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Research

Case–control study of household contacts to examine immunological protection from *Bordetella pertussis* transmission — study protocol

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Abstract

Background: There is mounting evidence that the recent resurgence of pertussis in many countries is in part related to the acellular vaccine, which has been administered in Canada since 1997. This vaccine elicits a different cell-mediated immune response than the previously used whole-cell vaccine, and its effectiveness wanes over time. The aim of this study is to understand the immunological, demographic and clinical factors that mediate protection from pertussis on exposure.

Methods: This is a household case–control study protocol. Following notification of an index case in a household, a study team will conduct a home visit to collect data and biological specimens. The study team will return to the household 8 weeks from the onset of illness in the index case. The Th1, Th2 and Th17 responses, cytokine expression, IgG subclass, blood cell counts and presence of *Bordetella pertussis* will be determined. We will use laboratory and statistical analyses to determine immunological differences between contacts who are infected with *B. pertussis* and contacts who remain healthy, and to determine which clinical and demographic covariates are associated with a reduced risk of infection.

Interpretation: The results of this study will be essential for understanding the immune response required for protection from infection with *B. pertussis* and will contribute to our understanding of the shortcomings of the current vaccine.

Pertussis (whooping cough), caused by the bacterium *Bordetella pertussis*, is one of the most poorly controlled vaccine-preventable diseases,[1] with peaks in incidence roughly every 2–5 years.[2,3] In Canada, 1000–3000 cases of pertussis are reported annually.[2] The highest incidence is among infants less than 1 year of age, in whom severe disease can develop.[3] Owing to safety concerns regarding the whole-cell pertussis (wP) vaccine,[4] many countries have switched to an acellular pertussis (aP) vaccine within the last 2 decades.[3,5] In Canada, the aP vaccine has been administered since 1997/98.[3] Although the aP vaccine is associated with fewer adverse events than the wP vaccine, its shortcomings have been underscored following a global resurgence of pertussis, which included large outbreaks in the United Kingdom and the United States.[6,7] In Canada, significant outbreaks of pertussis have occurred over the past 3–4 years, but the pattern is inconsistent between and even within provinces.[8] Recent studies in children vaccinated exclusively with the aP vaccine have shown that vaccine effectiveness declines rapidly with time.[9–11] This is suspected to be at least in part due to the

Competing interests: Otto Vanderkooi has conducted maternal immunization studies for pertussis in collaboration with the Canadian Centre for Vaccinology, Sanofi and GlaxoSmithKline. Scott Halperin has received grants and contracts from and has served on ad hoc advisory boards for Sanofi Pasteur and GlaxoSmithKline. No other competing interests were declared.

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underlying immunological characteristics, with differential protection derived from the aP vaccine compared to the wP vaccine or previous infection. There is evidence that cell-mediated immunity is essential for protection from disease,[12,13] but currently our understanding of the immune response to *B. pertussis* and its effect on infection and transmission is incomplete. A recent study indicates that cell-mediated immunity of the Th1 type as well as the Th17 type is essential for bacterial clearance of *B. pertussis*.[14] However, the aP vaccine produces a Th2 response but a weak Th1 and Th17 response. The implications of these findings are unclear and controversial, as the components of human immunity that determine susceptibility to transmission have not been adequately studied.

Immunological studies of pertussis acquisition in humans, including elucidation of the immune correlates of protection, are essential to gain a thorough understanding of the limitations of current pertussis vaccine formulations.[15]

As members of the Canadian Immunization Research Network, our mandate is to develop and test methodologies related to the evaluation of vaccines and perform outbreak-responsive research. Households are the most common setting for *B. pertussis* transmission, with a secondary attack rate of up to 80% in susceptible contacts.[3,16] The varying ages and vaccination histories of household members and their close contact with one another provide an ideal setting in which to examine *B. pertussis* immunity. We designed a study protocol to investigate the level of cell-mediated immunity that protects household contacts from infection after exposure to pertussis in the household. Our 2 research aims are to: 1) determine the humoral and cell-mediated immunological indicators, including Th1, Th2 and Th17 responses, in contacts that are associated with reduced risk of acquisition of pertussis from a case in the household and 2) identify the clinical and demographic features of cases and the environmental factors that are associated with the risk of *B. pertussis* transmission to household contacts. We hypothesize that people who do not acquire pertussis after household exposure have a different immune response to pertussis exposure than those who do acquire pertussis, and that a fuller understanding of how that immunity is different will help inform the future development of more protective vaccines.

Methods

Study design

The study design is a household contact case–control study (Figure 1). Measured outcomes include cellular and humoral immunological indicators as well as the clinical, demographic and environmental factors that are associated with *B. pertussis* transmission to household contacts.

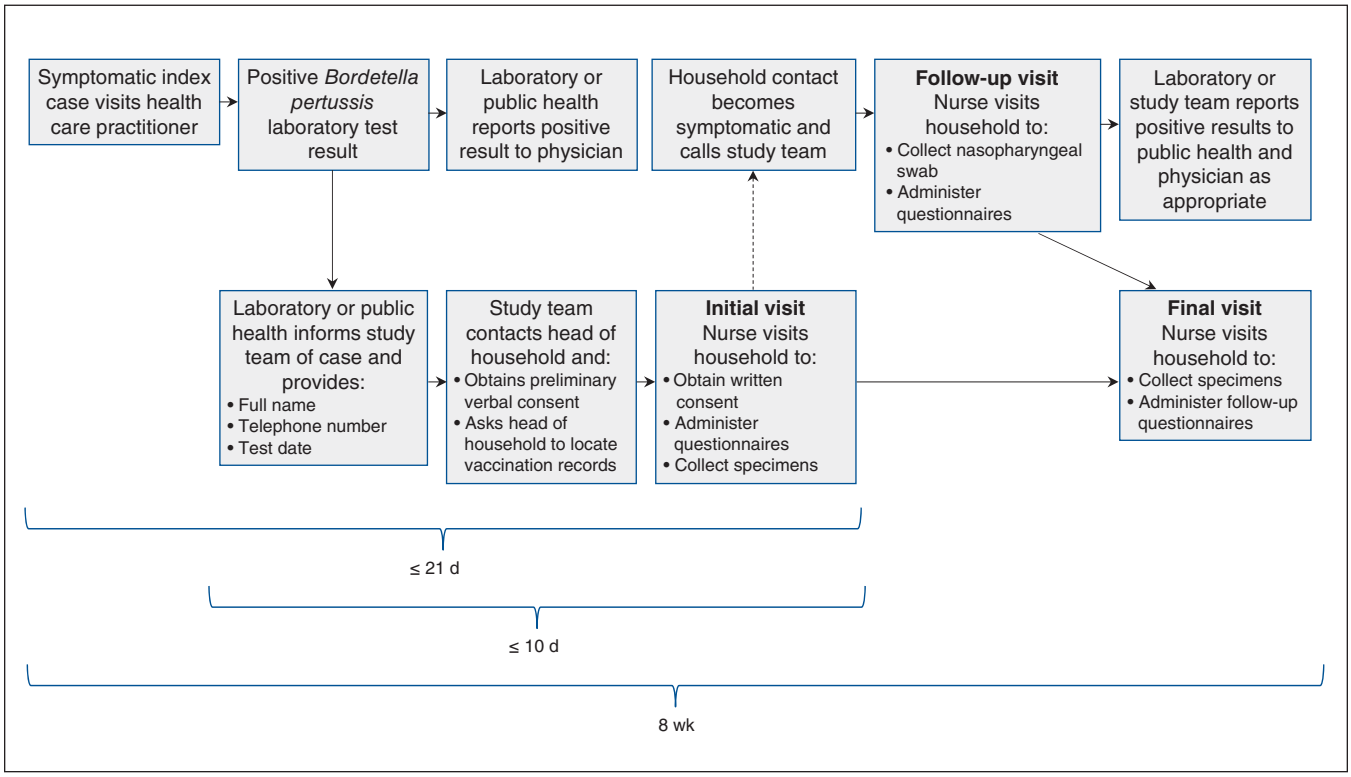

Figure 1: Workflow of pertussis household immunology study. Following a positive *Bordetella pertussis* laboratory test result and preliminary verbal consent, the study nurse will visit the household to collect specimens, optimally within 21 days of symptom onset and 10 days of a positive laboratory test result in the index case. Symptomatic study participants will contact the study team, who will arrange for a nurse to visit the household and collect specimens and data. The study nurse will return 8 weeks after the onset of symptoms in the index case to collect another set of specimens and data.

Sample size calculations

Sample size calculations are based on assumptions of having 12% Th17 cell response in symptomatic infected people versus 0.5% Th17 cell response in asymptomatic/uninfected people. These estimates are based on a previous project using human peripheral blood mononuclear cells that showed a range of 0.5%–12% for Th17 cells (J.W., unpublished data, 2015). However, the people in that study were not infected with *B. pertussis*, and we hypothesize that, if they had been infected, the effect size may have been greater (necessitating a smaller sample). To detect a difference of at least 11.5% in Th17 cell response between groups, we will aim to recruit 72 households, assuming an average of 2 consenting contacts per household. With an estimated secondary attack ratio of about 50%, this will result in 72 contact-cases and 72 contact-controls. Pilot experiments will be carried out before the start of the study to further optimize the sample size.

Feasibility criteria for study initiation

The study will be launched when an outbreak reaches a level of at least 12 cases of pertussis detected per week in a specific jurisdiction (we define a jurisdiction as a region that shares a public health infrastructure, for example, a public health unit, health zone or province). This assumes a 25% recruitment rate to achieve 3 households per week over a 6-month period (roughly 24 wk) from a single jurisdiction, for a total of 72 households. Detailed descriptions of the study feasibility criteria can be found in Appendix 1 (available at www.cmajopen.ca/content/5/4/E872/suppl/DC1).

Case definitions

Case definitions for pertussis cases (i.e., contact-cases) and noncases (i.e., contact-controls) for the study are listed in Table 1.

Identification of index case

In Canada, pertussis is routinely diagnosed and reported to the requesting physician and local public health agencies by laboratories as part of routine surveillance.[3] Index cases should therefore be identified rapidly, either by the diagnostic laboratory or the participating public health agency. If the research team is not embedded within the local public health authority, it will work closely with these groups to facilitate this.

Household recruitment

Once an index case has been identified through a positive result of a polymerase chain reaction test for *B. pertussis*[17] (Table 2), the research team will contact the head of the household (defined here as the first adult in the house reachable by telephone) to determine whether the household is eligible to participate in the study using a household screening questionnaire (Appendix 2, available at www.cmajopen.ca/content/5/4/E872/suppl/DC1).

Individual household members are ineligible for the study if any factors exist that would alter their risk of acquiring pertussis, including if they are immunocompromised, pregnant (and therefore more likely to have received prophylaxis treatment) or less than 1 year of age, have been sick with a

Table 1: Case and control definitions

Study participant	Definition
Index case	The first person in the household in whom laboratory-confirmed *Bordetella pertussis* infection is diagnosed by means of PCR. The index case may not be the first case in the household but is the first case that comes to the attention of public health authorities.
Primary case and coprimary case	The first member of the household to be infected with *B. pertussis*. This person may or may not be the index case. A coprimary case is a household member who becomes sick within 1 wk of symptom onset in the index case. Both case types must be epidemiologically linked and/or laboratory-confirmed.
Household contact	Any study-eligible person living in the household or regularly providing care in the home from 2 wk before onset of illness in the index case and who will still be living or providing care there within 8 wk of onset of illness in the index case. A child's caregiver is also considered a household contact.
Contact-cases	Household contacts who experience symptoms compatible with pertussis after the initial assessment and in whom pertussis is diagnosed by a positive PCR test result within 8 wk of disease onset in the index case.
Contact-controls	Household contacts who do not have a positive laboratory test result for pertussis during the entire study period (i.e., from 8 wk of disease onset in the index case) and remain asymptomatic during that period.
Asymptomatic PCR-positive contact	A PCR-positive household contact who does not manifest pertussis symptoms in the 2 wk before to 8 wk after onset of illness in the index case. Although it will be impossible to rule out whether these people are the primary case in the household, we will include them in the study and will account for this at the analysis stage.

Note: PCR = polymerase chain reaction.

Table 2: Biological specimens

Specimen no.	Amount	Purpose	Testing requirements	Collection schedule
S1	45 mL of blood (in adults) collected in 5–6 sodium citrate or sodium heparin tubes, enabling subsequent PBMC isolation Once isolated, PBMCs can be stored in 2–6 cryotubes (1.5-mL) at a concentration of 10–15 million cells per millilitre	To restimulate PBMCs and measure the Th1, Th2 and Th17 responses as well as cytokine expression and immune suppressors	PBMCs will be processed immediately and frozen at –80°C	Will be collected from all contacts during initial and final visits
S2	5 mL of blood collected in 1 serum separator tube Once separated by centrifugation, serum samples can be stored in 2 cryotubes (1.5-mL)	To measure IgG subclasses and gain information about antibody-mediated immunity and type of cell-mediated immunity	Serum samples will be centrifuged, aliquoted into a fresh tube and frozen at –80°C	Will be collected from all contacts during initial and final visits
S3	2 mL of blood using 1 green-top blood collection tube	To measure complete blood count and leukocyte count	Samples will be tested at a local hospital or frozen at –80°C	Will be collected from all contacts during initial and final visits
S4	Nasopharyngeal swab in phosphate-buffered saline	To diagnose infection by means of PCR[17] and to measure mucosal cytokine production	Before testing, the sample will be stored at 4°C to prevent nucleic acid and cytokine degradation; it will be tested as soon as possible If possible, PCR testing should take place at a clinical or public health laboratory to allow reporting of positive cases to public health authorities Nucleic acid remaining following PCR testing should be stored at –80°C Should specimen remain following PCR testing, the sample media will be split into 2 aliquots before being sent to the laboratory for measurement of cytokines	Will be collected from all contacts during initial and final visits; will be collected from symptomatic contacts during follow-up visit

Note: PBMC = peripheral blood mononuclear cell, PCR = polymerase chain reaction.

cough recently (i.e., epidemiologically linked cases) or have received prophylaxis treatment for pertussis (e.g., azithromycin). Apart from infants less than 1 year of age, who should not be included in the study because of the immaturity of their immune system and also because they may receive prophylaxis on exposure, there is no age restriction for the index case. However, depending on the jurisdiction in which the study takes place and the policies of the local research ethics board, there may be restrictions on the age of participation for contacts. Guidelines regarding provision of consent and collection of invasive biological specimens vary between different research ethics boards. In addition, there may be limits placed on the volume of blood that may be collected from children. At least 2 eligible household members, excluding the index case, are required for the household to be eligible for the study.

Consent

Verbal consent for participation and for a home visit will be obtained from the head of the household during the screening telephone call. At the start of the home visit, each eligible person willing to participate in the study will provide written informed consent.

Data collection

Initial household visit

The initial home visit will occur within 21 days of the onset of coughing symptoms and within 10 days of the positive laboratory test result in the index case, balancing the need to sample contacts before symptom onset with study feasibility. Participating household members will be encouraged to locate their vaccination documentation in advance.

Questionnaires

After obtaining written informed consent, the research nurse will administer questionnaires to study participants. The index case and any coprimary cases will be asked questions about medical and vaccination history as well as demographic questions (Appendix 3, available at www.cmajopen.ca/content/5/4/

E872/suppl/DC1). The household contacts will be asked a series of screening questions to ensure that they remain eligible for study participation and symptom free (Appendix 4, available at www.cmajopen.ca/content/5/4/E872/suppl/DC1), after which demographic information, data on vaccination history, and information about the proximity and duration of contact with the index or coprimary case will be captured (Appendix 5, available at www.cmajopen.ca/content/5/4/E872/suppl/DC1).

Biological specimen collection

After administering the questionnaire, the research nurse will collect all specimens (Table 2) from all consenting eligible contacts in the household, complying with local limitations placed on the total volumes that can be collected from children. Since characterization of the immune response in natural infection has been previously reported[18] and is not 1 of the aims of this study, no specimens will be collected from the index or coprimary case. Blood will be drawn first (S1–S3), followed by collection of a nasopharyngeal swab (S4). Collected blood will be used to characterize the immune response of contacts and to compare the immune response between contacts who subsequently have a positive result of testing for *B. pertussis* (i.e., contact-cases) and those who do not (i.e., contact-controls).

Follow-up visit for secondary cases (contact-cases)

Household contacts who are participating in the study will be asked to call the study nurse should pertussis symptoms develop within 8 weeks from the onset of illness in the index case. The 8-week period was chosen because it covers more than 2 serial intervals for *B. pertussis* infection, i.e., the time between symptom onset in a case and symptom onset in the exposed contacts.[19] Research nurses will return to the home to collect a nasopharyngeal swab from symptomatic contacts (or obtain test results from the contact's physician, if testing has already occurred). No blood specimens will be collected from symptomatic contacts during this visit. Research nurses will also administer a questionnaire to symptomatic contacts (Appendix 6, available at www.cmajopen.ca/content/5/4/E872/suppl/DC1).

Final visit

All contact-cases and contact-controls will be asked to undergo blood drawing (S1–S3) and nasopharyngeal swab sampling again at 8 weeks from the onset of illness in the index case, to confirm that no clinically unapparent infection occurred. Research nurses will also administer a follow-up questionnaire to assess whether symptoms were present (Appendix 6).

Laboratory analyses

The Th1, Th2 and Th17 responses, cytokine expression, IgG subclass, blood cell counts and presence of *B. pertussis* will be determined. The technical details of the laboratory analyses are described in Appendix 7 (available at www.cmajopen.ca/content/5/4/E872/suppl/DC1).

Statistical analysis

We will compare the demographic, clinical and immunological characteristics of household contacts who become infected with those of household contacts who do not become infected (i.e., contact-cases v. contact-controls). We will also assess the extent of asymptomatic infection and determine whether asymptomatic and symptomatic contact-cases differ demographically and immunologically. Differences in proportions and medians will be tested with the χ^2 test, Fisher exact test or Wilcoxon–Mann–Whitney test. We will use a univariate regression model to test the significance of each covariate for the outcome of *B. pertussis* infection (asymptomatic or symptomatic).

For aim 2, we will use a conditional logistic regression model to identify which clinical, demographic and environmental covariates are associated with the risk of *B. pertussis* infection. As part of aim 1, we will adjust for these covariates and vaccination status in a model that incorporates the immunological indicators to understand which of these indicators are associated with a reduced risk of *B. pertussis* infection. We will also compare the immune response between contact-cases and contact-controls to determine whether differences exist. Analysis will be performed with the use of SAS software (SAS Institute Inc.).

Data management

All data will be collected with the use of standardized paper or electronic data forms (e.g., FluidSurveys) and will be transferred into a shared, password-protected Microsoft Excel file at the end of the study. Records will be securely destroyed 7 years after the conclusion of the study.

Dissemination of results

Following data analysis and interpretation, we will notify study participants of our results in lay language. We will publish our findings in a peer-reviewed journal, present our results at scientific conferences and ensure that our results are available to federal and provincial/territorial decision-makers and knowledge users.

Ethical and legal considerations

This project has undergone a full review by the Ethics Review Board of Public Health Ontario and has been determined to be ethically acceptable, with a note that, in the event of an outbreak, full details will be required for expedited review and approval of the project(s). Further information is available from the corresponding author. Any jurisdiction applying this protocol will have to seek and comply with local ethical and legal guidelines for access to study participants, informed consent and assent, and the collection of specimens from study participants. In additional, jurisdictions may have their own guidelines around how and where data are managed and stored.

Interpretation

We present a study protocol designed to assess the immunological, environmental, demographic and clinical factors that

are associated with the risk of acquiring pertussis in a household setting. The results of this study will broaden our understanding of the immunological factors that protect against infection and can then contribute to improving the effectiveness of the current vaccine. During the study protocol development phase, our team identified several feasibility criteria that need to be considered. Through this process, we have noted several potential study sites across Canada that meet these criteria, including Toronto, Vancouver and Montréal — all large urban areas. Having protocols, ethics approval and legal agreements in place for each potential jurisdiction ahead of an outbreak would be optimal. An alternative option for ensuring we reach our sample size is to conduct the study in several outbreak jurisdictions in parallel or recruit sporadic cases as they occur. However, this approach would increase costs substantially and may create bias within the study relating to data collection and methods for specimen testing. It would also further complicate an already complex protocol.

In addition to logistical considerations, several other challenges exist for conducting this type of study. It may be difficult to recruit eligible households within the short 21-day window for sample collection, specifically if the index case is in an adult. Adults with pertussis may present with mild or atypical symptoms, resulting in a delayed or missed diagnosis, which would cause late recruitment of the household into the study. Because pertussis is highly infectious, even with a timely diagnosis it is possible that some household contacts may become infected before we have an opportunity to collect baseline specimens.

It may also be difficult to obtain consent from household members to participate in the study. We hope that this will be minimized by using an experienced research team. The invasive nature of the sampling may cause reluctance to participate in the study, particularly for children and asymptomatic people. Finally, obtaining an accurate vaccination history may be challenging, especially for adult participants, as records may be absent or not updated.

Conclusion

We expect that the information gained from this study will be valuable for acquiring a better understanding of cell-mediated immunity in protection from *B. pertussis* infection. This is essential knowledge as we search for a better alternative to the current vaccine.

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