PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Burden of Whooping Cough in China (PertussisChina): Study Protocol of a Prospective, Population-based Case-control Study
AUTHORS	YU, Jianxing; He, Hanqing; Zhang, Yanyang; Gao, Yuan; Chen, Chuanwei; Xu, Juan; Xu, Li; Zhang, Xiaoxiao; Zhou, Qianqian; Zhu, Yao; Tang, Xuewen; Guo, Yonghao; Chen, Zhiping; Shao, Zhujun

VERSION 1 – REVIEW

REVIEWER	Miller, Elizabeth London School of Hygiene & Tropical Medicine, Infectious Disease Epidemiology
REVIEW RETURNED	01-Aug-2021

GENERAL COMMENTS	This paper describes the protocol for a prospective study to determine the burden of pertussis in the catchment area of two study hospitals in China. There appears to have been a recent resurgence of pertussis in China possibly associated (among other potential factors) with the introduction of acellular-based pertussis vaccines in recent years (eg see line 37 page 4) but the study objectives (starting line 42 page 5) do not mention any vaccine-related objectives. It was difficult to understand the purpose of the case control study and how this would lead to an understanding of the reasons for the apparent resurgence of pertussis in China. The rationale for the HUAS only became clear after reviewing the CRFs in the supplementary appendix and while I can see how this study can generate burden of disease estimates it is unclear how this data will be used to help understand the reasons behind the apparent resurgence of pertussis in recent years in China as depicted in Figure 1 of the paper. Abstract: It is unclear from the abstract what the objectives of the prospective study are and how this relates to issues such as “adjusting immunisation schedules and recommendations” as mentioned in the introduction. The purpose of the case control study mentioned in the methods and analysis section is unclear. There needs to be results and conclusion sections on the abstract. Summary: This section does not discuss the limitations of the study nor is there any mention of the HUAS which would seem important in estimating the proportion of individuals with persistent cough who access hospital care in the two study areas. Methods and Analysis: Objectives of the study: These are related to estimating pertussis incidence by age and setting in the study population served by the study hospitals. The role of the HUAS only becomes clearer later on and it remains unclear how by itself the study will help understand

	whether there has been an increase in pertussis and if so its potential cause. Study population: I am unclear whether the 41 health care facilities are hospital based or not. Laboratory testing: What is the justification for using a 2-fold rise in anti-PT titres or a sample with an anti-PT titre of at least 40IU/mL with the Virion/Serion assay as evidence of confirmation of pertussis infection? Is there supporting laboratory data for these criteria? It is also unclear what the study hypothesis is for this case-control study. Discussion: The role of the study in relation to optimising the use of vaccines in China is unclear as the prospective study seems only designed to estimate the burden of disease from pertussis in these two areas of China. Overall I found this a confusing paper as it wasn't clear at the outset what was being done and why as the introduction mentioned pertussis resurgence and evaluating interventions. In fact it seems that this prospective study is just attempting to ascertain the burden of pertussis in the population by testing individuals who present at the study hospitals with persistent cough and looking for laboratory evidence of pertussis infection in these cases compared with healthy controls. The authors then via the HUAs survey estimate what proportion of the population have such symptoms and of these what proportion present to one of the study hospitals in an attempt to estimate the burden of pertussis in the population. This is an interesting approach but the method isn't stated clearly at the outset and only becomes evident by reading the whole paper and supplementary appendices. It is unclear to me how the study will help understand the potential reasons for the "rise of pertussis " as stated in the Abstract Introduction as it seems just focused on the current burden of pertussis as presenting in hospital or the community. This is a worthy objective and one which will provide a baseline for comparison with future the interventions but this is not clear from the way the paper is written.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1

Q1. This paper describes the protocol for a prospective study to determine the burden of pertussis in the catchment area of two study hospitals in China. There appears to have been a recent resurgence of pertussis in China possibly associated (among other potential factors) with the introduction of acellular-based pertussis vaccines in recent years (eg see line 37 page 4) but the study objectives (starting line 42 page 5) do not mention any vaccine-related objectives. It was difficult to understand the purpose of the case control study and how this would lead to an understanding of the reasons for the apparent resurgence of pertussis in China. The rationale for the HUAS only became clear after reviewing the CRFs in the supplementary appendix and while I can see how this study can generate burden of disease estimates it is unclear how this data will be used to help understand the reasons behind the apparent resurgence of pertussis in recent years in China as depicted in Figure 1 of the paper.

RE: We appreciate the reviewer's good comments. We acknowledge that the way of our writing in the manuscript is misleading. The primary objective of the study is to measure the burden of pertussis by

age group (i.e. children, adolescents and adults) and settings (i.e. in community, outpatient and inpatient), specifically, the prospectively measured incidence based on laboratory confirmation of Bordetella Pertussis infections. We re-wrote our Abstract and Background to make this point clear to the reader (please refer to lines 89-112, pages 5-6).
We agree that the study could not investigate the reasons for the rising incidence of pertussis in China, and it will be the goal of the future study, say by conducting case-control study via test-negative design or characterizing strains prevalence in different vaccine era (ie. DTwP period and cDTaP period). In fact, PertussisChina is a pilot of a laboratory-based and population-based active surveillance system, 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 in the country. We are planning to use this network for evaluating, prioritizing and optimizing the use of vaccines and for the development of new interventions against a list of bacterial infections (Bordetella Pertussis, Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Group B Streptococcus, Group A Streptococcus etc.) in future. Thank you for your good suggestions.

Q2. Abstract:
It is unclear from the abstract what the objectives of the prospective study are and how this relates to issues such as “adjusting immunisation schedules and recommendations” as mentioned in the introduction. The purpose of the case control study mentioned in the methods and analysis section is unclear. There needs to be results and conclusion sections on the abstract.

RE: We appreciate the reviewer’s comments. The primary objective of the study is to measure the burden of pertussis by age group (i.e. children, adolescents and adults) and settings (i.e. in community, outpatient and inpatient), specifically, the prospectively measured incidence based on laboratory confirmation of Bordetella Pertussis infections. We have re-organized the Abstract to make this point clear (please refer to lines 27-32, page 2).

Q3. Summary:
This section does not discuss the limitations of the study nor is there any mention of the HUAS which would seem important in estimating the proportion of individuals with persistent cough who access hospital care in the two study areas.

RE: Done. We included HUAS and limitations of the study in the Summary (please refer to lines 63-75, page 4). Thank you.

Q4. Objectives of the study: These are related to estimating pertussis incidence by age and setting in the study population served by the study hospitals. The role of the HUAS only becomes clearer later on and it remains unclear how by itself the study will help understand whether there has been an increase in pertussis and if so its potential cause.

RE: Accepted. The primary objective of the study is to measure the burden of pertussis by age group (i.e. children, adolescents and adults) and settings (i.e. in community, outpatient and inpatient), specifically, the prospectively measured incidence based on laboratory confirmation of Bordetella Pertussis infections. We agree that the study could not investigate the reasons for the rising incidence of pertussis in China. We have re-organized our background to make this point clear to the reader (please refer to lines 89-112, pages 5-6). Thank you.

Q5. Study population: I am unclear whether the 41 health care facilities are hospital based or not.

RE: Yes, there are a total of 65 hospitals at the two sites, including 24 hospitals (three tertiary care, four secondary care, and 17 primary care hospitals) in Yiwu and 41 hospitals (five secondary care

and 36 primary care hospitals) in Yongcheng. The private clinics and physicians' office are not investigated in the study. However, the information on health care behavior of residents to these health care facilities will be collected in HUAS. We have made this clear in the method (please refer to lines 145-150, page 7). Thank you.

Q6. Laboratory testing:
What is the justification for using a 2-fold rise in anti-PT titres or a sample with an anti-PT titre of at least 40IU/mL with the Virion/Serion assay as evidence of confirmation of pertussis infection? Is there supporting laboratory data for these criteria?

RE: Yes, there are several supporting materials for using the criteria in the manuscript. In the study, we used combined criteria for diagnosing of pertussis, "i.e. a 2-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". Both conditions must be met (i.e. 2-fold increase, and ≥40 IU/ml for at least one time point). They are based on the following considerations.
First, since the illness course of typical pertussis is long and our study included patient at a late course (i.e. after cough ≥14 days), the room for anti-Ptx IgG antibody to rise is limited at the late course (as pertussis antibody concentrations rise quickly after renewed contact with the bacteria, especially for adults). Several studies used a 2-fold increase of anti-Ptx IgG antibody for paired serum in previous studies (Wirsing et al. Lancet.1995; Strebel et al. J Infect Dis. 2001; and Wirsing et al. J Infect Dis. 1996). To increase sensitivity of ELISA assay, we choose to use a 2-fold or greater rise in anti-Ptx IgG antibody.
Second, because of the limited accuracy of ELISA at low values, a criterion for the minimal level to be reached in the second serum is included in most definitions, specifically for IgG-Ptx ELISA, a minimally 20 CBER EU/ml or 20 WHO IU/ml were required (Meade et al. 1995.Pediatrics). To avoid the influence caused by low values of anti-Ptx IgG antibody, we included a minimal level for the paired serum at one time point.
Third, Riffelmann et al. studied the Performance of the Virion/Serion assay in one study (Riffelmann et al. J Clin Microbiol. 2010). According to Riffelmann et al., the concentration of anti-Ptx IgG antibody over 100 IU/ml obtained from adults and adolescents are indicative of recent contact with Bordetella Pertussis whereas values below 40 IU/ml exclude the possibility of infection. The values between 40-100 IU/ml are inconclusive, and a second serum at late course or testing IgA activity to Ptx can assist the diagnosis of pertussis. Besides, several seroprevalence studies on anti-Ptx IgG antibody found a very low mean concentration of ~6 IU/ml in Chinese population (Meng Q et al. BMC pediatrics 2019; Ning Y, et al. Disease Surveillance 2017). Based on low prevalence of population anti-Ptx IgG antibody and the performance of ELISA assay kits (Virion/Serion), we included a minimal level of 40 IU/ml of serum titer for one time point of serum assays.
Base on the reviewer's comments, we further discussed our diagnostic criteria of the serological assays with other researchers and got some suggestions. Since Zee et al.'s review suggested that a 3-fold cutoff point had the higher specificity and cumulative sensitivity plus specificity than a 2-fold cutoff point (Anneke van der Zee et al. Clin Microbiol Rev. 2015), and children who contracted culture-confirmed pertussis in the year following vaccination with a Ptx-containing vaccine, the diagnostic sensitivity of increases of IgG-Ptx in paired sera was much lower (Trollfors et al. Clin Microbiol Infect.2003), we modified our diagnosis criteria as "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" (please refer to lines 361-364, page 14), and provided two references for this change.

Q7. It is also unclear what the study hypothesis is for this case-control study.

RE: Thank you. The case-control study in the study is not designed to investigate the efficacy of vaccine. Instead, the case-control study will be used 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. To make this point clear, in the revised manuscript we gave the objective of case-control study (please refer to lines 160-162, page 7) and its rationales (please refer to lines 494-498, page 19).

Q8. Discussion: The role of the study in relation to optimising the use of vaccines in China is unclear as the prospective study seems only designed to estimate the burden of disease from pertussis in these two areas of China.

RE: Accepted. We re-organized the manuscript as suggested. The primary objective of the study is to measure the burden of pertussis by age group (i.e. children, adolescents and adults) and settings (i.e. in community, outpatient and inpatient). And the rationales of the study are as follows, “Most epidemiological data on pertussis in China came from a passive reporting system, the National Notifiable Infectious Disease Surveillance System (NNIDSS). Because of limited diagnosis and incompleteness of reporting, underreporting was substantial in NNIDSS (≥90%) and the burden of pertussis remained underrecognized. 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. Moreover, 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, 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. Partly due to knowledge gaps existed in age-specific burden of pertussis, NIAC suspended its decision on this issue”. We have re-written background and objectives to make these point clear (please refer to lines 89-112, pages 5-6). Thank you.

Q9. Overall, I found this a confusing paper as it wasn’t clear at the outset what was being done and why as the introduction mentioned pertussis resurgence and evaluating interventions. In fact, it seems that this prospective study is just attempting to ascertain the burden of pertussis in the population by testing individuals who present at the study hospitals with persistent cough and looking for laboratory evidence of pertussis infection in these cases compared with healthy controls. The authors then via the HUAs survey estimate what proportion of the population have such symptoms and of these what proportion present to one of the study hospitals in an attempt to estimate the burden of pertussis in the population. This is an interesting approach but the method isn’t stated clearly at the outset and only becomes evident by reading the whole paper and supplementary appendices. It is unclear to me how the study will help understand the potential reasons for the “rise of pertussis “as stated in the Abstract Introduction as it seems just focused on the current burden of pertussis as presenting in hospital or the community. This is a worthy objective and one which will provide a baseline for comparison with future the interventions but this is not clear from the way the paper is written.

RE: Accepted. We appreciate the reviewer’s good comments. We re-organized our manuscript according to the reviewer’s suggestions. To avoid misunderstanding, we stated our study objectives clear in the Background section (please refer to lines 89-112, pages 5-6); we rewrite the Abstract (please refer to lines 26-59, pages 2-3); and the Overview of the study design (please refer to lines 155-167, pages 7-8). We sincerely hoped that this revised version addressed the concerns and requirements of the reviewer successfully. Thank you.

VERSION 2 – REVIEW

REVIEWER	Miller, Elizabeth London School of Hygiene & Tropical Medicine, Infectious Disease Epidemiology

REVIEW RETURNED	29-Nov-2021

GENERAL COMMENTS	This revised version addresses all the issues raised in my initial review. I note that the authors modified the serological criteria for confirming recent B pertussis infection to improve specificity. I wonder whether they could consider retaining the original criteria for a sensitivity analysis?

VERSION 2 – AUTHOR RESPONSE

Reviewer #1:
Q1. I note that the authors modified the serological criteria for confirming recent B pertussis infection to improve specificity. I wonder whether they could consider retaining the original criteria for a sensitivity analysis.
Re: Accepted. We added sensitivity analysis as suggested in the Statistical analysis section (please refer to page 16, lines 415-417). Thank you!