Subclinical pertussis in incompletely vaccinated and unvaccinated infants

A. Ramkissoon, H. M. Coovadia, W. E. K. Loening

Incidental to a phase II study of acellular and whole-cell pertussis vaccines involving 342 infants who were clinically observed from birth until the age of 9 months, subclinical pertussis was retrospectively diagnosed in 10 infants on the basis of serological evidence. IgG and IgA to filamentous haemagglutinin (FHA), pertussis toxin (PT) and agglutinogens 2 and 3 (AGG2,3) were assayed by enzyme-linked immunosorbent assay (ELISA) in serum obtained at birth and at 2, 4, 6 and 9 months of age. All 10 infants had ⩾ 4-fold rises in at least two different pertussis IgG antibodies. Nine of the 10 infants had ⩾ 4-fold increases in all three IgG antibodies measured. One infant had ⩾ 4-fold increases in IgG-FHA and IgG-AGG2,3 but not IgG-PT. Seven infants had raised IgA antibodies to PT and FHA and 4 infants had raised IgA antibodies to AGG2,3. Subclinical infection provoked differing degrees of antibody production in response to multiple antigens.

Subclinical infection was detected in both unvaccinated infants (4) and in infants who had been vaccinated from 2 months of age with either acellular (4) or whole-cell vaccines (2). Subjects were 8 months of age or younger and only 1 had completed primary vaccination. Other infections of infancy were commonly detected; 4 infants had upper respiratory disease about the time of subclinical pertussis. None had a household member with symptomatic pertussis.

Likelihood of subclinical infection was related to significantly lower levels of maternally acquired pertussis IgG-AGG2,3 antibodies but not associated with infants' nutritional status. Subclinical pertussis is described in very young babies at an age when the disease is most severe, and therefore has implications for infant morbidity and mortality; it is also relevant to disease surveillance and vaccine efficacy studies.

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Subclinical pertussis is important for a number of reasons: it may cause silent spread of infection in the community and among household contacts; it may be a major cause of unrecognised infant morbidity and mortality; and it distorts surveillance data and influences vaccine efficacy studies (although the latter are conventionally defined in terms of protection against clinical disease). Furthermore, this muted expression of disease consolidates our information on the

Department of Paediatrics and Child Health, University of Natal, Durban

A. Ramkissoon, B.SC., M.SC. (MED.SC.), PH.D. (MED.)

H. M. Coovadia, M.D., F.C.P. (S.A.), M.SC. (IMM.)

W. E. K. Loening, M.B. CH.B., F.C.P. (PAED.) (S.A.)

clinical spectrum of pertussis and raises interesting possibilities with regard to incidence and prevalence of the disease.

There is persuasive evidence that pertussis infection without disease (subclinical whooping cough) does in fact occur. Recent studies have shown subclinical disease in vaccinated infants, health personnel and medical employees, and 'inner-city children'.[1-3] One-third to two-thirds of cases of pertussis infection in vaccinated contacts are subclinical.[4-7]

Most evidence of subclinical infection has been established among vaccinated persons (older children and adults), given conventional whole-cell pertussis vaccines in richer industrialised countries.

Incidental to a phase II study of the immunogenicity and safety of acellular and whole-cell pertussis vaccines, we detected subclinical pertussis among 10 of 342 infants under the age of 9 months on the basis of serological evidence which was retrospectively analysed. We analysed various factors in these babies which might account for development of infection without disease. Special features of the study which we wish to emphasise are: (*i*) pertussis remained subclinical in unvaccinated babies; (*ii*) subclinical infection followed incomplete primary vaccination with either acellular or whole-cell pertussis vaccines; and (*iii*) the problem is described in very young black babies at an age when clinical disease is most severe, and therefore has implications for infant morbidity and mortality.

Methods

Study design

The data analysed in this paper were collected as part of a large, open, uncontrolled study of the antibody responses and post-vaccination events following primary acellular and whole-cell pertussis vaccination; 345 healthy full-term newborn infants from Kwa Mashu, a peri-urban suburb of Durban, inhabited exclusively by blacks, were enrolled in the study after informed consent had been obtained from the parents.

On entry, infants were assigned in sequence of birth to 1 of 3 groups of 115 children each, for receipt of either whole-cell (1 group) or acellular diphtheria-tetanus-pertussis (DTP) vaccine (2 groups) according to routine vaccination schedules at 2, 4 and 6 months of age. In addition, at birth one group of acellular vaccine recipients had received a neonatal dose of this vaccine and the second group, a saline placebo injection.

All infants received trivalent oral polio vaccine (TOPV) and BCG at birth. The parents were asked to return to the clinic when the infants were 2, 4, 6 and 9 months of age.

Blood samples were obtained at birth (mothers' blood and cord blood) and immediately before vaccination at every clinic visit. Sera thus obtained were coded and frozen at –20°C until antibody assays could be performed.

Three vaccine-unrelated deaths occurred in infants younger than 2 months of age. All had been assigned to the whole-cell vaccine group, and therefore had received only BCG and TOPV at birth. Of the 342 remaining subjects, 75% returned at 2 months of age, 68% at 4 months, 58% at 6 months and 51% at 9 months.

Clinical assessment

All infants were monitored for intercurrent illnesses and vaccine-associated symptoms up to the age of 9 months through home visits by a community health nurse with access to tertiary health care. Parents recorded post-vaccination events on a specially designed questionnaire. These were reviewed at each clinic visit by study personnel.

In addition, all infants underwent assessment of nutritional status and physical examination by a paediatrician at each clinic visit at 2, 4, 6 and 9 months. Mothers were questioned about the occurrence and nature of any clinical problems and household contacts of pertussis at this time. Data were recorded immediately on subjects' charts.

Serological assays

Serum IgG and IgA antibodies to filamentous haemagglutinin (FHA), pertussis toxin (PT) and agglutinogens 2 and 3 (AGG2,3) were assayed by micro-ELISA at the Centre for Applied Microbiology and Research, Public Health Laboratory Services, Porton, UK, by the author. The ELISA procedure used was essentially as described by Rutter *et al.*[8]

Antigens. FHA and PT for use as antigens in the assays were purchased from the Research Foundation for Microbial Diseases of Osaka University, Japan. Co-purified AGG2,3 was provided by Dr A. Robinson, Porton, UK.

Reference serum. Japanese Reference Pertussis Anti-serum (human) was a gift from the Research Foundation for Microbial Diseases of Osaka University, Japan. It was supplied from a single lot and contained 250 ELISA units of PT-IgG antibody and 400 ELISA units of FHA-IgG antibody to pertussis per millilitre. The reference serum was assigned a value of 400 ELISA units/ml of IgG anti-AGG2,3. The unitage of the test serum relative to the reference serum was calculated by means of parallel-line assays. Results were expressed in ELISA units/ml.

All sera from one individual were tested in the same assays on the same day. In some cases the quantity of serum was not sufficient to carry out all the tests required and therefore the number of samples giving rise to the data shown in the tables are not uniform.

Definition of subclinical pertussis

Pertussis infection was defined as 'symptomatic' on observation (or recent history) of prolonged paroxysmal cough, whoop, or cough with associated vomiting, cyanosis, apnoea, subconjunctival haemorrhage, epistaxis or peri-orbital oedema. In the absence of these well-recognised clinical signs serological evidence of 'probable pertussis' infection was defined as a significant, i.e. a $\geq$ 4-fold increase in value of IgG antibodies to AGG2,3, and to either or both PT and FHA between two consecutive serum samples taken 8 - 12 weeks apart. 'Definite' serological evidence of pertussis infection was defined as the presence of IgA antibodies to FHA and/or PT, in addition to a $\geq$ 4-fold increase in value of IgG to AGG2,3 and to either or both PT and FHA.

In fact, we found at the completion of the study that all 210 infants had 'definite' serological evidence of pertussis.

Assessment of characteristics of infants with subclinical infection

The roles of various factors which may have contributed to asymptomic infection were analysed, viz.: household contacts; type of antibody response (clinical v. subclinical; vaccinated v. subclinical); maternally acquired antibody levels; vaccination status (number of vaccine doses received); age and gender; and nutritional status.

Age- and vaccine-matched infants with neither 'definite'

nor 'probable' evidence of pertussis infection served as a control group.

Statistical methods

Calculations of results and analysis of ELISA antibody values were performed on logarithmically transformed data. Geometric mean antibody values and standard error were calculated. Unpaired and paired *t*-tests were used respectively to compare mean cord and maternal antibody values between clinical groups and change in an individual's values over time. Sample size was too small to allow statistical evaluation of differences in pre-infection, peak and final antibody titres in infected infants compared with uninfected cohorts. Statistical significance at a 95% confidence level was $P < 0,05$ (2-tailed).

Results

Subclinical pertussis cases

Subclinical pertussis infection was diagnosed in 10 infants during the study period. Three of the infants had received whole-cell vaccine and 7 acellular vaccine. No study subject had pertussis diagnosed clinically or had a definite history of disease contact. Patient details are shown in Table I.

Serology

Whole-cell pertussis vaccination of uninfected age-matched infants in the present study provoked antibody responses against AGG2,3 but not against PT and FHA. Acellular pertussis vaccination resulted in significant PT and FHA antibody titres, but little or no response to AGG2,3 in the study population.[9]

Antibody profiles of the 10 infants who had 'subclinical pertussis', i.e. a ⩾ 4-fold increase in IgG antibodies to AGG2,3 and to either PT or FHA or both are shown in Figs 1 - 3. The subclinical pertussis led to stimulation of antibody responses to multiple antigens of *Bordetella pertussis.*

Antibody levels attained were significantly higher for all pertussis antibody assays than those detected in age-matched unexposed vaccines ($P < 0,001$), and hence could

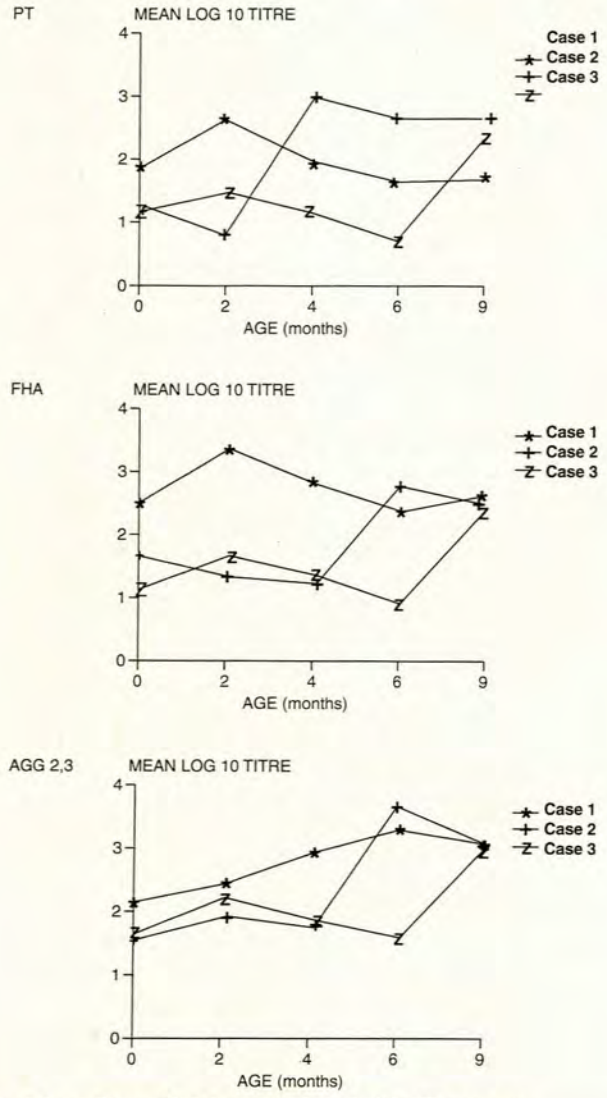

Fig. 1. Log_{10} titres of IgG to pertussis toxin (PT), filamentous haemagglutinin (FHA) and agglutinogens 2 and 3 (AGG2,3) in recipients of whole-cell pertussis vaccine with subclinical pertussis.

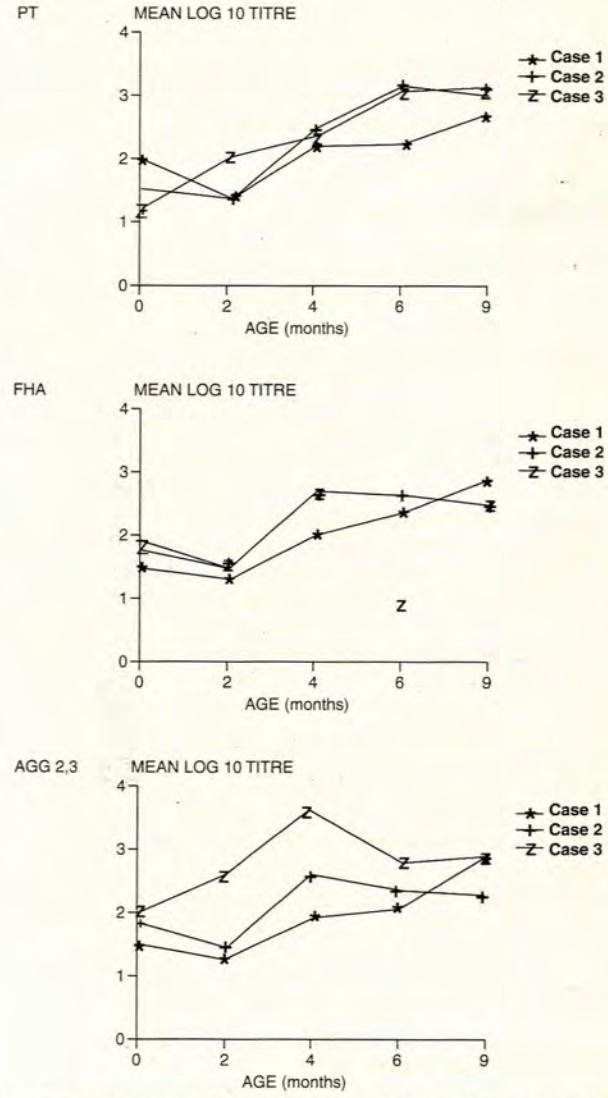

Fig. 2. Log_{10} titres of IgG to PT, FHA and AGG2,3 in 3 recipients of acellular pertussis vaccine with subclinical pertussis aged 2 - 4 months.

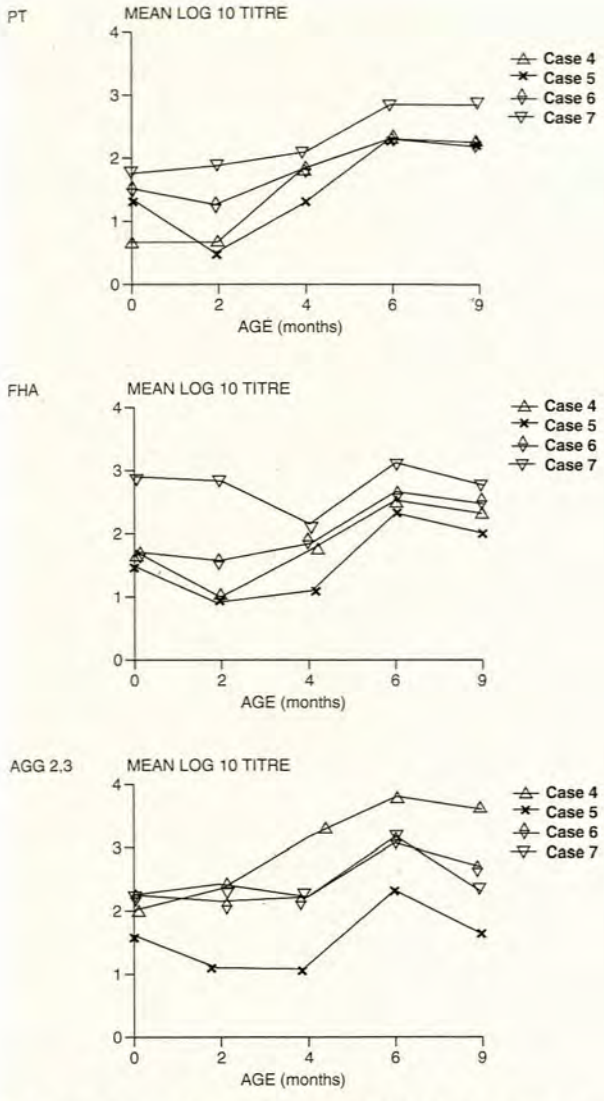

Fig. 3. Log_{10} titres of IgG to PT, FHA and AGG2,3 in 4 recipients of acellular pertussis vaccine with subclinical pertussis aged 4 - 6 months.

not be attributed to vaccination.[9] Nine of the 10 infants had ⩾ 4-fold increases in all three IgG antibodies measured. One infant had ⩾ 4-fold increases in IgG antibodies to FHA and AGG2,3, but not PT.

Pertussis antibody concentrations in serum taken prior to subclinical pertussis infection were not significantly lower in children who had subsequent serological evidence of infection compared with those who did not.

Peak IgG anti-PT levels (at the time of subclinical pertussis) ranged from ⩾ 2,9 to ⩽ 135-fold higher, peak IgG anti-FHA ⩾ 4 to ⩽ 27-fold higher, and peak IgG anti-AGG2,3 ⩾ 5 to ⩽ 51-fold higher than those in age- and vaccine-matched uninfected infants.

Serum IgA antibodies to PT and FHA were detected in all 10 infants, but 3 had only a very limited rise in IgA antibodies. IgA responses to AGG2,3 occurred less frequently than to FHA and PT: 4 of the 10 infants (40%) had demonstrable IgA-anti AGG2,3 antibodies (3 of these were acellular vaccine recipients).

Table I. Patient details

Case*	Sex	No. of vaccine doses prior to peak response	Peak antibody response (age in mo.)	Post-vaccination events (age in mo.)	Clinical problems (age in mo.)
1	F	0	2	nil	URTI (2)
2	F	1	4	nil	S (4, 6)
3	M	3	8	nil	URTI (6)
					S (6, 9)
4	F	0	2	LOA, C (6)	URTI (2, 6)
5	M	0	2	nil	URTI (2)
					S (0, 6, 9)
					GIT (6)
6	M	0	2	nil	LRTI (9)
					S (6)
7	F	1	4	nil	S (4)
8	F	1	4	C (4)	URTI (2, 6)
					F (2)
					GIT (9)
9	M	1	4	nil	GIT (6)
10	F	1	4	nil	URTI (4)
					GIT (6)

* Cases 1 - 3 — whole-cell vaccinees, cases 4 - 10 — acellular vaccinees (commenced at 2 months).
LRTI = lower respiratory tract infection; URTI = upper respiratory tract infection; S = skin infection; F = fever; GIT = diarrhoea; LOA = loss of appetite; C = excessive crying.

Placentally derived IgG antibodies to FHA, PT and AGG2,3 were present in cord blood of all subjects (Table II). Cord IgG-AGG2,3 titres were significantly lower ($P = 0,048$) in infected infants than in uninfected infants. All but 1 of the 10 infants had cord blood anti-AGG2,3 levels which were significantly lower than those of uninfected infants.

Table II. Maternal and cord blood pertussis IgG antibody titres in 10 infants with subclinical pertussis and in their mothers

	Pertussis antibody		
	PT	FHA	AGG2,3
Subjects with subclinical pertussis			
Maternal	29,7 ± 6,6 (10)*	170,2 ± 80,9 (10)	362,3 ± 169,2 (10)
Cord	33,5 ± 8,1 (10)	119,6 ± 53,6 (10)	134,7 ± 29,6† (10)
Subjects without subclinical pertussis			
Maternal	45,7 ± 5,9 (224)	79,4 ± 6,3 (223)	367,3 ± 82,9 (220)
Cord	46,8 ± 5,5 (225)	87,1 ± 8,9 (222)	366,9 ± 113,3 (220)

* Geometric mean titre (*N*).
† Cord IgG AGG2,3 was significantly lower in infected subjects ($P < 0,05$).

Cord blood anti-PT levels were lower in 8 of the 10 infants under study than in infants who did not develop subclinical infection and were higher in 2 of the 10 subjects. Cord blood anti-FHA levels were lower than those of uninfected infants in 8 of the 10 subjects, higher in 1 subject, and similar in 1 subject.

Age and gender

All infants were ⩽ 8 months of age at the time of subclinical pertussis infection; 4 infants were ⩽ 2 months old, 5 infants were between 2 and 4 months old, and 1 infant was between 6 and 8 months old. Infection occurred almost equally in girls and boys (6:4).[9]

Vaccination status

Only 1 of the 10 infants had completed primary vaccination (i.e. 3 doses of whole-cell DTP); 4 infants were unvaccinated and 5 had been vaccinated once (4 with accellular DTP and 1 with whole-cell DTP, all according to routine vaccination schedules commenced at 2 months of age).[9]

Other common infections of infancy and post-vaccination events

Four of the infants (40%) were reported to have had upper respiratory tract infections at or immediately prior to the age of peak antibody response (Table I). The percentage of non-infected infants with upper respiratory tract infections was 32,9% below 2 months of age, 37,3% between 2 and 4 months, 38,9% between 4 and 6 months, and 37,7% between 6 and 9 months. One infant developed a lower respiratory tract infection at 9 months of age, 7 months after subclinical infection. Other illnesses reported included infections of the skin (9), gastro-intestinal tract (5), eye (1), and anaemia (1). Children with serological evidence of *Bordetella* infection did not experience major post-vaccination events following subsequent doses. Two infants experienced minor symptoms (one had loss of appetite and excessive crying, and the second excessive crying only).

Nutritional status

Using NCHS standards, and the anthropometric indices of weight and length for age,[10] subjects were found to be adequately nourished at birth, at vaccination, and at the time of subclinical infection.

Number of contacts with study team prior to peak antibody response

Four infants had no contact, 5 had one contact and 1 had three contacts.

Discussion

Several investigators have suggested that age, pre-existing illness and immune status may modify the severity of infection and alter the clinical features of pertussis to atypical symptoms without whooping.[11-14]

The majority of infants in the present study were 4 months of age or younger at the time of injection. Most other studies of subclinical pertussis have been in older children and adults. Nutritional status was not found to be an important factor in subclinical disease.

No infected infant had illness with coughing of longer than 1 week's duration around the time of antibody rise. However, 4 infants (40%) were noted to have had signs of mild upper respiratory tract infection at this time which, although not significantly different from the occurrence in non-infected infants, may have been indicative of modified illness. Suppressed clinical expression in these infants may have resulted from partial protection by maternally acquired (present in all) or vaccine-induced (present in some) pertussis antibodies.[15]

The current study extends the knowledge of contagious spread of subclinical infection to young infants with high levels of circulating maternally acquired pertussis antibodies. Variable levels of PT, FHA and AGG2,3 IgG antibodies, ranging from low to high, were detected in maternal and cord sera, confirming findings of Phillips[16] that neonates are not protected by maternally derived immunity to pertussis. The presence of maternal antibodies is probably the end-result of natural infection (as the currently used whole-cell vaccine produced poor responses to PT and FHA) and indicates that pertussis is widespread in this community.

There is some evidence of the likelihood of transmission of infection from subclinical cases to vaccinated individuals in contact with them in the household, at health clinics or in the community.[4,7] The occurrence and magnitude of subclinical infection is unknown. In the past children with whooping cough were thought to be the source of family spread. Studies now show that most spread is due to older vaccinated persons with modified illness.[17-19] The importance of asymptomatic or mildly symptomatic individuals in silent transmission of infection is incompletely understood. The spontaneous acquisition of pertussis antibodies in 'inner city' areas where the disease is infrequently recognised is indicative of silent transmission.[1] Data from this study suggest that *B. pertussis* infection occurs in very young infants who are unvaccinated, incompletely vaccinated, or have had complete primary vaccination. This probably occurred through casual exposure to infected individuals. The source of contact was unidentified. No child had household or other known exposure to anyone with pertussis. Infants may have been infected by unrecognised or subclinical cases in the household or community, or during visits to the health clinic. At least 4 of the infants, who had been enrolled in the study at birth, had not visited the clinic prior to the subclinical episode.

It is known that pertussis vaccination provides better protection against disease than against infection.[4,19-21] The overall findings in this study, especially the detection of subclinical cases among infants incompletely and completely vaccinated with three doses of DTP, are in accordance with this effect of the vaccine.

There is nearly universal agreement that PT is responsible for the clinical manifestations of pertussis and is the essential protective antigen, although there are some case reports of convalescents who lack detectable serum anti-PT.[22] Antibodies to FHA may play a part in protection against pertussis when anti-PT antibodies are also present, but are not essential for recovery. Several studies have found that antibodies to FHA are not uniformly detected in convalescent sera.[23,24] Antibodies to FHA may indicate mild or asymptomatic infection with *B. pertussis*[25-27] or other *Bordetella* species, whereas antibodies to PT are produced only by *B. pertussis*. Anti-PT and anti-FHA are probably the most important antibodies in preventing adherence of *B. pertussis* to respiratory tract epithelium. The role of IgG-AGG2,3 antibodies in immunity to pertussis remains to be resolved, but in all likelihood is a supportive one. The significance of antibodies to pertussis-specific adenylate cyclase toxin in protection is not elucidated.

Both natural disease and whole-cell pertussis vaccination provoke serum antibody production against a variety of *B. pertussis* antigens.[28]

Pertussis infection is reported to induce significantly higher IgG-FHA titres and lower IgG-PT titres than those observed after either acellular or conventional whole-cell vaccination.[23,29-31] Furthermore, modest IgG agglutinogen antibody rises have been observed after whole-cell vaccination.[1]

All 10 infants in the present study had IgG-FHA and PT levels which were significantly higher than those of uninfected cohorts. Furthermore, all infected infants produced substantial IgG-AGG2,3 antibodies in response to infection with *B. pertussis.* Symptomatic infection is characterised by higher anti-PT antibodies, whereas in the asymptomatic infection, antibodies to FHA tended to be higher.[4,28] Antibody levels in the 1 symptomatic infant in the present study (data not reported) were in accordance with the above.

The role of serum IgA in pertussis has not been clearly defined. Raised IgA-FHA and PT antibody levels appear to be characteristic of infection and not vaccination, and thus may be used to differentiate between the two.[23] These antibodies may only appear 6 - 7 weeks after infection and may not develop in all infected individuals.[32,33] Burstyn *et al.*[33] and Winsnes *et al.*[23] reported serum IgA anti-PT in only 25% of infected individuals (but not in vaccinees). The majority of infants (70%) in this study produced a definite IgA response to FHA and PT; only 30% produced IgA-AGG2,3.

Some reports indicate that subclinical infections with *B. parapertussis* are common.[34,35] It is unlikely that the IgG antibody responses (and therefore the subclinical pertussis) were induced by infection with *B. parapertussis*, viral infection or nonspecific stimulation of pertussis antibodies. All infected infants had significant rises in antibodies to PT, which are not produced by *B. parapertussis*; furthermore, multiple pertussis antibodies were raised in infected infants. This is unlikely to have been due to other organisms. Other siblings or babies in the community were also not affected by the non-pertussis infections.

The alternative interpretation that these are maternal antibodies seems unlikely as the titres could not have resulted assuming a half-life of 3 - 4 weeks for maternal antibody.

Vaccine efficacy studies are based on clinically evident cases of pertussis and efficacy rates ranging from nil to 95% have been reported for whole-cell preparations.[21] The efficacy of acellular vaccines is currently under investigation. In the present study, pertussis infection was diagnosed in only 1 child prospectively on clinical grounds. Although not designed to determine the protective effect of the vaccines used (sample size being too small to make a definite connection) the detection of subclinical pertussis infection in this study highlights the limitations of clinical diagnostic criteria used in developed countries[6,14] for disease surveillance and pertussis vaccine efficacy studies, as infection and transmission of the organism may occur in the absence of detectable clinical clues.

The present study adds to the information on obscure causes of infant mortality; subclinical pertussis in the very young may be an unrecognised cause of post- and perinatal mortality and morbidity.[2,22] Classic disease symptoms are rare in very young infants. It is possible that infection may progress to disease with serious pathology in the absence of typical features in such cases and mortality from pertussis may hence be attributed to other causes. The magnitude of the antibody response to subsequent doses of acellular or whole-cell vaccine was no different in infected and uninfected infants.

Most other evidence of subclinical disease has been in recipients of conventional whole-cell vaccine. In the present study, infants vaccinated with both whole-cell and acellular vaccines were affected.

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