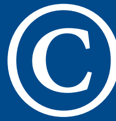


Det här verket är upphovrättskyddat enligt *Lagen (1960:729) om upphovsrätt till litterära och konstnärliga verk*. Det har digitaliserats med stöd av Kap. 1, 16 § första stycket p 1, för forskningsändamål, och får inte spridas vidare till allmänheten utan upphovsrättsinehavarens medgivande.

Alla tryckta texter är OCR-tolkade till maskinläsbar text. Det betyder att du kan söka och kopiera texten från dokumentet. Vissa äldre dokument med dåligt tryck kan vara svåra att OCR-tolka korrekt vilket medför att den OCR-tolkade texten kan innehålla fel och därför bör man visuellt jämföra med verkets bilder för att avgöra vad som är riktigt.

This work is protected by Swedish Copyright Law (*Lagen (1960:729) om upphovsrätt till litterära och konstnärliga verk)*. It has been digitized with support of Kap. 1, 16 § första stycket p 1, for scientific purpose, and may no be dissiminated to the public without consent of the copyright holder.

All printed texts have been OCR-processed and converted to machine readable text. This means that you can search and copy text from the document. Some early printed books are hard to OCR-process correctly and the text may contain errors, so one should always visually compare it with the images to determine what is correct.

GÖTEBORGS UNIVERSITET
GÖTEBORGS UNIVERSITETSBIBLIOTEK

diss 97.75

Studies of pertussis epidemiology and of serum antibody responses to *Bordetella pertussis* antigens after disease and vaccination

Jerker Isacson

Göteborg 1997

Biomedicinska biblioteket

diss 97.75

Avdelningen för Pediatrik, Institutionen för Särskilda Specialiteter,
Göteborgs Universitet

Studies of pertussis epidemiology and of serum antibody responses to *Bordetella pertussis* antigens after disease and vaccination

Akademisk avhandling

som för avläggande av medicine doktors examen vid Göteborgs Universitet
kommer att offentligen försvaras i Barnklinikens föreläsningssal I, Östra sjukhuset,
fredagen den 14 mars 1997, kl 13.00

av

Jerker Isacson
leg. läkare

Fakultetsopponent: Doc. Kjell Tullus, Stockholm

Avhandlingen baseras på följande arbeten:

I. Isacson J, Trollfors B, Taranger J, Zackrisson G, Lagergård T. How common is whooping cough in a nonvaccinating country? Pediatr Infect Dis J. 1993; 12:284-288

II. Isacson J, Trollfors B, Hedvall G, Taranger J, Zackrisson G. Response and decline of serum IgG antibodies to pertussis toxin, filamentous hemagglutinin and pertactin in children with pertussis. Scand J Inf Dis. 1995; 27:273-277.

III. Isacson J, Trollfors B, Taranger J, MacDowall I, Johansson J, Lagergård T Robbins JB. Safety, immunogenicity and an open, retrospective study of efficacy of a monocomponent pertussis toxoid vaccine in infants. Pediatr Infect Dis J. 1994; 13:22-27.

IV. Isacson J, Trollfors B, Taranger J, Lagergård T. Acquisition of IgG serum antibodies against two *Bordetella* antigens (filamentous hemagglutinin and pertactin) in children with no symptoms of pertussis. Pediatr Infect Dis J. 1995; 14:517-521

V. Isacson J, Trollfors B, Lagergård T, Taranger J. Comparison of a toxin neutralization assay and ELISA for determination of pertussis toxin antibodies. Serodiag Immunother Infect Dis. 1997; 8:163-167

Göteborg 1997

Studies of pertussis epidemiology and of serum antibody responses to *Bordetella pertussis* antigens after disease and vaccination

Jerker Isacson, MD. Department of Pediatrics, Göteborg University,
SU/Östra sjukhuset, S-416 85 Göteborg, Sweden

Abstract: This thesis focuses on the incidence of pertussis in Sweden and the study of serum antibody responses after disease and after vaccination with a pertussis toxoid (Ptxd) vaccine. IgG antibodies against pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin were measured using ELISA and toxin neutralizing antibodies using the Chinese hamster ovary (CHO) cell assay.

Two hundred and twenty-six of 372 (61%) nonvaccinated, 10-year-old children had a history of pertussis. PT antibodies were detected in 91% and 64% of children with and without a history of pertussis, respectively. In all, 80% of the children had PT antibodies. All had pertactin antibodies, and all but 3 had FHA antibodies.

In 89 children with clinical pertussis sera were obtained after 0, 1, 3 and 12 months duration of symptoms. **I**: Of 54 children with confirmed pertussis 83% had increases in PT, 74% in FHA, but only 54% in pertactin antibodies. No child had an increase in pertactin antibodies alone. Significant decreases were seen in 63%, 17% and 52% of the children, respectively. **II**: Of the remaining 35 children 17 (49%) had significant increases in PT and/or FHA, but only 6 (17%) of children in pertactin antibodies.

Infants were vaccinated with Ptxd at 3, 5 and 12 (n=76) or 3, 5 and 7 (n=69) months of age. No serious reactions occurred. Local reactions were mild and not more frequent than from a diphtheria-tetanus vaccine given concomitantly. All 145 children had PT IgG and neutralizing antibodies after the second injection. All had antibodies at 2 and 3 years of age. A retrospective controlled study at 3 years of age indicated that the vaccine conferred significant protection.

The acquisition of FHA and pertactin antibodies in Ptxd vaccinated children with no symptoms of pertussis were studied. **I**: Five consecutive sera obtained between 3 months and 3 years of age were available from 71 children. After a decline of maternally derived antibodies there was a slight but significant increase in IgG antibodies against both antigens. At 3 years of age all had FHA, and 78% had pertactin antibodies. **II**: At 3 years of age the FHA IgG titres were significantly higher in children with (n = 12), than in children without (n = 97), family exposure to pertussis.

PT-IgG (ELISA) and neutralizing antibodies (CHO-cell assay) were compared in 796 sera. There was a linear correlation between assays ($r=0.84$, $p<0.0001$). Spearman's Rho=0.82. Analysis of variance showed significant interaction.

<u>In conclusion</u>, 61% of 10-year-old children had a history of pertussis and 91% of them had PT IgG antibodies. Clinical pertussis induced PT and FHA and less frequently, pertactin antibody responses. A PT or FHA antibody decline can be used as a diagnostic tool. FHA and pertactin serum antibodies develop in young children without history of pertussis and can be detected in almost all 10-year-old children. A Ptxd vaccine is immunogenic and safe, and conferred substantial protection. PT neutralizing antibodies correlate with PT serum IgG.

Key words: Pertussis, epidemiology, serological markers, pertussis toxin, filamentous hemagglutinin, FHA, pertactin, pertussis toxoid vaccine, CHO-cell assay, ELISA.

ISBN 91-628-2393-0

Department of Pediatrics, SU/Östra sjukhuset

GÖTEBORGS UNIVERSITETSBIBLIOTEK
14000 000826492

Studies of pertussis epidemiology and of serum antibody responses to *Bordetella pertussis* antigens after disease and vaccination

Jerker Isacson

Göteborg 1997

BIOMEDICINSKA
BIBLIOTEKET

ISBN 91-628-2393-0

—Forty-two! yelled Loonquawl. Is that all you've got to show for seven and a half million years' work?

—I chequed it very thoroughly, said the computer, and that quite definitely is the answer. I think the problem, to be quite honest with you, is that you've never actually known what the question is.

—But it was The Great Question! The Ultimate Question of Life, the Universe and Everything, howled Loonquawl.

—Yes, said Deep Thought with the air of one who suffers fools gladly, but what actually *is* it?

Douglas Adams, 1979

(The Hitch Hiker's Guide to the Galaxy)

ABSTRACT

This thesis focuses on the incidence of pertussis in Sweden and the study of serum antibody responses after disease and after vaccination with a pertussis toxoid (Ptxd) vaccine. IgG antibodies against pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin were measured using ELISA and toxin neutralizing antibodies using the Chinese hamster ovary (CHO) cell assay.

Two hundred and twenty-six of 372 (61%) nonvaccinated, 10-year-old children had a history of pertussis. PT antibodies were detected in 91% and 64% of children with and without a history of pertussis, respectively. In all, 80% of the children had PT antibodies. All had pertactin antibodies, and all but 3 had FHA antibodies.

In 89 children with clinical pertussis sera were obtained after 0, 1, 3 and 12 months duration of symptoms. **I**: Of 54 children with confirmed pertussis 83% had increases in PT, 74% in FHA, but only 54% in pertactin antibodies. No child had an increase in pertactin antibodies alone. Significant decreases were seen in 63%, 17% and 52% of the children, respectively. **II**: Of the remaining 35 children 17 (49%) had significant increases in PT and/or FHA, but only 6 (17%) of children in pertactin antibodies.

Infants were vaccinated with Ptxd at 3, 5 and 12 (n=76) or 3, 5 and 7 (n=69) months of age. No serious reactions occurred. Local reactions were mild and not more frequent than from a diphtheria-tetanus vaccine given concomitantly. All 145 children had PT IgG and neutralizing antibodies after the second injection. All had antibodies at 2 and 3 years of age. A retrospective controlled study at 3 years of age indicated that the vaccine conferred significant protection.

The acquisition of FHA and pertactin antibodies in Ptxd vaccinated children with no symptoms of pertussis were studied. **I**: Five consecutive sera obtained between 3 months and 3 years of age were available from 71 children. After a decline of maternally derived antibodies there was a slight but significant increase in IgG antibodies against both antigens. At 3 years of age all had FHA, and 78% had pertactin antibodies. **II**: At 3 years of age the FHA IgG titers were significantly higher in children with (n=12), than in children without (n=97), family exposure to pertussis.

PT-IgG (ELISA) and neutralizing antibodies (CHO-cell assay) were compared in 796 sera. There was a linear correlation between assays ($r=0.84$, $p<0.0001$). Spearman's Rho=0.82. Analysis of variance showed significant interaction.

In conclusion, 61% of 10-year-old children had a history of pertussis and 91% of them had PT IgG antibodies. Clinical pertussis induced PT and FHA and less frequently, pertactin antibody responses. A PT or FHA antibody decline can be used as a diagnostic tool. FHA and pertactin serum antibodies develop in young children without a history of pertussis and can be detected in almost all 10-year-old children. A Ptxd vaccine is immunogenic and safe, and conferred substantial protection. PT neutralizing antibodies correlate with PT serum IgG.

Key words: Pertussis, epidemiology, serological markers, pertussis toxin, filamentous hemagglutinin, FHA, pertactin, pertussis toxoid vaccine, CHO-cell assay, ELISA.

ISBN 91-628-2393-0

LIST OF PAPERS

The thesis is based on the following papers, referred to by their Roman numbers:

I. Isacson J, Trollfors B, Taranger J, Zackrisson G, Lagergård T. How common is whooping cough in a nonvaccinating country? Pediatr Infect Dis J. 1993; 12:284-288

II. Isacson J, Trollfors B, Hedvall G, Taranger J, Zackrisson G. Response and decline of serum IgG antibodies to pertussis toxin, filamentous hemagglutinin and pertactin in children with pertussis. Scand J Inf Dis. 1995; 27:273-277.

III. Isacson J, Trollfors B, Taranger J, MacDowall I, Johansson J, Lagergård T, Robbins JB. Safety, immunogenicity and an open, retrospective study of efficacy of a monocomponent pertussis toxoid vaccine in infants. Pediatr Infect Dis J. 1994; 13:22-27.

IV. Isacson J, Trollfors B, Taranger J, Lagergård T. Acquisition of IgG serum antibodies against two *Bordetella* antigens (filamentous hemagglutinin and pertactin) in children with no symptoms of pertussis. Pediatr Infect Dis J. 1995; 14:517-521

V. Isacson J, Trollfors B, Lagergård T, Taranger J. Comparison of a toxin neutralization assay and ELISA for determination of pertussis toxin antibodies. Serodiag Immunother Infect Dis. 1997; 8:163-167

CONTENTS

ABSTRACT....4
LIST OF PAPERS....5
ABBREVIATIONS....7
PROLOGUE....8
BACKGROUND
- First descriptions of pertussis and the causative organism....9
- Epidemiology....9
- Pathogenesis and symptoms....10
- Treatment....12
- The genus *Bordetella*....12
- Virulence factors of *B. pertussis*....14
- Diagnosis of pertussis....18
- Vaccination....21

AIMS....28
SUBJECTS....29
METHODS
- Interviews, worksheets, questionnaires....31
- Vaccination procedure....32
- Culture of *B. pertussis*....32
- ELISA for determination of antibodies to PT, FHA and pertactin....32
- Pertussis toxin neutralization assay....34
- Criteria for retesting of sera....35
- Pertussis toxoid vaccine....35
- Statistics....36

ETHICS....36
RESULTS
- History of pertussis and serological markers of disease....37
- Antibody response and decline after clinical pertussis....39
- Pertussis toxoid vaccine;
  - adverse events, immunogenicity and effect against pertussis....42
- Acquisition of FHA and pertactin antibodies....45
- Correlation between neutralization assay and ELISA....47

DISCUSSION
- Pertussis epidemiology....49
- Antibody titers after clinical pertussis....53
- Acquisition of FHA and pertactin antibodies....56
- Vaccination with a pertussis toxoid vaccine....57
- Correlation of neutralizing antibodies and PT IgG antibodies....61
- Comparison of IgG antibodies to *B. pertussis* in the different studies....62

SUMMARY AND CONCLUSIONS....64
EPILOGUE....67
ACKNOWLEDGEMENTS....68
REFERENCES....69
PAPERS I-V....70

ABBREVIATIONS

ADP	adenosine diphosphate
AIDS	aquired immunodeficiency syndrome
ATP	adenosine triphosphate
BCG	Bacillus Calmette-Guérin
cAMP	cyclic adenosine monophosphate
CHO-cell	Chinese hamster ovary-cell
CNS	central nervous system
DNA	deoxyribonucleic acid
DT	Diphtheria-Tetanus
DTP	Diphtheria-Tetanus-(whole-cell) Pertussis
ELISA	enzyme linked immunosorbent assay
FHA	filamentous hemagglutinin
GM	geometric mean
IgG, IgA, IgM	immunoglobulin G, — A, — M
kD	kilo Dalton
MD	Maryland
NICHD	National Institute of Child Health and Human Development
OD	optical density
PCR	polymerase chain reaction
PT	pertussis toxin
Ptxd	pertussis toxoid
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TCA	tricloacetic acid
US (USA)	United States
WHO	World Health Organisation

PROLOGUE

Pertussis has puzzled physicians and scientists since the first descriptions of the disease. The severe, sometimes fatal symptoms of typical pertussis and the prolonged, recurring course of the disease, which does not readily respond to any known remedy including eradication of the causative organism, have frightened parents and caused consternation in the medical profession. Vaccination with whole-cell pertussis vaccine has substantially reduced the incidence of the disease in most countries, but there has also been great concern about severe neurological symptoms from this type of vaccine. The contribution of different antigens to the induction of protection has not been clarified. Owing to difficulties in standardisation, many vaccines have been shown to be ineffective after widespread use. This resulted in the resurgence of pertussis, as was the case in Sweden during the 1970s.

Both M. Pittman's hypothesis from 1979 that pertussis is a toxin mediated disease and the purification of antigens of *Bordetella pertussis* (the causative organism) have had profound effects, and an enormous amount of pertussis research has been performed in recent years. In Sweden there have been unique opportunities for studies of pertussis and new pertussis vaccines thanks to the high incidence of pertussis since the 1970s and the fact that there was no recommended vaccination against pertussis from 1980 through 1995.

In this thesis, an experimental monocomponent pertussis toxoid vaccine was evaluated to see if it was justified for use in a large scale double-blind efficacy trial. Studies of the incidence of pertussis in unvaccinated children of antibody responses against *B. pertussis* after disease, and of the prevalence of antibodies against *B. pertussis* in healthy children were performed to obtain and evaluate basic epidemiological and serological data before the reintroduction of general vaccination against pertussis in Sweden. Finally, two methods for antibody determination were compared.

BACKGROUND

First descriptions of pertussis and the causative organism

The Swedish Academy of Sciences *Almanac* from 1754 contains the first known description of symptoms which can definitely be recognised as typical pertussis. The author was the Swedish physician Nils Rosén von Rosenstein. In 1764 he published this series of almanacs in slightly revised form as a textbook of paediatrics, which was translated into English in 1776 [1]. Von Rosenstein mentions that: "Its (pertussis) first appearance in Sweden cannot be determined with any certainty; but in France it began in the year 1414." and that ".....it was a new disease to our ancestors in Europe, and probably was conveyed to them either from Africa or the East-Indies, where it was rooted before" [1].

The etiologic agent, then referred to as *Bacillus pertussis*, was first described and cultured by Bordet and Gengou in 1906 [2]. It went on being debated well into the 1930s whether a virus infection had a dual relationship in causing the disease. It was then clearly shown in 1933 by the MacDonald spouses, physicians who inoculated their own four children with the bacterium, that it could cause all the typical symptoms of pertussis by itself, and that vaccination seemed to protect the two children who were vaccinated [3].

Epidemiology of pertussis

In Sweden, national mortality statistics have been available since 1749 [1,4]. Morbidity statistics have been available since 1856, when a clinical reporting system was initiated, in which cases of infectious diseases such as pertussis were reported by county primary care physicians. This system continued until 1989. Beginning in 1977 it was supplemented by reports from all microbiological laboratories [4]. All departments of infectious diseases and of paediatrics have been reporting hospitalised patients with pertussis since 1981, and since 1982 a sample of child health centers have reported the annual incidence of pertussis based on information from parents [4].

The first reported epidemic of severe pertussis occurred in Sweden in 1769 [1]. As in other countries epidemics tend to occur with 3 to 4 year intervals irrespective of vaccination status [4-12]. An average of 2712 children died of pertussis each year from 1749 through 1764 in Sweden and Finland, which was then one country with a total population of about 2.3 million inhabitants [1].

As social conditions improved there was a sharp decline in mortality. From 1911 to 1945 mortality from pertussis in children under 6 years of age declined from approximately 740 to 70 deaths per year [13].

Morbidity was still high, with 5000 to 20,000 reported cases annually in a population of about 6.5 million inhabitants. Epidemiological studies in the prevaccination era indicated that 30% to 40% of 4-year-old children in Stockholm had a history of pertussis [14,15] as did 50% to 60% of children at 12 to 13 years of age [15,16]. There was only an additional 0.6% to 1.4% of clinical cases up to the age 30 years [15].

General vaccination with a whole-cell vaccine was introduced in Sweden from 1953 and resulted in a gradual decline in the number of reported cases. By the late 1960s only around 1000 cases were reported annually [4]. However, despite a continuing high vaccination rate of about 90% the reported pertussis incidence gradually returned to prevaccination levels during the late 1970s [5,17-19]. It has later been shown that the Swedish vaccine had become ineffective owing to changes in the culture and inactivation procedures made in the late 1960s [5].

In 1979 the general vaccination against pertussis was discontinued [4]. The number of positive cultures in recent years have been about 11,000 per year (122/100,000 inhabitants per year), [20] and active epidemiological studies have shown a high incidence of clinical pertussis in young children in different parts of the country [19,21].

Many other countries have reporting systems for pertussis similar to the Swedish system. However, all systems have weaknesses and result in underestimation of the true number of cases. Table 1 shows reported incidence figures and case fatality figures from Sweden and some comparable countries. The results of a surveillance study from rural Kenya are included for comparison [22].

Pathogenesis and symptoms

B. pertussis is spread by droplets from coughing patients. Asymptomatic carriers are very rare [23-25] but individuals, often partially or fully vaccinated, with mild or no symptoms may transiently harbour the bacteria or have serologic evidence of exposure [26-31]. The disease is highly contagious with transmission rates in susceptible populations ranging from 25–50% in schools to 70–>90% in household contacts [32].

The bacterium attaches to the respiratory epithelium of the airways. After an incubation period of 7 to 10 days the disease begins with a catarrhal stage resembling a common cold. Symptoms continue with a dry hacking cough which, after about seven days, changes to a paroxysmal stage of increasing severity which culminates after about one week. During the coughing attacks inspiration is usually impossible and the patient may become cyanotic. At the end of the paroxysm, in the typical case, a long drawn inspiration is accompanied by a whoop.

Table 1. Incidence of pertussis, case fatality rates and vaccination coverage during comparable time periods in different countries.

Country	Years	Incidence (per 100,000/year)	Case fatality (% of cases)	Vaccination coverage (%)	Reference
USA	80-89	1.2	0.4	80	[8]
	92-94	2.0	0.2	80	[9]
DDR	80-86	1.2	0.07	90	[33]
Holland	80-86	4.6	0.02	97	[33]
Italy	80-87	28	0.05	11-22	[7]
Denmark	80-86	52.7	0.03	88	[33]
Sweden*	80-85	75	0.008	2-3**	[4]
	86-94	122	0.005		[20]
Kenya	74-76	1900	1.3	12	[22]

* 80-85; culture or serology confirmed cases, 86—94; culture confirmed cases.
** Vaccination coverage estimate from study I. No general vaccination since 1979.

The paroxysmal stage may last for only a few days up to several weeks. Gradually symptoms become less severe, but may persist fore more than 6 months. The symptoms are very variable, and in the prevaccination era about 25% of patients had atypical or abortive pertussis [34,35].

The disease often causes great distress to the child and its family and markedly influences their social life [36-38]. Children under one year of age are often hospitalised. In Sweden, (with endemic pertussis), 7% of all culture verified cases, and 31% of culture verified cases of less than one year of age were hospitalised in the period 1981-83 [4]. Similar figures have been reported from Canada [10]. During an epidemic in Cincinnati in 1993, 31% of all cases were hospitalised [39] and overall about 70% of children under one year of age with pertussis are hospitalised in the USA [9].

The symptoms in infants are usually more severe than in older children and can be atypical with cyanosis, apnea and bradycardia without whoops or severe coughing [40-44]. Some infants require intensive care and mechanical ventilation [4,45-48]. Pertussis related deaths in infants may be mistaken for cases of Sudden Infant Death Syndrome [49]. Still, in Sweden, most infants less than 6 months of age with pertussis have an uncomplicated course of disease [50].

There is an increased risk of concomitant bacterial infection, e.g. pneumonia and otitis media, during a pertussis infection [26,51]. The

high mortality in pertussis in developing countries is mainly attributable to secondary infections [52-54].

Treatment

Eradication of the bacteria is most readily obtained by treatment with erythromycin [55,56] although resistant strains have recently been reported [57]. However, the eradication of bacteria does not influence the course of the disease unless antibiotic treatment is started in the catarrhal stage [58,59] or at least within 7 days of onset of cough [8]. There are indications that early treatment with erythromycin may result in a lesser antibody response [60].

Treatment with hyperimmune serum (i.e. serum containing high levels of antibodies against *B. pertussis*) was tried with apparent positive effect in the 1930s and 1940s, but later studies showed little or no effect [61]. Recently a controlled study using serum with a high pertussis toxin IgG content has shown a reduction in the duration of whoops in treated patients [62] and another study indicated a beneficial effect of hyperimmune serum in severely ill patients [63].

The genus *Bordetella*

Seven species of the genus *Bordetella* are currently recognised. Genetic studies indicate that the *Bordetella* species relatively recently evolved from a common ancestor [64,65] which is consistent with the fact that pertussis was not mentioned in ancient writings [66].

Bordetella pertussis

B. pertussis is a small, strictly aerobic, nutritionally fastidious Gram-negative rod. Its only known host is man, although an outbreak of pertussis in chimpanzees in close contact with humans has been described [67]. Respiratory infections can be induced in primates, rabbits, rats and mice [68-71]. The symptoms in animals other than primates, however do not resemble those of pertussis in humans. In humans the bacteria attach to cilia of the respiratory epithelial cells where they multiply. They do not invade the alveoli or the underlying tissue nor do they enter the cells. However, live bacteria have been recovered from inside human macrophages [72]. *B. pertussis* has also been described as entering cells in tissue cultures and the respiratory tract in rats [71]. It is not known whether this ability to enter and survive inside certain cells has any clinical implication, but it may be a way of avoiding the humoral immune response of the host [72,73].

B. pertussis produces a number of toxins and other virulence factors, discussed below [32,74,75].

Bordetella parapertussis

This species resembles *B. pertussis* but can be distinguished in terms of its growth characteristics and biological properties. The organism was first isolated in 1937 [76]. It is a pathogen in man but causes infection also in sheep [77]. A recent genetic study indicates that *B. parapertussis* in man and sheep are of different strains and thus comprise two distinct populations [78]. *B. parapertussis* is sometimes recovered from patients during pertussis epidemics although it has generally been regarded as a rather uncommon cause of a mild pertussis-like disease [79]. It has recently been shown that symptoms of a *B. parapertussis* infection may resemble typical pertussis [80,81]. Occasionally patients with severe symptoms have been reported [39,82]. A two-year active surveillance study in Czechoslovakia found that 1.73% of 9983 children (77% vaccinated with whole-cell vaccine) had positive *B. parapertussis* cultures [83]. Of these children 94% had mild or no clinical symptoms and 2/173 children had paroxysmal coughing. Surprisingly, only 4 children had positive *B. pertussis* cultures during the the time of the study [83]. More recent studies where *B. pertussis* and *B. parapertussis* cultures were obtained indicate that *B. parapertussis* may be a more prevalent cause of cough in certain areas or time periods (9%-30% of *Bordetella* isolates) [81,82,84-86] than in others (1.4%-6% of *Bordetella* isolates) [39,79,80,87-89].

Bordetella bronchiseptica

The organism was first described in 1910. In mammals the organism causes respiratory infections, e.g. atrophic rhinitis in piglets and acute tracheobronchitis in dogs. The species is not a normal pathogen in man, although sporadic cases may occur in susceptible individuals [90-92].

Similarities between *B. pertussis*, *B parapertussis* and *B. bronchiseptica*

It is known that infections with *B. parapertussis* may occur simultaneously with *B. pertussis* infection [86] and it was more common for *B. parapertussis* infections to follow *B. pertussis* infections than the reverse [79]. A recent experimental study indicates that *B. pertussis* infections may facilitate *B. parapertussis* infections [93]. *B. pertussis* and *B. parapertussis* have been cultured during disease from different members of the same family [82].

It has been shown that *B. parapertussis* and *B. bronchiseptica*, like *B. pertussis*, express the surface antigens FHA and pertactin [94,95]. The virulence factors dermonecrotic toxin, tracheal cytotoxin and adenylate cyclase-hemolysin are also produced by the three species. *B. parapertussis* and *B. bronchiseptica* contain genes encoding for pertussis toxin, but they are not expressed and thus no toxin is produced [64,96].

The organisms are immunologically distinct as shown by clinical reports and animal studies. *B. parapertussis* infections are reported to occur in individuals vaccinated with *B. pertussis* whole-cell vaccine or who have a previous history of pertussis [79,83], and culture proven infection with *B. parapertussis* does not protect against infection with *B. pertussis* [88,97]. Animal studies of cross-protection with sera from whole-cell vaccinated rabbits [98] and with pertussis toxin, FHA, pertactin and adenylate cyclase-hemolysine antisera from *B. pertussis* and *B. parapertussis* in a murine model show little or no cross-protection [99].

Other *Bordetella* species

Four new *Bordetella* species have been described in recent years. *B. avium* was described in 1984 [100] and *B. hinzii* in 1995 [101]. Both cause respiratory infections in poultry. *B. hinzii* has been isolated from patients with AIDS [101,102]. The organism also chronically colonized a patient with cystic fibrosis and was isolated from the respiratory tract of a farmer [65]. *B. holmesii* was described in 1995 [103]. It differs from previously described *Bordetella* species in that all 15 strains described (the first were received in 1983) were isolated from human blood and not the respiratory tract [103]. Many patients in the primary series and in a later case report were immuno-compromised [104]. The most recent member of the genus is *B. trematum*, described in October 1996 [65]. Ten isolates, mainly from the Culture Collection, Göteborg University, obtained from leg and arm wounds in 1980 and 1983 and from 2 patients with chronic otitis media were characterised.

Virulence factors of *B. pertussis*

At least 49 different antigens have been described using immunoelectrophoresis [105]. A number of these have been further identified as virulence factors [32,74,75]. It has been shown that *B. pertussis* undergoes changes, from phase I to phase IV, during growth in vitro with the simultaneous loss of the expression of virulence factors [75]. The expression of the virulence factors is regulated by the vir or bvg locus which encodes a two-component transmembrane regulator [106] that responds to environmental stimuli [107]. The vir locus is also involved in the response to antibiotics by the control of phase transition [108].

Pertussis toxin

Animal studies

Many physiological responses were noted when cultures or supernatants of *B. pertussis* cultures were injected into experimental animals [109]. Similar responses were seen in animals with experimental respiratory *B. pertussis* infection [110]. The causes of these effects were denoted as

lymphocytosis-promoting factor, histamine-sensitising factor, pertussigen, islet cell-activating protein and protective antigen [32,75,105,111]. Recent animal studies indicate that the increased susceptibility to bacteriological infection during pertussis may in part be caused by pertussis toxin [112].

Patophysiological effects in humans

The Leukocytosis, particularly lymphocytosis, in children with pertussis was described as early as 1897 [109]. It can be marked, but is an inconsistent finding, particularly in early or atypical disease and in infants under 6 months old [113]. The biochemical changes in humans with pertussis have been little studied but some investigations have shown hypoglycaemia, [114] and inhibition of the hyperglycaemic response to epinephrine [115]. In a more recent controlled study of 24 patients with pertussis, slight hyperinsulinaemia but no hypoglycaemia was seen [116]. Intravenous injection of "Islet activating protein" in 5 adult volunteers resulted in an enhanced insulin secretory response which lasted 1–2 months [117]. A lymphocytosis promoting factor was isolated in 1976 [118] and evidence was gathered indicating that the lymphocytosis and other effects were mediated by a single antigen.

The concept of pertussis toxin

In 1979 Margaret Pittman proposed that the different effects were mediated by an exotoxin and that this toxin, denoted pertussis toxin, was the cause of the symptoms of pertussis and the mediator of prolonged immunity, analogous, for example, with the diphtheria toxin [74,105].

A few years later the structure of pertussis toxin was described [119]. It is in conformity with the A-B model of protein exotoxins (cholera, Shigella, pertussis and Escherichia coli heat-labile toxins) with a light "A" subunit exhibiting the enzymatic (toxic) activity and a heavy "B" subunit consisting of 5–7 components which attach to the receptors of the target cells [120]. Pertussis toxin consists of six subunits: S1 being the enzymatically active (A) subunit and S2-S4 and S3-S4 joined by S5 making up the binding (B) subunit [119]. One S1 chain (A1) contains enzymatic activity and the other (A2) anchor the A1 chain to S5 of the B subunit [121-123]. The genes encoding the toxin subunits have been cloned and sequenced [124,125] and mutants producing immunogenic but enzymatically inactive pertussis toxin have been constructed [126]. *Bordetella* parapertussis and *B. bronchiseptica* contain transcriptionally silent genes encoding pertussis toxin [64,96].

Function of pertussis toxin

Some of the effects of pertussis toxin on the cell are mediated by ADP-ribosylation of the inhibitory guanyl nucleotide-binding regulatory protein (Gi) of the adenyl cyclase system, resulting in an increase in the activity of the system and resistance to inhibitory regulation [120,127]. How this results in the different biological effects elicited by pertussis toxin in experimental animal models and in the symptoms of pertussis is incompletely understood. Some effects are probably mediated by inhibition of stimulatory G proteins in some cells [128] or through other mechanisms such as blocking of arachidonate release and calcium mobilization by some mediators in some cells [75]. Patients with pertussis have increased levels of tumor necrosis factor α and interleukins, which may be involved in the disease process [129]. Pertussis toxin also seems to have adjuvant activity since pertussis toxin deficient mutants are not able to bind to ciliary respiratory cells [130] or initiate infection [131]. Pertussis toxoid is used alone in monocomponent vaccines and included in all multicomponent acellular pertussis vaccines.

Filamentous hemagglutinin

FHA is a 220 kD surface protein not associated with fimbriae [132,133]. It is a major adhesin [134] and mutants deficient in FHA could not bind to human ciliated respiratory cells in vitro [130]. In the experimental setting antibodies directed against FHA block the adherence of *B. pertussis* to different cells and the FHA mediated agglutination of erythrocytes [135]. Other factors apart from FHA and pertussis toxin are also needed for attachment, as indicated by some studies [130]. Antibodies directed against FHA, induced by mucosal immunization, protect mice against sublethal and lethal respiratory challenge with *B. pertussis* [70,136,137]. However, FHA serum antibodies do not protect mice against intracerebral challenge [138]. FHA is included in all multicomponent acellular pertussis vaccines.

Pertactin

Pertactin was previously called 69 kD outer membrane protein. It is a nonfimbrial protein associated with the bacterial membrane [139]. A *Bordetella* resistance to killing (brk) gene with sequence homology to pertactin has been described [140]. Pertactin (like FHA and pertussis toxin) is an important adhesin [141] and may be involved in the invasion of macrophages and other cells. It has no apparent toxic effects. Pertactin induces agglutinating antibodies in mice [139], which protect them against respiratory challenge with *B. pertussis* [142,143]. It also gives them an increased protection against intracerebral challenge when injected intraperitoneally in combination with pertussis toxin and FHA compared with the two last mentioned antigens alone, but only in high

pertactin vaccine doses [142]. Pertactin is included in most multicomponent acellular pertussis vaccines.

Agglutinogens and Fimbriae

The agglutinogens are protein surface antigens which have been used as serologic markers. Seven agglutinogens have been attributed to *B. pertussis* [32] but traditionally agglutinogens 1, 2 and 3 have been used to define different strains or "serotypes". They are found in the combinations 1-2, 1-3 and 1-2-3. Two antigenically distinct fimbriae are produced by *B. pertussis*. It has been claimed that they correspond to agglutinogens 2 and 3 [132,144]. Protection after vaccination with whole-cell vaccines correlated with the antibody response against agglutinogens [145]. Fimbriae, corresponding to agglutinogens 2 and 3 have been included in some experimental multicomponent acellular pertussis vaccines [146] based on the experience of whole-cell vaccines and reports that they induce protection in mice [147].

Adenylate cyclase-hemolysin

Adenylate cyclase-hemolysin is a protein exotoxin with the ability to enter eucaryotic cells. The toxin has inherent adenylate cyclase activity and is activated by calmodulin. The biological effect is mediated by an increase in the conversion of endogenous ATP to cAMP [148,149]. It can inhibit cellular immunfunctions, e.g. by inhibition of chemotaxis, phagocytosis and natural killer cell lysis of target cells. It also causes hemolysis. Both hemolytic and adenyl cyclase activity is required for *B. pertussis* to initiate infection [131]. The toxin induces protection in the mouse intracerebral and respiratory models [150]. Serum antibodies against a second form of adenylate cyclase called adenylate cyclase enzyme have been identified. It has only enzymatic activity and no effect on eucaryotic cells [75].

Dermonecrotic toxin

Dermonecrotic (heat-labile) toxin is a very potent protein toxin [151]. It is mainly located in the cytoplasm and is released when the cells are disrupted. In experimental animals the toxin causes dermal lesions after injection, atrophy of the spleen, vasoconstriction and death at high doses. The biochemical mechanism of the toxin is unknown, but it can be inhibited by corticosteroids.

Tracheal cytotoxin

Tracheal cytotoxin is a glycopeptide derived from the cell envelope and released into the culture supernatant during growth. It inhibits DNA production, causes ciliary stasis, and has a marked cytopathic effect [152-154].

Lipopolysaccharide

The lipopolysaccharide of *B. pertussis* elicits responses similar to liopopolysaccharides of other Gram-negative bacteria when injected into experimental animals, e.g. pyrogenicity and histamine sensitisation [75]. In man it may contribute to the initial slight fever and to local reactions, but it does not cause an acute phase response (increased erythrocyte sedimentation rate or C-reactive protein) in children with pertussis [129].

Diagnosis of pertussis

Introduction

Pertussis can be diagnosed by: 1. clinical case definitions, [155] 2. isolation of the bacteria, 3. identification of the bacteria by different immunological methods, 4. identification of specific *B. pertussis* genome by PCR, or 5. identification of an increase in antibodies directed against *B. pertussis* antigens. No method is perfect and the criteria used for the diagnosis of pertussis have to be specified. In epidemiological studies and in vaccination studies the definition of pertussis is crucial since different definitions yield different results. At a meeting sponsored by WHO in 1991 the following primary case definition was recommended [156].

A. ≥ 21 days of paroxysmal cough

and one or more of the following

B. 1. Positive culture for *B. pertussis*

2. Serological evidence of *Bordetella*-specific infection by a significant rise in IgG or IgA antibodies against pertussis toxin, FHA or agglutinogens 2 and 3.

3. Household contact with a *B. pertussis* confirmed case occurring within 28 days before or after the onset of illness in the trial child.

The study of pertactin and of differences in antibody responses in infected and in vaccinated subjects were considered research priorities [156].

Culture

Culture was long the only way to diagnose pertussis, except for the clinical diagnosis in typical cases. *B. pertussis* is most readily obtained from nasopharyngeal mucus obtained by a swab or a fine suction catheter [157-159].

The culture medium first used by Bordet and Gengou was a peptone free glycerine-potato-blood agar which still is in use, [2] sometimes with slight modifications [160]. Different media have been developed. They have the advantage of longer shelf life, but none have definitely proved better [160,161] although the charcoal horse blood ("Regan-Lowe") agar [162] or a modified Steiner-Scholte medium [163] in some studies seemed superior. The medium developed by Lacey has the advantage of being highly selective for the cultivation of *Bordetella* and in that it can be stored, but it is somewhat growth-inhibiting, especially as regards *B. parapertussis* [160](Annex 1 by B W Lacey).

Enzyme linked immunosorbent assay

A new method for detection and quantification of specific antibodies, ELISA, was described in the early 1970s [164,165]. The method was simplified owing to the use of microtiter plates instead of antigen-coated tubes [166] and further to the introduction of 96-well microtiterplates and automatic photometers in the early 1980s. The separation and purification of FHA and pertussis toxin [118,167] made it possible to develop more specific and sensitive methods of detection of antibodies against *B. pertussis*. During recent years ELISAs for the detection of immunoglobulins to many different pertussis antigens have been described [168].

The amount of antibodies in a sample can be expressed in different ways. The most common are end-point titer or units/ml.

End-point titer

The end-point titer is defined as the highest serum dilution corresponding to a pre-determined optical density (OD) (or "extinction") above the background value of a blank tested on the same microtiter plate. A reference tested on the same microtiter plate will increase reproducibility [169]. Without a reference serum the end-point titer method has been shown to have less reproducibility than variations of the parallel line method (see below) [170,171]. A computer can be connected to the photometer and with the proper software automatically calculate the end-point titer from the registered OD values. Previously, as in study I of this thesis, the OD values were manually plotted on a sheet and the end-point titer calculated graphically.

Units/ml

Another way to estimate the antibody concentration is to compare the regression line of the test serum with the corresponding line of a reference serum, which has a known concentration of antibody. The result is expressed in units/ml. Different relationships between the

reference serum and the test serum have been used, but the most common ones are variations of the parallel line assay [172]. In the line assays, the slope of a line through the points formed by the logarithm of absorbencies plotted against dilution steps in a specified interval for the test serum and the reference serum are used in a calculation. In an evaluation of the non-parallel, the parallel and the reference line methods used in an ELISA for pertussis toxin, the reference line method showed the highest reproducibility [170,171].

The results calculated by parallel or reference line methods can be expressed in µg/ml, if the antibody concentration in the reference serum is known, [173,174] in international units (IU)/ml, if such have been defined [175,176] or in arbitrary units [177]. Immunoglobulins against pertussis toxin, FHA and pertactin are expressed in arbitrary units since no international standard has been defined [177].

Toxin neutralization assays

Serum antibodies against bacterial toxins can be estimated by different neutralization assays [178]. In contrast to ELISA, these assays measure a function of the antibodies: their ability to neutralize the toxic activity of the antigen. The toxin neutralization tests take advantage of the effects of the toxin exerted on different animal systems [175,179] or on cell cultures in vitro [176,180]. The methods usually require serial dilution of the test serum and the results are usually expressed in end-point titers, which are defined as the highest serum dilution which inhibits toxin activity by 50% or 100%. The titers can be recalculated into IU/ml if the results of the test serum are compared with those of a reference serum with a defined unitage tested in the same assay [175,176]. For PT neutralizing antibodies no international unit is defined, but the tested sera can be compared with a reference serum tested concomitantly.

Serological markers of disease

Previously used methods have employed either whole-cells or crude extracts as the diagnostic antigen. Highly purified components of *B. pertussis* have only been generally available during the last decade [172]. Studies I, II and IV explore the prevalence and response of serum antibodies directed against PT, FHA and pertactin. Previous studies have shown a response of IgG, IgM and IgA antibodies directed against pertussis toxin and FHA after clinical pertussis [181-191]. The IgG response against pertussis toxin is mainly in the IgG1 subclass and, to a lesser extent, in the IgG3 subclass [192]. Studies of monoclonal antibodies have indicated that the response is mainly directed against the enzymatically active S1 subunit of pertussis toxin [182,193]. The antibody response after vaccination with the pertussis toxoid vaccine (III) is mainly in the IgG1 and IgG4 subclass and only rarely in the IgG3 subclass [192]. The clinical significance of this difference is not known.

It is indicated by earlier studies that IgG antibodies against pertactin develop after disease [182,194]. The antibody responses against other *B. pertussis* antigens are even less studied. There are conflicting results concerning the response to outer membrane proteins other than pertactin, [182,193,195] but there are indications that infection with *B. pertussis* elicits an adenylate cyclase-hemolysin antibody response [195,196] and a response against agglutinogens (fimbriae) 2 and 3 [183] or crude fimbriae [197].

It is generally conceived that a pertussis infection usually confers prolonged, but not always lifelong immunity [66,198-200]. The longevity of specific antibody responses have not been much studied and there are obvious methodological problems [184]. The antibody titers recorded in adolescents and adults may be the result of boosters from repeated exposure to *B. pertussis* present in society [184,200].

Vaccination

Whole-cell vaccines

Early experiences

Vaccination was considered a treatment of manifest pertussis already a few years after the isolation of the bacterium by Bordet and Gengou. Dr Graham in Philadelphia in 1912 described in detail 24 patients with typical pertussis treated by him, with believed success in 71% of the cases [201]. The first field studies of prophylactic vaccination were performed in the Faroe islands in 1923 and 1929 [202]. A thorough review of most of the whole-cell vaccine studies up to 1986 was performed by Fine and Clarkson in 1987 [203]. The efficacy estimates were very variable from close to 0 up to 90% [203].

Vaccination programmes

All developed countries have national vaccination programmes but there is great diversity in the kinds of vaccines offered, the age at the injections and the number of doses given [204,205]. Vaccination with a whole-cell vaccine was introduced in most developed countries during the 1950s and 1960s after large field studies in Great Britain and the USA [34,145].

In Sweden a whole-cell pertussis vaccine was produced by the National Bacteriological Laboratory and used for general vaccination between 1953 and 1979. Three doses were administered at 3, 4.5 and 6 months of age. Owing to an ineffective Swedish vaccine during the 1970s following changes in the vaccine production [5] and a wide spread suspicion that whole-cell vaccines had serious rare neurological side effects [16,206,207] the Swedish vaccine was withdrawn and the general vaccination against pertussis discontinued in 1979 [4]. An

imported plain whole-cell vaccine from Wellcome, and later Pasteur Merieux has been available on temporary license [208]. A maximum of 6000 doses were distributed annually to a population of approximately 100,000 newborns per year during the 1980s (A-M Ahlbom, National Bacteriological Laboratory, personal communication). Internationally the vaccination rates have shown great variations with time and between countries, but all vaccinating countries, with the exception of Japan who changed to acellular vaccines in 1981, [209] have continued with whole-cell vaccines for their primary vaccinations. Only since 1996 have acellular vaccines been registered for primary vaccinations in some countries, among them Sweden.

Standardisation of Whole-cell vaccines

Double blind randomised clinical trials were initiated by the British Medical Research Council after the Second World War. A number of studies showed that different vaccines could confer substantial protection but varied considerably in their efficacy. Protection in children correlated with the ability of the vaccine to protect mice against intracerebral infection with *B. pertussis* ("potency"). There was also a correlation with the agglutinin response in mice and, to a lesser extent, in children but this was not a consistent finding [35,145,210]. The potency was shown to correlate with the number of bacteria in the vaccine [211]. More recent studies have shown the potency test in mice to correlate with IgG against pertussis toxin ($r=0.95$, $p<0.001$). A lesser correlation was found with IgG against FHA ($r=0.78$) or adenylate cyclase-hemolysin ($r=0.68$) [212].

In 1964, WHO established an international standard for pertussis whole-cell vaccine based on the intracerebral potency test in mice and the number of bacteria in the vaccine estimated using the opacity test [213]. In Sweden, however, this standard was not applied until 1979.

British studies indicated that the low effectiveness of the British vaccine in the 1960s was associated with a change in *B. pertussis* serotype in the society [214-217]. WHO updated its guidelines in 1979 to recommend the inclusion of all three serotypes in the vaccine. A shift in the predominant serotype was also seen in Sweden after the cessation of vaccination from serotypes 1-2-3, predominant in the vaccination era, to type 1-2 which accounted for 80% of isolates in 1995 [218]. Despite the results from the original British studies, [210] from studies of pertussis toxoid and FHA containing acellular pertussis vaccines [219], and the positive experience with acellular vaccines in Japan not containing agglutinogens [220], some few vaccinologists still advocate agglutinogens as the main inducer of protection against disease [221].

It has previously been claimed that modern pertussis whole-cell vaccines vary in quality, safety and efficacy, [222-224] in part due to

production and standardisation difficulties, and recent studies have shown differences in the antibody response and in the rate of adverse reactions to different whole-cell vaccines used in the USA and Canada [225-227].

Efficacy of whole-cell vaccines

The whole-cell vaccines have generally been regarded as effective and, indeed, most studies have shown the vaccines to be efficacious [34,35,145,228-230] and effective in reducing the numbers of reported cases of pertussis [4,11,12,33,231-233]. However, the results of the studies are often difficult to evaluate and reviewers have concluded that the true efficacy of studied whole-cell vaccines is often impossible to assess [203,234].

More recent studies of pertussis in vaccinated and unvaccinated patients have indicated an efficacy between 60% and >80% [9,228,235-237] Numerous reports of pertussis epidemics in vaccinated children and adults have given reason to question the high efficacy estimates in some studies of whole-cell vaccines [6,10,19,26,39,238-240].

It was early noted that efficacy decreased with time after vaccination and in a study from Michigan there were no protection seen after 11 years [199]. Similar figures are reported from Denmark [33]. A study from the United Kingdom showed a decline from 100% efficacy in 1-year-old children to 46% in 7-year-olds [241]. Stewart, in a review in 1983, concluded that the British and US vaccines were insufficiently effective [242]. It is now generally agreed that the protective effect of the vaccine is rather short lived and that pertussis occurs epidemically in vaccinating countries particularly among adolescents and adults [30,31,243-246].

Adverse reactions to vaccination

The main concern about whole-cell vaccines has not been their degree of efficacy but the suspicion that they could cause serious neurological side effects and some times permanent brain damage and death [206,207,247-249]

A large national British study, "the National Childhood Encephalopathy Study", calculated the attributable risk of neurological sequele one year after vaccination to be 1:310,000 injections [250-252]. The report warned that the figure had to be interpreted with "extreme caution" owing to "....the wide confidence intervals and the broad nature of the underlying assumptions" [251].

A review of the data during a trial in court in London in 1988 under the "Vaccine Damage Payment Act 1979" led to a revision of some of the cases and inclusion of more cases and controls than in the original

(interim) report [253,254]. Statistical calculations using the complete and revised data did not indicate an increased risk of death or permanent neurological damage [254,255] and the judge concluded that " ...the plaintiffs had failed to satisfy him on the balance of probability that pertussis vaccine can cause permanent brain damage in young children, though it was still possible that it could" [253]. Scientific reviewers have come to the same conclusion [256], and the US National Academy of Sciences' Institute of Medicine also concluded that evidence indicated a causal relation between febrile but not afebrile seizures and Diphtheria-Tetanus-(whole-cell) Pertussis (DTP) vaccination [248]. Another large study of 38,000 vaccinated children did not show an increased risk of neurological sequele [257]. A trace of doubt still lingers among laymen and governments [258], but the risk, if any, of severe sequelae has by all calculations been regarded as less than the risk of severe complications from the disease itself [248,259-262].

Less serious reactions were studied in 15,752 children given US licensed DTP vaccine and 784 children given diphtheria-tetanus (DT) vaccine [261]. Of these children 195 DTP and 110 DT recipients were enrolled in a double-blind DTP versus DT vaccine study. The frequency of local and systemic reactions within 48 hours of vaccination in the double-blinded group is shown in table 2.

Table 2. Adverse reactions to diphtheria-tetanus-(whole-cell)pertussis (DTP) and to diphtheria-tetanus (DT) vaccines administered intramuscularly to children in a double-blind study. Data from Cody et. al., 1981 [261].

Reaction	DTP group (%) n = 195	DT group (%) n = 110
Redness	31.3	6.4
Swelling	36.4	9.1
Pain	47.2	10.9
Fever ≥38°C	39.8	12.5
Drowsiness	29.7	13.6
Fretfulness	60.0	25.5
Vomiting	4.6	1.8
"Anorexia"	18.5	5.5
Persistent crying	8.7	4.6

The frequency of reactions was similar in the whole group of children compared to the blinded group [261]. All differences between the DTP and the DT groups were significant except for vomiting and high pitched screaming. Nine convulsions, 9 hypotonic hyporesponsive episodes and 17 children with high-pitched unusual crying were reported in the whole DTP group. A recent double-blind study of two US licensed whole-

cell pertussis vaccines showed them to differ substantially both in immunogenicity and reactogenicity [227]. The rates of adverse events are not directly comparable but were probably lower than the rates reported by Cody, (Table 2). The differences in reactogenicity of the same vaccine in different studies may be attributable to differences in study design and also to variations among vaccine lots or changes over time in vaccine production techniques [227].

Acellular vaccines

Early experience

Acellular pertussis vaccine is not a new idea. One of the 7 vaccines evaluated in the large British field trials in the 1950s was the Pillemer antigenic fraction containing "only a small fraction of the whole bacillus" in a complex with autoclaved human red cell stromata [145]. This vaccine was highly potent in the mouse intracerebral protection test but produced a lower agglutinin antibody response than the whole-cell vaccines. However, it induced a high degree of immunity in children and was more protective than a whole-cell vaccine [145]. Owing to a higher frequency of local and systemic reactions (and the death of Professor Pillemer ?) it was not further pursued. An extracted pertussis vaccine from Eli Lilly Co. was marketed in the USA from 1962 until 1977 under the name "TriSolgen". The vaccine was never well characterised or evaluated [32,263].

The Japanese experience

In February 1975 pertussis vaccination was temporary discontinued in Japan after two deaths of infants in temporal association with pertussis whole-cell vaccination [264]. Vaccination was reintroduced at two years of age in April the same year but the vaccination figures fell to 10% and the pertussis incidence increased [264,265]. In the fall of 1981 acellular vaccines containing FHA and pertussis toxin detoxified by formaldehyde were introduced for mass vaccination from the age of 2 years [265,266]. From 1989 the acellular vaccines have been used for vaccination of infants in Japan. However, in 1990 only about 10% of infants younger than 12 months were vaccinated [267] Since the introduction of acellular vaccines a steady decline in the incidence of pertussis has been reported [266,267].

Acellular vaccines have been manufactured by six different companies and although they differ in their antigen content they have been regarded as equivalent by the Japanese government [268]. The vaccines can be divided into two groups according to their antigen content: the Takeda type contains a larger amount of FHA, a small amount of pertussis toxin and substantial amounts of other substances such as

agglutinogen; the Biken type contains a larger amount of pertussis toxin, a smaller amount of FHA and no other substances [268].

Recent experiences

No large efficacy studies of the Japanese vaccines currently in use were performed before the start of general vaccination [32]. Two acellular pertussis vaccines developed by the National Institutes of Health, Japan were evaluated in Sweden in the 1980s in a large double-blind placebo controlled efficacy study. One vaccine contained 36μg pertussis toxoid alone, the other 22.5μg of pertussis toxoid and FHA, respectively, in a volume of 0.5 ml [219]. Two injections of 0.5 ml were given subcutaneously at approximately 10 week intervals to 5 to 11 months old children.

Efficacy of the PT and PT-FHA vaccines were 54% and 69%, respectively, for any cough and positive *B. pertussis* culture and 79% and 80%, respectively, for culture confirmed cases with cough for more than 30 days [219]. The efficacy estimated by passive follow up for 3 years after unblinding were 71% and 81%, respectively, for parentally reported cases and 79% and 92%, respectively, for culture confirmed cases with >30 days of cough [269]. The efficacy for clinical disease after household exposure was 82% for the PT vaccine and 58% for the PT-FHA vaccine [270].

A number of studies of experimental acellular pertussis vaccines have been performed since then [146,271-282]. In all studies the vaccines have elicited a high serum antibody response and the side effects have been mild. In studies comparing different acellular and whole-cell vaccines the adverse reactions have, with few exceptions, been much milder and less frequent and the antibody responses more prominent in the recipients of the acellular vaccines and the acellular vaccines have been more efficacious than the whole-cell vaccines [283,284].

Table 3 shows the frequency of adverse reactions for the US licensed Lederle whole-cell vaccine and the mean of 13 different mono and multicomponent acellular vaccines tested in a multicenter study sponsored by the United States Department of Health and Human Services [146].

Potency of acellular vaccines

It is recognized that even minute traces of active pertussis toxin in a vaccine will result in a pronounced potentiation of the protective effect of the vaccine as evaluated by the mouse intracerebral potency test [285,286]. Comparisons or standardisation by this test of acellular vaccines that may contain traces of active pertussis toxin if not completely and irreversibly detoxified are, therefore, meaningless. The

use of rabbits, mice or rats in respiratory infection models or the use of in vitro tests with different cell lines have been discussed. No model has yet been deemed appropriate. Measurement of serum antibody titers may not be a possible method since there are indications that antibody titers may not correlate with protection [219,270,275].

Table 3. Frequency of adverse reactions to 13 acellular mono and multicomponent acellular pertussis vaccines and a US licensed whole-cell vaccine. The vaccines were injected intramuscularly together with DT vaccine. Data from the Nationwide Multicenter Acellular Pertussis Vaccine Trial [287]. Differences are significant except for vomiting.

Reaction		13 acellular vaccines (mean %)	whole-cell vaccine (%)
Injection site pain	moderate	6.5	25.9
	severe	0.4	14.3
Injection site swelling >20mm		4.2	22.4
Injection site redness >20mm		3.3	16.4
Fussiness, moderate		12.4	29.1
Persistent crying		4.7	12.4
Use of antipyretic		55.9	83.3
Drowsy		42.7	62.0
"Anorexia"		21.7	35.0
Vomiting		12.6	13.7

AIMS

The aims of this study were to:

Study the incidence of clinical pertussis in children in a nonvaccinating, industrialised country.

Compare anamnestic information about clinical pertussis with the presence of serum antibodies to three antigens of *B. pertussis*; pertussis toxin, FHA and pertactin.

Characterise the response and decline of serum IgG antibodies against pertussis toxin, FHA and pertactin in children with clinical pertussis and evaluate the role of serology for the diagnosis of pertussis.

Study the immunogenicity and safety of an experimental monocomponent pertussis toxoid vaccine in infants.

Study the effect of the same vaccine against pertussis in a nonrandomised trial to see whether a large-scale efficacy trial of the vaccine would be justified.

Study the acquisition of IgG antibodies against two *Bordetella* antigens, FHA and pertactin, in children with no symptoms of pertussis.

Compare a functional assay (toxin neutralization) with an assay of affinity and concentration (ELISA) for pertussis toxin antibodies.

SUBJECTS

The five studies were performed in three study populations which included healthy children (I), children with pertussis (II) and children vaccinated with pertussis toxoid (III, IV, V).

1. Healthy children (I)

In 1984, 400 children from the 1980 birth cohort were selected using a random number list from the computer-based birth register of Göteborg, Sweden to be part of a pertussis incidence study [19]. The children represent about 10% of the 1980 Göteborg birth cohort of 4384 children. In 1989–1990 all children were traced and the parents of all but 13 children, who had left Sweden, could be contacted. The majority of the children still lived in Göteborg. Ten families had unlisted telephone numbers and did not answer a letter (plus reminder) explaining the purpose of the study. Thus, the parents of 377 children could be interviewed about the child's history of pertussis during 1990 after the child's 10th birthday. Five children had been vaccinated against pertussis. Of the 372 nonvaccinated children, 195 (52%) agreed to donate a venous blood sample for determination of IgG antibodies against pertussis toxin, FHA and pertactin. IgM and IgA antibodies against pertussis toxin were determined in sera with undetectable IgG antibodies.

2. Children with pertussis (II)

Children with symptoms suggestive of pertussis who were brought for medical attention from September 1987 to December 1990 at the Department of Paediatrics, Göteborg and at two paediatric outpatient clinics (Västra Frölunda and Askim) were invited to participate. A first serum sample was obtained from 165 children with possible pertussis. After the first follow up a total of 121 children were considered to have clinical pertussis with at least 3 weeks of paroxysmal cough and were willing to participate in the study. Sera were obtained at the first visit and 1, 3 and 12 months later from 71 children in Göteborg and 18 children at the outpatient clinics. These 89 children constitute the patient population. The median (range) duration of coughs when a serum was obtained were: Serum 1, 2 (0-8) weeks, Serum 2, 7 (4-17) weeks, Serum 3, 17 (12-37) weeks and Serum 4, 56 (47-86) weeks. Four sera were obtained from 52 (58%) children and 3 sera from 37 children. The median age was 18 months (range 1 month to 7 years). The serum samples were analysed for IgG antibodies against pertussis toxin, FHA and pertactin.

3. Vaccinated children (III, IV, V)

Healthy, full term infants of Swedish speaking parents with a telephone in their home were recruited consecutively from child health centres in Göteborg (n=64), Västra Frölunda (n=30), Mölndal (n=18) and Borås (n=34), Sweden to the safety and immunogenicity study of a pertussis toxoid vaccine (III). One hundred and forty-six children were recruited but one child left the study after 2 vaccinations since the parents did not want to submit the child to further blood samples. The remaining 145 children were vaccinated at 3, 5 and 12 months of age (n=76) or at 3, 5 and 7 months of age (n=69).

Serum samples were obtained from all children before the first and third vaccinations and 1 month after the third vaccination. The parents of 142/145 children (3 had moved from Sweden) were interviewed concerning symptoms of pertussis when the children were 2 and 3 years old and blood samples were obtained from 102 (70%) and 109 (75%) children, respectively (in 82 cases from the same child). All sera were analysed for IgG antibodies against pertussis toxin and for pertussis toxin neutralizing antibodies. IgM and IgA antibodies were determined in the first 3 sera from 20 randomly selected children from each group.

The study was not originally designed to contain an unvaccinated control group, but a retrospective study of pertussis at 3 years of age was performed, comparing the history of the 142 vaccinated children with that of 284 unvaccinated age matched controls (2 controls per vaccinated child) living in the same areas as the study children.

Sera obtained from the vaccinated children were analysed for IgG antibodies against FHA and pertactin (IV). In part one of the study 5 consecutive samples obtained between 3 and 36 months of age from 71 children were available. In part 2 sera from 109 3-year old children were available. The antibody titers from 12 children exposed to pertussis in the family were compared to the titers of the 97 unexposed children.

In the study comparing antibodies measured by a neutralization assay and ELISA (V) all available sera from children participating in the vaccine study (III) were used. Sera from 47 children participating in a safety and immunogenicity study of the same Ptxd vaccine in combination with tetanus and diphtheria toxoid vaccines were included [288]. From the 192 children a total of 796 sera in which both ELISA and neutralization assay were performed were available. No child contributed less than 3 sera.

METHODS

Interviews, work-sheets, questionnaires (I, II, III, IV)

Paper I

The study of the epidemiology of pertussis (I) was performed using a structured questionnaire with different questions pertaining to symptoms related to pertussis, diagnosis of pertussis, age at time of symptoms or diagnosis, family structure, pertussis in the family and vaccination against pertussis. The questions were asked over the telephone by the author of this thesis or by a specially trained nurse. The answers were registered on the work sheet during the interview. In a few cases the interview or part of it had to be repeated owing to inconsistencies or because the parent answering on the first occasion (usually the father) was unfamiliar with the child's medical history. Parents of children donating blood samples were asked at the time of the sample if the child had had "whooping cough" or symptoms compatible with pertussis. No child had pertussis in the time period between the telephone interview and the sampling and no parent gave a different answer at the telephone interview and the sampling.

Paper II

A combined questionnaire and work sheet was used in the study of antibody response and decline after pertussis (II). Children with suspected pertussis were recruited to a first serum sample and nasopharyngeal culture of *B. pertussis*. The physicians involved were instructed to ask for and note relevant information about the disease in the children's medical records. Three to 4 weeks later the parents were interviewed by telephone concerning their child's symptoms (cough, whooping attacks, vomiting) exposure to pertussis, concurrent disease, medication and previous vaccination against pertussis. Information about treatment with erythromycin or other antibiotics and hospitalisation was obtained from the child's medical record.

Paper III and IV

Several questionnaires and work sheets were used in the vaccine study (III). The parents were instructed to measure the child's temperature with a rectal thermometer 6, 24 and 48 hours after each vaccination and to inspect the injection sites daily. If the child's temperature rose they were instructed to continue takeing the temperature twice daily until it normalised. The parents were also instructed to measure any redness and/or swelling and to record the results daily on a work sheet together with any unusual reaction or symptom the child may have had. Three and 7 days after each vaccination the parents were interviewed by

telephone, using a structured questionnaire, about local reactions, temperature, convulsions, unusual or persistent crying, hypotonic-hyporesponsive episodes or any unusual reaction of the child.

The retrospective study of pertussis in vaccinated children and age-matched controls (III) was performed using a structured questionnaire concerning symptoms compatible with pertussis and exposure to pertussis. If a child with a cough or suspected pertussis had been seen by a physician, a letter was sent to the physician (with the parents' permission) asking for laboratory results and details of the symptoms. The information was also used in the study of acquisition of FHA and pertactin antibodies after Ptxd vaccination (IV, part 2).

Vaccination procedure (III)

The infants were given 0.5 ml of the Ptxd vaccine administered as a deep subcutaneous injection in the anterolateral part of the thigh according to the vaccination procedure for DT and inactivated polio vaccine in Sweden. DT vaccine was given in the other thigh on the same occasions as the Ptxd vaccine to children vaccinated at 3, 5 and 12 months of age. Polio vaccine was not given earlier than 7 days after the Ptxd vaccine at the child health center.

Culture of *B. pertussis*

Cultures were obtained with pernasal calciumalginate swabs. The sample was immediately transferred to a tube containing modified Stuart transport medium. The samples were transported to the Department of Clinical Bacteriology in Göteborg who perform all *B. pertussis* cultures from samples obtained in the Göteborg region. During the course of this study Lacey's culture medium was used by the laboratory.

ELISA for determination of serum antibodies to pertussis toxin

A modification of a previously described method [186] for detection of immunoglobulins to PT was used [169]. By precoating the microtiter plates with fetuin (a glycoprotein obtained from fetal calf serum) the sensitivity and the specificity of the ELISA was increased, since PT has a high and probably unique affinity to fetuin, [289] specifically to branched N-acetyl glucosamine residues [290]. The plates were incubated overnight after the test sera had been added and the conjugate was incubated for 4 hours. Pertussis toxin was obtained from Amvax, Inc., Beltsville, MD, USA. Its purity and biological activity was characterised using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), tricloacetic acid (TCA) precipitation, PT-

ELISA, hemagglutination and histamine sensitising activity and lymphocytosis-promoting activity in mice (Amvax Inc. data on file).

Sera in all studies were tested in duplicate in eight, 3-fold dilutions starting with the dilution 1/10. Sera from the same individual in a study were tested on the same microtiter plate. A blank and a reference serum were tested concomitantly. The reference serum in all studies was the United States Food and Drug Administration reference pertussis antiserum lot 3. The end-point titer of the reference serum was 10,000 and the unitage 200/ml. The OD-values of the sera to be tested were measured when the reference serum on the microtiter plate had reached the OD corresponding to the assigned titer value of the reference serum. The results were expressed in end-point titers and (studies III and V) units/ml. The end-point titer was defined as the highest serum dilution showing an OD of 0.2 above the OD of the blank. Units were calculated using the reference line method [170,171].

The reproducibility of the methods was calculated (T. Lagergård, The Gothenburg Pertussis Vaccine Study work manual for serological methods). The intra-assay variation was 14% for units and 17% for end-point titers and the inter-assay variation 26% and 28%, respectively. The intra-individual variations of 20 paired sera were also calculated and 3 standard deviations of the difference between sera was 2.2-fold. A 3-fold difference between acute and convalescent sera was considered significant.

ELISA for determination of serum antibodies to FHA

The method is very similar to the PT-ELISA but uses no precoating of the plates. It is a modification of a previously described method [189,291]. It was used with the same modification as the PT-ELISA regarding the incubation time of tested sera and of conjugate. Sera were tested in the same way as in the PT-ELISA. The purified FHA was obtained from Institut Pasteur-Mérieux, Marcy l'Étoile, France. Its purity, specificity and absence of pertussis toxin was demonstrated by SDS-PAGE, the CHO-cell assay, absence of lymphocytosis activity in mice and cross-enzyme-linked immunosorbent assay with pertussis toxin (F. Arminjon, Institut Pasteur-Mérieux, personal communication). The same reference serum as in the PT-ELISA was used; the United States Food and Drug Administration reference pertussis antiserum lot 3. The end-point titer of the reference serum was 10,000 and the units 200/ml. Results were expressed in end-point titers and units as described. The intra-assay variation were 12% and 21%, respectively and the inter-assay variation 29% and 27%, respectively. The intra-individual variation was the same as for the PT-ELISA, and a 3-fold difference between acute and convalescent sera was considered significant.

ELISA for determination of serum antibodies to pertactin

The pertactin ELISA has previously been described [194]. It is similar to the PT and FHA ELISA. Unspecific binding of immunoglobulin was blocked by incubation with 0.1% bovine serum albumin. Pertactin was obtained from SmithKline Biologicals, Rixensart, Belgium. Its purity was characterised using SDS-PAGE, Western blot and isoelectric focusing analysis. Trace amounts of adenylate cyclase-hemolysin, pertussis toxin, dermonecrotic toxin and endotoxins were looked for with specific assays [142]. Sera in all studies were tested according to the same procedures as in the PT and FHA-ELISA. The reference serum was the United States Food and Drug Administration pertussis antiserum lot 4. It is assigned 90 units/ml of pertactin IgG antibodies and the end-point titer was 700. The intra-assay and intra-individual variations were similar to those of the PT and FHA-ELISA (data on file) and a 3-fold difference between acute and convalescent sera was considered significant.

Pertussis toxin neutralization assay

The pertussis toxin neutralization assay is based on the specific ability of pertussis toxin to cause a clustering of Chinese hamster ovary-cells (CHO-cells) which can be inhibited by pertussis toxin neutralizing antibodies [180,292]. The method used has previously been described [293]. Patient sera were tested in duplicate in eight, 2-fold dilutions starting with the dilution 1/5 in 96-well tissue culture plates. CHO-cells were cultivated according to standard procedures. A 95% or greater viability of the cells was accepted for the assay. The working cell suspension contained $1x10^5$ cells/ml. Pertussis toxin was obtained by Amvax Inc. (see PT-ELISA for details). It was used in a working dilution of 5 ηg/ml. The reference used was the same as in the PT and FHA-ELISA; the United States Food and Drug Administration reference pertussis antiserum lot 3. The reference serum had a neutralizing titer of 1600 (starting with the dilution 1/50). However, it should be pointed out that lot 3 is a control serum but not an official reference serum with assigned unitage for the CHO-cell assay. After 2 hours incubation of CHO-cells and patient sera the medium was changed. This makes it possible to virtually avoid death of cells owing to unspecific factors in the patient sera, a well known phenomenon [293]. The plates were then incubated for 48 hours and the inhibition of the clustering induced by pertussis toxin was determined by the same experienced laboratory assistant using an inverted light microscope. The titer was defined as the reciprocal of the highest serum dilution (i.e. the dilution before the addition of the CHO-cells) giving 50% or more inhibition of the clustering of the cells. On a separate control plate dilutions of the reference serum and of pertussis toxin and cell growth controls were tested together with each run of patient sera.

Criteria for retesting of sera

Details for retesting of sera by ELISA or CHO-cell assay are specified in the Gothenburg Pertussis Vaccine Study work manual for serological methods. Basically, sera were retested if evident technical failure was present or if references, controls, blanks or assayed sera were out of range of stipulated values.

Pertussis toxoid vaccine

The pertussis toxoid vaccine was originally developed at the National Institute of Child Health and Human Development (NICHD) at the National Institutes of Health (NIH), Bethesda MD, USA. [294] The vaccine was produced by Selcore Laboratories (now Amvax Inc., Beltsville, MD, USA). The procedure was roughly as follows (Amvax Inc., data on file): The organism, *Bordetella pertussis* strain CS (a Chinese production strain for cellular pertussis vaccine), was cultivated in a 100 litre fermentor for 40 to 48 hours. A sample was taken to determine the purity of the culture by Gram stain and by culture on different media. The supernatant was removed and the toxin adsorbed to Affi-Gel Blue. The adsorbed toxin was removed by washing of the Affi-Gel Blue in a column. Collected fractions were analysed for pertussis toxin, and the toxin containing fractions were adsorbed to a column containing fetuin-sepharose. The procedure of washing and collection of toxin containing fractions was repeated several times until a pure fraction with a high pertussis toxin concentration was obtained.

Homogeneity was characterised using SDS-PAGE, protein content, hemagglutination activity, histamine sensitising activity and lymphocytosis-promoting activity. The pertussis toxin was irreversibly detoxified by exposure to hydrogen peroxide (H_2O_2). This treatment results in a measurable irreversible oxidation of 3 amino acids of the pertussis toxin protein; cystein, methionine and tyrosine. About 40% of the antigenicity of the pertussis toxin is retained [294]. The vaccine contained 25 µg of pertussis toxoid adsorbed to 0.5 mg aluminium hydroxide (Al(OH)3) in a volume of 0.5 ml.

Statistics

Statistical calculations were performed on a personal computer using the program Statistical Analysing Systems (SAS) in the vaccine study (III) and Stat View 4.02 or 4.5 (Abacus Concepts, Inc., Berkeley, CA) in the other studies. Statistical expertise was consulted when needed. Parametric and non-parametric tests were used as deemed appropriate. Comparisons between groups were performed with the unpaired t-test and/or Mann-Whitney U-test. Comparisons within groups were performed with the paired t-test and/or Wilcoxon signed rank test. Proportions were compared with the Chi-square test. Linear and polynomial regression, Spearmans correlation and analysis of variance were performed in the study of correlation between ELISA and neutralization assay (V).

ETHICS

All parents of participating children consented to allowing their child to participate in a study after having received oral information about the study. Written information was also given before parents consented to their child's participation in the study of antibody response after clinical pertussis (II) and in the vaccine studies (III and reference [288]). All studies were approved by the Ethics Committee of Göteborg University. The vaccine studies were approved by the Swedish Medical Products Agency, the National Institutes of Health, Bethesda, MD (protocol 86-CH-93 and 91-CH-67) and the United States Food and Drug Administration (BB-IND-2329 and 3853).

RESULTS

Incidence of clinical pertussis (I)

The incidence per year and the cumulative incidence of clinical pertussis in 10-year-old nonvaccinated children in Göteborg is presented in Figure 1. Of the 372 children 226 (61%) had experienced whooping cough by the time they were 10 years old according to their parents. The median age at the time of disease was 4 years.

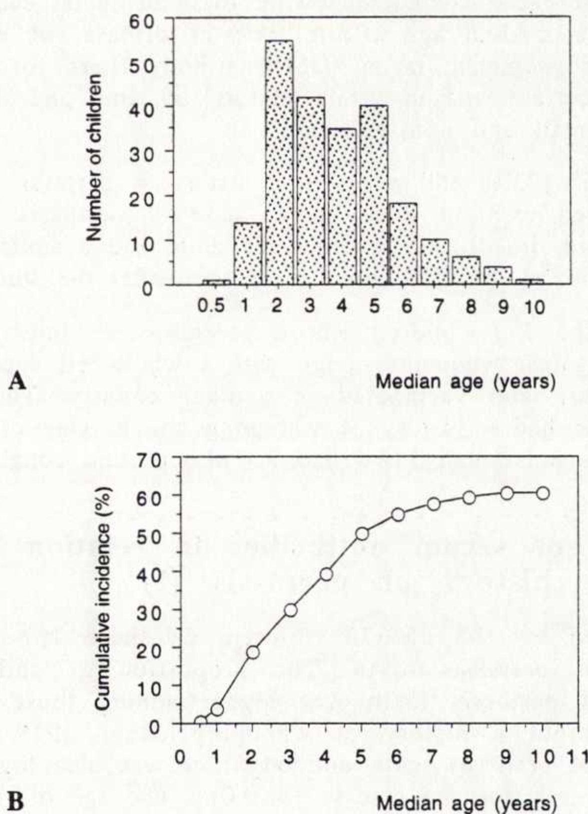


Figure 1. Number of children with a history of pertussis in relation to approximate median age at the time of disease (A) and cumulative incidence of clinical pertussis in relation to approximate median age at time of disease (B).

All children with a history of pertussis had had symptoms compatible with the diagnosis. The median time of paroxysmal coughing was 6 weeks (range 4 to 28 weeks). One hundred and eighty-seven children (83%) had had whooping attacks and 150 children (67%) had had cough

with vomiting. One hundred and sixty-six children (73%) had been seen by a doctor during the disease and in 68 (30%) cases the disease had been verified by culture. Five children (2%) had been hospitalised during the disease, which was verified from the children's hospital records. The median duration of hospitalisation was 2 days, range 1 to 5 days.

Many parents did not recollect the duration of the cough or the time of the disease with great accuracy, but in 93 (41%) cases the parent was quite sure of these facts, sometimes owing to coincidental social events. The differences in median age at the time of disease, of duration of symptoms and of geometric mean (GM) antibody titers for groups of children with a certain and uncertain history of time and duration of symptoms were small and nonsignificant.

The parents of 165 (73%) children with a history of pertussis stated that the child had been exposed to a known case of pertussis before the disease of the child. In 101 (45%) cases the child had a sibling with the same disease before, at the same time as or soon after the study child.

Five (1.3%) of the 377 children whose parents were interviewed had been vaccinated against whooping cough with a whole-cell vaccine and 3 of them were born and vaccinated in another country. Three of the vaccinated children had a history of whooping cough. One of them had whoops and prolonged coughs, two had no whoops and coughs of short duration.

Presence of serum antibodies in relation to history of pertussis (I)

Sera were donated by 195 of 372 children. Of these 157 (80%) had antibodies against pertussis toxin. The proportion of children with antibodies against pertussis toxin was higher among those with than among those without a history of whooping cough (91% vs. 64%, $p<0.001$). The GM pertussis toxin antibody titer was also higher among the children with a history of disease ($p<0.01$). The age of the child at the time of disease or the severity of the disease did not differ between the children with and without detectable pertussis toxin IgG antibodies. IgM and IgA antibodies against pertussis toxin were assayed for, but not detected, in sera from all children with undetectable pertussis toxin IgG antibodies.

Table 4 shows the prevalence and the GM IgG titers of antibodies against the three *B. pertussis* antigens in the 195 children who donated serum.

All 195 children had antibodies against pertactin and 192 (98%) against FHA. The GM antibody titers against the two antigens were similar in

children with and without a history of whooping cough and in children with (n=157) and without (n=38) detectable antibodies against pertussis toxin (GM FHA; 658 versus 296, pertactin; 151 versus 187, respectively).

Table 4. Serum IgG antibodies against pertussis toxin, FHA and pertactin in 195 nonvaccinated 10-year-old children.

	History of clinical pertussis		
	Yes (n=119)	No (n=76)	Total (n=195)
Prevalence of IgG antibodies			
Pertussis toxin	108 (91)* a**	49 (64) b	157 (80)
FHA	117 (98)	75 (99)	192 (98)
Pertactin	119 (100)	76 (100)	195 (100)
Geometric mean IgG antibody titers			
Pertussis toxin	539 c	91 d	269
FHA	723	577	662
Pertactin	171	198	181

* Numbers in parentheses, percent.

** a vs. b, $p < 0.001$; c vs. d, $p < 0.01$

Serum antibodies in children with clinical pertussis (II)

All 89 children fulfilled the WHO clinical pertussis criterion of at least 21 days of paroxysmal cough [156]. They are reported in two groups.

1. Children with culture confirmed pertussis (n=48) or culture confirmed pertussis in a family member (n=6)

Antibody response

Of all 54 children with directly or indirectly culture confirmed pertussis 45 (83%) had significant increases in pertussis toxin antibodies and 40 (74%) in FHA antibodies, while only 29 (54%) had significant increases in pertactin antibodies, (pertactin versus PT and FHA, $p < 0.05$). Table 5 shows the median and range of IgG antibody titers in relation to time after onset of cough in 89 sera obtained within 8 weeks of onset in the 54 children. Of the 19 children from whom the first serum was obtained within 14 days of symptoms, 9 (47%) had detectable pertussis toxin antibodies and 13 (68%) had detectable FHA antibodies. All had detectable pertactin antibodies.

Table 5. Median serum pertussis toxin, FHA and pertactin IgG titers in 89 sera obtained within 8 weeks after onset of cough from 54 children with culture-confirmed pertussis or culture-verified family exposure in relation to disease duration (from 35 children two sera are included).

	Median (range) antibody titers		
Disease duration	<2 weeks	2-4 weeks	5-8 weeks
Number of sera	19	33	37
Pertussis toxin-IgG	5 (<10-891)[a]*	1788 (<10-63,096)[b]	25,119(<10-100,000)[c]
FHA-IgG	25 (<10-224)[d]	158 (<10-2818)[e]	1585 (71-14,125)[f]
Pertactin-IgG	112 (18-1259)[g]	200 (11-23,442)[h]	1413 (45-25,119)[i]

* a vs. b, p <0.005; b vs. c, d vs. e, e vs. f, g vs. i, h vs. i, p <0.001; g vs. h=ns.

Significant increases in IgG antibodies against all three antigens were seen in 25 (46%) of the children, in pertussis toxin and FHA IgG titers in 12 (22%), and in pertussis toxin or FHA IgG titers alone or in combination with pertactin IgG titer in 11 (20%) of the children. No child had a response in pertactin antibodies alone.

The median values of the highest antibody titers, all obtained within 6 months of onset of cough were as follows: pertussis toxin 22,387, FHA 1995 and pertactin 1318. Two children had maximum pertussis toxin titers below 1000 (arbitrary cut-off) while 16 had FHA, and 25 had pertactin IgG maximum titers below 1000.

Six children had antibodies against all three antigens but no significant antibody response. The median value of the highest IgG pertussis toxin, FHA and pertactin titers for these children were 4050, 3025 and 2925, respectively.

In most children the peak antibody titers occurred 5 to 8 weeks after onset of symptoms. There were, however, wide inter-individual variations both in the interval between onset and the peak and in the magnitude of the IgG antibody response to the three antigens.

Antibody decline.

Table 6 shows the median and range of the highest IgG antibody titers measured during the first 12 weeks after onset, compared with sera obtained after at least 50 weeks from the 39 children from whom such sera were obtained. The antibody titers against all 3 antigens decreased significantly. The decrease in median FHA IgG titer was less pronounced (1.8-fold) than that in pertussis toxin and pertactin IgG titers (4-5–fold). Thirty-four of 54 (63%) children had significant decreases in pertussis

toxin IgG titers, 28 (52%) in pertactin IgG titers but only 9 (17%) children in FHA IgG titers.

Table 6. Decrease in median IgG titers between sera obtained ≤12 and ≥50 weeks after onset of cough in 39 children with culture-confirmed pertussis or culture-verified family exposure. If two sera were available ≤12 weeks after onset of cough, the serum with the higher titer was selected.

	Median (range) antibody titer in relation to duration since first cough	
	≤12 weeks	≥50 weeks
Pertussis toxin-IgG	22,387 (<10-100,000)[a] *	4169 (224-25,704)[b]
FHA-IgG	1995 (71-14,125)[c]	1122 (28-12,589)[d]
Pertactin-IgG	1259 (56-25,119)[e]	282 (22-6607)[f]

* a vs. b, e vs. f, $p<0.0001$; c vs. d, $p<0.01$

The response and decline of pertussis toxin, FHA and pertactin IgG antibody titers in relation to median time after onset of cough are depicted in figure 2. It should be noted that the duration of symptoms when a serum was obtained varied among children as presented in the "Patients" section, and there was some overlapp in the duration of symptoms between children donating the first and second serum and the second and third serum.

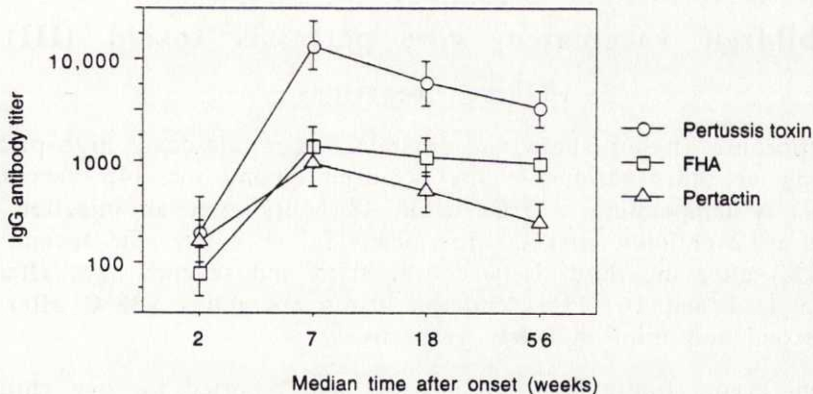


Figure 2. Geometric mean and 95% confidence interval (error bars) of serum IgG antibodies against pertussis toxin, FHA and pertactin in 54 children with culture-confirmed pertussis or culture-confirmed family exposure in relation to median time after onset of cough.

Subgroup comparisons

There were no significant differences in the maximum pertussis toxin, FHA or pertactin IgG titers or in the titers one year after onset of disease related to age (<6 months or older), erythromycin treatment, sex or hospitalisation.

2. Children with negative cultures (n=25) or from whom cultures were not obtained (n=10) and without culture confirmed family exposure to pertussis

Antibody response

Of the 35 children, 17 (49%) had significant increases in pertussis toxin and/or FHA IgG antibodies and thus fulfilled the serological criteria for the WHO definition of pertussis. Fourteen (40%) had significant increases in pertussis toxin antibodies and 15 (43%) in FHA antibodies. Only 6 (17%) children had increases in pertactin IgG antibodies, all with concomitant increases in pertussis toxin or FHA antibodies or both.

Antibody decline

Of the 18 children without significant increases in IgG antibodies significant decreases in pertussis toxin, FHA or pertactin IgG titers were seen in 11 (61%), 6 (33%), and 7 (39%) respectively. Five children had no significant pertussis toxin or FHA antibody decrease. Four of them had detectable pertussis toxin IgG antibodies (range <10-5012) or FHA IgG antibodies (range 16-1000) or both in all or some of their sera. All 5 had pertactin IgG antibodies (range 32-25,119).

Children vaccinated with pertussis toxoid (III)

Adverse reactions

No hypotonic hyporesponsive episodes, convulsions, high-pitched screaming or other serious events occurred among the 145 vaccinated children. A temperature >38°C within 48 hours after an injection was recorded in 2 children after the first injection, in 2 after the second and in 5 (3%) after the third. Between the third and seventh days after an injection 1, 4 and 16 (11%) children had temperatures >38°C after the first, second and third injection, respectively.

Persistent crying (more than 30 minutes) was reported for one child in Group 1 after the third vaccination, when pertussis toxoid vaccine only was administered, and for 3 children in Group 2 who received DT vaccine in the other leg at the same time. A total of 2 and 5 children cried for more than 30 minutes after the first and second vaccinations, respectively. Unusual fretfulness during 1 to 3 days was reported for 15% to 30% of children after vaccinations. Other recorded side effects

including crying, sleeping or vomiting more than usual, and eating less than usual were recorded for 0% to 20% of children after vaccinations (the higher frequency for "sleeping more than usual"). Most reported events tended to occur more frequently after the second or third vaccination.

Table 7 show the numbers of children with local reactions. The frequencies of redness, swelling and pain were similar to those after a DT vaccine given concomitantly in the other thigh in children vaccinated at 3, 5 and 12 months of age.

Table 7. Numbers of children with local reactions of 145 children vaccinated with pertussis toxoid (Ptxd) and diphtheria-tetanus toxoids (DT) in different legs. (Group 1: n=69; Group 2: n=76).

	Redness ≥2cm		Swelling ≥2cm		Tenderness, 1–3 days	
Vaccine	Ptxd	DT	Ptxd	DT	Ptxd	DT
After injection 1 (Group 1+2)	16	13	10	11	21	14
After injection 2 (Group 1+2)	25	56	19	35	19	28
After injection 3 (Group 1)	14	—*	9	—*	4	—*
After injection 3 (Group 2)	18	20	15	18	17	20

* The third injection of DT not given at the same time as pertussis toxoid in this group.

Antibody response

Figure 3 shows the pertussis toxin IgG antibodies in the two groups vaccinated at 3, 5 and 7 (Group 1) or at 3, 5 and 12 (Group 2) months of age. In 59 of 144 (41%) prevaccination sera antibodies in low titers (GM 93, range 10–2,512) probably of maternal origin, were detected. All children had IgG and pertussis toxin neutralizing antibodies against pertussis toxin in sera obtained at the time of the third injection, 2 months (Group 1) or 7 months (Group 2) after the second injection. After the third injection a significant antibody response was seen in both groups. One month after the injection (Serum 3) the GM IgG titers were 4611 (range 1259–17,783) in Group 1 and 7256 (range 794–316,228) in group 2 (Group 1 vs. Group 2; $p<0.001$).

The GM neutralizing antibody titers were 175 (range 80–>640) and 270 (range 80–>640) in Group 1 and Group 2, respectively.

At three years of age (serum 5) the GM IgG antibody titers were 180 (range 12–2512) and 330 (13–2188) in Group 1 and Group 2, respectively (Group 1 vs. Group 2; $p <0.01$).

IgM antibodies were found in 33 of 40 samples after the third injection. The highest IgM titer was 417. IgA antibodies were detected only after the third injection in 9 of 40 samples. The highest IgA titer was 57.

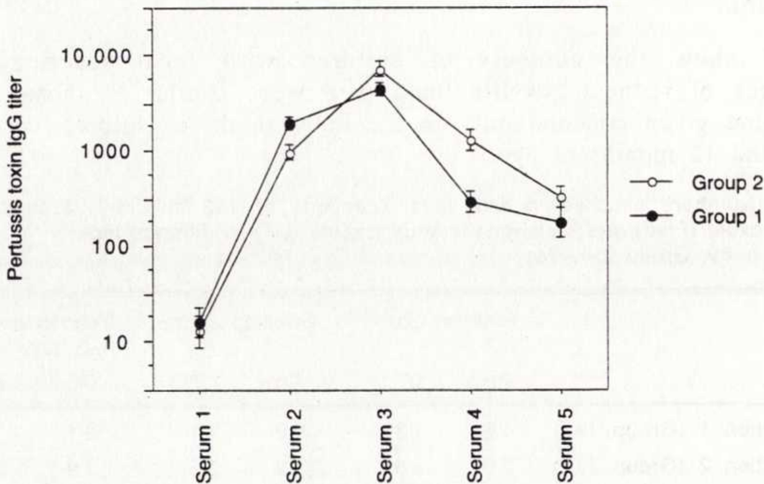


Fig 3. Geometric mean and 95% confidence intervals (error bars) of serum IgG antibodies against pertussis toxin in 145 children vaccinated with a monocomponent pertussis toxoid vaccine at 3, 5 and 7 months (Group 1) or at 3, 5 and 12 months (Group 2) of age. *Symbols* indicate samples (serum 1—5) obtained from children in Group 1 at 3, 7, 8, 24 and 36 (●) or from children in Group 2 at 3, 12, 13, 24 and 36 (○) months of age.

Retrospective comparison of pertussis in vaccinated children and in control children matched for age and area of residence

None of the vaccinated children had pertussis by 3 years of age according to the clinical definition of at least 6 weeks of paroxysmal cough with whooping attacks, vomiting or both. A 1-year-old child coughed for 2 weeks and a 2-year-old child for 4 weeks without whoops or vomiting. From one of them *B. pertussis* was cultured and the other had significant increases in pertussis toxin and FHA antibodies in paired sera. Sixteen vaccinated children had siblings with pertussis according to the definition, in 6 cases culture verified. Two of the vaccinated, family-exposed children developed verified pertussis as described above. The other 14 developed no cough according to their parents.

Of the 284 control children 57 (20%) had experienced pertussis according to our definition. The parents of 37 children claimed that a diagnosis of pertussis had been made by a doctor, which was verified from clinical records in 35 cases. In 22 cases cultures were taken, and *B. pertussis* was isolated from 12 of them.

Table 8 show the numbers of vaccinated children and controls with pertussis according to our definition.

Table 8. Age distribution of children with pertussis* according to their parents among 284 nonvaccinated children and of mild but laboratory-verified *Bordetella pertussis* infection among 142 children vaccinated with a monocomponent pertussis toxoid vaccine.

Age (months)	Nonvaccinated (n = 284)	Vaccinated (n = 142)
3–11	6	0
12–23	28	1 (2 weeks cough)
24–35	23	1 (4 weeks cough)
Total	57	2

* Defined as ≥6 weeks of cough with whooping attacks, vomiting or both.

Acquisition of FHA and pertactin antibodies (IV)

Part 1

The FHA and pertactin IgG antibody titers in 71 Ptxd vaccinated children without evidence of *B. pertussis* infection from whom 5 consecutive sera were obtained between 3 and 36 to 39 months of age are presented in figure 4.

All but one child had FHA antibodies, and 51 (72%) had pertactin antibodies (probably of maternal origin) in the first serum sample. The antibody titers declined initially, but from about 1 year of age there were small but significant increases in GM antibody titers against both antigens. The GM antibody titers (with ranges in parenthesis) for all 71 children at 2 and 3 years of age were 102 (11 to 7943) and 151 (14 to 6457), respectively, for FHA and 25 (<10 to 398) and 26 (<10 to 871), respectively, for pertactin IgG antibodies. Four children had undetectable pertactin antibodies in all 5 sera. None had undetectable FHA antibodies in all 5 sera. At 3 years of age all children had detectable FHA antibodies, whereas 58 of 71 (82%) children had detectable pertactin antibodies.

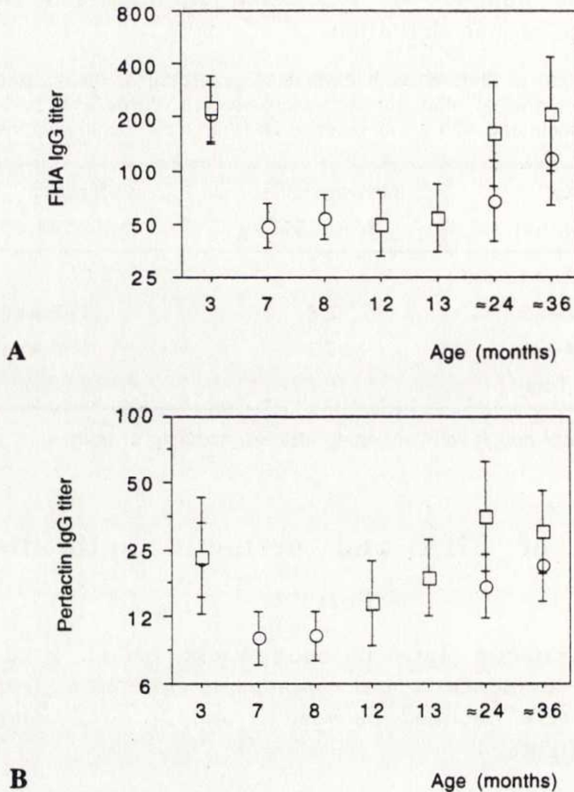


Figure 4. Geometric mean and 95% confidence intervals (error bars) of serum IgG antibodies against FHA (A) and pertactin (B) in 71 children vaccinated with a monocomponent pertussis toxoid vaccine and with no history of pertussis. Symbols indicate samples from children vaccinated at 3, 5 and 7 (○) or 3, 5 and 12 (□) months of age. Note the different scales of the y-axis in A and B.

Part 2

The GM IgG serum antibody titers at 3 years of age in 12 children exposed to pertussis by siblings and in 97 children without family exposure to pertussis is shown in table 9. The exposed children had significantly higher GM FHA antibody titers than the unexposed children (407 vs. 87, $p<0.05$). There were no significant differences in pertactin or pertussis toxin antibody titers.

Seven (6%) children had undetectable FHA antibodies and 20 (18%) had undetectable pertactin antibodies. These children were proportionally distributed between the 2 groups. All children had detectable pertussis toxin antibodies.

Table 9. Serum pertussis toxin, FHA and pertactin IgG titers at 3 years of age in 109 asymptomatic children who had been vaccinated with a monocomponent pertussis toxoid vaccine in relation to family exposure to pertussis.

	Geometric mean titers	
Antigen	Family exposure to pertussis (n=12)	No family exposure to pertussis (n=97)
Pertussis toxin	204 (102, 417)*	257 (200, 324)
FHA	407 [a] (115, 1445)	87 [b] (58, 132)
Pertactin	23 (11, 47)	31 (23, 40)

* Numbers in parentheses, 95% confidence intervals.
a vs. b, p<0.05

Comparison of a toxin neutralization assay and ELISA for determination of pertussis toxin antibodies (V)

The pertussis toxin IgG end-point titers for the 796 sera ranged from <10 to 65,610. The GM was 566 and the median 1000. The titers expressed in units/ml ranged from <1 to 1540. The GM was 19 and the median 18. The neutralizing antibody titers ranged from <5 to >640. The GM was 44 and the median 40.

Comparison of end-point titer and units/ml

Figure 5 show that there was a high degree of linear correlation between IgG antibodies expressed in end-point titer or units/ml ($r=0.97$, $p< 0.0001$).

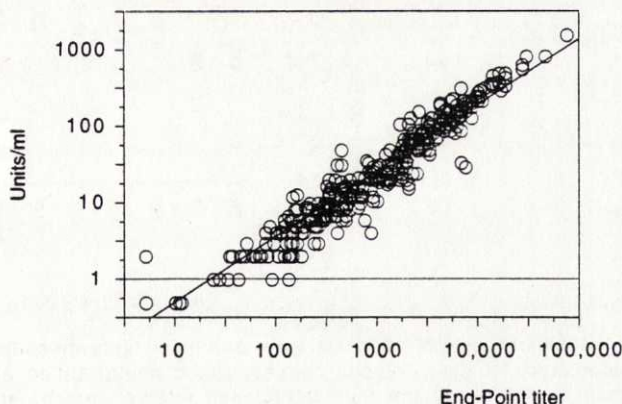


Figure 5. Simple regression model of pertussis toxin IgG antibodies expressed in end-point titer and units/ml in 796 sera from children vaccinated with a pertussis toxoid vaccine. A circle may represent more than one identical value.

Comparison of neutralizing antibodies and IgG antibodies

Comparisons of toxin neutralizing antibodies and IgG antibodies expressed in end-point titer or units/ml yielded similar results. Only comparisons with end-point titer are reported below. The optimum fit was a polynomial regression of the second power using log10 transformation of both variables (r=0.87), but it did not differ substantially from a simple linear regression model with r=0.84 ($p<0.0001$). Spearman's Rho corrected for ties =0.82.

Sera with a neutralizing antibody titer of <5 could be predicted from an end-point titer <10 in 78/90 (87%) cases. Sera with a neutralizing antibody titer of >640 could be predicted from an end-point titer >10,000 (arbitrary value) in 8/21 (38%) cases. The corresponding values for predicting ELISA end-point titers from neutralizing antibody titers were 45/93 (48%) and 8/40 (20%), respectively.

Analysis of variance showed a significant interaction between end-point titer and neutralizing antibody titer ($p<0.0001$) presented in Figure 6. Post hoc testing with Fischer's protected least significant difference test showed a significant difference ($p<0.05$) between groups, except between groups with a neutralizing antibody titer ≥320 and between the 2 groups with a neutralizing antibody titer of 160 and >640.

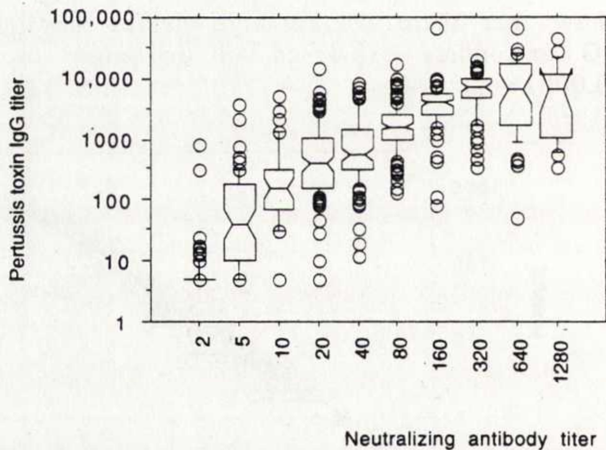


Figure 6. Interaction box plot of pertussis toxin end-point titers measured using ELISA as effect of the titer steps of the CHO-cell pertussis toxin neutralization assay. Boxes show 25th and 75th percentiles and the 95% confidence interval (notch) around the median. Error bars show 10th and 90th percentiles. Values outside the percentile interval are plotted separately. A circle may represent more than one identical value. Sera with a neutralizing antibody titer <5 were assigned the value of the previous titer step and sera with an antibody titer >640 were assigned the value of the subsequent titer step.

DISCUSSION

Pertussis epidemiology

Incidence of pertussis

It is shown in study I that 61% of 10-year-old, nonvaccinated children had a history of pertussis and that their parents could describe symptoms compatible with the disease. This cumulative incidence is similar to the pre-vaccination figures of 64% and 51% reported by Ström from Stockholm in 12-to-13-year-old children born 1939 and 1949. [14,15] Similar figures have been reported from Italy, a country with a low vaccination rate of about 8–12% [7,295,296]. The cumulative incidence at approximately 4 years of age (about 30%) is very similar to the figures reported in a previous Swedish retrospective interview study of young children born in 1980 [21].

The cumulative incidence of clinical pertussis thus seems to be as high as it was some 50 years ago, in the prevaccination era. If there was a decline in the incidence of pertussis before the vaccination era, as has been claimed by some [259,297] it does not seem to have continued during the latter part of the century. Nor have improved socioeconomic conditions had an influence, as was indicated in previous studies [15]. The histories of the children in study I indicate that most had an uncomplicated, although sometimes protracted course of the disease since only 5 (2%) (2 of them culture verified) of the children had been hospitalised and the records showed that none of them had been severely affected. This figure is less than the proportion of hospitalised culture verified cases reported for the years 1981–83 (7%) [4] and in a study from 1986 (5%) [38]. The frequency and duration of cough, whoops and vomiting are similar to those reported in a longitudinal study of 61 Swedish children with culture verified pertussis [38], and the parents' information on the child's symptoms in the present study thus seems fairly reliable.

Serologic evidence of pertussis

The parental information of a child's positive history of clinical pertussis is reliable, as shown in this study, albeit in a nonvaccinating country with endemic clinical pertussis. In 108/119 (91%) children with a history of pertussis IgG antibodies against pertussis toxin was detected. Previous studies from Sweden [21,298] and Italy [295,296] have shown similar figures. Cross-reacting antibodies against pertussis toxin have not been described, and although *B. parapertussis* may cause a pertussis-like disease [80], the organism does not produce pertussis toxin [64]. The prevalence of pertussis toxin antibodies in a nonvaccinated individual is thus a reliable sign of infection with *B. pertussis*. Children with a positive history of pertussis but undetectable

pertussis toxin IgG may have had another respiratory disease mistaken for pertussis. It is also possible that with time the antibodies may decrease below the level of detection in some patients.

A majority of children with a negative history of pertussis, 49/76 (64%), had pertussis toxin IgG antibodies. In some cases the parents may have forgotten an uncomplicated whooping cough but it is unlikely that they would forget if the child had typical disease, considering its impact on families [38]. Probably these children had a mild or abortive form of pertussis. Early studies indicated that about 27% of nonvaccinated children had mild or subclinical pertussis [34], and similar figures have been reported in nonvaccinated children in Italy [295,296] and Sweden [21]. In the present study 49/195 (25%) children had serological evidence of unrecognised pertussis. The data can also be interpreted to mean that in 49/157 (31%) of children with serological evidence of *B. pertussis* infection (detectable pertussis toxin IgG antibodies) the exposure to the organism resulted in a mild or subclinical infection.

IgG antibodies against pertussis toxin were detected in a total of157 (80%) of the 195 10-year-old children donating blood samples. This means that this proportion of children had had a *B. pertussis* infection. It is interesting that the prevalence of pertussis toxin IgA antibodies (an antibody class not induced by vaccination with pertussis vaccine [183,299]) was detected in 67% and 63%, respectively, of whole-cell vaccinated American and partly (approximately 40%) vaccinated German young men [245]. That study, and other studies from England and the USA, [6,243] indicate that pertussis infection is almost as prevalent in pertussis whole-cell vaccinating countries as in Sweden. The incidence of clinical infection, i.e. typical whooping cough, is much lower in vaccinating countries as indicated by the morbidity statistics in various countries. This is probably due to the fact that vaccination with whole-cell vaccines and acellular vaccines modifies the disease and results in less severe symptoms compared with nonvaccinated controls [34,35,269,275]. This can also be interpreted to mean that the vaccines protect more against disease than against infection [6].

Indications of the pertussis incidence after childhood

It is not possible to draw firm conclusions from study I about the number of children who will have detectable antibodies or a history of pertussis at adult age. The decreasing slope of the cumulative incidence curve (Figure 1B) and the study by Ström in the prevaccinating era [15] indicate that only a small additional proportion of children will have clinical pertussis during adolescence or as adults. A study from Italy also showed that few clinical cases were reported above 10 years of age, but the seroprevalence of pertussis toxin IgG increased from 83% in 10-to-11-year-old children to 95% in the 18-to-19-year-olds [295]. It is

unknown whether the high seroprevalence but low reported incidence of clinical pertussis reflects a milder form of disease in higher age groups, as in previously vaccinated adults [31,244,246], or is a result of underreporting in these age groups. Culture verified pertussis in Swedish adults does not, however, have a mild course. Of 174 patients 82% had whooping attacks and 65% had to stay at home from work for 2 to 4 weeks [200].

Presence of antibodies against FHA and pertactin

It was an unexpected finding that all children had detectable pertactin IgG antibodies and almost all (98%) had IgG antibodies against FHA. Furthermore, there was no difference in the GM FHA and pertactin antibody titers between children with or without a history of pertussis. In a Swedish study from 1982 the seroprevalence of FHA IgG antibodies in 350 healthy persons (a large proportion assumed to have been vaccinated with whole-cell vaccine) aged 0 to >60 years were investigated [94]. Only 25% of children had detectable FHA IgG at 2 years of age although 80–90% had received 3 doses of whole-cell vaccine. A larger proportion of older children had FHA antibodies and about 90% of teenagers had detectable FHA IgG. In another Swedish study 36% of 175 nonvaccinated children 1–4 years old without a history of pertussis had antibodies against FHA, and 14% had antibodies against pertussis toxin [298].

Methodological impact on serological results

In ELISA studies of serum antibodies a cut-off point is arbitrarily selected or is based on some statistical value. If a high cut-off point (i.e. a high dilution of the sample) is used, sera with lower antibody concentrations will be considered negative. In studies of serum antibody responses against pertussis toxin and FHA using the same ELISAs as in the present study, titers below the dilution 1:100 (the first dilution) were arbitrarily considered negative [188,189]. Other initial dilutions have been used, e.g. 1:36 [183] and in some incidence studies the sera have been tested in single dilutions, e.g. 1:100 [295] or 1:1000 [94]. It could be argued that a higher cut-off point should have been used in the ELISAs in the studies of this thesis. However, since no correlation between protection and serum antibody titers have been defined [219,270,275], and since it is possible that antibody concentrations with time decrease to undetectable levels, it was justifiable to analyse sera in a first dilution of 1:10. Since highly purified antigens were used, a blank was analysed in all assays and a cut-off point of 0.2 OD above the OD of the blank was used, any unspecific binding was considered to be minimal and not to interfere with the calculations of the titers in sera with low antibody concentrations. In recent large vaccine studies a minimal level of detection has been set at 1 or 2 units/ml [283,284,300].

These levels correspond to an end-point titer of 3–5 according to the present study of antibody correlations (V).

Hypothesis about antibody prevalence and protection

The study shows that virtually all 10-year-old, nonvaccinated children in Sweden have acquired IgG antibodies against FHA and pertactin, irrespective of history of pertussis. There was no difference in the antibody titers of children with and without a history of pertussis. Since most children with pertussis had the disease several years before the time of the sampling, the antibody titers would be expected to be low. However, children with a history of pertussis had significantly higher pertussis toxin IgG titers than children without a history of pertussis.

This could be interpreted to mean that pertussis toxin, but not FHA or pertactin IgG, is a major mediator of prolonged immunity against disease. This is consistent with previous observations. In a Swedish study of serum IgG antibody titers against pertussis toxin, FHA and lipopolysaccharide, sera were obtained a median of 220 days before culture verified pertussis in 94 female patients. [301] The authors concluded: ".... that antibodies to different antigens all participate in protection against disease but that antibodies to pertussis toxin play a major role." The authors of another Swedish study of pertussis toxin and FHA IgG, IgM and/or IgA antibodies in 79 children whose parents were interviewed 2 to 4 years later about their children's history of pertussis reached the same conclusion [298]. It is possible that antibodies induced by other antigens (e.g. fimbriae) of *B. pertussis* contribute at least to short term protection, as indicated by vaccine studies [283].

Crossreacting antibodies

Since about 20% of children in study I had IgG antibodies against FHA and pertactin but undetectable pertussis toxin IgG, IgA or IgM, it is possible that the FHA and pertactin antibodies were induced by other organisms than *B. pertussis*. This is discussed below.

Antibody titers after clinical pertussis

Antibody responses

Pertussis toxin and FHA

It has already been shown that antibodies against pertussis toxin and FHA develop in most patients after a *B. pertussis* infection. However, most studies have had a short follow-up or few included patients and have usually not looked at the dynamics of the response and the decline of antibodies. In many studies a majority of patients have previously

been vaccinated with whole-cell vaccine. Only a few small studies have looked at the pertactin antibody responses. The study of antibody responses to pertactin was recently defined as a research priority by a meeting organized by WHO [156].

Study II confirms that almost all nonvaccinated children (83%) with culture confirmed infection or family exposure to pertussis have a significant IgG antibody response against pertussis toxin. The proportion of children with an IgG antibody response against FHA was somewhat lower (74%). This indicates that the diagnostic sensitivity of pertussis toxin IgG serology is somewhat higher than of FHA IgG serology. Almost identical figures of pertussis toxin and FHA antibody responses have been reported in other Swedish studies [188,189,191].

Pertactin

Only 54% of the children had an increase in pertactin antibodies and none of them had an increase in pertactin IgG alone. Neither did any of the remaining children with clinical pertussis not verified by culture or family exposure according to the WHO case definitions [156] have an increase in pertactin IgG alone. The conclusion is that pertactin antibodies can be detected very shortly after onset of symptoms of pertussis and that the addition of pertactin IgG serology to the case definition would not have increased the number of positive pertussis diagnoses.

The conclusions of this thesis about pertactin are supported by the results of previous studies. In a study of 19 infants with pertussis only 1 had pertactin antibodies in the convalescent serum measured by Western blot [302]. In a small study including 6 nonvaccinated children none had pertussis toxin antibodies in the initial serum but all had pertussis toxin antibodies in the convalescent sera detected by immunoblotting. In contrast, very low levels of pertactin antibodies were detected in almost all sera, irrespective of duration of symptoms [182]. Another study also detected pertactin IgG in sera from all 206 1-to-4-year-old children studied. However, in this study children with a history of pertussis had higher GM pertactin IgG antibody titers than children without a history of pertussis [194].

Subgroup comparison

There were no significant differences in the antibody responses of children <6 months old or older indicating that maternal antibodies (if present) did not interfere with the antibody responses. In study III, 41% of the 3-months-old children had maternally derived pertussis toxin IgG antibodies. In study IV, 100% and 72% of children had FHA and pertactin IgG serum antibodies, respectively, at 3 months of age. Nor were there any differences in the antibody responses of children

irrespective of whether they were treated with erythromycin. In a previous study, children treated with erythromycin within 14 days of onset of symptoms had somewhat lower pertussis toxin IgG antibody titers than children not treated [60]. In that study the symptoms of the children are not reported. In the present study all children had clinical pertussis with at least 3 weeks of cough.

Influence of duration of symptoms

As could be expected, the possibility of detecting an increase in antibody titer was dependent on the duration of disease when the first sample was obtained. In most children the peak of antibody titers occurred 5 to 8 weeks after onset of cough. To diagnose an increase in antibody titers the first serum has to be obtained some weeks before the peak. This is not always possible in the clinical setting since the onset of disease is often insidious and patients may not seek professional medical help until the cough has persisted for some time or the patient has come to the convulsive stage. It should also be observed that in vaccinated children who have been primed against the antigen(s) in the vaccine, there will be a much faster secondary antibody response.

Antibody decreases

We postulated that a significant decline in antibody titers could be used as a diagnostic criterion in patients who come late for diagnosis. A significant decline in pertussis toxin, FHA and pertactin IgG was seen in 63%, 17% and 52% of children with verified pertussis, respectively. In most children without detectable antibody responses but with significant antibody decreases the first serum was obtained around the time of the peak. There was a more pronounced decline in pertussis toxin and pertactin IgG titers (4–5-fold) than in FHA IgG titers (1.8-fold). This decline was noted 1 to several months following the onset of symptoms.

If significant pertussis toxin and FHA IgG decreases were included in the diagnostic criteria the diagnosis would have been verified in 94% (84/89) of children with the clinical diagnosis of pertussis instead of 80% using only increases in pertussis toxin and FHA IgG. Thus in a patient coming for diagnosis 4 to 5 weeks after onset of cough it may be possible to confirm the diagnosis by obtaining a convalescent serum at least 4 weeks after the first serum.

Duration of antibodies

It is not possible to draw any firm conclusions about the decline of antibodies during subsequent years. In a few children in study II antibody titers declined after an initial response and then a second response was detected against one or more antigens one year after the

infection, without the children having had clinical or suspected pertussis. This indicates that exposure to pertussis in society induced an antibody response without causing clinical infection. The same was indicated from the children in study IV who were vaccinated with a pertussis toxoid vaccine and were later exposed to pertussis by siblings. The group with family exposure had higher GM FHA IgG antibody titers than the unexposed group. Since virtually all 10-year-old-children in study I had detectable FHA and pertactin IgG antibodies and the antibody levels were similar in children with and without a history of pertussis it seems plausible that the FHA IgG antibodies of the children with clinical pertussis (II) eventually declines to similar low levels and that pertactin IgG remains low. The pertussis toxin antibodies probably remain higher, despite the marked decline during the first year compared with FHA IgG, as indicated by the higher pertussis toxin IgG titers in the 10-year-olds with a history of pertussis (I).

Acquisition of FHA and pertactin antibodies without evidence of pertussis

In a previous study, 40/150 (27%) of non-vaccinated children 1 to 4 years old without a history of pertussis and without detectable pertussis toxin IgG antibodies had detectable FHA IgG antibodies [298]. The same children (a total of 266) were included in another study and, as mentioned above, all these children had detectable pertactin IgG antibodies [194]. The finding that almost all 10-year-old children in Study I also had FHA and pertactin IgG, irrespective of history of pertussis, raised the question of whether the antibodies were induced by exposure to *B. pertussis* or other crossreacting antigens.

Maternal antibodies

All but one of 71 children had FHA IgG antibodies in the initial sera obtained at 3 months of age, which declined in the later sera (IV). The same was observed in five infants in a previous study of FHA IgG antibodies, [94] and is the usual finding regarding maternally derived serum antibodies. About 70% of children had detectable pertactin IgG in their initial sera. The vaccine study (III) showed that 41% of children had maternally derived pertussis toxin antibodies.

Antibody increases

From about 1 year of age there were small but significant increases in geometric mean FHA and pertactin IgG antibody titers. The group of children exposed to pertussis in the family had a higher GM FHA IgG antibody titer but similar GM pertussis toxin and pertactin antibody titers. This indicates that exposure to *B. pertussis* results in exposure to

adhesins and the exposure results in an antibody response against FHA. Study II indicates that pertactin is less immunogenic than FHA and results in a more transient response. Since the children were protected by pertussis toxin IgG induced by vaccination and pertussis toxin seems to be an important colonisation factor for *B. pertussis* [131], the exposure to the organism may have been too transient to induce a detectable response to pertactin and pertussis toxin.

Crossreacting antibodies

Some of the children without family exposure to pertussis may have been exposed to pertussis in day-care centers or anywhere in society since pertussis is prevalent as shown by study I. *B. parapertussis* and *B. bronchiseptica* have pertactin and FHA, which induce cross-reacting antibodies [94,95,133]. Some children may have been exposed to *B. parapertussis*. This organism is, however, an uncommon cause of whooping cough-like disease in the Göteborg area. *B. parapertussis* was recovered in 1.4% of all *Bordetella* isolates at the Department of Clinical Bacteriology in Göteborg from September, 1991 to October, 1994 (G. Zackrisson, personal communication). *B. bronchiseptica* is an extremely rare cause of respiratory infection in Sweden as judged by 2421 samples, all negative for *B. bronchiseptica* by culture and/or PCR, obtained from patients with possible pertussis [20].

It has been suggested that cross-reacting antibodies against FHA may be induced by non-encapsulated *Haemophilus influenzae*. This organism has surface antigens similar to FHA and antibodies to FHA of *B. pertussis* recognise FHA of *H. influenzae* [303]. The organism is frequently found in children with or without signs of infection [304-307]. However, in a recent study of 16 patients aged 34 to 88 years with non-typable (non-encapsulated) *H. influenzae* verified pneumonia none of the patients had a significant increase in FHA IgG [308]. This indicates that infections with *H. influenzae* may have little clinical relevance for the prevalence of FHA IgG in the children in Studies I and IV, although studies of children with verified non-encapsulated *H. influenzae* infection are needed to rule out the possibility.

FHA has been shown to mimic the ligand for the leukocyte adhesion molecule CR3 on vascular endothelial cells, which promotes leukocyte transmigration into tissues. In an experiment, antibodies against FHA interfered with leukocyte transmigration and increased blood-brain barrier permeability [309]. The implications of this regarding the disease and vaccination with FHA are not known. It is not known whether crossreacting antibodies to CR3 can contribute to the high prevalence of FHA antibodies, but this seems highly speculative. Autoantibodies sometimes arise after vaccination due to sharing of aminoacid sequences between "foreign" and self antigens [310]. The risk

of inducing autoimmune FHA-CR3 antibodies with vaccines containing FHA should be taken into consideration.

No cross-reacting organisms to pertactin of *B. pertussis* other than *B. parapertussis* and *B. bronchiseptica* have been described. The high prevalence of pertactin antibodies in children irrespective of history of pertussis in this and other studies indicates that other organisms than *Bordetella* may have pertactin or antigens similar to pertactin.

Vaccination with a pertussis toxoid vaccine

Adverse reactions

Systemic reactions

No serious reactions occurred and no children left the study owing to adverse reactions. The proportion of children with a temperature >38 °C after an injection was similar to that of children vaccinated with DT vaccine only [261] and of children vaccinated with 13 different acellular vaccines [287]. Other systemic reactions: crying, drowsiness, anorexia and vomiting, were infrequent. Fussiness and sleeping more than usual were somewhat more common, but not more frequent than in children vaccinated with a DT vaccine only [261,275].

The normal temperature of an infant varies during the day and night and may reach temperatures close to 38°C during active periods [311]. After vaccination with whole-cell pertussis vaccine the rectal temperature may be elevated during the night compared with the child's normal temperature, although not evident as fever [312]. It is possible that the activity of the child or the time of the day when the temperature is taken could influence the results when a cut-off point of 38°C is used. An increase in temperature may also occur by chance, due due to a common viral infection, etc. Some of the children in the vaccine study (III) with an increase in temperature had signs of a common cold.

Behavioural changes noted after vaccination may also be part of normal variations. In a large study of reactions after vaccination an unblinded control group was included [313]. The control children received no injections. Still, behavioural changes were noted in 15% of controls. Irritability was noted in 15% of the control children and in 26% of vaccinated children ($p>0.1$) [313]. In controlled, blinded studies this normal variation is of little importance. Rare serious events, e.g. fatal infections, may, however, cause concern about the investigated vaccine [219].

Local reactions

No serious local reactions occurred in the present study. The frequency of local reactions was low (0%–11%) and similar to that of the DT vaccine administered concomitantly in the other thigh (Table 8) and to the frequencies reported for DT vaccines in other studies [261,275].

Direct comparison with other acellular pertussis vaccines is difficult owing to differences in study design. Key parameters to be used in acellular pertussis vaccine studies have recently been defined to facilitate comparisons [314]. All studied acellular vaccines were considerably less reactogenic than whole-cell vaccines, but there are variations between acellular vaccines attributable to the different antigens in the vaccine and to their immunogenicity, to the amount of each antigen, and to other substances contained in the vaccine [287]. The method of inactivating pertussis toxin may also be of importance. Many vaccines include formalin treated pertussis toxin, which may result in incomplete inactivation of the toxin [111].

It is known that subcutaneous administration of vaccines tends to cause more redness and swelling than intramuscular administration [315]. This must be considered when comparing results from clinical trials with different methods of vaccine administration. In most studies the pertussis vaccine was injected intramuscularly. In Sweden, all vaccines in the general vaccination program for children before 1996 were injected deep subcutaneously (BCG vaccine excepted). The vaccines of the first Swedish efficacy trial of acellular pertussis vaccines were administered subcutaneously [219] like the present pertussis toxoid vaccine. An approximate comparison of local reactions after the second vaccination with the vaccines in that trial and the present pertussis toxoid vaccine study indicates that the present vaccine may be somewhat more reactogenic.

Other components than pertussis toxoid may contribute to the adverse reactions, as indicated by the significantly higher frequency of local reactions to the PT-FHA vaccine in comparison with the PT vaccine in the first Swedish trial, despite the higher pertussis toxoid content of the monocomponent vaccine [219].

A comparison of local and systemic reactions after vaccination with the pertussis toxoid vaccine (III) and 13 other mono and multicomponent acellular vaccines combined with DT vaccine in a US trial is presented in table 10.

Table 10. Reactions within 48 hours of the first, second or third subcutaneous (s.c.) injection with the pertussis toxoid vaccine (Ptxd) (III) or "by the third evening" of the first, second or third intramuscular (i.m.) injection with one of 13 acellular vaccines in a US multicenter acellular pertussis vaccine trial. Data from Decker, 1995 [287].

Reaction	Ptxd s.c. injections (range, %)	13 acellular vaccines i.m. injections (range, %)
Redness or Swelling >2 cm	3–27	0–6
Tenderness/Pain	6–22	0–7
Fussiness	16–30	2–10
Sleeping more/Drowsy	10–21	5–31
Vomiting	4–12	1–13
Temperature > 38°C	1.4–3.4	0–3.5

In general, local reactions tended to be reported more frequently with the present vaccine, probably owing to the subcutaneous administration. Systemic reactions were seen in similar frequencies. When the pertussis toxoid vaccine combined with DT toxoids was administered intramuscularly in US infants, a lower incidence of local reactions was observed than when the same vaccine was administered subcutaneously in Swedish infants (data on file, Amvax Inc., Beltsville, MD, USA).

Induction of antibodies

All children in the present study (IV) had an antibody response after the two initial vaccinations. The vaccine induced a pronounced IgG antibody response against pertussis toxin after the third vaccination and although the antibodies declined with time, all children donating sera had detectable IgG antibodies at 2 and 3 years of age. Similar to the results from older children vaccinated with the vaccine, there was a slight IgM response and virtually no IgA response [316]. Slight or no IgM and IgA responses have previously been reported after vaccination with whole-cell or acellular pertussis vaccines [172,181,245,317].

Maternally derived serum antibodies are known to interfere with the antibody response of infants after some vaccinations, including acellular pertussis vaccines [318,319]. In this study the 59 children with detectable maternal antibodies in sera obtained at 3 months of age and the 85 without (sera not available from 1 child) had similar antibody responses and pertussis toxin antibody GM titer at 3 years of age (data not shown). Many of the children with high initial pertussis toxin antibody titers also had high antibody titers in sera at 3 years of age. Since the youngest children are most at risk from pertussis, vaccination at a young age is desirable.

Booster doses of diphtheria and tetanus toxoid vaccines are recommended for long lasting protection. In Sweden, a fourth dose of DT is given at the age of 10. It seems probable that booster doses of pertussis toxoid vaccine will also be needed. Booster doses of whole-cell pertussis vaccines have not been given after the age of 7 years due to the reactogenicity of such vaccines. But e.g. in the USA 5 doses are recommended; 4 during the first 18 months of life and a fifth dose at 4–6 years of age [320].

Pertussis in vaccinated and unvaccinated children

The diagnosis of pertussis at 2 and 3 years of age was based on the retrospective parental information on symptoms. It is possible that some of the control children with pertussis according to our clinical definition had a pertussis-like cough due to some agent other than *B. pertussis*. However, pertussis is a disease familiar to Swedish parents, owing to the high incidence of disease (study I). As shown in study I and a previous study of prevalence of pertussis toxin and FHA antibodies in relation to history of pertussis [298], the parental information is reliable. The incidence of pertussis in the control group (20%) was similar to the incidence at the age of 3 years estimated from study I and another Swedish study [21].

The difference in the proportion of children with typical pertussis with ≥6 weeks of cough in unvaccinated and vaccinated children (20% vs. 0%) indicated that this vaccine could confer substantial protection against severe disease. It is known from previous studies that pertussis whole-cell and acellular vaccines protect more against severe disease than against mild disease or infection [203,321]. This should also be valid for the present pertussis toxoid vaccine. It was found that among the vaccinated children there were 2 cases of confirmed pertussis with a shorter duration of cough, 2 and 4 weeks respectively. Thus, a different clinical case definition, e.g. the WHO definition of ≥3 weeks of paroxysmal cough [156] or inclusion of serology and cultures would have resulted in other numbers of children in the respective groups.

Correlation of pertussis toxin neutralizing and pertussis toxin IgG antibodies

Toxin neutralization assays measure antibody ability to neutralize the effect of the toxin excerted in certain animal or cell-culture systems. For many toxin-mediated bacterial infections neutralizing antibodies correlate with protection against disease. ELISA measures antibody concentration and antibody affinity [322]. In pertussis vaccine studies both assays have usually been performed since neutralizing antibodies are often assumed to have greater clinical relevance than ELISA. For

diphtheria and tetanus toxin neutralizing antibodies minimum protective levels have been defined [323,324]. Protective levels have not been defined for pertussis toxin neutralizing or IgG antibodies, [219,270,275] but there is evidence that serum antibodies against pertussis toxin are protective [62,63,298,301]. The role of cell-mediated immunity has not been much studied in children, but animal studies indicate that CD4+ Th 1 cells are of importance [325,326].

Generally speaking, ELISA is less time-consuming, less labour-intensive, less expensive and less subjective than neutralization assays. It would be positive if ELISA alone contributed sufficient information about the pertussis toxin antibody response.

The results of this study show that there is a significant correlation between pertussis toxin neutralizing antibodies measured by the CHO-cell assay and pertussis toxin IgG antibodies measured by ELISA ($r = 0.84$). However, it was not possible to predict the neutralizing antibody titer from IgG antibody titers in an individual serum with great accuracy. There was a better correlation at low and intermediate antibody concentrations than at high antibody concentrations. One explanation for this could be that at high antibody concentrations there may not be a larger proportion of pertussis toxin IgG antibodies involved in toxin neutralization, but they may still have sufficient affinity for the solid phase of the ELISA.

IgA and IgM antibodies may be toxin neutralizing, but were not assayed because the IgA and IgM antibody response after pertussis toxoid vaccination is virtually negligible compared with the IgG response as shown in the present vaccine study (III) and previous reports [172,181,245,317].

In analogy with the findings in this study, a good correlation has been shown between antibodies against tetanus and diphtheria toxin measured using ELISA and toxin neutralizing antibodies measured using animal or cell-culture assays [175,176,179,323,324]. The linear correlation ($r=0.84$) is in the same range as reported between diphtheria antibodies measured using a toxin neutralization assay and ELISA ($r=0.81$) [176]. Similar correlations were also recently reported in a study [327] of acellular and whole-cell pertussis vaccines: Spearman's Rho=0.59 to 0.85 (Rho=0.82 in the present study).

A comparison of two methods (end-point titer and parallel line) of calculating pertussis toxin antibody titers measured using ELISA was also performed. In this study there was a significant correlation ($r=0.96$) between the two methods of calculaton. A low correlation between the methods has previously been reported [170,171]. The high correlation in this study can be explained by the use of a reference serum assayed

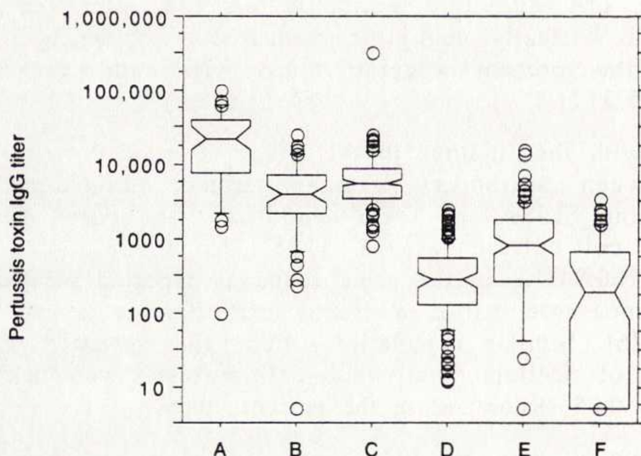
concomitantly on each microtiter plate since this increases the reproducibility of the assay [169].

Comparison of IgG antibodies to *B. pertussis* in healthy children, and after disease and vaccination

Since the same ELISAs for pertussis toxin, FHA and pertactin antibodies were used in all studies and performed at the same laboratory it can be assumed that conditions were relatively constant, although the sera from the different studies were assayed over a period of 3 years. Comparison between the results of different studies is therefore possible, although the comparison must be interpreted with some caution.

The pertussis toxin, FHA and pertactin IgG antibodies were assayed in 195 10-year-old unvaccinated children with and without a history of pertussis (I), in 89 children aged 1 month to 7 years with clinical pertussis (II) and in 71 children vaccinated with a pertussis toxoid vaccine at 3, 5 and 7, or 3, 5 and 12 months of age and who contributed 5 consecutive sera (III, part 1).

Figure 7 show the antibody titers for the different groups of children.



a

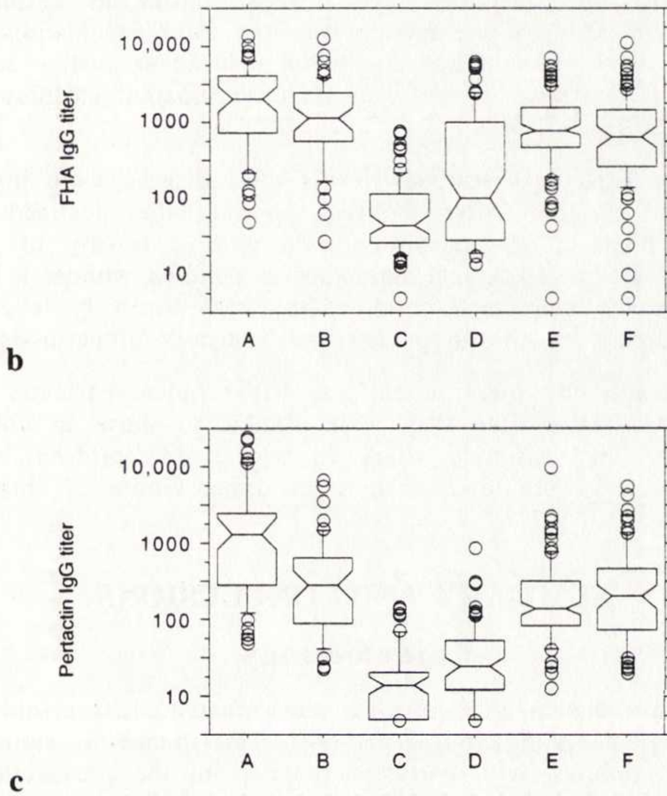


Figure 7. Box plot of pertussis toxin (a), FHA (b) and pertactin (c) IgG antibody titers in sera donated by 4 groups of children. Boxes show 25th and 75th percentiles and the 95% confidence interval (notch) around the median. Error bars show 10th and 90th percentiles. Outliers are presented individually. A circle may represent more than one identical value.

A: Sera from 54 children with culture confirmed pertussis or culture confirmed family exposure to pertussis obtained after a median of 17 weeks after onset of cough, and

B: sera from the same children obtained after a median of 56 weeks after onset.

C: Sera from 71 children, 8 or 13 months of age, obtained one month after the third injection with a pertussis toxoid vaccine, and

D: sera from the same children at 3 years of age and without a history of pertussis (III).

E: Sera from 119 10-year old children with a history of pertussis (I).

F: Sera from 76, 10-year old children without a history of pertussis (I).

Pertussis toxin IgG antibody levels were similar in children with pertussis and in children one month after the third vaccination with the Ptxd vaccine. At 3 years of age the toxoid induced antibodies declined to levels similar to those in 10-year-old-unvaccinated children with a history of pertussis.

The maximum FHA IgG antibody levels after disease were higher than in 10 year old children. After one year the antibodies declined to levels approaching those of 10-year-old-children with a history of pertussis. At 3 years of age the FHA IgG antibodies in children without a history of pertussis increased and some children had FHA antibody levels in the range of children with clinical pertussis or a history of pertussis.

Pertactin IgG antibody titers in children with clinical pertussis declined, and one year after disease they were similar to those in children 10 years of age. The antibody titers in 3-year-old children without a history of pertussis were lower than in the other groups of children.

SUMMARY AND CONCLUSIONS

Epidemiology

The study showed that 61% of 372 unvaccinated, 10-year-old Swedish children had a history of clinical pertussis. This number is similar to the proportion of children with pertussis reported in the prevaccination era in Sweden, indicating that socioeconomic changes in society during the last 50 years have not led to a decrease in the incidence of clinical pertussis.

In 91% of cases, the history could be confirmed by the presence of serum antibodies against pertussis toxin, a protein exotoxin produced by *Bordetella pertussis* only. Of children without a history of pertussis, 64% had pertussis toxin IgG antibodies, indicating that they had experienced subclinical or mild disease. The antibody titers were higher in children with, than in children without a history of pertussis. In all, 80% of the 10-year-old children had pertussis toxin antibodies as serological evidence of a *B. pertussis* infection.

Virtually all 10-year-old children had antibodies against FHA and pertactin, two surface located adhesins of *B. pertussis*. The antibody titers were similar in children with or without a history of pertussis. FHA and pertactin antibodies may have been induced by exposure to *B. pertussis* but may also have been induced by *B. parapertussis* which in Göteborg, is an uncommon cause of a generally mild, pertussis-like disease. Other microorganisms with cross-reacting antigens may have contributed to the high prevalence of FHA and pertactin IgG antibodies.

The clinical significance of cross-reacting antigens in children, e.g. FHA of *Haemophilus influenzae*, remains to be investigated.

In conclusion, 61% of 10-year-old, unvaccinated children had a history of pertussis. In 91% of them this could be confirmed by pertussis toxin serum antibodies. Of children without a history of pertussis 25% had pertussis toxin antibodies, indicating that they had had a mild form of disease. FHA and pertactin antibodies could be detected in almost all sera, irrespective of history of pertussis.

Antibodies after clinical pertussis

A significant pertussis toxin IgG antibody response was detected in 83% of the 54 children with clinical, culture confirmed pertussis or culture confirmed family exposure. A somewhat lower (74%) proportion of children had a FHA IgG antibody response, indicating that the diagnostic sensitivity of pertussis toxin IgG serology is somewhat higher than of FHA IgG serology.

Only 54% of children had a pertactin IgG antibody response and no child had an increase in pertactin IgG alone. Thus, the inclusion of pertactin IgG increases in the diagnostic criteria does not increase the sensitivity of serological diagnosis of pertussis.

A significant decline in pertussis toxin and FHA antibody titers were seen in 63% and 17% of children, respectively. The inclusion of significant decreases in pertussis toxin or FHA IgG antibody titers increased the proportion of children diagnosed by serology from 80% to 94% of 89 children with a clinical diagnosis of pertussis. The significant decline of pertussis toxin and FHA IgG antibodies after clinical pertussis should be added to the serological criteria of pertussis in children coming for a diagnosis 5–8 weeks after onset of symptoms, when the antibody peak has generally been passed.

FHA and pertactin antibodies without pertussis

Of 71 children vaccinated with a pertussis toxoid vaccine and with no symptoms of pertussis, all acquired serum IgG antibodies against FHA and 58 (82%) against pertactin by 3 years of age. Most had maternally derived antibodies in the first sera at 3 months of age, which then declined. From about 1 year of age there were small but significant increases in GM antibody titers, and in up to 30% of individual sera, against both antigens. The mere presence of IgG serum antibodies against FHA or pertactin is thus not a reliable indication of previous *B.*

pertussis infection, and significant increases may be caused by other organisms than *B. pertussis*.

The 12 children exposed to pertussis by siblings had significantly higher FHA IgG antibody titers at 3 years of age than 97 children not exposed to pertussis in the family. The pertussis toxin and pertactin antibody titers were similar in exposed and unexposed children. This indicates that exposure to *B. pertussis* can result in an FHA antibody increase without causing signs of infection.

Pertussis toxoid vaccine

All 145 children vaccinated at 3, 5 and 7, or 3, 5 and 12 months of age with a vaccine composed of pertussis toxin inactivated by hydrogen peroxide had detectable pertussis toxin IgG antibodies after the second vaccination. There was a significant response in sera obtained one month after the third vaccination in both groups of children. Children vaccinated at 3, 5 and 12 months of age had significantly higher GM pertussis toxin IgG antibody titers than children vaccinated at 3, 5 and 7 months of age. There were no serious systemic reactions. Local reactions were mild, and the frequency of local reactions was similar to the frequency of local reactions to a DT vaccine injected concomitantly in the other thigh at 3, 5 and 12 months of age.

An open, retrospective comparison of pertussis, defined as ≥ 6 weeks of cough with whooping attacks, vomiting or both, at 3 years of age in 142 vaccinated children and 284 age matched controls living in the same areas as the study children were performed. Of controls, 57 (20%) had experienced pertussis according to the definition. Sixteen vaccinated children were exposed by siblings to pertussis according to the clinical definition. Two of them had laboratory verified *B. pertussis* infection and a cough of 2 and 4 weeks duration. None of the study children had pertussis according to the clinical definition.

In conclusion, the results showed the vaccine to be safe, highly immunogenic and to confer substantial protection against typical pertussis. It was found to be justified to perform a large-scale double blind efficacy trial of the vaccine [275].

Pertussis toxin IgG and neutralizing antibodies

From 192 children vaccinated with the pertussis toxoid vaccine (III) 796 sera assayed for pertussis toxin IgG antibodies by ELISA and pertussis toxin neutralizing antibodies by CHO-cell assay were available for statistical comparison. There was a significant linear correlation

(r=0.84, p<0.0001) and analyses of variance showed significant interaction between antibodies measured by the two methods. However, an antibody titer obtained by one method could generally not be accurately used to predict a titer for the other method in an individual serum.

In conclusion, the study showed a significant correlation between pertussis toxin neutralizing antibodies and pertussis toxin antibodies measured using ELISA. In studies of antibody responses after vaccination with pertussis toxoid PT IgG ELISA alone can be used for pertussis toxin antibody determinations.

EPILOGUE

During 1995 and 1996 results of three double-blind placebo-controlled acellular vaccine trials were published [275,283,284]. The monocomponent pertussis toxoid vaccine, studied in the present thesis (III), and four different vaccines with two to five components each were tested in other parts of Sweden and in Italy. All acellular pertussis vaccines contained pertussis toxoid, and the multicomponent vaccines also contained one or more of the following antigens: FHA, pertactin and fimbriae 2 and 3. In all studies the acellular pertussis vaccines were combined with DT. All three studies included a control group of children who received only DT. Two studies included the US licensed Connaught whole-cell pertussis vaccine [283,284].

All studies showed that the acellular pertussis vaccines were considerably less reactogenic than whole-cell pertussis vaccines but the studies were too small to conclude with certainty that the acellular vaccines are less likely than whole-cell vaccines to cause rare but serious side effects, such as hypotonic hyporesponsive episodes and "persistent crying".

All acellular vaccines induced significant protection against pertussis with efficacy estimates (using the WHO definition of pertussis, page 18 [156]) varying between 59% and 85%. Although there were many similarities between the studies, it is not possible to draw any conclusions about differences in efficacy between the different acellular vaccines or as to whether the inclusion of other antigens in addition to pertussis toxoid confer improved protection compared with pertussis toxoid alone. Differences in study design, methods of sampling and modifications of the WHO definition used in the different studies exacerbate detailed comparison. Furthermore, the serological assays used in all studies included antibody determinations against pertussis toxin and FHA. Since all multicomponent vaccines included both pertussis toxoid and FHA, studies using these vaccines could not

determine antibodies against an antigen not included in the vaccines. The Göteborg trial on the other hand, which studied the monocomponent vaccine, could use a nonvaccine antigen, FHA, for serology.

One of the most unexpected findings was that the whole-cell pertussis vaccine which was included in two of the studies had a very low efficacy, 36-48%. Proponents of whole-cell vaccines now argue that the particular whole-cell vaccine was probably not representative for other whole-cell vaccines.

In June 1995 a mass vaccination project of the monocomponent pertussis toxoid vaccine began in the Göteborg area (population more than 600,000 inhabitants). The aim is to see wether the incidence of pertussis can be substantially reduced in the study area. Infants are vaccinated with the pertussis toxoid combined with DT toxoids at 3, 5 and 12 months of age. Children older than one year are offered the pertussis toxoid alone at the same intervals as infants. In January 1997, about 60% of children born between1990 and 1996 had received at least one injection, and 30% had received three injections. There are indications that the incidence of pertussis began to decrease during the second quarter of 1996, i.e. when the project had been ongoing for 9 months. Further follow-up of the pertussis incidence will be made during the coming years.

In Sweden, two different acellular pertussis vaccines combined with diphtheria and tetanus toxoids were licenced during 1996, the monocomponent pertussis toxoid vaccine discussed in this thesis and a 3-component vaccine (PT + FHA + pertactin). In Denmark, the monocomponent pertussis vaccine combined with DT and an inactivated polio vaccine has been used in the general vaccination program for infants at 3, 5 and 12 months of age since the beginning of 1997.

Conclusion

Available data clearly demonstrate the complexity of pertussis immunology and vaccination. Acellular pertussis vaccines are definitely less reactogenic than whole-cell vaccines, but the issue of rare serious adverse events has not yet been solved. Efficacy studies show that acellular vaccines are at least as efficacious as whole-cell vaccines and, even though there is no consensus about the antigen(s) to be included, the acellular vaccines can and should replace the whole-cell vaccines.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to:

Birger Trollfors, my supervisor, for his never ending support and enthusiasm for this study and for his untiring effort to confer to me some of his clear-sighted knowledge of medical research.

John Taranger, for valuable advise and constructive criticism and for contributions to all parts of this study.

Teresa Lagergård, for letting me work at her laboratory, for teaching me the secrets of the ELISA and for valuable contributions to the studies.

Gunilla Zackrisson, for all the work with the ELISA in the first studies.

My other coauthors; **Gunnel Hedvall**, for her friendly and encouraging support in the first initiated study and for contribution of patients, **Jan Johansson** and **Ian MacDowall**, for their generous support and contribution of patients in the vaccine study, and **John Robbins**, NICHD, for support and valuable advice.

Ingemar Kjellmer, Professor and encouraging overseer of the project.

Maja Berg, Eva Gunnarsson and **Anne Kinnunen**, for skillful performance of the ELISA and (Eva) of the CHO-cell assay, and for sharing your knowledge with me during the days in the laboratory.

Asta Eringe, for interviewing parents and always having a high spirit.

Nina Knutsson, Anna Lena Jönsson, Ann-Kristin Forsell, and **Wenche Thyrén,** for contacting parents and obtaining blood samples.

All nurses at the Department of Pediatrics, SU/Östra sjukhuset, the pediatric clinics in Borås and Mölndal, the pediatric out patient clinics in Västra Frölunda, Askim, Backa and Biskopsgården who contacted patients and collected blood samples during various parts of this study.

Margareta Ryden, for her kindness and help with many practical things.

Valter Sundh, for performing the statistical analyses of the vaccine study.

Berit Bähr and her colleges at the Laboratories of Immunology and of Clinical Chemistry at the Department of Pediatrics for generous help with blood sampling and taking care of hundreds of sera.

The personal of the **Medical Library**, SU/Östra sjukhuset for always being very helpful and kind.

Ildiko Marky, my medical chief and supervisor at the Division of Pediatric Oncology for letting me take time of to finish this work, and together with **Kirsti Pekkanen** and all other colleges and staff of ward 22 for friendship and an encouraging and stimulating working environment.

Kristina "**Kicki**", my beloved wife. **Sara, Josefin** and **Arvid**, my children. Without your support this work would never have been completed.

All wonderful children and their parents who saw the studies through; answered all the questions, filled in the forms, contributed serum. My sincere thanks are due to You.

The Research Fund of the Department of Pediatrics, the Göteborg Medical Society, the Petter Silfverskiöld Memorial Foundation, the Wilhelm and Martina Lundgren Foundation for Scientific Research, the Foundation "Samariten" and the National Institute of Child Health and Human Development contract NO1-HD-2905, who contributed generous economical support.

REFERENCES

1. Rosén von Rosenstein N. Underrättelser om barnsjukdomar och deras botemedel (The diseases of children and their remedies). The Nutrition Foundations' Reprints. Johnson Reprint Corporation. New York. 1977. Anders Sparrman (transl.).
2. Bordet J, Gengou O. Le microbe de la coqueluche. Ann Inst Pasteur 1906; 20:731-741
3. MacDonald H, MacDonald E J. Experimental pertussis. J Infect Dis 1933; 53:328-330
4. Romanus V, Jonsell R, Bergquist S-O. Pertussis in Sweden after the cessation of general immunization in 1979. Pediatr Infect Dis J 1987; 6:364 - 371
5. Taranger J. Kikhosta och kikhostevaccination - ett vaccinationspolitiskt dilemma. Läkartidningen 1982; 79:1935-1938
6. Fine P E, Clarkson J A. The recurrence of whooping cough: possible implications for assessment of vaccine efficacy. Lancet 1982; i:666-669
7. Binkin N J, Salmaso S, Tozzi A E, Scuderi G, Greco D. Epidemiology of pertussis in a developed country with low vaccination coverage: the Italian experience. Pediatr Infect Dis J 1992; 11:653-661
8. Farizo K M, Cochi S L, Zell E R, Brink E W, Wassilak S G, Patriarca P A. Epidemiological features of pertussis in the United States, 1980-1989. Clin Infect Dis 1992; 14:708-719
9. Centers for disease control and prevention. Pertussis - United States, January 1992-June 1995. MMWR 1995; 44:525-529
10. Halperin S A, Bortolussi R, MacLean D, Chisholm N. Persistence of pertussis in an immunized population: results of the Nova Scotia enhanced pertussis surveillance program. J Pediatr 1989; 115:
11. Miller E, Vurdien J E, White J M. The epidemiology of pertussis in England and Wales. Communicable disease report 1992; 2 Review No13:152-154
12. Kimura M, Kuno-Sakai H. Epidemiology of pertussis in Japan. Tokai J Exp Clin Med 1988; 13 Suppl:1-7
13. Ström J. Dödlighet och dödsorsaker hos spädbarn och småbarn mellan 1 och 5 år i Sverige från 1911-1945. Nord Med 1949; 41:915-921
14. Ström J. Om insjukningsfrekvensen i våra vanligaste epidemiska infektionssjukdomar belyst av förhållandena i Stockholm (About the incidense of our most common epidemic infectious diseases exemplified by the situation in Stockholm) in Swedish. Socialmed tidskrift 1959; 9:347-354
15. Ström J. Social development and declining incidence of some common epidemic diseases in children. Acta Paediatr Scand 1967; 56:159-163
16. Ström J. Is universal vaccination against pertusis always justified? Brit Med J 1960; 1184 - 1186
17. Rabo E. Kikhostans återkomst. Läkartidningen 1975; 72:2036-2038
18. Taranger J. Mild clinial course of pertussis in Swedish infants of today (letter). Lancet 1982; 1:1360
19. Krantz I, Taranger J, Trollfors B. Estimating incidence of whooping cough over time: a cross-sectional recall study of four Swedish birth cohorts. Int J Epidemiol 1989; 18:959-963
20. Reizenstein E. Laboratory diagnosis of *Bordetella* infection. DNA hybridization and PCR validated against serology and culture in a phase 3 vaccine efficacy trial. (Thesis). Microbiology and Tumorbiology Center, Karolinska Institute, Swedish Institute for Infectious Disease Control, Stockholm, Sweden. 1996
21. Mark A, Granström M. Cumulative incidence of pertussis in an unvaccinated preschool cohort based on notifications, interview and serology. Eur J Epidemiol 1991; 7:121-126

22. Mahieu J M, Muller A S, Voorhoeve A M, Dikken H. Pertussis in a rural area of Kenya: epidemiology and a preliminary report on a vaccine trial. Bull World Health Org 1978; 56:773-780
23. Linnemann Jr C C, Bass J W, Smith M H D. The carrier state in pertussis. Am J Epidemiol 1968; 88:422-427
24. Krantz I, Alestig K, Trollfors B, Zackrisson G. The carrier state in pertussis. Scand J Infect Dis 1986; 18:121-123
25. Field L H, Parker C D. Pertussis outbreak in Austin and Travis County, Texas, 1975. J Clin Microbiol 1977; 6:154-160
26. Huovila R. Clinical symptoms and complications of whooping cough in children and adults. Acta Paediatr Scand 1983; Suppl 298:13-20
27. Bass J W. Is there a carrier state in pertussis? (letter). Lancet 1987; i:96
28. Long S S, Lischner H W, Deforest A, Clark J L. Serologic evidence of subclinical pertussis in immunized children. Pediatr Infect Dis J 1990; 9:700-705
29. Long S S, Welkon C J, Clark J L. Widespread silent transmission of pertussis in families: Antibody correlates of infection and symptomatology. J Infect Dis 1990; 161:480-486
30. Aoyama T, Takeuchi Y, Goto A, Iwai H, Murase Y, Iwata T. Pertussis in adults. AJDC 1992; 146:163-166
31. Cromer B A, Goydos J, Hackell J, Mezzatesta J, Dekker C, Mortimer E A. Unrecognized pertussis infection in adolescents. Am J Dis Child 1993; 147:575-577
32. Cherry J D, Brunell P A, Golden G S, Karzon D T. Report of the task force on pertussis and pertussis immunization-1988. Pediatrics 1988; 81 (6 Suppl):939-984
33. Nielsen A, Olesen Larsen S. The epidemiology of whooping cough in Denmark before and after the introduction of vaccination for whooping cough. Vaccine efficacy and "herd immunity" (In Danish. Eng. abstract). Ugeskr Laeger 1990; 152:597-604
34. Kendrick P, Eldering G. A study in active immunication against pertussis. Am J Hyg 1939; 29:133-153
35. The prevention of whooping-cough by vaccination. A Medical Research Council investigation. Br Med J 1951; June 30:1463-1471
36. Johnston I D A, Hill M, Anderson H R, Lambert H P. Impact of whooping cough on patients and their families. Br Med J 1985; 290:1636-1638
37. Conway S P, Philips R R. Morbidity in whooping cough and measles. Arch Dis Child 1989; 64:1442-1445
38. Mark A, Granström M. Impact of pertussis on the afflicted child and family. Pediatr Infect Dis J 1992; 11:554-557
39. Christie C D C, Marx M L, Marchant C D, Reising S F. The 1993 epidemic of pertussis in Cincinnati. Resurgence of disease in a highly immunized population of children. N Engl J Med 1994; 331:16-21
40. Strangert K. Clinical course and prognosis of whooping-cough in Swedish children during the first six months of life. Scand J Infect Dis 1970; 2:45-48
41. Vincent J M, Wack R P, Person D A, Bass J W. Pertussis as the cause of recurrent bradycardia in a young infant. Pediatr Infect Dis J 1991; 10:340-342
42. Congeni B L, Orenstein D M, Nankervis G A. Three infants with neonatal pertussis. Clin Pediatrics 1978; 17:113-118
43. Christie C D C, Baltimore R S. Pertussis in neonates. Am J Dis Child 1989; 143:1199-1202
44. Heininger U, Stehr K, Cherry J D. Serious pertussis overlooked in infants. Eur J Pediatr 1992; 151:342-343
45. Johnston I D A, Anderson H R, Lambert H P. The severity of whooping cough in hospitalised children - is it declining? J Hyg Camb 1985; 94:151-161

46. Gillis J, Grattan-Smith T, Kilham H. Artificial ventilation in severe pertussis. Arch Dis Child 1988; 63:364-367

47. Southall D P, Thomas M G, Lambert H P. Severe hypoxaemia in pertussis. Arch Dis Child 1988; 63:598-605

48. Tam A Y-C, Yeung C-Y. Severe neonatal pertussis treated by salbutamol. Arch Dis Child 1986; 61:600-602

49. Nicoll A, Gardner A. Whooping cough and unrecognised postperinatal mortality. Archives of Disease in Childhood 1988; 63:41-47

50. Trollfors B. Clinical course of whooping cough in children younger than six months. Acta Paediatr Scand 1979; 68:323-328

51. Postels-Multani S, Schmitt H J, Wirsing von König C H, Bock H L, Bogaerts H. Symptoms and complications of pertussis in adults. Infection 1995; 23:139-142

52. UNICEF. State of the world's children 1992. Oxford University Press. New York. 1992. (14)

53. Bart K J, Feng-Ying K. Vaccine-preventable disease and immunization in the developing world. Pediatr Clin North Am 1990; 37:735-756

54. Garenne M, Ronsmans C, Campbell H. The magnitude of mortality from acute respiratory infections in children under 5 years in developing countries. Wld hlth statist quart 1992; 45:180-191

55. Bass J W. Erythromycin for treatment and prevention of pertussis. Pediatr Infect Dis J 1986; 5:154-157

56. Hoppe J E, and the erythromycin study group. Comparison of erythromycin estolate and erythromycin ethylsuccinate for treatment of pertussis. Pediatr Infect Dis J 1992; 11:189-193

57. Lewis K, Saubolle M A, Tenover F C, Rudinsky M F, Barbour S D, Cherry J D. Pertussis caused by an erythromycin-resistant strain of *Bordetella pertussis*. Pediatr Infect Dis J 1995; 14:388-391

58. Bergquist S-O, Bernander S, Dahnsjö H, Sundelöf B. Erythromycin in the treatment of pertussis: a study of bacteriologic and clinical effects. Pediatr Infect Dis J 1987; 6:458-461

59. de Serres G, Boulianne N, Duval B. Field effectiveness of erythromycin prophylaxis to prevent pertussis within families. Pediatr Infect Dis J 1995; 14:969-975

60. Granström G, Granström M. Effect of erythromycin treatment on antibody responses in pertussis. Scand J Infect Dis 1994; 26:453-457

61. Balagtas R C, Nelson K E, Levin S, Gotoff S P. Treatment of pertussis with pertussis immune globulin. J Pediatr 1971; 79:203-208

62. Granström M, Olinder-Nielsen A M, Holmblad P, Mark A, Hanngren K. Specific immunoglobulin for treatment of whooping cough. Lancet 1991; 338:1230-1233

63. Ichimaru T, Ohara Y, Hojo M, Miyazaki S, Harano K, Totoki T. Treatment of severe pertussis by administration of specific gamma globulin with high titers anti-toxin antibody. Acta Paediatr 1993; 82:1076-1078

64. Arico B, Rappuoli R. *Bordetella parapertussis* and *Bordetella bronchiseptica* contain transcriptionally silent pertussis toxin genes. J Bacteriol 1987; 169:2847-53

65. Vandamme P, Heyndrickx H, Vancanneyt M, Hoste B, de Vos P, Falsen E, Kersters K, Hinz K H. *Bordetella trematum* sp. nov., isolated from wounds and ear infections in humans, and reassessment of *Alcaligenes denitrificans* Rüger and Tan 1983. Int J Syst Bacteriol 1996; 46:849-858

66. Gordon J E, Hood R L. Whooping cough and its epidemiological anomalies. Am J Med Sci 1951; 222:333-361

67. Gustavsson O E A, Röken B O, Serrander R. An epizootic of whooping cough among chimpanzees an a zoo. Folia Primatol 1990; 55:45-50

68. Ashworth L A E, Fitzgeorge R B, Irons L I, Morgan C P, Robinson A. Rabbit nasopharyngeal colonozation by *Bordetella pertussis*: the effects of immunization on clearance and on serum and nasal antibody levels. J Hyg Camb 1982; 88:475-486

69. Preston N W, Timewell M, Carter E J. Experimental pertussis infection in the rabbit: similarities with infection in primates. J Infect 1980; 2:227-235

70. Cahill E S, O'Hagan D T, Illum L, Redhead K. Mice are protected against *Bordetella pertussis* infection by intra-nasal immunization with filamentous haemagglutinin. Fems Microbiol Lett 1993; 107:211-6

71. Woods D E, Franklin R, Cryz Jr S J, Ganss M, Peppler M, Ewanowich C. Development of a rat model for respiratory infection with *Bordetella pertussis*. Infect Immun 1989; 57:1018-1024

72. Friedman R L, Nordensson K, Wilson L, Akporiaye E T, Yocum D E. Uptake and intracellular survival of *Bordetella pertussis* in human macrophages. Infect Immun 1992; 60:4578-4585

73. Hazenbos W L, Geuijen C A W, van den Berg B M, Mooi F R, van Furt R. *Bordetella pertussis* fimbriae bind to human monocytes via the minor fimbrial subunit FimD. J Infect Dis 1995; 171:924-929

74. Pittman M. The concept of pertussis as a toxin-mediated disease. Pediatr Infect Dis J 1984; 3:467-486

75. Weiss A A, Hewlett E L. Virulence factors of *Bordetella pertussis*. Ann Rev Microbiol 1986; 40:661-86

76. Eldering G, Kendrick P. Bacillus parapertussis: a species resembling both bacillus pertussis and bacillus bronchisepticus but identical with neither. J Bacteriol 1938; 35:561-572

77. Porter J F, Connor K, Donachie W. Isolation and characterization of *Bordetella parapertussis*-like bacteria from ovine lungs. Microbiology 1994; 140:255-261

78. van der Zee A, Groenendijk H, Peeters M, Mooi F. The differentiation of *B. parapertussis* and *B. bronchiseptica* from man and animals as determined by DNA polymorphism mediated by two different insertion sequence elements suggests their phylogenetic relationship. Int J Syst Bacteriol 1996; accepted:

79. Lautrop H. Epidemics of *parapertussis*: 20 years observations in Denmark. Lancet 1971; 1:1195-1198

80. Heininger U, Stehr K, Schmitt-Grohe´ S, Lorenz C, Rost R, Christenson P D, Überall M, Cherry J D. Clinical characteristics of illness caused by *Bordetella parapertussis* compared with illness caused by *Bordetella pertussis*. Pediatr Infect Dis J 1994; 13:306-309

81. Wirsing von König C H, Finger H. Role of pertussis toxin in causing symptoms of *Bordetella parapertussis* infection. Eur J Clin Microbiol Infect Dis 1994; 13:455-458

82. Linneman Jr C C, Perry E B. *Bordetella parapertussis*. Recent experience and a review of the literature. Am J Dis Child 1977; 131:560-563

83. Borska K, Simkovicova M. Studies on the circulation of *Bordetella pertussis* and *Bordetella parapertussis* in populations of children. J Hyg Epid Microbiol 1972; 16:159-172

84. Hoppe J E, Preston N W. Whooping cough in West Germany (letter). Lancet 1985; ii:776

85. Mertsola J. Mixed outbreak of *Bordetella pertussis* and *Bordetella parapertussis* infection in Finland. Eur J Clin Microbiol 1985; 4:123-128

86. Reizenstein E, Lindberg L, Möllby R, Hallander H O. Validation of a nested *Bordetella* PCR in a pertussis vaccine trial. J Clin Microbiol 1996;

87. Isacson J, Trollfors B, Taranger J, Lagergård T. Acquisition of IgG serum antibodies against two *Bordetella* antigens (filamentous hemagglutinin and pertactin) in children with no symptoms of pertussis. Pediatr Infect Dis J 1995; 14:517-521

88. Lautrop H. Observations on *parapertussis* in Denmark, 1950-1957. Acta Path Microbiol Scand 1958; 43:255-266

89. Hallander H O, Storsaeter J, Möllby R. Evaluation of serology and nasopharyngeal cultures for diagnosis of pertussis in a vaccine efficacy trial. J Inf Dis 1991; 163:1046-1054

90. Gueirard P, Weber C, Coustumier A, Guiso N. Human *Bordetella bronchiseptica* infection related to contact with infected animals: persistance of bacteria in host. J Clin Microbiol 1995; 33:2002-2006

91. Woolfrey B F, Moody J A. Human infections associated with *Bordetella bronchiseptica.* Clin Microbiol Rev 1991; 4:243-255

92. Musser J M, Bemis D A, Ishikawa H, Selander R K. Clonal diversity and host distribution in *Bordetella bronchiseptica.* J Bacteriol 1987; 169:2793-2803

93. Kawai H, Aoyama T, Murase Y, Tamura C, Imaizumi A. A casual relationship between *Bordetella pertussis* and *Bordetella parapertussis* infections. Scand J Infect Dis 1996; 28:377-381

94. Granström M, Lindberg A A, Askelöf P, Hederstedt B. Detection of antibodies in human serum against the fimbrial hemagglutinin of *Bordetella pertussis* by enzyme linked immunosorbent assay. J Med Microbiol 1982; 15:85-96

95. Charles I G, Li L J, Strugnell R, Beesly K, Romanos M, Novotny P, Heron I, Jensen M, Manclark C R, Brennan M J, Fairweather N F. Repeat sequense motifs constitute the immunodominant regions of the p.69 protein, pertactin, from *Bordetella pertussis*: Comparison with repeat sequences from *B. parapertussis* and *B. bronchiseptica*. In: Manclark C R (ed.), Proceedings of the sixth international symposium on pertussis. Department of Health and Human Services, United States Public Health Service. Bethesda, MD. 1990. DHHS Publication No (FDA) 90-1164. 408 pages. pp 136-140

96. Marchitto K S, Smith S G, Locht C, Keith J M. Nucleotide sequence homology to pertussis toxin gene in *Bordetella bronchiseptica* and *Bordetella parapertussis*. Infect Immun 1987; 55:497-501

97. Taranger J, Trollfors B, Lagergård T, Zackrisson G. Parapertussis infection followed by pertussis infection. (Letter). Lancet 1994; 344:1703

98. Saran G, Angra S S, Balasubrahmanyan M. Serological and protective activities of pertussis, parapertussis and bronchiseptica vaccines. Indian J Med Res 1981; 74:815-820

99. Khelef N, Danve B, Quentin-Millet M-J, Guiso N. *Bordetella pertussis* and *Bordetella parapertussis*: two immunologically distinct species. Infect Immun 1993; 61:486-490

100. Kersters K, Hinz K-H, Hertle A, Segers P, Lievens A, Siegmann O, De Ley J. *Bordetella avium* sp. nov., isolated from the respiratory tracts of turkeys and other birds. Int J Syst Bacteriol 1984; 34:56-70

101. Vandamme P, Hommez J, Vancanneyt M, Monsieurs M, Hoste B, Coockson B, Wirsing von König C H, Kersters K, Blackall P J. *Bordetella hinzii* sp. nov., isolated from poultry and humans. Int J Syst Bacteriol 1995; 45:37-45

102. Cookson B T, Vandamme P, Carlson L C, Larson A M, Sheffild J V L, Kersters K, Spach D H. Bacteremia caused by a novel *Bordetella* species, "*B. hinzii*". J Clin Microbiol 1994; 32:2569-2571

103. Weyant R S, Hollis D G, Weaver R E, Amin M F M, Steigerwalt A G, O´Connor S P, Whitney A M, Daneshvar M I, Moss C W, Brenner D J. *Bordetella holmesii* sp. nov., a new Gram-negative species associated with septicemia. J Clin Microbiol 1995; 33:1-7

104. Lindquist S W, Weber D J, Mangum M E, Hollis D G, Jordan J. *Bordetella holmesii* sepsis in an asplenic adolescent. Pediatr Infect Dis J 1995; 14:813-815

105. Pittman M. Pertussis toxin: the cause of the harmful effects and prolonged immunity of whooping cough. A hypotesis. Rev Infect Dis 1979; 1:401-412

106. Roy C R, Miller J F, Falkow S. The bvgA gene of *Bordetella pertussis* encodes a transcriptional activator recuired for coordinate regulation of several virulence genes. J Bacteriol 1989; 171:6338-6344

107. Melton A R, Weiss A A. Environmental regulation of expression of virulence determinants in *Bordetella pertussis*. J Bacteriol 1989; 171:6206-6212

108. Tuomanen E, Schwartz J, Sande S. The vir locus affects the response of *Bordetella pertussis* to antibiotics: phenotypic tolerance and control of autolysis. J Infect Dis 1990; 162:560-563

109. Munoz J J. Biological activities of pertussigen (pertussis toxin). In: Sekura R Moss J Vaughan M. (eds.), Pertussis Toxin. Academic Press Inc. New York. 1985. 1-18

110. Pittman M, Furman B L, Wardlaw A C. *Bordetella pertussis* respiratory tract infection in the mouse: pathophysological responses. J Infect Dis 1980; 142:56-66

111. Robbins J B, Pittman M, Trollfors B, Lagergård T, Taranger J, Schneerson R. Primum non nocere: a pharmacologically inert pertussis toxoid alone should be the next pertussis vaccine. Pediatr Infect Dis J 1993; 12:795-807

112. Samore M H, Siber G R. Effect of pertussis toxin on susceptibility of infant rats to Haemophilus influenzae type b. J Infect Dis 1992; 165:945-948

113. Brooksaler F, Nelson J D. Pertussis. A reappraisal and report of 190 confirmed cases. Am J Dis Child 1967; 114:389-396

114. Regan J C, Tolstoouhov A. Relations of acid base equilibrium to the pathogenesis and treatment of whooping cough. NY State J Med 1936; 36:1075-1087

115. Badr-El-Din M K, Mazloum A G H, El-Towesy Y A, Kassem A S, Abdel-Moneim M A, Amr Abassy A. The beta-adrenergic receptors in pertussis. J Trop Med Hyg 1976; 79:213-217

116. Furman B L, Walker E, Sidey F M, Wardlaw A C. Slight hyperinsulinaemia but no hypoglycaemia in pertussis patients. J Med Microbiol 1988; 25:183-186

117. Toyota T, Kai Y, Kakizaki M, Sakai A, Goto Y, Yajima M, Ui M. Effects of islet-activating protein (IAP) on blood glucose and plasma insulin in healthy volunteers (phase I studies). Tohoku J Exp Med 1980; 130:105-116

118. Arai H, Sato Y. Separation and characterization of two distinct hamagglutinins contained in purified leucocytosis-promoting factor from *Bordetella pertussis*. Biochim Biophys Acta 1976; 444:765-782

119. Tamura M, Nogimori K, Murai S, Yajima M, Ito K, Katada T, Ui M, Ishii S. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. Biochemistry 1982; 21:5516-5522

120. Berkowitz F E. Bacterial exotoxins: how they work. Pediatr Infec Dis J 1989; 8:42-47

121. Burns D L, Hausman S Z, Lindner W, Robey F A, Manclark C R. Structural characterization of pertussis toxin A subunit. J Biol Chem 1987; 262:17677-17682

122. Burns D L. Subunit structure and enzymatic activity of pertussis toxin. Microbiol Sciences 1988; 5:285-287

123. Cieplak W, Burnette N W, Mar V L, Kaljot K T, Morris C F, Chen K K, Sato H, Keith J M. Identification of a region in the S1 subunit of pertussis toxin that is required for enzymatic activity and that contributes to the formation of a neutralizing antigenic determinant. Proc Natl Acad Sci 1988; 85:4667-4671

124. Nicosia A, Perugini M, Francini C, Casagli M C, Borri M G, Antoni G, Almoni M, Neri P, Ratti G, Rappuoli R. Cloning and sequencing of the pertussis toxin genes: operon structure and gene duplication. Proc Natl Acad Sci USA 1986; 83:4631-4635

125. Locht C, Keith J. Pertussis toxin gene: nucleotide sequence and genetic organization. Science 1986; 232:1258-1264

126. Bartoloni A, Pizza M, Tagliabue A, Rappuoli R. Engineering bacterial toxins for the development of a new vaccine against whooping cough. Pharmcol Res 1989; 21 supplement 2:19-25

127. Moss J, Vaughan M, Hewlett E L. Pertussis toxin-catalyzed ADP-ribosylation: effects on coupling of inhibitory receptors to adenylate cyclase. In: Sekura R Moss J Vaughan M. (eds.), Pertussis toxin. Academic Press Inc. New York. 1985. 105-147

128. Griese M, Sideropoulou O, Reinhardt D. Impaired formation of the second messenger cAMP im mononuclear blood cells of children with pertussis. Pediatr Res 1989; 25:209-213

129. Torre D, Zeroli C, Giola M, Fiori G P, Nespoli L, Daverio A, Ferrario G, Martegani R. Acute-phase proteins and levels of interleukin 1b, interleukin 6, tumor necrosis factor α, and interleukin 8 in children with pertussis. AJDC 1993; 147:27-29

130. Tuomanen E, Weiss A. Characterization of two adhesins of *Bordetella pertussis* for human ciliated respiratory-epithelial cells. J Infect Dis 1985; 152:118-125

131. Khelef N, Sakamoto H, Guiso N. Both adenylate cyclase and hemolytic activities are required by *Bordetella pertussis* to initiate infection. Microb Pathog 1992; 12:227-235

132. Ashworth L A E, Irons L I, Dowsett A B. Antigenic relationship between serotype specific agglutinogen and fimbriae of *Bordetella pertussis*. Infect Immun 1982; 37:1278-1281

133. Irons L I, Ashworth L A E, Wilton-Smith P. Heterogeneity of the filamentous hemagglutinin of *Bordetella pertussis* studied with monoclonal antibodies. J Gen Microbiol 1983; 129:2769-2778

134. Robinson A, Irons L I, Seabrook R N, Pearce A, Matheson M, Funnel S G P. Structure-function studies of *Bordetella pertussis* fimbriae. In: Manclark C R (ed.), Proceedings of the sixth international symposium on pertussis. Department of Health and Human Services, United States Public Health Service. Bethesda MD. 1990. DHHS Publication No (FDA) 90-1164. 408 pages. pp 126-135

135. Leininger E, Probst P G, Brennan M J, Kenimer J G. Inhibition of *Bordetella pertussis* filamentous hemagglutinin-mediated cell adherence with monoclonal antibodies. Fems Microbiol Lett 1993; 106:31-38

136. Shahin R D, Amsbaugh D F, Leef M F. Mucosal immunization with filamentous hemagglutinin protects against *Bordetella pertussis* respiratory infection. Infect Immun 1992; 60:1482-8

137. Roberts M, Cropley I, Chatfield S, Dougan G. Protection of mice against respiratory *Bordetella pertussis* infection by intranasal immunization with P.69 and FHA. Vaccine 1993; 11:866-72

138. Sato H, Sato Y. *Bordetella pertussis* infection in mice: correlation of specific ant ibodies against two antigens, pertussis toxin, and filamentous hemagglutinin with mouse protectivity in an intracerebral or aerosol chalange system. Infect Immun 1984; 46:415-421

139. Brennan M J, Li Z M, Cowell J L, Bisher M E, Steven A C, Novotny P, Manclark C R. Identification of a 69 kilodalton nonfimbrial protein as an agglutinogen of *Bordetella pertussis*. Infect Immun 1988; 56:3189-3195

140. Fernandez R C, Weiss A. Cloning and sequencing of a *Bordetella pertussis* serum resistance locus. Infect immun 1994; 62:4727-4738

141. Leininger E, Kenimer J G, Brennan M J. Surface proteins of *Bordetella pertussis*: role in adherence. In: Manclark C R (ed.), Proceedings of the Sixth International Symposium on Pertussis. Department of Health and Human Services, United States Public Health Service. Bethesda, MD. 1990. DHHS Publication No (FDA) 90-1164. 408 pages. pp 100-105

142. Capiau C, Carr S A, Hemling M E, Plainchamp D, Conrath K, Hauser P, Simoen E, Comberbach M, Roelants P, Desmons P, Permanne P, Pétre J O. Purification, Characterization and Immunological Evaluation of the 69-kDa Outer membrane protein of *Bordetella pertussis*. In: Manclark C R (ed.), Proceedings of the Sixth International Symposium on Pertussis. Department of Health and Human Services, United States Public Health Service. Bethesda, MD. 1990. DHHS Publication No (FDA) 90-1164. 408 pages. pp 75-85

143. Shahin R D, Brennan M J, Li Z M, Meade B D, Manclark C R. Characterization of the protective capacity and immunogenicity of the 69kDa outer membrane protein of *Bordetella pertussis*. J Experimental Med 1990; 171:63-73

144. Ashworth L A E, Dowsett A B, Irons L I, Robinson A. The location of surface antigens of *Bordetella pertussis* by immuno-electron microscopy. Dev Biol Standard 1985; 61:143-151

145. Vaccination against whooping-cough. The final report to the whooping-cough immunization committee of the medical research council and to the medical officers of health for Battersea and Wandsworth, Bradford, Liverpool and Newcastle. Brit Med J 1959; 994-1000

146. Decker M D, Edwards K M E. Report of the nationwide multicenter acellular pertussis trial. Pediatrics 1995; 96 (supplement):547-603

147. Robinson A, Gorringe A R, Funnell S G, Fernandez M. Serospecific protection of mice against intranasal infection with *Bordetella pertussis*. Vaccine 1989; 7:321-324

148. Mooi F R. Virulence factors of *Bordetella pertussis*. Antonie van Leeuwenhoek 1988; 54:

149. Hanski E. Invasive adenylate cyclase toxin of *Bordetella pertussis*. Trends Biochem Sci 1989; 14:459-463

150. Guiso N, Szatanik M, Rocancourt M. *Bordetella* adenylate cyclase: a protective antigen against lethality and bacterial colonization in murine respiratory and intracerebral models. In: Manclark C R (ed.), Proceedings of the sixth international symposium on pertussis. Department of Health and Human Services, United States Public Health Service. Bethesda, MD. 1990. DHHS Publication No. (FDA) 90-1164. 408 pages. pp 207-211

151. Zhang Y L, Sekura R D. Purification and characterization of the heat-labile toxin of *Bordetella pertussis*. Infect Immun 1991; 59:3754-3759

152. Cookson B T, Cho H-L, Herwaldt L A, Goldman W E. Biological activities and chemical composition of purified tracheal cytotoxin of *Bordetella pertussis*. Infect Immun 1989; 57:2223-2229

153. Goldman W E, Collier J L, Cookson B T, Marshall G R, Erwin K M. Tracheal cytotoxin of *Bordetella pertussis*: biosynthesis, structure and specificity. In: Manclark C R (ed.), Proceedings of the sixth international symposium on pertussis. Department of Health and Human Services, United States Public Health Service. Bethesda, MD. 1990. DHHS Publication No. (FDA) 90-1164. 408 pages. pp 5-12

154. Cundell D R, Kanthaukumar K, Taylor G W, Goldman W E, Flak T, Cole P J, Wilson R. Effect of tracheal cytotoxin from *Bordetella pertussis* on human neutrophil function in vitro. Infect Immun 1994; 62:639-643

155. Patriarca P A, Biellik R J, Sanden G, Burstyn D G, Mitchell P D, Silverman P R, Davis J P, Manclark C R. Sensitivity and specificity of clinical case definitions for pertussis. Am J Public Health 1988; 78:833-836

156. WHO meeting on case definition of pertussis. Geneva, 10-11 January 1991. MIM/EPI/PERT/91.1

157. Gehanno P, Lenoir G, Barry B, Bons J, Boucot I, Berche P. Evaluation of nasopharyngeal cultures for bacteriologic assessment of acute otitis media in children. Pediatr Infect Dis J 1990; 15:329-332

158. Bejuk D, Begovac J, Bace A, Kuzmanovic-Sterk N, Aleraj B. Culture of *Bordetella pertussis* from three upper respiratory tract specimens. Pediatr Infect Dis J 1995; 14:64-65

159. Hallander H O, Reizenstein E, Renemar B, Rasmuson G, Mardin L, Olin P. Comparison of Nasopharyngeal aspirates with swabs for culture of *Bordetella pertussis*. J Clin Microbiol 1993; 31:50-52

160. Lautrop H. Laboratory diagnosis of whooping-cough or *Bordetella* infections. Bull Wld Hlth Org 1960; 23:15-35

161. Hoppe J E. Methodes for isolation of *Bordetella pertussis* from patients with whooping cough. Eur J Clin Microbiol Infedt Dis 1988; 7:616-620

162. Hoppe J E, Vogl R. Comparison of three media for culture of *Bordetella pertussis*. Eur J Clin Microbiol 1986; 5:361-363

163. Aoyama T, Murase Y, Iwata T, Imaizumi A, Suzuki Y, Sato Y. Comparison of blood-free medium (Cyclodextrin solid medium) with Bordet-Gengou medium for clinical isolation of *Bordetella pertussis*. J Clin Microbiol 1986; 23:1046-1048

164. Engvall E, Pearlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry 1971; 8:871-874

165. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. J Immunol 1972; 109:129-135

166. Voller A, Bidwell D, Huldt G, Engvall E. A microplate method of enzyme-linked immunosorbent assay and its application to malaria. Bull Wld Hlth Org 1974; 51:209-211

167. Morse S I, Morse J H. Isolation and properties of the leucocytosis and lymphocytosis-promoting factor of *Bordetella pertussis*. J Exp Med 1976; 143:1483-1502

168. Voller A, Bidwell D. Enzyme-linked immunosorbent assay. In: Rose NR Friedman H Fahey JL. (eds.), Manual of clinical laborayory immunology, 3'edition. American Society for Microbiology. Washington DC. 1986. 99-109

169. Zackrisson G, Lagergård T, Lönnroth I. An enzyme-linked immunosorbent assay method for detection of immunoglobulins to pertussis toxin. Acta path microbiol immunol scand 1986; 94:227-231

170. Möllby R, Gadler H, Möllby H, Hallander H. Serological analysis in the pertussis vaccine trial. In: A clinical trial of acellular pertussis vaccines in Sweden, Technical Report. National Bacteriological Laboratory, Sweden. 1988.

171. Reizenstein E, Hallander H O, Blackwelder W C, Kühn I, Ljungman M, Möllby R. Comparison of five calculation modes for antibody ELISA procedures using pertussis serology as a model. J Immunol Methods 1995; 183:279-290

172. Manclark C R, Meade B D, Burstyn D G. Serological response to *Bordetella pertussis*. In: Rose NR Friedman H Fahey JL. (eds.), Manual of clinical laboratory immunology, 3'edition. American Society for Microbiology. Washington DC. 1986. 388-394

173. Sutton A, Vann W F, Karpas A B, Stein K E, Schneerson R. An avidin-biotin-based ELISA for quantitation of antibody to bacterial polysaccharides. J Immunol Meth 1985; 82:215-224

174. Phipps D C, West J, Eby R, Koster M, Madore D V, Quataert S A. An ELISA employing a Haemophilus influenzae type b oligosaccaride-human serum albumin conjugate correlates with the radioantigen binding assay. J Immunol Methods 1990; 135:121-128

175. Melville-Smith M E, Seagroatt V A, Watkins J T. A comparison of enzyme-linked immunosorbent assay (ELISA) with the toxin neutralization test in mice as a method for the estimation of tetanus antitoxin in human sera. J Biol Stand 1983; 11:137-144

176. Lagergård T, Trollfors B, Claesson B A, Karlberg J, Taranger J. Determination of neutralizing antibodies and specific immunoglobulin isotype levels in infants after vaccination against diphteria. Eur J Clin Microbiol Infect Dis 1992; 11:341-345

177. Relyveld E, Oato N H, Huet M, Gupta R K. Determination of antibodies to pertussis toxin in working reference preparations of anti-pertussis sera from various national control laboratories. Biologicals 1992; 20:67-71

178. Peters S M, Bellanti J A. Neutralization assays. In: Rose NR Friedman H Fahey JL. (eds.), Manual of Clinical Laboratory Immunology, 3'edition. American Society for Microbiology. Washington, DC. 1986. 67-71

179. Melville-Smith M, Balfour A. Estimation of Corynebacterium diphteriae in human sera: a comparison of an enzyme-linked immunosorbent assay with the toxin neutralisation test. J Med Microbiol 1988; 25:279-283

180. Gillenius P, Jäätmaa E, Askelöf P, Granström M, Tiru M. The standardization of an assay for pertussis toxin and antitoxin in microplate culture of Chinese hamster ovary cells. Journal of Biological Standardization 1985; 13:61-66

181. Macaulay M E. The IgM and IgG response to *Bordetella pertussis* vaccination and infection. J Med Microbiol 1981; 14:1 -7

182. Thomas M G, Redhead K, Lambert H P. Human serum antibody responses to *Bordetella pertussis* infection and pertussis vaccination. J Infect Dis 1989; 159:211-218

183. Thomas M G, Ashworth L A E, Miller E, Lambert H P. Serum IgG, IgA and IgM responses to pertussis toxin, filamentous hemagglutinin and agglutinogens 2 and 3 after infection with *Bordetella pertussis* and immunization with whole-cell pertussis vaccine. J Infect Dis 1989; 160:838-845

184. Tomoda T, Ogura H, Kurashige T. The longevity of the immune response to filamentous hemagglutinin and pertussis toxin in patients with pertussis in a semiclosed community. J Infect Dis 1992; 166:908-910

185. Tomoda T, Ogura H, Kurashige T. Immune responses to *Bordetella pertusis* infection and vaccination. J Infect Dis 1991; 163:559-563

186. Burstyn D G, Baraff L J, Peppler M S, Leake R D, St Geme Jr J, Manclark C R. Serological response to filamentous hamagglutinin and lymphocytosis-promoting toxin of *Bordetella pertussis*. Infect Immun 1983; 41:1150-1156

187. Viljanen M K, Ruuskanen O, Granberg C, Salmi T T. Serological diagnosis of pertussis: IgM, IgA and IgG antibodies against *Bordetella pertussis* measured by enzyme-linked immunosorbent assay (ELISA). Scand J Infect Dis 1982; 14:117-122

188. Zackrisson G, Krantz I, Lagergård T, Larsson P, Sekura R, Sigurs N, Taranger J, Trollfors B. Antibody response to pertussis toxin in patients with clinical pertussis measured by enzyme-linked immunosorbent assay. Eur J Clin Microbiol Infect Dis 1988; 7:149-154

189. Zackrisson G, Arminjon P, Krantz I, Lagergård T, Sigurs N, Taranger J, Trollfors B. Serum antibody response to filamentous hemagglutinin in patients with clinical pertussis measured by an enzyme-linked immunosorbent assay. Eur J Clin Microbiol Infect Dis 1988; 7:764-770

190. Granström M, Granström G, Lindfors A, Askelöf P. Serologic diagnosis of whooping cough by an enzyme-linked immunosorbent assay using fimbrial hemagglutinin as antigen. J Infect Dis 1982; 146:741-745

191. Granström G, Wretlind B, Salenstedt C-R, Granström M. Evaluation of serologic assays for diagnosis of whoopingh cough. J Clin Microbiol 1988; 26:1818-1823

192. Zackrisson G, Lagergård T, Trollfors B. Subclass composition of immunoglobulin G to pertussis toxin in patients with whooping cough, in healthy individuals, and in recipients of a pertussis toxoid vaccine. Journal of Clinical Microbiology 1989; 27:1567-1571

193. Redhead K. Serum antibody responses to the outher membrane proteins of *Bordetella pertussis*. Infect Immun 1984; 44:724-729

194. Trollfors B, Zackrisson G, Taranger J, Lagergård T. Serum antibodies against a 69-kilodalton outer membrane protein, pertactin, from *Bordetella pertussis* in nonvaccinated children with and without a history of clinical pertussis. J Pediatr 1992; 120:924-926

195. Arciniega J L, Hewlett E L, Johnson F D, Deforest A, Wassilak S G F, Onorato I M, Manclark C R, Burns D L. Human serologic response to envelope-associated proteins and adenylate cyclase toxin of *Bordetella pertussis*. J Infect Dis 1991; 163:135-142

196. Arciniega J L, Hewlett E L, Edwards K M, Burns D L. Antibodies to *Bordetella pertussis* adenylate cyclase toxin in neonatal and maternal sera. Fems Immunol Med Microbiol 1993; 6:325-30

197. Viljanen M K, Mertsola J, Kuronen T, Ruuskanen O. Class specific antibody response to lymphocytosis promoting factor (LPF) and fimbrie (F) in pertussis. Develop Biol Standard 1985; 61:337-340

198. Mannerstedt G. Pertussis in adults. J Pediatr 1934; 5:596-600

199. Lambert H J. Epidemiology of a small pertussis outbreak in Kent County, Michigan. Public Health Report 1965; 80:365-369

200. Trollfors B, Rabo E. Whooping cough in adults. Br Med J 1981; 283:696-697

201. Graham E E. The treatment of pertussis with vaccine. Am J Dis Child 1912; 3:41-49

202. Madsen T. Vaccination against whooping cough. JAMA 1933; 101:187-188

203. Fine P E M, Clarkson J A. Reflections on the efficacy of pertussis vaccines. Rev Infect Dis 1987; 9:866-883

204. Schmitt H J, Wagner S. Pertussis vaccines - 1993. Eur J Pediatr 1993; 152:462-466

205. Mark A. Studies on adjustments in the Swedish DTP vaccination programme (Thesis). University of Göteborg, Sweden. Dept of Pediatrics. 1993

206. Ström J. Further experience of reactions, especially of a cerebral nature, in conjunction with triple vaccination: a study based on vaccinations in Sweden 1959-65. Brit Med J 1967; 320-323

207. Kulenkampff M, Schwartzman J S, Wilson J. Neurological complications of pertussis inoculation. Arch Dis Child 1974; 49:46-49

208. Socialstyrelsen: Skoglund G. Kikhosta - profylax och behandling (in Swedish) Whooping cough - prophylaxis and treatment. Meddelandeblad 1982; 23/82. Dnr FAP 3 3354-670/82

209. Kimura M, Kuno-Sakai H. Immunization system in Japan: its history and present situation. Acta Paediatr Jpn 1988; 30:109-126

210. Vaccination against whooping-cough. Relation between protection in children and results of laboratory tests. A report to the whooping-cough immunization committee of the medical research council and to the medical officers of health for Cardiff, Leeds, Leyton, Manchester, Middlesex, Oxford, Poole, Tottenham, Walthamstow, and Wembley. BMJ 1956; 2:454-462

211. Pittman M. Variability of the potency of pertussis vaccine in relation to the number of bacteria. J Pediatr 1954; 45:57-68

212. Dellepiane N I, Manghi M A, Eriksson P V, di Paola G, Cangelosi A. Pertussis whole cell vaccine: relation between intracerebral protection in mice and antibody response to pertussis toxin, filamentous hemagglutinin and adenylate cyclase. Int J Med Microbiol Virol Parasitol Infect Dis 1992; 277:65-73

213. WHO expert committee on biological standardization. Sixteenth Report. Annex 1. Requirements for pertussis vaccine. Wld Hlth Org techn Rep Ser 1964; 274:24-40

214. Perkins F T. Vaccination against whooping-chough. BMJ 1969; 2:429-430

215. Efficacy of whooping-cough vaccines used in the United Kingdom before 1968. Final report to the director of the public health laboratoy service by the public health laboratory service whooping-cough committe and working party. Br Med J 1973; 259-262

216. Preston N W. Type-specific immunity against whooping-cough. BMJ 1963; 2:724-726

217. Preston N W. Efectiveness of pertussis vaccines. BMJ 1965; 2:11-13

218. Tiru M, Askelöf P, Granström M, Hallander H. *Bordetella pertussis*. Serotype of clinical isolates in Sweden during 1970—1995 and influence of vaccine efficacy studies. In: International Symposium on Pertussis Vaccine Trials. Oct. 30—Nov. 1. Rome, Italy. 1995.

219. Ad Hoc Group for the Study of Pertussis Vaccines. Placebo controlled trial of two acellular pertussis vaccines in Sweden: protective efficacy and adverse events. Lancet 1988; 955-960. [Erratum, Lancet 1988;1:1238]

220. Isomura S. Efficacy and safety of acellular pertussis vaccine in Aichi prefecture, Japan. Pediatr Infect Dis J 1988; 7:258-262

221. Preston N W, Matthews R C. Immunological and bacteriological distinction between parapertussis and pertussis (letter). Lancet 1995; 345:463-464

222. Hopkins R S. Reactions to DTP vaccine, by lot and manufacturer: results of a survey in Montana. In: Manclark C H and Hill J C (eds.), International symposium on pertussis. United States Department of Health, Education, and Welfare. Public Health Service. National Institutes of Health. Bethesda, MD. 1978. DHEW publication No. (NIH)79-1830. pp 300-303

223. Griffith A H. Reactions after pertussis vaccine: a manufacturer's experiences and difficulties since 1964. BMJ 1978; 1:809-815

224. Zakharova M S, Malivanova O M, Sokolovskaya A D, Shavrova E N, Kapustik L A. The results of study of pertussis component of DTP vaccine produced in USSR and some other countries. J Hyg Epidemiol Microbiol Immunol 1981; 25:439-448

225. Edwards K M, Decker M D, Halsey N A, Koblin B A, Townsend T, Auerbach B, Karzon D T. Differences in antibody response to whole-cell pertussis vaccines. Pediatrics 1991; 88:1019-1023

226. Baker J D, Halperin S A, Edwards K, Miller B, Decker M, Stephens D. Antibody response to *Bordetella pertussis* antigens after immunization with American and Canadian whole-cell vaccines. J Pediatr 1992; 121:523-527

227. Steinhoff M C, Reed G F, Decker M D, Edwards K M, Englunf J A, Pichichero M E, Rennels M B, Anderson E L, Deloria M A, Meade B D. A randomized comparison of reactogenicity and immunogenicity of two whole-cell pertussis vaccines. Pediatrics 1995; 96 (supplement):567-570

228. Efficasy of pertussis vaccination in England. Report from the PHLS Epidemiological Research Laboratory and 21 Area Health Authorities. Br Med J 1982; 285:357-359

229. Bell J A. Pertussis profylaxis with two doses of alum-precipitated vaccine. Public Health Rep 1941; 56:1535-1546

230. Centers for disease control. Pertussis - United States 1982-1983. MMWR 1984; 33:673-575

231. Mertsola J, Viljanen M K, Ruuskanen O. Current status of pertussis and pertussis vaccination in Finland. Annals Clin Research 1982; 14:253-259

232. Centers for disease control. Pertussis. MMWR 1987; 36:168-171

233. Mebel S, Dittman S. Experiences with pertussis vaccination in GDR. Develop Biol Standard 1979; 43:101-106

234. Trollfors B. *Bordetella pertussis* whole cell vaccines - efficacy and toxicity. Acta Paediatr Scand 1984; 73:417-425

235. Onorato I M, Wassilak S G, Meade B. Efficacy of whole-cell pertussis vaccine in preschool children in the United States [see comments]. Jama 1992; 267:2745-9

236. Fine P E M, Clarkson J A, Miller E. The efficasy of pertussis vaccines under conditions of household exposure. Further analysis of the 1978-80 PHLS/ERL study in 21 Area Health Authorities in England. Int J Epidemiol 1988; 17:635-642

237. Kenyon T A, Izurieta H, Shulman S T, Rosenfeld E, Miller M, Daum R, Strebel P M. Large outbreak of pertussis among young children in Chicago, 1993: investigation of potential contributing factors and estimation of vaccine effectiveness. Pediatr Infect Dis J 1996; 15:655-661

238. Wilson A T, Henderson I R, Moore E J H, Heywood S N. Whooping-cough: difficulties in diagnosis and ineffectiveness of immunization. Brit Med J 1965; 2:623-626

239. Ditchburn R K. Whooping cough after stopping pertussis immunisation. Br Med J 1979; 1:1601-1603

240. Mink C M, Sirota N M, Nugent S. Outbreak of pertussis in a fully immunized adolescent and adult population. Arch Pediatr Adolesc Med 1994; 148:153-157

241. Jenkinson D. Duration of effectiveness of pertussis vaccine: evidence from a 10 year community study. Br Med J 1988; 296:612-614

242. Stewart G T. Whooping cough and pertussis vaccine. Br Med J 1983; 287:287-289

243. Bass J W, Wittler R R. Return of epidemic pertussis in the United States. Pediatr Infect Dis J 1994; 13:343-345

244. Morgan Mink C, Cherry J D, Christenson P, Lewis K, Pineda E, Shlian D, Dawson J A, Blumberg D A. A search for *Bordetella pertussis* infection in university students. Clin Infect Dis 1992; 14:464-71

245. Cherry J D, Beer T, Chartrand S A, DeVille J, Beer E, Olsen M A, Christenson P D, Moore C V, Stehr K. Comparison of values of antibody to *Bordetella pertussis* antigens in young German and American men. Clin Infect Dis 1995; 20:1271-1274

246. Rosenthal S, Strebel P, Cassiday P, Sanden G, Brusuelas K, Wharton M. Pertussis among adults during the 1993 outbreak in Chicago. J Infect Dis 1995; 171:

247. Stewart G T. Toxicity of pertussis vaccine: frequency and probability of reactions. J Epidemiol Commun Health 1979; 33:150-156

248. Cowan L D, Griffin M R, Howson C P, Katz M, Johnston Jr R B, Shaywitz B A, Fineberg H V. Acute encephalopathy and chronic neurological damage after pertussis vaccine. Vaccine 1993; 11:1371-1379

249. Hoffman H J, Hunter J C, Damus K, Pakter J, Peterson D R, van Belle G, Hasselmeyer E G. Diphteria-tetanus-pertussis immunization and sudden infant death: Results of the National Institute of Child Health and Human Development cooperative epidemiological study of sudden infant death syndrome risk factors. Pediatrics 1987; 79:598-611

250. Miller D L, Ross E M. National childhood encephalopaty study: an interim report. Br Med J 1978; 992-993

251. Miller D L, Ross E M, Alderslade R, Bellman M H, Rawson N S. *B. pertussis* immunisation and serious acute neurological illness in children. Br Med J 1981; 282:1595-1599

252. Miller D, Madge N, Diamond J, Wadsworth J, Euan R. Pertussis immunisation and serious acute neurological illnesses in children. Br Med J 1993; 307:1171-1176

253. Brahams D. Pertussis vaccine: court finds no justification for association with permanent brain damage (medicine and the law). Lancet 1988; i:837

254. Bowie C. Lessons from the pertussis vaccine court trial. Lancet 1990; 335:397-399

255. Griffith A H. Permanent brain damage and pertussis vaccination: is the end of the saga in sight? Vaccine 1989; 7:199-210

256. Wentz K R, Marcuse E K. Diphteria-tetanus-pertussis vaccine and serious neurological illness: an updateed review of the epidemiological evidence. Pediatrics 1991; 87:287-297

257. Griffin M R, Ray W A, Mortimer E A, Fenichel G M, Schaffner W. Risk of seizures and encephalopathy after immunization with the diphteria-tetanus-pertussis vaccine. JAMA 1990; 263:1641-1645

258. Brahams D. Pertussis vaccine litigation (correspondent). Lancet 1990; 335:905-906 (comment by Eggington p 1162)

259. Miller D L, Alderslade R, Ross E M. Whooping cough and whooping cough vaccine: the risks and benefits debate. Epidemiol Rev 1982; 4:1-24

260. Koplan J P, Schoenbaum S C, Weinstein M C, Fraser D W. Pertussis vaccine - an analysis of benefits, risks and costs. New Eng J Med 1979; 301:906-911

261. Cody C L, Baraff L J, Cherry J D, Marcy M S, Manclark C R. Nature and rates of adverse reactions associated with DTP and DT immunizations in infants and children. Pediatrics 1981; 68:650-660

262. Mortimer E A, Jones P K. An evaluation of pertussis vaccine. Rev Infect Dis 1979; 1:927-932

263. Edwards K M, Karzon D T. Pertussis Vaccines. Pediatr Clin N Am 1990; 37:549-566

264. Kanai K. Japan's experience in pertussis epidemiology and vaccination in the past thirty years. Japan J Med Sci Biol 1980; 33:107-143

265. Sato Y, Kimura M, Fukumi H. Development of a pertussis component vaccine in Japan. Lancet 1984; 122-126

266. Kimura M, Kuno-Sakai H. Pertussis vaccines in Japan. Acta Paediatr Jpn 1988; 30:143-153

267. Kimura M, Kuno-Sakai H. Current epidemiology of pertussis in Japan. Pediatr Infect Dis J 1990; 9:705-709

268. Kimura M, Kuno-Sakai H. Developments in pertussis immunisation in Japan. Lancet 1990; 336:30-32

269. Storsaeter J, Olin P. Relative efficacy of two acellular pertussis vaccines during three years of passive surveillance. Vaccine 1992; 10:142-144

270. Storsaeter J, Blackwelder W C, Hallander H O. Pertussis antibodies, protection, and vaccine efficacy after household exposure. Am J Dis Child 1992; 146:167-72

271. Edwards K M, Bradley R B, Decker M D, Palmer P S, Van Savage J, Taylor C J, Dupont W D, Hager C C, Wright P F. Evaluation of a new highly purified pertussis vaccine in infants and children. J Infect Dis 1989; 160:832-837

272. Pichichero M E, Francis A B, Marsocci S M, Green J L, Disney F A, Meschievitz C. Safety and immunogenicity of an acellular pertussis vaccine booster in 15- to 20-month-old children previously immunized with acellular or whole-cell pertussis vaccine as infants [see comments]. Pediatrics 1993; 91:756-60

273. Pichichero M E, Green J L, Francis A B, Marsocci S M, Lynd M, Litteer T. Comparison of a three-component acellular pertussis vaccine with whole cell pertussis vaccine in two-month-old children. Pediatr Infect Dis J 1994; 13:193-196

274. Bernstein H H, Rothstein E P, Pichichero M E, Francis A B, Kovel A J, Disney F A, Green J L, Marsocci S M, Lynd A M, Wood G C, et a l. Clinical reactions and immunogenicity of the BIKEN acellular diphtheria and tetanus toxoids and pertussis vaccine in 4- through 6-year-old US children [published errata appear in Am J Dis Child 1992 Jul;146(7):802 and 1992 Sep;146(9):1084]. Am J Dis Child 1992; 146:556-9

275. Trollfors B, Taranger J, Lagergård T, Lind L, Sundh V, Zackrisson G, Lowe C U, Blackwelder W, Robbins J B. A placebo-controlled trial of a pertussis-toxoid vaccine. N Engl J Med 1995; 333:1045-1050

276. Halperin S A, Eastwood B, Baretto L, Mills E, Blatter M, Reisinger K, Bader G, Keyserling H, Roberts E A, Guasparini R, Medd L, Humphreys G. Safety and immunogenicity of two acellular pertussis vaccines with different pertussis toxoid and filamentous hemagglutinin content in infants 2-6 months old. Scand J Infect Dis 1995; 27:279-287

277. Podda A, Carapella D L E, Titone L, Casadei A M, Cascio A, Bartalini M, Volpini G, Peppoloni S, Marsili I, Nencioni L, Rappuoli R. Immunogenicity of an acellular pertussis vaccine composed of genetically inactivated pertussis toxin combined with filamentous hemagglutinin and pertactin in infants and children. J Pediatr 1993; 123:81-4

278. Podda A, De Luca E C, Titone L, Casadei A M, Cascio A, Peppoloni S, Volpini G, Marsili I, Nencioni L, Rappuoli R. Acellular pertussis vaccine composed of genetically inactivated pertussis toxin: safety and immunogenicity in 12- to 24- and 2- to 4-month-old children. J Pediatr 1992; 120:680-5

279. Feldman S, Perry S, Andrew M, Jones L, Moffitt J E. Comparison of acellular (B type) and whole-cell pertussis-component diphtheria-tetanus-pertussis vaccines as the first booster immunization in 15- to 24-month-old children. J Pediatr 1992; 121:857-61

280. Blumberg D A, Mink C M, Cherry J D, Reisinger K S, Blatter M M, Congeni B L, Dekker C L, Stout M G, Mezzatesta J R, Scott J V, Christenson P D. Comparison of an acellular pertussis-component diphteria-tetanus-pertussis (DTP) vaccine with a whole-cell pertussis-component (DTP) vaccine in 17- to 24 month-old children, with measurement of 69-kilodalton outer membrane protein antibody. J Pediatr 1990; 117:46-51

281. Blennow M, Granström M, Jäätmaa E, Olin P. Primary immunization of infants with an acellular pertussis vaccine in a double-blind randomized clinical trial. Pediatrics 1988; 82:293-299

282. Kamiya H, Nii R, Matsuda T, Yasuda N, Christenson P D, Cherry J D. Immunogenicity and reactogenicity of Takeda acellular pertussis -component diphteria-tetanus-pertussis vaccine in 2- and 3-month-old children in Japan. Am J Dis Child 1992; 146:1141-1147

283. Gustafsson L, Hallander H O, Olin P, Reizenstein E, Storsaeter J. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. N Engl J Med 1996; 334:349-355

284. Greco D, Salmaso S, Mastrantonio P, Giuliano M, Tozzi A E, Anemona A, Ciofi-degli-Atti M L, Giammanco A, Panei P, Blackwelder W C, Klein D L, Wassilak S G, the Progetto Pertosso Working Group. A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. Progetto Pertosse Working Group. N Engl J Med 1996; 334:341-348

285. Gupta R K, Saxena S N, Sharma S B, Ahuja S. Protection of mice inoculated with purified pertussis toxin and filamentous haemagglutinin against intracerebral challange with live *Bordetella pertussis*. Vaccine 1990; 8:289

286. Robinson A, Funnell S G. Potency testing of acellular pertussis vaccines. Vaccine 1992; 10:139-41

287. Decker M D, Edwards K M, Steinhoff M C, Rennels M B, Pichichero M E, Englund J A, Anderson E A, Deloria M A, Reed G F. Comparison of 13 acellular pertussis vaccines: adverse reactions. Pediatrics 1995; 96 (supplement):557-566

288. Taranger J, Trollfors B, Lagergård T, Isacson J, Lowe C, Robbins J. Immunogenicity of a new triple toxoid vaccine against diphteria, tetanus and pertussis. In: First european paediatric congress, 9th—12th March. Paris, France. 1994. Abstract book. IMM 38

289. Askelöf P, Gillenius P. Effect of lymphocytosis-promoting factor from *Bordetella pertussis* on cerebellar cyclic GMP levels. Infect Immun 1982; 36:958-961

290. Sekura R D, Zhang Y-l. Pertussis toxin: structural elements involved in the interaction with cells. In: Sekura R Moss J Vaughan M (eds.), Pertussis toxin. Academic Press Inc. New York. 1985. pp 45-64

291. Askelöf P, Granström M, Gillenius P, Lindberg A A. Purification and characterisation of a fimbrial haemagglutinin from *Bordetella pertussis* for use in an enzyme-linked immunosorbent assay. J Med Microbiol 1982; 15:73-83

292. Hewlett E, Sauer K, Myers G, Cowell J, Guerrant R. Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. Infect Immun 1983; 40:1198-1203

293. Trollfors B, Krantz I, Sigurs N, Taranger J, Zackrisson G, Roberson R. Toxin-neutralizing antibodies in patients with pertussis, as determined by an assay using Chinese hamster ovary cells. J Infect Dis 1988; 158:991-995

294. Sekura R D, Zhang Y-l, Roberson R, Acton B, Trollfors B, Tolson N, Shiloach J, Bryla D, Muir-Nash J, Koeller D, Schneerson R, Robbins J B. Clinical, metabolic, and antibody responses of adult volonteers to an investigational vaccine composed of pertussis toxin inactivated by hydrogen peroxide. J Pediatr 1988; 113:806-813

295. Giammanco A, Chiarini A, Stroffolini T, De Mattia D, Chiaramonte M, Moschen M E, Mura I, Rigo G, Taormina S, Sarzana A, Mazza G, Scarpa B. Seroepidemiology of pertussis in Italy. Rev Infect Dis 1991; 13:1216-1220

296. Stroffolini T, Giammanco A, De Crescenzo L, Lupo F, Nicosia V, Torres G, Valenza A R, Cascio A, Taormina S, Nisticò L, Chiarini A. Prevalence of pertussis IgG antibodies in children in Palermo, Italy. Infection 1989; 17:280-283

297. Bassili W R, Stewart G T. Epidemiological evaluation of immunisation and other factors in the control of whooping cough. Lancet 1976; 1:471-474

298. Zackrisson G, Taranger J, Trollfors B. History of whooping cough in nonvaccinated Swedish children, related to serum antibodies to pertussis toxin and filamentous hemagglutinin. J Pediatr 1990; 116:190-194

299. Meade B D, Mink C M, Manclark C R. Serodiagnosis of pertussis. In: Manclark C R (ed.), Proceedings of the Sixth International Symposium on Pertussis. Department of Health and Human Services United States Public Health Service. Bethesda, MD. 1990. DHSS publication no (FDA) 90-1164. 408 pages. pp 322-329

300. Edwards K M, Meade B D, Decker m D, Reed G F, Rennels M B, Steinhoff M C, Anderson E L, Englund J A, Pichichero M E, Deloria M A, Deforest A. Comparison of 13 acellular pertussis vaccines: overview and serologic response. Pediatrics 1995; 96:548-557

301. Granström M, Granström G. Serological correlates in whooping cough. Vaccine 1993; 11:445-448

302. Guiso N, Grimprel G, Anjak I, Be'gue' P. Western blot analysis of antibody responses of young infants to pertussis infection. Eur J Clin Microbiol Infect Dis 1993; 12:596-600

303. Barenkamp S J. Outer membrane proteins and lipopolysaccharides of nontypeable Haemophilus influenzae. J Infect Dis 1992;

304. Branefors-Helander P, Nylén O, Jeppsson P H. Acute otitis media, A bacteriological study. ORL J Otorinolaryngol relat spec 1972; 34:281-295

305. Ingvarsson L, Lundgren K, Ursing J. The bacterial flora in the nasopharynx in healthy children. Acta Otolaryngol, Suppl 1982; 386:94-96

306. Söderström M, Hovelius B, Schalén C. Decreased absence due to infectious diseases in children at two day care centres over an eight-year interval. Acta Paediatr Scand 1990; 79:454-460

307. Weiss A, Brinser J H, Nazar-Stewart V. Acute conjunctivitis in childhood. J Pediatr 1993; 122:10-14

308. Trollfors B, Burman L, Lagergård T, Leinonen M, Taranger J. No cross-reactivity with filamentous hemagglutinin of *Bordetella pertussis* in sera from patients with nontypable Haemophilus influenzae pneumonia. Pediatr Infect Dis J 1996; 15:558-559

309. Tuomanen E I, Prasad S M, George J S, Hoepelman A I, Ibsen P, Heron I, Starzyk R M. Reversible opening of the blood-brain barrier by anti-bacterial antibodies. Proc Natl Acad Sci U S A 1993; 90:7824-8

310. Ada G L. The immunological principles of vaccination. Lancet 1990; 335:523-526

311. Anderson E S, Petersen S A, Wailo M P. Factors influencing the body temperature of 3-4 month old infants at home during the day. Arch Dis Child 1990; 65:1308-1310

312. Rawson D, Petersen S A, Wailoo M P. Rectal temperature of normal babies the night after first diphteria, pertussis and tetanus vaccination. Arch Dis Child 1990; 65:1305-1307

313. Cherry J D. The epidemiology of pertussis and pertussis immunization in the United Kingdom and the United States: a comparative study. Curr Probl Pediatr 1984; 14:1-78

314. Pichichero M E, Christy C, Decker M D, Steinhoff M C, Edwards K M, Rennels M B, Anderson E L, Englund J A. Defining the key parameters for comparing reactions among acellular and whole-cell pertussis vaccines. Pediatrics 1995; 96:588-592

316. Krantz I, Sekura R, Trollfors B, Taranger J, Zackrisson G, Lagergård T, Schneerson R, Robbins J. Immunogenicity and safety of a pertussis vaccine composed of pertussis toxin inactivated by hydrogen peroxide, in 18- to 23-month-old children. J Pediatr 1990; 116:539-543

317. Heininger U, Cherry J D, Christenson P D. Comparative study of Lederle/Takeda acellular and Lederle whole-cell pertussis-component diphteria-tetanus-pertussis vaccines in infants in Germany. Vaccine 1994; 12:81-86

318. Booy R, Aitken S J, Taylor S, Tudor- Williams G, Macfarlane J A, Moxon E R, Ashworth L A E, Mayon-White R T, Griffiths H, Chapel H M. Immunogenicity of combined diphtheria, tetanus, and pertussis vaccine given at 2, 3, and 4 months versus 3, 5, and 9 months of age [see comments]. Lancet 1992; 339:507-510

319. Schmitt H J, Schuind A, Knuf M, Beutel K, Schulte-Wissermann H, Gahr M, Schult R, Folkens J, Rauh W, Bogaerts H, Bork H L, Clemens R. Clinical experience of a tricomponent acellular pertussis vaccine combined with diphteria and tetanus toxoids for primary vaccination in 22,505 infants. J Pediatr 1996; 129:695-701

320. American Academy of Pediatrics. Committee on Infectious Diseases. 1994 Red Book: Report of the Committee on Infectious Diseases. Twenty-third edition. Peter G. (ed.). 1994.

321. Blackwelder W C. Acellular pertussis vaccine efficacy determined from clinical criteria. In: Manclark C R (ed.), Proceedings of the sixth international symposium on pertussis. Department of Health and Human Services, United States Public Health Service. Bethesda, MD. 1990. DHHS Publication No (FDA) 90-1164. 408 pages. pp 295-298

322. Butler J, Feldbush T, McGivern P, Stewart N. The enzyme-linked immunosorbent assay (ELISA): A measure of antibody concentration or affinity? Immunochemistry 1978; 15:131-136

323. Ipsen J. Circulating antitoxin at onset of diphteria in 425 patients. J Immunol 1946; 54:325-347

324. Christenson B, Böttiger M. Epidemiology and immunity to tetanus in Sweden. Scand J Infect Dis 1987; 19:429-435

325. Mills K H, Barnard A, Watkins J, Redhead K. Cell-mediated immunity to *Bordetella pertussis*: role of Th1 cells in bacterial clearance in a murine respiratory infection model. Infect Immun 1993; 61:399-410

326. Redhead K, Watkins J, Barnard A, Mills K H. Effective immunization against *Bordetella pertussis* respiratory infection in mice is dependent on induction of cell-mediated immunity. Infect Immun 1993; 61:3190-8

327. Meade B D, Lynn F, Reed G F, Mink C M, Romani T A, Deforest A, Deloria M A. Relationships between functional assays and enzyme immunoassays as measurements of responses to acellular and whole-cell pertussis vaccines. Pediatrics 1995; 96:595-600

På grund av upphovsrättsliga skäl kan vissa ingående delarbeten ej publiceras här.
För en fullständig lista av ingående delarbeten, se avhandlingens början.

Due to copyright law limitations, certain papers may not be published here.
For a complete list of papers, see the beginning of the dissertation.

GÖTEBORGS UNIVERSITET

Tryckt & Bunden
Vasastadens Bokbinderi AB
1997