Diphtheria, pertussis, and tetanus serosurvey in Saudi children

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ABSTRACT

Objective: To evaluate immune protection against vaccine-preventable diseases targeted by the Expanded Program of Immunization in Saudi Arabia.

Methods: The study was carried out from September 2001 to February 2002. Using multistage sampling techniques, samples were collected from 5 regions of Saudi Arabia and sent for laboratory assay from the following age groups; 50 samples at 12 months, 50 at 6 years, and 100 at 17 years. Sera were assayed for diphtheria, tetanus, and pertussis. Sero neutralization was used for anti-diphtheria antibody assay, while enzyme linked immunoassay was used for anti-tetanus, anti-filament hemoagglutination (anti-FHA), and anti-pertussis titer (anti-PT) antibody assay.

Results: This survey showed that 100% of children had protection levels (≥0.01 IU/ml) against diphtheria at one year, 100% at 6 years, and 93.7% at 17 years. For tetanus, 95.9% had protection levels (≥0.1 IU/ml) at one year, 100% at 6 years, and 98.9% at 17 years. The geometric mean titer (GMT) of anti-FHA is 22 at one year, 29 at 6 years, and 24 IU/ml at 17 years, while the GMT of anti-PT is 36 at one year, 18 at 6 years, and 11 IU/ml at 17 years.

Conclusion: Children at one, 6, and 17 years are well protected against diphtheria, pertussis, and tetanus.

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Serologic data on diseases that are preventable by vaccines are useful in evaluating the success of immunization programs, and can help to identify susceptible subgroups. The presence of serum antibodies does not always mean that immunity exists, but reflects a previous encounter with microorganisms. So, immunity evaluation in serological surveys depends on a predefined level for protection, but this protective level is defined somewhat arbitrarily. Diphtheria is a bacterial disease in which the clinical manifestations result from the action of an exotoxin produced by *Corynebacterium diphtheriae*. Immunity to Diphtheria is antibody-mediated and depends primarily on antibodies against the toxin. Although there are many techniques to measure diphtheria antibodies, neutralization and enzyme-linked immunoassay (ELISA) are the common methods used. A circulating antibody level of ≥0.01 IU/ml determined by neutralization test in animal or cell culture is believed to be protective, and can provide clinical immunity against the disease.[1] According to data collected from outbreaks and in some studies that used *in vitro* techniques, a level of 0.01-0.09 IU/ml is considered to give basic protection while 0.1 IU/ml is considered protective.[2] Tetanus is caused by the action of a highly potent neurotoxin, which is produced during the growth of the anaerobic bacterium *Clostridium tetani*. Tetanus vaccine as a mono vaccine or in combination (diphtheria, pertussis, tetanus [DPT]), is produced by the inactivation of the tetanus toxin by formaldehyde to produce tetanus toxoid. Immunity to tetanus is produced only by immunization. To measure immune response *in vitro*, passive hemagglutination, ELISA or radioimmunoassay is used. The ELISA is the most commonly used method in serological surveys. A level of 0.01 IU/ml is considered as the minimum protective level in surveys, although there are some reports of tetanus occurring in persons with antitoxin levels greater than 0.01 IU/ml.[3,4] Recent studies on the immunochemistry of *Bordetella pertussis* (*B.*

pertussis) have resulted in a better understanding of the multiple biological activities and the pathogenesis of the organism. These studies also resulted in isolation and characterization of several biologically active substances and demonstrated that they are the determinant of immunity following disease or vaccination. Antigens of pertussis include, pertussis toxin (PT), adenylate cyclase (AC), filamentous hemagglutinin (FHA), agglutinogens (AGG), lipopolysaccharide endotoxins (LPS) and others. There is no reliable measure of immunity to pertussis, although ELISA is used widely in serological surveys with no definite protection level. Vaccination with the whole cell vaccine results in an increase in the ELISA antibody titers to all known antigens of *B. pertussis*.[5] Serological surveys are important tools to generate antibody prevalence data for national immunization policy evaluation and to discover and fill gaps or windows of susceptibility between different age groups. This paper will present the results of the DPT serological survey that was carried out during the year 2002 in different age groups in Saudi Arabia. Measles, mumps, and rubella results were published earlier.[6]

Methods. The study was approved by the Ministry of Health Ethical Committee, and the study was explained and informed consent was collected. In a cross-sectional study, 100 children at the age of 6 months, 12 months, 18 months, 6 years, 13 years, and 17 years were recruited to evaluate antibody levels against diseases targeted by the immunization.[6] A multistage sampling technique was used to draw the sample. In the first stage: The Kingdom was divided into 5 regions: northern, southern, eastern, western, and central regions. In each region, one health province was selected randomly. The following provinces were selected: Tabuk (northern), Gizan (southern), Al-Gatif (eastern), Madina (western) and Al-Qassim (central). In the second stage, 20 primary health care (PHC) centers and schools in their vicinity were selected randomly. Sample distribution in each region was carried out according to proportion of total population living in that region: 5 PHCs from Qassim, 2 from Tabuk, 3 from Gazan, 3 from Gatif, and 7 from Madinah. In the third stage, for each PHC, 5 blood samples from each age group: 6 months, 12 months, 18 months, 4 years, 6 years, 13 years, and 17 years, were collected. For DPT antibody assay, a sub-sample of 50 at the age of 12 months, 6 years, and 100 at the age of 17 years were selected randomly. Primary DPT vaccination was given at 2, 4, and 6, months. Children aged 6 years were given 2 boosters, at 18 months, and preschool. Fieldwork was conducted during the period September 2001 to February 2002. Sera were sent to the Sanofi-Pasteur laboratory in France for DPT antibody assay. The ELISA was used for laboratory assay of pertussis (anti FHA and anti PT) and anti-tetanus antibody. Sero neutralization was used for laboratory assay of anti-diphtheria antibody. Children with anti-diphtheria antibody level less than 0.01 IU/ml are considered susceptible, while 0.01-<0.1 IU/ml is considered the basic protection range. Full protection is achieved if they have a level of ≥0.1 IU/ml. The cutoff level for tetanus protection is ≥0.1 IU/ml.

Data were entered and analyzed by the Statistical Package for Social Sciences, version 13. Results are presented as proportion of children with a cutoff point of protection and as geometric mean titer (GMT) value for each age group.

Results. **Table 1** shows the distribution of children according to different levels of immunity against diphtheria. The highest level of protection is recorded at the age of 6 years compared to other age groups (p=0.0001). Adding the second and the third category together shows that 100% have protective levels (≥0.01 IU/ml) against diphtheria at one year, 100% at 6 years, and 93.7% at 17 years. The GMT of anti-diphtheria antitoxin is 0.1 IU/ml at one year, 2.3 IU/ml at 6 years, and 0.12 IU/ml at 17 years. The GMT (of anti-diphtheria, antitoxin) at 6 years is significantly higher than the other 2 groups (p=0.0001). The proportion of children with protective level (≥0.1 IU/ml) against tetanus exceeds 95% in all age groups without any significant differences (**Table 2**), but GMT was significantly higher in the 6 years age group compared to the other age groups. (p=0.0001). A significant decrease in anti-PT is noticed with the highest rate at the age of one year. This is not observed in the anti-FHA (p=0.0001) (**Table 3**).

Discussion. Comparing our results with other studies should consider several factors that include: schedule, the potency of the vaccine used, the time since the last dose, and the age at vaccination. Serum

Table 1 - Proportion of children with different protection ranges against diphtheria in different age group.

Protection IU/ml	Age (year) n (%)		
	1	6	17
<0.01	-	-	6 (6.3)
0.01 - <0.1	30 (61.2)	5 (10)	34 (35.8)
≥0.1	19 (38.8)	45 (90)	55 (57.9)
Total	**49**	**50**	**95**

Table 2 - Proportion of children with tetanus protection level 0.1 IU/ml.

Parameter	Age groups (year)			Total n (%)	*P* value
	1	6	17		
≥0.1 IU/ml n (%)	47 (95.9)	49 (100.0)	93 (98.9)	**189 (98.4%)**	0.229
Mean	1.983367	18.625245	2.850137	**6.635155**	
Total number	49	49	95	**193**	
Standard deviation	3.1777459	45.8056669	2.7048922	**24.0820822**	
Geometric mean	0.977389	4.903500	1.831667	**2.005250**	0.0001

Table 3 - Mean antibody levels against filamentous hemagglutinin (FHA) and pertussis titer (PT).

Age	FHA	PT
1 year		
n	50	50
M±SD	57.48±107.026	127.44±239.052
Geometric mean	22.55	36.42
6 years		
n	50	50
M±SD	66.12±87.993	46.18±60.601
Geometric mean	29.84	18.51
17 years		
n	95	95
M±SD	60.38±108.185	34.52±63.358
Geometric mean	24.89	11.02
Total		
n	195	195
M±SD	61.11±102.616	61.33±137.274
Geometric mean	25.43	17.10
p-value	0.912	0.0001

diphtheria anti-toxin after vaccination shows a steep decline, immediately after vaccination, followed by an exponential fall-off. Comparing studies carried out in the 1940-50s then 1980s shows that the anti-diphtheria anti-toxin level in school children has been steadily declining, although the number of doses of diphtheria vaccines administered remained the same. Tetanus antitoxin concentration does not show such decline. The current lower diphtheria immunity among school children compared to earlier years may be due to less exposure.[7]

In a study from the early 1990s, 97.5% (153/157) of Saudi infants at 6 months of age and after one month from the third DPT dose showed a protection level of ≥0.01 IU/ml against diphtheria.[8] Another study in the same age group showed a similar proportion of protection, (98%).[9] In the same age group (6 months), the diphtheria component of Federal Drug Association DPT formula (12.5 Lf), although it contained only 50% of the World Health Organization (WHO) formula (25 Lf), gives a 100% protection of ≥0.1 IU/ml compared to 86.8% in the WHO DPT formula.[10] Tetanus antitoxin response and duration of immunity depends on the number of doses and age of vaccination. In our results, **Table 2** shows that at the age of one year a drop to below 0.1 IU/ml protective level can occur, 4% in our study. As for GMT, at one year after 3 doses, our results (0.97 IU/ml) are comparable to other studies.[11] Previous studies in Saudi Arabia showed 100% protective level (≥0.1 IU/ ml) at the age of 6 months, one month after the third dose.[8,9]

After the third dose, each additional dose given within at least one-year intervals prolongs the duration of immunity. Immunity will last for 10 years after the fourth dose and for 20 years after the fifth. This is also reflected in our results, as 98.9% at age 17 years still have >0.1 IU/ml, although most probably they did not receive any dose after the fifth dose given at the age of 6 years.

When we compare our results with other studies, we have to consider that there are no reliable methods to measure immunity to pertussis. Also, whole cell pertussis vaccine from various manufacturers differs considerably in stimulating production of antibodies.[12] **Table 3** shows the decrease in mean anti-PT antibodies from one to 17 years. It is lower at 6 years, after 5 doses, compared to one year, after only 3 doses. This is different from anti-FHA antibodies where the level at 6 years is the highest compared to one or 17 years. We have no explanation for the decline in PT with age, particularly, why PT is lower at the age of 6 years compared to one year.

This study shows the importance of continuous monitoring of the immunity of children and adults. Children at school entry are fully protected against DPT, while adolescents at the age of 17 years need further attention. Booster doses against diphtheria and tetanus may be needed before leaving the secondary schools or before entering university level. Our results support the need to study the expanding program of immunization in Saudi Arabia to cover older age groups.

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