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Original article

# Evaluation of serum anti-pertussis toxin IgA antibodies for the diagnosis of Bordetella pertussis infection in young children

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ABSTRACT

*Background:* The determination of serum anti-pertussis toxin (PT) IgG antibodies is recommended for the diagnosis and surveillance of pertussis. However, the diagnostic power of anti-PT IgG can be hampered by possible interference from previous vaccinations. We aim to assess if anti-PT IgA antibodies can be well induced by *Bordetella pertussis* (*B. pertussis*) infections in children, and their capacity to improve pertussis serodiagnosis.

*Methods:* Serum samples from 172 hospitalized children younger than 10 years old with confirmed pertussis were tested. Pertussis was confirmed by culture, PCR and/or serology. Anti-PT IgA antibodies were determined with commercial ELISA kits.

*Results:* Sixty-four (37.2 %) subjects had anti-PT IgA antibodies greater than or equal to 15 IU/ml, and 52 (30.2 %) of them had anti-PT IgA antibodies greater than or equal to 20 IU/ml. No children with negative anti-PT IgG (less than 40 IU/ml) were observed to have anti-PT IgA antibodies greater than or equal to 15 IU/ml. Of patients younger than one year of age, about 50 % had an IgA antibody response. Moreover, the proportion of subjects with anti-PT IgA antibodies greater than or equal to 15 IU/ml among PCR negative subjects was significantly higher than that among PCR positive subjects (76.9 % vs 35.5 %).

*Conclusions:* The determination of anti-PT IgA antibodies does not seem to have added value for the serodiagnosis of pertussis in children older than one year of age. However, for infants, determination of serum anti-PT IgA antibodies appears to be useful for the diagnosis of pertussis especially when PCR and culture are negative. The results should be interpreted with caution as the number of subjects included in this study was limited.



## Introduction

Pertussis, or whooping cough, is a highly contagious disease of the respiratory tract, caused by *Bordetella pertussis* (*B. pertussis*) bacteria. The disease occurs in all age groups. It is most dangerous for young infants. Although there is high vaccination coverage, reported pertussis incidences have increased in many countries and regions during the past few decades, which is called pertussis resurgence [1,2]. In addition, many seroepidemiological studies indicated that the reported incidence of pertussis is most likely underestimated [3]. Several possible reasons have been considered, one of which might be the result of incomplete identification of pertussis cases in the past [4]. In countries with high vaccination rates, most infected adolescents and adults are atypical cases, and some of them can even have asymptomatic infections [5].

The diagnosis of pertussis includes clinical diagnosis and laboratory diagnosis. Since clinicians lack experience in making diagnosis based on atypical symptoms, laboratory methods are more important. At present, laboratory methods recommended for the diagnosis of pertussis include *B. pertussis* cultures, PCR techniques

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for nucleic acid detection of *B. pertussis*, and serological methods for the detection of anti-PT IgG antibodies. The sensitivity and specificity of the above-mentioned methods vary, depending on many factors such as the status of vaccination, timing of specimen collection and the onset of symptoms [6]. PCR and culture are maximally sensitive during the first 2–3 weeks after the onset of this disease [4,7]. However, symptoms are usually non-specific in the early onset of the disease, patients especially adolescents and adults with atypical clinical symptoms often delay seeing a doctor. Diagnosis can become difficult beyond the best period of sample collection for culture and PCR. Therefore, serological tests play an important role in the diagnosis of pertussis and have been widely used in several European countries and Australia. [8–11]. The sensitivity and specificity of serum anti-PT IgG antibodies are higher than those classes of immunoglobulins [4,6,7], and single-sample based ELISA has been investigated for diagnosing pertussis in different countries with different vaccination policies [10]. However, PT is included in all types of currently used acellular vaccines, and anti-PT IgG antibodies are induced by acellular vaccination as well as natural pertussis infection. Therefore, detection of anti-PT IgG antibodies cannot completely distinguish the rise in antibody concentration caused by vaccination from that caused by natural infection, and subjects who have received acellular vaccine within a year should be taken into account when the serology result is interpreted [9]. Clearly, additional parameters have been evaluated for their capacity to improve pertussis serodiagnosis. In Australia, there has been a gradual shift from whole cell of *B. pertussis* IgA assays to specific anti-PT IgG assays. However, most laboratories in Australia continue to test for IgA either alone or in combination with the anti-PT IgG assay [12,13]. Recently, the serodiagnostic tests Novagnost *Bordetella pertussis* IgA and IgM have been approved for use as in vitro diagnostic assays in Japan [14]. So far, the clinical use of anti-PT IgA antibodies for the diagnosis of pertussis is still under debate [12,15].

In China, the vaccination strategy is primarily administered with three doses of combined diphtheria–tetanus–pertussis (DTP) vaccines at the ages of 3, 4, and 5 months, with a booster dose given at 18–24 months. The acellular pertussis (aP) vaccine has been in use since 2007, and completely replaced the whole pertussis (wP) vaccine by 2013. Similarly, with high vaccination coverage, the incidence of pertussis has continued to rise over the following years. The reported pertussis cases are based mainly on clinical diagnosis, and laboratory confirmation of *B. pertussis* infection is not routinely used in China. To certify if serum anti-PT IgA antibodies is well induced in infants and children after *B. pertussis* infection, and whether the determination of anti-PT IgA antibodies can provide valuable information for the serological diagnosis, we collected serum samples from 172 hospitalized children with confirmed pertussis from 2016 to 2018 at Xi'an Children's Hospital in Xi'an, China, and evaluated the role of anti-PT IgA antibodies in comparison of anti-PT IgG antibodies as well as of PCR and culture results.

## Materials and methods

### *Study population and specimen collection*

Sera were collected and stored (at −80℃) from 172 hospitalized children (median: 14.5 months; age range 1.4–129 months) with suspected pertussis from 2016 to 2018 at Xi'an Children Hospital in Xi'an, China. Of the 172 subjects, 105 are male and 67 are female. Basic information and the history of vaccination of each subject were collected, such as age, gender, length of cough, date of admission to the hospital and sampling, and vaccination time. These children were diagnosed as having pertussis by clinical criteria as described previously [16]. All the children showed at least one of the typical symptoms, including paroxysmal spastic cough, whooping and vomiting after coughing. Total leukocytes number and lymphocytes ratio in their blood were obviously increased. Nasopharyngeal swabs and sera from all the cases were collected. The pertussis infections were further confirmed by at least one positive result of bacterial culture, PCR and/or serum anti-PT IgG antibodies.

### *Bordetella pertussis culture and PCR*

Culture and PCR of nasopharyngeal swabs were used. A 3-plex real-time PCR targeting IS*481*, hIS*1001* and pIS*1001* and a simplex real-time PCR targeting *ptxS1* were performed [17], and the primers and amplification protocols used were the same as described by Tatti et al. [18]. PCR results that were positive for IS*481* and/or *ptxS1* but negative for hIS*1001* and pIS*1001* were interpreted as having *B. pertussis* infection.

### *Serological Tests*

Concentrations of anti-PT IgG and IgA antibodies were determined by Serion ELISA classic immunoassays (Institut Virion/Serion GmbH, Germany). Briefly, the sera were diluted by 1:100 and tested in duplicate for the presence of total anti-PT IgG and IgA antibodies. The tests were calibrated using WHO international standard serum 06/140, and the antibody results obtained from the tests were expressed in international units per milliliter (IU/ml).

The lower limit of detection for anti-PT IgG was 5 IU/ml. The anti-PT IgG levels were categorized as negative, borderline and positive when values obtained were less than 40, 40–100 and greater than or equal to 100 IU/ml, respectively, according to the manufacturer's instructions. Concentration of anti-PT IgG greater than or equal to 100 IU/ml was defined as positive serology to indicate a recent pertussis infection, except for those who have received a pertussis vaccine within a year.

For anti-PT IgA antibodies, the lower limit was 10 IU/ml, and the negative, borderline and positive cut-off values were less than 15, 15–20 and greater than or equal to 20 IU/ml, respectively, according to the manufacturer's instructions. The diagnostic cut-off value is based on greater than or equal to 15 IU/ml for anti-PT IgA antibodies, to indicate a pertussis infection.

### *Statistical analysis*

Statistical analysis was performed using the GraphPad Prism 7 version (San Diego, CA, USA) and SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). Serum anti-PT IgG and IgA concentrations were analyzed by a normality test. Normally distributed continuous variables of the two or more groups were compared using a Student's t-test or One-Way ANOVA. Non-normally distributed continuous variables from the two or more groups were compared using the Mann-Whitney U test or Kruskal-Wallis test. The Chi-square test was used to evaluate differences in the proportion of subjects calculated on anti-PT IgG and IgA in different age groups or under different PCR statuses. Values of $p < 0.05$ with two sides were considered statistically significant.

## Results

Of the 172 Chinese children with diagnosed pertussis, 170 samples were tested with *B. pertussis* culture and 8 (4.7 %) showed positive results. PCR was performed on 84 samples, of which 53 (63.1 %) were reported positive. All 172 serum specimens had been tested for anti-PT IgG antibodies. One hundred and sixty-nine (98.3 %) subjects had anti-PT IgG antibodies greater than or equal to 40 IU/ml and 99 (57.6 %) had anti-PT IgG antibodies greater than or equal to 100 IU/ml. Of 82 subjects whose samples were tested by all the three

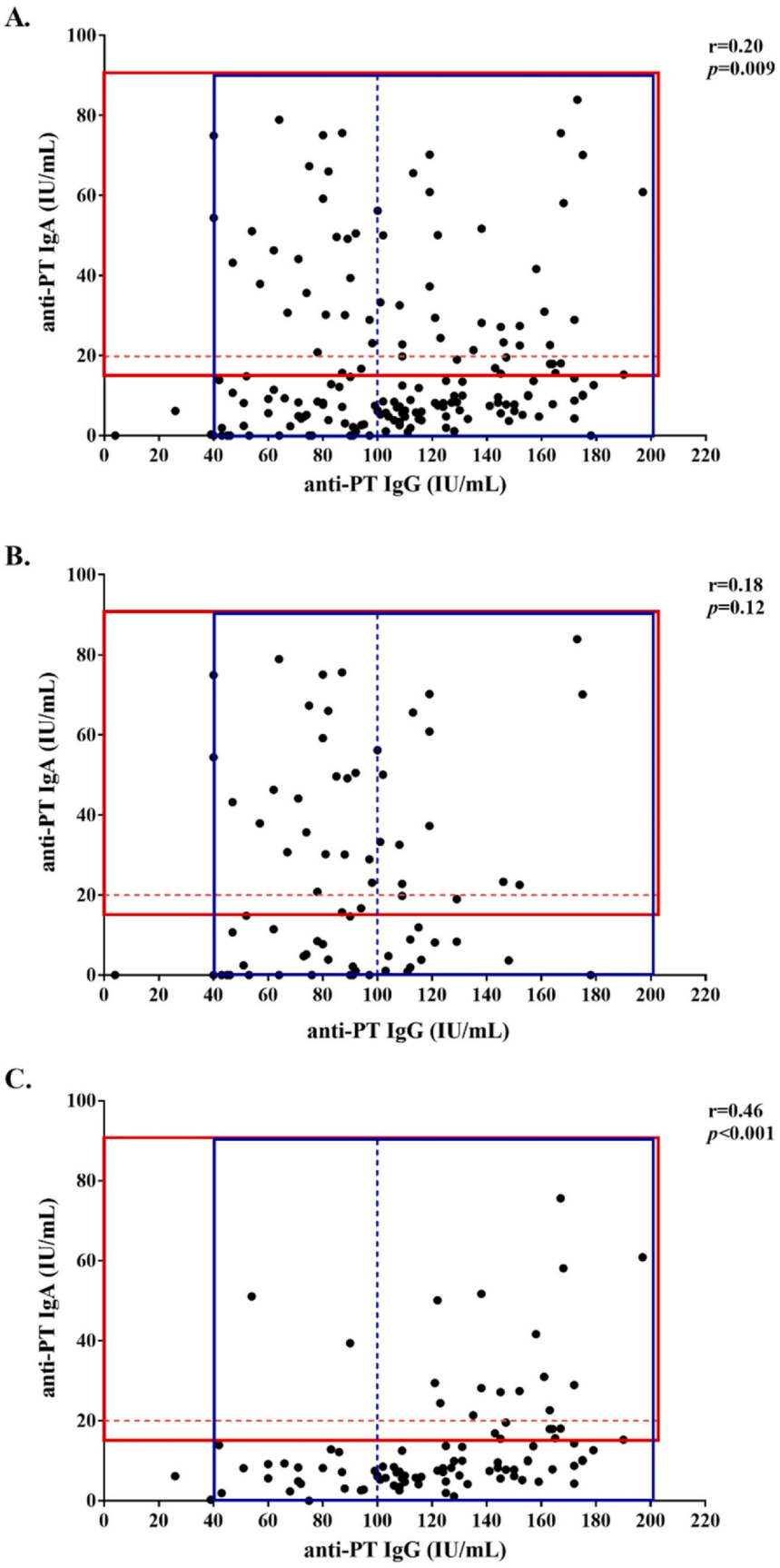


**Fig. 1.** Concentrations of anti-PT IgA and IgG antibodies in sera from study subjects. Each point represents a subject. A. includes results from all 172 children with pertussis (n = 172); B. includes results from children younger than 12 months (n = 76); C. includes results from children older than 12 months (n = 96). The red rectangle includes subjects with anti-PT IgA antibodies ≥ 15 IU/ml; red dotted line indicates the cut-off value of anti-PT IgA antibodies ≥ 20 IU/ml; The blue rectangle includes subjects with anti-PT IgG antibodies ≥ 40 IU/ml; blue dotted line indicates the cut- off value of anti-PT IgG antibodies≥ 100 IU/ml. r, Spearman's rank correlation coefficients, and *p* values for all subjects.

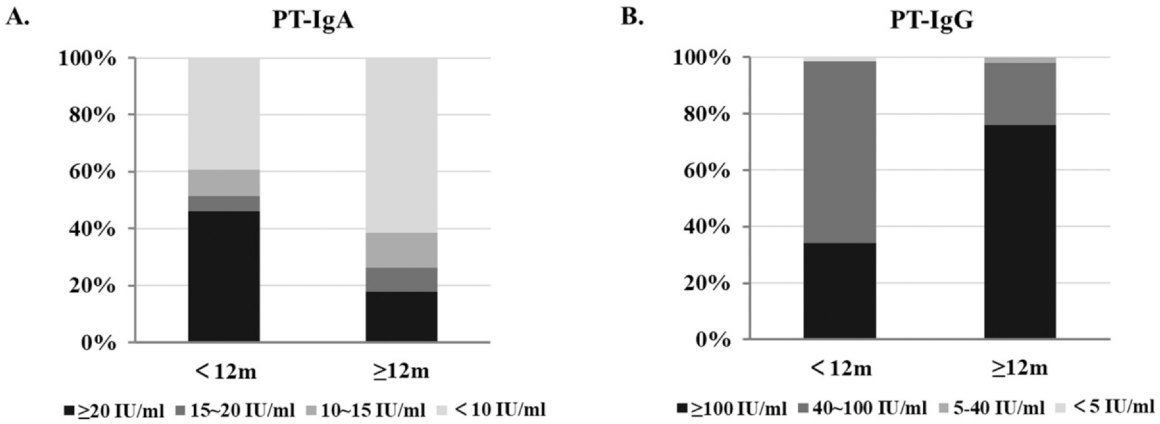


**Fig. 2.** Distribution of serum anti-PT IgA/IgG antibody concentrations in children with pertussis of different age groups. The numbers of serum specimens with different anti-PT IgA/IgG antibody concentrations in each age group were calculated, and the data are shown as percentages. A. includes results of anti-PT IgA. B. includes results of anti-PT IgG antibodies.

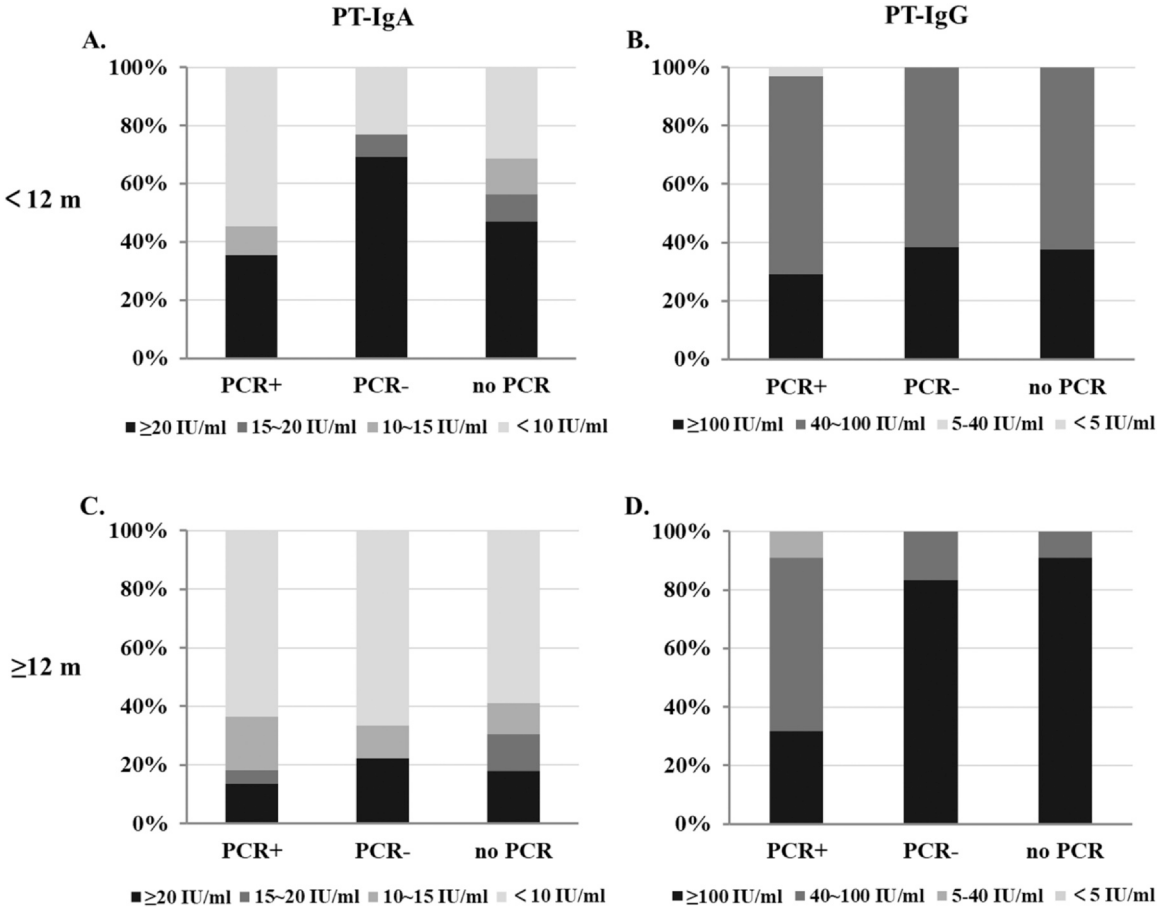


**Fig. 3.** Distribution of serum anti-PT IgA/IgG antibody concentrations in children with pertussis under different *B. pertussis* PCR status. The numbers of serum specimens with different anti-PT IgA/IgG antibody concentrations in each group were calculated, and the data are shown as percentages. A. includes results of anti-PT IgA in children younger than 12 months of age. B. includes results of anti-PT IgG in children younger than 12 months of age. C. includes results of anti-PT IgA in children older than 12 months of age. D. includes results of anti-PT IgG in children older than 12 months of age.

tests, 53 (64.6 %) were PCR positive, 8 (9.8 %) were culture positive and 10 (12.2 %) were seropositive.

Distribution of anti-PT IgA and IgG antibodies among all patients is shown in Fig. 1. Sixty-four (37.2 %) of the 172 subjects had anti-PT IgA antibodies greater than or equal to 15 IU/ml, including 38 (22.1 %) subjects with anti-PT IgG greater than or equal to 100 IU/ml and 26 (15.1 %) subjects with anti-PT IgG between 40 and 100 IU/ml. Among those 64 children, fifty-two (30.2 %) subjects had anti-PT IgA antibodies greater than or equal to 20 IU/ml and 12 (7.0 %) had anti-PT IgA antibodies between 15 IU/ml and 20 IU/ml. No children with negative anti-PT IgG ( < 40 IU/ml) were found to have anti-PT IgA antibodies greater than or equal to 15 IU/ml. The correlations between the concentrations of anti-PT IgA and IgG were positive, and the Spearman correlation coefficients were 0.20 ($p$ = 0.009) (Fig. 1A). When further grouped by age, it was found that no significant correlation between anti-PT IgA and IgG antibodies was noticed among

children younger than 12 months. Thirty-nine (51.3 %) of the 76 infants had anti-PT IgA greater than or equal to 15 IU/ml, including 15 (19.7 %) subjects with anti-PT IgG greater than or equal to 100 IU/ml and 24 (31.6 %) subjects with anti-PT IgG between 40 IU/ml and 100 IU/ml (Fig. 1B). Unlike that in children younger than 12 months, correlations between anti-PT IgA and IgG antibodies among children over 12 months were positive (r = 0.46, $p < 0.001$). twenty-five (26.0 %) of the 96 children had anti-PT IgA greater than or equal to 15 IU/ml, including 23 (24.0 %) subjects with anti-PT IgG greater than or equal to 100 IU/ml and only 2 (2 %) subjects with anti-PT IgG between 40 IU/ml and 100 IU/ml (Fig. 1 C).

Therefore, the proportion of subjects with anti-PT IgA antibodies greater than or equal to 15 IU/ml in infants younger than 12 months of age were higher than that in children older than 12 months of age (51.3 % vs 26.0 %, $p = 0.001$). The proportion of subjects with anti-PT IgA antibodies greater than or equal to 20 IU/ml in children younger than 12 months of age was also higher than that in children older than 12 months of age (46.1 % vs 17.7 %, $p < 0.001$) (Fig. 2A). In contrast, no statistical difference in the proportion of subjects with anti-PT IgG antibodies greater than or equal to 40 IU/ml was observed among these age groups. However, the proportion of subjects with anti-PT IgG antibodies greater than or equal to 100 IU/ml in subjects younger than 12 months of age was lower than that in subjects older than 12 months of age (34.2 % vs 76.0 %, $p < 0.001$) (Fig. 2B).

In 76 children younger than 12 months of age, further grouped by *B. pertussis* PCR status, the proportion of subjects with anti-PT IgA antibodies greater than or equal to 15 IU/ml among PCR negative subjects (11/13, 76.9 %) was significantly higher than among PCR positive subjects (11/31, 35.5 %) ($p = 0.012$). A statistical difference was also found in the proportion of subjects with anti-PT IgA antibodies greater than or equal to 20 IU/ml (9/13, 69.2 % vs 11/31, 35.5 %, $p = 0.04$) (Fig. 3 A). There was no statistical difference in the proportion of subjects with anti-PT IgG antibodies greater than or equal to 40 IU/ml nor 100 IU/ml among different PCR statuses (Fig. 3B). In 96 children older than 12 months of age, no statistical differences were observed in the proportion of subjects with anti-PT IgA antibodies greater than or equal to 15 IU/ml nor 20 IU/ml (Fig. 3 C). The proportion of subjects with anti-PT IgG antibodies greater than or equal to 100 IU/ml among PCR negative subjects (15/18, 83.3 %) was significantly higher than among PCR positive subjects (7/22, 31.8 %) ($p = 0.001$) (Fig. 3D).

Of 76 children younger than 12 months of age, forty-four were tested by both PCR and ELISA. Of the 44 children, 31 were proven to be PCR positive for *B. pertussis*. Of the 31 children with PCR positive for *B. pertussis*, eleven had anti-PT IgA greater than or equal to 15 IU/ml and nine had anti-PT IgG greater than or equal to 100 IU/ml. Of the 13 children with PCR negative for *B. pertussis*, ten had anti-PT IgA greater than or equal to 15 IU/ml and only five had anti-PT IgG greater than or equal to 100 IU/ml (Tables 1 and 2).

One hundred and sixty-eight children had information on their primary immunization status. As shown in Table 3, the age of children vaccinated with 3 doses (median: 33.5) was higher than that of unvaccinated children (median: 3.7, $p < 0.001$) and children vaccinated with 1–2 doses (median: 6.8, $p < 0.001$). No difference in the average concentration of anti-PT IgA was found between vaccinated and unvaccinated children nor children with different immunization time, although the concentration decreased with the increase in immunization times. On the contrary, the average concentration of anti-PT IgG was found higher in children vaccinated with 3 doses (median:125.0) than in unvaccinated children (median: 85.0, $p < 0.001$) and children vaccinated with 1–2 doses (median: 80.0, $p < 0.001$).

**Table 1**
Detection of anti-PT IgA antibodies by ELISA compared to PCR in children younger than 12 months.

| PCR result | Anti-PT IgA ≥ 15 IU/ml | Anti-PT IgA < 15 IU/ml | Total |
|---|---|---|---|
| Positive | 11 | 20 | 31 |
| Negative | 10 | 3 | 13 |
| Total | 21 | 23 | 44 |

**Table 2**
Detection of anti-PT IgG antibodies by ELISA compared to PCR in children younger than 12 months.

| PCR result | Anti-PT IgG ≥ 100 IU/ml | Anti-PT IgG < 100 IU/ml | Total |
|---|---|---|---|
| Positive | 9 | 22 | 31 |
| Negative | 5 | 8 | 13 |
| Total | 13 | 31 | 44 |

## Discussion

In our previous seroepidemiological investigations, a cut-off value of 40 IU/ml was used to evaluate anti-PT IgG seroprevalence in China [19–22]. According to de Melker et al. and Riffelmann et al., anti-PT IgG levels over 100 IU/ml are indicative of recent contact with *B. pertussis*, whereas values below 40 IU/ml might exclude the possibility of infection [15,23]. In the guideline from the EU Bordetella Experts Group, it was recommended that a dual cut-off between 50 IU/ml and 120 IU/ml is advisable for diagnosis from a single serum sample [9]. Various cut-off values for anti-PT IgG antibodies have been used in different countries [10], it is believed that higher specificity will be obtained by using a higher cut-off but with a lower sensitivity. However, cut-offs for anti-PT IgA antibodies are less well standardized. The proposed cut-offs ranged from 10 to 20 IU/ml, considering their relatively high specificity and low sensitivity [8,9,12,24]. In this study, similar results were obtained on the proportion of subjects with anti-PT IgA antibodies greater than or equal to 15 IU/ml and 20 IU/ml. It is suggested that the IgA assay was not significantly affected by the inclusion of equivocal results (15–20 IU/ml) as positive or negative, whether 15 IU/ml or 20 IU/ml is used as the cut-off value to indicate infection will cause a relatively slight impact.

It was reported that anti-PT IgA is frequently induced after infection in children older than 4 years, adolescents and adults, but is a less reliable marker for *B. pertussis* infection than anti-PT IgG [24]. In this study, all the children with pertussis who had anti-PT IgA antibodies greater than or equal to 15 IU/ml also had anti-PT IgG antibodies greater than or equal to 40 IU/ml. In addition, among the patients older than 12 months of age, the proportion of subjects with anti-PT IgA antibodies greater than or equal to 15 IU/ml was lower than that of subjects with anti-PT IgG antibodies greater than or equal to 100 IU/ml, and nearly all the subjects with anti-PT IgA antibodies greater than or equal to 15 IU/ml had anti-PT IgG antibodies greater than or equal to 100 IU/ml. Our results suggested that for children older than one year of age, the use of anti-PT IgG for serodiagnosis is more sensitive than anti-PT IgA. The combination of anti-PT IgA and anti-PT IgG resulted in no change as compared to the use of anti-PT IgG alone.

However, although almost 100 % of subjects had anti-PT IgG antibodies greater than or equal to 40 IU/ml, the proportion of subjects with anti-PT IgG antibodies greater than or equal to 100 IU/ml was low (~20 %) before 6 months of age and increased to more than 50 % after 6–12 months of age (data not shown). It is interesting to find that, the proportion of subjects with anti-PT IgA antibodies greater than or equal to 15 IU/ml was higher than that of subjects with anti-PT IgG antibodies greater than or equal to 100 IU/ml in patients before 12 months of age, and nearly half of the patients (24/49) with anti-PT IgG antibodies between 40 and 100 IU/ml had anti-PT IgA antibodies greater than or equal to 15 IU/ml. Most of the

**Table 3**
Concentration of anti-PT IgA and IgG antibodies in children with recorded primary immunization status (n = 168).

| Characteristic | Unvaccinated n = 39 | 1–2 doses n = 31 | 3 doses n = 98 |
|---|---|---|---|
| Age (median, IQR, months) | 3.7 (2.4–5.8)[a] | 6.8 (5.0–9.0)[b] | 33.5 (15.0–66.3) |
| Anti-PT IgA (median, IQR, IU/ml) | 30.1 (0.0–65.6) | 13.9 (3.7–32.6) | 8.5 (5.7–17.9) |
| Anti-PT IgG (median, IQR, IU/ml) | 85.0 (62.0–97.0)[c] | 80.0 (51.0–108.0)[d] | 125.0 (101.8–152.0) |

IQR: Interquartile range.
[a] Unvaccinated children vs children vaccinated with 3 doses of aP, $p < 0.001$.
[b] Children vaccinated with 1–2 doses vs 3 doses, $p < 0.001$.
[c] Unvaccinated children vs children vaccinated with 3 doses of aP, $p < 0.001$.
[d] Children vaccinated with 1–2 doses vs 3 doses, $p < 0.001$.

children with diagnosed pertussis in this study were recorded with 1–3 dose of primary immunization. It is difficult to interpretate anti-PT concentrations in recently vaccinated person. As suggested by May et al. [8], a significant anti-PT IgA or IgG response to vaccination is usually relatively short-lived and single high-titer (> 94 IU/ml) anti-PT IgG results can still have diagnostic value for children of < 10 years of age. Whereas, if vaccinated within the last 3–6 months, intermediate levels of anti-PT IgG (e.g., 65–94 IU/ml) should be interpreted with caution. Therefore, in this study, cut-off of anti-PT IgA equal to 15 IU/ml might provide further diagnostic information for infants younger than 12 months especially those with anti-PT IgG antibodies between 40 and 100 IU/ml.

Since PCR positivity is definite evidence for pertussis infection, the percentages of different serological profiles were also compared by PCR status between different age groups. Similar to culture, PCR positivity occurred mostly in subjects within 2–3 weeks from the onset of paroxysmal cough [25] and anti-PT IgA antibody is generally positive after that time [13,26]. In this study, the average cough days when these children came to the hospital were 16.6 and 21.2 in PCR positive and negative cases respectively (data not shown). The proportion of subjects with anti-PT IgA greater than or equal to 15 IU/ml was higher in PCR negative cases than in positive cases in children younger than 12 months of age. On the contrary, a higher proportion of subjects with anti-PT IgG greater than or equal to 100 IU/ml in PCR negative cases was mainly observed in children older than 12 months of age. Therefore, to confirm *B. pertussis* infection beyond the sensitive period for culture and PCR in previously undiagnosed patients, detecting serum anti-PT IgA antibodies might be useful for diagnosis of pertussis in children younger than 12 months of age.

Children with pertussis in our study are younger than 10 years old, and about two thirds of whom (64.4 %) are younger than 2 years old. It is well recognized that IgA responses are not robust in young children, especially those younger than 1 year, and this was demonstrated even in older children until 12 years old [27–29]. Both the response and prevalence of anti-PT IgA tend to increase with age [12,27,28]. However, our results do not seem to meet these findings. A higher positivity rate of anti-PT IgA was found in children younger than 12 months.

Although the determination of anti-PT IgG antibodies has shown ideal sensitivity compared to IgA, primary wP or aP vaccinations in the first 6 months of life induce IgM and IgG antibodies but do not induce IgA antibodies. Since the study was conducted in 2016–2018, all children younger than 2 years should have received the aP vaccine. Those anti-PT IgA antibodies detected are most likely due to infection. In this study, the average age of children increases with the vaccination time, indicating that most children receive immunization according to the vaccination procedure. It was found that anti-PT IgG antibody concentration increased with immunization time, but anti-PT IgA antibody concentration seemed to decrease as immunization dose increased. Therefore, IgA antibodies detected after primary vaccination might be highly specific for infection [30–34]. It was reported that detection of anti-PT IgA antibodies is useful to discriminate between infection and vaccination in recently vaccinated subjects with elevated anti-PT IgG [24]. In addition, maternal transferred antibodies in infants younger than 3 months will interfere with diagnosis of actual infections. According to seroepidemiologic studies conducted in Beijing, China, about 2–4 % of newborns have anti-PT IgG antibodies higher than or equal to 40 IU/ml [35,36]. We have also recently shown that 1.1 % of Chinese infants younger than 3 months of age who attended annual health examinations have anti-PT IgG greater than or equal to 40 IU/ml [21]. We have also determined anti-PT IgA antibodies in 65 heathy children younger than 1 year (Data not shown). All of these infants had not detectable anti-PT IgA antibodies, whereas four (aged from 3 to 7 months) (6.2 %) had anti-PT IgG antibodies greater than or equal to 40 IU/ml and two (3.1 %) had anti-PT IgG greater than or equal to 100 IU/ml. Since the vaccination background of these health children were not available, we also can't identify the anti-PT IgG antibodies derived from vaccination or maternal transferred antibodies. Von Linstow et al. conducted a study in Denmark in which they measured anti-PT IgA and IgG antibodies in sera from 203 1-year-old children who had received one to three doses of a mono-component PT toxoid vaccine. Based on the detection of anti-PT IgA antibodies, 5 % of healthy children had serological evidence of *B. pertussis* infection during their first year of life [37]. However, until now, there have been few studies on the serodiagnosis conducted among infants under 1 year old. In our study, although an IgA antibody response occurs in only ~50 % of infected children younger than 1 year, determination of anti-PT IgA antibodies may be useful to distinguish antibodies derived from recent infection, primary vaccination or even maternally transferred antibodies.

## Conclusions

Our results suggested that for children older than one year of age determination of serum anti-PT IgG antibodies is important for the serodiagnosis of pertussis, and the determination of anti-PT IgA antibodies does not help with the diagnosis. However, for children younger than one year of age determination of serum anti-PT IgA antibodies appears to be useful for diagnosis of pertussis especially when PCR/culture are negative. Since the number of subjects included in this study is limited, patients included in our study were diagnosed by both clinical and laboratory diagnosis, instead of single gold-standard and cut-off value used in this study is based on literatures and manufacturers' recommendation. Although we noticed the anti-PT IgA might play an assistant role in diagnosis, we still need to be very cautious, further studies with large cohorts and in populations with different background of priming are needed.

## Ethical approval

The study protocol was approved by the Ethics Committee of Capital Medical University (2020SY008) and Xi'an Children Hospital (20200022) and exempted from signing informed consent.

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## CRediT authorship contribution statement

**Zhiyun Chen:** Methodology, Investigation, Formal analysis, Writing—original draft. **Xiaoguai Liu:** Resources, Investigation. **Yuxiao Zhang:** Methodology, Investigation. **Xiaokang Peng:** Resources, Investigation. **Nan Zhang:** Investigation. **Ning Chen:** Project administration. **Yarong Li:** Conceptualization, Methodology, Resources. **Qiushui He**: Conceptualization, Methodology, Writing – Review & Editing; Funding acquisition.

## Declaration of Competing Interest

The author has no conflict of interest to declare.

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