higher in study participants who had lived in East-Berlin before 1989 as compared to those from West-Berlin. Of the latter group, 25% had no protection against diphtheria. This proportion was 13% in East-Berlin ( $p < 0.0001$ ). Such differences, however, were only found in the age groups  $<40$  years (Table 1).

Tetanus immunity was also associated with the site of residence in univariate analysis. Of the 27 individuals without any protection against tetanus ( $<0.01$  IU/ml), 26 had lived in West-Berlin prior to 1989 ( $p < 0.0001$ ). However, full protection against tetanus ( $\geq 0.1$  IU/ml) was more common in West-Berlin than in East-Berlin among individuals  $<40$  years old (Table 1).

We also found differences in poliomyelitis immunity between East-Berlin and West-Berlin. In the age group  $<40$  years the immunity levels were significantly higher in individuals from West-Berlin (Table 2). These differences were most pronounced for the poliomyelitis types 2 and 3. The immunity levels against all three types together differed significantly between participants from West-Berlin (62%) and East-Berlin (52%) ( $p < 0.001$ ).

### 3.3. Multivariate analysis

The results of the logistic regression analysis are shown in Tables 3–5. Diphtheria immunity (full protection) remained significantly higher in individuals

Table 1  
Differences in immunity levels against diphtheria and tetanus among blood donors by site of residence (East-Berlin, West-Berlin)

Age group (years)	% of participants fully protected against			
	diphtheria		tetanus	
	East- Berlin	West- Berlin	East- Berlin	West- Berlin
$< 30$ ( $n = 500$ )	86	68***	81	86
30–39 ( $n = 593$ )	74	53***	74	83**
40–49 ( $n = 500$ )	44	47	75	72
$\geq 50$ ( $n = 486$ )	45	47	61	50*

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

Table 2  
Differences in immunity levels against poliomyelitis among blood donors by site of residence

	% of participants fully protected against poliomyelitis					
	type 1		type 2		type 3	
	East- Berlin	West- Berlin	East- Berlin	West- Berlin	East- Berlin	West- Berlin
Age group (years)						
< 30 ( <i>n</i> = 500)	86	92	79	92***	64	84***
30–39 ( <i>n</i> = 593)	77	89***	72	90***	67	80***
40–49 ( <i>n</i> = 500)	88	93	72	79	76	82
≥ 50 ( <i>n</i> = 486)	90	84	69	70	76	65

\*\*\*  $p < 0.001$ .

from East-Berlin (adjusted odds ratio (OR) 1.47, 95% confidence interval (CI) 1.22–1.77) and decreased significantly by age. When the interaction term ‘age/site of residence’ was added to the regression model, living in East-Berlin was only an independent determinant of diphtheria immunity in the age group < 30 years (adjusted OR 2.98, 95% CI 1.84–4.50), and in the age group 30–39 years (adjusted OR 2.51, 95% CI 1.77–3.56) (Table 3).

After adjusting for age and gender, immunity (full protection) against tetanus was not associated with site of residence. However, females (adjusted OR 0.44, 95% CI 0.35–0.53) and older individuals were less likely to have protective antibodies against tetanus toxin (Table 4).

Immunity against all three types of poliomyelitis was associated with site of residence (living in East-Berlin: adjusted OR 0.55, 95% CI 0.46–0.67), age (age group ≥ 50 years: adjusted OR 0.48, 95% CI 0.37–0.63) and gender (females: adjusted OR 1.48, 95% CI 1.23–1.79) (Table 5).

### 3.4. Self-reported vaccinations in the previous 10 years and immunity levels

Of all participants, 17% reported that they had been vaccinated against diphtheria in the previous 10 years. The corresponding figures were 59% for tetanus and 21% for poliomyelitis. The immunity levels indicating full protection in these groups with self-reported vaccination were 78% for diphtheria, 81% for tetanus and 76% for poliomyelitis (all three types) and in the groups without vaccination in the previous 10 years 56% for diphtheria, 59% for tetanus and 51% for poliomyelitis (all  $p < 0.0001$ ). The associations between self-reported vaccinations and the actual immune status did not differ significantly by site of residence.

## 4. Discussion

The study indicates that a high proportion of the adult population in Berlin is not sufficiently protected

Table 3  
Determinants of full protection against diphtheria (antitoxin level ≥ 0.1 IU/ml) (logistic regression)

Variable (baseline)	Adjusted odds ratio for diphtheria immunity	95% CI	<i>p</i> -value
Age (< 30 years)			
30–39 years	0.50	0.38–0.66	< 0.001
40–49 years	0.22	0.17–0.29	< 0.001
≥ 50 years	0.24	0.18–0.32	< 0.001
Gender (males)			
Females	1.14	0.94–1.39	0.18
Interaction			
Site of residence: East-Berlin versus West-Berlin			
In age group < 30 years	2.98	1.84–4.50	< 0.001
In age group 30–39 years	2.51	1.77–3.56	< 0.001
In age group 40–49 years	0.87	0.61–1.24	n.s.
In age group ≥ 50 years	0.90	0.62–1.31	n.s.

n.s., not significant; CI, confidence interval.

Table 4  
Determinants of full protection against tetanus (antitoxin level  $\geq 0.1$  IU/ml) (logistic regression)

Variable (baseline)	Adjusted odds ratio for tetanus immunity	95% CI	<i>p</i> -value
Age (< 30 years)			
30–39 years	0.73	0.54–0.98	0.04
40–49 years	0.58	0.42–0.79	< 0.001
$\geq 50$ years	0.23	0.17–0.31	< 0.001
Site of residence (West-Berlin)			
East-Berlin	1.03	0.84–1.27	n.s.
Gender (Males)			
Females	0.44	0.34–0.53	< 0.001

against diphtheria, tetanus and poliomyelitis. We identified some important determinants of protection. The immunity levels against diphtheria and tetanus are continuously decreasing by age. Protection is relatively good in the age group < 30 years. However, in the participants older than 50 years, only 46% are adequately protected against diphtheria and 56% against tetanus. The immunity levels may be even lower in individuals older than 70 years who are excluded from blood donation. The selection of blood donors for the study population may have introduced some bias, e.g. the results may overestimate the immunisation levels since unremunerated blood donors may be more health conscious than other individuals. Our results are in accordance with studies from other industrialised countries. In London, 38% of blood donors had no antibodies against diphtheria toxin [12]. This proportion increased to 53% among those aged 50–59 years. In other studies from England, France and Italy, the prevalences of insufficient protection

Table 5  
Determinants of full protection against all three types of poliomyelitis (antibody titre  $\geq 1:8$ ) (logistic regression)

Variable (baseline)	Adjusted odds ratio for immunity against poliomyelitis (all three types)	95% CI	<i>p</i> -value
Age (< 30 years)			
30–39 years	0.85	0.67–1.09	n.s.
40–49 years	0.78	0.61–1.02	0.06
$\geq 50$ years	0.48	0.37–0.63	< 0.001
Site of residence (West-Berlin)			
East-Berlin	0.55	0.46–0.67	< 0.001
Gender (Males)			
Females	1.48	1.23–1.79	< 0.001

against diphtheria among adults ranged from 33 to 57% [13–15]. In a large representative study in the United States, the proportion of participants fully protected against tetanus declined to less than 50% in the  $\geq 60$  age group [16]. In another study from the United States among an elderly population, a high proportion of the participants lacked adequate immunity against tetanus and diphtheria [17].

In our total study population, the immunity against tetanus was much better than against diphtheria. This difference was most pronounced in the younger age groups in West-Berlin. General practitioners and medical doctors in the emergency departments of hospitals tend to readily vaccinate patients with wounds against tetanus and sometimes these vaccinations are not indicated. On the other hand, the physicians frequently miss these opportunities to assess the diphtheria vaccination status of their patients and to give them a booster with the Td vaccine or a primary series of vaccination against diphtheria if indicated.

Gender was independently associated with immunity against tetanus and poliomyelitis. Females were significantly less likely to be protected against tetanus. This is probably due to the fact that a high proportion of males are serving in the army as young adults where vaccination is mandatory. Moreover, males are more prone to injuries during work and leisure activities which require medical care. In this context, tetanus booster vaccinations are common. Another study from West-Germany found an even larger difference in tetanus immunity between females and males [18]. Conversely, females were more likely to have full protection against poliomyelitis. They may have been more compliant to the oral vaccination programmes which have been regularly offered by the public health services.

We found striking differences between individuals from East- and West-Berlin with respect to diphtheria immunity [8]. The diphtheria antitoxin prevalence was significantly higher among individuals from East-Berlin. However, the stratified and multivariate analysis showed that these differences only existed in the two younger age groups. This finding is explained by the different vaccination strategies in East- and West-Germany. In East-Germany, vaccination against diphtheria and tetanus was mandatory for young children and a mandatory booster vaccination was performed at age 8. This resulted in relatively high immunity levels in the age groups up to 40 years. Booster vaccinations for adults were not performed because the Td vaccine was not available. To maintain long-lasting immunity, a booster vaccination in adulthood appears to be necessary for many individuals. In West-Berlin, there is evidence that a relatively high coverage of over 90% of the primary vaccination

series against tetanus, diphtheria and poliomyelitis was achieved in young children [19]. However, booster vaccinations in later childhood were only recommended but not systematically performed. This may explain the relatively low immunisation levels against diphtheria in the participants from West-Berlin.

Immunisation coverage against all three types of the poliomyelitis virus was higher in individuals from West-Berlin in the age group <50 years. A previous seroepidemiological multicentre study in Germany revealed substantial regional variations of immunity levels [20]. In accordance to our results, in some of the study centres in East-Germany relatively low immunity levels were found. This may also be due to the differences in the vaccination policies. In East-Germany, the basic vaccination schedule against poliomyelitis in early childhood consisted of three doses of monovalent vaccine only which was given consecutively against type 1, type 2 and type 3. Only for the booster vaccinations at age 2 and 8 the trivalent vaccine was used. This may not have sufficed to achieve long-term immunity. In West-Germany, three trivalent doses are given to children in the first year of life on a voluntary basis, and booster vaccinations are recommended at age 10. Until 1990 there were public vaccination campaigns against poliomyelitis and the oral vaccine was provided free of charge to the general population by the public health services. These programmes probably reached high proportions of older children and younger adults as indicated by the relatively high immunity levels against poliomyelitis in this group in West-Germany [21].

Interestingly, a low proportion of the individuals reported that they had been vaccinated against diphtheria and poliomyelitis in the previous 10 years. Overall, the self-reported vaccinations in the previous 10 years correlate well with the actual levels of immunity. However, these associations are not very strong and a substantial percentage of those who stated that they had been vaccinated in the previous 10 years are not adequately protected against the respective diseases. One reason may be incorrect recall of the date and the type of the previous vaccinations. Thus, data on self-reported vaccinations cannot be regarded as valid proxy measures for the actual immunisation levels. Another possible explanation is that some of the individuals vaccinated with a single dose in the previous 10 years had never received a full primary series of vaccination.

The results of our study show the need to improve the immunity levels for each of the three diseases. The situation is particularly worrying for diphtheria where 18% of the population are not protected at all and a further 22% have uncertain protection. The data from East-Berlin also show that mandatory vaccinations in childhood may lead to relatively good immunisation

levels in younger adults. Immunity wanes, however, with age and an effective vaccination policy for adults is needed. With respect to poliomyelitis it may be sufficient to booster only specific groups at risk (e.g. travellers to developing countries) because no autochthonous cases of poliomyelitis have been reported in Germany in recent years. Systematic booster vaccinations for both tetanus and diphtheria (Td), should be organised for those who had received a full primary series of three doses. Moreover, individuals who never received an adequate series of primary immunisation should be identified when they are in contact with health care personnel. In Germany as probably in many other industrialised countries, general practitioners could play a major role in this effort. This would require specific training of medical students in vaccination issues which is lacking so far and continued information and training of medical doctors. This should be accompanied by public campaigns including the mass media to increase the awareness of the general population for the necessary vaccinations.

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