JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Seroprevalence of antibodies for pertussis and diphtheria among people leaving or entering China: a cross-sectional study

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Abstract

Introduction: Despite high population immunity, pertussis remains one of the leading causes of vaccine-preventable deaths worldwide. The aim of this study was to determine the seroprevalence of IgG antibodies to pertussis toxin (PT) and diphtheria among the adult male population leaving or entering China.

Methodology: Blood samples were obtained from 240 Chinese and 207 African healthy adults that were leaving and entering China, respectively. Serum IgG antibodies against PT (anti-PT IgG) and diphtheria were determined.

Results: The mean concentration of anti-PT IgG antibodies was 13.82 IU/mL and 18.11 IU/mL for the leaving and entering populations, respectively. None of the studied Chinese leaving China were seropositive for pertussis. Of the 240 subjects leaving China, 209 (87.1%) had anti-diphtheria antibody concentrations of ≥ 0.1 IU/mL and 31 (12.9%) had antibody concentrations between 0.01 and 0.099 IU/mL. Eleven (5.31%) of the studied Africans entering China had anti-PT IgG antibodies higher than 30 IU/mL and thus were considered seropositive for pertussis. Of the 207 Africans entering China, antibody concentrations of ≥ 0.1 IU/mL were found in 164 subjects (79.2%) while 43 (20.8%) had antibody concentrations between 0.01 and 0.099 IU/mL.

Conclusions: Almost all Chinese adult men leaving China and most African men entering China have very low serum antibody levels of pertussis. Furthermore, the antibody level of diphtheria among these two populations was low among adults. A larger population study is needed to determine whether booster vaccinations against pertussis and diphtheria should be considered for adults in China and also for Africans entering China.

Key words: Pertussis; diphtheria; serum IgG antibody.

J Infect Dev Ctries 2019; 13(5):394-399. doi:10.3855/jidc.10147

(Received 07 January 2018 – Accepted 12 February 2019)

Introduction

Pertussis and diphtheria are two important vaccine-preventable diseases. In China, the main vaccines used in the prevention of these diseases are DTwP and DTaP. DTaP vaccines have a lower efficacy than DTwP vaccines against pertussis [1-3]. Primary vaccination consists of three doses of DTP (DTwP or DTaP) in the 3rd, 4th and 5th months of life, and a fourth dose received at 18–24 months, with Diphtheria-Tetanus vaccine (DT) booster doses being recommended at 7 and 12 years. DTwP was introduced in 1978 and was replaced by DTaP in 2007 because of its potentially severe adverse reaction. In China, primary vaccination with DTP (DTwP or DTaP) is mandatory, while the DT booster doses are not. Since 2002, the immunization coverage rate in China for three doses of the DTP vaccination in childhood has been more than 90% [4]. In 2011, the immunization coverage for four doses of the vaccine was over 99% [5]. No diphtheria has been reported in China recent years. However, the number of pertussis cases has increased in China from 1612 in 2009 to 6658 in 2015.

Despite the immunisation effect on pertussis, there were 195,000 deaths from this disease worldwide in 2008, and most of the deaths occurred in developing countries, according to World Health Organization (WHO) estimates [6]. Studies have suggested that there are approximately 48.5 million annual cases of pertussis worldwide [7-8]. Moreover, the incidence of pertussis has increased in adolescents and adults, who are a significant source of infection to neonates and younger infants [9-11]. Between 2010 and 2012, there were large outbreaks in the UK and North America [12-14].

Pertussis, therefore, remains one of the leading causes of vaccine-preventable deaths in the world today.

The lack of diphtheria vaccination in adolescents and adults might make them potentially at increased risk for diphtheria. Therefore, it is important to evaluate antibody levels against diphtheria in adult populations. In several previous studies, very low concentrations of anti-pertussis toxin (PT) antibodies were observed in the Chinese population [3,5,15]. In this study, we wanted to determine the concentrations of IgG antibodies against PT and diphtheria among the populations of adult men leaving or entering China to gain an insight into the seroepidemiology of pertussis and diphtheria in exported labour workers and the entering population in China.

Methodology

Study setting and subjects

Healthy male adults attending health check-up clinics were enrolled in a cross-sectional seroepidemiological study in China between 2011 and 2015. All individuals were either leaving or entering China. Individuals with any signs of respiratory disease or immunocompromised conditions were excluded. The leaving population was Chinese, and the entering population was African. Of the 240 male subjects leaving China, 19 belonged to the 21-30 age group, 76 to the 31-40 age group, 120 to the 41-50 age group, and 25 to the 51-60 age group (Table 1). Of the 207 male subjects entering China, 45 belonged to the 16-20 age group, 109 belonged to the 21-30 age group, 39 to the 31-40 age group, 12 to the 41-50 age group, and two to the 51-60 age group (Table 2). Basic demographic and epidemiological data (e.g., age, gender, address, and cough symptoms lasting more than one week) were recorded.

Laboratory methods

Three to five mL of venous blood was drawn. Serum was extracted from the blood samples within 2 h of arrival to local laboratories and stored at -20°C until transported refrigerated to the laboratory. IgG antibodies against PT of *B. pertussis* and *C. diphtheria* toxin were measured quantitatively with a commercial ELISA kit (Serion ELISA classic, InstitutVirion\serion GmbH, Würzburg, Germany), according to the manufacturer's protocol. In the *B. pertussis* IgG antitoxins ELISA kit, the US reference pertussis antiserum (Human) lot3 (IgG anti-pertussis toxin) of the Food and Drug Administration (FDA) was used as the reference standard. The results for anti-PT IgG were interpreted as positive, negative and equivocal following the kit instructions. Subjects bearing more than 30 IU/mL of IgG against PT were considered seropositive, below 20 IU/mL as seronegative, and between 20 IU/mL and 30 IU/mL as equivocal. For diphtheria, three levels of immunity to diphtheria were defined: anti-diphtheria toxin antibody levels of ≤ 0.01 IU/mL = 'no immune protection or seronegativity or susceptibility'; antibody levels of 0.011–0.099 IU/mL = 'basic immunity or low seropositivity or basic protection'; and ≥ 0.1 IU/mL = 'full protection or seropositivity'.

Statistical analysis

Data analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago, USA). For statistical analysis, subjects were categorised into four groups according to their age: 21-30, 31-40, 41-50, and 51-60 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 plots.

Results

IgG antibodies against pertussis toxin from the Chinese male population leaving China

The mean concentration of anti-PT IgG antibodies among 240 Chinese male subjects leaving China was 13.82 IU/mL (95% Confidence Interval (CI): 13.19-14.43). None (0%) of these subjects had anti-PT IgG antibody levels higher than 30 IU/mL, and thus none were considered seropositive to *B. pertussis*. There

Table 1. Anti-pertussis toxin and anti-diphtheria antibodies in 240 Chinese subjects leaving China.

Age group	No of subjects	Status of anti-PT antibodies			Status of anti-diphtheria antibodies	
		seropositive	seronegative	equivocal	0.01-0.09 IU/mL	≥ 0.1 IU/mL
21-30	19	0	19	0	1	18
31-40	76	0	65	11	14	62
41-50	120	0	97	23	13	107
51-60	25	0	20	5	3	22

were 201 (83.75%) and 39 (16.25%) of the subjects had antibody concentrations that were categorised as seronegative or equivocal, respectively (Table 1, Figure 1).

Low anti-PT IgG concentrations were observed in all age groups. The mean anti-PT IgG levels were 9.84 IU/mL in subjects aged 21-30 years (95% CI: 8.22-11.46), 13.48 IU/mL in those aged 31-40 years (95% CI: 12.39-14.57), 14.60 IU/mL in those aged 41-50 years (95% CI: 13.74-15.47), and 14.07 IU/mL in the 51-60 age group (95% CI: 11.86-16.29). The mean concentration of anti-PT IgG antibodies was significantly lower in subjects aged 21-30 years than those aged 31-40 years or those aged 41-50 years ($P = 0.014$ and 0.001, respectively). No difference in the mean concentration of anti-PT IgG antibodies was observed among the other age groups ($P \geq 0.05$).

IgG antibodies against diphtheria from the Chinese male population leaving China

All 240 Chinese male subjects leaving China had anti-diphtheria antibody concentrations of ≥0.01 IU/mL (immune protection). Antibody concentrations of ≥ 0.1 IU/mL (full protection) were found in 209 subjects (87.1%), while 31 (12.9%) had antibody concentrations between 0.01 and 0.099 IU/mL (basic protection). In each age group, the percentage of subjects with full protection was highest (94.7%) in the 21-30 age group, and the lowest (81.6%) was found in the 31-40 age group (Table 1, Figure 2).

The mean concentration of anti-diphtheria antibodies was 0.20 IU/mL (95% CI, 0.19-0.21) for all Chinese male subjects leaving China. The corresponding concentrations were 0.19 IU/mL for those aged 21-30 years (95% CI, 0.16-0.22), 0.18 IU/mL for those aged 31-40 years (95% CI, 0.17-0.20), 0.22 IU/mL for those aged 41-50 years (95% CI, 0.20-0.23), and 0.19 IU/mL for those aged 51-60 years (95% CI, 0.16-0.22). No difference in the mean concentration of anti-diphtheria antibodies was observed among the age groups ($P \geq 0.05$).

Figure 1. Concentrations (A) and cross-sectional distribution (B) of anti-PT IgG antibodies in 240 healthy male Chinese subjects leaving China by age (21-60 years of age).

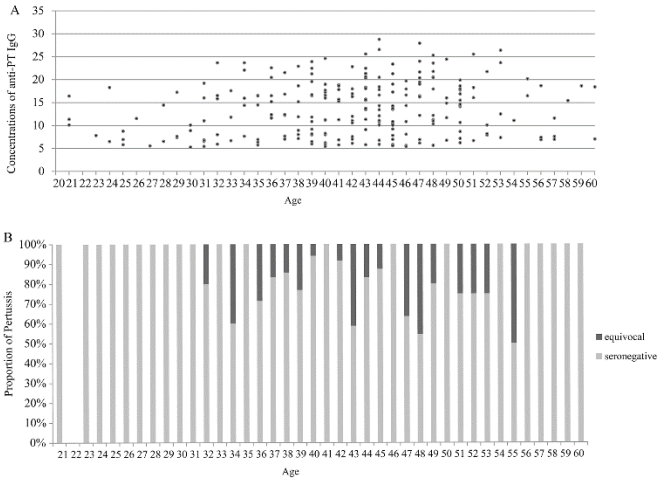


Figure 2. Concentrations (A) and cross-sectional distribution (B) of anti-diphtheria IgG antibodies in 240 healthy Chinese subjects leaving China by age (21-60 years of age).

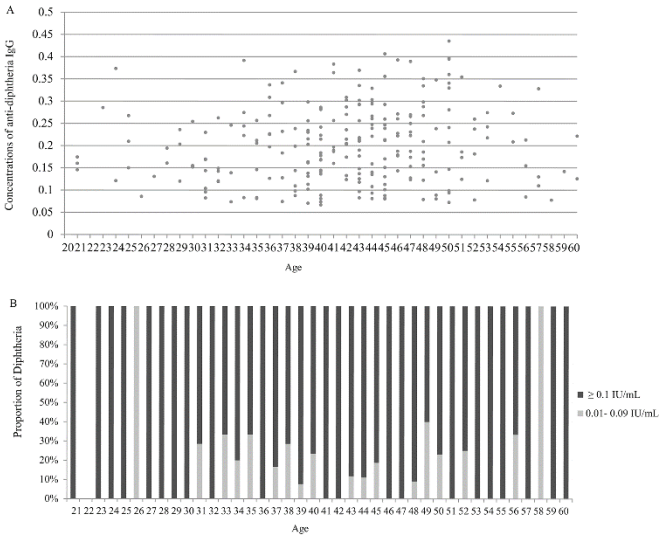


Table 2. Anti-pertussis toxin and anti-diphtheria antibodies in 207 African subjects entering China.

Age group	No of subjects	Status of anti-PT antibodies			Status of anti-diphtheria antibodies	
		seropositive	seronegative	equivocal	0.01-0.09 IU/mL	≥ 0.1 IU/mL
16-20	45	3	36	6	9	36
21-30	109	7	92	10	25	84
31-40	39	1	31	7	7	32
41-50	12	0	9	3	2	10
51-60	2	0	1	1	0	2

IgG antibodies against pertussis toxin from the African male population entering China

The mean anti-PT IgG antibody concentration for the 207 African subjects entering China was 18.11 IU/mL (95% Confidence Interval (CI): 17.29-19.01). Eleven (5.31%) of these subjects had anti-PT IgG antibody concentrations higher than 30 IU/mL and, thus, were considered seropositive to *B. pertussis*, while 169 (81.64%) and 27 (13.04%) had antibody concentrations that were negative or equivocal, respectively (Table 2, Figure 3).

Low anti-PT antibody concentrations were observed in all age groups. The mean anti-PT IgG level was 17.09 IU/mL in subjects aged 16-20 years (95% CI: 15.42-18.76), 23.68 IU/mL for those aged 21-30 years (95% CI: 12.48-34.89), 14.02 IU/mL for those aged 31-40 years (95% CI: 13.68-14.36), 21.45 IU/mL for those aged 41-50 years (95% CI: 18.94-24.04), and 18.76 IU/mL for the 51-60-year-old age group (95% CI: 10.69-26.84). No difference in the mean anti-PT IgG antibody concentration was observed among the other age groups ($P \geq 0.05$).

IgG antibodies against diphtheria from the African male population entering China

All 207 African male subjects entering China had anti-diphtheria antibody concentrations of ≥ 0.01 IU/mL (immune protection). Antibody concentrations of ≥ 0.1 IU/mL (full protection) were found for 164 subjects (79.2%), while 43 (20.8%) had antibody concentrations of between 0.01 and 0.099 IU/mL (basic protection). In each age group, the percentage of subjects with full protection was highest (100%) in the 51-60 age group, and lowest (77.1%) in the 21-30 age group (Table 2, Figure 4).

The mean anti-diphtheria antibody concentration was 0.17 IU/mL (95% CI, 0.16-0.18) in all male subjects entering China. The corresponding concentrations were 0.17 IU/mL for those aged 16-20 years (95% CI, 0.15-0.20), 0.16 IU/mL for those aged 21-30 years (95% CI, 0.15-0.17), 0.17 IU/mL for those aged 31-40 years (95% CI, 0.15-0.20), 0.18IU/mL for those aged 41-50 years (95% CI, 0.14-0.22), and 0.17IU/mL for those aged 51-60 years (95% CI, 0.14-0.20). No difference in the mean anti-diphtheria antibody concentrations was observed among age groups ($P \geq 0.05$).

Discussion

In China, little is known about the seroprevalence of *B. pertussis* antibodies in adults travelling abroad. In this study, we demonstrated low seroprevalence of specific anti-PT IgG in the adult male population between 21 and 60 years, and only a few Africans aged 16-53 years entering China had anti-PT IgG antibodies. In a previous report, 5% of adults aged 18-50 years in Shandong province and 8.91% of healthy individuals aged 0-95 years in Guangdong province tested positive for anti-PT IgG antibodies [5]. These studies, together with ours, suggest that adult pertussis is not uncommon in China, and booster vaccinations against pertussis should be considered for this population. On the other hand, our results were different seroprevalence rates of

Figure 3. Concentrations (A) and cross-sectional distribution (B) of anti-PT IgG antibodies in 207 African subjects entering China by age (16-53 years of age).

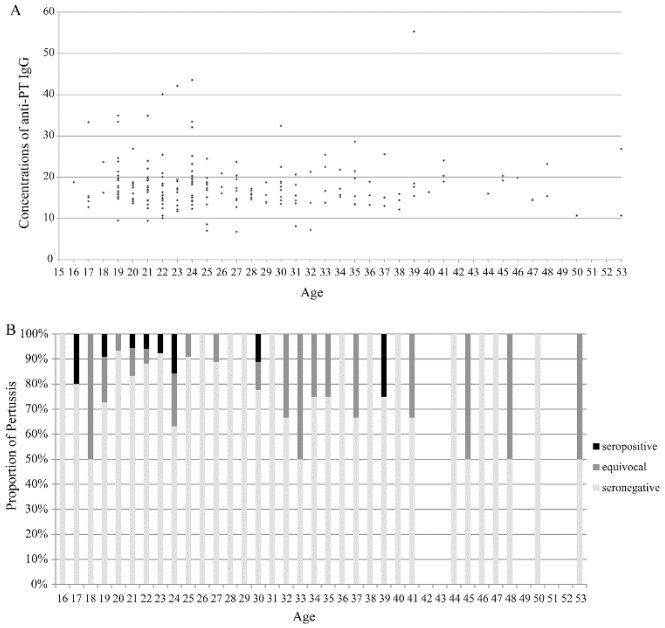
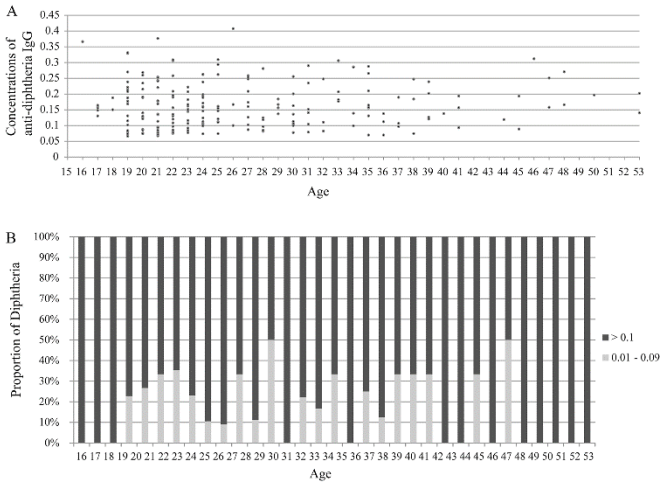


Figure 4. Concentrations (A) and cross-sectional distribution (B) of anti-diphtheria IgG antibodies in 207 African male subjects entering China by age (16-53 years of age).



30-97% in other reported from China [16] and other countries [17-23], which can be explained by different methods, study population, vaccine, and immunisation strategies among the studies.

In this study, the coating antigen used in the commercial ELISA kits was highly purified PT. It is known that antibodies directed against PT are specific to *B. pertussis* infections. However, several seroprevalence studies conducted earlier in China used an agglutination assay to evaluate immunity to pertussis in healthy people. Agglutination with *B. pertussis* cells mainly measures IgM antibodies to its outer surface antigens such as fimbriae, pertactin and lipooligosaccharides. Because of its low sensitivity and specificity, the agglutination assay has been replaced by ELISA in many countries. This might explain why high seroprevalence rates of 30-97% in adults were reported earlier in China [16,24,25]. In previous studies with the same commercial kit and criterion, 11% of the studied population ≥ 20 years old would have a serology strongly indicative or indicative of recent exposure to *B pertussis* [5,15]. However, applying these same criteria, none of the subjects studied here is positive. This may be due to the different studied population. Therefore, it is necessary to carry out a larger population study, taking into account the gender, region, age, nationality, and occupation and so on, in order to clarify the immune status of pertussis and diphtheria in the Chinese population.

Antibodies directed against PT are specific for *B. pertussis* infections. For vaccinations against pertussis in China, three primary doses and one booster dose are given at two years. Booster vaccinations against pertussis have not been introduced in adolescents and adults yet. It is known that the vaccine-induced antibodies begin to wane 3-5 years after the last dose of vaccination, and immunity to the pertussis vaccine diminishes to 0%-20% over a 10-year interval [26-27]. Therefore, high levels of anti-PT antibodies observed in subjects were most likely caused by *B. pertussis* infections. In this study, 11 subjects entering China had high anti-PT antibodies levels, suggesting having been infected with *B. pertussis*.

Conclusion

In conclusion, our study indicated that almost all Chinese adult men aged 21-60 years leaving China and most African aged 16-53 years entering China have very low serum antibody levels of pertussis. Although a high proportion of the studied subjects had a protective level of immunity against diphtheria, the antibody level was low among adults. A limitation of this study is the relatively low number of healthy subjects recruited, with a study population from a single city and only men were included in the study. A multi-centre seroepidemiology investigation should be carried out to reveal the overall picture of the seroprevalence of antibodies to pertussis and diphtheria among healthy adults in China as well as to determine whether booster vaccinations against pertussis and diphtheria should be considered for adults in China and also for Africans entering China.

Acknowledgements

This study was supported in part by the national key research and development plan (Grant No. 2016YCF1200705) and National Science and Technology Major Project (Grant No. 2018ZX10201001) from the Ministry of Science and Technology of the People's Republic of China.

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Conflict of interests: No conflict of interests is declared.