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Increased susceptibility to pertussis in adults at childbearing age as determined by comparative seroprevalence study, China 2010–2016

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SUMMARY

Objectives: This study was aimed to determine pertussis specific serum PT-IgG antibodies in healthy individuals during 2010 and 2015/2016 in Beijing, China.

Methods: A total of 3884 healthy individuals were included: 3058 aged 3–69 years randomly selected from an epidemiological survey conducted in 2010, and 826 aged 20–39 years selected from those who attended an annual medical examination in 2015/2016. Serum PT-IgG antibodies were determined using the Serion/Virion ELISA kits.

Results: Of 3058 subjects in 2010, 167 (5.5%) and 39 (1.3%) subjects had PT-IgG antibodies ≥40 IU/ml and ≥100 IU/ml, respectively. No differences were observed among different age groups. Altogether, 26.2% had undetectable PT-IgG antibodies (<5 IU/ml), and the highest undetectable rate of 56.8% was found in children aged 3–5 years. When the age group of 20–39 years was compared between the two periods, no difference was found in seroprevalence of PT-IgG antibodies ≥ 40 IU/ml (5.1% vs. 4.0%). However, an undetectable rate of PT-IgG antibodies was significantly higher in 2015/2016 than that in 2010 (57.4% vs. 29.1%, $p < 0.001$).

Conclusions: Our results showed that about 5% of individuals had PT-IgG antibodies indicative of a recent infection, and adults at childbearing age have an increased risk to pertussis in China.

Introduction

Pertussis is a respiratory infectious disease caused by the Gram negative bacterium *Bordetella pertussis*. The number of pertussis cases in many countries with a high vaccination coverage has increased in the last decade. Pertussis has become a common vaccine preventable disease throughout the world. The typical symptoms of pertussis are often seen in unvaccinated children. In order to reduce the morbidity and mortality caused by pertussis, whole cell pertussis vaccines (wP) have been used in many countries since the 1950s. The wP vaccines are composed of complete inactivated whole cell bacteria.[1] In view of the side reactions caused by wP vaccines, acellular pertussis vaccines (aP) were developed and have been in use since the 1980s.[2] To date, aP vaccines have almost completely replaced wP vaccines in most developed countries. Despite the high coverage of vaccines, the incidence of pertussis has been increasing throughout the world and large outbreaks have been reported in several countries.[3–7] The causes for the reemergence of pertussis are multiple. The potential factors may include: (1) the increased awareness, diagnosis and surveillance of pertussis; (2) the limited effects and the faster waning of immunity after the change from wP to aP; (3) adaptation of *B. pertussis* to vaccine-induced immunity.[8] It is commonly accepted that among these factors the waning of immunity plays a leading role in the resurgence of pertussis.[9,10]

Pertussis can occur in all ages. Because adolescents and adults often have asymptomatic pertussis, their incidences are much underestimated.[4,11–13] It is well known that adolescents and adults have become the reservoir of pertussis and the main source of transmission of the bacteria to vulnerable infants. While the classical symptoms are uncommon among adolescents and adults, they do tend to have a prolonged cough.[14] The current laboratory methods used for diagnosis of pertussis include bacterial culture, PCR and serology.[15] The sensitivity of these methods varies in different stages of the disease. Furthermore, serology is widely

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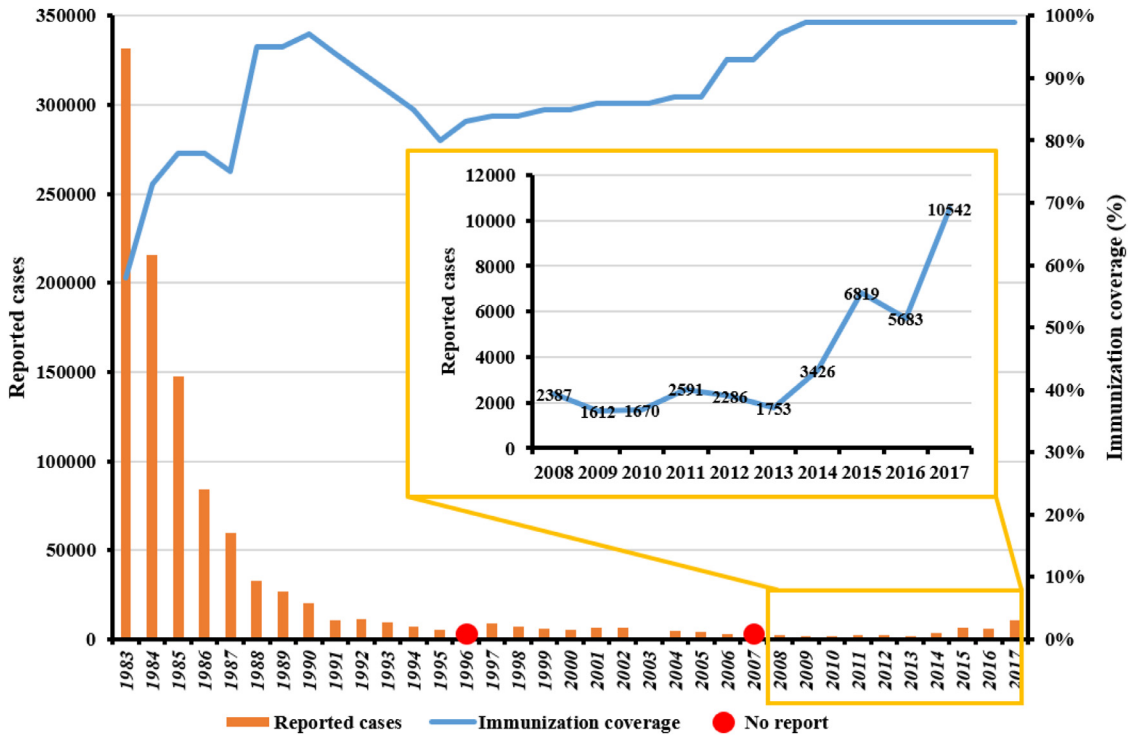

Fig. 1. Number of reported pertussis cases and DTP3 coverage in China, 1980-2017[18,19]a,b
[a] WHO vaccine-preventable disease: monitoring system. 2018 global summary: http://apps.who.int/immunization_monitoring/globalsummary/countries?countrycriteria%5Bcountry%5D%5B%5D=CHN#.
[b] National Health and Family Planning Commission of the People's Republic of china. Epidemic situations of legal infectious disease in china: http://www.nhfpc.gov.cn/jkj/s3578/201802/de926bdb046749abb7b0a8e23d929104.shNHaFPCotPsRocEsolidi.

used to study the seroprevalence of pertussis at population level and to assess vaccine effectiveness.[7,16]

In China, pertussis vaccination was introduced in the early 1960s. From 2007 onwards, both DTwP and DTaP vaccines were in use. From 2013 on, only the DTaP vaccines are in use. According to the data published by the National Institutes for Food and Drug Control, Beijing, China, there are two types of DTaP vaccines available in China: one type made by Chinese institutions containing pertussis toxin (PT) and filamentous hemagglutinin (FHA), and the other made by GSK containing three components (PT, FHA and pertactin) or by Sanofi Pasteur containing two components (PT and FHA). The antigens included in Chinese aP vaccines are co-purified from the culture of *B. pertussis*.[17] In 1982 infants were primarily administered with three doses of DTwP vaccines, at age of 3, 4, 5 months, and a booster dose is given at 18–24 months. The vaccination program has remained unchanged since then. The vaccination coverage was low before the 1980s and only 58% in 1983. Since 2009, the immunization coverage of the primary three doses has been increasing, being greater than 99% (Fig. 1).[18]

Pertussis is a notable infectious disease in China. Although the vaccination coverage has been high, the reported number of cases has been increasing since 2008 (Fig. 1).[18,19] The reasons for the increased number of reported cases are multiple, including increased awareness of clinicians, and improved diagnosis and surveillance of pertussis. However, both the clinical and laboratory diagnostic criteria of pertussis have remained unchanged since 2007. In addition, laboratory diagnostic methods (culture, PCR and ELISA serology) are not widely used in China. Most of the reported cases were infants and the clinically diagnosed. Several seroprevalence studies conducted in different regions of China indicate that the incidence of pertussis is most likely underestimated. However, in these studies, the number of subjects included in each age group was not sizable. In addition, these studies were only focused on samples collected at a single time point. The aim of this study was to determine pertussis specific serum anti-pertussis toxin (anti-PT) IgG antibodies in healthy individuals recruited during two periods of 2010 and 2015/2016 in Beijing, China. This study with a large number of samples provided us with a unique opportunity to estimate and compare the "true" incidences of pertussis among Chinese populations during 2010 and 2015/2016. An emphasis was put on the group of adults who are at a childbearing age.

Materials and methods

Study subjects

A total of 3884 individuals were included in the present study: 3058 aged 3–69 years randomly selected from a large-scale epidemiological survey conducted in Beijing in 2010, and 826 aged 20–39 years who attended an annual medical examination in 2015/2016. The survey in 2010 was conducted at the Center for Disease Control and Prevention (CDC) in the Chaoyang District, Beijing. Beijing is the capital of the People's Republic of China, with 21 million permanent residents. The Chaoyang District is the largest area of Beijing, having about one fifth of the population of Beijing. The design of the study and detailed information of blood samples were described previously.[4] In short, from about 14,500 samples collected in 2010, 3058 were selected from an age group of 3–69 years. Of the 3058, 1425 were males and 1633 were females. Subjects were divided into 8 age groups: 3–5 years, 6– years, 10–19 years, 20–29 years, 30–39 years, 40–49 years, 50–59 years and 60–69 years. To investigate the change of seroprevalence in the population at childbearing age in recent years, individuals who attended an annual medical examination at the CDC in the Chaoyang District were recruited (600 subjects aged 20–59 years in 2015 and 1890 subjects in 2016). These individuals who attended the annual

Table 1
The geometric mean concentration (GMC) and positivity rate of PT-IgG antibodies between genders in different age groups, Beijing, China, 2010 ($n = 3058$)[a].

Group		*n*	GMC (95%CI)	Median (95%CI)	≥40 IU/ml		≥100 IU/ml	
			(IU/ml)	(IU/ml)	n	Positive (%)	n	Positive (%)
3–69	Total	3058	8.33 (8.06–8.60)	7.45 (7.21–7.72)	167	5.4	39	1.3
	Male	1425	8.11 (7.71–8.53)	7.08 (6.69–7.41)	92	6.5[b]	23	1.6
	Female	1633	8.54 (8.19–8.90)	7.85 (7.45–8.32)	75	4.6	16	1.0
3–5	Total	148	5.11 (4.46–5.86) [c]	4.36 (3.66–5.07)	6	4.1	0	0
	Male	81	5.10 (4.15–6.13)	4.59 (3.82–5.30)	3	3.7	0	0
	Female	67	5.12 (4.15–6.33)	3.66 (3.46–5.36)	3	4.5	0	0
6–9	Total	287	6.37 (5.65–7.18)	5.13 (4.57–5.46)	20	7.0	7	2.4
	Male	149	5.92 (4.99–7.01)	4.84 (4.24–5.57)	9	6.0	3	2.0
	Female	138	6.90 (5.83–8.16)	5.22 (4.72–5.93)	11	8.0	4	2.9
10–19	Total	446	7.10 (6.49–7.76)	5.54 (5.14–6.02)	26	5.8	9	2.0
	Male	240	6.51(5.75–7.37)	5.07 (4.46–5.81)	13	5.4	6	2.5
	Female	206	7.85 (6.91–8.92)	6.03 (5.38–7.34)	13	6.3	3	1.5
20–29	Total	457	8.12 (7.45–8.84)	7.29 (6.64–8.28)	25	5.5	4	0.9
	Male	201	7.57 (6.60–8.68)	6.27 (5.48-7.30)	15	7.5	2	1.0
	Female	256	8.57 (7.70–9.54)	8.45 (7.17–9.32)	10	3.9	2	0.8
30–39	Total	440	8.93 (8.19–9.75)	8.44 (7.59–9.50)	21	4.9	5	1.1
	Male	181	8.14 (7.06–9.37)	7.23 (6.07–8.32)	11	6.1	2	1.1
	Female	259	9.53 (8.54–10.65)	9.45 (8.39–10.66)	10	3.9	3	1.2
40–49	Total	459	9.18 (8.53–9.87)	8.52 (7.66–9.33)	28	6.1	4	0.9
	Male	205	9.46 (8.40–10.66)	8.76 (7.64–10.31)	16	7.8	3	1.5
	Female	254	8.95 (8.17–9.80)	8.19 (7.09–9.53)	12	4.7	1	0.4
50–59	Total	414	9.57 (8.90–10.29)	9.01 (8.25–9.84)	14	3.4	3	0.7
	Male	185	10.51 (9.36–11.81)	9.88 (8.28–11.78)	8	4.3	3	0.5
	Female	229	8.87 (8.10–9.71)	8.62 (7.66–9.55)	6	2.6	0	0
60–69	Total	407	10.7 (9.78–11.69)	9.83 (9.00–10.76)	27	6.6	7	1.7
	Male	183	11.96 (10.32–13.87)	11.01 (9.00–13.32)	17	9.3	4	2.2
	Female	224	9.76 (8.77–10.87)	9.38 (8.12–10.24)	10	4.5	3	1.3

CI: confidence interval.
[a] A cut-off ≥100 IU/ml indicates a recent infection and that ≥40 IU/ml indicates a recent infection in a few years.
[b] A significant difference in positivity rate was found between men and women of all age groups ($P = 0.024$).
[c] A significant difference in GMC and median of PT-IgG antibodies was found between age groups of 60–69 years and 3–5 years ($P < 0.001$).

medical examination were residents in the Chaoyang District. A total of 228 and 598 subjects aged 20–39 years were selected from those in 2015 and 2016, respectively. Of the 826 subjects in 2015 and 2016, 377 were males and 449 were females. The age, gender and date of sampling were collected. However, the information on the vaccination status of these study subjects was not collected. All samples were stored at −80 °C until analysis.

The study protocol was approved by the Ethics Committee of Capital Medical University and the CDC, Chaoyang District, Beijing and the signed informed consent was obtained.

Laboratory testing

The concentrations of IgG antibodies against PT were measured using commercial ELISA kits (Institut Virion/Serion GmbH, Germany), according to the manufacturer's instructions. The results of PT-IgG antibodies in 897 subjects aged at 20–39 years in the 2010 survey was published.[4] The interpretation of the results was described previously.[4] Concentration of PT-IgG antibodies greater than or equal to 100 IU/ml indicates a recent infection within a year, and a concentration greater than or equal to 40 IU/ml indicates an infection within a few years, and lower than 40 IU/ml is considered as negative. The cut off of the test was 5 IU/ml, therefore those who have PT-IgG antibodies lower than 5 IU/ml were interpreted as undetectable.

Statistical analysis

Statistical analyses were performed using Microsoft Excel and SPSS v.19,0 (IBM Corp, Armonk, NY). The distinction of serum PT-IgG antibodies in different age groups and genders were expressed as geometric mean concentration (GMC) with 95% confidence interval (CIs), using one-way analysis of variance (ANOVA). The proportion of subjects with undetectable PT-IgG antibodies between the study periods 2010 and 2015/2016 was compared by the Chi-square test. Values of $P < 0.05$ with two sides were considered statistically significant.

Results

Of 3058 serum samples collected in 2010, the GMC and median of PT-IgG antibodies were 8.33 IU/ml (95%CI: 8.06–8.60) and 7.45 IU/ml (95%CI: 7.21–7.72) (Table 1). The highest GMC and median of PT-IgG antibodies were observed in subjects aged 60–69 years (GMC: 10.70 IU/ml, 95%CI: 9.78–11.69; median: 9.83 IU/ml, 95%CI: 9.00–10.76), and the lowest in children aged 3–5 years (GMC: 5.11 IU/ml, 95%CI: 4.46–5.86; median: 4.36 IU/ml, 95%CI: 3.66–5.07) ($P<0.001$). No significant differences in the GMC of PT-IgG antibodies were found in other age groups and between genders in each age group. Altogether, 167 (5.5%) subjects had PT-IgG antibodies concentrations ≥40 IU/ml, indicative of a recent pertussis infection within a few years, and 39 (1.3%) had PT-IgG antibodies concentrations ≥100 IU/ml indicating a recent infection within a year. No significant differences in positivity rate (≥40 IU/ml) were observed among all of the age groups (Table 1). However, there was a significant difference in the positivity rate between men and women of all age groups (6.5% vs. 4.6%, $p = 0.024$). No significant differences in positivity rate were observed between genders in each age group. The rate of undetectable PT-IgG antibodies was highest in the age group of 3–5 years (56.8%) and lowest in the age group of 60–69 years (16.5%) ($P < 0.001$). There was a clear decrease in the rate of undetectable PT-IgG antibodies as the age of subjects increased (Fig 2, $p<0.001$).

We next compared the seroprevalence of PT-IgG antibodies in age groups of 20–39 years between those collected in 2010 and 2015/2016. The seroprevalence of PT-IgG antibodies (≥ 40 IU/ml) was 5.1% (46/897) in 2010 and 4.0% (33/826) in 2015/2016. The number of samples with PT-IgG antibodies ≥ 100 IU/ml was 9 (1%)

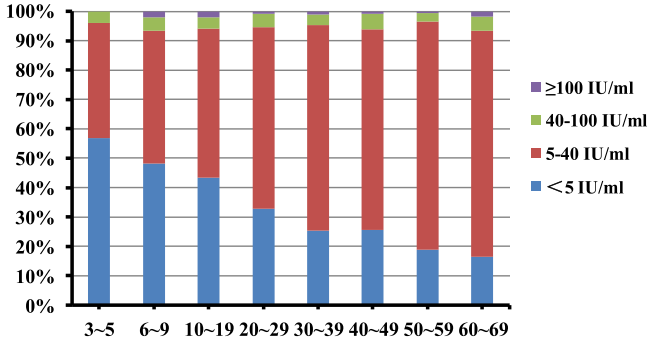

Fig. 2. The rate of undetectable PT-IgG antibodies among different age groups, Beijing, China, 2010 ($n = 3058$)[a]
[a]A cut-off<5 IU/ml indicates the antibody was undetectable. A clear decrease in the rate of undetectable PT-IgG antibodies was found with increasing age ($p<0.001$).

in 2010 and 5 (0.6%) in 2015/2016 (Fig. 3(A)). There were no significant differences in seroprevalence in age groups of 20–29 years and 30–39 years between the two periods (Fig. 3(B)). The percentage of subjects with PT-IgG antibodies ≥ 40 IU/ml in 2010 was 3.9% in women and 6.8% in men ($P = 0.05$), whereas the significant difference was not observed in 2015/2016 (Fig. 3(C)).

Of the 897 subjects aged 20–39 years in 2010, 29.1% had undetectable PT-IgG antibodies. In the same age group in 2015/2016, 57.4% subjects had undetectable PT-IgG antibodies. There was a significant difference in the rate of undetectable PT-IgG antibodies between the two periods ($p<0.001$) (Fig. 4).

No difference in the rate of undetectable PT-IgG antibodies was observed between those aged 20–29 years and 30–39 years in 2010 or 2015/2016. In addition, there were no differences in the rate of population with PT-IgG antibodies ≥ 100 IU/ml, ≥ 40 IU/ml and <5 IU/ml between 20 and 29 years and 30–39 years or genders collected in 2015 and 2016.

Discussion

In this study, we examined pertussis specific serum PT-IgG antibodies in healthy individuals during two periods 2010 and 2015/2016 in Beijing, China. The study included a large number of subjects and enabled us to estimate and compare the "true" incidence of pertussis among Chinese populations during different time periods.

To our best knowledge, such a cross-sectional and longitudinal sero-surveillance study in China has not been previously published. Several cross-sectional seroepidemiology studies conducted in different regions in China during 2010–2015 have shown that 1.17–9.90% of the population have had evidence of a recent infection of pertussis.[20–24] Consistent with these studies, we found that about 5% of the population had been recently infected by pertussis, confirming that pertussis is common and occurs in all ages in China. According to the official notification report by the National Health and Family Planning Commission (NHFPC) of China, the reported number of pertussis cases was 1764 in 2010. Compared to our results, the reported number was severely lower. Therefore, the true incidence of pertussis is significantly underestimated in China. One main reason of this could be due to the increased number of atypical cases in adolescents and adults. The other reason could be due to the lack of laboratory diagnostic methods, such as culture, PCR and ELISA serology. In countries with high vaccination coverages, most of the patients in adolescents and adults present a prolonged non-specific cough.[25] This could lead to misdiagnosis and the failure of the investigation of pertussis. In China, most of the reported pertussis cases are infants and the clinically diagnosed, indicating that only those infants with typical symptoms are diagnosed and notified in China.

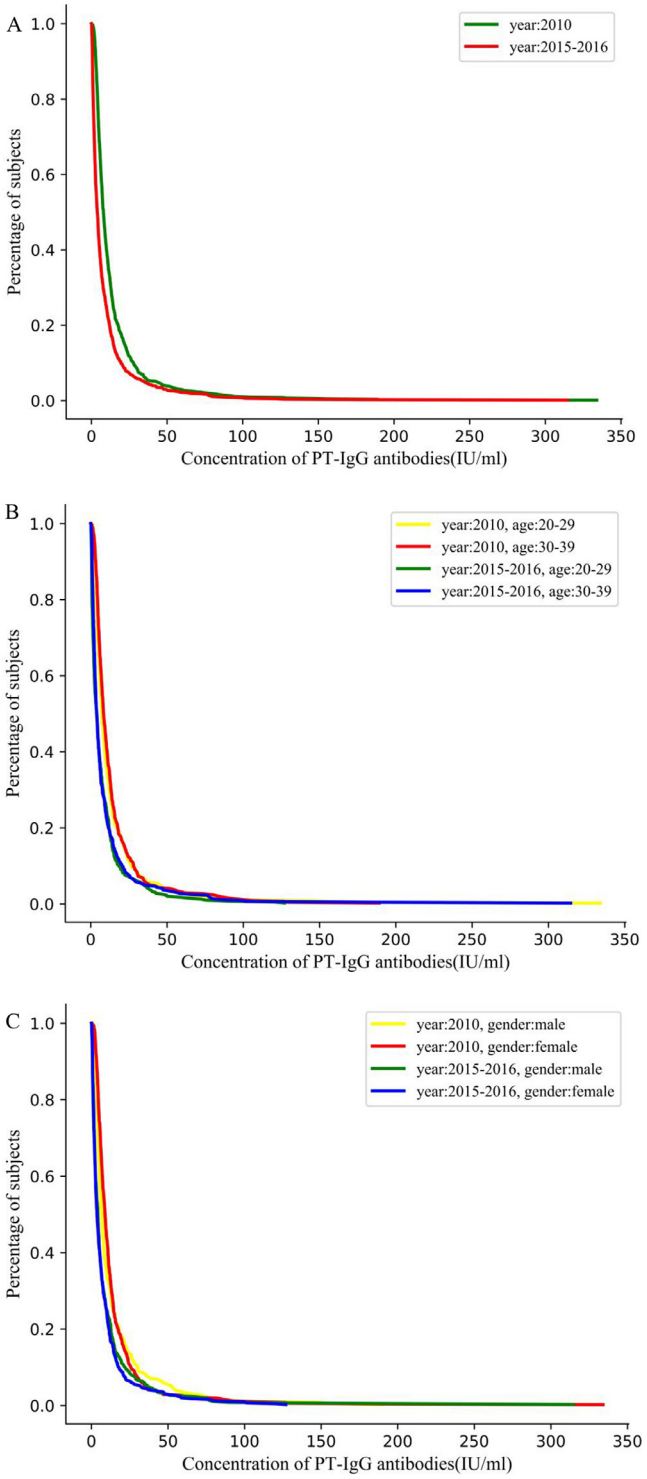

Fig. 3. Reverse cumulative distribution of PT-IgG antibodies in adults at child bearing age, Beijing, China between 2010 ($n = 897$) and 2015/2016 ($n = 826$)[a]
[a]A cut-off ≥100 IU/ml indicates a recent infection and that ≥40 IU/ml indicates a recent infection in a few years.

Pertussis toxin is only produced by *B. pertussis*. Therefore, the determination of specific PT-IgG antibodies is recommended.[26,27] Since PT is included in all of the aP vaccines, serology should not be recommended for individuals who are vaccinated within 12 months. Our investigation was based on the detection of specific serum PT-IgG antibodies using the commercial ELISA kit, and the lowest age group included in this study population was 3–5 years,

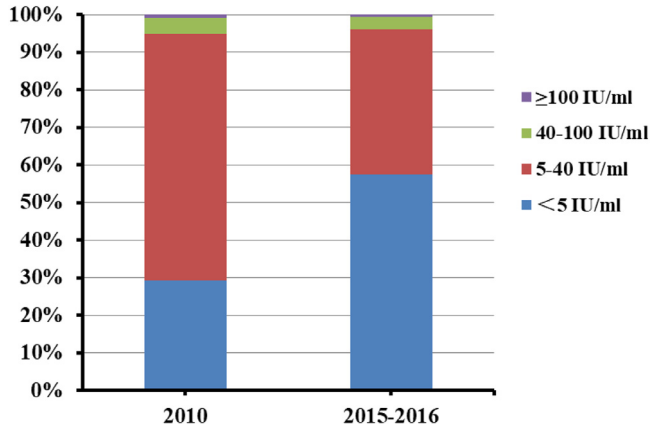

Fig. 4. Proportions of adults aged 20–39 years who had detectable and undetectable PT-IgG antibodies, Beijing, China, 2010 ($n=897$) and 2015/2016 ($n=826$)[a]
[a]Proportion of adults who had undetectable of PT-IgG antibodies was significantly higher in adults in 2015/2016 compared to those in 2010 ($p<0.001$).

who had not been vaccinated against pertussis within at least a year. Therefore, the PT-IgG antibodies detected should come from a pertussis infection rather than a vaccination.

It is known that the increased incidence of pertussis is partly due to the waning of immunity after vaccination. It is estimated that the duration of immunity wanes within 4–12 years in children after vaccination.[28] However, recent study suggests that protection from the DTaP begins to wane soon after vaccination.[9] The DTaP vaccine has been introduced to China since 2008, and has gradually replaced the use of DTwP. Therefore, the children aged 3–5 years enrolled in our study were vaccinated with DTwP. Unfortunately, the vaccination records in these children were not available, and we could not estimate whether the highest rate of undetectable PT-IgG antibodies was due to waning immunity after vaccination with DTwP, or due to the status of un-vaccination. It should be kept in mind that the vaccination coverage of the first 3 primary doses in China has been increased from 85% to 99% between 2000 and 2017.[18]

In China, the reported pertussis cases have been increasing since 2008, with over 10,000 cases being reported in 2017.[19] In this study, we found the rate of undetectable PT-IgG antibodies has significantly increased in adults who are at a childbearing age from 2010 to 2015/2016. In 2015/2016, almost 60% of adults do not have any PT-IgG antibodies, suggesting that they are susceptible to pertussis. It is well known that parents are the main source of transmission of a pertussis disease to infants. The finding may partly explain the increased incidence of pertussis in 2017 in this country.

In conclusion, our results indicated that pertussis is endemic and common in China. Although vaccination coverage has been high in this country, the protection provided by the current vaccination schedule is not optimal, as evidently shown in that about 4–5% of individuals had positive PT-IgG antibodies during the two study periods. Further, a large proportion of children at 3–5 years and adults at a childbearing age do not have specific PT-IgG antibodies, suggesting that they are becoming vulnerable to pertussis. In order to reduce the incidence of pertussis in infants and young children, the use of cell-free vaccines during pregnancy is widely used in many developed countries. To protect against pertussis in infants, the vaccination strategy in China should be reconsidered.

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Conflict of interests

The authors declare no conflicts of interests.

Authors' contribution

Qiushui He conceived and designed the study. Yuxiao Zhang, Zhiyun Chen, Nan Zhang and Ning Chen performed experiments. Yuxiao Zhang, Zhiyun Chen and Qiushui He analyzed data. Jianhong Zhang, Jing Zhang and Suming Li were responsible for sample collection. Yuxiao Zhang, Zhiyun Chen and Qiushui He wrote the manuscript. All authors reviewed and approved the final manuscript.

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