Seroprevalence of Pertussis Antibodies among Adolescents in Israel

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Abstract

Background: There is an increasing number of reports of pertussis among older children and adults. The development and licensure of an acellular pertussis vaccine offer the possibility of adult vaccination against the disease. Information on immunity to pertussis in this age group is needed before any vaccination policy can be considered.

Objectives: To study the seroepidemiology of pertussis antibodies in a random sample of adolescents.

Methods: Serum IgG antibodies to whole-cell lysate of *Bordetella pertussis* were measured by enzyme-linked immunosorbent assay in sera of 533 Israeli military recruits aged 17–18 years. Epidemiologic variables were collected by a questionnaire and analyzed for correlation with pertussis antibodies.

Results: Of the sera tested 58.6% were positive for pertussis IgG antibodies, while 35.4% were negative and 6% were borderline. The seropositivity rate was significantly higher among females and non-smokers than among males and smokers. Serum samples of subjects found negative to *Bordetella pertussis* on recruitment were tested again, using the same ELISA assay, 2–3 years later. Seroconversion during the 3 year military service was detected in 12.5% of 40 subjects. Using the pertussis toxin as the antigen in a subsample of 160 sera, the seroprevalence was lower than that detected by the whole-cell lysate on the same sera (45% vs. 58%).

Conclusions: A significant part of the adolescent population in Israel has low titer of serum IgG antibodies to the multiple antigens of *B. pertussis*. The relatively low concentration of anti-pertussis antibodies, together with the serological evidence of exposure to the disease indicates that booster immunization with the acellular pertussis vaccine of military recruits should be considered after more information on the incidence of clinical cases of pertussis will be available.

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Pertussis is an endemic and epidemic disease that affects all age groups in countries where the immunization rate is low as well as in countries where high levels of immunizations exists [1,2]. There is an increasing number of such infections reported in older children and adults [3–5]. Still, the most vulnerable age group is young infants who may develop severe complications including pneumonia, encephalopathy and seizures, that may result in death. In the last few years evidence has accumulated showing significant morbidity in adults as well. In this age group there is no typical course of the disease, and the most common manifestations are prolonged cough along with other signs and symptoms of upper respiratory tract infection. In several reports, 12–32% of adults with prolonged cough have been diagnosed as having pertussis [6]. They play an important role in the transmission of pertussis to infants, and are considered to be the main reservoir of the organism.

Seroepidemiological studies show that the immunity level, as induced either by the whole-cell vaccine or after natural infection, wanes over time [7]. High immunity lasts about 3 years, and then gradually declines for 12 years [8]. In spite of the growing recognition of the importance of pertussis in adults, the whole-cell vaccine cannot be combined as a booster with diphtheria and tetanus toxoids because of the relatively high rates of adverse effects. The acellular vaccines containing one or more of the main components of *Bordetella pertussis* are currently in clinical use in various countries including the U.S. Four acellular vaccines are already approved for use in infants [9]. These were found to be immunogenic in adults, exhibiting significantly less local and systemic side effects [10].

Waning immunity, the increasing recognition of pertussis as an important cause of adult respiratory diseases, and the role of adults in transmitting the disease to infants and children indicate a potential advantage in providing the new vaccine to adolescents and young adults.

In Israel, pertussis is common despite a high rate of immunization (over 90%) [11] with the whole-cell vaccine. The acellular vaccine is not yet in clinical use, and susceptible age groups that can be a target for such a vaccine are not yet defined. Since Israel Defense Force recruits (18-

ELISA = enzyme-linked immunosorbent assay

year-old males and females) may be inadequately protected, an important question is whether the acellular vaccine should be combined with dT as a booster for new army recruits. The result of introducing the acellular vaccine may lower the respiratory morbidity in adolescents and young adults as well as in other age groups. The aim of this study was to determine the seroprevalence of pertussis antibodies among adolescents, who are a possible target population for future immunization with the acellular pertussis vaccine.

Materials and Methods

Data collection

The IDF Medical Corps continuously draws a systematic, representative blood sample of male and female recruits for routine surveillance of immunity to infectious diseases. At the time of enrollment these recruits undergo a short interview and are asked to give a blood sample. At discharge from service the participants are identified and undergo a similar process. Relevant questions in the interview relate to demographic data, including age, gender, and country of birth of the recruit and his/her parents. All interviews are performed by nurses of the IDF Army Health Branch. Missing data and additional information are retrieved from the central computerized military personnel file. The entire process has been authorized by the IDF Medical Corps Committee for Research on Human Subjects, and a signed informed consent is obtained from each participant. It was not obtained specifically for this study.

The induction into military duty is compulsory in Israel for Jewish men and women. About 30% of women are exempt from service, mainly due to religious reasons. Among men, about 90% are recruited. Therefore the sampling frame used among the recruits is more representative of the total Jewish male population with a possible selection bias among the females. The country of origin was defined by the father's birthplace (or, when this was Israel, the paternal grandfather's birthplace). Europe (excluding Turkey), the Americas, Australia and South Africa were defined as West. All other birthplaces, except Israel, were mentioned separately.

Laboratory procedure

Blood samples were drawn from a random sample of Israeli young adult male and female military recruits and were allowed to clot at room temperature for one hour, then cooled in a refrigerator for 1–2 hours before being centrifuged. Sera were stored at -20°C until tested for IgG antibodies to pertussis. The concentration of IgG antibodies to *Bordetella pertussis* was measured by ELISA using a commercial kit (InVitro Diagnostika GmbH, Germany). Briefly, microtiter wells as a solid phase were coated with a lysate of *B. pertussis*, and sera at a dilution of 1:100 were then added to the wells. After five washing steps to remove unbound material, anti-human IgG conjugated to peroxidase was added. The microwells were rewashed (five cycles) and a colorless substrate system (TMB/H2O2) was added. The substrate was hydrolyzed to a blue color, which changes to yellow after stopping the reaction by sulfuric acid. The optical density at 450 nm, which was directly proportional to the amount of anti-pertussis lysate IgG antibodies in the specimen, was measured by an ELISA microtiter plate reader (EL 340, Biotek Instruments Inc., Winooski, USA). Each serum was tested in duplicate and the average OD result was calculated in U/ml according to a fixed factor determined from a positive and a negative control. Duplicates were analyzed only when the measured optical density difference was less than 10%. As recommended by the manufacturer, a value below 60 U/ml was interpreted as negative, between 60 and 70 U/ml as borderline, and above 70 U/ml as positive. The sensitivity and specificity of the kit provided by the manufacturer are 98% and 85% respectively.

Forty samples of subjects found negative for *B. pertussis* IgG antibodies upon enrollment to the IDF were tested again (using the same ELISA assay) upon their release 2–3 years later, to estimate the rate of seroconversion during their service.

A total of 160 random sera were tested in parallel by ELISA for IgG antibodies to *B. pertussis* lysate antigen and pertussis toxin as the sole antigen, using a second commercial kit from the same company. The sensitivity and specificity for the second kit are 85% and 93% respectively.

Statistical analysis

The Chi-square test was used to evaluate statistical significance of differences between proportions in the univariate analyses. Multiple logistic regression analysis was used to determine correlates of pertussis antibodies while controlling for potentially confounding factors.

Results

A representative sample of 567 recruits who joined the Israel Defense Forces between 1 January and 31 December 1993 were included in the study. All were born between 1974 and 1975.

The sera of 332 (58.6%) of the subjects were positive for pertussis IgG antibodies, while 201 (35.4%) were negative. Excluded from further analysis were 34 subjects (6%) who had borderline levels to pertussis IgG antibodies. After exclusion, the study sample comprised 215 females and 318 males.

Factors associated with seropositivity by univariate analysis are summarized in Table 1. The seroprevalence of anti-pertussis antibodies was substantially higher in female than in male recruits (odds ratio 1.79, 95% confidence interval 1.24–2.58). A strong association was also found between smoking and positive pertussis antibodies (OR for non-smokers vs. smokers 1.54, 95% CI 1.06–

dT = diphtheria and tetanus toxoids
IDF = Israel Defense Force

Table 1. Seroprevalence of pertussis IgG antibodies by socio-demographic variables

Variable	No.	% seropositivity
Study population	533	62.29%
Gender		
Females	215	70.2%
Males	318	56.9%
P value	0.002	
Smoking status		
Non-smokers	368	65.5%
Smokers: 1–10 cigarettes/day	105	59.05%
Smokers: >10 cigarettes/day	60	48.33%
P value	0.029	
Ethnicity		
African origin	113	56.6%
Asian origin	131	64.9%
Israeli origin	29	41.4%
Western origin	253	65.5%
P value	0.037	

2.24). The proportion of seropositivities was related to the number of cigarettes smoked a day.

No association was found between the level of pertussis antibodies and either the number of siblings or parental education (number of school years completed by the father). However, the relationship between soldiers' education (number of school years) and immunity was significant ($P=0.024$); immunity increased with the number of school years. There was no association between the subjects' country of birth and pertussis immunity. However, a significant correlation was found between ethnic background and immunity (OR for western origin vs. Israeli origin 2.79, 95% CI 1.24–5.92).

Factors tested in the univariate analysis were entered into a logistic regression model, with dichotomous dependent variable — absence versus presence of anti-pertussis antibodies. Both gender and smoking remained significantly associated with seropositivity, while education lost its association with the presence of anti-pertussis antibodies.

Forty soldiers that had negative results at induction were tested again at discharge 2–3 years later. The assay used to assess seroconversion employed the whole-cell lysate as antigen. Eight of them (12.5%) showed seroconversion during their military service.

A total of 160 serum samples were tested in parallel for antibody response to the whole-cell lysate and to pertussis toxin as the sole antigen. Borderline results were excluded from analysis. The prevalence of antibodies to pertussis toxin was lower as compared with that determined against the whole-cell lysate (45%). The correlation between the results is summarized in Table 2. Of 143 samples, 47 and 52 were found negative and positive, respectively, by both assays. Moreover, 31 sera defined as positive by the whole-cell lysate were detected negative by the pertussis toxin assay, and 13 sera negative by the whole-cell lysate assay were positive by the pertussis toxin assay.

Table 2. the correlation between measuring serum pertussis antibodies against bacterial lysate and against pertussis toxin

Bacterial lysate	Pertussis toxin		
	Negative	Positive	All
Negative	47	13	60
Positive	31	52	83
All	78	65	143

Discussion

The results of this study indicate that a significant proportion of the Israeli adolescent population has inadequate antibody levels to *Bordetella pertussis* and therefore is probably unprotected against the disease. Indeed, the seroconversion detected suggests that exposure to pertussis occurred in this age group. Although the mechanism of protective immunity against *B. pertussis* has not yet been elucidated, and may involve humoral as well as cell-mediated immunity [12], on a population basis these serum anti-pertussis antibodies are still considered the best surrogate measure of immunity. Two recent studies [13,14] identified antibody to pertactin as being the most important, and both found that antibodies to pertussis toxin and fimbriae contributed to protection. However, antibody to filamentous hemagglutinin did not contribute to protection.

Pertussis infection has been reported to be more common among girls [3]. This gender difference was also found in advanced age [15]. In our study, the seroprevalence was significantly higher among females, suggesting either a better response to immunization or a higher incidence of infection during the adolescent period. The number of cigarettes smoked was associated with the level of immunity against pertussis, an observation that was also noted regarding immunity to measles [16]. Smoking was also related to a more rapid decline in influenza antibody titers after infection or vaccination with the influenza virus [17]. These findings can be explained by several laboratory studies revealing that smoking may lead to T cell anergy [18], suppression of the humoral response to inhaled antigens, and other alterations in B and T cell function [19].

Comparing the serological response to pertussis lysate and pertussis toxin, we found a lower prevalence of antibodies to pertussis toxin. The assay using the whole-cell lysate as antigen is expected to detect antibodies to all the *B. pertussis* antigens and therefore to be more sensitive than the pertussis toxin assay. This probably explains why 31 sera that were defined positive by the whole-cell lysate assay were found to be negative by the pertussis toxin assay. Since the specificity of the lysate assay is 85%, we cannot rule out the possibility that part of the seropositives may be the result of cross-reacting antibodies with other gram-negative organisms. The data showing that 13 sera negative by the whole cell lysate were positive by the pertussis toxin assay are more difficult to explain, although they may represent cross-reacting antibodies to proteins other that those present in the pertussis whole-cell lysate.

In a study evaluating pertussis in U.S. Marine Corps trainees [20], the ELISA IgG antibody to fimbriae 2/3 was the assay most likely to show changes between acute-phase and convalescent-phase samples. The authors suggested that this test was more useful than measuring antibodies to pertussis toxin for diagnosing pertussis in adults. We therefore believe that the antibody levels to the cell lysate (which also contains the pertussis toxin) is more relevant to the general estimation of immunity and infection in adolescents.

Data from pertussis outbreaks suggest that immunity to the whole-cell vaccine wanes 7–12 years after completion of immunization [7]. It is feasible that asymptomatic exposure or symptomatic infection occurs even earlier than 18 years, since the last booster dose of pertussis is given in Israel at 12 months.

To define the seroepidemiology of pertussis infection in Nashville, TN, levels of antibody to pertussis toxin and filamentous hemagglutinin were tested by enzyme immunoassay in 585 serum samples from healthy subjects aged 1 to 65 [21]. The results demonstrated a peak in PT and FHA titers in the 4 to 6 year age group, concurrent with the administration of a booster dose of pertussis vaccine. A second larger peak was noted in the 13 to 17 year age group, however, suggesting that pertussis infection is frequent during the adolescent period.

Our results indicate that 12.5% of the soldiers who were seronegative for pertussis at enrollment seroconverted during their military service. This suggests that *Bordetella pertussis* could be an important cause of upper respiratory morbidity in this age group. However, our findings are based on a small group of subjects, and further study is needed to validate this conclusion. Moreover, these results provide only an estimate of exposure to *B. pertussis* during military service and are not necessarily evidence of symptomatic infection. Information on pertussis-associated symptoms during military service is not required or collected at discharge from the army.

The evidence for waning immunity, the recognition that *Bordetella pertussis* is an important causative agent of respiratory infections in young adults, and the role of adults as a reservoir for transmission of the disease to infants and children, all point to the potential benefit of immunizing adults. Vaccination of adults with acellular pertussis vaccine is well tolerated and is highly immunogenic [10].

In Israel, the compliance to infant immunization is high. Therefore, it seems logical to decrease infection rates by reducing the reservoir. A booster of anti-tetanus and anti-diphtheria to adolescents might reduce morbidity among both young adults and other age groups. To reach a decision regarding the necessity for a booster injection of an acellular pertussis vaccine in young adults, data on the incidence of clinical cases of pertussis in this population may also be needed. In addition, the safety, immunogenicity, and efficacy of acellular pertussis vaccine in young adults should be further studied before implementation of the vaccine in this age group.

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