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Seroprevalence of antibodies to pertussis and diphtheria among healthy adults in China

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Summary *Objectives:* Despite extensive childhood immunization, pertussis remains one of the world's leading causes of vaccine-preventable deaths. Incidence of pertussis in adolescents and adults has increased in many countries despite high vaccination coverage. In China, booster vaccinations against diphtheria, tetanus and pertussis are not used in adults, and little is known about pertussis incidence in the age group. The aim of this study was to determine seroprevalence of IgG antibodies to pertussis toxin (PT) and diphtheria among adults in China.
Methods: Blood samples were obtained from 210 healthy adults aged 18–50 years in Weifang city, China during the period of May and June 2010. Serum IgG antibodies against PT (anti-PT IgG) and diphtheria were determined by the commercial ELISA kits, respectively. According to the kit, concentration of anti-PT IgG higher than 30 IU/mL was considered positive. An antibody concentration of $\geq$0.1 IU/mL was defined as evidence of seroprotection against diphtheria.
Results: The mean concentration of anti-PT IgG antibodies was 9.95 IU/mL (95% confidence interval (CI) 8.45–11.44). Eleven (5.24%) of the studied subjects were proved to be seropositive to pertussis. Of the 210 subjects, 161 (76.6%) had anti-diphtheria antibody concentration $\geq$0.1 IU/mL and 49 (23.3%) had the antibody concentration between 0.01 and 0.099 IU/mL.
Conclusions: Our study indicated that about 5% of adults aged 18–50 years had positive anti-PT IgG antibodies, suggesting that adult pertussis is not uncommon in China. Although a high proportion of studied subjects had a protective level of immunity against diphtheria, the antibody level decreased with the increasing age of adults. Booster vaccinations against pertussis should be considered in adults in China.

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Background

Diphtheria and pertussis are vaccine-preventable respiratory diseases. In China, use of whole cell pertussis vaccine combined with diphtheria and tetanus toxoids (DTwP) was started in 1980s. Since 2007, a combined diphtheria-tetanus-acellular pertussis vaccine (DTaP) has been introduced. Both DTwP and DTaP vaccines are now in use in China. In several regions of China including Shandong province and Beijing, DTwP is administered in the 3rd, 4th and 5th months of life. A booster dose of DTwP or DTaP is given at 18—24 months, and the other two doses of DT vaccine are given at 7 and 12 years. According to China official country estimates, the immunization coverage rates and number districts achieved with 3 doses of DTP vaccination in childhood have been more than 90% since 2002.[1]

Vaccination against diphtheria has resulted in less than 10 diphtheria cases reported during the period of 2003—2010 in China. In recent years, however, several imported cases of *Corynebacterium diphtheriae* infection were reported in the United States, France and neighbor countries.[2,3] The lack of diphtheria vaccination in adolescents and adults might make them potentially at increased risk to imported diphtheria in China. Therefore, it is important to evaluate antibody levels of diphtheria in the adult populations.

Immunization against pertussis has resulted in a significant decrease in morbidity and mortality of this disease in the world. However, over the past two decades, pertussis continued to be a global concern with increased incidence in many countries including Argentina, Australia, Canada, Italy, Japan, the Netherlands, Switzerland and the USA.[4] Moreover, incidence of pertussis has increased in adolescents and adults, who are the significant source of infection to neonates and younger infants.[5—9] The Strategic Advisory Group of Experts (SAGE) in World Health Organization (WHO) recommended that the mortality caused by vaccine-preventable diseases should be reduced by two thirds by 2015 compared to that in 2000.[10]

In China, pertussis is a reportable infectious disease and the number of reported cases has been decreasing. Pertussis is clinically diagnosed, and laboratory methods such as ELISA serology are not used. Since the 1990s, incidence has been less than 1 case per 100,000 population.[11,12] In 2008, only 2387 cases were reported in China. Because adults and adolescents often have atypical symptoms and do not usually seek physicians, the true incidence of pertussis is most likely underestimated.[13]

Pertussis toxin (PT) is the most specific antigen for pertussis and cross-reacting antigens have not been described.[14,15] Therefore, IgG antibodies against PT is a specific indicator of active or recent *Bordetella pertussis* infection.[16,17] In this study, we wanted to determine conentrations of IgG antibodies to PT and diphtheria among 18—50 years old adults population in Weifang city of Shandong province, in order to gain an insight into seroepidemiology of pertussis and diphtheria in China.

Methods

Study setting and subjects

Healthy adults aged 18—50 years attending health checkup clinic were enrolled in a cross-sectional seroepidemiological study in Weifang city (with a population of 8.5 millions) of Shandong province from May to June 2010. All individuals were asymptomatic while entering the study. Individuals with any sign of respiratory diseases or immunocompromised conditions were excluded. A total of 210 subjects were recruited and classified into three age groups: 18—24 years, 25—34 years and 35—50 years. Of the 210 subjects, 98 were men and 112 women (gender ratio 1.14). Basic demographic and epidemiological data, for example, age, gender, address and cough symptom lasting more than one week was recorded.

Laboratory methods

3—5 mL of venous blood was drawn. Serum was extracted from blood samples within 1—2 h after arrival to local laboratories and stored at −20 °C until transported to the laboratory of China CDC using cold chain. IgG antibodies against PT of *B. pertussis* were measured quantitatively by a commercial ELISA kit (Virion\serion GmbH, Würzburg, Germany), according to the manufacturer's protocol. In the ELISA kit, the US reference pertussis antiserum (Human) lot3 (IgG anti-pertussis toxin) of the Food and Drug Administration (FDA) is used as reference standard. Results of anti-PT IgG were interpreted as positive, negative and equivocal following the instruction of kit's manual. Subjects bearing more than 30 IU/mL of IgG against PT were considered seropositive, below 20 IU/mL as seronegative, between 20 IU/mL and 30 IU/mL as equivocal.

Antibodies of *C. diphtheriae* were tested quantitatively by CE-marked sandwich ELISAs (IBL International, Hamburg, Germany), according to the manufacturer's instruction. For diphtheria, the classification into specific titer ranges for the evaluation of seroprotection was based on practical and theoretical considerations. Anti-diphtheria antibody concentration $\leq$0.01 IU/mL was defined as no immune protection or seronegativity and susceptibility. A value of $>$0.01 IU/mL was given for a secure individual protection. For safety reasons a tenfold higher value (0.1 IU/mL) than the internationally specified minimum titer for a prophylaxis was recommended.

Statistical analysis

Data analysis was performed using SPSS 13.0 software (SPSS Inc, Chicago, USA). For statistical analysis, subjects were categorized into three groups according to their age: 18—24, 25—34, 35—50 years. The prevalence of seropositivity and mean concentration of anti-PT IgG and anti-diphtheria IgG in all subjects and each group were descriptively calculated. Mean levels of IgG antibodies between the age groups were examined by one-way ANOVA. A P value $<$0.05 was considered significant. The serological change trends of anti-PT and anti-diphtheria IgG antibodies by age were demonstrated by scatter figures.

Ethical considerations

This study was approved by the Institutional Review Board (IRB) of Chinese Center for Disease Control and Prevention. Informed consent was received from all subjects before the blood samples were collected.

Results

Of the 210 subjects, 64 belonged to the age group of 18—24 years, 68 to the age group of 25—34 years and 78 to the age group of 35—50 years.

IgG antibodies against pertussis toxin

The mean concentration of anti-PT IgG antibodies among 210 subjects was 9.95 IU/mL (95% Confidence Interval (CI): 8.45—11.44). Eleven (5.24%) of the studied subjects had anti-PT IgG antibodies higher than 30 IU/mL and were thus considered seropositive to *B. pertussis*, while 184 (87.62%) and 15 (7.14%) subjects had concentration of antibodies fell into negative and equivocal category respectively (Table 1). Very low concentration of anti-PT antibodies were observed in all age groups. The mean anti-PT IgG levels were 9.98 IU/mL in subjects aged 18—24 years (95% CI, 7.25—12.93), 8.56 IU/mL (95%CI, 6.11—11.00) for those aged 25—34 years and 11.13 IU/mL (95%CI, 8.68—13.59) for 35—50 years age group (Figure 1 left). No difference in the mean concentration of anti-PT IgG antibodies was observed among the age groups ($P = 0.367$). Local regression (loess) analysis was used to estimate serological trends since the data did not conform to a single overall linear regression model. No clear difference in levels of anti-PT IgG was noticed among the subjects studied (Figure 2 left).

Antibody against diphtheria

None of the 210 subjects had an anti-diphtheria antibody concentration $\leq$0.01 IU/mL (no immune protection). An antibody concentration of $\geq$0.1 IU/mL (full protection) were found in 161 subjects (76.6%), while 49 (23.3%) had an antibody concentration between 0.01 and 0.099 IU/mL (basic protection). In each age group of subjects, percentage of the subjects with full protection was highest (87.5%) in 18—24 years age group, whereas the lowest (74.4%) was found in 35—50 years age group (Table 1). The mean concentration of anti-diphtheria antibodies was 0.61 IU/mL (95% CI, 0.511—0.71) in all subjects. The corresponding concentrations were 0.94 IU/mL (95% CI, 0.74—1.15) for those aged 18—24 years, 0.79 IU/mL (95% CI, 0.48—0.86) for those aged 25—34 years and 0.28 IU/mL (95% CI, 0.21—0.36) for those aged 35—50 years (Figure 1 right). The mean concentration of anti-diphtheria antibodies was significantly lower in subjects aged 35—50 years than those aged 25—34 years or those aged 18—24 years ($P = 0.00$ and 0.002, respectively). The graph of loess analysis also showed two peaks in concentration of anti-diphtheria antibodies among subjects aged 18—34 years (Figure 2 right).

Discussion

In China, little is known about seroprevalence of *B. pertussis* antibodies in adults. In this study, we demonstrated a 5.24% seroprevalence of specific anti-PT IgG in the adult population between 18 and 50 years. Moreover, the seroprevalence observed was independent of the age of study subjects.

This result distinguished less than the range of seroprevalence rates of 30—97% in adults reported from China and various other countries.[11,18—23] These can be explained by applied different methods, studied population, different vaccine and various vaccination program. Concentration of anti-PT IgG in most of subjects was relatively low fluctuating between 10 IU/mL and 20 IU/mL. However, the seroprevalence of pertussis in other western countries showed similar results with this study,[7,24] especially, the results of the adult pertussis trial (APERT) Study demonstrated that even among unimmunized controls with illness, 0.7%—5.7% had *B. pertussis* infection, which was evaluated with anti-PT IgG and IgA in-house ELISA.[7]

Antibodies directed against PT are specific for *B. pertussis* infections. For vaccinations against pertussis in China, three primary doses and one booster dose given at 2 years are used. Booster vaccinations against pertussis have not been introduced in adolescents and adults yet. It is known that the vaccine-induced antibodies began to wane 3—5 years after the last dose of vaccination, and immunity to pertussis vaccine diminished to 0%—20% over a 10-year interval,[14,18] Therefore, high anti-PT antibodies observed in 11 (5.24%) subjects were most likely caused by *B. pertussis* infections.

In this current study, 26 (12.38%) subjects had either diagnostic or equivocal anti-PT antibodies, suggesting that *B. pertussis* still circulates in the societies, especially in the adult population. However, these subjects having had anti-

Table 1 Anti-pertussis toxin and anti-diphtheria antibodies in 210 healthy adults.

Age group	No of subjects	Status of anti-PT antibodies (%)			Status of anti-diphtheria antibodies (%)	
		seropositive[a]	seronegative[b]	equivocal[c]	0.01—0.09 IU/mL	$\geq$0.1 IU/mL
18—24	64	5 (7.81)	53 (82.81)	6 (9.38)	8 (12.5)	56 (87.5)
25—34	68	2 (2.94)	64 (94.11)	2 (2.94)	21 (30.9)	47 (69.1)
35—50	78	4 (5.12)	67 (85.90)	7 (8.97)	20 (25.6)	58 (74.4)
Total	210	11 (5.24)	184 (87.62)	15 (7.14)	49 (23.3)	161 (76.6)

[a] The antibody level of anti-pertussis IgG more than 30 IU/mL.
[b] The antibody level of anti-pertussis IgG less than 20 IU/mL.
[c] The antibody level of anti-pertussis IgG within the range between 20 IU/mL and 30 IU/mL.

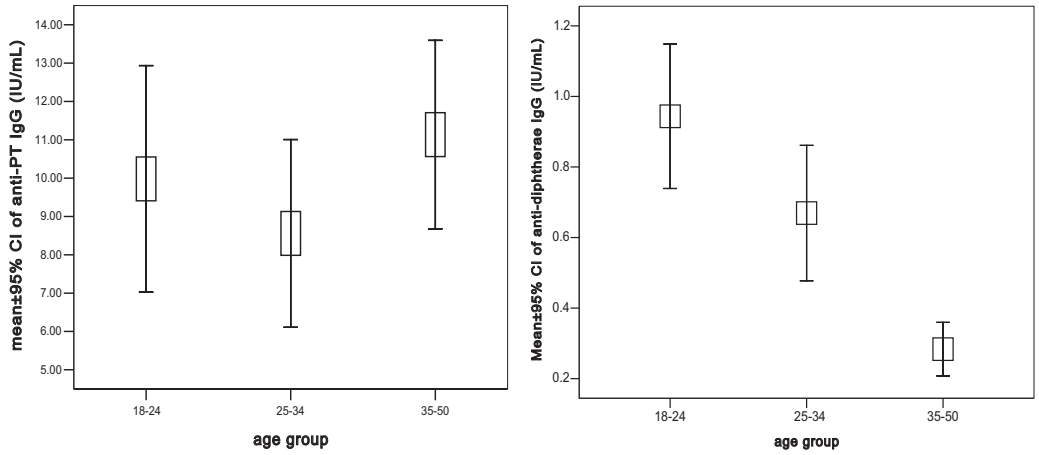

Figure 1 . Mean concentrations (±95% confidence interval) of anti-PT IgG and anti-diphtheria IgG antibodies by age group. Left for pertussis, right for diphtheria.

PT antibodies did not report to have coughing at the time of sampling, This finding is consistent with many previous studies.[25,26] It is known that the adults are the major source of infection to infants who are too young to receive all doses of vaccine (<6 months of age). Moreover, the adults with atypical symptoms might be also potential source to transmit pertussis to other adults.

The EU Pertstrain group consists of representatives of the Bordetella reference laboratories in their respective EU countries. Recently, the group recommended that a single or dual diagnostic cut-off for single-serum serology using IgG anti-PT between 50 and 120 IU/mL should be used according to countries as a proof of a recent infection with *B. pertussis* and provided that the patient was not vaccinated during last 12 months([27]). In this study, only four (1.82%) subjects with anti-PT antibodies ≥50 IU/mL were considered to have recent infection. This could be explained that it was not feasible to apply the cut-off for diagnostic purpose in China. The dual cut-off selected was based on studies carried out in countries where preschool, adolescent and/or adult boosters have already used. In China, however, no booster doses were used in adolescent and/or adult groups. Therefore, the level of anti-PT antibodies in these age groups might be lower in China than those countries with different booster immunizations. On the other hands, it is also possible that due to lack of the booster immunizations, more natural infections occur in China and the level of anti-PT antibodies might be thus higher than that in countries with booster immunizations. Furthermore, it should be kept in mind that the concentration of anti-PT antibodies measured in the ELISA kits used in this study was not equivalent to that recommended by the EU pertstrain group. To further understand seroepidemiology of pertussis in China, large multi-center studies should be conducted, and a diagnostic cut-off should be established for the Chinese populations in which different vaccination programme against pertussis is used.

In the present study, none of the studied subjects had a diphtheria antitoxin level of ≤0.01 IU/mL (indicating as having no immune protection). In some countries, the proportion of individuals susceptible to diphtheria with antibody concentration of <0.01 IU/mL varied from 5.3% to 37.6%.[28–34] Compared with the threshold (75%) indicated by Dadswell[35] as sufficient to prevent an outbreak of diphtheria, the percentages of 76.6% full protected subjects and 23.3% basic protected subjects were significantly above

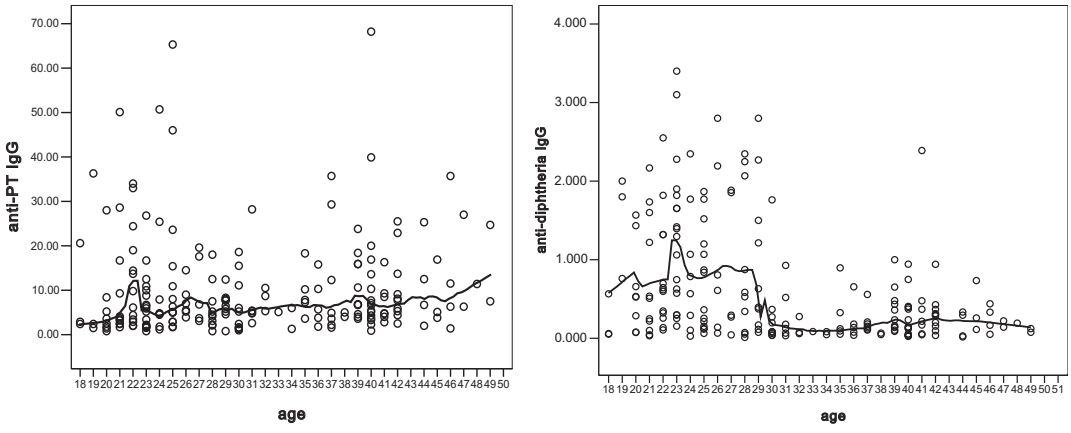

Figure 2 Concentrations of anti-PT and anti-diphtheria IgG antibodies in 210 healthy subjects by age (18–50 years of age). Each symbol represent one subject. Solid line presents mean level of IgG using local regression analysis; left: IgG to PT; right: IgG to diphtheria.

the safety limits. The results are in agreement with two similar studies carried out in Beijing (91.59%) and Jiangsu province (81.99%) in China.[36,37] The difference in the results between China and some countries might be due to different vaccination programs and booster schedules used, awareness of the disease among population, and different study designs and methods. Schneerson's study pointed out that the ELISA test have led to an overestimation of the subjects really protected, since the antibodies revealed might not always be efficient.[38]

In Shandong province where the present study took place, booster vaccine of diphtheria and tetanus is given to individuals at 7 years old and adolescents who enter secondary school and university students in first year. The vaccine coverage is more than 95% in Shandong province. According to WHO, following the primary immunization series, the average duration of protection is about 10 years, booster dose of diphtheria toxin beyond infancy and early school age are required to maintain protective immunity.[39] These might explain the peaks observed in adults aged between 18 and 34 years old.

Both of the results of ANOVA and loess analyses demonstrated that there was an overall trend of waning immunity with time. Although the adults aged 35—50 years old who are not immunized against diphtheria and they also had a high concentration of protective diphtheria antibody, this was considered to be due to the effect of inapparent or apparent natural diphtheria infection during the period of absent diphtheria vaccine. Similar findings have also been observed in several other countries.[40—42]

In this study, the coating antigen used in the commercial ELISA kits were highly purified PT. It is known that antibodies directed against PT are specific to *B. pertussis* infections. However, several seroprevelance studies conducted earlier in China used agglutination assay to evaluate immunity of pertussis in healthy people.[11,37,43] Agglutination with *B. pertussis* cells mainly measures IgM antibodies to its outer surface antigens such as fimbriae, pertactin and lipooligossacharide. Because of its low sensitivity and specificity, agglutination assay has been replaced by ELISA in many countries. This might explain why the high seroprevalence rates of 30—97% in adults were reported earlier in China.

A limitation of this study was the relatively low number of healthy subjects recruited, with a study population from a single city. In Germany, the performance of 11 commercial ELISA kits including the one used in this present study was recently compared by using two WHO Reference preparations,[44] the former FDA/CBER reference preparations, serum samples from patients with clinically suspected pertussis, and serum samples from patients having received a Tdap vaccination. An in-house ELISA with purified PT served as a reference method. They found that only kits using PT as a coating antigen showed overall good sensitivity and specificity compared to their in-house ELISA, suggesting that our results obtained by using the commercial ELISA kits are true.

Conclusions

Our study indicated that about 5% of adults aged 18—50 years had positive anti-PT IgG antibodies, suggesting that adult pertussis is not uncommon in China, Although a high proportion of the studied subjects had a protective level of immunity against diphtheria, the antibody level decreased with the increasing age. Booster vaccinations against pertussis should be considered in adults in China.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Q Zhang, FL Han, QY Liu and ZJ Shao planned the study. BQ Zhang and Q Nie were in charge of data collection and blood samples collection. Q Zhang carried out the immunoassays and performed the statistical analysis. Q Zhang drafted and edited the manuscript. Q He participated in data analysis and edited the manuscript.

All authors read and approved the final manuscript.

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