A boarding school outbreak of pertussis in adolescents: value of laboratory diagnostic methods

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SUMMARY

Culture for *Bordetella pertussis* (*B. pertussis*) is the traditional gold standard for laboratory diagnosis of pertussis but is insensitive, especially later in the course of illness and in vaccinated persons. Interpretation of serology is limited by the lack of an appropriate reference standard. An outbreak of pertussis in a crowded boarding-school dormitory allowed evaluation of laboratory correlates of infection. Questionnaires, serum samples and throat swabs were collected from members of the exposed group. Serum samples from unexposed controls of a similar age group were used for comparison. *B. pertussis* PCR was performed on throat swabs, and sera were tested for IgA antibodies against whole-cell (WC) *B. pertussis* antigen and IgG antibodies to pertussis toxin (PT). The Centers for Disease Control and Prevention definition for pertussis was used to define clinical cases. We evaluated the use of a previously published cut-off for PT IgG of 125 EIA units (EU)/ml. Completed questionnaires were obtained from 115 students, of whom 85 (74%) reported coughing symptoms, including 32 (28%) who met the clinical case definition for pertussis. *B. pertussis* was detected by PCR in 17 (15%) and WC IgA in 22 (19%) students; neither correlated with symptoms, but dormitory of residence strongly predicted PCR status. The mean PT IgG geometric mean concentration, in this situation of high pertussis exposure, correlated with severity of symptoms and was significantly higher in both symptomatic and asymptomatic children exposed during the outbreak ($P < 0.001$) than in control children. A cut-off for PT IgG of 125 EU/ml was too high in an outbreak situation to be sensitive enough to identify pertussis cases. A case of pertussis in a crowded boarding-school dormitory resulted rapidly in an outbreak. Serology and PCR were useful in identifying the outbreak and commencing disease control measures. The use of serology has mostly been evaluated in community serosurveys, where it is not possible to determine if immunity reflects vaccination, asymptomatic disease or symptomatic disease. This outbreak gave us the opportunity to evaluate the value of serology and PCR in the presence of confirmed exposure to pertussis.

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INTRODUCTION

Diagnosis of pertussis is problematical, particularly in adolescents and adults in whom symptoms are

atypical and presentation is often delayed [1–4]. Culture of *Bordetella pertussis* is the traditional gold standard for diagnosis, but is less sensitive in older children and adolescents than in infants because of delayed presentation and previous immunity. Detection of *B. pertussis* DNA by polymerase chain reaction (PCR) partially overcomes these problems but also has limited sensitivity, especially with late presentations [5]. Serology is increasingly used to diagnose pertussis in older age groups, but cannot distinguish reliably between natural and vaccine-induced immunity. Delay in diagnosis also limits the utility of paired sera to detect an antibody rise.

Both natural immunity and vaccination result in protection for a limited period of time [6]. Exposure to *B. pertussis* is generally believed to be frequent, with associated boosting of antibody levels. However, detection of elevated titres to one or more pertussis antigens, most commonly pertussis toxin (PT) in sero-epidemiological studies may have limited correlation with symptom severity [7–10].

A pertussis outbreak in a boarding school for boys with an enrolment of over 900 students, in Sydney, Australia, and a contemporaneous serological study in a population of similar age without known pertussis exposure, offered an opportunity to examine many of these issues. The boys were living in a crowded dormitory, such that exposure to *B. pertussis* from coughing residents over the period prior to investigation was assumed to be universal. The control students, of similar age, were participants in a vaccine trial from other schools.

The aim of our study was to describe the outbreak and examine the performance of various diagnostic tests for pertussis, in the context of uniform, extensive exposure to *B. pertussis* and to compare serological parameters with an age-matched control group.

METHODS

Outbreak investigation

The first pertussis case in a year 8 student in the boarding school was notified on 10 November 2000 and three more suspected cases, also in year 8, were notified on 20 November. A subsequent outbreak investigation showed that the majority of cases were among year 8 students, aged 13–15 years, with the suspected index case developing symptoms on 10 October 2000. The detailed outbreak investigation was confined to year 8 boarders, as there were few cases outside of this group. Erythromycin prophylaxis was initially given only to close friends of and students in neighbouring beds to affected students, but was extended to all year 8 students over the period 27–30 November. Pupils were asked to complete a written questionnaire and a throat swab and blood sample were taken at the same time. A second blood sample was taken 3 months later.

The Centers for Disease Control and Prevention (CDC) case definition for pertussis [11] was used to define a clinical case. This definition requires $\geqslant 2$ weeks' cough plus one or more of: paroxysms, *or* post-tussive vomiting, *or* inspiratory whoop (without any other cause), *or* epidemiological link to a laboratory-confirmed pertussis case. Children who had a cough but did not meet the CDC definition were also counted in the epidemic curve, as they are likely to be true cases in the context of a confirmed outbreak.

Survey

An initial questionnaire was administered to all students on 23 November, asking about socio-demographic details, cough symptoms in the past 4 weeks, exposure to students with cough, dormitory and study arrangements within the boarding school, past medical history and vaccination history. Students were asked to complete the questionnaires themselves, but received assistance from public health nurses if necessary. As the school closed for the extended summer vacation on 7 December, a follow-up questionnaire addressing the resolution of the cough, side-effects of erythromycin and childhood immunization status was sent to parents in mid-December 2000. Parents were asked to consult official immunization records if possible. Parental recall of immunization history was poor, with a response of 'unknown/immunization record not available' in 60% (73/122) of cases. Therefore, immunization history was obtained from school records.

Laboratory tests

Written, informed consent was obtained from all parents or guardians. A team of doctors and nurses visited the school and collected throat swabs and 10 ml of blood from the students on 23 November 2000. A second visit was made on 9 March 2001 to collect convalescent sera.

Children aged 12–14 years participating in a separate study of cough prevalence, combined within

a hepatitis B vaccine immunogenicity study [12], were used as community controls. This study involved a convenience sample from four secondary schools in Sydney of 440 high-school students, whose mean age was 13 ± 0.4 S.D., comparable to the outbreak population. Sera were collected in November 2000, and parents completed a questionnaire concerning cough history in the previous 12 months and pertussis immunization history.

Serology

IgA against whole-cell pertussis antigen (WC IgA)

Sera were tested for IgA, using *B. pertussis* IgA ELISA kit (PanBio, Australia, catalogue no. BPA-300), in which the antigen is an extract of WC *B. pertussis*. The manufacturer's instructions were followed. Test and control sera were diluted 1:100. Cut-off calibrators were tested in triplicate. After addition of reagents, incubation and washing, results were calculated by dividing the optical density (OD) of the serum by the mean OD of the cut-off calibrator, and multiplying this by 10. Interpretation, according to manufacturer's recommendations, was as follows: <9 units negative, 'no evidence of recent infection', repeat after 7–14 days; >11 units positive, 'suggests a recent infection'; 9–11 units equivocal. Any equivocal results, which are uncommon, were repeated and classified as positive, negative or equivocal, according to the result of the second test.

IgG against pertussis toxin (PT IgG)

PT IgG levels were measured at the University of Palermo, Italy, by enzyme-linked immunosorbent assay (ELISA), which is a standardized assay for pertussis within the European Sero-Epidemiology Network (ESEN) [13, 14]. Due to cost and other logistic issues, not all serum samples could be tested. A random, blinded selection of 95 out of 114 initial samples and 38 out of 56 paired samples were tested.

Polymerase chain reaction (PCR)

Throat swabs were taken from all students who completed the first questionnaire on 23 November for pertussis PCR testing, at the Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Australia. Throat swabs were processed using the Roche Respiratory Specimen Preparation Kit (Roche, Basel, Switzerland; catalogue no. 0756903) according to manufacturer's instructions. Samples were stored at $-20\ ^{\circ}C$ prior to testing. PCR was performed using primers described by Glare et al., which target a repetitive sequence in *B. pertussis* and generate a 153 bp amplicon [15]. The single amplification PCR conditions were optimized in-house. Final concentrations of reagents in the reaction mix were: 10 mM Tris–HCl (pH 8·3); 50 mM KCl; 1·5 mM MgCl$_2$; 0·01% gelatin; 100 μM dNTPs; 200 nM BP1 and BP2 primers; 1·0 U AmpliTaq Gold DNA polymerase (PerkinElmer, Mulgrave, Victoria, Australia). The thermal profile for the reaction was: 95 $^{\circ}C$ for 10 min, followed by 50 cycles of 96 $^{\circ}C$ for 10 s, 63 $^{\circ}C$ for 1 min and 72 $^{\circ}C$ for 30 s with a final extension step at 72 $^{\circ}C$ for 5 min. Controls for monitoring specimen inhibition (50/50 mixture of positive control and specimen), episodes of contamination and assay sensitivity were included in each assay. Amplicons were identified using agarose gel electrophoresis and ethidium bromide staining. An amplicon of exactly the same length to that of the positive control was read as a positive result.

Data analysis

On the basis of the information provided in the questionnaires, pupils were placed into one of three clinical categories: those with no cough; those who satisfied the US CDC clinical case definition for pertussis; and those reporting a cough but not meeting the case definition [16]. The CDC clinical case definition for pertussis is cough for 14 days or longer, accompanied by one or more of bouts of coughing, post-cough vomiting or inspiratory whoop. For the purpose of the analysis, individuals who reported they were still coughing at the time of the second questionnaire were assumed to have stopped coughing on that day.

Serological results (for PT IgG) were transformed using log to the base 10, and geometric mean concentration (GMC) [with 95% confidence interval (CI)] was used as measure of central tendency. As the distribution of serological results remained non-normal after log transformation, non-parametric statistical tests were used. The Kruskal–Wallis H test was used to test the statistical significance of differences between PT IgG GMC in the three clinical categories. The Mann–Whitney U test was used to test the statistical significance of differences between PT

Fig. 1. One of the affected dormitories.

Fig. 2. Epidemic curve of pertussis by date of cough onset, in boarding-school children ($n = 85$). □, Cough >2 weeks, not CDC; ■, meets CDC definition.

IgG GMC of the symptomatic and asymptomatic groups. Wilcoxon matched-pairs signed rank sum was used to test the statistical significance of differences in PT IgG GMCs between paired serum samples.

The statistical significance of differences in proportions was tested using a χ^2 test or Fisher's exact test if any cells in 2×2 tables had an expected frequency of <5.

A cut-off of 125 EIA units (EU)/ml of PT IgG was used to define recent pertussis infection, as described by De Melker et al. [17]. De Melker found that a single sample of anti-PT IgG of >125 Italian EU/ml was indicative of recent (within 6 months) infection with *B. pertussis*. Because both de Melker's study and ours used the ESEN standardized assay, the value of this cut-off can be compared directly between the two studies.

Univariate and multivariate logistic regression analysis (Egret software [18]) was used to determine predictors of meeting the CDC clinical case definition for pertussis.

RESULTS

The first four reported cases were all boarders in a relatively crowded dormitory. There were 122 boy boarders in three dormitories, all aged between 13 and 15 years (median 14·2 years). The close proximity of beds in the dormitories is illustrated by Figure 1. Completed questionnaires were obtained for 115 out of 122 boys (94%). All students boarded for at least two nights per week in one of three 30- to 40-bed dormitories with 75 out of 115 (65%) boarding seven nights a week. The index case and the other three reported cases boarded in Dormitory A.
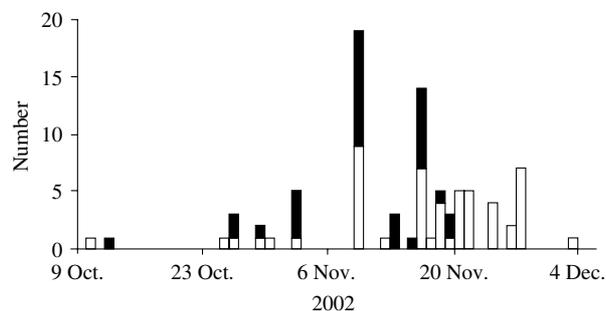
A throat swab (for PCR) was obtained from 114 out of 115 boys, at least one blood sample was tested in 95 out of 115 and paired blood samples were tested in 31% (38/122).

The epidemic curve is shown in Figure 2. The first case occurred on 10 October, and the peak of cases was in November. Of the 115 who completed questionnaires, 74% (85/115) reported coughing symptoms over the period covered by the two questionnaires. The overall mean duration of coughing symptoms was 13 days, and 32 individuals (28%) met the clinical case definition for pertussis. Of these, 15 students were in Dormitory A, and nine and eight students respectively in adjacent dormitories B and C. The median interval from onset of coughing symptoms to first serum specimen was 19 days (range 2–41 days). The median interval from onset of symptoms to second serum specimen was 113 days (range 100–134 days).

There was no significant association between fulfilling the clinical case definition for pertussis and the following variables: previous vaccination against pertussis ($P = 0\cdot11$), reporting a previous diagnosis of pertussis ($P = 0\cdot68$) or having ever been diagnosed with asthma ($P = 0\cdot17$).

Seventeen pupils (15%) had a positive PCR result and 22 (19%) had positive tests for IgA antibody to WC pertussis antigen (there were no equivocal results). There was no significant difference between the clinical categories in the proportion PCR- or WC IgA-positive (Table). A positive PCR result appeared to be more likely when the testing was done close to the date of symptom onset, including some ($n = 6$) that were positive before symptom onset. The odds ratio for a positive PCR result for tests taken within 7 days of symptom onset was 2·7 (95% CI 0·59–14, $P = 0\cdot15$). There was no significant difference in the

Table. *Results of diagnostic tests by clinical category*

| Diagnostic test | Clinical cough category | | | |
	No cough	Cough, not fulfilling CDC case definition (*n* = 53)	Cough, fulfilling CDC case definition (*n* = 32)	*P* value
PCR +ve/ tested (%)	6/30 (20)	4/52 (8)	7/32 (22)	0·14†
WC IgA +ve/ tested (%)	5/23 (22)	10/48 (21)	7/24 (29)	0·64†
PT IgG >125 EU/tested (%)	0/23 (0)	6/48 (12)	8/24 (33)	<0·01†
PT IgG GMC* (95% CI)	15 (11–21)	31 (21–47)	70 (37–1323)	0·001‡

WC, whole-cell pertussis antigen; PT, pertussis toxin; GMC, geometric mean concentration; EU, EIA units.

CDC case definition is: cough for 14 days or longer, accompanied by one or more of bouts of coughing, post-cough vomiting, inspiratory whoop or an epidemiological link to another case.

* Geometric mean concentration, first serum specimen.

† χ^2 test.

‡ Kruskal–Wallis test.

mean IgG titres between PCR-positive (89·8 EU) and PCR-negative (104·5 EU) subjects.

When the first serum specimen only (*n* = 95) was considered, the mean PT IgG concentration differed significantly between clinical groups, increasing from 15 in asymptomatic children to 70 in those who met the CDC case definition for pertussis (Table). The mean PT IgG concentration in those who had a cough but did not meet the clinical case definition was also higher than in those who had no cough, but this difference was not statistically significance (*P* = 0·07).

Paired serum specimens were available from 10 boys, who met the CDC clinical case definition for pertussis. In these cases, the mean PT IgG concentration of the first sample was significantly higher (*P* = 0·01) than that of the second. For the other clinical categories, there was no significant difference between the mean PT IgG concentrations in the first and second samples.

Serum samples taken in November 2000 were available from 287 control children aged 12–15 years who reported that they had not had a coughing illness lasting 14 days or more in the preceding year. The age of the control group (median 14·0 years) was slightly, but significantly younger than that of the outbreak group (median age 14·2 years) (*P* < 0·001). Forty-seven per cent of the control group were female, whilst all of the outbreak group were male.

When compared to the PT IgG GMC in the control group, the proportions of students in the outbreak group with a GMC greater than two standard deviations above the control group value were: 54% (13/24) in the group who met the CDC case definition for pertussis, 32% (14/44) in the group with cough who did not meet the CDC definition, and 0% (0/22) in the asymptomatic group.

When compared to the 287 control subjects, the PT IgG GMC was significantly higher in all clinical groups involved in the outbreak (*P* < 0·001, Fig. 3). The GMC of the control group was 2·7; the asymptomatic outbreak group, 17·4; the outbreak group with cough, 33; and the outbreak group who met the CDC case definition, 71. Finally, 17 out of 95 (18%) of the boarding-school cohort had PT IgG levels >125 EU/ml, compared to only 3 out of 287 (1%) in the control group (OR 20·6, 95% CI 5·5–91, *P* < 0·0001).

In univariate analysis, the only significant predictor of a positive PCR result was residing in dormitory A, in which the index case and the largest number of clinical cases resided (OR 4·7, 95% CI 1·4–15·3). Age, PT IgG >125 EU/ml and past pertussis vaccination were not significant predictors of a positive PCR result. In multivariate logistic regression, dormitory of residence remained the only significant variable (data not shown).

DISCUSSION

School children are an important risk group for pertussis transmission, with 18 out of 20 outbreaks of pertussis over one year in one US state occurring in
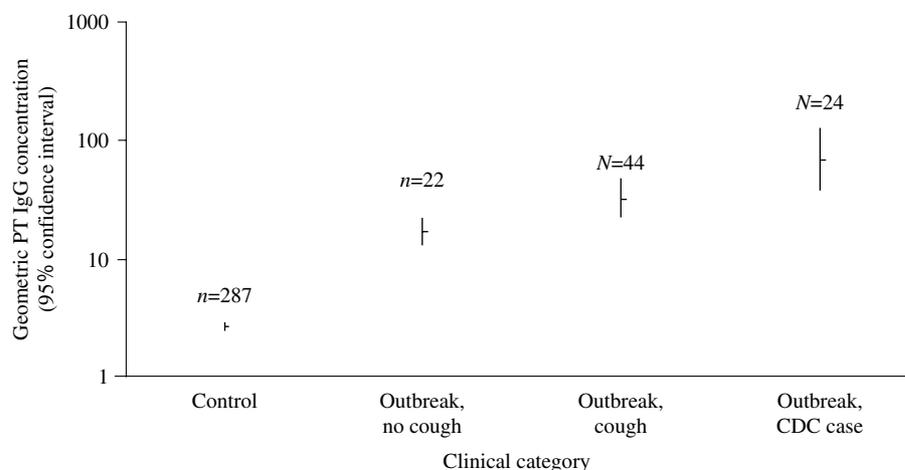
Fig. 3. Geometric mean PT IgG concentration by clinical categories for control and outbreak populations.

schools [19]. This potential for pertussis outbreaks exists even in highly vaccinated school populations [20, 21]. In the special group of adolescents reported here, who were exposed to pertussis in a crowded, closed environment, 74% developed coughing symptoms, and 28% met a standard case definition for pertussis. There was a clear correlation between the severity of symptoms and PT IgG antibody levels. The GMC was significantly higher in the outbreak population compared with community controls, and within the outbreak population, it increased with increasing severity of clinical symptoms.

In a non-epidemic period, using a population sample, de Melker et al., in The Netherlands, found that a single serum anti-PT IgG level of >125 EU/ml was indicative of recent pertussis, but levels decayed rapidly, over time, with high levels lasting an average of 4·4 months [17]. The low number of subjects in this study with a PT Ig titre over this cut-off in this setting suggests that a lower cut-off may be appropriate in an outbreak situation, as the pre-test probability of infection was very high. The cut-off suggested by de Melker might be more appropriate for a general population survey than for a universally exposed subpopulation in an acute outbreak.

As in our study, during an institutional outbreak of pertussis in The Netherlands, only a minority of people with clinical pertussis had positive PCR or cultures, but a high proportion (85%) had serological evidence of pertussis [22]. Others have found that PCR for pertussis is significantly more sensitive than culture and highly specific, in that it is consistently negative in healthy, unexposed controls [15, 23]. Positive PCR results in exposed subjects without

cough, indicates subclinical infection. The sensitivity of PCR for detecting *B. pertussis* in this study was limited by the timing of the available samples, but our results show that PCR was more likely to be positive when specimens were collected within a week (before or after) onset of symptoms. This is a similar limitation to culture, but the advantage of PCR is the simpler methods for handling of specimens. Univariate analysis also showed that a positive PCR result was predicted by dormitory of residence, with students in the same dormitory as the index case being almost five times more likely to have a positive PCR result. However, the same analysis showed that PCR status was not predicted by serology or clinical symptoms.

An IgA assay against WC pertussis antigen was the only commercially available serological test for pertussis diagnosis at the time of this study. It is widely used in Australia for diagnosis of pertussis, especially in older children and adults. We have previously demonstrated that the test is relatively insensitive but, in individual cases, has a high positive predictive value for coughing illness, which fulfils the case definition for pertussis [24]. However, in this highly exposed population, it did not discriminate between symptomatic and subclinical infection.

The study had several other limitations. First, not all sera were tested for PT IgG, introducing the potential for bias. However, they were selected randomly for testing, thereby reducing this risk. Second, there were some differences between the control and outbreak groups, in age, gender and sample timeframe, which may bias the results. The controls were on average 1–2 years older than the outbreak

population, were of mixed gender and sera were collected a month earlier. If there was less pertussis during that time, the effect of this may be to over-estimate the difference in the GMC of PT IgG between the outbreak and control groups. Third, it was not possible to obtain a reliable immunization history for 60% of the children, whose parents did not have records, and could not recall details of previous vaccinations. These children were born between 1985 and 1987, when WC pertussis vaccine was used and only ~70% of infants received three or more doses, but without accurate data, we were unable to examine the relationship between vaccination status and risk of disease.

The early diagnosis of pertussis in this school by the laboratory methods described, and the prompt initiation of disease control strategies such as isolation and antibiotic use, may have prevented the outbreak from spreading outside the boarding school and beyond the affected age group.

The use of serology has mostly been evaluated in community serosurveys, where it is not possible to determine if evidence of serological immunity reflects vaccination, asymptomatic disease or symptomatic disease. Many studies of diagnostic tests for pertussis have relied on past history of exposure to pertussis or have sought to differentiate causes of non-specific coughing illness [5, 8, 17, 25]. This outbreak gave us the rare opportunity to evaluate the value of serology and PCR in the presence of confirmed, intense and universal exposure to pertussis. These data, showing the correlation between elevated serological titres and clinical symptoms and evidence of asymptomatic pertussis infection, as demonstrated by PCR, will contribute to interpretation of findings in population-based serosurveillance studies of pertussis transmission.

ACKNOWLEDGEMENTS

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