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Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study

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ABSTRACT

Introduction

Despite high vaccination coverage with three-dose diphtheria-tetanus-pertussis (DTP3), a "rise of pertussis" emerged and poses threat to public health in China. To understand the potential reasons behind this rise, precise incidence of laboratory-confirmed pertussis and characteristics of strains from rigorously conducted, prospective, population-based studies can be useful, namely evaluating or optimizing interventions, developing new interventions, and adjusting immunization schedule and recommendations.

Methods and analysis

This paper describes the study design of a one-year, prospective, age-stratified, and population-based case-control study, including sites selection, study population, case ascertainment and enrolment, control recruitment, follow-up of case, microbiological methods, data collection, quality control activities, and statistical methods used to generate incidence estimates. During June 2021 through May 2022, we aim to enroll 1,000 suspected pertussis cases (i.e. chronic/persistent cough) and 2,000 frequency matched healthy controls in a well characterized population, which would cover approximately 2.23 million censused populations at two sites. Our primary study outcome, the laboratory-confirmed *Bordetella Pertussis* infection, will be determined by a comprehensive laboratory methods and procedures (i.e. culture, PCR, and serological tests) in both cases and controls at enrolment and during 60-day's follow-up visits.

Ethics and dissemination

This study has been approved by Chinese Center for Disease Control and Prevention's Institutional Review Board. Upon completion, the results from this study will provide valuable scientific data and some new insights into the incidence, etiology, and risk factors for severe sequelae of pertussis to academic societies and the public health authorities who is currently struggling and fighting against this burdensome disease worldwide.

Keywords: Bordetella pertussis, Case-Control Studies, Incidence, China

Summary

Strengths and limitations of this study

- A population-based study, appoximately 1,000 suspected pertussis cases and 2,000 frequency matched healthy controls will be enrolled in a well characterized population covering approximately 2.23 million censused populations at two sites of China during June 2021 through May 2022.
- A laboratory-based study, in which a comprehensive laboratory methods and procedures (i.e. culture, PCR, and serological tests) will be used to confirm our primary study outcome, i.e. *Bordetella Pertussis* infection, allowing us to specifically measure pertussis disease burden.
- A case-control study, in which, the prevalence and population attributable fraction (AF) of *Bordetella Pertussis* infection will be acquired.
- A follow-up study, in which all cases will be followed up to 60-days to collect interesting events (i.e. adverse clinical outcomes of hospitalization or death) at 2, 4 and 8 weeks after enrolment.
- The incidence of laboratory-confirmed pertussis will be acquired by age groups (children, adolescents and adults), and by settings (community, outpatient and inpatient).

BACKGROUND

Whooping cough (pertussis) is a highly contagious respiratory disease caused by *Bordetella Pertussis*[1 2]. Despite a high vaccine coverage of third dose diphtheria-tetanus-pertussis vaccine (DTP3)[3], the "resurgence of pertussis" in recent years has posed a great threat to global public health[4-6], as well as to Chinese infants[7 8]. The WHO estimates that pertussis kills about 160,700 children under 5 years old worldwide each year[9]. In China, three types of pertussis vaccines are available till March 31, 2021, i.e. the co-purified diphtheria and tetanus toxoids and acellular pertussis (cDTaP, used for routine immunization), DTaP/Hib (Minhai Biotechnology Co., Ltd., Beijing, China)[10] and DTaP-IPV/Hib (Sanofi Pasteur, Lyon, France)[11 12]. The coverage of DTP3 remained high above 99% for children throughout the 2010s[13 14], but no school-age, adolescent or adult immunization (e.g. maternal vaccination) and post-exposure prophylaxis are recommended in the country[15]. The reported incidence of pertussis has risen from 0.12 per 100,000 in 2013 to 2.14 per 100,000 in 2019 (Figure 1). The rise was mainly concentrated in infants less than 1 year old (103.89 per 100,000 in 2019), and the underlying causes are unknown. It was suspected that multifactorial causes might have contributed to the rise[4], e.g. the change of the whole-cell pertussis vaccine to acellular vaccine in national immunization program[15 16], the adaption of the bacterium to evade immunization induced by vaccine, the increased diagnosis with the introduction of the PCRs in clinical practices, the raised awareness of reporting in doctors, as well as the rise in circulation of the pathogen among older children, adolescents and adults whose immunity from vaccination or natural infection has waned[17].

Most epidemiological data on pertussis in China come from a passive reporting system, the National Notifiable Infectious Disease Surveillance System (NNIDSS)[18]. Because of limited diagnosis and incompleteness of reporting[8 19 20], underreporting is substantial in the system and the burden of pertussis remains underrecognized. Moreover, some important data such as clinical, laboratory and vaccine information are not available, which is unfavorable for evaluating the effectiveness of vaccine and implementing of other disease control and prevention programs (such as adult vaccination, diagnostic

tests and post-exposure prophylaxis of pertussis). Rigorously conducted, prospective, population-based studies can be used to strengthen the NNIDSS, by providing information on the burden of laboratory-confirmed pertussis, strains distribution, risk factors for severe sequelae and case fatality, and most importantly, to assist health authority in China to investigate the reasons behind the rising incidence of pertussis and prioritize health research investments and vaccine development.

We designed the PertussisChina study, a one-year, prospective, age-stratified, population-based longitudinal cohort (active surveillance) and case-control study, which will enroll suspected pertussis patients (i.e. chronic/persistent cough) seeking healthcare in several selected participating hospitals (SHs) at two sites of China, covering approximately 2.23 million censused population. This article describes the study design, including sites selection, study population, case ascertainment and enrolment, control recruitment, follow-up of cases and controls, microbiological methods (i.e. culture, PCR, and serological tests), data collection, quality control activities, and statistical methods used to generate incidence estimates of pertussis. We then further discuss the strengths and weaknesses of the study design.

METHODS AND ANALYSIS

Objectives of the study

The primary objective of the study is to measure the incidence of laboratory-confirmed pertussis by age groups (children, adolescents and adults), and by settings (community, outpatient and inpatient). The secondary objectives are: 1) to describe the distribution of disease severity and outcomes across age groups; 2) to describe the patterns and factors of under-detection and under-reporting of pertussis; 3) to study the carrier (colonization) status of the *B. pertussis* in the upper respiratory tract of healthy controls, and the serum levels of anti-pertussis toxin antibodies (anti-Ptx IgG) in both patients and healthy people; and 4) to create a repository of well-characterized clinical specimens and *B. pertussis* isolates that can be used in future studies.

Study sites and population

Site Selection Criteria

Sites are selected based on the following criteria: 1) have strong willingness to participate; 2) have capability and resources to conduct ongoing surveillance, namely staffs to facilitate specimen collection and case investigation, previous experience in disease surveillance, infrastructures to secure data collection and specimen storage or transportation; and 3) provide a full list of healthcare facilities in the area and the information of built-in hospital information system in the facilities. Currently, there are two sites in the study, including Yongcheng, Henan and Yiwu, Zhejiang (Figure 2).

Study population

In 2019, Yiwu had a permanent population of 821,000 (47,000 were children under five years of age) served by 24 health care facilities. Most hospital admissions (≥80% of the total number) occurred in the three large tertiary hospitals, including a children's hospital and two general hospitals; meanwhile, Yongcheng had a permanent population of 1,411,000 (94,000 were children under five years of age) served by 41 health care facilities. Most hospital admissions occurred in the five large secondary hospitals, including four general hospitals and a maternal and pediatric hospital. In total, the two sites cover a total of 2.23 million permanent population in the study area.

Study overview and design

In order to achieve our study objectives, we will conduct the following study activities at the two sites from June 2021 through May 2022, including, 1) a Healthcare Utilization and Attitudes Survey (HUAS) and a census data updating to define study population (i.e. incidence denominator), so as to set up a sampling frame for the case-control study and selecting participating hospitals (i.e. SH) for case recruitment; 2) the case-control study to acquire the prevalence of *B. pertussis* infection among suspected pertussis cases and healthy controls, as well as the calculation of population

attributable fraction (AF) indicating the proportion of cases that can be prevented if *B. pertussis* was totally removed from the population; and 3) the retrieval of electronic medical records (EMRs) from hospital information system to validate the number of suspected pertussis case patient (chronic/persistent cough) enrolled in SHs (i.e. incidence numerator) (Figure 3).

Defining and calibrating study population

Census data updating

Population census data at the two sites will be collected and updated during the study period. Population census is conducted every ten years in China and the nearest one is in 2020. However, a intermittent survey of 1% sampling of the total population would be performed to update population census data every year between the two census. We will retain the up-to-date population data from the National Bureau of Statistics. Moreover, the population birth, mortality, and population migration is recorded by the local government. We will also contact the local health bureau quarterly to access these data to give a precise estimation of population size in the two sites.

Healthcare Utilization and Attitudes Surveys (HUAS)

HUAS will be conducted prior to recruiting cases and controls at the two sites, which will serve three purposes, 1) to set up a sampling frame for the case-control study; 2) to select SHs in which prospective enrolment of cases will be conducted; and 3) to provide estimates of the population coverage for SHs and healthcare seeking behavior weights applied in estimating pertussis incidence.

In summary, a population-based cross-sectional study, with an age-stratified sample of 3,000 children aged 0-59 mo and 6,000 adolescents/adults aged ≥5 years, will be conducted in the community of the two sites. The sample size was calculated based on: i) for children, a monthly prevalence of cough illness, π=1% (estimated from the reported incidence of lower respiratory tract infection of 0.15 per child year[21]), allowable error (δ=0.5%), significant level (α=0.05), and design effect (deff=2); ii) and for adolescents/adults, a monthly prevalence of cough illness, π=3.3%[22], allowable

error (δ=0.66%), significant level (α=0.05), and design effect (deff=2).

A complex sampling method will be used to select survey respondents as follows. Firstly, a probability proportionate to size sampling will be used to randomly select 50 clusters (e.g. communities or villages) in the site's administrative regions. At the second sampling stage in selected communities, quota sampling will be used to recruit interviewee. The quota required in each age stratum was calculated based on the age distribution of the population in the site and the number of surveys allocated to each cluster. Trained work staff will go to the selected communities to conduct face-to-face surveys at several locations (residential areas, kindergartens and children's vaccination clinics) Monday to Sunday during daytime in the study period. All residents living in the communities or villages for at least half a year prior to survey are eligible for and invited to participate in the interview. After the quota required in each age group is complete, the interviews will stop.

The following questions (Supplementary table 1 & table 2) are asked to respondents, 1) the occurrence and length of cough illness in the previous month prior to survey, 2) healthcare-seeking behavior regarding the self-reported cough illness for the most recent episodes and the sources of healthcare facilities; and 3) the willingness to seek healthcare and where would they choose to visit for an assumptive cough illness.

Based on the HUAS and census data, hospitals at which over 80% of respondents in each site choose to attend when hospital admission is required will be selected as our SHs. In case healthcare providers in the site change their practice or scope of service during our study period, for example the opening of new hospitals or the establishment of new branches of existing hospitals, an abbreviated HUAS with a smaller sample of 1,000 will be administered at the middle or the end of the year during which cases are recruiting at SHs.

Case-control study

Case definition of suspected pertussis

Patients will be classified as suspected pertussis cases and offered to participate if they present chronic/persistent cough defined as cough of ≥2 weeks duration with one or

more of the following symptoms, 1) paroxysmal cough; 2) inspiratory whoop; or 3) post-tussive vomiting; Or, for children aged <1 years-old, cough (regardless of cough duration) accompanied by one or more of the following symptoms, 1) apnea; 2) paroxysmal cough; 3) inspiratory whoop; or 4) post-tussive vomiting.

We will exclude patients presenting with gastroesophageal reflux, spastic bronchitis, and clearly diagnosed tuberculosis, mycoplasma/chlamydia infection, or chronic sinusitis. Adults/adolescents with a measured body temperature of ≥38.5 °C at enrolment will also be excluded.

Sample Size Considerations

We planned to enroll approximately 250 suspected cases and 2 matched controls for each case in each age stratum (i.e. children under 5 years, and adolescents/adults aged ≥5 years) at each site, which would add up to approximately 1000 suspected cases and 2000 controls at the two sites. We calculated the above sample size based on a prevalence of *B. pertussis* in chronic/persistent cough of 20% (range=12%-32%) [23-25], an allowable error of 5% and a significant level of 0.05. This sample size would have a 90% power (two sided α =0.05) to detect an odds ratio (OR) of 2 between case and control for a site and age stratum-specific comparison, if the true prevalence of *B. pertussis* is 20% in case; or an OR of 3, if the true prevalence is 10%. Although the carrier state of *B. pertussis* is transient in family contacts[26 27], *B. pertussis* is rarely identified in healthy people [28 29], and we expected a larger OR of ≥ 2 in the study. This sample size means that the laboratory would process average 115 samples per week, which is feasible and acceptable for our laboratories.

Case Registry, Ascertainment and Enrollment

Case registry, ascertainment and enrollment will be conducted in SHs during the study period. Clinicians or trained nurses working in selected departments of the SHs (i.e. respiratory, pediatric, infectious disease, and emergency department) will carry out case registry of suspected pertussis cases every weekday (i.e. Monday through Sunday) except national holidays. Each outpatient visits and new hospital admission seeking healthcare in above departments will be screened for the eligibility of inclusion using

the inclusion & exclusion criteria of the suspected case definition of pertussis. Eligible ones will be ascertained and recorded by study coordinator who assist with doctors in SHs in enrolling cases using a standardized case reporting form (CRF) (Supplementary Table 3). We planned to enroll all hospital admissions and the first 1-3 outpatients each week in each hospital. After obtaining informed consent, study staff will conduct enrollment interviews, and collect nasopharyngeal (N/P) and blood specimens for each enrolled case.

Controls selection

At the middle of the study year when the sample size of cases reaches a half of the total, a control is recruited in community of the study sites using approximate frequency matching, based on the following criteria, 1) similar proportion in sex strata; 2) similar proportion in age strata, i.e. <1 year, 1-5 years, 6-19 years, 20-64 years and ≥65 years; 3) a control/case ratio of 2:1; and 4) no cough, running nose, shortness of breath, dyspnea or other respiratory symptoms at enrolment nor have a record of healthcare for respiratory disease in previous 6 months before recruitment.

60-day follow-up of case

We will follow cases from the time of enrollment to a maximum time period of 60 days after enrollment. Follow-up will be conducted at 2nd, 4th and 8th weeks after enrollment, with face-to-face interview if patient is currently hospitalized, or one telephone call each follow-up time if patient is discharged from hospital. At each follow-up visit/phone call, the study staff will ask about cough or other respiratory or systemic illness symptoms in the period since the last contact. If case is still symptomatic (cough) during follow-up, they will be encouraged to visit their doctor who enrolled them in the SHs within 24h of contact. The doctor will checkup the patient's health status and collect the swab and serum samples during the visit. If an enrolled patient does not want to visit the SHs, the study staff will arrange a household visit to collect the sample in the home.

Data collection from cases and controls

At enrolment, trained physicians and the study coordinator will conduct face-to-face

interview to collect socio-demographic, clinical and epidemiological data from cases and controls using a standardized CRF (Supplementary Table 4). Demographic information includes household size (defined as a group of people who share a dinner table), average household income, rural or urban residence, age, alcohol consumption and smoking exposure, and occupation etc. A clinician will also examine all cases to document clinical signs and symptoms at enrollment, including cough characteristics [duration, paroxysms, post-tussive vomiting, exacerbation at night], body temperature, respiratory rate, heart rate, seizure, apnea, and other general respiratory symptoms, non-prescription antibiotic usage before visiting the doctor, blood test results and chest x-ray examinations. Vaccination history (i.e. band, dosing, procedure and time of administration) of children aged ≤14 years is also collected by linkage of his/her individual records on immunization in the national database (Childhood Immunization Information Management System,CIIMS)[30] or checking of vaccination certificate.

During follow-up visits, data on any current cough or respiratory symptoms, subjective severity of illness, illness duration, functional impairment, whether medical care was sought, and outcomes since the last visits will be collected using CRFs (Supplementary Table 5). At the end of follow-up, medical charts of each hospitalized case will be reviewed by study staff to collect information on antibiotic treatment and outcomes during hospitalization (i.e. mechanical ventilation, ICU transfer, and death) (Supplementary Table 6).

The retrieval of electronic medical records and Validation of the total number of suspected pertussis case

Since our case registry and enrolment is conducted in selective departments (i.e. respiratory, pediatric, infectious disease and emergency departments) and on workdays, it is essential that the total number of hospital admissions and outpatient/emergency department visits for chronic/persistent cough illness in the whole of hospital is retrieved from SH's EMRs, and used to validate the number of suspected pertussis case encountered in SH (i.e. the numerator of incidence). Specifically, all hospital discharges or ambulatory visits in SHs coded for diagnosis

under the International Classification of Diseases 10th Revision (ICD-10) codes A37, J00-J22, J40-J47, R05, R09.2, P22, P28.2, P28.3, P28.4, and P28.5 will be monitored on a daily basis as registry cases, by hospital departments. At the end of the month, all the records with the above diagnosis codes will be abstracted from hospital information system (HIS) of the SHs. This data will be validated by prospectively counting data in the selective departments that conduct case enrolment to make a precise estimate of the total number of chronic/persistent cough illness outcomes in the studied population. Namely, through linking and comparing between the number of registered cases and the number of suspected pertussis case patients enrolled in the selected departments, we will calculate the W_{case}. With this W_{case}, we will narrow down the ICD-based EMRs records to the total number of suspected pertussis cases in SHs.

Laboratory investigation

Specimen collection and transport

When patients meet our suspected pertussis case definition or are recruited controls, they, as well as symptomatic (cough) cases during follow-up contacts, will be sampled within 24 hours. Physicians or nurses in SHs will be trained to collect nasopharyngeal swabs (N/P) and whole blood sample. Dacron or nylon swab will be used to collect N/P specimen to facilitate culture and PCR tests for *B.pertussis*[31]. Collected swab specimens will be plated onto selective agar or placed in transport medium (Charcoal Agar, Thermo Fisher Scientific Inc.) immediately after sampling at the SHs. Whole blood without adding any anticoagulants (>4ml for participants aged 5 years and older, and ≥2 ml for children aged <5 years) will be collected, and centrifuged to separate serum within 24h of collection. All collected swab and sera samples will be transported to the central laboratory of Chinese Center for Disease Control and Prevention (China CDC), using a cold box to maintain a temperature of 4°C. During transportation, samples are packaged and transported in accordance with the provision of International Civil Aviation Organization (ICAO) document Doc9284 and UN3373

Processing and storage of specimen

Upon arrival at the laboratory of China CDC, swab samples will be processed and prepared into three aliquots of swab supernatant, so will serum samples be. One of these aliquots will be analyzed and the other two aliquots will be kept for future analyses. All aliquots will be stored at -70°C temperature until the time of analysis.

Laboratory testing

In the laboratory of China CDC, Charcoal Agars will be cultured to isolate *B. pertussis* using standard method recommended by China CDC[32] and World Health Organization[33]. Swab supernatant will be analyzed for *B. pertussis, B. parapertussis, B. bronchiseptica* and *B. holmesii* using polymerase chain reaction (PCR) as recommended by US CDC [34 35]. Sera samples that have a minimum volume of $\geq$ 1 ml will be tested for Anti-Ptx IgG titer using a commercially available diagnostic kit (Virion\Serion, Wurzburg, Germany) according to the manufacturer's recommendations. To validate our laboratory methods and testing results, external quality assurance testing will be conducted to reach agreements with a reference laboratory on *Bordetellae* prior to study start. For serology testing, we use standard from the National Institute for Biological Standards and Control, London, UK, (*https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=18/146*); and for PCR assays, the Wisconsin State Laboratory of Hygiene, Wisconsin, U.S. (*http://www.slh.wisc.edu/proficiency/training-and-competency/*).

Suspected pertussis cases and controls that have *B. pertussis* Isolated, positive tests of swabs in any of samples collected during enrolment and follow-up, or have a 2-fold or greater rise in anti-PT IgG antibody between sequential sera samples with at least one time point higher than 40 IU/ml of serum titer would be considered laboratory-confirmed pertussis.

Data flow, management and analysis

The data collected in the study are centrally managed at China CDC, using an online data platform (*http://eddc.chinacdc.cn/dap/*). The completed CRFs will be entered

into the information system by local study staff at the two sites and uploaded to data server through encrypted transmission via a Virtual Private Network set up by China CDC. The entered records are regularly checked for completeness, consistency, and logical errors by data manager and the site's co-principle investigator who is responsible for authorization, integrity, security, and backup of database during data collection.

Statistical analysis

The collected data processing and key indicators based on which we calculate incidence are shown in figure 4. We will calculate the incidence of pertussis by age group and by settings with the following formula.

$$Hospitalization\ \ incidence\ rate = \sum \frac{S_i^{inpatient} \times W_i^{case} \times AF_i}{N_i \times W_i^{cover} \times W_{sampling} \times C_i}$$

$$Outpatient\ \ incidence\ rate = \sum \frac{S_i^{outpatient} \times W_i^{case} \times AF_i}{N_i \times W_i^{cover} \times W_{sampling} \times C_i}$$

$$Community\ incidence\ rate = \frac{Outpatient\ \ incidence\ rate}{r_i}$$

Where, $S_i^{inpatient}$ and $S_i^{outpatient}$ indicates the registered number of inpatients and outpatient visits of cough illnesses at age group i, as obtained from HIS. W_i^{case} is the weight used to adjust $S_i^{inpatient}$ and $S_i^{outpatient}$ to meet our case definition in age group i. This weight is calculated from the results of the prospective case-control study as a ratio of suspected pertussis cases over registered cases of cough illnesses at the selective departments of SHs. $W_{sampling}$ is the weight used to adjust holidays in which we do not conduct active case enrollment (we only conduct case enrollment in weekdays, not holidays). It is calculated as the total days that we conduct active study divided by 365.25. N_i is the population size in age group i in census year 2020. W_i^{cover} is the weight used to adjust catchment population overlapping between participating hospitals from HUAS in age group i. It is calculated as the ratio of inhabitants who actually have reported seeking medical care in the participating hospitals for the last episodes of their cough illness over the inhabitants who have the willingness of healthcare-seeking in the participating hospitals, as obtained from the HUAS study. C_i is the proportion of population covered by participating hospitals in age group i, as

measured in the HUAS study. It is calculated as the proportion of residents who report having the willingness of healthcare-seeking in the participating hospitals over the total no. of residents responded. r_i is the proportion of community residents reporting seeking health-care for their most recent episode of cough illnesses in age group i as measured in the HUAS study. AF_i is the population attributable fraction of chronic/persistent cough due to *B. pertussis* infection in age group i, calculated based on case-control study using unconditional logistic regression model, as follows:

$$\log_e(OR) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_k x_k$$

$$OR = \exp(\beta_k)$$

$$AF_i = Pr(\textit{Bordetella pertussis} \mid \text{Chronic cough})\left(1 - \frac{1}{OR}\right)$$

Note: $Pr(\textit{Bordetella pertussis} \mid \text{Chronic cough}) = P_i$ is the prevalence of *B. pertussis*, calculated by dividing the number of laboratory-confirmed pertussis with the total number of chronic/persist cough tested. $x_1, x_2, x_3, \ldots, x_k$ are variables associated with the occurrence of chronic/persistent cough, including the presence of *B. pertussis* and other social and environmental factors significant at $p < 0.1$ in univariate analysis. OR is the odds ratio.

The 95% CI of incidence is calculated with bootstrap method with 1000 replications. Besides incidence estimates, we will also explore factors associated with severe pertussis (defined as a composite outcome of death, sepsis, invasive ventilation and Intensive Care Unit transfer), by using multivariable logistic regression. Factors significantly associated with severe pertussis at $p < 0.1$ in univariate analysis will be included in the model. The median age of children with pertussis will be calculated by type of vaccinee, and factors predicting the age of pertussis breakthrough among children who had received DTP vaccination early in their life will be also studied by using Cox proportional hazards regression models.

ETHICS AND DISSEMINATION

This study is designed an observational study. The risk of harm is minimal and adverse medical events are not anticipated from the procedures involved in the study. The

study protocol, CRF, and consent form have been sent to and approved by China CDC's Institutional Review Board (reference no. ICDC-2019012).

The primary risk to participants is the loss of confidentiality. To help maintain confidentiality, all study investigators will sign a confidentiality agreement and receive appropriate ethics training. All interviews will be conducted at the study investigator's office, and signed consent forms and completed survey forms will be locked in a secure file cabinet at the end of each day. A very limited number of trained study staff can have the key to the locked file cabinets. Participation in every aspect of the study will be voluntary, and for all new data collection, participants will be asked to provide written informed consent. Besides, collection of specimens may cause mild discomfort to the subject during the procedure, especially drawing blood from young children. To minimize invasive procedures during sample collection, swab and blood specimens will be collected by aseptic technique and we encourage the use of leftover sera during routine medical care at the time point of enrolment.

As a benefit of participating in the study, participants with pertussis will receive senior doctor consultation during treatment on how to limit transmissions among family members and co-workmates; Patients enrolled in the study will have access to antibiotic susceptibility testing results should they have *B. pertussis* isolates acquired. This will give a guide on empirical antibiotic usages for physicians; moreover, the data generated in the study will be valuable to determine the burden of pertussis and explore risk factors for illness attributable to severe pertussis in children as well as adolescents/adults, which can be used by public health departments, healthcare providers and scientific group in China to inform policies making, implement disease control and prevention (i.e. vaccination) and improve patient care, both at the sites level and national level. In general, the minimal risks associated with physical discomfort during blood and N/P sample collection are offset by the great benefit associated with the study's ability to inform pertussis prevention and control strategies in China.

DISCUSSION

PertussisChina is an innovative and a pilot of a laboratory-based and population-based

active surveillance platform for vaccine-preventable bacterial diseases (VPBD) in China, which endeavors to establish a network of laboratories and hospitals using comparable and unified standards to provide up-to-date disease burden estimates and disease determinants for evaluating, prioritizing and optimizing the use of vaccines and for the development of new interventions against bacterial infections in the country. Pertussis is the first one of the several bacterial infections that we are planning to take this approach. In 2019, pertussis was one of the top ten diseases with highest burden in children younger than 10 years[36]. In response to the changing epidemiology of pertussis in China[7 8 37 38], the 2019 summon of the National Immunization Advisory Committee submitted a motion to its members urging the modification of the current immunization schedule of pertussis vaccine administered at 3, 4, 5 and 18-24 months[39], to vaccinate children at 2, 4, 6 and 18-24 months instead and to add a 5th booster dose at 4-6 years of age. To provide up-to-date evidence on disease burden of pertussis, this study will focus on age-specific incidence based on laboratory confirmation and will fill the data gaps on prospectively and actively collected incidence data and key information on illness severity and outcomes. We are expecting that data from this study can be served as background information augmenting NIDSS to inform NIAC's recommendations on children vaccination and further quantify the benefit of adolescent/adult vaccination to protect infants from severe outcomes in future. There are several strengths of the study.

In this one year study, we will enroll suspected chronic/persistent cough patients (for infants aged less than 1 year, cough regardless of duration) from health care facilities in two sites of China, covering a censused population of 2.23 million. The catchment population utilizing health-care services at the SHs are well characterized and defined by HUAS, providing an unbiased estimates of age-stratified total person-times observed in the cohort. The prevalence of cough in regarding of illness duration and proportion of people who do not seek healthcare are measured retrospectively by HUAS. Thus by comparing between data generated from HUAS in community and case registry in SHs, we will able to measure incidence by settings (i.e. community, outpatient and inpatient), especially those in communities whose symptoms are mild or atypical after the waning of vaccine-induced immunity or those no healthcare are

sought[2]. Besides, all hospitalizations suspected of pertussis will be actively searched and prospectively enrolled in a timely manner, serving as a complete and representative sample of pertussis occurred in the interested population that would have induced minimal selection bias. As for milder cases in ambulatory settings, sampling of patients with chronic/persistent cough in outpatient setting to conduct laboratory investigation is preferred. Misclassification of cases or recall bias will be minimized by the complex laboratory procedures (i.e. culture, PCR, and serology combined), unified data collection tools (i.e. CRFs) and data collection process, i.e. the 60-day of follow-up during which interesting events (e.g. 2-fold titer raising) will be closely monitored by sequential sera samples. Using laboratory-confirmed pertussis as the outcome will allow us to specifically measure pertussis disease burden. To account for asymptomatic carriage of *B. pertussis*, we will recruit healthy control to investigate the proportion of population carrying *B. pertussis* in their upper respiratory tract and sero-positivity, which could be useful for calculating population attributable fraction (AF) to adjust rate estimates. In addition, the prospective cohort will provide valuable follow-up data related to risk factors for severe illness (i.e. adverse clinical outcomes of hospitalization or death). Collection of the vaccination history (including band, dosing, procedure and time of administration) from study participants will help explore the breakthrough rates of *B. pertussis* infection among different type of vaccinee, by linkage of study subjects $\leq$14 years old with his/her individual records on immunization in the national database. Finally, we will abstract EMR data from hospital information system, which serves as a complete and accurate record of cough illness outcomes occurred in SHs. The retrospectively collected EMR data will be validated by prospectively counting cases eligible for inclusion at selective departments of SHs on a daily basis. Using data from the EMR will allow us to determine the size of outpatient and emergency department visits for cough illness in the studied population. For most of adults and fully immunized children and adolescents, their illness is generally mild and is most likely to be encountered at the ambulatory settings in which the diagnostic capacity is generally lacking.

Aside from acquiring incidence estimates, the prevalence and distribution of *B. pertussis* strains circulating in the population will be determined and characterized,

which are reported to be evolving under the selection pressure from both vaccine and antibiotics in previous studies [40] and are important data for the development of novel vaccine or new therapeutics in the country. For example, as a benefit of the study, we will create a representative national and well characterized repository of strains and specimens that can be shared with other investigators for future research, the main antigenic and genotypic features of *B. pertussis* will be characterized by sequencing or other bio-molecular methods.

We realized that there are several limitations worthy of note in our study. Firstly, we will not identify all pertussis that occur in our studied population since our case definition will not capture atypical and asymptomatic manifestations associated with *B. pertussis* infection. For example, previous studies showed that about 17.4% children[41] and 20% adolescents/adults[42] with *B. pertussis* infection had a cough duration less than 3 weeks, and other symptoms/signs used in the case definition, like spasmodic cough (63%), post-tussive vomiting (42%) and whoops (8%), were infrequently presented in adults[43], which will make incidence underestimated. It is argued that no symptom is sufficiently predictive for diagnosing pertussis[44] and there was no case definition that has been proposed for purpose of studying disease burden of pertussis. After balancing at the sensitivity and specificity of case definition commonly recommended by WHO, the U.S. and others[45-47] and the available laboratory capacity and resources in the study, we finally adopted the current case definition that can be used to facilitate comparison of results between studies and countries. Second, our study period is a little short. Since pertussis has showed a cyclic pattern and peaked every 3-5 years[2 48], our study will not capture this feature. Moreover, our study are going to recruit cases in 2021-2022, right after COVID-19 pandemic. As the epidemiology of many respiratory infections have been reported changing as a result of widely implementation of nonpharmaceutical interventions (e.g. wearing masks, social distancing, and personal health protection)[49 50] and the detained coverage of vaccines used in Expanded Program on Immunization during the pandemic[51]. The impacts of COVID-19 outbreak on incidence estimates of pertussis are not foreseeable in the study. Future studies are upcoming depending on the results of this pilot. Finally, China is a big country with large variations in population

density and across different climate, geographic and economic regions. Although we have paid careful attention to variables, like DTP3 vaccine coverage, childhood mortality and health-care delivery pattern when selecting study sites, regions with the highest and lowest reported incidence of pertussis are generally not included. This may also influence the generalizability of the incidence estimates to extrapolate to other regions.

In summary, PertussisChina is an innovative study that uses unified protocol to generate up-to-date high-quality incidence data on pertussis. The study design can secure the precision of data collection and provide insights into the prospectively conducted studies that designed to augment passive surveillance in countries where resources is limited and data is currently lacking. When completed, the results coming out this study will provide valuable scientific data on the incidence, etiology, and risk factors for severe sequelae of pertussis to academic societies and the public health authorities, who is currently struggling and fighting against this burdensome disease worldwide.

Contributors

ZS is the lead and corresponding authors who conceptualized and designed the protocol and critically revised the manuscript. JY, HH and YZ took part in design of the protocol, wrote the first draft and contributed equally to this work. JX, LX, YG and ZC participated in the design of the protocol and wrote the statistical analysis plan. CC, XZ, YZ, WT and QZ commented on and revised drafts of the manuscript. All authors contributed to reviewing, revising, and approving the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication

Not required.

Ethics approval

This study has been approved by Chinese Center for Disease Control and Prevention's Institutional Review Board (reference no. ICDC-2019012).

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Figure Legends

Figure 1. Incidence of reported pertussis from NNIDSS, China, 1952-2019. Abbreviations: DTwP, combined diphtheria, tetanus toxoid and whole-cell pertussis vaccine; cDTaP, co-purified diphtheria, tetanus toxoid and acellular pertussis vaccine; National Notifiable Infectious Disease Surveillance System (NNIDSS).

Figure 2. Location and population size of study sites included in PertussisChina study

Figure 3. Flow diagram of major study activities

Figure 4. Data flow chart and key indicators used to calculate incidence of pertussis

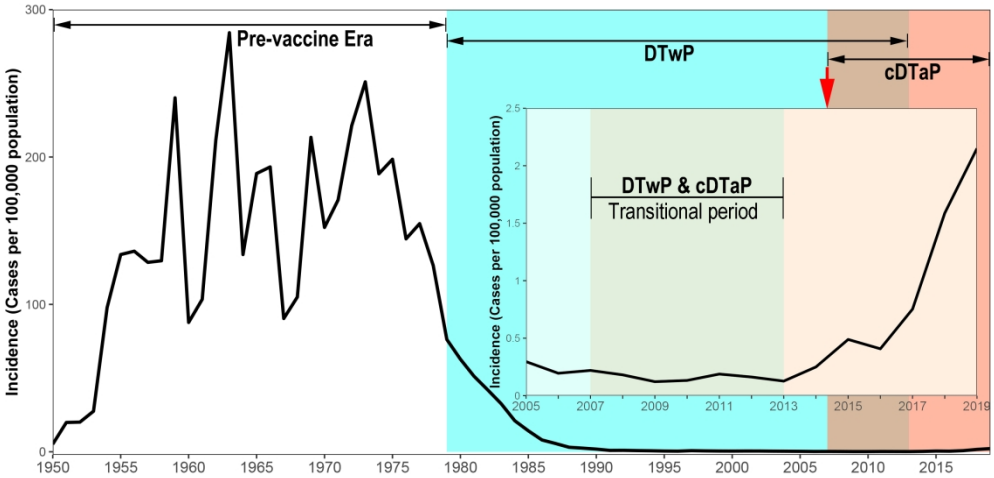

Figure 1. Incidence of reported pertussis from NNIDSS, China, 1952-2019

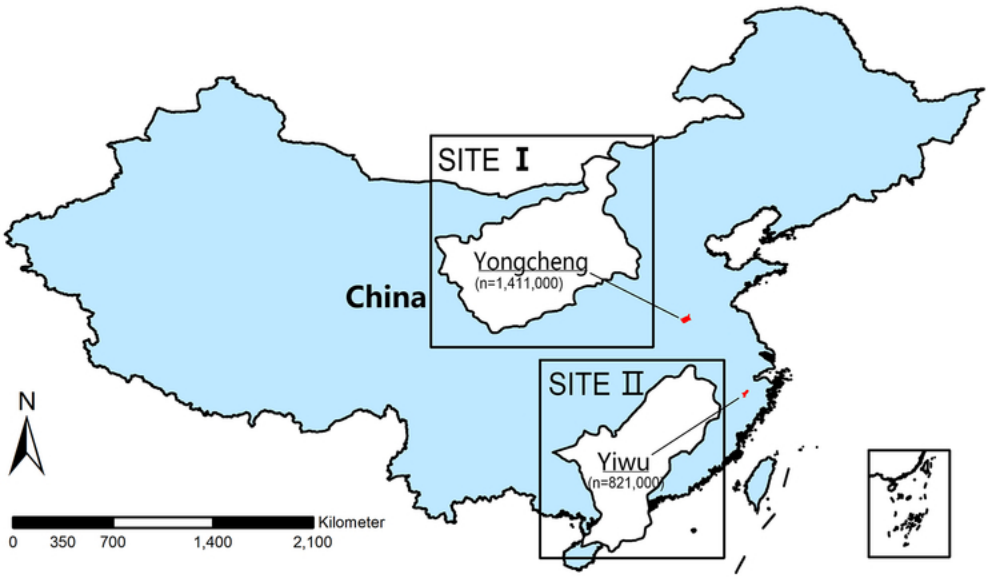

Figure 2. Location and population size of study sites included in PertussisChina study

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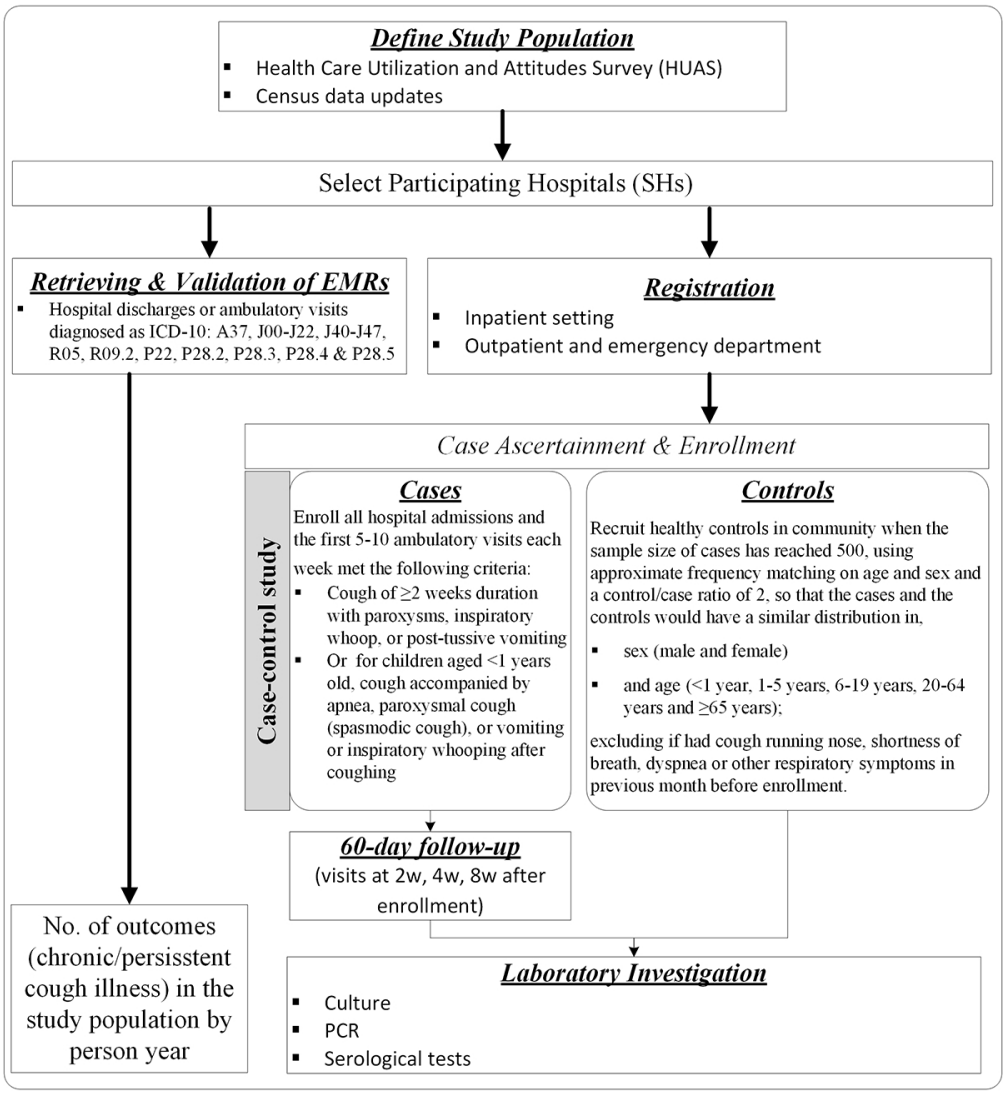

Figure 3. Flow diagram of major study activities

101x110mm (300 x 300 DPI)

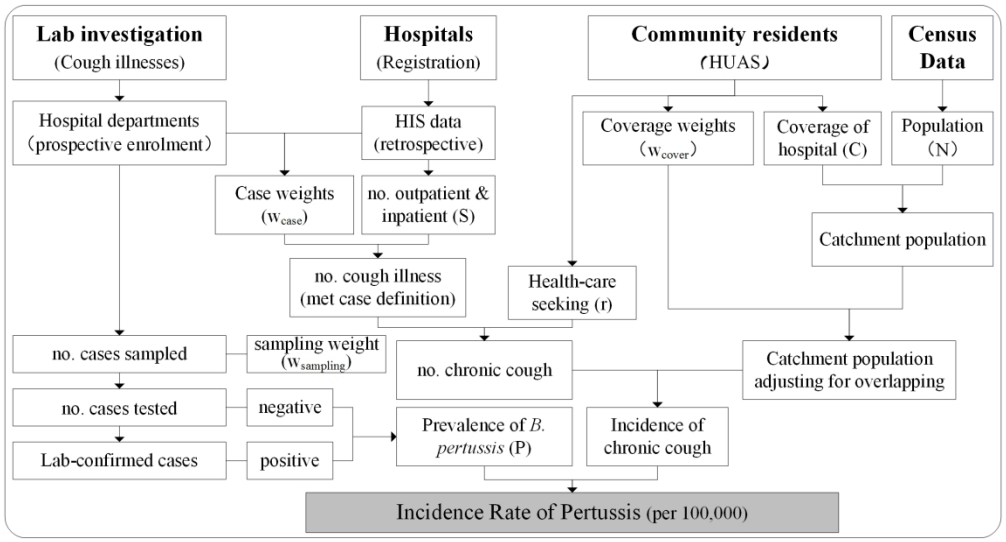

Figure 4. Data flow chart and key indicators used to calculate incidence of pertussis

187x100mm (300 x 300 DPI)

Supplementary Appendix

Title: Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study

Running head: PertussisChina Study, 2020

Tables & Forms

- Supplementary Table 1. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among children under 5 years old
- Supplementary Table 2. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among persons aged 5 years and older
- Supplementary Table 3. Case screening and ascertainment form
- Supplementary Table 4. Baseline information of case & control (CRF—T0)
- Supplementary Table 5. Follow-up information of case (CRF—T2w/T4w/T8w)
- Supplementary Table 6. Outcome of case at the end of follow-up (CRF—Tend)

Supplementary Table 1. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among children under 5 years old

Greetings! We are the staff of Center for Disease Control and Prevention. We sincerely invite you to participate in this questionnaire survey. The purpose of the survey is to study the utilization and attitudes of community residents towards health-care services of chronic/persistent cough, so that we can better serve you in the future.

This survey is anonymous. Your answers to these questions are kept confidential. You can choose whether to participate in the survey voluntarily or not. Refusal of the survey will not have any adverse consequences on you personally and your children. If you agree to the participate, please read each question carefully and fill in the answer on your own. Thank you for your support and cooperation!

Part I. Basic Information

1.1 Site:_____ Site ID:_____

1.2 Your length of time (years & months) living in the site (e.g. Yiwu or Yongcheng):

□ less than 6 months □six months and over

1.3 Current address: _____ county _____ street _____community/village

1.4 Type of respondents in relation to the studied subject/children:

□ mother □ father □ grandma/grandpa □ others_____

1.5 Date of Birth: □□□□/□□/□□ (yyyy/MM/dd)

1.6 Gender: □ Male □ Female

1.7 Ethnicity: □ Han □ others_____

1.8 Did your child attend school? □ yes □ no

1.9 Your occupation (of the respondent who answered the question):

□students □housework or unemployed □retired people □commercial & service sector workers □ food handler or employees of food industry □specialists, including teacher, medical personnel and workers □agriculture, forestry, animal husbandry and fishery workers □ others_____

1.10 Your educational attainment (of the respondent who answered the question).

□primary school or illiteracy □middle school □high school

□technical secondary school □college level and above

1.11 Including yourself, there are_____members in your family (defined as those who shared the same dining table in the house)?

Of which, there are_____children under five years old.

1.12 Is there any smokers or ex-smokers in your family? □ yes □ no

Part II. self-perceived illness and health-care seeking behavior

2.1 Did your children experienced cough during the past one month prior to our interview? □ no □ yes

2.1.1 If yes, how long did the cough last?

□ <1 week □ 1-2 weeks □ 3-4 weeks □ 5-8 weeks □ >8 weeks

2.1.2 If yes, what is the clinical characteristics of the last episode of cough?

□paroxysmal cough □vomiting after coughing □whooping cough

□cough worsening during the night □vomiting after coughing

□productive cough with large amount of sputum □dry cough

□cough with blood in sputum □others_____

2.1.3 If yes, what is the other concomitant symptoms?

□productive cough □running nose □fever (body temperature ≥ 37.2°C)

□belching □acid reflux □irritable and crying □vomiting □headache

□tachypnea □earache □sore throat □dyspnea □abdominal pain

□arthralgia □chest pain □myalgia □fatigue □lethargy

□burn after sternum □without any other discomfort □others_____

2.1.4 If yes, what do you think is the most probable cause of your cough?

□respiratory tract infection □inhalation of foreign objects in the respiratory tract

□COPD exacerbation □asthma exacerbation □recurrent tuberculosis

□chronic cardiopulmonary disease □lung cancer □inhalation of cold air

□chronic bronchitis □bronchiectasis □I don't know □others_____

2.2 Did your child visit a doctor or seek healthcare during the last episode of cough?

□ no □ yes

2.2.1 If yes, where did your child see a doctor?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

□Suxi Township Health Center □Beiyuan Community Health Center

□Shangxi Township Health Center □Dachen Township Health Center

□Houzhai Community Health Center □Chi'an Township Health Center

□Chengxi Community Health Center □Niansanli Community Health Center

□Yiwu Huashan Rehabilitation Hospital □Jiangdong Community Health Center

□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

□Yongcheng People's Hospital □Yongcheng Central Hospital

□Yongmei Group General Hospital □Henan Shenhuo Group General Hospital

□Yongcheng Maternal & Child Health Hospital □Yucheng Township Health Center

□Chenji Township Health Center □ Gaozhuang Township Health Center

□Yongcheng Mangdang Hospital □ Lizhai Township Health Center

□Yongcheng Second People's Hospital □ Liuhe Township Health Center

□Yanji Township Health Center □ Dawangji Township Health Center

□Longgang Township Health Center □ Shunhe Township Health Center

□Peiqiao Township Health Center □ Huaihai Community Health Center

□Huangkou Township Health Center □ Maqiao Township Health Center

□Jiangkou Township Health Center □ Houling Township Health Center

□Chenguanzhuang Township Health Center □ Taiqiu Township Health Center

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

2.2.1 If no, why did not your child see a doctor?

□Symptoms are mild, no need to see a doctor

□The hospital is too far from home and the transportation is inconvenient

□Drugs purchased in pharmacies □Distrust the doctor

□Unaffordable high medical expenses

□Hospital facilities and environment were poor

□others_____

2.3 Was your child hospitalized for the last episode of cough? □ no □ yes

2.3.1 If yes, where was your child hospitalized?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

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□Yiwu Tianxiang Medical Group Dongfang Hospital

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□Zhejiang Children's Hospital □Village clinics or private clinics

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□Mangshan Township Health Center □ Luanhu Township Health Center

□others

Part III. Attitudes towards health-care utilization

(Next, we will ask some questions about the actions you might take under some hypothetical situations that do not need to actually happen.)

3.1 If your child keeps coughing for 2 weeks but does not get better, and you have decided to see a doctor, which one of the following medical institutions would you choose to go?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

3.2 If the doctor recommends that your child be hospitalized, which one of the following medical institutions would you choose?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

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(For Yongcheng site, please select the following)

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

Part IV. Other questions

4.1 Has your child ever received the following vaccines?

□influenza vaccine □pneumococcal vaccine □Haemophilus influenzae vaccine

□Vaccines containing pertussis components (i.e. DTP)

4.1.1 If received vaccines containing pertussis (i.e. DTP), what kind of the vaccine?

□cDTaP □ DTaP/Hib □ DTaP-IPV/Hib

4.2 Your family's average annual income (Chinese Yuan) is,

□<50,000 □50,000-90,000 □100,000-190,000 □200,000-490,000 □≥500,000

4.3 Your phone number is_____

Thank you very much for taking your time. The information you provided in this interview is very valuable to help us improve our work. Wish you a happy life!

Time of survey started: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Time of survey ended: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Investigator: _____ Supervisor: _____

Supplementary Table 2. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among persons aged 5 years and older

Greetings! We are the staff of Center for Disease Control and Prevention. We sincerely invite you to participate in this questionnaire survey. The purpose of the survey is to study the utilization and attitudes of community residents towards health-care services of chronic/persistent cough, so that we can better serve you in the future.

This survey is anonymous. Your answers to these questions are kept confidential. You can choose whether to participate in the survey voluntarily or not. Refusal of the survey will not have any adverse consequences on you personally and your children. If you agree to the participate, please read each question carefully and fill in the answer on your own. Thank you for your support and cooperation!

Part I. Basic Information

1.1 Site:_____ Site ID:_____

1.2 Your length of time (years & months) living in the site (e.g. Yiwu or Yongcheng):

□ less than 6 months □six months and over

1.3 Current address: _____ county _____ street _____ community/village

1.4 Type of respondents in relation to the studied subject:

□respondent himself is the study subject □ others_____

1.5 Date of Birth: □□□□/□□/□□ (yyyy/MM/dd)

1.6 Gender: □ Male □ Female

1.7 Ethnicity: □ Han □ others_____

1.8 Your occupation:

□students □housework or unemployed □retired people □commercial & service sector workers □ food handler or employees of food industry □specialists, including teacher, medical personnel and workers □agriculture, forestry, animal husbandry and fishery workers □ others_____

1.9 Did you ever contact with dust/chemical materials in the working environment in the past one year, such as those encountered by workers using pneumatic drills at

construction sites, miners, painters, benzene solvents in leather production, etc.

□ no □ yes

1.10 Your educational attainment:

□primary school or illiteracy □middle school □high school

□technical secondary school □college level and above

1.11 Including yourself, there are_____members in your family (defined as those who shared the same dining table in the house)?

Of which, there are_____children under five years old.

1.12 Are you smoker or ex-smoker? □ yes □ no

Part II. self-perceived illness and health-care seeking behavior

2.1 Did you experienced cough during the past one month prior to our interview?

□ no □ yes

2.1.1 If yes, how long did the cough last?

□ <1 week □ 1-2 weeks □ 3-4 weeks □ 5-8 weeks □ >8 weeks

2.1.2 If yes, what is the clinical characteristics of the last episode of cough?

□paroxysmal cough □vomiting after coughing □whooping cough

□cough worsening during the night □vomiting after coughing

□productive cough with large amount of sputum □dry cough

□cough with blood in sputum □others_____

2.1.3 If yes, what is the other concomitant symptoms?

□productive cough □running nose □fever (body temperature ≥ 37.2°C)

□belching □acid reflux □irritable and crying □vomiting □headache

□tachypnea □earache □sore throat □dyspnea □abdominal pain

□arthralgia □chest pain □myalgia □fatigue □lethargy

□burn after sternum □without any other discomfort □others_____

2.1.4 If yes, what do you think is the most probable cause of your cough?

□respiratory tract infection □inhalation of foreign objects in the respiratory tract

□COPD exacerbation □asthma exacerbation □recurrent tuberculosis

□chronic cardiopulmonary disease □lung cancer □inhalation of cold air

□chronic bronchitis □bronchiectasis □I don't know □others_____

2.2 Did you see a doctor or seek healthcare during the last episode of cough? □ no □ yes

2.2.1 If yes, where did you see a doctor?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

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□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

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□others_____

2.2.1 If no, why did not you see a doctor?

□Symptoms are mild, no need to see a doctor

□The hospital is too far from home and the transportation is inconvenient

□Drugs purchased in pharmacies □Distrust the doctor

□Unaffordable high medical expenses

□Hospital facilities and environment were poor

□others_____

2.3 Were you hospitalized for the last episode of cough? □ no □ yes

2.3.1 If yes, where were you hospitalized?

(For Yiwu site, please select the following)

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□others_____

Part III. Attitudes towards health-care utilization

(Next, we will ask some questions about the actions you might take under some hypothetical situations that do not need to actually happen.)

3.1 If you keep coughing for 2 weeks but does not get better, and you have decided to see a doctor, which one of the following medical institutions would you choose to go?

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□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

3.2 If the doctor recommends that you should be hospitalized, which one of the following medical institutions would you choose?

(For Yiwu site, please select the following)

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□others_____

Part IV. Other questions

4.1 Have you ever received the following vaccines?

□influenza vaccine □pneumococcal vaccine □Haemophilus influenzae vaccine

□Vaccines containing pertussis components (i.e. DTP)

4.1.1 If received vaccines containing pertussis (i.e. DTP), what kind of the vaccine?

□cDTaP □ DTaP/Hib □ DTaP-IPV/Hib

4.2 Your family's average annual income (Chinese Yuan) is,

□<50,000 □50,000-90,000 □100,000-190,000 □200,000-490,000 □≥500,000

4.3 Your phone number is_____

Thank you very much for taking your time. The information you provided in this interview is very valuable to help us improve our work. Wish you a happy life!

Time of survey started: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Time of survey ended: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Investigator: _____ Supervisor:_____

Supplementary Table 3. Case screening and ascertainment form

Name of Hospital_____ Departments_____

Name of patient		Sex	☐ male ☐female
Birthdate		Ethnicity	
Current address		Phone number	
Date of illness onset		Date of admission	
Date of written informed consent signed			

Lists of inclusion & exclusion criteria	yes	no
Inclusion criteria:		
Patient regardless of ages:		
1.cough of ≥2 weeks duration;	☐	☐
2.had one or more of the following symptoms;	☐	☐
– paroxysmal cough;	☐	
– inspiratory whoop;	☐	
– post-tussive vomiting.	☐	☐
Infants less than one year old		
1.cough (regardless of cough duration);	☐	☐
2.had one or more of the following symptoms;	☐	☐
– paroxysmal cough;	☐	
– inspiratory whoop;	☐	
– post-tussive vomiting.	☐	
– apnea	☐	
Written informed consent signed	☐	☐
If you answer "No" to any of the above, the patient cannot enter the study.		
Exclusion criteria:		
1.not a permanent residents (lived longer than 6 months) at the site;	☐	☐
2.gastroesophageal reflux;	☐	☐
3.spastic bronchitis;	☐	☐
4.diagnosed tuberculosis;	☐	☐
5.lung mycoplasma/chlamydia infection;	☐	☐
6.chronic sinusitis;	☐	☐
7.Adults/adolescents with a measured body temperature of ≥38.5 °C	☐	☐
8. researchers considered not suitable for participating in the study	☐	☐
If you answer Yes" to any of the above, the patient cannot enter the study.		
Whether the patient is included in the study	☐	☐
If no, what is the reason for not included?		
– not meet the inclusion & exclusion criteria;	☐	☐
– Refuse to participate ;	☐	☐
If yes, what is the patient identifier no.? _____	☐	☐

Supplementary Table 4. Baseline information of case & control (CRF—T0)

Patient identifier no.:_____ Type: □Inpatient □outpatient □Control

Name：_____（or **Parents' name**：_____） **Sex：**□ male □ female **Ethnicity**：□ han □ other_____

Birthdate：____/____/____(YYYY/mm/dd) **Preterm baby：**□ yes，(gestational age_____weeks) □ no

Address*：_____ **mobile phone**：_____

Family member No.：_____persons **No. of children under five in the family：**_____children

Smokers in the family：□ yes，(_____persons) □ no **Smoking：**□ yes □ no **Drinking：**□ yes □ no

Occupation：

□kindergarten children□preschool children□students□teacher□childcarer□food industry worker□commercial service

□medical staff□ worker□migrant worker□farmer□herdsman□fishermen□cadre□retired□housewife□others□unknown

Clinical characteristics (within 24h before and after admission)

Illness onset Date：_____(YYYY/mm/dd) **Primary diagnosis**：_____ **Diagnose date**：_____(YYYY/mm/dd)

Hospitalized：□ yes □ no **Admission diagnosis**：_____ **Admission date**：_____(YYYY/mm/dd)

□cough（Starting date____/____/____[YYYY/mm/dd]，duration_____days）

□post-tussive vomiting□paroxysmal cough□whooping cough□apnea□cyanosis□fever（body temperature_____℃）

□seizure □cough worsening in night □productive cough

Sputum color：yellow/white/black/glass like □hemoptysis□chills□headache□myalgia□sore throat□joint pain□chest pain

□sweat□shortness-of-breath□running nose□lachrymation□fatigue□other(_____)

Complications：□pneumonia（radiographical evidence：□yes □ no）□atelectasis □pulmonary hypertension

□encephalopathy□seizure□others（_____）

Blood tests：WBC_____×10^9/L; L_____×10^9/L; N_____×10^9/L; Plt_____×10^9/L; Hb_____g/L; CRP_____mg/L; GLU_____mmol/L

Physical check：body temperature：_____℃ **Breath rate**：_____breath/min **Heart rate：**_____beats/min

Systolic/diastolic blood pressure：_____/_____mmHg **Pulse oximetry**: sPO_2 (if any):_____%

Lung auscultation：□dry rale □wet rale **Consciousness**：clear/lethargy/irritable/delirium/convulsions/coma

Treatment (one week before admission)

（1）Drug name：Please give the name of the drug, or the trade name if it is a fixed compound preparation

（2）Category: A=antibiotic (1. Amoxicillin; 2. Amoxicillin-clavulanic acid; 3. Ampicillin; 4. Azithromycin; 5. Ceftriaxone; 6. Cefuroxime; 7. Ciprofloxacin; 8. Clarithromycin; 9. Doxycycline; 10. Erythromycin; 11. Penicillin; 12. Tetracycline; 13. Compound sulfamethoxazole); B=antiviral drugs; C=steroid hormone drugs

（3）Route：1=oral, 2=intravenous injection, 3=intravenous drip, 4=intramuscular injection, 5=inhalation, 6=other

（4）Frequency：1= continuous，2=intermittent

Drug name (1)	Category (2)	Route (3)	Daily dose		Frequency (4)	Starting date (YYYY/mm/dd)	Stop date (YYYY/mm/dd)
			dose	unit			

Vaccination history of DTP3 (for children aged under 14 years old)

Source of data：☐ Vaccination certificate; ☐ linkage with national database; ☐ self-reports;

Reasons of unvaccinated：1. Contraindications; 2. Under the age of vaccination; 3. Missed vaccination time; 4. Parents refused to vaccination; 5. migrating population; 6. Don't know; 7. Others_____

dose	lot number	producer	dosage		site	Date (YYYY/mm/dd)	Reasons of unvaccinated
			dose	unit			
1							
2							
3							

hospital：_____ **reporter**：_____ **Date of reporting**：____/____/____(YYYY/mm/dd)

Supplementary Table 5. Follow-up information of case (CRF—T_{2w}/T_{4w}/T_{8w})

Patient identifier no.:_____ Type: ☐Inpatient ☐outpatient

Name：_____（or **Parents' name**：_____） **Sex：**☐ male ☐ female

Illness onset date：____/____/____(YYYY/mm/dd) **Admission date：**____/____/____(YYYY/mm/dd)

Follow-up date：____/____/____(YYYY/mm/dd) Weeks of follow-up:☐ 2 wks ☐ 4 wks ☐ 8 wks

Follow-up method: ☐ hospital visits ☐ telephone interview

Outcomes

Survival: ☐ yes ☐ no Date of death:____/____/____(YYYY/mm/dd) death diagnosis：_____

Hospitalized： ☐ yes ☐ no Re-admitted into hospital after discharge: ☐ yes ☐ no

Reasons for re-admission：Pneumonia/heart failure/cardiogenic shock/encephalopathy/Seizure/other_____

Lost to follow-up: ☐ yes ☐ no (refers to 3 consecutive calls to patients on different working days but no answers at all)

Clinical characteristics (during follow-up visits)

☐cough（Starting date____/____/____[YYYY/mm/dd]，duration_____days）

☐post-tussive vomiting ☐paroxysmal cough ☐whooping cough ☐apnea ☐cyanosis ☐fever（body temperature_____℃）

☐seizure ☐cough worsening in night ☐productive cough

Sputum color：yellow/white/black/glass like ☐hemoptysis ☐chills ☐headache ☐myalgia ☐sore throat ☐joint pain ☐chest pain

☐sweat ☐shortness-of-breath ☐running nose ☐lachrymation ☐fatigue ☐other(_____)

Blood tests：WBC_____×10^9/L; L_____×10^9/L; N_____×10^9/L; Plt_____×10^9/L; Hb_____g/L; CRP_____mg/L; GLU_____mmol/L

Physical check：body temperature：_____℃ **Breath rate**：_____breath/min **Heart rate：**_____beats/min

Systolic/diastolic blood pressure：_____/_____mmHg **Pulse oximetry**: sPO_2 (if any):_____%

Lung auscultation：□dry rale □wet rale **Consciousness**：clear/lethargy/irritable/delirium/convulsions/coma

Patient specimen collection

Specimen collected： ☐ yes ☐ no **Date of sampling**：____/____/____(YYYY/mm/dd)

Type of specimen： Nasopharyngeal swab ☐ amounts：_____

Whole blood ☐ quantity：_____ ml

Reasons for not sampling： ☐ without coughing symptoms for 1 week ☐refusal to sampling

Hospital：_____ **investigator**：_____ **Date of follow-up**：____/____/____(YYYY/mm/dd)

Supplementary Table 6. Outcome of case at the end of follow-up (CRF—T_{end})

Patient identifier no.:_____ Type: ☐Inpatient ☐outpatient

Name： _____（or **Parents' name**：_____） **Sex：** ☐ male ☐ female **Illness onset date：** ____/____/____ (YYYY/mm/dd)

Admission date： ____/____/____(YYYY/mm/dd) **Discharge date：** ____/____/____(YYYY/mm/dd)

Discharge diagnosis： primary diagnosis _____

secondary diagnosis **1.**_____ **2.**_____ **3.**_____

Treatment during hospitalization

Admitting into ICU： ☐ yes ☐ no ____/____/____(YYYY/mm/dd)

1.**Transfer in date**____/____/____(YYYY/mm/dd) **Transfer out date**____/____/____(YYYY/mm/dd)

2.**Transfer in date**____/____/____(YYYY/mm/dd) **Transfer out date**____/____/____(YYYY/mm/dd)

3.**Transfer in date**____/____/____(YYYY/mm/dd) **Transfer out date**____/____/____(YYYY/mm/dd)

Oxygen therapy： ☐ yes ☐ no duration：_____days

Invasive ventilation： ☐ yes ☐ no duration：_____days (invasive ventilation refers to tracheal intubation or tracheotomy)

Non-invasive ventilation: ☐ yes ☐ no duration：_____days

Oscillating respirator： ☐ yes ☐ no duration：_____days

ECMO or interventional lung adjuvant therapy（iLA） ☐ yes ☐ no **date of treatment start：**____/____/____(YYYY/mm/dd)

Renal replacement therapy/dialysis： ☐ yes ☐ no **date of treatment start：**____/____/____(YYYY/mm/dd)

Exchange transfusion： ☐ yes ☐ no **date of treatment start：**____/____/____(YYYY/mm/dd)

Leukophoresis or leukoreduction therapy： ☐ yes ☐ no **date of treatment start：**____/____/____(YYYY/mm/dd)

Drugs

（1）Drug name：Please give the name of the drug, or the trade name if it is a fixed compound preparation

（2）Category: A=antibiotic (1. Amoxicillin; 2. Amoxicillin-clavulanic acid; 3. Ampicillin; 4. Azithromycin; 5. Ceftriaxone; 6. Cefuroxime; 7. Ciprofloxacin; 8. Clarithromycin; 9. Doxycycline; 10. Erythromycin; 11. Penicillin; 12. Tetracycline; 13. Compound sulfamethoxazole); B=antiviral drugs; C=steroid hormone drugs

（3）Route：1=oral, 2=intravenous injection, 3=intravenous drip, 4=intramuscular injection, 5=inhalation, 6=other

（4）Frequency：1= continuous，2=intermittent

Drug name (1)	Category (2)	Route (3)	Daily dose		Frequency (4)	Starting date (YYYY/mm/dd)	Stop date (YYYY/mm/dd)
			dose	unit			

Clinical characteristics

Symptoms/signs：

☐cough（Starting date____/____/____[YYYY/mm/dd]，duration_____days）

☐post-tussive vomiting ☐paroxysmal cough ☐whooping cough ☐apnea ☐cyanosis ☐fever（body temperature_____℃）

☐seizure ☐cough worsening in night ☐productive cough

Sputum color：yellow/white/black/glass like ☐hemoptysis ☐chills ☐headache ☐myalgia ☐sore throat ☐joint pain ☐chest pain

☐sweat ☐shortness-of-breath ☐running nose ☐lachrymation ☐fatigue ☐other(_____)

Complications：

☐Viral pneumonia ☐Cardiac arrest ☐Bacterial pneumonia ☐Bacteremia ☐Acute lung injury/ARDS ☐Heart infection

☐Coagulation disorders ☐Pneumothorax ☐Anemia ☐Pleural effusion ☐Acute kidney injury ☐Myolysis

☐Bronchiolitis ☐Gastrointestinal hemorrhage ☐Meningitis ☐Pancreatitis ☐Epilepsy ☐Arrhythmia ☐Liver insufficiency ☐stroke ☐Hyperglycemia ☐Hypoglycemia ☐Congestive Heart Failure ☐other (_____)
Prognosis
☐**Cured** ☐**Improved and be discharged** ☐**Transferred to other hospital** **reasons for transfer** : Community rehabilitation/other (_____) ☐**Give up treatment** **reasons for give-up**：Economic reasons/illness exacerbation/other (_____) ☐**Death** **date of death**：____/____/____(YYYY/mm/dd) **death diagnosis**：_____
Hospital：_____ **investigator**：_____ **Date of record**：____/____/____(YYYY/mm/dd

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Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study

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Running Title: PertussisChina Study, 2020

Title: Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study

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ABSTRACT

Introduction

Pertussis is one of the top ten diseases of children under 10 years of age, and the few vaccine-preventable diseases who is on a rise in China in recent years; however, the true burden of pertussis, including age-stratified incidence and risk factors of severe sequelae, are underrecognized. We aim to estimate the health burden of laboratory-confirmed pertussis by age groups, considering the setting of illness onset (i.e. in community, outpatient and inpatient), in a Chinese population (~2.23 million in total) at two sites.

Methods and analysis

This paper describes the study design of a one-year, prospective, age-stratified, and population-based case-control study, including site selection, study population, case registry, ascertainment and enrolment, control recruitment, follow-up of case, microbiological methods, data collection, quality control activities, and statistical methods used to generate incidence estimates. During June 2021 through May 2022, registry of suspected pertussis cases (namely chronic/persistent cough) will be conducted in several participating hospitals (SHs) at the two sites, which are selected based on Healthcare Utilization and Attitudes Surveys (HUAS) carried out before study initiation. A case-control study will be conducted in the SHs and we aim to enroll a total of 1,000 suspected pertussis cases (i.e. all hospital admissions and the first 1-3 outpatient visits each week each hospital) and 2,000 frequency matched healthy controls in community. Our primary study outcome, the laboratory-confirmed *Bordetella Pertussis* infection, will be determined by a comprehensive laboratory methods and procedures (i.e. culture, PCR, and serological tests) in both cases and controls at enrolment and during 60-day's follow-up visits. Finally, data from HUAS (i.e. population size), case registry (i.e. the total number of suspected pertussis cases), and case-control study (i.e. the prevalence or population attributable fraction of *Bordetella Pertussis*) will be combined to calculate incidence and its 95% confidence interval through bootstrap method. Epidemiological analyses will be conducted to determine the risk factors associated with severe sequelae of pertussis.

Ethics and dissemination

This study has been approved by Chinese Center for Disease Control and Prevention's Institutional Review Board (no. ICDC-202110). Results will be disseminated via academic

presentations and publication in peer-reviewed journals, and will provide valuable scientific data and some new insights into the incidence, etiology, and risk factors for severe sequelae of pertussis to academic societies and the public health authorities who is currently struggling and fighting against this burdensome disease worldwide.

Keywords: *Bordetella pertussis*, Case-Control Studies, Incidence, China

SUMMARY

Strengths and limitations of this study

- PertussisChina is a population-based study at two sites, covering approximately 2.23 million populations defined through conducting Healthcare Utilization and Attitudes Surveys (HUAS) in community.
- PertussisChina is a laboratory-based study, in which comprehensive laboratory methods (i.e. culture, PCR, and serological tests) and procedures (i.e. 60-days follow-up) will be used to specifically measure pertussis disease burden.
- PertussisChina is a case-control study in which the prevalence and population attributable fraction (AF) of *Bordetella Pertussis* infection can be ready acquired.
- All cases will be prospectively followed up to 60-days to collect interesting events (i.e. adverse clinical outcomes of hospitalization or death) at 2, 4 and 8 weeks after enrolment.
- Limitations are that our incidence might be underestimated and cannot be extrapolated to represent the whole country due to the insensitive case definition used, short study period and relatively small population covered.

BACKGROUND

Whooping cough (pertussis) is a highly contagious respiratory disease caused by *Bordetella Pertussis*[1 2]. Despite a high vaccine coverage of third dose diphtheria-tetanus-pertussis vaccine (DTP3)[3], the "resurgence of pertussis" in recent years has posed a great threat to global public health[4-6], as well as to Chinese infants[7 8]. In 2019, pertussis was one of the top ten diseases with highest burden in children younger than 10 years[9], and the World Health Organization estimates that pertussis kills about 160,700 children under 5 years old worldwide each year[10]. In China, three types of pertussis vaccines are available till Octorber 31, 2021, i.e. the co-purified diphtheria and tetanus toxoids and acellular pertussis (cDTaP, used for routine immunization), DTaP/Hib (Minhai Biotechnology Co., Ltd., Beijing, China)[11] and DTaP-IPV/Hib (Sanofi Pasteur, Lyon, France)[12 13]. The coverage of DTP3 remained high above 99% for children throughout the 2010s[14 15], and the reported incidence of pertussis has been risen from 0.12 per 100,000 in 2013 to 2.14 per 100,000 in 2019 (Figure 1). Unlike the other countries who had experience resurgence of pertussis, especially in adolescents/adults, primarily due to the waning of vaccine induced immunity[16-20], China observed no such changes of age distribution [21]. The rise of pertussis in China was mainly concentrated in infants less than 1 year old, and less than 5% of reported pertussis were adolescents and adults.

Since most epidemiological data on pertussis in China came from a passive reporting system, the National Notifiable Infectious Disease Surveillance System (NNIDSS)[22], underreporting was substantial in the system (≥90%) because of limited diagnosis and incompleteness of reporting[8 23 24]. And the burden of pertussis remained underrecognized. It has been suggested that immunizing schoolchildren is the key for curtailing transmission of pertussis in population[18]. Due to a substantial knowledge gaps existed in age-specific burden of pertussis (i.e. incidence and severity), no adolescent or adult immunization are recommended in the country[25]. Moreover, some important data such as clinical, laboratory and vaccine information are also not available, which is unfavorable for evaluating the effectiveness of vaccine and implementing of other disease control and prevention programs (such as adult

vaccination, diagnostic tests and post-exposure prophylaxis of pertussis). Rigorously conducted, prospective, population-based studies can be used to strengthen the NNIDSS, by providing information on the burden of laboratory-confirmed pertussis, strains distribution, risk factors for severe sequelae and case fatality, and most importantly, to assist health authority in China to allocate health resources, prioritize health research investments, optimize interventions (i.e. vaccination) and innovate vaccine development.

We designed the PertussisChina study, a one-year, prospective, age-stratified, population-based longitudinal cohort and case-control study, which will enroll suspected pertussis patients (i.e. chronic/persistent cough) seeking healthcare in several selected participating hospitals (SHs) at two sites of China, covering approximately 2.23 million censused population. This article describes the study design, including sites selection, study population, case registry, ascertainment and enrolment, control recruitment, follow-up of cases and controls, microbiological methods (i.e. culture, PCR, and serological tests), data collection, quality control activities, and statistical methods used to generate incidence estimates of pertussis. We then further discuss the strengths and weaknesses of the study design.

METHODS AND ANALYSIS

Objectives of the study

The primary objective of the study is to measure the incidence of laboratory-confirmed pertussis by age groups (children, adolescents and adults), and by settings (community, outpatient and inpatient). The secondary objectives are: 1) to describe the distribution of disease severity and outcomes across age groups; 2) to describe the patterns and factors of under-detection and under-reporting of pertussis; 3) to study the carrier (colonization) status of the *B. pertussis* in the upper respiratory tract of healthy controls, and the serum levels of anti-pertussis toxin antibodies (anti-Ptx IgG) in both patients and healthy people; and 4) to create a repository of well-characterized clinical specimens and *B. pertussis* isolates that can be used in future studies.

Study sites and population

Site Selection Criteria

Sites are selected based on the following criteria: 1) have strong willingness to participate; 2) have capability and resources to conduct ongoing surveillance, namely staffs to facilitate specimen collection and case investigation, previous experience in disease surveillance, infrastructures to secure data collection and specimen storage or transportation; and 3) provide a full list of healthcare facilities in the area and the information of built-in hospital information system in the facilities. Currently, there are two sites in the study, including Yongcheng, Henan and Yiwu, Zhejiang (Figure 2).

Study population

In 2019, Yiwu had a permanent population of 821,000 (47,000 were children under five years of age) served by 24 health care facilities (i.e. three tertiary care, four secondary care, and 17 primary care hospitals). Most hospital admissions (≥80% of the total number) occurred in the three large tertiary hospitals, including a children's hospital and two general hospitals; meanwhile, Yongcheng had a permanent population of 1,411,000 (94,000 were children under five years of age) served by 41 health care facilities (i.e. five secondary care and 36 primary care hospitals). Most hospital admissions occurred in the five large secondary care hospitals, including four general hospitals and a maternal and pediatric hospital. In total, the two sites cover a total of 2.23 million permanent population in the study area.

Study overview and design

In order to achieve our study objectives, we will conduct the following study activities at the two sites from June 2021 through May 2022, including, 1) a Healthcare Utilization and Attitudes Survey (HUAS) and a census data updating to define study population (i.e. incidence denominator), so as to set up a sampling frame for the case-control study and selecting participating hospitals (i.e. SH) for case registry and case recruitment; 2) the case-control study to acquire the prevalence of *B. pertussis* infection among suspected pertussis cases and healthy controls, as well as the calculation of population attributable fraction (AF) indicating the proportion of cases

that can be prevented if *B. pertussis* was totally removed from the population; and 3) case registry and the retrieval of electronic medical records (EMRs) from hospital information system to provide and validate the total number of suspected pertussis case patient (chronic/persistent cough) encountered in the SHs (i.e. incidence numerator) (Figure 3).

Defining and calibrating study population

Census data updating

Population census data at the two sites will be collected and updated during the study period. Population census is conducted every ten years in China and the nearest one is in 2020. However, an intermittent survey of 1% sampling of the total population would be performed to update population census data every year between the two censuses. We will retain the up-to-date population data from the National Bureau of Statistics. Moreover, the population birth, mortality, and population migration are recorded by the local government. We will also contact the local health bureau quarterly to access these data to give a precise estimation of population size in the two sites.

Healthcare Utilization and Attitudes Surveys (HUAS)

HUAS will be conducted prior to recruiting cases and controls at the two sites, which will serve three purposes, 1) to set up a sampling frame for the case-control study; 2) to select SHs in which prospective enrolment of cases will be conducted; and 3) to provide estimates of the population coverage for our SHs and healthcare seeking behavior weights applied in estimating pertussis incidence in community.

In summary, a population-based cross-sectional study, with an age-stratified sample of 3,000 children aged 0-59 mo and 6,000 adolescents/adults aged ≥5 years, will be conducted in the community of the two sites. The sample size was calculated based on: i) for children, a monthly prevalence of cough illness, π=1% (estimated from the reported incidence of lower respiratory tract infection of 0.15 per child year[26]), allowable error (δ=0.5%), significant level (α=0.05), and design effect (deff=2); ii) and for adolescents/adults, a monthly prevalence of cough illness, π=3.3%[27], allowable error (δ=0.66%), significant level (α=0.05), and design effect (deff=2).

A complex sampling method will be used to select survey respondents as follows. Firstly, a probability proportionate to size sampling will be used to randomly select 50 clusters (e.g. communities or villages) in the site's administrative regions. At the second sampling stage in selected communities, quota sampling will be used to recruit interviewee. The quota required in each age stratum was calculated based on the age distribution of the population in the sites and the number of surveys allocated to each cluster. Trained work staff will go to the selected communities to conduct face-to-face surveys at several locations (residential areas, kindergartens and children's vaccination clinics) Monday to Sunday during daytime in the study period. All residents living in the communities or villages for at least half a year prior to survey are eligible for and invited to participate in the interview. After the quota required in each age group is complete, the interviews will stop.

The following questions (Supplementary table 1 & table 2) are asked to respondents, 1) the occurrence and length of cough illness in the previous month prior to survey, 2) healthcare-seeking behavior regarding the self-reported cough illness for the most recent episodes and the sources of healthcare facilities; and 3) the willingness to seek healthcare and where would they choose to visit for an assumptive cough illness.

Based on the HUAS and census data, hospitals at which over 80% of respondents in each site choose to attend when hospital admission is required will be selected as our SHs. In case healthcare providers in the site change their practice or scope of service during our study period, for example the opening of new hospitals or the establishment of new branches of existing hospitals, an abbreviated HUAS with a smaller sample of 1,000 will be administered at the middle or the end of the year during which cases are recruiting at SHs.

Case-control study

Case definition of suspected pertussis

Patients will be classified as suspected pertussis cases and offered to participate if they present chronic/persistent cough defined as cough of ≥2 weeks duration with one or more of the following symptoms, 1) paroxysmal cough; 2) inspiratory whoop; or 3) post-tussive vomiting; Or, for children aged <1 years-old, cough (regardless of cough

duration) accompanied by one or more of the following symptoms, 1) apnea; 2) paroxysmal cough; 3) inspiratory whoop; or 4) post-tussive vomiting.

We will exclude patients presenting with gastroesophageal reflux, spastic bronchitis, and clearly diagnosed tuberculosis, mycoplasma/chlamydia infection, or chronic sinusitis. Adults/adolescents with a measured body temperature of ≥38.5 °C at enrolment will also be excluded.

Sample Size Considerations

We planned to enroll approximately 250 suspected cases and 2 matched controls for each case in each age stratum (i.e. children under 5 years, and adolescents/adults aged ≥5 years) for laboratory investigation at each site, which would add up to approximately 1000 suspected cases and 2000 controls at the two sites. We calculated the above sample size based on a prevalence of *B. pertussis* in chronic/persistent cough of 20% (range=12%-32%) [28-30], an allowable error of 5% and a significant level of 0.05. This sample size would have a 90% power (two sided α =0.05) to detect an odds ratio (OR) of 2 between case and control for a site and age stratum-specific comparison, if the true prevalence of *B. pertussis* is 20% in case; or an OR of 3, if the true prevalence is 10%. Although the carrier state of *B. pe*rtussis is transient in family contacts[31 32], *B. pertussis* is rarely identified in healthy people [33 34], and we expected a larger OR of ≥ 2 in the study. This sample size means that the laboratory would process average 115 samples per week, which is feasible and acceptable for our laboratories.

Case Registry, Ascertainment and Enrollment

Case registry, ascertainment and enrollment for suspected case will be conducted in SHs during the study period. Clinicians or trained nurses working in selected departments of the SHs (i.e. respiratory, pediatric, infectious disease, and emergency department) will carry out case registry of suspected pertussis cases every weekday (i.e. Monday through Sunday) except national holidays. Each outpatient visits and new hospital admission seeking healthcare in above departments will be screened for the eligibility of inclusion using the inclusion & exclusion criteria of the suspected case definition of pertussis by clinicians. Eligible ones will be ascertained and recorded as suspected case by study coordinator who assist with clinicians in SHs in enrolling cases

using a standardized case reporting form (CRF) (Supplementary Table 3). Among the suspected pertussis case recorded in SHs, convenient sampling method will be used to recruit cases for case-control study. We aim to enroll all hospital admissions and the first 1-3 outpatient visits each week in each hospital. After obtaining informed consent, study staff will conduct enrollment interviews, and collect nasopharyngeal (N/P) and blood specimens for each enrolled case.

Controls selection

At the middle of the study year when the sample size of cases reaches a half of the total (i.e. n=500), a control is recruited in community of the study sites using approximate frequency matching, based on the following criteria, 1) similar proportion in sex strata; 2) similar proportion in age strata, i.e. <1 year, 1-5 years, 6-19 years, 20-64 years and ≥65 years; 3) a control/case ratio of 2:1; and 4) no cough, running nose, shortness of breath, dyspnea or other respiratory symptoms at enrolment nor have a record of healthcare for respiratory disease in previous three months before recruitment.

60-day follow-up of case

We will follow cases from the time of enrollment to a maximum time period of 60 days after enrollment. Follow-up will be conducted at 2nd, 4th and 8th weeks after enrollment, with face-to-face interview if patient is currently hospitalized, or one telephone call each follow-up time if patient is discharged from hospital. At each follow-up visit/phone call, the study staff will ask about cough or other respiratory or systemic illness symptoms in the period since the last contact. If case is still symptomatic (i.e. cough) during follow-up, they will be encouraged to visit their doctor who enrolled them in the SHs within 24h of contact. The doctor will checkup the patient's health status and collect the swab and serum samples during the visit. If an enrolled patient does not want to visit the SHs, the study staff will arrange a household visit to collect the samples in the home.

Data collection from cases and controls

At enrolment, trained clinicians and the study coordinator will conduct face-to-face interview to collect socio-demographic, clinical and epidemiological data from cases

and controls using a standardized CRF (Supplementary Table 4). Demographic information includes household size (defined as a group of people who share a dinner table), average household income, rural or urban residence, age, alcohol consumption and smoking exposure, and occupation etc. A clinician will also examine all cases to document clinical signs and symptoms at enrollment, including cough characteristics [duration, paroxysms, post-tussive vomiting, exacerbation at night], body temperature, respiratory rate, heart rate, seizure, apnea, and other general respiratory symptoms, non-prescription antibiotic usage before visiting the doctor, blood test results and chest x-ray examinations. Vaccination history (i.e. band, dosing, procedure and time of administration) of children aged ≤14 years is also collected by linkage of his/her individual records on immunization in the national database (Childhood Immunization Information Management System, CIIMS)[35] or checking of vaccination certificate.

During follow-up visits, data on any current cough or respiratory symptoms, subjective severity of illness, illness duration, functional impairment, whether medical care was sought, and outcomes since the last visits will be collected using CRFs (Supplementary Table 5).

At the end of follow-up, medical charts of each hospitalized case will be reviewed by study staff to collect information on antibiotic treatment and outcomes during hospitalization (i.e. mechanical ventilation, ICU transfer, and death) (Supplementary Table 6).

The retrieval of electronic medical records and Validation of the total number of suspected pertussis case

Since our case registry and enrolment is conducted in selective departments (i.e. respiratory, pediatric, infectious disease and emergency departments) and on workdays in SHs, it is an incomplete record of the total number of suspected cases encountered in the whole hospital. It is essential to calibrated the registered number of suspected cases to equal the total. To do this, all hospital discharges or ambulatory visits coded for diagnosis under the International Classification of Diseases 10th Revision (ICD-10) codes A37, J00-J22, J40-J47, R05, R09.2, P22, P28.2, P28.3, P28.4, and P28.5 will be monitored on a daily basis as registry case, by hospital departments.

At the end of the month, the complete EMRs records with the above diagnosis codes in the whole hospital will be abstracted from hospital information system (HIS) of the SHs. This data will be used to calibrate the prospectively counting data of suspected case in the selective departments that conduct case enrolment to make a precise estimate of the total number of chronic/persistent cough illness outcomes in the studied population. Namely, through linking and comparing between the number of registry cases and the number of suspected pertussis case registered in the selected departments, we will calculate the W_{case}. With this W_{case}, we will narrow down the ICD-based EMRs records to the total number of suspected pertussis cases met our case definition in SHs (i.e. the numerator of incidence).

Laboratory investigation

Specimen collection and transport

When patients meet our suspected pertussis case definition or are recruited controls, they, as well as symptomatic (cough) cases during follow-up contacts, will be sampled within 24 hours. Clinicians or nurses in SHs will be trained to collect nasopharyngeal swabs (N/P) and whole blood sample. Dacron or nylon swab will be used to collect N/P specimen to facilitate culture and PCR tests for *B. pertussis*[36]. Collected swab specimens will be plated onto selective agar or placed in transport medium (Charcoal Agar, Thermo Fisher Scientific Inc.) immediately after sampling at the SHs. Whole blood without adding any anticoagulants (>4ml for participants aged 5 years and older, and ≥2 ml for children aged <5 years) will be collected, and centrifuged to separate serum within 24h of collection. All collected swab and sera samples will be transported to the central laboratory of Chinese Center for Disease Control and Prevention (China CDC), using a cold box to maintain a temperature of 4°C. During transportation, samples are packaged and transported in accordance with the provision of International Civil Aviation Organization (ICAO) document Doc9284 and UN3373

Processing and storage of specimen

Upon arrival at the laboratory of China CDC, swab samples will be processed and prepared into three aliquots of swab supernatant, so will serum samples be. One of these aliquots will be analyzed and the other two aliquots will be kept for future

analyses. All aliquots will be stored at -70°C temperature until the time of analysis.

Laboratory testing

In the laboratory of China CDC, Charcoal Agars will be cultured to isolate *B. pertussis* using standard method recommended by China CDC[37] and World Health Organization[38]. Swab supernatant will be analyzed for *B. pertussis, B. parapertussis, B. bronchiseptica* and *B. holmesii* using polymerase chain reaction (PCR) as recommended by US CDC [39 40]. Sera samples that have a minimum volume of ≥ 1 ml will be tested for Anti-Ptx IgG titer using a commercially available diagnostic kit (Virion\Serion, Wurzburg, Germany) according to the manufacturer's recommendations. To validate our laboratory methods and testing results, external quality assurance testing will be conducted to reach agreements with a reference laboratory on *Bordetellae* prior to study start. For serology testing, we use standard from the National Institute for Biological Standards and Control, London, UK, (*https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=18/146*); and for PCR assays, the Wisconsin State Laboratory of Hygiene, Wisconsin, U.S. (*http://www.slh.wisc.edu/proficiency/training-and-competency/*).

Suspected pertussis cases and controls that have *B. pertussi*s Isolated, positive tests of swabs in any of samples collected during enrolment and follow-up, or for persons three years of age and over have a 3-fold or greater rise in anti-Ptx IgG antibody between sequential sera samples with at least one time point higher than 40 IU/ml of serum titer would be considered laboratory-confirmed pertussis.[36 41]

Data flow, management and analysis

The data collected in the study are centrally managed at China CDC, using an online data platform (*http://eddc.chinacdc.cn/dap/*). The completed CRFs will be entered into the information system by local study staff at the two sites and uploaded to data server through encrypted transmission via a Virtual Private Network set up by China CDC. The entered records are regularly checked for completeness, consistency, and logical errors by data manager and the site's co-principle investigator who is responsible for authorization, integrity, security, and backup of database during data collection.

Statistical analysis

The collected data processing and key indicators based on which we calculate incidence are shown in figure 4. We will calculate the incidence of pertussis by age group and by settings with the following formula.

$$Hospitalization\ \ incidence\ rate = \sum \frac{S_i^{inpatient} \times W_i^{case} \times AF_i}{N_i \times W_i^{cover} \times C_i}$$

$$Outpatient\ \ incidence\ rate = \sum \frac{S_i^{outpatient} \times W_i^{case} \times AF_i}{N_i \times W_i^{cover} \times C_i}$$

$$Community\ incidence\ rate = \frac{Outpatient\ \ incidence\ rate}{r_i}$$

Where, $S_i^{inpatient}$ and $S_i^{outpatient}$ indicates the registered number of inpatients and outpatient visits of cough illnesses at age group i, as obtained from HIS. W_i^{case} is the weight used to adjust $S_i^{inpatient}$ and $S_i^{outpatient}$ to meet our case definition in age group i. This weight is calculated from the results of the prospective case-control study as a ratio of suspected cases over registered cases of cough illnesses at the selective departments of SHs. N_i is the population size in age group i in census year 2020. W_i^{cover} is the weight used to adjust catchment population overlapping between participating hospitals from HUAS in age group i. It is calculated as the ratio of community residents who have the reported seeking medical care in the participating hospitals for the last episodes of their cough illness over the residents who have the willingness of healthcare-seeking in the participating hospitals, as obtained from the HUAS study. C_i is the proportion of population covered by participating hospitals in age group i, as measured in the HUAS study. It is calculated as the proportion of residents who report having the willingness of healthcare-seeking in the participating hospitals over the total no. of residents responded. r_i is the proportion of community residents reporting seeking health-care for their most recent episode of cough illnesses in age group i as measured in the HUAS study. AF_i is the population attributable fraction of chronic/persistent cough due to *B. pertussis* infection in age group i, calculated based on case-control study using unconditional logistic regression

model, as follows:

$$\log_e(OR) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_k x_k$$

$$\mathrm{OR} = \exp(\beta_k)$$

$$\mathrm{AF_i} = \Pr(\textit{Bordetella pertussis} \mid \text{Chronic cough})\ (1 - \frac{1}{OR})$$

Note: Pr(*Bordetella pertussis*|Chronic cough) = P_i is the prevalence of *B. pertussis*, calculated by dividing the number of laboratory-confirmed pertussis with the total number of chronic/persist cough tested. x_1, x_2, x_3, …, x_k are variables associated with the occurrence of chronic/persistent cough, including the presence of *B. pertussis* and other social and environmental factors significant at p < 0.1 in univariate analysis. OR is the odds ratio.

The 95% CI of incidence is calculated with bootstrap method with 1000 replications. Besides incidence estimates, we will also explore factors associated with severe pertussis (defined as a composite outcome of death, sepsis, invasive ventilation and Intensive Care Unit transfer), by using multivariable logistic regression. Factors significantly associated with severe pertussis at p < 0.1 in univariate analysis will be included in the model. The median age of children with pertussis will be calculated by type of vaccinees, and factors predicting the age of pertussis breakthrough among children who had received DTP vaccination early in their life will be also studied by using Cox proportional hazards regression models.

ETHICS AND DISSEMINATION

This study is designed an observational study. The risk of harm is minimal and adverse medical events are not anticipated from the procedures involved in the study. The study protocol, CRF, and consent form have been sent to and approved by China CDC's Institutional Review Board (reference no. ICDC-202110).

The primary risk to participants is the loss of confidentiality. To help maintain confidentiality, all study investigators will sign a confidentiality agreement and receive appropriate ethics training. All interviews will be conducted at the study investigator's

office, and signed consent forms and completed survey forms will be locked in a secure file cabinet at the end of each day. A very limited number of trained study staff can have the key to the locked file cabinets. Participation in every aspect of the study will be voluntary, and for all new data collection, participants will be asked to provide written informed consent. Besides, collection of specimens may cause mild discomfort to the subject during the procedure, especially drawing blood from young children. To minimize invasive procedures during sample collection, swab and blood specimens will be collected by aseptic technique and we encourage the use of leftover sera during routine medical care at the time point of enrolment.

As a benefit of participating in the study, participants with pertussis will receive senior doctor consultation during treatment on how to limit transmissions among family members and co-workmates; Patients enrolled in the study will have access to antibiotic susceptibility testing results should they have *B. pertussis* isolates acquired. This will give a guide on empirical antibiotic usages for physicians; moreover, the data generated in the study will be valuable to determine the burden of pertussis and explore risk factors for illness attributable to severe pertussis in children as well as adolescents/adults, which can be used by public health departments, healthcare providers and scientific group in China to inform policies making, implement disease control and prevention (i.e. vaccination) and improve patient care, both at the sites level and national level. In general, the minimal risks associated with physical discomfort during blood and N/P sample collection are offset by the great benefit associated with the study's ability to inform pertussis prevention and control strategies in China. Upon completion, results from this study will be disseminated via academic presentations and publication in peer-reviewed journals.

DISCUSSION

PertussisChina is an innovative and a pilot of a laboratory-based and population-based active surveillance platform for vaccine-preventable bacterial diseases (VPBD) in China, which endeavors to establish a network of laboratories and hospitals using comparable and unified standards to provide up-to-date disease burden estimates and disease determinants for evaluating, prioritizing and optimizing the use of

vaccines and for the development of new interventions against bacterial infections in the country. Pertussis is the first one of the several bacterial infections that we are planning to take this approach. In response to the changing epidemiology of pertussis in China[7 8 42 43], the 2019 summon of the National Immunization Advisory Committee submitted a motion to its members urging the modification of the current immunization schedule of pertussis vaccine administered at 3, 4, 5 and 18-24 months[44], to vaccinate children at 2, 4, 6 and 18-24 months instead and to add a 5th booster dose at 4-6 years of age; however, partly due to knowledge gaps existed in age-specific burden of pertussis, NIAC suspended its decision on this issue. To provide up-to-date evidence on disease burden of pertussis, this study will focus on age-specific incidence based on laboratory confirmation and will fill the data gaps on prospectively and actively collected incidence data and key information on illness severity and outcomes. We are expecting that data from this study can be served as background information augmenting NIDSS to inform NIAC's recommendations on children vaccination and further quantify the benefit of adolescent/adult vaccination to protect infants from severe outcomes in future. There are several strengths of the study.

In this one-year study, we will enroll suspected chronic/persistent cough patients (for infants aged less than 1 year, cough regardless of duration) from health care facilities in two sites of China, covering a censused population of 2.23 million. The catchment population utilizing health-care services at the SHs are well characterized and defined by HUAS, providing unbiased estimates of age-stratified total person-times observed in the cohort. The prevalence of cough in regarding of illness duration and proportion of people who do not seek healthcare are measured retrospectively by HUAS. Thus by comparing between data generated from HUAS in community and case registry in SHs, we will able to measure incidence by settings (i.e. community, outpatient and inpatient), especially those in communities whose symptoms are mild or atypical after the waning of vaccine-induced immunity or those no healthcare are sought[2]. Besides, all hospitalizations suspected of pertussis will be actively searched and prospectively enrolled in a timely manner in our SHs, serving as a complete and representative sample of pertussis occurred in the interested population that would have induced minimal selection bias. As for milder cases in ambulatory settings, sampling of patients

with chronic/persistent cough in outpatient setting to conduct laboratory investigation is preferred. Misclassification of cases or recall bias will be minimized by the complex laboratory procedures (i.e. culture, PCR, and serology combined), unified data collection tools (i.e. CRFs) and data collection process, i.e. the 60-day of follow-up during which interesting events (e.g. 3-fold titer raising) will be closely monitored by sequential sera samples. Using laboratory-confirmed pertussis as the outcome will allow us to specifically measure pertussis disease burden. To account for asymptomatic carriage of *B. pertussis*, we will recruit healthy control to investigate the proportion of population carrying *B. pertussis* in their upper respiratory tract and sero-positivity, which could be useful for calculating population attributable fraction (AF) to adjust rate estimates. In addition, the prospective cohort will provide valuable follow-up data related to risk factors for severe illness (i.e. adverse clinical outcomes of hospitalization or death). Collection of the vaccination history (including band, dosing, procedure and time of administration) from study participants will help explore the breakthrough rates of *B. pertussis* infection among different type of vaccinees and investigate reasons of vaccination failure, by linkage of study subjects ≤14 years old with his/her individual records on immunization in the national database. Finally, we will abstract EMR data from hospital information system, which serves as a complete and accurate record of cough illness outcomes occurred in SHs. The retrospectively collected EMR data will be validated by prospectively counting cases eligible for inclusion at selective departments of SHs on daily basis. Using data from the EMR will allow us to determine the size of outpatient and emergency department visits for cough illness in the studied population. For most of adults and fully immunized children and adolescents, their illness is generally mild and is most likely to be encountered at the ambulatory settings in which the diagnostic capacity is generally lacking.

Aside from acquiring incidence estimates, the prevalence and distribution of *B. pertussis* strains circulating in the population will be determined and characterized, which are reported to be evolving under the selection pressure from both vaccine and antibiotics in previous studies [45] and are important data for the development of novel vaccine or new therapeutics in the country. For example, as a benefit of the study, we

will create a representative national and well characterized repository of strains and specimens that can be shared with other investigators for future research, the main antigenic and genotypic features of *B. pertussis* will be characterized by sequencing or other bio-molecular methods.

We realized that there are several limitations worthy of note in our study. Firstly, we will not identify all pertussis that occur in our studied population since our case definition will not capture atypical and asymptomatic manifestations associated with *B. pertussis* infection. For example, previous studies showed that about 17.4% children[46] and 20% adolescents/adults[47] with *B. pertussis* infection had a cough duration less than 3 weeks, and other symptoms/signs used in the case definition, like spasmodic cough (63%), post-tussive vomiting (42%) and whoops (8%), were infrequently presented in adults[48], which will make incidence underestimated. It is argued that no symptom is sufficiently predictive for diagnosing pertussis[49] and there was no case definition that has been proposed for purpose of studying disease burden of pertussis. After balancing at the sensitivity and specificity of case definition commonly recommended by WHO, the U.S. and others[50-52] and the available laboratory capacity and resources in the study, we finally adopted the current case definition that can be used to facilitate comparison of results between studies and countries. Second, our study period is a little short. Since pertussis has showed a cyclic pattern and peaked every 3-5 years[2] [16], our study will not capture this feature. Moreover, our study is going to recruit cases in 2021-2022, right after COVID-19 pandemic. As the epidemiology of many respiratory infections have been reported changing as a result of widely implementation of nonpharmaceutical interventions (e.g. wearing masks, social distancing, and personal health protection)[53] [54] and the detained coverage of vaccines used in Expanded Program on Immunization during the pandemic[55]. The impacts of COVID-19 outbreak on incidence estimates of pertussis are not foreseeable in the study. Future studies are upcoming depending on the results of this pilot. Finally, China is a big country with large variations in population density and across different climate, geographic and economic regions. Although we have paid careful attention to variables, like DTP3 vaccine coverage, childhood mortality and health-care delivery pattern when selecting study sites, regions with the

highest and lowest reported incidence of pertussis are generally not included. This may also influence the generalizability of the incidence estimates to extrapolate to other regions.

In summary, PertussisChina is an innovative study that uses unified protocol to generate up-to-date high-quality incidence data on pertussis. The study design can secure the precision of data collection and provide insights into the prospectively conducted studies that designed to augment passive surveillance in countries where resources is limited and data is currently lacking. When completed, the results coming out this study will provide valuable scientific data on the incidence, etiology, and risk factors for severe sequelae of pertussis to academic societies and the public health authorities, who is currently struggling and fighting against this burdensome disease worldwide.

Contributors

ZS is the principal investigator on this study who conceived and critically revised the manuscript. JY, HH and YZ conceptualized and designed the study, wrote the first draft and contributed equally to this work. YG, JX, LX, and YG designed the laboratory methods. XZ, QZ, YZ and XT wrote the statistical analysis plan. CC and ZC commented on and revised drafts of the manuscript. All authors contributed to reviewing, revising, and approving the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication

Not required.

Ethics approval

This study has been approved by Chinese Center for Disease Control and Prevention's Institutional Review Board (reference no. ICDC-202110).

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Figure Legends

Figure 1. Incidence of reported pertussis from NNIDSS, China, 1952-2019. A cDTaP was introduced into national immunization program to replace DTwP in 2007 and the transition was fully completed in 2013. Abbreviations: DTwP, combined diphtheria, tetanus toxoid and whole-cell pertussis vaccine; cDTaP, co-purified diphtheria, tetanus toxoid and acellular pertussis vaccine; National Notifiable Infectious Disease Surveillance System (NNIDSS).

Figure 2. Location and population size of study sites included in PertussisChina study

Figure 3. Flow diagram of major study activities

Figure 4. Data flow chart and key indicators used to calculate incidence of pertussis

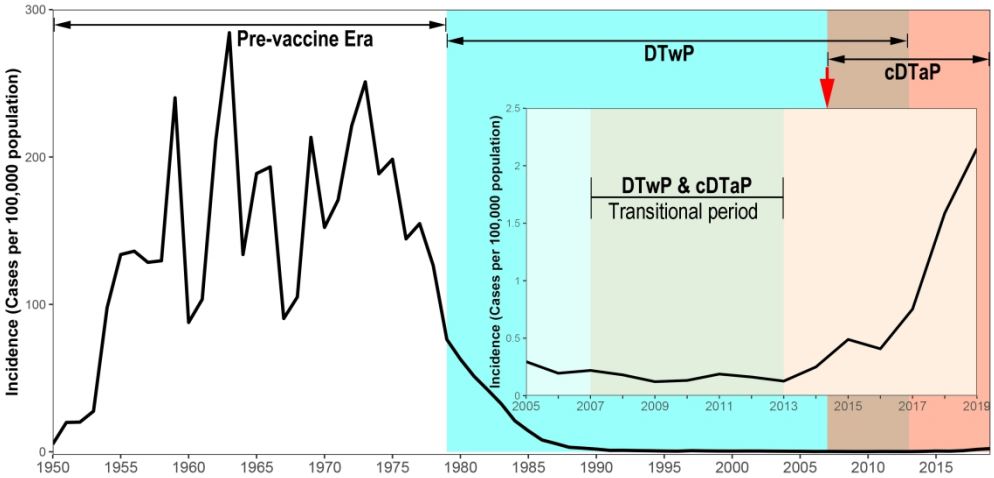

Figure 1. Incidence of reported pertussis from NNIDSS, China, 1952-2019

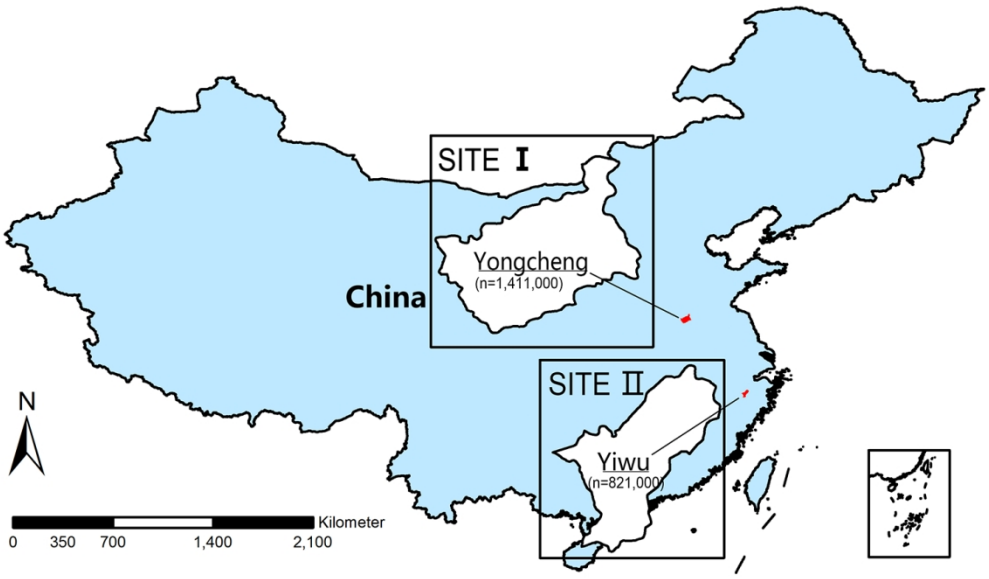

Figure 2. Location and population size of study sites included in PertussisChina study

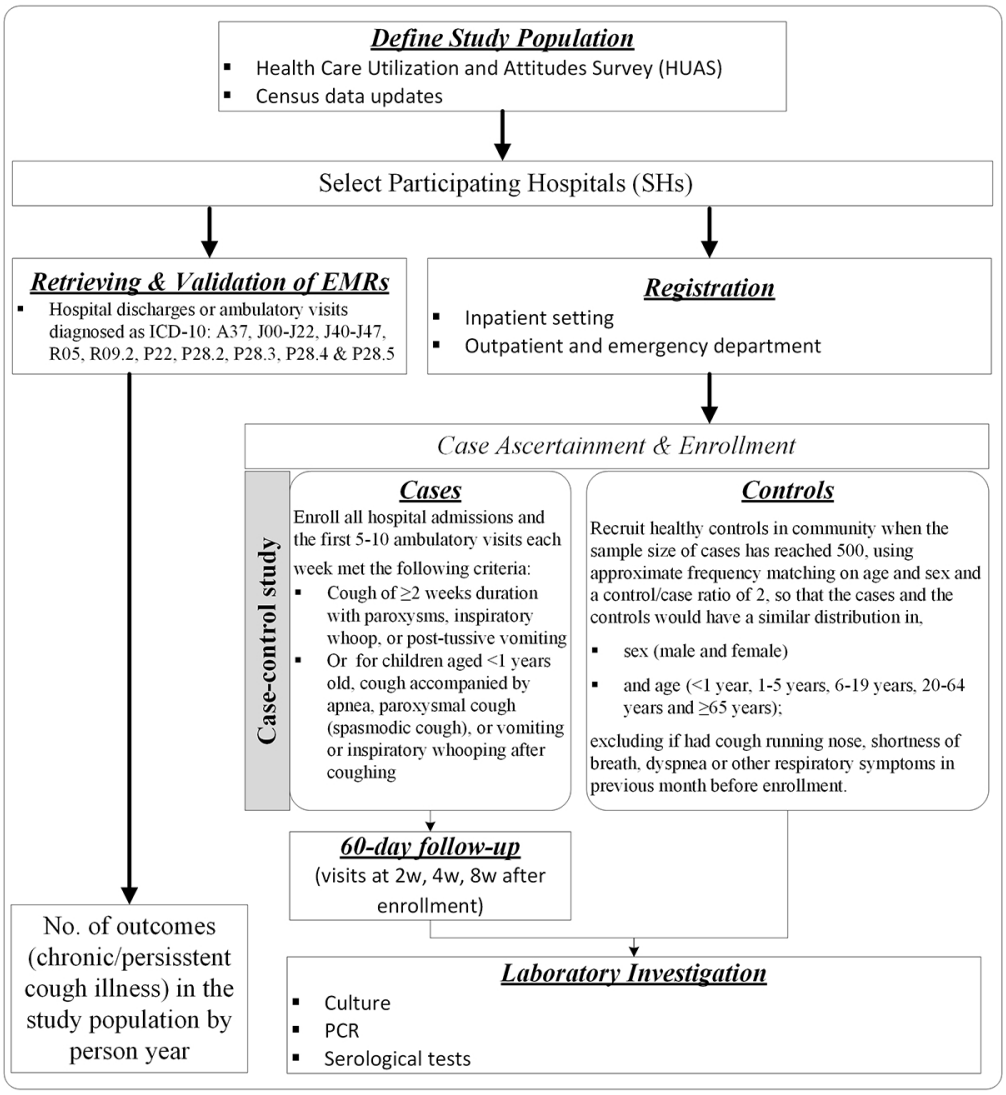

Figure 3. Flow diagram of major study activities

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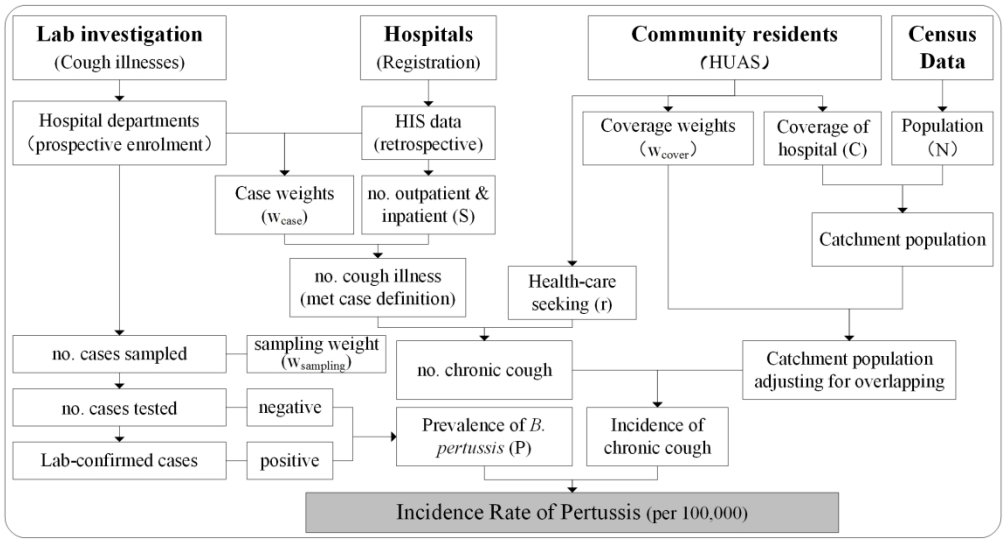

Figure 4. Data flow chart and key indicators used to calculate incidence of pertussis

187x100mm (300 x 300 DPI)

Supplementary Appendix

Title: Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study

Running head: PertussisChina Study, 2020

Tables & Forms

- Supplementary Table 1. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among children under 5 years old
- Supplementary Table 2. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among persons aged 5 years and older
- Supplementary Table 3. Case screening and ascertainment form
- Supplementary Table 4. Baseline information of case & control (CRF—T0)
- Supplementary Table 5. Follow-up information of case (CRF—T2w/T4w/T8w)
- Supplementary Table 6. Outcome of case at the end of follow-up (CRF—Tend)

Supplementary Table 1. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among children under 5 years old

Greetings! We are the staff of Center for Disease Control and Prevention. We sincerely invite you to participate in this questionnaire survey. The purpose of the survey is to study the utilization and attitudes of community residents towards health-care services of chronic/persistent cough, so that we can better serve you in the future.

This survey is anonymous. Your answers to these questions are kept confidential. You can choose whether to participate in the survey voluntarily or not. Refusal of the survey will not have any adverse consequences on you personally and your children. If you agree to the participate, please read each question carefully and fill in the answer on your own. Thank you for your support and cooperation!

Part I. Basic Information

1.1 Site:_____ Site ID:_____

1.2 Your length of time (years & months) living in the site (e.g. Yiwu or Yongcheng):

□ less than 6 months □six months and over

1.3 Current address: _____ county _____ street _____community/village

1.4 Type of respondents in relation to the studied subject/children:

□ mother □ father □ grandma/grandpa □ others_____

1.5 Date of Birth: □□□□/□□/□□ (yyyy/MM/dd)

1.6 Gender: □ Male □ Female

1.7 Ethnicity: □ Han □ others_____

1.8 Did your child attend school? □ yes □ no

1.9 Your occupation (of the respondent who answered the question):

□students □housework or unemployed □retired people □commercial & service sector workers □ food handler or employees of food industry □specialists, including teacher, medical personnel and workers □agriculture, forestry, animal husbandry and fishery workers □ others_____

1.10 Your educational attainment (of the respondent who answered the question).

□primary school or illiteracy □middle school □high school

□technical secondary school □college level and above

1.11 Including yourself, there are_____members in your family (defined as those who shared the same dining table in the house)?

Of which, there are_____children under five years old.

1.12 Is there any smokers or ex-smokers in your family? □ yes □ no

Part II. self-perceived illness and health-care seeking behavior

2.1 Did your children experienced cough during the past one month prior to our interview? □ no □ yes

2.1.1 If yes, how long did the cough last?

□ <1 week □ 1-2 weeks □ 3-4 weeks □ 5-8 weeks □ >8 weeks

2.1.2 If yes, what is the clinical characteristics of the last episode of cough?

□paroxysmal cough □vomiting after coughing □whooping cough

□cough worsening during the night □vomiting after coughing

□productive cough with large amount of sputum □dry cough

□cough with blood in sputum □others_____

2.1.3 If yes, what is the other concomitant symptoms?

□productive cough □running nose □fever (body temperature ≥ 37.2°C)

□belching □acid reflux □irritable and crying □vomiting □headache

□tachypnea □earache □sore throat □dyspnea □abdominal pain

□arthralgia □chest pain □myalgia □fatigue □lethargy

□burn after sternum □without any other discomfort □others_____

2.1.4 If yes, what do you think is the most probable cause of your cough?

□respiratory tract infection □inhalation of foreign objects in the respiratory tract

□COPD exacerbation □asthma exacerbation □recurrent tuberculosis

□chronic cardiopulmonary disease □lung cancer □inhalation of cold air

□chronic bronchitis □bronchiectasis □I don't know □others_____

2.2 Did your child visit a doctor or seek healthcare during the last episode of cough?

□ no □ yes

2.2.1 If yes, where did your child see a doctor?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

□Suxi Township Health Center □Beiyuan Community Health Center

□Shangxi Township Health Center □Dachen Township Health Center

□Houzhai Community Health Center □Chi'an Township Health Center

□Chengxi Community Health Center □Niansanli Community Health Center

□Yiwu Huashan Rehabilitation Hospital □Jiangdong Community Health Center

□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

□Yongcheng People's Hospital □Yongcheng Central Hospital

□Yongmei Group General Hospital □Henan Shenhuo Group General Hospital

□Yongcheng Maternal & Child Health Hospital □Yucheng Township Health Center

□Chenji Township Health Center □ Gaozhuang Township Health Center

□Yongcheng Mangdang Hospital □ Lizhai Township Health Center

□Yongcheng Second People's Hospital □ Liuhe Township Health Center

□Yanji Township Health Center □ Dawangji Township Health Center

□Longgang Township Health Center □ Shunhe Township Health Center

□Peiqiao Township Health Center □ Huaihai Community Health Center

□Huangkou Township Health Center □ Maqiao Township Health Center

□Jiangkou Township Health Center □ Houling Township Health Center

□Chenguanzhuang Township Health Center □ Taiqiu Township Health Center

□Wolong Township Health Center □ Huicun Township Health Center

□Yongcheng Traditional Chinese Medicine Hospital

□Shibali Township Health Center □ Xuehu Township Health Center

□Mamu Township Health Center □ Xinqiao Township Health Center

□Xunyang Township Health Center □ Shuangqiao Township Health Center

□Yongcheng Jiangkou Yongji Hospital □ Miaoqiao Township Health Center

□Yongcheng Tuberculosis Hospital □ Tiaohe Township Health Center

□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

2.2.1 If no, why did not your child see a doctor?

□Symptoms are mild, no need to see a doctor

□The hospital is too far from home and the transportation is inconvenient

□Drugs purchased in pharmacies □Distrust the doctor

□Unaffordable high medical expenses

□Hospital facilities and environment were poor

□others_____

2.3 Was your child hospitalized for the last episode of cough? □ no □ yes

2.3.1 If yes, where was your child hospitalized?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

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□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

□Yongcheng People's Hospital □Yongcheng Central Hospital

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□Mangshan Township Health Center □ Luanhu Township Health Center

□others

Part III. Attitudes towards health-care utilization

(Next, we will ask some questions about the actions you might take under some hypothetical situations that do not need to happen.)

3.1 If your child keeps coughing for 2 weeks but does not get better, and you have decided to see a doctor, which one of the following medical institutions would you choose to go?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

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□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

□Yongcheng People's Hospital □Yongcheng Central Hospital

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□Jiangkou Township Health Center □ Houling Township Health Center

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□Wolong Township Health Center □ Huicun Township Health Center

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□Yongcheng Tuberculosis Hospital □ Tiaohe Township Health Center

□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

3.2 If the doctor recommends that your child be hospitalized, which one of the following medical institutions would you choose?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

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□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

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□Chenguanzhuang Township Health Center □ Taiqiu Township Health Center

□Wolong Township Health Center □ Huicun Township Health Center

□Yongcheng Traditional Chinese Medicine Hospital

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□Xunyang Township Health Center □ Shuangqiao Township Health Center

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

Part IV. Other questions

4.1 Has your child ever received the following vaccines?

□influenza vaccine □pneumococcal vaccine □Haemophilus influenzae vaccine

□Vaccines containing pertussis components (i.e. DTP)

4.1.1 If received vaccines containing pertussis (i.e. DTP), what kind of the vaccine?

□cDTaP □ DTaP/Hib □ DTaP-IPV/Hib

4.2 Your family's average annual income (Chinese Yuan) is,

□<50,000 □50,000-90,000 □100,000-190,000 □200,000-490,000 □≥500,000

4.3 Your phone number is_____

Thank you very much for taking your time. The information you provided in this interview is very valuable to help us improve our work. Wish you a happy life!

Time of survey started: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Time of survey ended: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Investigator: _____ Supervisor: _____

Supplementary Table 2. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among persons aged 5 years and older

Greetings! We are the staff of Center for Disease Control and Prevention. We sincerely invite you to participate in this questionnaire survey. The purpose of the survey is to study the utilization and attitudes of community residents towards health-care services of chronic/persistent cough, so that we can better serve you in the future.

This survey is anonymous. Your answers to these questions are kept confidential. You can choose whether to participate in the survey voluntarily or not. Refusal of the survey will not have any adverse consequences on you personally and your children. If you agree to the participate, please read each question carefully and fill in the answer on your own. Thank you for your support and cooperation!

Part I. Basic Information

1.1 Site:_____ Site ID:_____

1.2 Your length of time (years & months) living in the site (e.g. Yiwu or Yongcheng):

□ less than 6 months □six months and over

1.3 Current address: _____ county _____ street _____ community/village

1.4 Type of respondents in relation to the studied subject:

□respondent himself is the study subject □ others_____

1.5 Date of Birth: □□□□/□□/□□ (yyyy/MM/dd)

1.6 Gender: □ Male □ Female

1.7 Ethnicity: □ Han □ others_____

1.8 Your occupation:

□students □housework or unemployed □retired people □commercial & service sector workers □ food handler or employees of food industry □specialists, including teacher, medical personnel and workers □agriculture, forestry, animal husbandry and fishery workers □ others_____

1.9 Did you ever contact with dust/chemical materials in the working environment in the past one year, such as those encountered by workers using pneumatic drills at

construction sites, miners, painters, benzene solvents in leather production, etc.

□ no □ yes

1.10 Your educational attainment:

□primary school or illiteracy □middle school □high school

□technical secondary school □college level and above

1.11 Including yourself, there are_____members in your family (defined as those who shared the same dining table in the house)?

Of which, there are_____children under five years old.

1.12 Are you smoker or ex-smoker? □ yes □ no

Part II. self-perceived illness and health-care seeking behavior

2.1 Did you experienced cough during the past one month prior to our interview?

□ no □ yes

2.1.1 If yes, how long did the cough last?

□ <1 week □ 1-2 weeks □ 3-4 weeks □ 5-8 weeks □ >8 weeks

2.1.2 If yes, what is the clinical characteristics of the last episode of cough?

□paroxysmal cough □vomiting after coughing □whooping cough

□cough worsening during the night □vomiting after coughing

□productive cough with large amount of sputum □dry cough

□cough with blood in sputum □others_____

2.1.3 If yes, what is the other concomitant symptoms?

□productive cough □running nose □fever (body temperature ≥ 37.2°C)

□belching □acid reflux □irritable and crying □vomiting □headache

□tachypnea □earache □sore throat □dyspnea □abdominal pain

□arthralgia □chest pain □myalgia □fatigue □lethargy

□burn after sternum □without any other discomfort □others_____

2.1.4 If yes, what do you think is the most probable cause of your cough?

□respiratory tract infection □inhalation of foreign objects in the respiratory tract

□COPD exacerbation □asthma exacerbation □recurrent tuberculosis

□chronic cardiopulmonary disease □lung cancer □inhalation of cold air

□chronic bronchitis □bronchiectasis □I don't know □others_____

2.2 Did you see a doctor or seek healthcare during the last episode of cough?

□ no □ yes

2.2.1 If yes, where did you see a doctor?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

□Suxi Township Health Center □Beiyuan Community Health Center

□Shangxi Township Health Center □Dachen Township Health Center

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□Chengxi Community Health Center □Niansanli Community Health Center

□Yiwu Huashan Rehabilitation Hospital □Jiangdong Community Health Center

□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

□Yongcheng People's Hospital □Yongcheng Central Hospital

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□Chenguanzhuang Township Health Center □ Taiqiu Township Health Center

□Wolong Township Health Center □ Huicun Township Health Center

□Yongcheng Traditional Chinese Medicine Hospital

□Shibali Township Health Center □ Xuehu Township Health Center

□Mamu Township Health Center □ Xinqiao Township Health Center

□Xunyang Township Health Center □ Shuangqiao Township Health Center

□Yongcheng Jiangkou Yongji Hospital □ Miaoqiao Township Health Center

□Yongcheng Tuberculosis Hospital □ Tiaohe Township Health Center

□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

2.2.1 If no, why did not you see a doctor?

□Symptoms are mild, no need to see a doctor

□The hospital is too far from home and the transportation is inconvenient

□Drugs purchased in pharmacies □Distrust the doctor

□Unaffordable high medical expenses

□Hospital facilities and environment were poor

□others_____

2.3 Were you hospitalized for the last episode of cough? □ no □ yes

2.3.1 If yes, where were you hospitalized?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

□Suxi Township Health Center □Beiyuan Community Health Center

□Shangxi Township Health Center □Dachen Township Health Center

□Houzhai Community Health Center □Chi'an Township Health Center

□Chengxi Community Health Center □Niansanli Community Health Center

□Yiwu Huashan Rehabilitation Hospital □Jiangdong Community Health Center

□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

□Yongcheng People's Hospital □Yongcheng Central Hospital

□Yongmei Group General Hospital □Henan Shenhuo Group General Hospital

□Yongcheng Maternal & Child Health Hospital □Yucheng Township Health Center

□Chenji Township Health Center □ Gaozhuang Township Health Center

□Yongcheng Mangdang Hospital □ Lizhai Township Health Center

□Yongcheng Second People's Hospital □ Liuhe Township Health Center

□Yanji Township Health Center □ Dawangji Township Health Center

□Longgang Township Health Center □ Shunhe Township Health Center

□Peiqiao Township Health Center □ Huaihai Community Health Center

□Huangkou Township Health Center □ Maqiao Township Health Center

□Jiangkou Township Health Center □ Houling Township Health Center

□Chenguanzhuang Township Health Center □ Taiqiu Township Health Center

□Wolong Township Health Center □ Huicun Township Health Center

□Yongcheng Traditional Chinese Medicine Hospital

□Shibali Township Health Center □ Xuehu Township Health Center

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□Yongcheng Tuberculosis Hospital □ Tiaohe Township Health Center

□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

Part III. Attitudes towards health-care utilization

(Next, we will ask some questions about the actions you might take under some hypothetical situations that do not need to happen.)

3.1 If you keep coughing for 2 weeks but does not get better, and you have decided to see a doctor, which one of the following medical institutions would you choose to go?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

□Suxi Township Health Center □Beiyuan Community Health Center

□Shangxi Township Health Center □Dachen Township Health Center

□Houzhai Community Health Center □Chi'an Township Health Center

□Chengxi Community Health Center □Niansanli Community Health Center

□Yiwu Huashan Rehabilitation Hospital □Jiangdong Community Health Center

□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

□Yongcheng People's Hospital □Yongcheng Central Hospital

□Yongmei Group General Hospital □Henan Shenhuo Group General Hospital

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□Yongcheng Tuberculosis Hospital □ Tiaohe Township Health Center

□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

3.2 If the doctor recommends that you should be hospitalized, which one of the following medical institutions would you choose?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

□Suxi Township Health Center □Beiyuan Community Health Center

□Shangxi Township Health Center □Dachen Township Health Center

□Houzhai Community Health Center □Chi'an Township Health Center

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□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

(For Yongcheng site, please select the following)

□Yongcheng People's Hospital □Yongcheng Central Hospital

□Yongmei Group General Hospital □Henan Shenhuo Group General Hospital

□Yongcheng Maternal & Child Health Hospital □Yucheng Township Health Center

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

Part IV. Other questions

4.1 Have you ever received the following vaccines?

□influenza vaccine □pneumococcal vaccine □Haemophilus influenzae vaccine

□Vaccines containing pertussis components (i.e. DTP)

4.1.1 If received vaccines containing pertussis (i.e. DTP), what kind of the vaccine?

□cDTaP □ DTaP/Hib □ DTaP-IPV/Hib

4.2 Your family's average annual income (Chinese Yuan) is,

□<50,000 □50,000-90,000 □100,000-190,000 □200,000-490,000 □≥500,000

4.3 Your phone number is_____

Thank you very much for taking your time. The information you provided in this interview is very valuable to help us improve our work. Wish you a happy life!

Time of survey started: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Time of survey ended: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Investigator: _____ Supervisor: _____

Supplementary Table 3. Case screening and ascertainment form

Name of Hospital_____ Departments_____

Name of patient			Sex	☐ male ☐female	
Birthdate			Ethnicity		
Current address			Phone number		
Date of illness onset		Date of admission			
Date of written informed consent signed					
Lists of inclusion & exclusion criteria				yes	no
Inclusion criteria:					
Patient regardless of ages:					
1.cough of ≥2 weeks duration;				☐	☐
2.had one or more of the following symptoms;				☐	☐
– paroxysmal cough;				☐	
– inspiratory whoop;				☐	
– post-tussive vomiting.				☐	
Infants less than one year old					
1.cough (regardless of cough duration);				☐	☐
2.had one or more of the following symptoms;				☐	☐
– paroxysmal cough;				☐	
– inspiratory whoop;				☐	
– post-tussive vomiting;				☐	
– apnea.				☐	
Written informed consent signed				☐	☐
If you answer "No" to any of the above, the patient cannot enter the study.					
Exclusion criteria:					
1.not a permanent resident (lived less than 6 months at the site);				☐	☐
2.gastroesophageal reflux;				☐	☐
3.spastic bronchitis;				☐	☐
4.diagnosed tuberculosis;				☐	☐
5.lung mycoplasma/chlamydia infection;				☐	☐
6.chronic sinusitis;				☐	☐
7.adults/adolescents with a measured body temperature of ≥38.5 °C;				☐	☐
8. researchers considered not suitable for participating in the study.				☐	☐
If you answer Yes" to any of the above, the patient cannot enter the study.					
Whether the patient is included in the study				☐	☐
If no, what is the reason for not included?					
– not meet the inclusion & exclusion criteria;				☐	☐
– Refuse to participate ;				☐	☐
If yes, what is the patient identifier no.? _____				☐	☐

Supplementary Table 4. Baseline information of case & control (CRF—T0)

Patient identifier no.:_____ Type: ☐Inpatient ☐outpatient ☐Control

Name：_____（or **Parents' name**：_____） **Sex：**☐ male ☐ female **Ethnicity**：☐ Han ☐ other_____

Birthdate：____/____/____(YYYY/mm/dd) **Preterm baby：**☐ yes，(gestational age_____weeks) ☐ no

Address*：_____ **mobile phone**：_____

Family member No.：_____persons **No. of children under five in the family：**_____children

Smokers in the family：☐ yes，(_____persons) ☐ no **Smoking：**☐ yes ☐ no **Drinking：**☐ yes ☐ no

Occupation：

☐kindergarten children ☐preschool children ☐students ☐teacher ☐childcares ☐food industry worker ☐commercial service

☐medical staff ☐factory workers ☐migrant worker ☐farmer ☐herdsman ☐fishermen ☐cadre ☐retired ☐housewife

☐others ☐unknown

Clinical characteristics (within 24h before and after admission)

Illness onset Date：_____(YYYY/mm/dd) **Primary diagnosis**：_____ **Diagnose date**：_____(YYYY/mm/dd)

Hospitalized：☐ yes ☐ no **Admission diagnosis**：_____ **Admission date**：_____(YYYY/mm/dd)

☐cough（Starting date____/____/____[YYYY/mm/dd]，duration_____days）

☐post-tussive vomiting ☐paroxysmal cough ☐whooping cough ☐apnea ☐cyanosis ☐fever（body temperature_____℃）

☐cough worsening in night ☐productive cough; Sputum color：yellow/white/black/glass like

☐seizure ☐hemoptysis ☐chills ☐headache ☐myalgia ☐sore throat ☐joint pain ☐chest pain

☐sweat ☐shortness-of-breath ☐running nose ☐lachrymation ☐fatigue ☐other(_____)

Complications：☐pneumonia（radiographical evidence：☐yes ☐ no）☐atelectasis ☐pulmonary hypertension

☐encephalopathy ☐seizure ☐others（_____）

Blood tests：WBC_____×10^9/L; L_____×10^9/L; N_____×10^9/L; Plt_____×10^9/L; Hb_____g/L; CRP_____mg/L; GLU_____mmol/L

Physical check：body temperature：_____℃ **Breath rate**：_____breath/min **Heart rate：**_____beats/min

Systolic/diastolic blood pressure：_____/_____mmHg **Pulse oximetry**: sPO_2 (if any):_____%

Lung auscultation：□dry rale □wet rale **Consciousness**：clear/lethargy/irritable/delirium/convulsions/coma

Treatment (one week before admission)

（1）Drug name：Please give the name of the drug, or the trade name if it is a fixed compound preparation

（2）Category: A=antibiotic (1. Amoxicillin; 2. Amoxicillin-clavulanic acid; 3. Ampicillin; 4. Azithromycin; 5. Ceftriaxone; 6. Cefuroxime; 7. Ciprofloxacin; 8. Clarithromycin; 9. Doxycycline; 10. Erythromycin; 11. Penicillin; 12. Tetracycline; 13. Compound sulfamethoxazole); B=antiviral drugs; C=steroid hormone drugs

（3）Route：1=oral, 2=intravenous injection, 3=intravenous drip, 4=intramuscular injection, 5=inhalation, 6=other

（4）Frequency：1= continuous，2=intermittent

Drug name (1)	Category (2)	Route (3)	Daily dose		Frequency (4)	Starting date (YYYY/mm/dd)	Stop date (YYYY/mm/dd)
			dose	unit			

Vaccination history of DTP3 (for children aged under 14 years old)

Source of data：☐vaccination certificate ☐linkage with national database ☐ self-reports

Reasons of unvaccinated：1. Contraindications; 2. Under the age of vaccination; 3. Missed vaccination time; 4. Parents refused to vaccination; 5. migrating population; 6. Don't know; 7. Others_____

dose	lot number	producer	dosage		site	Date (YYYY/mm/dd)	Reasons of unvaccinated
			dose	unit			
1							
2							
3							

Patient specimen collection

Specimen collected： ☐ yes ☐ no **Date of sampling**：____/____/____(YYYY/mm/dd)

Type of specimen： Nasopharyngeal swab ☐ amounts：_____

Whole blood ☐ quantity：_____ ml

Hospital：_____ **Investigator**：_____ **Date of reporting**：____/____/____(YYYY/mm/dd)

Supplementary Table 5. Follow-up information of case (CRF—T_{2w}/T_{4w}/T_{8w})

Patient identifier no.:_____ Type: ☐Inpatient ☐outpatient

Name：_____（or **Parents' name**：_____） **Sex：**☐ male ☐ female

Illness onset date：____/____/____(YYYY/mm/dd) **Admission date：**____/____/____(YYYY/mm/dd)

Follow-up date： ____/____/____(YYYY/mm/dd) **Weeks of follow-up:**☐ 2 wks ☐ 4 wks ☐ 8 wks

Follow-up method: ☐ hospital visits ☐ home visits

Outcomes

Survival: ☐ yes ☐ no Date of death:____/____/____(YYYY/mm/dd) death diagnosis：_____

Hospitalized： ☐ yes ☐ no **Re-admitted into hospital after discharge:** ☐ yes ☐ no

Reasons for re-admission：Pneumonia/heart failure/cardiogenic shock/encephalopathy/Seizure/other_____

Lost to follow-up: ☐ yes ☐ no (refers to 3 consecutive phone calls to patients on different working days but no answers at all)

Clinical characteristics (during follow-up visits)

☐cough（Starting date____/____/____[YYYY/mm/dd]，duration_____days）

☐post-tussive vomiting ☐paroxysmal cough ☐whooping cough ☐apnea ☐cyanosis ☐fever（body temperature_____℃）

☐cough worsening in night ☐productive cough; Sputum color：yellow/white/black/glass like

☐seizure ☐hemoptysis ☐chills ☐headache ☐myalgia ☐sore throat ☐joint pain ☐chest pain

☐sweat ☐shortness-of-breath ☐running nose ☐lachrymation ☐fatigue ☐other(_____)

Blood tests：WBC_____×10^9/L; L_____×10^9/L; N_____×10^9/L; Plt_____×10^9/L; Hb_____g/L; CRP_____mg/L; GLU_____mmol/L

Physical check：body temperature：_____℃ **Breath rate**：_____breath/min **Heart rate：**_____beats/min

Systolic/diastolic blood pressure：_____/_____mmHg **Pulse oximetry**: sPO_2 (if any):_____%

Lung auscultation：□dry rale □wet rale **Consciousness**：clear/lethargy/irritable/delirium/convulsions/coma

Patient specimen collection

Specimen collected： ☐ yes ☐ no **Date of sampling**：____/____/____(YYYY/mm/dd)

Type of specimen： Nasopharyngeal swab ☐ amounts：_____

Whole blood ☐ quantity：_____ ml

Reasons for not sampling： ☐ without coughing symptoms for 1 week ☐refusal to sampling

Hospital：_____ **Investigator**：_____ **Date of follow-up**：____/____/____(YYYY/mm/dd)

Supplementary Table 6. Outcome of case at the end of follow-up (CRF—T_{end})

Patient identifier no.:_____ Type: ☐inpatient ☐outpatient

Name：_____(or **Parents' name**：_____) **Sex：**☐ male ☐ female **Illness onset date：**__/__/__(YYYY/mm/dd)

Admission date：__/__/__(YYYY/mm/dd) **Discharge date：**__/__/__(YYYY/mm/dd)

Discharge diagnosis： primary diagnosis _____

secondary diagnosis **1.**_____ **2.**_____ **3.**_____

Treatment during hospitalization

Admitting into ICU： ☐ yes ☐ no __/__/__(YYYY/mm/dd)

1.**Transfer in date**__/__/__(YYYY/mm/dd) **Transfer out date**__/__/__(YYYY/mm/dd)

2.**Transfer in date**__/__/__(YYYY/mm/dd) **Transfer out date**__/__/__(YYYY/mm/dd)

3.**Transfer in date**__/__/__(YYYY/mm/dd) **Transfer out date**__/__/__(YYYY/mm/dd)

Oxygen therapy： ☐ yes ☐ no duration：_____days

Invasive ventilation： ☐ yes ☐ no duration：_____days (invasive ventilation refers to tracheal intubation or tracheotomy)

Non-invasive ventilation: ☐ yes ☐ no duration：_____days

Oscillating respirator： ☐ yes ☐ no duration：_____days

ECMO or interventional lung adjuvant therapy（iLA） ☐ yes ☐ no **date of treatment start：**__/__/__(YYYY/mm/dd)

Renal replacement therapy/dialysis： ☐ yes ☐ no **date of treatment start：**__/__/__(YYYY/mm/dd)

Exchange transfusion： ☐ yes ☐ no **date of treatment start：**__/__/__(YYYY/mm/dd)

Leukophoresis or leukoreduction therapy： ☐ yes ☐ no **date of treatment start：**__/__/__(YYYY/mm/dd)

Drugs

（1）Drug name：Please give the name of the drug, or the trade name if it is a fixed compound preparation

（2）Category: A=antibiotic (1. Amoxicillin; 2. Amoxicillin-clavulanic acid; 3. Ampicillin; 4. Azithromycin; 5. Ceftriaxone; 6. Cefuroxime; 7. Ciprofloxacin; 8. Clarithromycin; 9. Doxycycline; 10. Erythromycin; 11. Penicillin; 12. Tetracycline; 13. Compound sulfamethoxazole); B=antiviral drugs; C=steroid hormone drugs

（3）Route：1=oral, 2=intravenous injection, 3=intravenous drip, 4=intramuscular injection, 5=inhalation, 6=other

（4）Frequency：1= continuous，2=intermittent

drug name (1)	category (2)	route (3)	daily dose		frequency (4)	starting date (YYYY/mm/dd)	stop date (YYYY/mm/dd)
			dose	unit			

Clinical characteristics

Symptoms/signs：

☐cough（Starting date__/__/__[YYYY/mm/dd]，duration_____days）

☐post-tussive vomiting ☐paroxysmal cough ☐whooping cough ☐apnea ☐cyanosis ☐fever（body temperature_____℃）

☐cough worsening in night ☐productive cough; Sputum color：yellow/white/black/glass like

☐seizure ☐hemoptysis ☐chills ☐headache ☐myalgia ☐sore throat ☐joint pain ☐chest pain

☐sweat ☐shortness-of-breath ☐running nose ☐lachrymation ☐fatigue ☐other(_____)

Complications：

☐viral pneumonia ☐cardiac arrest ☐bacterial pneumonia ☐bacteremia ☐acute lung injury/ARDS ☐heart infection

☐coagulation disorders ☐pneumothorax ☐anemia ☐pleural Effusion ☐acute kidney injury ☐myolysis

☐bronchiolitis ☐gastrointestinal hemorrhage ☐meningitis ☐pancreatitis ☐epilepsy ☐arrhythmia

☐liver insufficiency ☐stroke ☐hyperglycemia ☐hypoglycemia ☐congestive Heart Failure
☐other (_____)

Patient Prognosis

☐**cured**
☐**improved and be discharged**
☐**transferred to other hospital** **reasons for transfer**：community rehabilitation/other (_____)
☐**give up treatment** **reasons for give-up**：economic reasons/illness exacerbation/other (_____)
☐**death** **date of death**：____/____/____(YYYY/mm/dd) **death diagnosis**：_____

Hospital：_____ **Investigator**：_____ **Date of record**：____/____/____(YYYY/mm/dd

BMJ Open

Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study

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Title: Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study

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ABSTRACT

Introduction

Pertussis is one of the top ten diseases of children under 10 years of age, and the few vaccine-preventable diseases who is on a rise in China in recent years; however, the true burden of pertussis, including age-stratified incidence and risk factors of severe sequelae, are underrecognized. We aim to estimate the health burden of laboratory-confirmed pertussis by age groups, considering the setting of illness onset (i.e. in community, outpatient and inpatient), in a Chinese population (~2.23 million in total) at two sites.

Methods and analysis

This paper describes the study design of a one-year, prospective, age-stratified, and population-based case-control study, including site selection, study population, case registry, ascertainment and enrolment, control recruitment, follow-up of case, microbiological methods, data collection, quality control activities, and statistical methods used to generate incidence estimates. During June 2021 through May 2022, registry of suspected pertussis cases (namely chronic/persistent cough) will be conducted in several participating hospitals (SHs) at the two sites, which are selected based on Healthcare Utilization and Attitudes Surveys (HUAS) carried out before study initiation. A case-control study will be conducted in the SHs and we aim to enroll a total of 1,000 suspected pertussis cases (i.e. all hospital admissions and the first 1-3 outpatient visits each week each hospital) and 2,000 frequency matched healthy controls in community. Our primary study outcome, the laboratory-confirmed *Bordetella Pertussis* infection, will be determined by a comprehensive laboratory methods and procedures (i.e. culture, PCR, and serological tests) in both cases and controls at enrolment and during 60-day's follow-up visits. Finally, data from HUAS (i.e. population size), case registry (i.e. the total number of suspected pertussis cases), and case-control study (i.e. the prevalence or population attributable fraction of *Bordetella Pertussis*) will be combined to calculate incidence and its 95% confidence interval through bootstrap method. Epidemiological analyses will be conducted to determine the risk factors associated with severe sequelae of pertussis.

Ethics and dissemination

This study has been approved by Chinese Center for Disease Control and Prevention's Institutional Review Board (no. ICDC-202110). Results will be disseminated via academic

presentations and publication in peer-reviewed journals, and will provide valuable scientific data and some new insights into the incidence, etiology, and risk factors for severe sequelae of pertussis to academic societies and the public health authorities who is currently struggling and fighting against this burdensome disease worldwide.

Keywords: *Bordetella pertussis*, Case-Control Studies, Incidence, China

SUMMARY

Strengths and limitations of this study

- PertussisChina is a population-based study at two sites, covering approximately 2.23 million populations defined through conducting Healthcare Utilization and Attitudes Surveys (HUAS) in community.
- PertussisChina is a laboratory-based study, in which comprehensive laboratory methods (i.e. culture, PCR, and serological tests) and procedures (i.e. 60-days follow-up) will be used to specifically measure pertussis disease burden.
- PertussisChina is a case-control study in which the prevalence and population attributable fraction (AF) of *Bordetella Pertussis* infection can be ready acquired.
- All cases will be prospectively followed up to 60-days to collect interesting events (i.e. adverse clinical outcomes of hospitalization or death) at 2, 4 and 8 weeks after enrolment.
- Limitations are that our incidence might be underestimated and cannot be extrapolated to represent the whole country due to the insensitive case definition used, short study period and relatively small population covered.

BACKGROUND

Whooping cough (pertussis) is a highly contagious respiratory disease caused by *Bordetella Pertussis*[1 2]. Despite a high vaccine coverage of third dose diphtheria-tetanus-pertussis vaccine (DTP3)[3], the "resurgence of pertussis" in recent years has posed a great threat to global public health[4-6], as well as to Chinese infants[7 8]. In 2019, pertussis was one of the top ten diseases with highest burden in children younger than 10 years[9], and the World Health Organization estimates that pertussis kills about 160,700 children under 5 years old worldwide each year[10]. In China, three types of pertussis vaccines are available till Octorber 31, 2021, i.e. the co-purified diphtheria and tetanus toxoids and acellular pertussis (cDTaP, used for routine immunization), DTaP/Hib (Minhai Biotechnology Co., Ltd., Beijing, China)[11] and DTaP-IPV/Hib (Sanofi Pasteur, Lyon, France)[12 13]. The coverage of DTP3 remained high above 99% for children throughout the 2010s[14 15], and the reported incidence of pertussis has been risen from 0.12 per 100,000 in 2013 to 2.14 per 100,000 in 2019 (Figure 1). Unlike the other countries who had experience resurgence of pertussis, especially in adolescents/adults, primarily due to the waning of vaccine induced immunity[16-20], China observed no such changes of age distribution [21]. The rise of pertussis in China was mainly concentrated in infants less than 1 year old, and less than 5% of reported pertussis were adolescents and adults.

Since most epidemiological data on pertussis in China came from a passive reporting system, the National Notifiable Infectious Disease Surveillance System (NNIDSS)[22], underreporting was substantial in the system (≥90%) because of limited diagnosis and incompleteness of reporting[8 23 24]. And the burden of pertussis remained underrecognized. It has been suggested that immunizing schoolchildren is the key for curtailing transmission of pertussis in population[18]. Due to a substantial knowledge gaps existed in age-specific burden of pertussis (i.e. incidence and severity), no adolescent or adult immunization are recommended in the country[25]. Moreover, some important data such as clinical, laboratory and vaccine information are also not available, which is unfavorable for evaluating the effectiveness of vaccine and implementing of other disease control and prevention programs (such as adult

vaccination, diagnostic tests and post-exposure prophylaxis of pertussis). Rigorously conducted, prospective, population-based studies can be used to strengthen the NNIDSS, by providing information on the burden of laboratory-confirmed pertussis, strains distribution, risk factors for severe sequelae and case fatality, and most importantly, to assist health authority in China to allocate health resources, prioritize health research investments, optimize interventions (i.e. vaccination) and innovate vaccine development.

We designed the PertussisChina study, a one-year, prospective, age-stratified, population-based longitudinal cohort and case-control study, which will enroll suspected pertussis patients (i.e. chronic/persistent cough) seeking healthcare in several selected participating hospitals (SHs) at two sites of China, covering approximately 2.23 million censused population. This article describes the study design, including sites selection, study population, case registry, ascertainment and enrolment, control recruitment, follow-up of cases and controls, microbiological methods (i.e. culture, PCR, and serological tests), data collection, quality control activities, and statistical methods used to generate incidence estimates of pertussis. We then further discuss the strengths and weaknesses of the study design.

METHODS AND ANALYSIS

Objectives of the study

The primary objective of the study is to measure the incidence of laboratory-confirmed pertussis by age groups (children, adolescents and adults), and by settings (community, outpatient and inpatient). The secondary objectives are: 1) to describe the distribution of disease severity and outcomes across age groups; 2) to describe the patterns and factors of under-detection and under-reporting of pertussis; 3) to study the carrier (colonization) status of the *B. pertussis* in the upper respiratory tract of healthy controls, and the serum levels of anti-pertussis toxin antibodies (anti-Ptx IgG) in both patients and healthy people; and 4) to create a repository of well-characterized clinical specimens and *B. pertussis* isolates that can be used in future studies.

Study sites and population

Site Selection Criteria

Sites are selected based on the following criteria: 1) have strong willingness to participate; 2) have capability and resources to conduct ongoing surveillance, namely staffs to facilitate specimen collection and case investigation, previous experience in disease surveillance, infrastructures to secure data collection and specimen storage or transportation; and 3) provide a full list of healthcare facilities in the area and the information of built-in hospital information system in the facilities. Currently, there are two sites in the study, including Yongcheng, Henan and Yiwu, Zhejiang (Figure 2).

Study population

In 2019, Yiwu had a permanent population of 821,000 (47,000 were children under five years of age) served by 24 health care facilities (i.e. three tertiary care, four secondary care, and 17 primary care hospitals). Most hospital admissions (≥80% of the total number) occurred in the three large tertiary hospitals, including a children's hospital and two general hospitals; meanwhile, Yongcheng had a permanent population of 1,411,000 (94,000 were children under five years of age) served by 41 health care facilities (i.e. five secondary care and 36 primary care hospitals). Most hospital admissions occurred in the five large secondary care hospitals, including four general hospitals and a maternal and pediatric hospital. In total, the two sites cover a total of 2.23 million permanent population in the study area.

Study overview and design

In order to achieve our study objectives, we will conduct the following study activities at the two sites from June 2021 through May 2022, including, 1) a Healthcare Utilization and Attitudes Survey (HUAS) and a census data updating to define study population (i.e. incidence denominator), so as to set up a sampling frame for the case-control study and selecting participating hospitals (i.e. SH) for case registry and case recruitment; 2) the case-control study to acquire the prevalence of *B. pertussis* infection among suspected pertussis cases and healthy controls, as well as the calculation of population attributable fraction (AF) indicating the proportion of cases

that can be prevented if *B. pertussis* was totally removed from the population; and 3) case registry and the retrieval of electronic medical records (EMRs) from hospital information system to provide and validate the total number of suspected pertussis case patient (chronic/persistent cough) encountered in the SHs (i.e. incidence numerator) (Figure 3).

Defining and calibrating study population

Census data updating

Population census data at the two sites will be collected and updated during the study period. Population census is conducted every ten years in China and the nearest one is in 2020. However, an intermittent survey of 1% sampling of the total population would be performed to update population census data every year between the two censuses. We will retain the up-to-date population data from the National Bureau of Statistics. Moreover, the population birth, mortality, and population migration are recorded by the local government. We will also contact the local health bureau quarterly to access these data to give a precise estimation of population size in the two sites.

Healthcare Utilization and Attitudes Surveys (HUAS)

HUAS will be conducted prior to recruiting cases and controls at the two sites, which will serve three purposes, 1) to set up a sampling frame for the case-control study; 2) to select SHs in which prospective enrolment of cases will be conducted; and 3) to provide estimates of the population coverage for our SHs and healthcare seeking behavior weights applied in estimating pertussis incidence in community.

In summary, a population-based cross-sectional study, with an age-stratified sample of 3,000 children aged 0-59 mo and 6,000 adolescents/adults aged ≥5 years, will be conducted in the community of the two sites. The sample size was calculated based on: i) for children, a monthly prevalence of cough illness, π=1% (estimated from the reported incidence of lower respiratory tract infection of 0.15 per child year[26]), allowable error (δ=0.5%), significant level (α=0.05), and design effect (deff=2); ii) and for adolescents/adults, a monthly prevalence of cough illness, π=3.3%[27], allowable error (δ=0.66%), significant level (α=0.05), and design effect (deff=2).

A complex sampling method will be used to select survey respondents as follows. Firstly, a probability proportionate to size sampling will be used to randomly select 50 clusters (e.g. communities or villages) in the site's administrative regions. At the second sampling stage in selected communities, quota sampling will be used to recruit interviewee. The quota required in each age stratum was calculated based on the age distribution of the population in the sites and the number of surveys allocated to each cluster. Trained work staff will go to the selected communities to conduct face-to-face surveys at several locations (residential areas, kindergartens and children's vaccination clinics) Monday to Sunday during daytime in the study period. All residents living in the communities or villages for at least half a year prior to survey are eligible for and invited to participate in the interview. After the quota required in each age group is complete, the interviews will stop.

The following questions (Supplementary table 1 & table 2) are asked to respondents, 1) the occurrence and length of cough illness in the previous month prior to survey, 2) healthcare-seeking behavior regarding the self-reported cough illness for the most recent episodes and the sources of healthcare facilities; and 3) the willingness to seek healthcare and where would they choose to visit for an assumptive cough illness.

Based on the HUAS and census data, hospitals at which over 80% of respondents in each site choose to attend when hospital admission is required will be selected as our SHs. In case healthcare providers in the site change their practice or scope of service during our study period, for example the opening of new hospitals or the establishment of new branches of existing hospitals, an abbreviated HUAS with a smaller sample of 1,000 will be administered at the middle or the end of the year during which cases are recruiting at SHs.

Case-control study

Case definition of suspected pertussis

Patients will be classified as suspected pertussis cases and offered to participate if they present chronic/persistent cough defined as cough of ≥2 weeks duration with one or more of the following symptoms, 1) paroxysmal cough; 2) inspiratory whoop; or 3) post-tussive vomiting; Or, for children aged <1 years-old, cough (regardless of cough

duration) accompanied by one or more of the following symptoms, 1) apnea; 2) paroxysmal cough; 3) inspiratory whoop; or 4) post-tussive vomiting.

We will exclude patients presenting with gastroesophageal reflux, spastic bronchitis, and clearly diagnosed tuberculosis, mycoplasma/chlamydia infection, or chronic sinusitis. Adults/adolescents with a measured body temperature of ≥38.5 °C at enrolment will also be excluded.

Sample Size Considerations

We planned to enroll approximately 250 suspected cases and 2 matched controls for each case in each age stratum (i.e. children under 5 years, and adolescents/adults aged ≥5 years) for laboratory investigation at each site, which would add up to approximately 1000 suspected cases and 2000 controls at the two sites. We calculated the above sample size based on a prevalence of *B. pertussis* in chronic/persistent cough of 20% (range=12%-32%) [28-30], an allowable error of 5% and a significant level of 0.05. This sample size would have a 90% power (two sided α =0.05) to detect an odds ratio (OR) of 2 between case and control for a site and age stratum-specific comparison, if the true prevalence of *B. pertussis* is 20% in case; or an OR of 3, if the true prevalence is 10%. Although the carrier state of *B. pe*rtussis is transient in family contacts[31 32], *B. pertussis* is rarely identified in healthy people [33 34], and we expected a larger OR of ≥ 2 in the study. This sample size means that the laboratory would process average 115 samples per week, which is feasible and acceptable for our laboratories.

Case Registry, Ascertainment and Enrollment

Case registry, ascertainment and enrollment for suspected case will be conducted in SHs during the study period. Clinicians or trained nurses working in selected departments of the SHs (i.e. respiratory, pediatric, infectious disease, and emergency department) will carry out case registry of suspected pertussis cases every weekday (i.e. Monday through Sunday) except national holidays. Each outpatient visits and new hospital admission seeking healthcare in above departments will be screened for the eligibility of inclusion using the inclusion & exclusion criteria of the suspected case definition of pertussis by clinicians. Eligible ones will be ascertained and recorded as suspected case by study coordinator who assist with clinicians in SHs in enrolling cases

using a standardized case reporting form (CRF) (Supplementary Table 3). Among the suspected pertussis case recorded in SHs, convenient sampling method will be used to recruit cases for case-control study. We aim to enroll all hospital admissions and the first 1-3 outpatient visits each week in each hospital. After obtaining informed consent, study staff will conduct enrollment interviews, and collect nasopharyngeal (N/P) and blood specimens for each enrolled case.

Controls selection

At the middle of the study year when the sample size of cases reaches a half of the total (i.e. n=500), a control is recruited in community of the study sites using approximate frequency matching, based on the following criteria, 1) similar proportion in sex strata; 2) similar proportion in age strata, i.e. <1 year, 1-5 years, 6-19 years, 20-64 years and ≥65 years; 3) a control/case ratio of 2:1; and 4) no cough, running nose, shortness of breath, dyspnea or other respiratory symptoms at enrolment nor have a record of healthcare for respiratory disease in previous three months before recruitment.

60-day follow-up of case

We will follow cases from the time of enrollment to a maximum time period of 60 days after enrollment. Follow-up will be conducted at 2nd, 4th and 8th weeks after enrollment, with face-to-face interview if patient is currently hospitalized, or one telephone call each follow-up time if patient is discharged from hospital. At each follow-up visit/phone call, the study staff will ask about cough or other respiratory or systemic illness symptoms in the period since the last contact. If case is still symptomatic (i.e. cough) during follow-up, they will be encouraged to visit their doctor who enrolled them in the SHs within 24h of contact. The doctor will checkup the patient's health status and collect the swab and serum samples during the visit. If an enrolled patient does not want to visit the SHs, the study staff will arrange a household visit to collect the samples in the home.

Data collection from cases and controls

At enrolment, trained clinicians and the study coordinator will conduct face-to-face interview to collect socio-demographic, clinical and epidemiological data from cases

and controls using a standardized CRF (Supplementary Table 4). Demographic information includes household size (defined as a group of people who share a dinner table), average household income, rural or urban residence, age, alcohol consumption and smoking exposure, and occupation etc. A clinician will also examine all cases to document clinical signs and symptoms at enrollment, including cough characteristics [duration, paroxysms, post-tussive vomiting, exacerbation at night], body temperature, respiratory rate, heart rate, seizure, apnea, and other general respiratory symptoms, non-prescription antibiotic usage before visiting the doctor, blood test results and chest x-ray examinations. Vaccination history (i.e. band, dosing, procedure and time of administration) of children aged ≤14 years is also collected by linkage of his/her individual records on immunization in the national database (Childhood Immunization Information Management System, CIIMS)[35] or checking of vaccination certificate.

During follow-up visits, data on any current cough or respiratory symptoms, subjective severity of illness, illness duration, functional impairment, whether medical care was sought, and outcomes since the last visits will be collected using CRFs (Supplementary Table 5).

At the end of follow-up, medical charts of each hospitalized case will be reviewed by study staff to collect information on antibiotic treatment and outcomes during hospitalization (i.e. mechanical ventilation, ICU transfer, and death) (Supplementary Table 6).

The retrieval of electronic medical records and Validation of the total number of suspected pertussis case

Since our case registry and enrolment is conducted in selective departments (i.e. respiratory, pediatric, infectious disease and emergency departments) and on workdays in SHs, it is an incomplete record of the total number of suspected cases encountered in the whole hospital. It is essential to calibrated the registered number of suspected cases to equal the total. To do this, all hospital discharges or ambulatory visits coded for diagnosis under the International Classification of Diseases 10th Revision (ICD-10) codes A37, J00-J22, J40-J47, R05, R09.2, P22, P28.2, P28.3, P28.4, and P28.5 will be monitored on a daily basis as registry case, by hospital departments.

At the end of the month, the complete EMRs records with the above diagnosis codes in the whole hospital will be abstracted from hospital information system (HIS) of the SHs. This data will be used to calibrate the prospectively counting data of suspected case in the selective departments that conduct case enrolment to make a precise estimate of the total number of chronic/persistent cough illness outcomes in the studied population. Namely, through linking and comparing between the number of registry cases and the number of suspected pertussis case registered in the selected departments, we will calculate the W_{case}. With this W_{case}, we will narrow down the ICD-based EMRs records to the total number of suspected pertussis cases met our case definition in SHs (i.e. the numerator of incidence).

Laboratory investigation

Specimen collection and transport

When patients meet our suspected pertussis case definition or are recruited controls, they, as well as symptomatic (cough) cases during follow-up contacts, will be sampled within 24 hours. Clinicians or nurses in SHs will be trained to collect nasopharyngeal swabs (N/P) and whole blood sample. Dacron or nylon swab will be used to collect N/P specimen to facilitate culture and PCR tests for *B. pertussis*[36]. Collected swab specimens will be plated onto selective agar or placed in transport medium (Charcoal Agar, Thermo Fisher Scientific Inc.) immediately after sampling at the SHs. Whole blood without adding any anticoagulants (>4ml for participants aged 5 years and older, and ≥2 ml for children aged <5 years) will be collected, and centrifuged to separate serum within 24h of collection. All collected swab and sera samples will be transported to the central laboratory of Chinese Center for Disease Control and Prevention (China CDC), using a cold box to maintain a temperature of 4°C. During transportation, samples are packaged and transported in accordance with the provision of International Civil Aviation Organization (ICAO) document Doc9284 and UN3373

Processing and storage of specimen

Upon arrival at the laboratory of China CDC, swab samples will be processed and prepared into three aliquots of swab supernatant, so will serum samples be. One of these aliquots will be analyzed and the other two aliquots will be kept for future

analyses. All aliquots will be stored at -70°C temperature until the time of analysis.

Laboratory testing

In the laboratory of China CDC, Charcoal Agars will be cultured to isolate *B. pertussis* using standard method recommended by China CDC[37] and World Health Organization[38]. Swab supernatant will be analyzed for *B. pertussis, B. parapertussis, B. bronchiseptica* and *B. holmesii* using polymerase chain reaction (PCR) as recommended by US CDC [39 40]. Sera samples that have a minimum volume of ≥ 1 ml will be tested for Anti-Ptx IgG titer using a commercially available diagnostic kit (Virion\Serion, Wurzburg, Germany) according to the manufacturer's recommendations. To validate our laboratory methods and testing results, external quality assurance testing will be conducted to reach agreements with a reference laboratory on *Bordetellae* prior to study start. For serology testing, we use standard from the National Institute for Biological Standards and Control, London, UK, (*https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=18/146*); and for PCR assays, the Wisconsin State Laboratory of Hygiene, Wisconsin, U.S. (*http://www.slh.wisc.edu/proficiency/training-and-competency/*).

Suspected pertussis cases and controls that have *B. pertussi*s Isolated, positive tests of swabs in any of samples collected during enrolment and follow-up, or for persons three years of age and over have a 3-fold or greater rise in anti-Ptx IgG antibody between sequential sera samples with at least one time point higher than 40 IU/ml of serum titer would be considered laboratory-confirmed pertussis.[36 41]

Data flow, management and analysis

The data collected in the study are centrally managed at China CDC, using an online data platform (*http://eddc.chinacdc.cn/dap/*). The completed CRFs will be entered into the information system by local study staff at the two sites and uploaded to data server through encrypted transmission via a Virtual Private Network set up by China CDC. The entered records are regularly checked for completeness, consistency, and logical errors by data manager and the site's co-principle investigator who is responsible for authorization, integrity, security, and backup of database during data collection.

Statistical analysis

The collected data processing and key indicators based on which we calculate incidence are shown in figure 4. We will calculate the incidence of pertussis by age group and by settings with the following formula.

$$Hospitalization\ \ incidence\ rate = \sum \frac{S_i^{inpatient} \times W_i^{case} \times AF_i}{N_i \times W_i^{cover} \times C_i}$$

$$Outpatient\ \ incidence\ rate = \sum \frac{S_i^{outpatient} \times W_i^{case} \times AF_i}{N_i \times W_i^{cover} \times C_i}$$

$$Community\ incidence\ rate = \frac{Outpatient\ \ incidence\ rate}{r_i}$$

Where, $S_i^{inpatient}$ and $S_i^{outpatient}$ indicates the registered number of inpatients and outpatient visits of cough illnesses at age group i, as obtained from HIS. W_i^{case} is the weight used to adjust $S_i^{inpatient}$ and $S_i^{outpatient}$ to meet our case definition in age group i. This weight is calculated from the results of the prospective case-control study as a ratio of suspected cases over registered cases of cough illnesses at the selective departments of SHs. N_i is the population size in age group i in census year 2020. W_i^{cover} is the weight used to adjust catchment population overlapping between participating hospitals from HUAS in age group i. It is calculated as the ratio of community residents who have the reported seeking medical care in the participating hospitals for the last episodes of their cough illness over the residents who have the willingness of healthcare-seeking in the participating hospitals, as obtained from the HUAS study. C_i is the proportion of population covered by participating hospitals in age group i, as measured in the HUAS study. It is calculated as the proportion of residents who report having the willingness of healthcare-seeking in the participating hospitals over the total no. of residents responded. r_i is the proportion of community residents reporting seeking health-care for their most recent episode of cough illnesses in age group i as measured in the HUAS study. AF_i is the population attributable fraction of chronic/persistent cough due to *B. pertussis* infection in age group i, calculated based on case-control study using unconditional logistic regression

model, as follows:

$$\log_e(OR) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_k x_k$$

$$\mathrm{OR} = \exp(\beta_k)$$

$$\mathrm{AF_i} = \Pr(\textit{Bordetella pertussis} \mid \text{Chronic cough})\ (1 - \frac{1}{OR})$$

Note: Pr(*Bordetella pertussis*|Chronic cough) = P_i is the prevalence of *B. pertussis*, calculated by dividing the number of laboratory-confirmed pertussis with the total number of chronic/persist cough tested. x_1, x_2, x_3, …, x_k are variables associated with the occurrence of chronic/persistent cough, including the presence of *B. pertussis* and other social and environmental factors significant at p < 0.1 in univariate analysis. OR is the odds ratio.

The 95% CI of incidence is calculated with bootstrap method with 1000 replications. Besides incidence estimates, we will also explore factors associated with severe pertussis (defined as a composite outcome of death, sepsis, invasive ventilation and Intensive Care Unit transfer), by using multivariable logistic regression. Factors significantly associated with severe pertussis at p < 0.1 in univariate analysis will be included in the model. The median age of children with pertussis will be calculated by type of vaccinees, and factors predicting the age of pertussis breakthrough among children who had received DTP vaccination early in their life will be also studied by using Cox proportional hazards regression models. For sensitivity analysis, we will use a two-fold or greater increase of anti-Ptx IgG antibody as the cut-off threshold for our serological assays and calculate incidence again.

ETHICS AND DISSEMINATION

This study is designed an observational study. The risk of harm is minimal and adverse medical events are not anticipated from the procedures involved in the study. The study protocol, CRF, and consent form have been sent to and approved by China CDC's Institutional Review Board (reference no. ICDC-202110).

The primary risk to participants is the loss of confidentiality. To help maintain

confidentiality, all study investigators will sign a confidentiality agreement and receive appropriate ethics training. All interviews will be conducted at the study investigator's office, and signed consent forms and completed survey forms will be locked in a secure file cabinet at the end of each day. A very limited number of trained study staff can have the key to the locked file cabinets. Participation in every aspect of the study will be voluntary, and for all new data collection, participants will be asked to provide written informed consent. Besides, collection of specimens may cause mild discomfort to the subject during the procedure, especially drawing blood from young children. To minimize invasive procedures during sample collection, swab and blood specimens will be collected by aseptic technique and we encourage the use of leftover sera during routine medical care at the time point of enrolment.

As a benefit of participating in the study, participants with pertussis will receive senior doctor consultation during treatment on how to limit transmissions among family members and co-workmates; Patients enrolled in the study will have access to antibiotic susceptibility testing results should they have *B. pertussis* isolates acquired. This will give a guide on empirical antibiotic usages for physicians; moreover, the data generated in the study will be valuable to determine the burden of pertussis and explore risk factors for illness attributable to severe pertussis in children as well as adolescents/adults, which can be used by public health departments, healthcare providers and scientific group in China to inform policies making, implement disease control and prevention (i.e. vaccination) and improve patient care, both at the sites level and national level. In general, the minimal risks associated with physical discomfort during blood and N/P sample collection are offset by the great benefit associated with the study's ability to inform pertussis prevention and control strategies in China. Upon completion, results from this study will be disseminated via academic presentations and publication in peer-reviewed journals.

DISCUSSION

PertussisChina is an innovative and a pilot of a laboratory-based and population-based active surveillance platform for vaccine-preventable bacterial diseases (VPBD) in China, which endeavors to establish a network of laboratories and hospitals using

comparable and unified standards to provide up-to-date disease burden estimates and disease determinants for evaluating, prioritizing and optimizing the use of vaccines and for the development of new interventions against bacterial infections in the country. Pertussis is the first one of the several bacterial infections that we are planning to take this approach. In response to the changing epidemiology of pertussis in China[7 8 42 43], the 2019 summon of the National Immunization Advisory Committee submitted a motion to its members urging the modification of the current immunization schedule of pertussis vaccine administered at 3, 4, 5 and 18-24 months[44], to vaccinate children at 2, 4, 6 and 18-24 months instead and to add a 5th booster dose at 4-6 years of age; however, partly due to knowledge gaps existed in age-specific burden of pertussis, NIAC suspended its decision on this issue. To provide up-to-date evidence on disease burden of pertussis, this study will focus on age-specific incidence based on laboratory confirmation and will fill the data gaps on prospectively and actively collected incidence data and key information on illness severity and outcomes. We are expecting that data from this study can be served as background information augmenting NIDSS to inform NIAC's recommendations on children vaccination and further quantify the benefit of adolescent/adult vaccination to protect infants from severe outcomes in future. There are several strengths of the study.

In this one-year study, we will enroll suspected chronic/persistent cough patients (for infants aged less than 1 year, cough regardless of duration) from health care facilities in two sites of China, covering a censused population of 2.23 million. The catchment population utilizing health-care services at the SHs are well characterized and defined by HUAS, providing unbiased estimates of age-stratified total person-times observed in the cohort. The prevalence of cough in regarding of illness duration and proportion of people who do not seek healthcare are measured retrospectively by HUAS. Thus by comparing between data generated from HUAS in community and case registry in SHs, we will able to measure incidence by settings (i.e. community, outpatient and inpatient), especially those in communities whose symptoms are mild or atypical after the waning of vaccine-induced immunity or those no healthcare are sought[2]. Besides, all hospitalizations suspected of pertussis will be actively searched and prospectively enrolled in a timely manner in our SHs, serving as a complete and representative

sample of pertussis occurred in the interested population that would have induced minimal selection bias. As for milder cases in ambulatory settings, sampling of patients with chronic/persistent cough in outpatient setting to conduct laboratory investigation is preferred. Misclassification of cases or recall bias will be minimized by the complex laboratory procedures (i.e. culture, PCR, and serology combined), unified data collection tools (i.e. CRFs) and data collection process, i.e. the 60-day of follow-up during which interesting events (e.g. 3-fold titer raising) will be closely monitored by sequential sera samples. Using laboratory-confirmed pertussis as the outcome will allow us to specifically measure pertussis disease burden. To account for asymptomatic carriage of *B. pertussis*, we will recruit healthy control to investigate the proportion of population carrying *B. pertussis* in their upper respiratory tract and sero-positivity, which could be useful for calculating population attributable fraction (AF) to adjust rate estimates. In addition, the prospective cohort will provide valuable follow-up data related to risk factors for severe illness (i.e. adverse clinical outcomes of hospitalization or death). Collection of the vaccination history (including band, dosing, procedure and time of administration) from study participants will help explore the breakthrough rates of *B. pertussis* infection among different type of vaccinees and investigate reasons of vaccination failure, by linkage of study subjects ≤14 years old with his/her individual records on immunization in the national database. Finally, we will abstract EMR data from hospital information system, which serves as a complete and accurate record of cough illness outcomes occurred in SHs. The retrospectively collected EMR data will be validated by prospectively counting cases eligible for inclusion at selective departments of SHs on daily basis. Using data from the EMR will allow us to determine the size of outpatient and emergency department visits for cough illness in the studied population. For most of adults and fully immunized children and adolescents, their illness is generally mild and is most likely to be encountered at the ambulatory settings in which the diagnostic capacity is generally lacking.

Aside from acquiring incidence estimates, the prevalence and distribution of *B. pertussis* strains circulating in the population will be determined and characterized, which are reported to be evolving under the selection pressure from both vaccine and

antibiotics in previous studies [45] and are important data for the development of novel vaccine or new therapeutics in the country. For example, as a benefit of the study, we will create a representative national and well characterized repository of strains and specimens that can be shared with other investigators for future research, the main antigenic and genotypic features of *B. pertussis* will be characterized by sequencing or other bio-molecular methods.

We realized that there are several limitations worthy of note in our study. Firstly, we will not identify all pertussis that occur in our studied population since our case definition will not capture atypical and asymptomatic manifestations associated with *B. pertussis* infection. For example, previous studies showed that about 17.4% children[46] and 20% adolescents/adults[47] with *B. pertussis* infection had a cough duration less than 3 weeks, and other symptoms/signs used in the case definition, like spasmodic cough (63%), post-tussive vomiting (42%) and whoops (8%), were infrequently presented in adults[48], which will make incidence underestimated. It is argued that no symptom is sufficiently predictive for diagnosing pertussis[49] and there was no case definition that has been proposed for purpose of studying disease burden of pertussis. After balancing at the sensitivity and specificity of case definition commonly recommended by WHO, the U.S. and others[50-52] and the available laboratory capacity and resources in the study, we finally adopted the current case definition that can be used to facilitate comparison of results between studies and countries. Second, our study period is a little short. Since pertussis has showed a cyclic pattern and peaked every 3-5 years[2 16], our study will not capture this feature. Moreover, our study is going to recruit cases in 2021-2022, right after COVID-19 pandemic. As the epidemiology of many respiratory infections have been reported changing as a result of widely implementation of nonpharmaceutical interventions (e.g. wearing masks, social distancing, and personal health protection)[53 54] and the detained coverage of vaccines used in Expanded Program on Immunization during the pandemic[55]. The impacts of COVID-19 outbreak on incidence estimates of pertussis are not foreseeable in the study. Future studies are upcoming depending on the results of this pilot. Finally, China is a big country with large variations in population density and across different climate, geographic and economic regions. Although we

have paid careful attention to variables, like DTP3 vaccine coverage, childhood mortality and health-care delivery pattern when selecting study sites, regions with the highest and lowest reported incidence of pertussis are generally not included. This may also influence the generalizability of the incidence estimates to extrapolate to other regions.

In summary, PertussisChina is an innovative study that uses unified protocol to generate up-to-date high-quality incidence data on pertussis. The study design can secure the precision of data collection and provide insights into the prospectively conducted studies that designed to augment passive surveillance in countries where resources is limited and data is currently lacking. When completed, the results coming out this study will provide valuable scientific data on the incidence, etiology, and risk factors for severe sequelae of pertussis to academic societies and the public health authorities, who is currently struggling and fighting against this burdensome disease worldwide.

Contributors

Zhujun Shao is the principal investigator on this study who conceived and critically revised the manuscript. Jianxing Yu, Hanqing He and Yanyang Zhang conceptualized and designed the study, wrote the first draft and contributed equally to this work. Yuan Gao, Juan Xu, Li Xu, and Yonghao Guo designed the laboratory methods. Xiaoxiao Zhang, Qianqian Zhou, Yao Zhu and Xuewen Tang wrote the statistical analysis plan. Chuanwei Chen and Zhiping Chen commented on and revised drafts of the manuscript. All authors contributed to reviewing, revising, and approving the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication

Not required.

Ethics approval

This study has been approved by Chinese Center for Disease Control and Prevention's Institutional Review Board (reference no. ICDC-202110).

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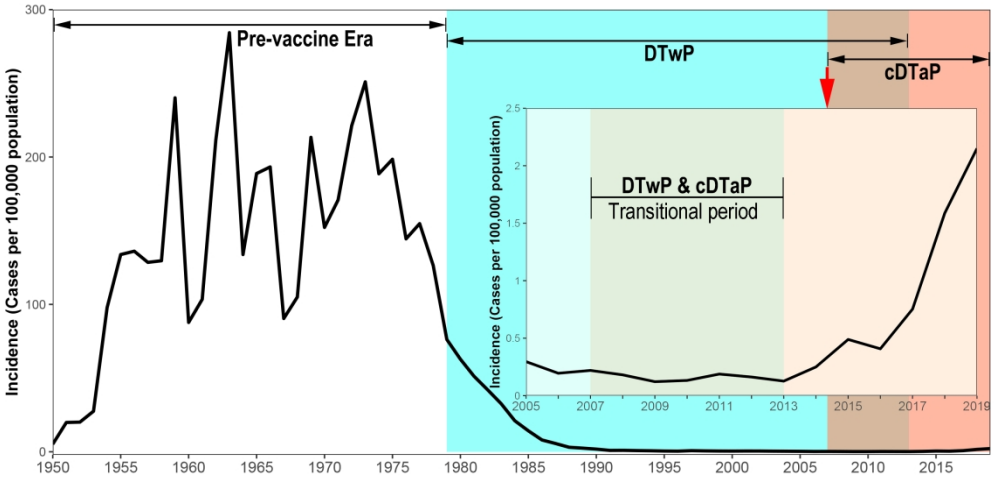
Figure Legends

Figure 1. Incidence of reported pertussis from NNIDSS, China, 1952-2019. A cDTaP was introduced into national immunization program to replace DTwP in 2007 and the transition was fully completed in 2013. Abbreviations: DTwP, combined diphtheria, tetanus toxoid and whole-cell pertussis vaccine; cDTaP, co-purified diphtheria, tetanus toxoid and acellular pertussis vaccine; National Notifiable Infectious Disease Surveillance System (NNIDSS).

Figure 2. Location and population size of study sites included in PertussisChina study

Figure 3. Flow diagram of major study activities

Figure 4. Data flow chart and key indicators used to calculate incidence of pertussis



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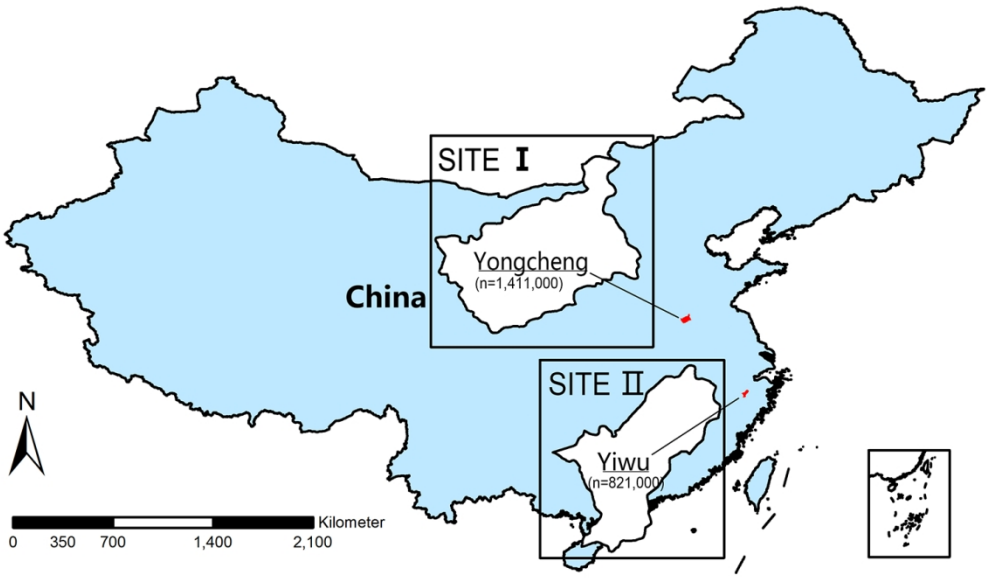

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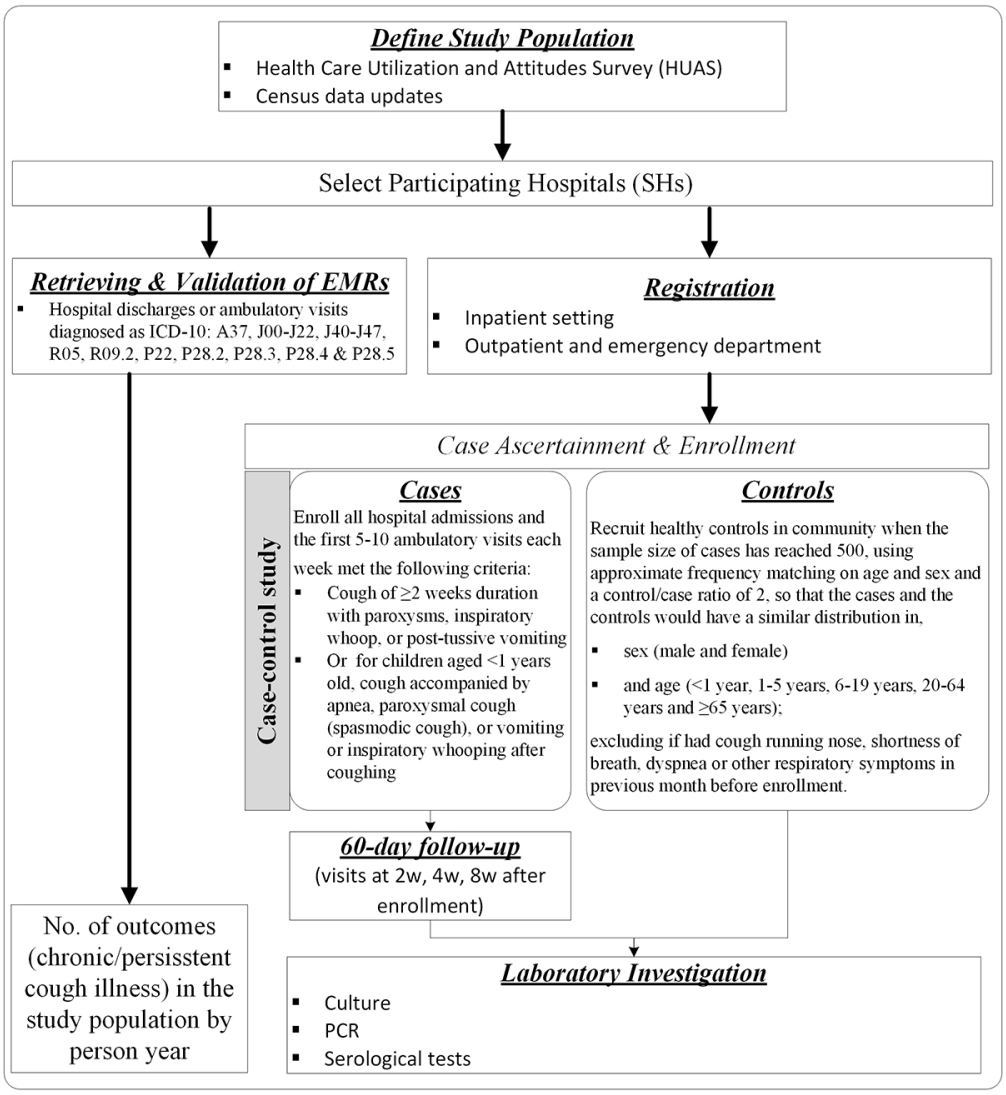

Figure 3. Flow diagram of major study activities

101x110mm (300 x 300 DPI)

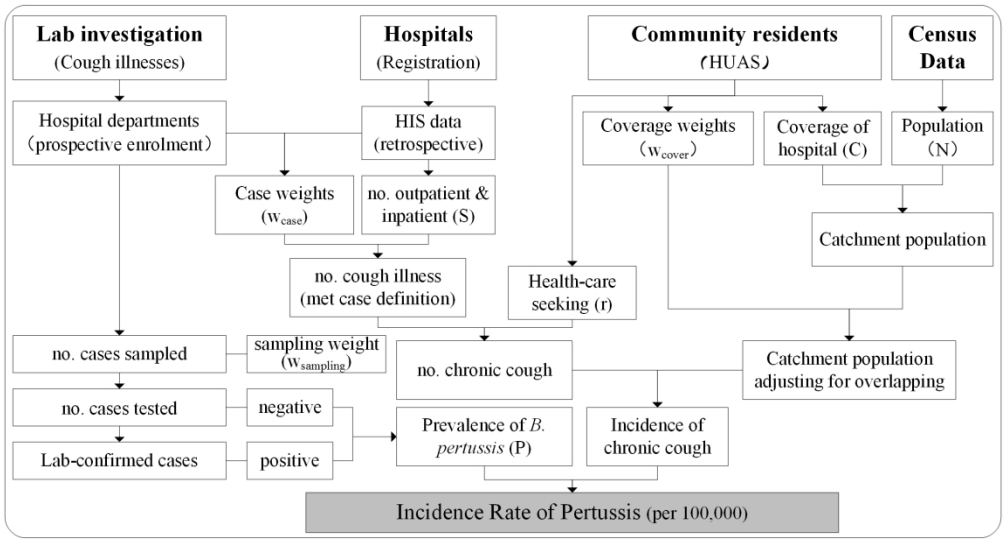

Figure 4. Data flow chart and key indicators used to calculate incidence of pertussis

187x100mm (300 x 300 DPI)

Supplementary Appendix

Title: Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study

Running head: PertussisChina Study, 2020

Tables & Forms

- Supplementary Table 1. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among children under 5 years old
- Supplementary Table 2. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among persons aged 5 years and older
- Supplementary Table 3. Case screening and ascertainment form
- Supplementary Table 4. Baseline information of case & control (CRF—T0)
- Supplementary Table 5. Follow-up information of case (CRF—T2w/T4w/T8w)
- Supplementary Table 6. Outcome of case at the end of follow-up (CRF—Tend)

Supplementary Table 1. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among children under 5 years old

Greetings! We are the staff of Center for Disease Control and Prevention. We sincerely invite you to participate in this questionnaire survey. The purpose of the survey is to study the utilization and attitudes of community residents towards health-care services of chronic/persistent cough, so that we can better serve you in the future.

This survey is anonymous. Your answers to these questions are kept confidential. You can choose whether to participate in the survey voluntarily or not. Refusal of the survey will not have any adverse consequences on you personally and your children. If you agree to the participate, please read each question carefully and fill in the answer on your own. Thank you for your support and cooperation!

Part I. Basic Information

1.1 Site:_____ Site ID:_____

1.2 Your length of time (years & months) living in the site (e.g. Yiwu or Yongcheng):

□ less than 6 months □six months and over

1.3 Current address: _____ county _____ street _____community/village

1.4 Type of respondents in relation to the studied subject/children:

□ mother □ father □ grandma/grandpa □ others_____

1.5 Date of Birth: □□□□/□□/□□ (yyyy/MM/dd)

1.6 Gender: □ Male □ Female

1.7 Ethnicity: □ Han □ others_____

1.8 Did your child attend school? □ yes □ no

1.9 Your occupation (of the respondent who answered the question):

□students □housework or unemployed □retired people □commercial & service sector workers □ food handler or employees of food industry □specialists, including teacher, medical personnel and workers □agriculture, forestry, animal husbandry and fishery workers □ others_____

1.10 Your educational attainment (of the respondent who answered the question).

□primary school or illiteracy □middle school □high school

□technical secondary school □college level and above

1.11 Including yourself, there are_____members in your family (defined as those who shared the same dining table in the house)?

Of which, there are_____children under five years old.

1.12 Is there any smokers or ex-smokers in your family? □ yes □ no

Part II. self-perceived illness and health-care seeking behavior

2.1 Did your children experienced cough during the past one month prior to our interview? □ no □ yes

2.1.1 If yes, how long did the cough last?

□ <1 week □ 1-2 weeks □ 3-4 weeks □ 5-8 weeks □ >8 weeks

2.1.2 If yes, what is the clinical characteristics of the last episode of cough?

□paroxysmal cough □vomiting after coughing □whooping cough

□cough worsening during the night □vomiting after coughing

□productive cough with large amount of sputum □dry cough

□cough with blood in sputum □others_____

2.1.3 If yes, what is the other concomitant symptoms?

□productive cough □running nose □fever (body temperature ≥ 37.2°C)

□belching □acid reflux □irritable and crying □vomiting □headache

□tachypnea □earache □sore throat □dyspnea □abdominal pain

□arthralgia □chest pain □myalgia □fatigue □lethargy

□burn after sternum □without any other discomfort □others_____

2.1.4 If yes, what do you think is the most probable cause of your cough?

□respiratory tract infection □inhalation of foreign objects in the respiratory tract

□COPD exacerbation □asthma exacerbation □recurrent tuberculosis

□chronic cardiopulmonary disease □lung cancer □inhalation of cold air

□chronic bronchitis □bronchiectasis □I don't know □others_____

2.2 Did your child visit a doctor or seek healthcare during the last episode of cough?

□ no □ yes

2.2.1 If yes, where did your child see a doctor?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

□Chouzhou Hospital of Yiwu □The Second People's Hospital of Yiwu

□The Third People's Hospital of Yiwu □Yiting township Health Center

□Suxi Township Health Center □Beiyuan Community Health Center

□Shangxi Township Health Center □Dachen Township Health Center

□Houzhai Community Health Center □Chi'an Township Health Center

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□Yiwu Huashan Rehabilitation Hospital □Jiangdong Community Health Center

□Futian Community Health Center □Yiwu Dermatology Hospital

□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

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□Yongcheng People's Hospital □Yongcheng Central Hospital

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□Yongcheng Maternal & Child Health Hospital □Yucheng Township Health Center

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2.2.1 If no, why did not your child see a doctor?

□Symptoms are mild, no need to see a doctor

□The hospital is too far from home and the transportation is inconvenient

□Drugs purchased in pharmacies □Distrust the doctor

□Unaffordable high medical expenses

□Hospital facilities and environment were poor

□others_____

2.3 Was your child hospitalized for the last episode of cough? □ no □ yes

2.3.1 If yes, where was your child hospitalized?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

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□Mangshan Township Health Center □ Luanhu Township Health Center

□others

Part III. Attitudes towards health-care utilization

(Next, we will ask some questions about the actions you might take under some hypothetical situations that do not need to happen.)

3.1 If your child keeps coughing for 2 weeks but does not get better, and you have decided to see a doctor, which one of the following medical institutions would you choose to go?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

3.2 If the doctor recommends that your child be hospitalized, which one of the following medical institutions would you choose?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

Part IV. Other questions

4.1 Has your child ever received the following vaccines?

□influenza vaccine □pneumococcal vaccine □Haemophilus influenzae vaccine

□Vaccines containing pertussis components (i.e. DTP)

4.1.1 If received vaccines containing pertussis (i.e. DTP), what kind of the vaccine?

□cDTaP □ DTaP/Hib □ DTaP-IPV/Hib

4.2 Your family's average annual income (Chinese Yuan) is,

□<50,000 □50,000-90,000 □100,000-190,000 □200,000-490,000 □≥500,000

4.3 Your phone number is_____

Thank you very much for taking your time. The information you provided in this interview is very valuable to help us improve our work. Wish you a happy life!

Time of survey started: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Time of survey ended: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Investigator: _____ Supervisor: _____

Supplementary Table 2. Survey questionnaire for healthcare utilization and attitudes survey of cough illness among persons aged 5 years and older

Greetings! We are the staff of Center for Disease Control and Prevention. We sincerely invite you to participate in this questionnaire survey. The purpose of the survey is to study the utilization and attitudes of community residents towards health-care services of chronic/persistent cough, so that we can better serve you in the future.

This survey is anonymous. Your answers to these questions are kept confidential. You can choose whether to participate in the survey voluntarily or not. Refusal of the survey will not have any adverse consequences on you personally and your children. If you agree to the participate, please read each question carefully and fill in the answer on your own. Thank you for your support and cooperation!

Part I. Basic Information

1.1 Site:_____ Site ID:_____

1.2 Your length of time (years & months) living in the site (e.g. Yiwu or Yongcheng):

□ less than 6 months □six months and over

1.3 Current address: _____ county _____ street _____ community/village

1.4 Type of respondents in relation to the studied subject:

□respondent himself is the study subject □ others_____

1.5 Date of Birth: □□□□/□□/□□ (yyyy/MM/dd)

1.6 Gender: □ Male □ Female

1.7 Ethnicity: □ Han □ others_____

1.8 Your occupation:

□students □housework or unemployed □retired people □commercial & service sector workers □ food handler or employees of food industry □specialists, including teacher, medical personnel and workers □agriculture, forestry, animal husbandry and fishery workers □ others_____

1.9 Did you ever contact with dust/chemical materials in the working environment in the past one year, such as those encountered by workers using pneumatic drills at

construction sites, miners, painters, benzene solvents in leather production, etc.

□ no □ yes

1.10 Your educational attainment:

□primary school or illiteracy □middle school □high school

□technical secondary school □college level and above

1.11 Including yourself, there are_____members in your family (defined as those who shared the same dining table in the house)?

Of which, there are_____children under five years old.

1.12 Are you smoker or ex-smoker? □ yes □ no

Part II. self-perceived illness and health-care seeking behavior

2.1 Did you experienced cough during the past one month prior to our interview?

□ no □ yes

2.1.1 If yes, how long did the cough last?

□ <1 week □ 1-2 weeks □ 3-4 weeks □ 5-8 weeks □ >8 weeks

2.1.2 If yes, what is the clinical characteristics of the last episode of cough?

□paroxysmal cough □vomiting after coughing □whooping cough

□cough worsening during the night □vomiting after coughing

□productive cough with large amount of sputum □dry cough

□cough with blood in sputum □others_____

2.1.3 If yes, what is the other concomitant symptoms?

□productive cough □running nose □fever (body temperature ≥ 37.2°C)

□belching □acid reflux □irritable and crying □vomiting □headache

□tachypnea □earache □sore throat □dyspnea □abdominal pain

□arthralgia □chest pain □myalgia □fatigue □lethargy

□burn after sternum □without any other discomfort □others_____

2.1.4 If yes, what do you think is the most probable cause of your cough?

□respiratory tract infection □inhalation of foreign objects in the respiratory tract

□COPD exacerbation □asthma exacerbation □recurrent tuberculosis

□chronic cardiopulmonary disease □lung cancer □inhalation of cold air

□chronic bronchitis □bronchiectasis □I don't know □others_____

2.2 Did you see a doctor or seek healthcare during the last episode of cough?

□ no □ yes

2.2.1 If yes, where did you see a doctor?

(For Yiwu site, please select the following)

□The Fourth Affiliated Hospital Zhejiang University School of Medicine

□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

□Yiwu Central Hospital □Yiwu Traditional Chinese Medicine Hospital

□Yiwu Tianxiang Medical Group Dongfang Hospital

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□Zhejiang Children's Hospital □Village clinics or private clinics

□others_____

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

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□others_____

2.2.1 If no, why did not you see a doctor?

□Symptoms are mild, no need to see a doctor

□The hospital is too far from home and the transportation is inconvenient

□Drugs purchased in pharmacies □Distrust the doctor

□Unaffordable high medical expenses

□Hospital facilities and environment were poor

□others_____

2.3 Were you hospitalized for the last episode of cough? □ no □ yes

2.3.1 If yes, where were you hospitalized?

(For Yiwu site, please select the following)

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□others_____

Part III. Attitudes towards health-care utilization

(Next, we will ask some questions about the actions you might take under some hypothetical situations that do not need to happen.)

3.1 If you keep coughing for 2 weeks but does not get better, and you have decided to see a doctor, which one of the following medical institutions would you choose to go?

(For Yiwu site, please select the following)

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□Zhongyuan Road Community Health Center □ Yongcheng Wuguanke Hospital

□Mangshan Township Health Center □ Luanhu Township Health Center

□others_____

3.2 If the doctor recommends that you should be hospitalized, which one of the following medical institutions would you choose?

(For Yiwu site, please select the following)

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□Yiwu Fuyuan Hospital □Yiwu Maternal and Children's Hospital

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□others_____

Part IV. Other questions

4.1 Have you ever received the following vaccines?

□influenza vaccine □pneumococcal vaccine □Haemophilus influenzae vaccine

□Vaccines containing pertussis components (i.e. DTP)

4.1.1 If received vaccines containing pertussis (i.e. DTP), what kind of the vaccine?

□cDTaP □ DTaP/Hib □ DTaP-IPV/Hib

4.2 Your family's average annual income (Chinese Yuan) is,

□<50,000 □50,000-90,000 □100,000-190,000 □200,000-490,000 □≥500,000

4.3 Your phone number is_____

Thank you very much for taking your time. The information you provided in this interview is very valuable to help us improve our work. Wish you a happy life!

Time of survey started: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Time of survey ended: □□□□/□□/□□:□□:□□ (yyyy/MM/dd hh:mm)

Investigator: _____ Supervisor: _____

Supplementary Table 3. Case screening and ascertainment form

Name of Hospital_____ Departments_____

Name of patient			Sex	☐ male ☐female	
Birthdate			Ethnicity		
Current address			Phone number		
Date of illness onset		Date of admission			
Date of written informed consent signed					
Lists of inclusion & exclusion criteria				yes	no
Inclusion criteria:					
Patient regardless of ages:					
1.cough of ≥2 weeks duration;				☐	☐
2.had one or more of the following symptoms;				☐	☐
– paroxysmal cough;				☐	
– inspiratory whoop;				☐	
– post-tussive vomiting.				☐	
Infants less than one year old					
1.cough (regardless of cough duration);				☐	☐
2.had one or more of the following symptoms;				☐	☐
– paroxysmal cough;				☐	
– inspiratory whoop;				☐	
– post-tussive vomiting;				☐	
– apnea.				☐	
Written informed consent signed				☐	☐
If you answer "No" to any of the above, the patient cannot enter the study.					
Exclusion criteria:					
1.not a permanent resident (lived less than 6 months at the site);				☐	☐
2.gastroesophageal reflux;				☐	☐
3.spastic bronchitis;				☐	☐
4.diagnosed tuberculosis;				☐	☐
5.lung mycoplasma/chlamydia infection;				☐	☐
6.chronic sinusitis;				☐	☐
7.adults/adolescents with a measured body temperature of ≥38.5 °C;				☐	☐
8. researchers considered not suitable for participating in the study.				☐	☐
If you answer Yes" to any of the above, the patient cannot enter the study.					
Whether the patient is included in the study				☐	☐
If no, what is the reason for not included?					
– not meet the inclusion & exclusion criteria;				☐	☐
– Refuse to participate ;				☐	☐
If yes, what is the patient identifier no.? _____				☐	☐

Supplementary Table 4. Baseline information of case & control (CRF—T0)

Patient identifier no.:_____ Type: ☐Inpatient ☐outpatient ☐Control

Name：_____（or **Parents' name**：_____） **Sex：**☐ male ☐ female **Ethnicity**：☐ Han ☐ other_____

Birthdate：____/____/____(YYYY/mm/dd) **Preterm baby：**☐ yes，(gestational age_____weeks) ☐ no

Address*：_____ **mobile phone**：_____

Family member No.：_____persons **No. of children under five in the family：**_____children

Smokers in the family：☐ yes，(_____persons) ☐ no **Smoking：**☐ yes ☐ no **Drinking：**☐ yes ☐ no

Occupation：

☐kindergarten children ☐preschool children ☐students ☐teacher ☐childcares ☐food industry worker ☐commercial service

☐medical staff ☐factory workers ☐migrant worker ☐farmer ☐herdsman ☐fishermen ☐cadre ☐retired ☐housewife

☐others ☐unknown

Clinical characteristics (within 24h before and after admission)

Illness onset Date：_____(YYYY/mm/dd) **Primary diagnosis**：_____ **Diagnose date**：_____(YYYY/mm/dd)

Hospitalized：☐ yes ☐ no **Admission diagnosis**：_____ **Admission date**：_____(YYYY/mm/dd)

☐cough（Starting date____/____/____[YYYY/mm/dd]，duration_____days）

☐post-tussive vomiting ☐paroxysmal cough ☐whooping cough ☐apnea ☐cyanosis ☐fever（body temperature_____℃）

☐cough worsening in night ☐productive cough; Sputum color：yellow/white/black/glass like

☐seizure ☐hemoptysis ☐chills ☐headache ☐myalgia ☐sore throat ☐joint pain ☐chest pain

☐sweat ☐shortness-of-breath ☐running nose ☐lachrymation ☐fatigue ☐other(_____)

Complications：☐pneumonia（radiographical evidence：☐yes ☐ no）☐atelectasis ☐pulmonary hypertension

☐encephalopathy ☐seizure ☐others（_____）

Blood tests：WBC_____×10^9/L; L_____×10^9/L; N_____×10^9/L; Plt_____×10^9/L; Hb_____g/L; CRP_____mg/L; GLU_____mmol/L

Physical check：body temperature：_____℃ **Breath rate**：_____breath/min **Heart rate：**_____beats/min

Systolic/diastolic blood pressure：_____/_____mmHg **Pulse oximetry**: sPO_2 (if any):_____%

Lung auscultation：□dry rale □wet rale **Consciousness**：clear/lethargy/irritable/delirium/convulsions/coma

Treatment (one week before admission)

（1）Drug name：Please give the name of the drug, or the trade name if it is a fixed compound preparation

（2）Category: A=antibiotic (1. Amoxicillin; 2. Amoxicillin-clavulanic acid; 3. Ampicillin; 4. Azithromycin; 5. Ceftriaxone; 6. Cefuroxime; 7. Ciprofloxacin; 8. Clarithromycin; 9. Doxycycline; 10. Erythromycin; 11. Penicillin; 12. Tetracycline; 13. Compound sulfamethoxazole); B=antiviral drugs; C=steroid hormone drugs

（3）Route：1=oral, 2=intravenous injection, 3=intravenous drip, 4=intramuscular injection, 5=inhalation, 6=other

（4）Frequency：1= continuous，2=intermittent

Drug name (1)	Category (2)	Route (3)	Daily dose		Frequency (4)	Starting date (YYYY/mm/dd)	Stop date (YYYY/mm/dd)
			dose	unit			

Vaccination history of DTP3 (for children aged under 14 years old)

Source of data：☐vaccination certificate ☐linkage with national database ☐ self-reports

Reasons of unvaccinated：1. Contraindications; 2. Under the age of vaccination; 3. Missed vaccination time; 4. Parents refused to vaccination; 5. migrating population; 6. Don't know; 7. Others_____

dose	lot number	producer	dosage		site	Date (YYYY/mm/dd)	Reasons of unvaccinated
			dose	unit			
1							
2							
3							

Patient specimen collection

Specimen collected： ☐ yes ☐ no **Date of sampling**：____/____/____(YYYY/mm/dd)

Type of specimen： Nasopharyngeal swab ☐ amounts：_____

Whole blood ☐ quantity：_____ ml

Hospital：_____ **Investigator**：_____ **Date of reporting**：____/____/____(YYYY/mm/dd)

Supplementary Table 5. Follow-up information of case (CRF—T_{2w}/T_{4w}/T_{8w})

Patient identifier no.:_____ Type: ☐Inpatient ☐outpatient

Name：_____（or **Parents' name**：_____） **Sex：**☐ male ☐ female

Illness onset date：____/____/____(YYYY/mm/dd) **Admission date：**____/____/____(YYYY/mm/dd)

Follow-up date： ____/____/____(YYYY/mm/dd) **Weeks of follow-up:**☐ 2 wks ☐ 4 wks ☐ 8 wks

Follow-up method: ☐ hospital visits ☐ home visits

Outcomes

Survival: ☐ yes ☐ no Date of death:____/____/____(YYYY/mm/dd) death diagnosis：_____

Hospitalized： ☐ yes ☐ no **Re-admitted into hospital after discharge:** ☐ yes ☐ no

Reasons for re-admission：Pneumonia/heart failure/cardiogenic shock/encephalopathy/Seizure/other_____

Lost to follow-up: ☐ yes ☐ no (refers to 3 consecutive phone calls to patients on different working days but no answers at all)

Clinical characteristics (during follow-up visits)

☐cough（Starting date____/____/____[YYYY/mm/dd]，duration_____days）

☐post-tussive vomiting ☐paroxysmal cough ☐whooping cough ☐apnea ☐cyanosis ☐fever（body temperature_____℃）

☐cough worsening in night ☐productive cough; Sputum color：yellow/white/black/glass like

☐seizure ☐hemoptysis ☐chills ☐headache ☐myalgia ☐sore throat ☐joint pain ☐chest pain

☐sweat ☐shortness-of-breath ☐running nose ☐lachrymation ☐fatigue ☐other(_____)

Blood tests：WBC_____×10^9/L; L_____×10^9/L; N_____×10^9/L; Plt_____×10^9/L; Hb_____g/L; CRP_____mg/L; GLU_____mmol/L

Physical check：body temperature：_____℃ **Breath rate**：_____breath/min **Heart rate：**_____beats/min

Systolic/diastolic blood pressure：_____/_____mmHg **Pulse oximetry**: sPO_2 (if any):_____%

Lung auscultation：□dry rale □wet rale **Consciousness**：clear/lethargy/irritable/delirium/convulsions/coma

Patient specimen collection

Specimen collected： ☐ yes ☐ no **Date of sampling**：____/____/____(YYYY/mm/dd)

Type of specimen： Nasopharyngeal swab ☐ amounts：_____

Whole blood ☐ quantity：_____ ml

Reasons for not sampling： ☐ without coughing symptoms for 1 week ☐refusal to sampling

Hospital：_____ **Investigator**：_____ **Date of follow-up**：____/____/____(YYYY/mm/dd)

Supplementary Table 6. Outcome of case at the end of follow-up (CRF—T_{end})

Patient identifier no.:_____ Type: ☐inpatient ☐outpatient

Name：_____(or **Parents' name**：_____) **Sex：**☐ male ☐ female **Illness onset date：**__/__/__(YYYY/mm/dd)

Admission date：__/__/__(YYYY/mm/dd) **Discharge date：**__/__/__(YYYY/mm/dd)

Discharge diagnosis： primary diagnosis _____

secondary diagnosis **1.**_____ **2.**_____ **3.**_____

Treatment during hospitalization

Admitting into ICU： ☐ yes ☐ no __/__/__(YYYY/mm/dd)

1.**Transfer in date**__/__/__(YYYY/mm/dd) **Transfer out date**__/__/__(YYYY/mm/dd)

2.**Transfer in date**__/__/__(YYYY/mm/dd) **Transfer out date**__/__/__(YYYY/mm/dd)

3.**Transfer in date**__/__/__(YYYY/mm/dd) **Transfer out date**__/__/__(YYYY/mm/dd)

Oxygen therapy： ☐ yes ☐ no duration：_____days

Invasive ventilation： ☐ yes ☐ no duration：_____days (invasive ventilation refers to tracheal intubation or tracheotomy)

Non-invasive ventilation: ☐ yes ☐ no duration：_____days

Oscillating respirator： ☐ yes ☐ no duration：_____days

ECMO or interventional lung adjuvant therapy（iLA） ☐ yes ☐ no **date of treatment start：**__/__/__(YYYY/mm/dd)

Renal replacement therapy/dialysis： ☐ yes ☐ no **date of treatment start：**__/__/__(YYYY/mm/dd)

Exchange transfusion： ☐ yes ☐ no **date of treatment start：**__/__/__(YYYY/mm/dd)

Leukophoresis or leukoreduction therapy： ☐ yes ☐ no **date of treatment start：**__/__/__(YYYY/mm/dd)

Drugs

（1）Drug name：Please give the name of the drug, or the trade name if it is a fixed compound preparation

（2）Category: A=antibiotic (1. Amoxicillin; 2. Amoxicillin-clavulanic acid; 3. Ampicillin; 4. Azithromycin; 5. Ceftriaxone; 6. Cefuroxime; 7. Ciprofloxacin; 8. Clarithromycin; 9. Doxycycline; 10. Erythromycin; 11. Penicillin; 12. Tetracycline; 13. Compound sulfamethoxazole); B=antiviral drugs; C=steroid hormone drugs

（3）Route：1=oral, 2=intravenous injection, 3=intravenous drip, 4=intramuscular injection, 5=inhalation, 6=other

（4）Frequency：1= continuous，2=intermittent

drug name (1)	category (2)	route (3)	daily dose		frequency (4)	starting date (YYYY/mm/dd)	stop date (YYYY/mm/dd)
			dose	unit			

Clinical characteristics

Symptoms/signs：

☐cough（Starting date__/__/__[YYYY/mm/dd]，duration_____days）

☐post-tussive vomiting ☐paroxysmal cough ☐whooping cough ☐apnea ☐cyanosis ☐fever（body temperature_____℃）

☐cough worsening in night ☐productive cough; Sputum color：yellow/white/black/glass like

☐seizure ☐hemoptysis ☐chills ☐headache ☐myalgia ☐sore throat ☐joint pain ☐chest pain

☐sweat ☐shortness-of-breath ☐running nose ☐lachrymation ☐fatigue ☐other(_____)

Complications：

☐viral pneumonia ☐cardiac arrest ☐bacterial pneumonia ☐bacteremia ☐acute lung injury/ARDS ☐heart infection

☐coagulation disorders ☐pneumothorax ☐anemia ☐pleural Effusion ☐acute kidney injury ☐myolysis

☐bronchiolitis ☐gastrointestinal hemorrhage ☐meningitis ☐pancreatitis ☐epilepsy ☐arrhythmia

☐liver insufficiency ☐stroke ☐hyperglycemia ☐hypoglycemia ☐congestive Heart Failure
☐other (_____)

Patient Prognosis

☐**cured**
☐**improved and be discharged**
☐**transferred to other hospital** **reasons for transfer**：community rehabilitation/other (_____)
☐**give up treatment** **reasons for give-up**：economic reasons/illness exacerbation/other (_____)
☐**death** **date of death**：____/____/____(YYYY/mm/dd) **death diagnosis**：_____

Hospital：_____ **Investigator**：_____ **Date of record**：____/____/____(YYYY/mm/dd