Seroprevalence of pertussis antibodies in 6–17-year-old students in Ahvaz, south-west Islamic Republic of Iran

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الانتشار المصلي لأضداد السعال الديكي لدى الطلاب بأعمار 6-17 عاماً في الأهواز، بجنوب غرب جمهورية إيران الإسلامية

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الخلاصة: على الرغم من أن السعال الديكي مرض مُعْدٍ يمكن توقيه باللقاح، فإن المناعة المُحدَثة باللقاح لا تبقى مدى الحياة، وينصح بإعطاء جرعات معزِّزة وفقاً للوبائيات الوطنية للمرض. لقد كان الهدف من هذه الدراسة تقييم مستويات الغلوبولين المناعي G للسعال الديكي لدى طلاب سن المدرسة في الأهواز، بجنوب غرب جمهورية إيران الإسلامية. ففي دراسة وصفية مستعرضة تم الحصول على عينات دم من 640 طالباً (382 من الفتيان و 258 من الفتيات) بأعمار 6-17 سنة خلال العام الدراسي 10-2011. وكان جميع الطلاب قد تلقوا مقرَّراً كاملاً من التلقيح ضد السعال الديكي بلقاح كامل الخلية في أعمار 2 و4 و6 و18 شهراً و4-6 سنوات. وباستخدام عتيدة مُقَايَسَةُ المُمْتَزِّ المَناعِيِّ المُرْتَبِطِ بالإِنْزِيْم للغلوبولين المناعي G للبورديتيلة تم الكشف عن الغلوبولين المناعي G للسعال الديكي لدى 301 (47.0٪) من الطلاب. ولم توجد فروق ذات دلالة إحصائية في مستويات الغلوبولين المناعي G للسعال الديكي بين الفتيات والفتيان أو بين الفئات العمرية المختلفة. وأظهرت النتائج أن المستوى العام لإيجابية المصل بالنسبة للغلوبولين المناعي G الخاص بالسعال الديكي لم يكن مقبولاً. مما يبيّن ضرورة التفكير بتلقيح معزِّز بأحد لقاحات السعال الديكي اللاخلوية لدى المراهقين و/ أو البالغين في منطقتنا.

ABSTRACT Although pertussis is a vaccine-preventable infection, vaccine-induced immunity is not lifelong and booster doses are recommended according to national disease epidemiology. The aim of this study was to evaluate pertussis-IgG levels in school-aged students in Ahvaz, south-west Islamic Republic of Iran. In a descriptive, cross-sectional study, blood samples were obtained from 640 students (382 boys and 258 girls) aged 6–17 years during 2010–2011. All students had received a full course of pertussis whole-cell vaccination at ages 2, 4, 6 and 18 months and 4–6 years. Using a *Bordetella* IgG ELISA kit, pertussis-IgG was detected in 301 (47.0%) students. No statistically significant differences in pertussis-IgG levels were found between girls and boys or across different age groups. The findings show that the overall level of pertussis-IgG seropositivity was unacceptable. Booster vaccination with an acellular pertussis vaccine should be considered in adolescents and/or adults in our region.

Séroprévalence des anticorps de la coqueluche chez des écoliers âgés de 6 à 17 ans à Ahvaz (sud-ouest de la République islamique d'Iran)

RÉSUMÉ Si la coqueluche est une infection évitable par la vaccination, l'immunité induite par le vaccin ne dure pas toute la vie et des doses de rappel sont recommandées en fonction de l'épidémiologie de la maladie dans le pays. L'objectif de la présente étude était d'évaluer les taux d'IgG anticoquelucheux chez des écoliers à Ahvaz (sud-ouest de la République islamique d'Iran). Dans une étude transversale descriptive, des échantillons de sang ont été prélevés chez 640 écoliers (382 garçons et 258 filles) âgés de 6 à 17 ans en 2010 et 2011. Tous les écoliers avaient reçu un cycle complet du vaccin anticoquelucheux à germes entiers à l'âge de 2, 4, 6 et 18 mois puis à 4–6 ans. À l'aide du kit ELISA spécialisé, des IgG anti-*Bordetella pertussis* ont été dépistés chez 301 écoliers, soit 47,0 %. Aucune différence statistiquement significative n'a été observée entre les taux d'IgG anticoquelucheux des filles et des garçons ou entre les différents groupes d'âges. Ces résultats révèlent que le taux global de séropositivité aux IgG anticoquelucheux est inacceptable. Une vaccination de rappel au moyen d'un vaccin anticoquelucheux acellulaire doit être envisagée chez les adolescents et/ou les adultes de notre région.

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Received: 22/07/13; accepted: 07/05/14

Introduction

Pertussis or whooping cough is a highly contagious, vaccine-preventable respiratory disease caused by *Bordetella pertussis* and less frequently by *B. parapertussis*. The illness occurs worldwide and affects all age groups *(1)*. National immunization programmes against pertussis, which were introduced in many countries in the mid-1950s, have dramatically decreased the incidence and complications of pertussis in children *(2)*. Despite universal pertussis vaccination in many countries, the circulation of *B. pertussis* has not been eliminated. There are reports from a large number of countries that the incidence of pertussis is increasing in adolescents and adults due to waning immunity *(3–7)*. These patients are a source of infection for infants and young children *(3)*.

Several explanations has been proposed for the re-emergence of pertussis in vaccinated children, including poor vaccine quality, improved diagnostic facilities, increased awareness of the disease and waning of vaccine-induced immunity *(4,8)*. The immunity following vaccination is not lifelong and it gradually decreases over 10–12 years *(9)*. Booster vaccination by an acellular vaccine has been recommended in some countries such as France, Germany and Canada. In 2006, the American Academy of Paediatrics published their recommendations for pertussis booster vaccination *(10)*.

Immunization by the triple diphtheria, tetanus and whole-cell pertussis vaccine has been applied in the Islamic Republic of Iran since the 1950s *(11,12)*. The vaccine is administered at the 2nd, 4th and 6th months of life, with booster doses at 18 months and between 4–6 years of age. Since recommendations for pertussis booster vaccination depend on the national disease epidemiology, we aimed to determine the seroprevalence of pertussis antibodies among school-aged children. The prevalence of pertussis antibody in different age groups has not been adequately studied in the Islamic Republic of Iran and the results of this study, together with future studies in other parts of the country, could help to inform policy decisions about pertussis booster vaccination.

Methods

Study design

In a descriptive, cross-sectional study blood samples were obtained from primary-school, junior high-school and high-school students aged 6–17 years in Ahvaz, south-west Islamic Republic of Iran from October 2010 to June 2011.

Sampling

We used the following sample size determination formula for estimating a proportion to calculate the required sample size, in which $\alpha = 0.05$, $P = 0.7$ (based on previous studies), $d = 0.05$ and design effect = 1.93 were considered. Finally, a sample of 623 students was estimated for the survey.

A multi-stage, stratified, cluster sampling design was used, in which at the first stage we classified all schools in 6 strata by sex of students and type of schools. Then, proportionally to size, 18 schools were randomly selected as the clusters. At the next stage, using simple random sampling, 1/3 of classes in each chosen school were selected. Finally, using systematic random sampling, 1/6 of students in each chosen classroom were selected for the study. Students with a history of chronic cough or evidence of acute respiratory tract infection were excluded from the study.

Before sampling, written informed consent was obtained from the students' parents. The study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences.

Data collection

All students had received a full course of pertussis whole-cell vaccination (Razi Institute of Iran) at the ages of 2, 4, 6 and 18 months and 4–6 years. Confirmation of vaccination history was done by observation of each student's vaccination card. The clinical information was collected using a questionnaire to students' parents for young children or by the students themselves in high school.

For the serological study, a venous blood sample of 3–5 mL was drawn from each child, centrifuged and stored at –20 °C until assayed. Immunoglobulin G (IgG) antibodies against a mixture of *B. pertussis* antigens (endotoxin, filamentous haemagglutinin and pertussis toxin) were measured using a commercially available enzyme-linked immunosorbent assay kit (IBL International GmbH).

According to the manufacturer's instructions for qualitative evaluation, the cut-off index was calculated from the mean optical densities (OD) of the sample and cut-off value. Samples with an OD within a range of 20% around the cut-off value (grey zone) are considered as borderline, samples with higher ODs are positive and samples with lower ODs are negative. Antibody levels > 24 U/mL were considered as positive, < 16 U/mL as negative and 16–24 U/mL as equivocal. Equivocal samples were rechecked and classified as positive or negative. Antibody levels are shown as geometric mean titres (GMTs).

Data analysis

The data were analysed using *SPSS* software, version 18.00. The chi-squared and Fisher exact tests were used for analysis. $P < 0.05$ was considered as statistically significant.

Results

Out of a total of 640 students, 258 (40.3%) were girls and 382 (59.7%) boys. The mean age of students was 12.1 (standard deviation 3.18) years, range 6–17 years. The participants

Table 1 Frequency of pertussis antibody seropositivity in children and geometric mean titres, by sex and age group

Variable	Pertussis antibody levels					
	Total	> 24 U/mL Positive		< 16 U/mL Negative		Geometric mean titre
	No.	No.	%	No.	%	(U/mL)
Total	640	301	47.0	339	53.0	25.0
Sex						
Boys	382	173	45.3	209	54.7	26.0
Girls	258	128	49.6	130	50.4	23.6
Age group (years)						
6–11	280	132	47.1	148	52.9	23.8
12–14	179	92	51.4	87	48.6	29.3
15–17	181	77	42.5	104	57.5	22.4

consisted of 280 (43.8%) elementary-school (aged 6–11 years), 179 (28.0%) junior high-school (aged 12–14 years) and 181 (28.2%) high-school students (aged 16–17 years).

IgG antibodies against *B. pertussis* were detected in 301 (47.0%) of the 640 samples analysed. The GMT of pertussis antibody in all samples was 25.0 U/mL (95% confidence interval: 20.8–29.2 U/mL). The GMT of pertussis antibodies in the boys and girls were 26.0 U/mL and 23.6 U/mL respectively ($P = 0.45$) (Table 1). Table 1 also shows the frequency of pertussis antibodies by age group. The level of pertussis antibodies did not show a statistically significant difference between the different age groups ($P = 0.22$).

Discussion

We documented a 47.0% seroprevalence of pertussis antibodies in students aged 6–17 years. The levels of pertussis antibodies did not change significantly across the different age groups Others studies from the Islamic Republic of Iran showed a pertussis seroprevalence of 60.6% in military recruits *(12)* and 47.6% in first-year medical students *(13)*.

Pertussis antibodies were detected in 47.1% of the primary-school students (6–11 years). Since these students had received the second booster dose of pertussis vaccine when they were 4–6 years old, the levels of pertussis antibodies were lower than expected. In one study from Turkey, 48.3% of children aged 4–6 years were positive for pertussis antibodies *(5)*, but in most studies, including another study from the Islamic Republic of Iran, children in this age group had higher levels of pertussis antibodies *(11,14–16)*. Possible reasons for low levels of pertussis antibodies in a relatively short period after vaccination include the quality of vaccine, lack of cold-chain preservation and poor vaccine administration technique.

In our study , 51.4% of high-school students were susceptible to pertussis. Because of waning of pertussis antibodies after vaccination *(6,7,17,18)*, we expected that the antibody level would be much lower in high-school students compared with younger students. The similar antibody level in both age groups indicates the presence of natural infection in the community, which increased the antibody levels in high-school students.

Similar to most studies, we found no significant difference in the level of pertussis antibodies between boys and girls *(17,19)*. However in one study girls had higher antibody levels than boys; however, the authors did not discuss any reasons for this difference *(9)*.

One limitation of our study was the use of a mixture of pertussis antigens. This may cross-react with antibodies produced by other bacteria present in the sera.

Conclusions

A significant portion of school-aged children in this area of the Islamic Republic of Iran were susceptible to pertussis. In addition to a reassessment of vaccination in 4–6 year-old children, a booster dose of an acellular pertussis vaccine in adolescents and/or adults is recommended *(20,21)*. Further studies are needed to evaluate the levels of pertussis antibodies in different age groups in other parts of the country to plan a national programme for pertussis booster vaccination.

Acknowledgements

This paper comprised part of the thesis of Homayon Yosefi, and financial support was provided by Ahvaz Jundishapur University of Medical Sciences. We thank all the participating students and their families. We also thank Mrs Shohreh Banidavoodi for preparation of the manuscript.

Funding: This study was supported by the Infectious and Tropical Diseases Research Center of Ahvaz Jundishapur University of Medical Sciences.

Competing interests: None declared.

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