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Severe *Bordetella pertussis* infection and vaccine issue in Chongqing, from 2012 to 2018

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ABSTRACT

Objective: Pertussis is a highly contagious respiratory illness mainly caused by the Gram-negative bacterium *Bordetella pertussis*. The infection of *B. pertussis* has been increasing and the current diagnosis of pertussis in children is challenging; little is known of *B. pertussis* infection in Chongqing.
Methods: There were 25,441 children (14,863 male and 10,578 female) with suspected pertussis enrolled in our retrospective study from December 2012 to November 2018. Then 800 children with suspected *B. pertussis* infection were randomly chosen to be evaluated by simultaneous amplification and testing in this prospective study.
Results: Infants younger than 12 months had the greatest burden of pertussis, and the incidence of pertussis in Chongqing appeared to have a periodic pattern. The problem of vaccine quality in China was more serious than previously reported based on the fluctuation of infection rates from 2012 to 2018. Simultaneous amplification and testing to detect *B. pertussis* RNA (Area Under Curve: 0.900 and Kappa value: 0.831) had better diagnostic performance than real-time PCR for *B. pertussis* DNA (Area Under Curve: 0.869 and Kappa value: 0.690).
Conclusions: We revealed the characteristics of *B. pertussis* infection and vaccine issues in Chongqing. Simultaneous amplification and testing could be a potential novel assay for measuring *B. pertussis* infection in the future.

Introduction

Pertussis is a highly contagious respiratory illness that is primarily caused by the Gram-negative bacterium *Bordetella pertussis* (*B. pertussis*) (Petridou et al., 2018). The infection rates range from 1% to 12% in developed countries and 5% to 50% in developing countries (Barkoff et al., 2015). Despite high childhood vaccination rates and coverage, pertussis infection rates have increased (Cassiday et al., 2000; Huang et al., 2017). Like other countries, a national mandatory immunization programme (NIP) targeting *B. pertussis* was initiated in China in 1978 (Meng et al., 2018). Two commercial pertussis-containing vaccines are licensed for immunization on the Chinese market. The first primary dose is inoculated at 3 months (Huang et al., 2017) following the 3-dose primary series schedule recommended by the World Health Organization (WHO), and a booster dose is given ≥6 months after the last primary dose (Chen and He, 2017). Although the vaccination rate of *B. pertussis* in China is high, the incidence of pertussis has increased in recent years, and pertussis remains a serious disease in Chinese children (Xu et al., 2014). The diagnosis of pertussis is challenging because other respiratory pathogens cause pertussis-like illnesses, and the clinical presentation of pertussis varies with age and vaccination status (Frumkin, 2013; Wirsing von Koenig, 2014).

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Several laboratory assays aid in the diagnosis of pertussis, including bacterial culture, serological assays and polymerase chain reaction (PCR). Bacterial culture is the "gold standard" pertussis diagnostic test. However, it exhibits very low sensitivity, and it is time-consuming (Wendelboe and Van Rie, 2006). Serological assays based on IgM and IgG detection provide a method for the identification of *B. pertussis* infection, but IgM results are not reliable in certain circumstances, especially children who may have insufficient circulating antibodies (Emery, 2013). Real-time PCR detection of *B. pertussis* DNA is widely used for the diagnosis of pertussis due to its time-saving characteristics, high sensitivity and specificity (Liu et al., 2014). Although it is easy to detect *B. pertussis* by real-time PCR in laboratories, a high false-positive rate of real-time PCR result exists compared to the clinical diagnosis (Loeffelholz, 2012). It is necessary to improve the diagnostic performance of laboratory assays for *B. pertussis*.

The clinical and molecular characteristics of pertussis were reported previously (Barkoff et al., 2015; Cassiday et al., 2000; Huang et al., 2017), but the real medical burden of pertussis and the effect of vaccination of pertussis in Chongqing is not clear. The present study analysed the characteristics of pertussis infection from December, 2012 to November, 2018 in Chongqing to establish and evaluate a more accurate method of simultaneous amplification and testing (SAT) to detect RNA of *B. pertussis* and improve the diagnosis of pertussis.

Materials and methods

Patients

We performed retrospective and prospective studies in the Children's Hospital of Chongqing Medical University, China from December 2012 to November 2018 (Figure 1). The inclusion criteria were presented in a previous report (Lee et al., 2018) and are listed in Table 1. Children with cough lasting more than 2 weeks were included in our study. Children with cough that lasted between 5 and 14 days and at least one symptom in the second part of Table 1 were included in our study. Otherwise, the child was excluded. Children with cough that lasted less than 5 days were excluded. There were 25,441 male and female inpatients with suspected pertussis infection between 14 days and 12 years old who were enrolled in the retrospective study. All of the pertussis was detected by culture and real-time PCR. A total of 4477 children, who were included in our study, were diagnosed as having pertussis. The standard for clinical diagnosis of pertussis was not only based on the criteria (Mbayei et al., 2018), but also confirmed as follows: (1) Culture for *B. pertussis* or PCR for Bordetella-DNA from respiratory samples were positive; (2) The results of sputum or blood culture proved that there was no other bacteria and fungi infection; (3) There was no evidence of *Mycoplasma pneumoniae* (MP) or *Chlamydia pneumoniae* (CP) infection, as verified by real-time PCR; and (4) There was no respiratory virus infection, as shown in Supplementary Table S1. The co-infection status of children in the retrospective study is shown in Supplementary Table S2. If a child was infected by any parasite mentioned above, then the child was included in our study but was not diagnosed as having pertussis. For the prospective study, respiratory specimens [sputum or bronchoalveolar lavage fluid (BALF)] of 8510 children were collected to confirm the inclusion criteria, and 800 of these children were chosen randomly based on a random number table. The proportion of samples chosen in each month were roughly the same as the total number of collected samples in each month from December 2017 to November 2018. The infectious status of all of the children included in the prospective study was also confirmed as described in the retrospective study. The sputum or BALF samples were collected using a sputum aspirator or fibrobronchus, and the collection was performed as shown in Supplementary Table S3.

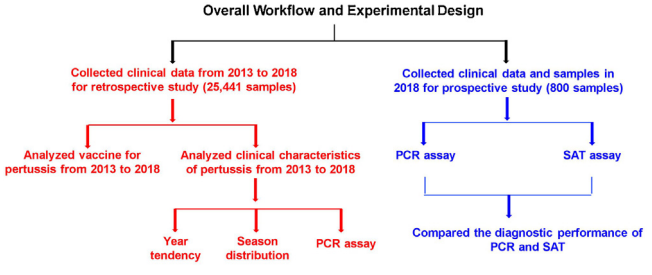

Figure 1. The overview of study design.
The clinical data of 25,441 samples included in our study from Dec 2012 to Nov 2018 was collected to analyze the status and the clinical characteristics of pertussis, which included the yearly tendency in Chongqing, season distributions and PCR performance, for retrospective study. All the samples in 2018 were collected and 800 of them were randomly chosen for prospective study, which aimed to establish and evaluate the new method-SAT assay for the diagnosis of pertussis.

The workflow and experimental design for this study are shown in Figure 1. The study was approved by the Ethics Committee of the Children's Hospital of Chongqing Medical University (Chcmu-20150008). Written informed consent was obtained from the parents or legal guardians of the patients.

Data collection

Clinical and laboratory data were collected from the medical records of the patients. The clinical data included sex, age, season, duration of illness, and symptoms. Laboratory data included sputum and blood culture results, real-time PCR results of MP, CP and *B. pertussis*, and respiratory virus antigen detection results.

B. pertussis *culture*

The culture was performed as described previously (Lee et al., 2018). Briefly, clinical specimens were plated onto Regan-Lowe agar plates with cephalexin. The small and pearly white colonies were selected for biochemical tests and plated on blood agar plates. *B. pertussis* culture infection was confirmed if there was no growth on blood agar plates and the following biochemical results were obtained: "oxidase+, nitrate-, urease-, motility-, HIT-no pigment, MacConkey-no growth or fermentation, citrate-no growth or colour change".

DFA screening for respiratory virus

Respiratory viruses were detected using a DFA kit (Diagnostic Hybrids, INC, Athens, USA) based on immunofluorescence. The assay was performed according to the manufacturer's instructions. A control glass slide was stained at the same time to ensure the reliability of test results for each assay. The experimental results were credible when the positive control hole was apple green, and the negative control hole was dark red. Multiple cells with an apple green colour in one reaction hole indicated that the virus present in the hole was positive. Dark red reaction holes were negative. Assays with only one cell with apple green or green fluorescence that blurred in the reaction hole were re-tested.

Real-time PCR for B. pertussis *DNA*

B. pertussis DNA was detected using a real-time PCR kit (Sansure Biotech Co., Ltd., Changsha, China) based on TaqMan

Table 1
The inclusion criteria for suspected BP-Infection children in this study.

Diagnosis sequence	Clinical symptoms
1	Cough duration >2 weeks
2	Cough duration 5–14 days with at least one of the following symptoms:
	Paroxysmal coughing
	Inspiratory whoop
	Post-tussive vomiting
	Apnoea
	Cyanosis
	Subconjunctival bleeding
	Leukocytosis

Note: BP: *Bordetella pertussis.*

probe PCR. The target sequence was in the *IS481* gene, and human *rnase* P gene was used as an internal control (Valero-Rello et al., 2018). The assay was performed according to the manufacturer's instructions. Forty cycles were performed for real-time PCR. The Ct value was less than 30, and the internal standard showed an S-shaped curve, which was regarded as positive. Ct values greater than 30 or no Ct value and an internal standard that showed an S-shaped curve were regarded as negative.

SAT for B. pertussis *RNA*

B. pertussis RNA was detected using an assay kit (RenDu Biotech Co., Ltd., Shanghai, China) based on SAT technology (Yang et al., 2007). The testing target was ptxA-mRNA of *B. pertussis* (Fry et al., 2009). The magnetic beads used to extract RNA were labelled with oligo-dT, and a specific nucleic acid capture fragment with oligo-dA was connected to the segment of oligo-dT. The fragment captured RNA specifically. The internal control was a synthetic RNA fragment, which was synthesized by RenDu Biotech Co., Ltd. and is the exclusive intellectual property of RenDu Biotech Co., Ltd. The control had no homology with common pathogens. The purity of RNA was detected based on ratios of optical density (OD): OD260/280 (2.0) and OD 260/230 (>2.0). According to Yang's study (Yang et al., 2007), target RNA was reverse transcribed under the action of a reverse transcriptase to synthesize a double-stranded DNA with T7 promoter. The T7 RNA polymerase used this double-stranded DNA as a template for transcription, and each double-stranded DNA was transcribed into 1000 copies of RNA within the amplification time in our study. The resulting RNA bound to a molecular beacon and fluoresced, which was detected using a fluorescence detector.

The protocol followed the manufacturer's instructions. Briefly, the RNA was purified and mixed with the reaction buffer without reverse transcriptase and polymerase. The mixture was kept at 60 °C for 10 min and 42 °C for 5 min, and the reverse transcriptase and polymerase were added. The entire reaction mixture was placed in a real-time PCR instrument (Hongshi Med Tech. Co., Ltd., Shanghai, China) with the following reaction conditions: 42 °C for 1 min in each cycle, and 40 cycles were performed. Ct values less than 30 and an internal standard showing an S-shaped curve were regarded as positive. Ct values greater than 30 or no Ct value and an internal standard showing an S-shaped curve were regarded as negative.

Statistics

Correlations between different *B. pertussis* nucleic acid assays and clinical diagnosis were tested by McNemar's test. The receiver operating characteristic curve (ROC) was used to evaluate the diagnostic performance of different *B. pertussis* nucleic acid assays. Data were analysed using SPSS version 13.0.

Results

The general characteristics for suspected B. pertussis *infection child patients*

There were 25,441 children with suspected *B. pertussis* infection enrolled in our retrospective study from December 2012 to November 2018. There was no significant difference in the number of children with suspected *B. pertussis* infection between the four seasons (Table 2). Because all the children were inoculated at 3 months (Huang et al., 2017), there was a group of children younger than 3 months. Pertussis is a threat to children, especially children under the age of five years (Forsyth et al., 2018), and infants younger than 12 months bear the greatest burden (Forsyth et al.,

Table 2
The characteristics of all the enrolled suspected BP-Infection children from December, 2012 to November, 2018.

	Suspected BP-Infection (%) (n = 25 441)
Onset Season	
Winter (Dec–Feb)	5177 (20.35)
Spring (Mar–May)	7475 (29.38)
Summer (Jun–Aug)	6545 (25.73)
Autumn (Sep–Nov)	6245 (24.55)
Age	
<3 M	3583 (14.08)
3–12 M	15 993 (62.86)
1–5 Y	5007 (19.68)
>5 Y	858 (3.37)
Gender	
Male	14 863 (58.42)
Female	10 578 (41.58)
Received vaccine against pertussis	
<3 M	3583 (14.08)
≥3 M	21 858 (85.92)
Cough duration before enrollment	
≤2 weeks	15 156 (59.57)
>2 weeks	10 285 (40.43)
Manifestations	
Paroxysmal coughing	15 181 (59.67)
Inspiratory whoop	6189 (24.33)
Post-tussive vomiting	3935 (15.47)
Apnoea	2853 (11.21)
Cyanosis	3254 (12.79)
Subconjunctival bleeding	158 (0.62)
Leukocytosis	22 354 (87.87)
Length of stay	
<4 days	20 611 (81.01)
4–10 days	4617 (18.15)
>10 days	213 (0.84)
Intensive care	
Yes	187 (0.74)
No	25 254 (99.26)
BP-infection measurement	
PCR positive	6491 (25.51)
Culture positive	4988 (19.61)

Note: BP: *Bordetella pertussis*; Dec: December; Feb: February; Mar: March; Jun: June; Aug: August; Sep: September; Nov: November.

2015). One and 5 years were two other truncations for age groups. The children were divided into 4 age groups: <3 months, 3–12 months, 1–5 years and >5 years. Most included children were 3–12 months old. More boys were enrolled than girls, and over 85% of the total children had ever received a pertussis-containing vaccine. Children younger than 3 months were not inoculated, but 100% of children older than 3 months were inoculated (Table 2). Most infants aged between 3 and 12 months received one dose of vaccine. The vaccine doses of the infants are shown in Supplementary Table S4.

To investigate the clinical characteristics of children with pertussis, the symptoms are shown in Table 3 by age group. The incidences of respiratory failure, hypoxia, cyanosis, apnoea and dehydration were higher in younger children, especially the age group of <3 M (Table 3). The incidences of post-tussive emesis and syncope were higher in older children, especially the age group of >5 Y.

Epidemiology of pertussis in Chongqing from 2013 to 2018

To investigate the tendency of pertussis in Chongqing, we first analysed the data of the 25,441 children included in our study for 6 successive years. The data showed that the number of samples detected as positive for *B. pertussis* DNA using PCR and the number of samples diagnosed as positive by clinical diagnosis criteria increased annually, regardless of the number of total included samples (Figure 2A). The ratio of PCR positivity, which was defined as the number of samples detected positive for *B. pertussis* DNA using PCR divided by the number of total included samples, also increased annually (Figure 2B). However, the ratio of clinical diagnosis to PCR, which was defined as the number of samples diagnosed as positive using the standard of clinical diagnosis mentioned in the first paragraph of the Materials and methods section, divided by the number of positive samples based on PCR for *B. pertussis* DNA, was approximate 70% annually during these 6 years (Figure 2B). These results demonstrated that the real number of *B. pertussis* infections increased annually, and the diagnostic efficacy of real-time PCR for *B. pertussis* DNA was stable during this period.

The relationships between *B. pertussis* infection and seasons are shown in Figure 2C. Winter was from December to February, spring was from March to May, summer was from June to August, and autumn was from September to November. The investigation period was December 2012 to November 2018. The ratios of positive PCR assays and clinical diagnoses were low in winter, high in spring and summer, peaked in summer, and low in autumn annually. *B. pertussis* infection in Chongqing was significantly seasonal, and the diagnostic efficacy of real-time PCR for *B. pertussis* DNA was stable in all seasons (Figure 2C).

The correlation between age group and B. pertussis *infection*

We focused on the age distribution of *B. pertussis* infection and found that the ratios of pertussis vaccinations of all children included ranged from 84.33% to 86.59% during the study period (Figure 3A). Overall, the vaccination rates were generally stable during the study period, especially for children older than 3 months, because 100% of these children were included in the NIP in China (Figure 3A).

Although the vaccination rates were generally stable during the period, the distributions of positive cases in each age group varied significantly. The ratio of positive cases in the <3 months group decreased annually. This tendency may be attributed to improvements in caring for babies by hospitals and families in Chongqing over time. In view of the stable vaccination rate as shown in Figure 3A, an effective vaccine should alter the number of positive cases in 3–12 months, 1–5 years and >5 year groups (Figure 3B), but the proportion of positive rates of 3–12 months, 1–5 year and >5 year groups should be stable. However, the proportion of positive cases in 1–5 year and >5 year groups increased from 7.35% to 27.45%, and from nearly 0% to 4.56% from 2013 to 2018, respectively, and the proportion in the 3–12 month group decreased from 70.59% to 58.39% from 2013 to 2018, respectively (Figure 3C). These results indicate a serious problem in the quality of inoculated vaccines and require further investigation.

Validation of the diagnosis effects of B. pertussis *assays*

Since *B. pertussis* was becoming increasingly serious in Chongqing, and the current detection method had a high false-positive rate (30%, Figure 2B), we planned to establish a new diagnostic method to detect *B. pertussis* RNA based on SAT technology. The results of clinical diagnoses are considered the "gold standard", and McNemar's test was used for the prospective study. The diagnostic performances of real-time PCR for *B. pertussis* DNA and SAT for *B. pertussis* RNA are shown in Table 4 based on McNemar's test. The Kappa values for the two assays were 0.690 and 0.831, respectively, which means that SAT had a higher coincidence rate with clinical diagnosis than real-time PCR. The diagnostic performances of both methods were also evaluated using ROC. The results demonstrated that the value of the area under curve (AUC) of SAT was 0.900 (95% CI, 0.861 to 0.939), which was higher than real-time PCR at 0.869 (95% CI, 0.832 to 0.906). These results indicate that SAT had a better diagnostic performance than real-time PCR for real *B. pertussis* infection.

Discussion

Pertussis is a vaccine-preventable disease that causes morbidity and mortality, particularly in infants and children under 5 years

Table 3
Clinical Characteristics of Patients with pertussis by Age Group in Chongqing, 2013-2018.

Symptoms	By age group				All ages
	< 3 M (n = 914) (%)	3–12 M (n = 4076) (%)	1–5 Y (n = 1279) (%)	>5 Y (n = 222) (%)	(n = 6491) (%)
Paroxysmal coughing	496 (54.27)	2563 (62.88)	698 (54.57)	116 (52.25)	3873 (59.67)
Inspiratory whoop	213 (23.30)	1105 (27.11)	246 (19.23)	15 (6.76)	1579 (24.33)
Respiratory failure	235 (25.71)	716 (17.57)	196 (15.32)	27 (12.16)	1184 (18.24)
Post-tussive emesis	132 (14.44)	582 (14.28)	247 (19.31)	43 (19.37)	1004 (15.47)
Hypoxia	154 (16.85)	351 (8.61)	113 (8.84)	14 (6.31)	632 (9.74)
Cyanosis	176 (19.26)	503 (12.34)	132 (10.32)	19 (8.56)	830 (12.79)
Apnea	215 (23.52)	412 (10.11)	91 (7.11)	10 (4.50)	728 (11.22)
Fever	357 (39.06)	1518 (37.24)	480 (37.53)	74 (33.33)	2429 (37.42)
Dehydration	32 (3.50)	89 (2.18)	34 (2.66)	3 (1.35)	158 (2.43)
Syncope	5 (0.55)	39 (0.96)	16 (1.25)	7 (3.15)	67 (1.03)

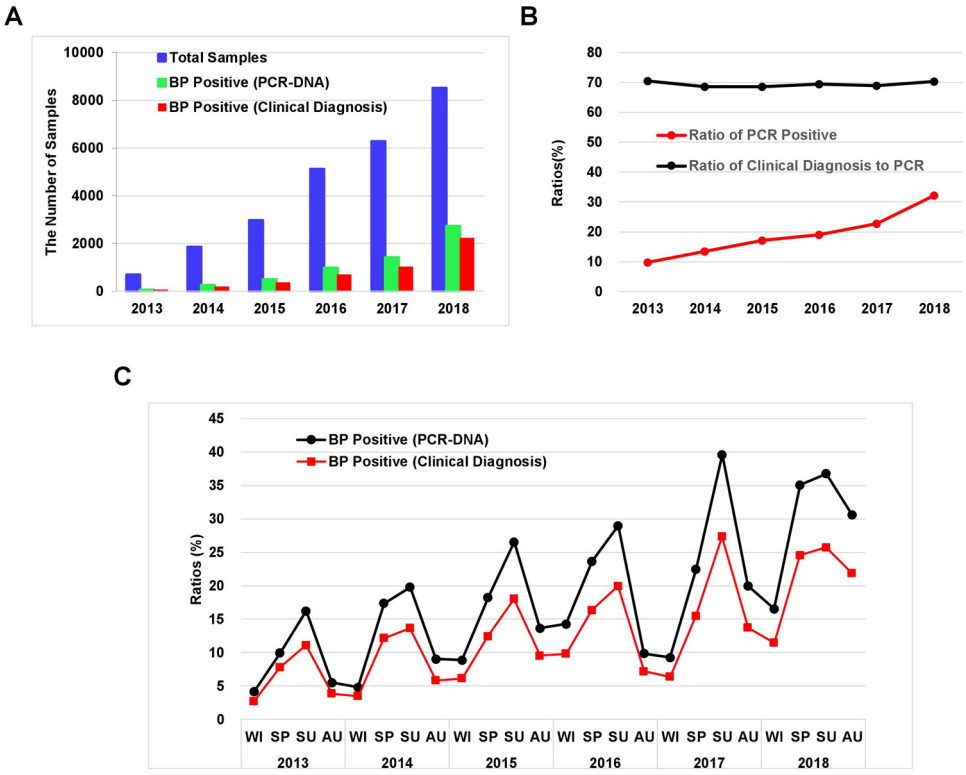

Figure 2. The PCR detection, clinical diagnosis and season distributions of pertussis from 2013 to 2018.
A: The number of all samples included in our study in each year (blue bar); The number of samples which were detected as positive by real-time PCR in each year (green bar); The number of samples which were diagnosed as pertussis as the criteria of clinical diagnosis (red bar).
B: The ratios of samples detected as positive by real-time PCR among all the included samples from 2013 to 2018 (red line); The ratios of positive samples confirmed by clinical diagnosis among all the samples detected as positive by real-time PCR from 2013 to 2018 (black line).
C: The season distribution of the ratios of samples detected as positive by real-time PCR among all the included samples in each season from 2013 to 2018 (black line).The season distribution of the ratios of positive samples confirmed by clinical diagnosis among all the included samples in each season from 2013 to 2018 (red line).
WI: winter; SP: spring; SU: summer; AU: autumn.

(Forsyth et al., 2018). Pertussis remains difficult to diagnose because of the variation in its clinical presentation and interference of pertussis-like illnesses (Frumkin, 2013; Wirsing von Koenig, 2014). We investigated the clinical characteristics of pertussis in Chongqing from December 2012 to November 2018 to establish a new assay to improve the diagnostic efficacy of pertussis.

Our study revealed that pertussis became increasingly serious in Chongqing annually (Figure 2A–C), and children suffering from pertussis were under 1 year and 5 years old and accounted for 70.72% (3166/4477) and 96.72% (4330/4477) of all children suffering from pertussis, respectively. The incidence of pertussis in Chongqing exhibited a periodic pattern, which was high in spring and summer, peaked in summer, and low in autumn and winter. The age distribution of *B. pertussis* infection and the pattern of the incidence of pertussis were quite similar to previous reports (World Health Organization, 2015; Oliveira et al., 2018; Guimarães et al., 2015). As Table 3 shows, the incidences of respiratory failure, hypoxia, cyanosis, apnoea and dehydration were higher in younger children, which may underlie the increased vulnerability of younger children to pertussis. The incidences of post-tussive emesis and syncope were higher in older children, which may be attributed to increased exercise in older children.

Although the vaccination rates of children in Chongqing were generally stable (Figure 3A), the proportion of *B. pertussis* infection-positive cases in 1–5 year and >5 year groups increased significantly, but the proportion of positive cases in the 3–12 month group decreased from 2013 to 2018 (Figure 3C). If the inoculated vaccines were effective, the proportion of positive rates of 3–12 month, 1–5 year and >5 year groups should vary only slightly. Until 2015, the proportion in the 3–12 month group was stable at approximately 70%, and the increased proportions in the 1–5 year and >5 year groups may be related to the decreased proportion in the <3 month group (Figure 3C). However, the proportion of *B. pertussis* infection in children younger than 3 months and children aged 3–12 months decreased from 2015 to 2018. The proportions of *B. pertussis* infection in children in the 1–5 year and >5 year groups increased. These results suggest vaccines against pertussis may be problematic because more children received all of the primary and booster doses of vaccine but remained vulnerable to *B. pertussis*.

The problematic vaccine for pertussis was due to changes in process parameters and equipment in 2018 (The Lancet, 2018). The positive rate of anti-pertussis toxin IgG in 944 healthy children in Chongqing was only 1.17% in 2018 (Yao et al., 2018). This low positive rate of anti-pertussis toxin may be related to the immunization programme, as reported previously (Yao et al., 2018), but it is more likely related to the vaccine quality based on our data. If an unqualified production process was the main reason for the change in the proportions of the different age groups, this condition would have been in place since at least 2014 because the proportions of 1–5 year and >5 year groups increased from 2015. Given the monopoly status of domestic vaccine companies in Chongqing and mainland China, increasing numbers of children over 3 months will suffer from pertussis in the next years because of this “unqualified production process”. All of the children who were inoculated with a pertussis vaccine since 2014 should

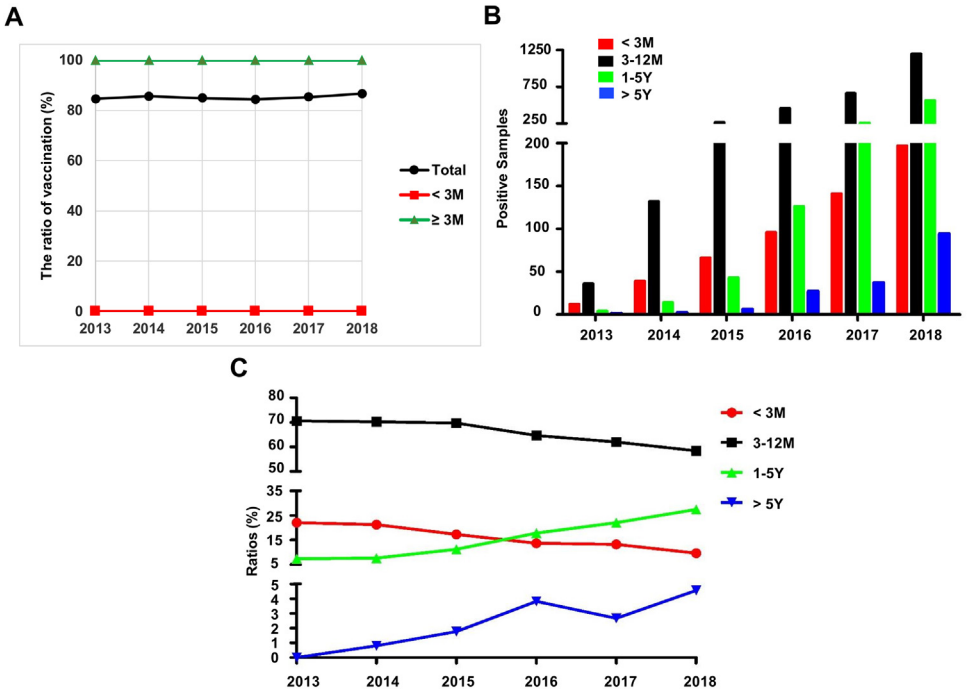

Figure 3. The vaccination rate and proportion of clinical positive samples of each age group from 2013 to 2018.
A: The ratios of the vaccination of children from 2013 to 2018.
B: The clinical positive samples of each age group from 2013 to 2018.
C: The proportion of clinical positive samples of each age group from 2013 to 2018.

Table 4
The correlation between different assays and clinical diagnosis for BP-Infection.

	Assay/Clinical Diagnosis				χ^2	*Kappa* value	*p* value
	+/+	−/+	+/−	−/−			
Real-time PCR on BP DNA*	272	16	106	406	66.3934	0.690	<0.0001
SAT on BP RNA*	234	54	6	506	38.4000	0.831	<0.0001

Note: *:McNemar's test; BP: *Bordetella pertussis*.

undergo antibody detection to determine whether they require re-inoculation.

To overcome the high false-positive rate of real-time PCR, we established a new assay based on SAT technology to improve the diagnostic performance of *B. pertussis* infection. Although the false-negative rate of SAT was slightly higher than real-time PCR, SAT greatly reduced the false-positive rate (Table 4). Taken together, the diagnostic performance of SAT was better than real-time PCR (Figure 4), and the higher false-negative rate of SAT may be attributed to the degradation of RNA. In the future, the sensitivity of SAT should be improved to reduce the false-negative rate.

In summary, we provide a better understanding of the incidence and clinical characteristics of pertussis in Chongqing. We revealed that the influence of a problematic vaccine for pertussis in China was much more serious than public reports (The Lancet, 2014). SAT for the detection of *B. pertussis* RNA had better diagnostic performance than traditional real-time PCR used to detect *B. pertussis* DNA.

Ethics statement

The study was approved by the Ethics Review Board of the Children's Hospital of Chongqing Medical University. Written informed consent was obtained from all the parents and guardians of children in this research.

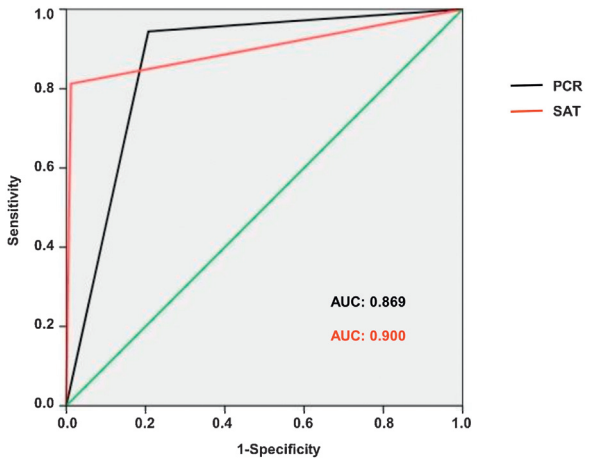

Figure 4. ROC analysis of the diagnostic performance of real-time PCR and SAT. Black curve and AUC value: real-time PCR for *Bordetella pertussis* DNA; Red curve and AUC value: SAT for *Bordetella pertussis* RNA.

Author contributions

The conception and design of the study: Lin Zou, Enmei Liu, Zhou Fu

Acquisition of data: Zhidai Liu, Shan Liu, Yi Shu, Zuqun Yang, Hongmei Xu, Qubei Li, Zhengxiu Luo, Jihong Dai

Analysis and interpretation of data: Zhidai Liu, Bin Peng

Drafting the article: Zhidai Liu, Lin Zou

Final approval of the version to be submitted: All the authors

Conflicts of interest

None of the authors has a conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.05.014.

References

Barkoff AM, Grondahl-Yli-Hannuksela K, He Q. Seroprevalence studies of pertussis: what have we learned from different immunized populations. Pathog Dis 2015;73: pii:ftv050.

Cassiday P, Sanden G, Heuvelman K, Mooi F, Bisgard KM, Popovic T. Polymorphism in *Bordetella pertussis* pertactin and pertussis toxin virulence factors in the United States, 1935-1999. J Infect Dis 2000;182:1402–8.

Chen Z, He Q. Immune persistence after pertussis vaccination. Hum Vaccin Immunother 2017;13:744–56.

Emery VC. CMV infected or not CMV infected: that is the question. Eur J Immunol 2013;43:886–8.

Forsyth K, Plotkin S, Tan T, Wirsing von König CH. Strategies to decrease pertussis transmission to infants. Pediatrics 2015;135:e1475–82.

Forsyth KD, Tan T, von König CW, Heininger U, Chitkara AJ, Plotkin S. Recommendations to control pertussis prioritized relative to economies: a Global Pertussis Initiative update. Vaccine 2018;36:7270–5.

Frumkin K. Pertussis and persistent cough: practical, clinical and epidemiologic issues. J Emerg Med 2013;44:889–95.

Fry NK, Duncan J, Wagner K, Tzivra O, Doshi N, Litt DJ, et al. Role of PCR in the diagnosis of pertussis infection in infants: 5 years' experience of provision of a same-day real-time PCR service in England and Wales from 2002 to 2007. J Med Microbiol 2009;58:1023–9.

Guimarães LM, Carneiro EL, Carvalho-Costa FA. Increasing incidence of pertussis in Brazil: a retrospective study using surveillance data. BMC Infect Dis 2015;15:442.

Huang HT, Gao ZG, Liu Y, Wang LJ, Liu YP, Zhang Y. Epidemiological characteristics and risk factors of the pertussis in infants <12 months of age in Tianjin, China. Biomed Environ Sci 2017;30:545–8.

Lee AD, Cassiday PK, Pawloski LC, Tatti KM, Martin MD, Briere EC, et al. Clinical Validation Study Group. Clinical evaluation and validation of laboratory methods for the diagnosis of *Bordetella pertussis* infection: culture, polymerase chain reaction (PCR) and anti-pertussis toxin IgG serology (IgG-PT). PLoS One 2018;13:e0195979.

Liu Z, Zhang P, Tang S, He X, Zhang R, Wang X, et al. Urine real-time polymerase chain reaction detection for children virus pneumonia with acute human cytomegalovirus infection. BMC Infect Dis 2014;14:245.

Loeffelholz M. Towards improved accuracy of *Bordetella pertussis* nucleic acid amplification tests. J Clin Microbiol 2012;50:2186–90.

Mbayei SA, Faulkner A, Miner C, Edge K, Cruz V, Peña SA, et al. Severe pertussis infections in the United States, 2011-2015. Clin Infect Dis 2018;, doi:http://dx.doi.org/10.1093/cid/ciy889.

Meng QH, Liu Y, Yu JQ, Li LJ, Shi W, Shen YJ, et al. Seroprevalence of maternal and cord antibodies specific for diphtheria, tetanus, pertussis, measles, mumps and rubella in Shunyi, Beijing. Sci Rep 2018;8:13021.

Oliveira SM, Gonçalves-Pinho M, Freitas A, Guimarães H, Azevedo I. Trends and costs of pertussis hospitalizations in Portugal, 2000 to 2015: from 0 to 95 years old. Infect Dis (Lond) 2018;50:625–33.

Petridou E, Jensen CB, Arvanitidis A, Giannaki-Psinaki M, Michos A, Krogfelt KA, et al. Molecular epidemiology of *Bordetella pertussis* in Greece, 2010-2015. J Med Microbiol 2018;67:400–7.

The lancet. Vaccine scandal and confidence crisis in China. Lancet 2018;392:360.

Valero-Rello A, Henares D, Acosta L, Jane M, Jordan I, Godoy P, et al. Validation and implementation of a diagnostic algorithm for DNA detection of *Bordetella pertussis*, *B. parapertussis* and *B. holmesii* in a pediatric referral hospital in Barcelona, Spain. J Clin Microbiol 2018;57: pii: e01231-18.

Wendelboe AM, Van Rie A. Diagnosis of pertussis: a historical review and recent developments. Expert Rev Mol Diagn 2006;6:857–64.

Wirsing von Koenig CH. Pertussis diagnostics: overview and impact of immunization. Expert Rev Vaccin 2014;13:1167–74.

World Health Organization. Pertussis vaccines: WHO position paper—September 2015. Wkly Epidemiol Rec 2015;90:433–58.

Xu Y, Wang L, Xu J, Wang X, Wei C, Luo P, et al. Seroprevalence of pertussis in China: need to improve vaccination strategies. Hum Vaccin Immunother 2014;10:192–8.

Yang JL, Schachter J, Moncada J, Habte D, Zerihun M, House JI, et al. Comparison of an rRNA-based and DNA-based nucleic acid amplification test for the detection of *Chlamydia trachomatis* in trachoma. Br J Ophthalmol 2007;91:293–5.

Yao N, Zeng Q, Wang Q. Seroepidemiology of diphtheria and pertussis in Chongqing, China: serology-based evidence of *Bordetella pertussis* infection. Public Health 2018;156:60–6.